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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 45, Issue of November 6, pp. 29577–29585, 1998

Printed in U.S.A.

Peroxisome Proliferator-activated Receptor α-Isoform Deficiency Leads to Progressive Dyslipidemia with Sexually Dimorphic Obesity and Steatosis*

(Received for publication, May 19, 1998, and in revised form, August 11, 1998)

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The α-isoform of the peroxisome proliferator-activated receptor (PPARα) is a nuclear transcription factor activated by structurally diverse chemicals referred to as peroxisome proliferators. Activators can be endogenous molecules (fatty acids/steroids) or xenobiotics (fibrate lipid-lowering drugs). Upon pharmacological activation, PPARα modulates target genes encoding lipid metabolism enzymes, lipid transporters, or apolipoproteins, suggesting a role in lipid homeostasis. Transgenic mice deficient in PPARα were shown to lack hepatic peroxisomal proliferation and have an impaired expression and induction of several hepatic target genes. Young adult males show hypercholesterolemia but normal triglycerides. Using a long term experimental set up, we identified these mice as a model of monogenic, spontaneous, late onset obesity with stable caloric intake and a marked sexual dimorphism. Serum triglycerides, elevated in aged animals, are higher in females that develop a more pronounced obesity than males. The latter show a marked and original centrilobular-restricted steatosis and a delayed occurrence of obesity. Fat cells from their liver express substantial levels of PPARγ2 transcripts when compared with lean cells. These studies demonstrate, in rodents, the involvement of PPARα nuclear receptor in lipid homeostasis, with a sexually dimorphic control of circulating lipids, fat storage, and obesity. Characterization of this pathological link may help to delineate new molecular targets for therapeutic intervention and could lead to new insights into the etiology and heritability of mammalian obesity.

Obesity, an increasing health problem in wealthy societies, has been causatively linked to hyperlipidemia, diabetes, hypertension, and atherosclerosis. Adipose cell hypertrophy and hyperplasia occur as the ultimate consequence of a disequilibrium in energy balance and exert adverse effects on longevity (1, 2). Several causal genetic determinants responsible for spontaneous monogenic obesity in mice (*ob*, *db*, *tub*, A^r, and *fat* genes) have been identified (reviewed in Ref. 3). In humans, a limited number of obese syndromes have been related to single gene disorders (*e.g.* Ahlstrom, Bardet Biedl, Cohen, Prader Willi). Recently, two mutations, affecting the leptin signal transduction pathway and leading to human early onset morbid obesity, have been characterized. They affect the *ob* gene (4), encoding leptin, and the leptin receptor gene (5), respectively. A mutation in the human prohormone convertase 1 gene, leading to childhood obesity, has been documented (6), and tissue-specific attenuation of the prohormone convertase 2 gene has been reported in two patients with Prader-Willi syndrome (7). Proconvertases act, proximally to carboxypeptidase E, in the pathway of post-translational processing of prohormones and neuropeptides, therefore associating this syndrome with the *fat*/*fat* murine phenotype. Prevalence of these mutations in the human obese population was reported as being rather limited (4, 5), and human counterparts of the *tub* and A^r murine defective loci have not been identified (8). Consequently, the etiology of numerous human obesity syndromes remains elusive. Results using transgenic models engineered to alter fat mass through manipulation of a specific metabolic protein or to alter energy intake or energy expenditure have been recently reviewed (9). They have reemphasized that obesity is most frequently a polygenic disorder. Considerable evidence points to the contribution of multiple genetic determinants to human obesity (10, 11). Additionally, the recent and rapid emergence of the obesity pandemic underlines the role of environmental factors.

Although several transcription factors can promote adipogenesis, their direct implication in mammalian obesity remains to be fully substantiated (3). Among them, peroxisome proliferator-activated receptor (PPARγ),[1] a nuclear receptor activated by the insulin-sensitizing drugs thiazolidinediones and oxidized linoleic acid metabolites (12), can trigger the differentiation of various progenitor cells of mesenchymal origin into adipocytes and the monocyte/macrophage differentiation (13). The α-isoform of the receptor, PPARα, is predominantly a hepatic transcription modulator activated by eicosanoids, fatty acids, and peroxisome proliferators (PP) (14–16) through direct interaction with the receptor as demonstrated in several reports (14, 17). Among PP are the widely prescribed lipid-lowering drugs of the fibrate (phenoxyisobutyrate) class. When administrated to rodents, the PP that are not genotoxic produce a liver-specific stereotypical response resulting in peroxisomal proliferation, hepatomegaly, and ultimately hepatocel-

* This work was supported by Région Midi Pyrénées Grant 9600469, Association pour la Recherche sur le Cancer Grant 1386, Centre National de la Recherche Scientifique Grant 96/C/04, and by the Fondation pour la Recherche Medicale. Parts of this work were reported at the XIIth International Symposium on Microsomes and Drug Oxidation, Montpellier, France, July 20–24, 1998. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; PP, peroxisome proliferator(s); bp, base pair(s); PCR, polymerase chain reaction; RT, reverse transcription; LDL, low density lipoprotein; VLDL, very low density lipoprotein; RXR, retinoid X receptor.

lular carcinoma (18). In humans, peroxisome proliferation and hepatocarcinoma are not observed, while beneficial serum triglyceride and cholesterol lowering by fibrates is retained (19).

PP trigger the coordinate transcriptional gene modulation of an array of proteins located in diverse cellular compartments (*e.g.* microsomal cytochrome P450 4A (20), the peroxisomal acyl-coenzyme A oxidase/bifunctional enzyme/3-ketoacyl-CoA thiolase (21, 22), the cytosolic acyl-CoA-binding protein/liver fatty acid-binding protein (L-FABP) (23, 24), and mitochondrial malic enzyme/acyl-CoA dehydrogenase (25, 26)). These observations established the pivotal role of PPARα in the regulation of lipid oxidation (peroxisomal and mitochondrial β-oxidation, microsomal ω-hydroxylation). Additionally, PPARα influences lipid trafficking by altering the gene expression of lipoproteine lipase and apolipoprotein C-III (apoC-III) in the liver, thus leading to a significant serum triglyceride lowering (27, 28). Consequently, PPARα could represent a key mediator of lipid homeostasis, as a physiological sensor of lipid levels (16) and a molecular regulator of an array of lipid metabolism-related genes.

Generation of PPARα knockout mice (PPARα −/−) established that the receptor is a prerequisite for hepatic peroxisome proliferation and coordinate induction of acyl-coenzyme A oxidase, bifunctional enzyme, thiolase, cytochrome P450 4A 1/3, and liver fatty acid-binding protein genes by clofibrate (29, 30). Furthermore, the role of PPARα in the duration of the inflammatory response induced by leukotriene B_4, a bona fide natural ligand of the receptor, was confirmed in these mice (15). This model, which exhibits reduced levels of high density lipoprotein cholesterol and apolipoprotein A-I (apoA-I), was thoroughly investigated under pharmacological conditions of treatment by the extremely potent PP Wy 14,643 (31). An additional set of hepatic genes (apoA-I, apoC-III) was shown to be under PPARα-dependent modulation by PP, suggesting a causal link with the hypotriglyceridemic capability of fibrates. Nevertheless, a provocative paradigm remained: how to reconcile the putative influence of PPARα on lipid metabolism with the absence of a documented, naturally occurring, null phenotype. In the absence of any pharmacological influence, our experimental procedures were designed to substantiate putative pathophysiological traits observed in both sexes. We report that PPARα deficiency leads to an original form of monogenic, late onset, spontaneous obesity with a stable caloric intake and a remarkable sexual dimorphism. The specificity and extrapolation of this phenotype will be discussed.

EXPERIMENTAL PROCEDURES

Animals and Diet—Care of mice was according to institutional guidelines. PPARα −/− mice originated from homologous recombinant 129Sv-derived cells. Chimeric males were initially backcrossed to C57BL/6 females. For the purpose of this study, several additional rounds of backcrossing were performed in our animal facility to increase the C57BL/6 genetic background (common to several murine obese phenotypes) and to generate the animals used. We developed an original PCR-based animal screening, using a set of primers located at both ends of exon 8 of the PPARα gene that amplified either 200 bp (wild type) or 1250 bp (knockout) from tail DNA (forward, 5′-ATCGGCCTGGCCTTCTAAAC-3′; backward, 5′-GTCTCTGTAGATCTCTTGC-3′). Mice were housed in conventional conditions, under 12 h/12 h dark/light cycles, each group being divided in two cages of five animals each. Sampling of animals was designed to reduce the influence of interfering parameters such as parental imprinting or litter specificity. C57BL/6 controls were from IFFA-CREDO (Les Oncins, France). Mice received a regular chow diet, 3200 kcal/kg (UAR, Epinay, France) containing 4.5% lipids. Water and food were available *ad libitum*. Prior to phenobarbital anesthesia and euthanasia, blood was collected at the orbital sinus.

Lipid Analysis—Upon collection, serum samples were stabilized (32) and stored 16 h at 4 °C prior to analysis. Serum lipids were evaluated as a single batch of samples on a Cobas FARA apparatus (Roche, Paris) using biochemical reagents (Bio-Merieux, Marcy l'Etoile). Hepatic lipids were extracted from liquid nitrogen-frozen samples from the same anatomical site (liver large lobe) and analyzed by gas chromatography (cholesterol) and biochemical test (triglycerides) (Bio-Merieux, Marcy l'Etoile, France) according to published protocols (33).

Liver Samples and Organ Dissociation—Following euthanasia, livers were dissected, and fragments of the large lobe were treated by a formaldehyde solution (10% in 1× phosphate-buffered saline) for 48 h prior to paraffin embedding. Hematoxilin/eosin-stained sections were 5 μm thick, while frozen sections for oil red-O staining, embedded in Tissue-Teck (Miles, Inc., Kankakee, IL), were 15 μm thick.

In situ liver dissociation was performed by collagenase perfusion according to published protocols (34) except that solutions and media were buffered with Hepes (pH 7.4). Flow rates for rinsing solution and dissociation solution were 5 and 4 ml/min, respectively. Cells resuspended in Dulbecco's modified Eagle's medium following filtration on a sterilized gauze were centrifuged (3 min, 120 × *g*); fat-loaded cells were collected as a distinct top ring.

RNA Purification and RT-PCR—Total RNA was prepared from 100 μg of fresh fragments of liver large lobe or 300 μl of dissociated cell suspension, homogenized in 1 ml of Trizol solution (Life Technologies, Paris) using a tissue homogenizer (Ika, Jena, Germany) with a 7-mm diameter probe. Four μg of RNAs were reverse transcribed with Moloney murine leukemia virus reverse transcriptase, and 250 ng were used for PCR with *Taq* polymerase (enzymes from Life Technologies). Mouse primer sequences and PCR parameters were as follows: PPARγ2, 5′-TGTTGACCCAGAGCATGTGCCTT-3′ (forward, PPARγ2-specific) and 5′-TCGTGTAGATGATCTCATGGACACCA-3′ (reverse, shared with PPARγ1) for 38 cycles; LHS, 5′-GCACTTCTGGAAAGCCTTCTGG-3′ (forward, exon 3) and 5′-GAAGGAGTTGAGCCATGAGGAGG-3′ (reverse, exon 7) for 45 cycles; aP2, 5′-ATGTGTGATGCCTTTGTGGGAACCTG-3′ (forward, exon 1) and 5′-CTCTTGTGGAAGTCACGCCTTTCA-3′ (reverse, exon 4) for 37 cycles; adipoQ, 5′-TGCAAGCTCTCCTGTTCCTCTTAACC-3′(forward) and 5′-TAGAGTCCCGGAATGTTGCAGTAGAAC-3′ (reverse) for 45 cycles; and rat albumin, 5′-CCTAGTCCTGATTGCCTTTTCCCAGT-3′ (forward) and 5′-CTCAACAAAGTCAGCAGCTATTGAGGG-3′ (reverse) for 40 cycles. Following gel electrophoresis and transfer on Gene Screen Plus membrane (DuPont, Les Ulis, France), hybridization was performed with random priming radiolabeled specific cDNA probes (Amersham Pharmacia Biotech, Orsay, France). Autoradiograms were quantified with a scanner (Bioimage, Roissy, France).

RESULTS

We randomly selected control and deficient mice from different litters for the purpose of this study. They were kept under identical conditions of housing and feeding and received a standard mouse chow diet (3200 kcal/kg) suitable for reproduction. Under these conditions, 7–11-week-old PPARα −/− mice, like those investigated in previous reports (29–31, 35), exhibit no phenotypic abnormalities and at this age are morphologically undistinguishable from wild type age-matched controls. Surprisingly, an apparent obesity developed specifically among 6–12-month-old breeders of the PPARα deficient colony kept under standard dietary conditions. To substantiate our supposition of a delayed pathophysiological trait in the PPARα −/− mice, we implemented a protocol aimed at monitoring the body weight gain relative to food intake and the adipose tissue mass at various critical locations (36–38). Weekly recordings of individual weight during 8 months revealed an increased body growth rate for PPARα-deficient mice compared with controls and a marked sexual dimorphism in the kinetics of weight gain (Fig. 1). By the age of 219 days, weight differences between PPARα −/− mice and controls were 40 and 18.6% for females and males, respectively. A durable, statistically significant difference of body weight was established between females of both genotypes by 69 days, while it occurred only at 168 days for males. Statistical analysis of daily food intake monitoring indicated no increase of caloric intake in PPARα −/− animals compared with wild type, when expressed per gram of body weight (Fig. 1). Relative body weight increase among aged PPARα-deficient animals was obvious with 200-day-old females (Fig. 2*A*).

To get insights into the source of the observed differential phenotype, animals followed for long term weight gain and food intake monitoring were euthanized at 240 days, and white

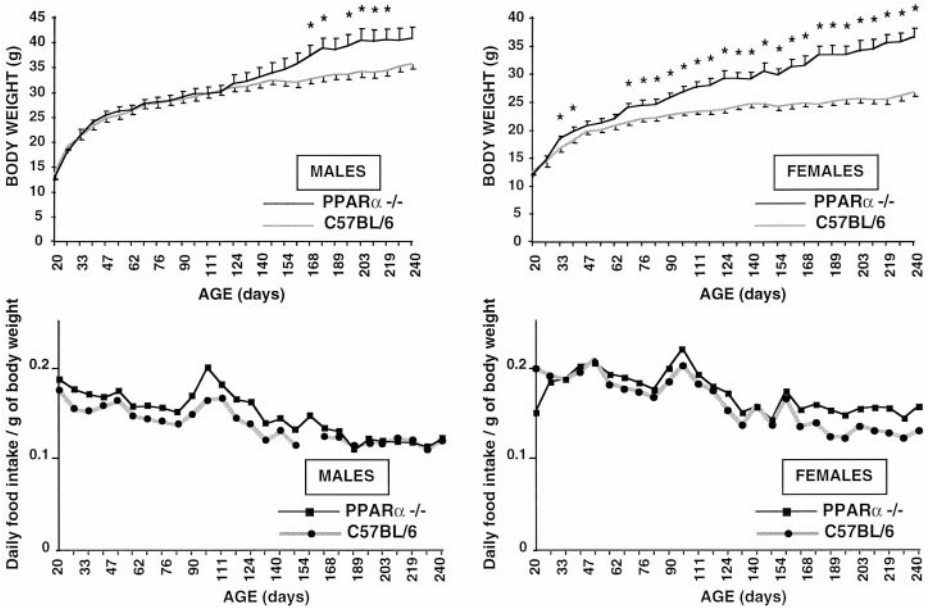

FIG. 1. *Top,* PPARα-deficient mice display a kinetics of body weight gain different from C57BL/6 controls over a 240-day-long study. PPARα −/− and C57BL/6 males and females (10 animals in each group) were individualy weighed weekly. Results are presented as means ± S.E. (PPARα −/− and C57BL/6, respectively). Statistical significance of observed differences between means of different groups was assessed by the Mann and Whitney statistical test and is represented by an *asterisk*. *Bottom*, daily food intakes were calculated weekly by mesuring the total food consumption of groups of five mice caged together. Results, per gram of body weight, are expressed as means of two calculations of the parameter, arising from two subgroups for each group of animals.

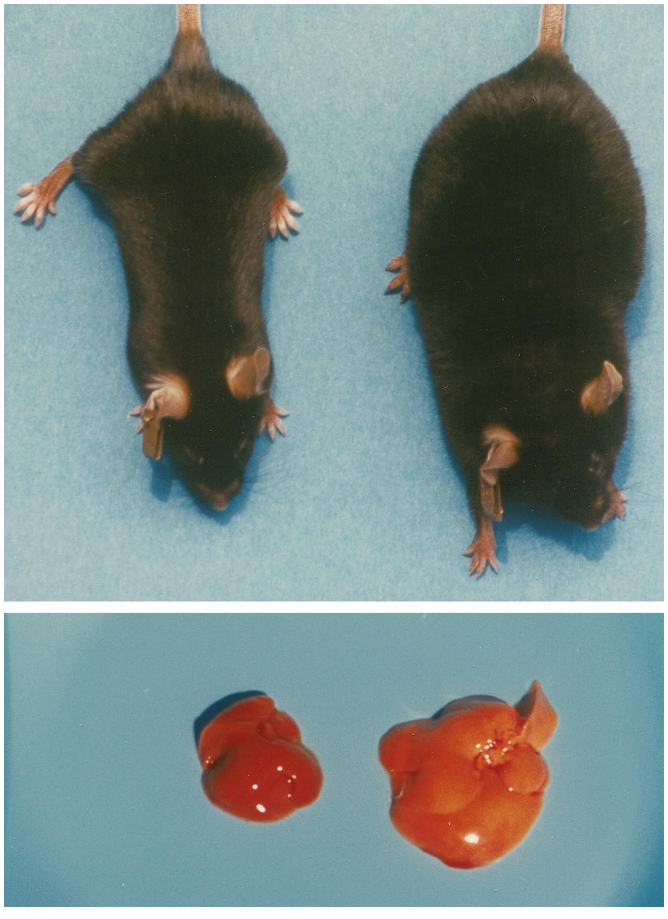

FIG. 2. *Top*, female PPARα knockout mice (*right*) display morphological signs of obesity when compared with age-matched (200 days) wild type C57BL/6 control (*left*). *Bottom*, livers of 240-day-old PPAR −/−-males (*right*) show considerable enlargement and beige steatosis-evocative color when compared with the liver from aged-matched wild type males (*left*).

adipose tissue was quantified at four anatomical sites: inguinal, reproductive (periovarian plus periuteral/periepididymal), interscapular, and retroperitoneal. Additionally, the liver and the interscapular brown adipose tissue were dissected. We compared the adipose tissue mass from PPARα-deficient mice to those from wild type controls for all samples and expressed it as a percentage of the wild type tissue mass (Table I). We observed that, with a single exception (male retroperitoneal white adipose tissue), all fat tissues from the various anatomical sites, including the interscapular brown adipose tissue, showed a statistically significant increase in PPARα −/− animals of both sexes compared with wild type controls. The total amount of dissected fat pads for PPARα −/− females and males represented 238 and 150% of C57BL/6 mice, respectively (Table I), thus confirming that a size increase of fat adipose tissue accounts for the higher body weight recorded in knockouts compared with controls. Also, in accordance with the sexual dimorphism of weight gain, substantially greater fat deposits were observed in deficient females compared with males.

Necropsy analyses revealed striking hepatic abnormalities exclusively in deficient males. All PPARα −/− males displayed a considerable hepatic enlargement, which was associated with a pale steatotic color (Fig. 2*B*). Detailed examination of the organ surface revealed a regular pattern of clearer dots expressed on all lobes (not shown). Liver weight analyses (Table I) showed a male-specific hepatomegaly. The liver weight from PPARα −/− males represented 125% of wild type organs, while no difference was noted when comparing livers from females. This spontaneously occurring abnormality, observed in all deficient male individuals, arose with a strong statistical significance ($p = 0.0005$, $n = 10$). The correlation between liver morphological changes and hepatomegaly in deficient males was strongly evocative of hepatic steatosis. To substantiate this hypothesis, a separate study was designed. PPARα −/− and control mice of both sexes, kept under the same standard diet conditions, were euthanized at 44, 116 (data not shown), 166, and 302 days to evaluate the kinetics of a putative lipid accumulation in the liver (Fig. 3). Histological analyses revealed a unique pattern of accumulation of intracellular fat droplets, clearly restricted to the centrilobular area of deficient male livers. At 44 days, no abnormality was detected. Numerous lipid microvesicules were seen already at 110 days in the cytoplasm of mono- and polynucleated parenchymal cells (Fig. 3*L*). The extent of lipid storage and the size of the fat droplets increased progressively with time. At 302 days, normal hepatocytes were restricted to periportal zones, while wide centrilobular areas evoked adipose tissue, indicating a cellular heterogeneity of the liver toward fat storage capability. This heterogeneity is highlighted by the lipid-specific oil red-O-pos-

TABLE I

PPARα null mutants at 240 days have increased adipose tissue mass and mutant males have increased hepatic index

Liver and discrete fat pads from five anatomical sites were dissected and weighed. WAT and BAT refer to white and brown adipose tissue, respectively. "Reproductive" refers to the epididymal and parametrial adipose pads of males and females. Total WAT weight represents the sum of the data reported in the table for the four WAT sites considered in the study. Results are means ± S.E., $n = 10$ for each group. Statistical relevance of data is assessed by p values.

	Liver weight	Inguinal WAT weight	Reproductive WAT weight	Interscapular WAT weight	Retroperitoneal WAT weight	Interscapular BAT weight	Total WAT weight
				% body weight			
Male							
Wild type	4.04 ± 0.12	0.98 ± 0.14	2.79 ± 0.24	0.37 ± 0.06	0.90 ± 0.12	0.35 ± 0.02	5.05 ± 0.44
PPARα-deficient	5.08 ± 0.22	1.99 ± 0.25	3.67 ± 0.27	0.73 ± 0.10	1.19 ± 0.27	0.51 ± 0.07	7.58 ± 0.63
Percentage of wild type	125.8% ($p = 0.0005$)	203.0% ($p = 0.0024$)	131.6% ($p = 0.0247$)	194.1% ($p = 0.0093$)	131.3% ($p = 0.2012$)	143.7% ($p = 0.046$)	150.1% ($p = 0.042$)
Female							
Wild type	4.47 ± 0.12	1.03 ± 0.21	2.39 ± 0.26	0.40 ± 0.04	0.40 ± 0.05	0.25 ± 0.03	4.24 ± 0.45
PPARα-deficient	4.62 ± 0.16	2.49 ± 0.38	5.83 ± 0.75	0.93 ± 0.12	0.71 ± 0.10	0.45 ± 0.09	10.09 ± 1.26
Percentage of wild type	103.3% ($p = 0.4673$)	240.9% ($p = 0.0036$)	243.9% ($p = 0.0004$)	229.1% ($p = 0.0006$)	177.1% ($p = 0.0035$)	179.1% ($p = 0.0528$)	238% ($p = 0.0004$)

itive staining of frozen liver sections of 302-day-old deficient males (Fig. 3*L*).

Preliminary studies conducted with 8–10-week-old males of both genotypes showed steady levels of serum triglycerides, while an increase of the cholesterol level in the deficient mice was noted (data not shown), in accordance with a previous report (31). When performed at 240 days, the analysis revealed increased levels of circulating triglycerides, cholesterol, and phospholipids in PPARα −/− mice compared with controls (Fig. 4). Deficient females exhibited a markedly high level of triglycerides, 250.5% of control values. By quantifying lipoproteins in these samples, we observed similar values for VLDL, LDL, and high density lipoproteins between deficient or wild type males or females with a single exception; the VLDL value in PPARα −/− females was 246% of wild type. When expressed as the ratio VLDL/LDL, deficient females show 291% of the wild type value (Fig. 4). Considering the high contribution of triglycerides in the composition of VLDL, triglycerides and VLDL values in deficient females are very corroborative data that suggest a VLDL increase as the primary cause for total triglyceride increase. Additional blood parameters such as transaminases, alkaline phosphatase, and urea were not different from C57BL/6 in PPARα −/− mice (data not shown). Lipids were quantified from frozen fragments of the liver large lobe. The results accurately correlate with histological and serological studies. Steatotic PPARα −/− male livers exhibit 10-fold more cholesteryl esters and 9.5-fold more triglycerides than controls. In deficient females, who presented the highest circulating levels in triglycerides but the mildest hepatic repercussions, these ratios were only 3.3- and 2.7-fold, respectively (Fig. 5).

Progressively, centrilobular hepatocytes from PPARα −/− males undergo extensive morphological alterations, which evoke a putative phenotypical evolution toward an adipocyte phenotype. To substantiate this hypothesis, the expression of adipocyte markers was assessed. PPARγ2 is a key adipogenic factor shown to be active in the *in vitro* transdifferentiation of fibroblasts and myoblasts (39, 40), cells with no inherent adipogenic potential. Although PPARγ2 expression is most abundant in adipocytes, transcripts have been reported to various extents in other organs (brown adipose tissue, adrenal gland, skeletal muscle) (41). Repeated PPARγ2-specific RT-PCR experiments, followed by Southern blotting and hybridization with a radiolabeled probe, consistently showed a constitutive hepatic expression of PPARγ2 for both genotypes and both sexes, in 240-day-old animals (data not shown). Although limited, a statistically significant higher expression of PPARγ2 was specifically detected in whole liver samples (lean plus steatotic cells) from PPARα −/− males compared with wild type males (Fig. 6*A*). Interestingly, this result was not observed when analyzing female whole liver samples (Fig. 6*A*). Considering the higher relative abundance of steatotic cells in male livers compared with females, a putative specific overexpression of PPARγ2 messengers in these fat cells might consequently account for the significantly stronger signal dectected from deficient male whole liver samples. But the extreme fat load of these hepatic cells constitutes a restricting constraint for *in situ* hybridization or immunohistochemical studies. To circumvent this technical limitation, we combined an *in situ* organ dissociation protocol with a cell floatation step to isolate the male-specific lipid-loaded cell subpopulation of the liver. Subsequently, its specific PPARγ2 messenger content was compared with the signal obtained from lean parenchymal cells collected in the centrifugation pellet of the corresponding organs. Six 240-day-old PPARα-deficient males underwent a liver collagenase perfusion to prepare single cell suspensions. Equal amounts of total RNAs from lipid-loaded cells (floating

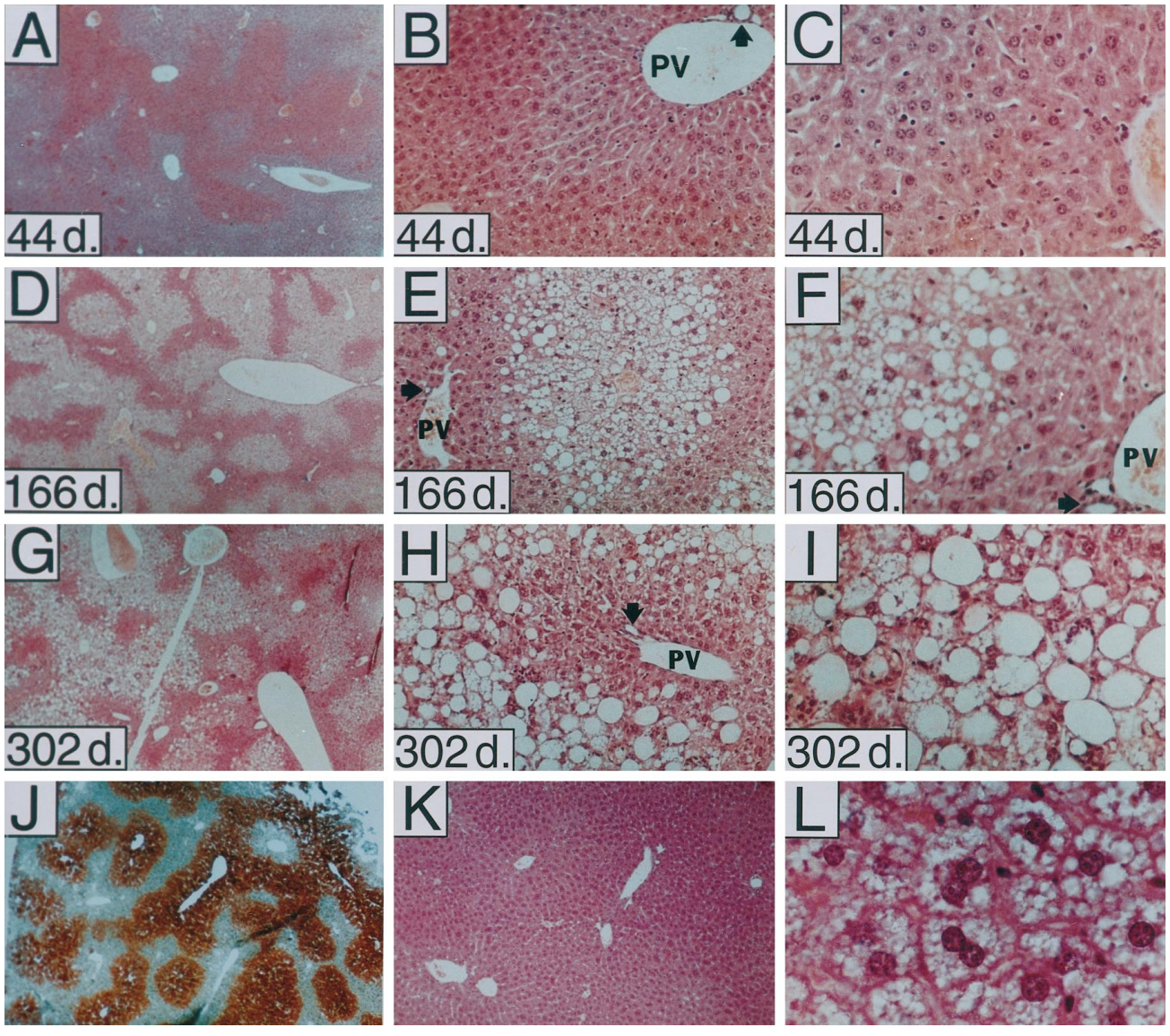

FIG. 3. **Histological analysis of paraffin-embedded liver samples from PPARα −/− male mice were performed at 44 (*A–C*), 166 (*D–F*), and 302 (*G–I*) days.** Evolutive steatosis in the centrilobular area of PPARα-deficient livers is obvious at days 166 and 302 when compared with the organ of a 295-day-old wild type male (*K*). All samples in this figure were prepared with H & E staining (lipid droplets appear as *white empty spaces*) except in *J*. Sample *J* was stained with the lipid-specific oil red-O dye to positively reveal lipids (in *dark red*) in a frozen section of a 302-day-old PPARα −/− liver. *L*, PPARα −/− mono- and polynucleated hepatocytes at 110 days show accumulation of microdroplets of lipids. The *arrows* indicate bile ducts. *PV*, portal vein. Magnifications were as follows: × 50 (*A*, *D*, *G*, and *J*), × 200 (*B*, *E*, and *H*), × 400 (*C*, *F*, and *I*), × 100 (*K*), and × 1000 (*L*).

top ring) or from lean cells (pellet) were simultaneously submitted to RT-PCR analysis. With the exception of sample 5 (confirmed by repeated experiments), male parenchymal PPARα-deficient hepatic cells show a substantially lower PPARγ2 signal than their liver corresponding fat cells (Fig. 6*B*). Using specific sets of primers (see "Experimental Procedures") in the same experimental procedure, we failed to detect any discrepancy between floating and pellet liver cells in the expression of three additional adipocyte markers (adipoQ (42), aP2 (43), and hormone-sensitive lipase (44)) (data not shown). Remarkably, albumin transcripts that are expressed in hepatocytes and absent from adipocytes were consistently detected in floating and pellet fractions from PPARα-deficient livers at comparable levels (Fig. 6*B*). HPRT amplifications were used to monitor the equivalence of RT product substrates in amplification experiments. Additionally, PPARγ2 transcripts were evaluated in these hepatic floating cells in comparison with age-matched wild type hepatocytes or adipocytes. In this experiment, PPARγ2 constitutive hepatic expression, in aged wild type mice, was identical to its initial determination (Fig. 6*A*). Interestingly, this expression was considerably increased in PPARα-deficient hepatic floating cells of the same age; nevertheless, it remained less than the levels observed in wild type adipocytes (Fig. 6*C*).

DISCUSSION

PPARα-deficient mice were initially investigated in pharmacological studies. The aim of our work was to document the *in vivo* implication(s) of this nuclear receptor in the control of biochemical pathway(s). A long term experimental set up allowed us to establish that the lack of this receptor is primarily responsible for the development of a late onset, sexually dimorphic, obese phenotype, exposing a physiological role.

We established the chronology of overweight occurrence in PPARα −/− mice and observed that a substantial increase in adipose tissue at five atomical locations was responsible for this obesity in 8-month-old mice kept under standard diet conditions. These results define an original model of monogenic

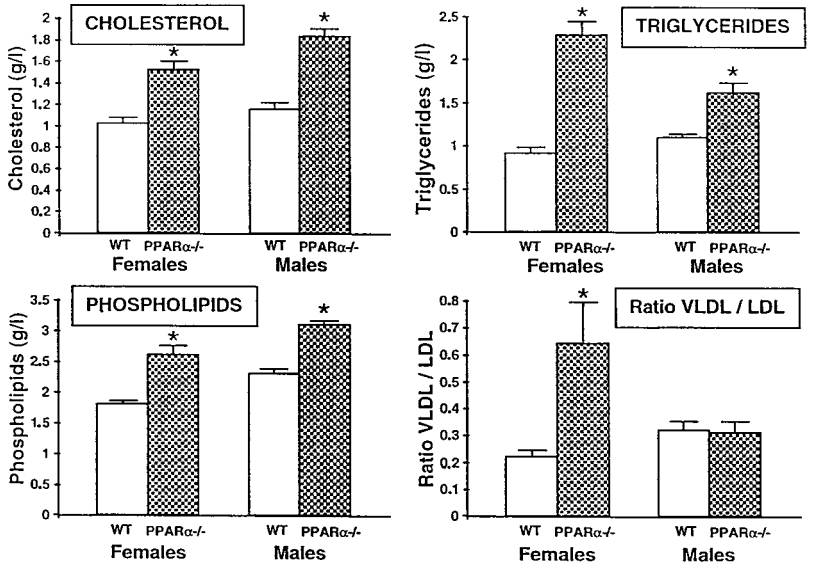

FIG. 4. **Circulating lipid analyses reveals dyslipidemia in PPARα-deficient animals; differences are observed between sexes.** Individual serum samples collected at 240 days were analyzed. Results for cholesterol, triglycerides, phospholipids, and VLDL/LDL ratios are expressed as means ± S.E. of 10 samples/group. Statistical relevance of discrepancies between groups, evaluated by variance analysis and Student *t* test, is expressed with an *asterisk*.

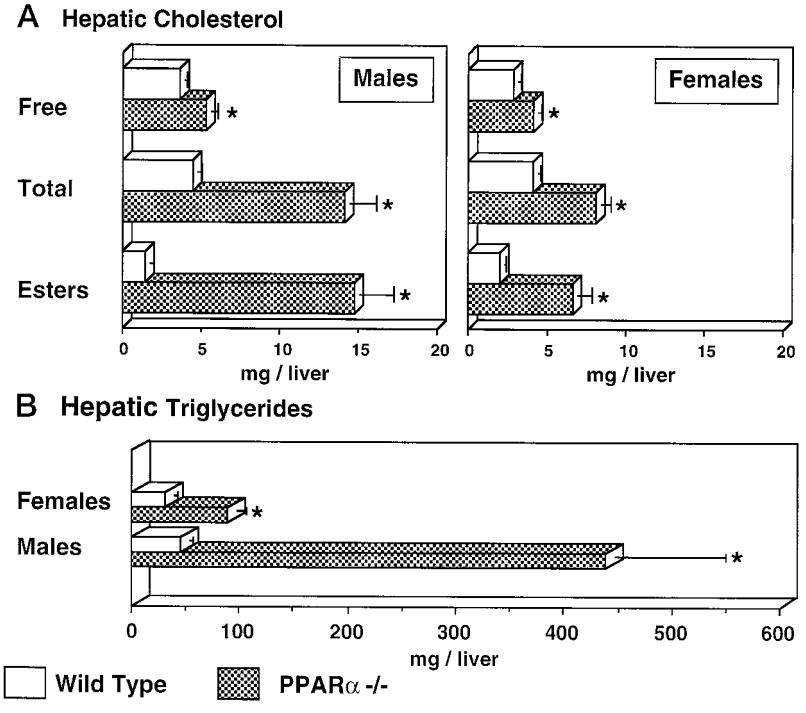

FIG. 5. **Lipid content analysis of livers from PPARα −/− and C57BL/6 wild type mice at 240 days.** *A*, free cholesterol, total cholesterol, and esters of cholesterol have been quantified in liquid nitrogen-frozen liver samples from 240-day-old wild type and PPARα-deficient mice of both sexes. Results are expressed as mean ± S.E. of 10 (wild type) or 9 (PPARα −/−) values. *B*, triglycerides were evaluated in the same samples. Results are presented as mean ± S.E. of 10 (wild type) or 9 (PPARα −/−) values. Statistical relevance of discrepancies between groups, evaluated by variance analysis and Student's *t* test, is expressed with an *asterisk*.

obesity that relies on the impairment of a pathway controlled by a key regulator of lipid processing and metabolism. Unlike juvenile onset obesity models (*ob*, *db*) where energy intake is disregulated mainly through neuronal network disturbances (45, 46), the maturity onset obesity in the PPARα −/− line resembles those in the tubby, agouti, and MC4-R-knockout phenotypes (47–49). Crucial differences with the latter three models are nonetheless noted: PPARα deficient mice were not hyperphagic; expression of the molecular target is not restricted to the brain; and the adaptive response to the deficiency, with a limited extent and sexual dimorphism of obesity, is a closer reminiscence of the pattern observed in human populations. Effects on male liver evoke the pathological histology of livers deficient in fatty acyl-CoA oxidase (50, 51), a rate-limiting enzyme in the peroxisomal β-oxidation of very long chain fatty acids, shown to be regulated, *in vivo*, by PPARα (29). Some phenotypic features that we observed (obesity and pale and enlarged liver) are also reported for STAT5b-deficient mice (52). STAT5b, a cytoplasmic transcription factor, plays a key role in regulating the sexual dimorphism of hepatic gene expression induced by pulsatile plasma growth hormone. This deficient phenotype, with impaired body growth rates and liver gene expression, suggests that STAT5b may be the major protein mediating the sexually dimorphic effect of growth hormone in the liver. Considering that PPARα deficiency leads to a pronounced sexually dimorphic hepatic phenotype, it could be of considerable interest to investigate, in the future, a putative cross-talk between growth hormone and PPARα transduction pathways. An oxysterol-sensitive receptor, LXR, has been identified that also signals through dimerization with the retinoid

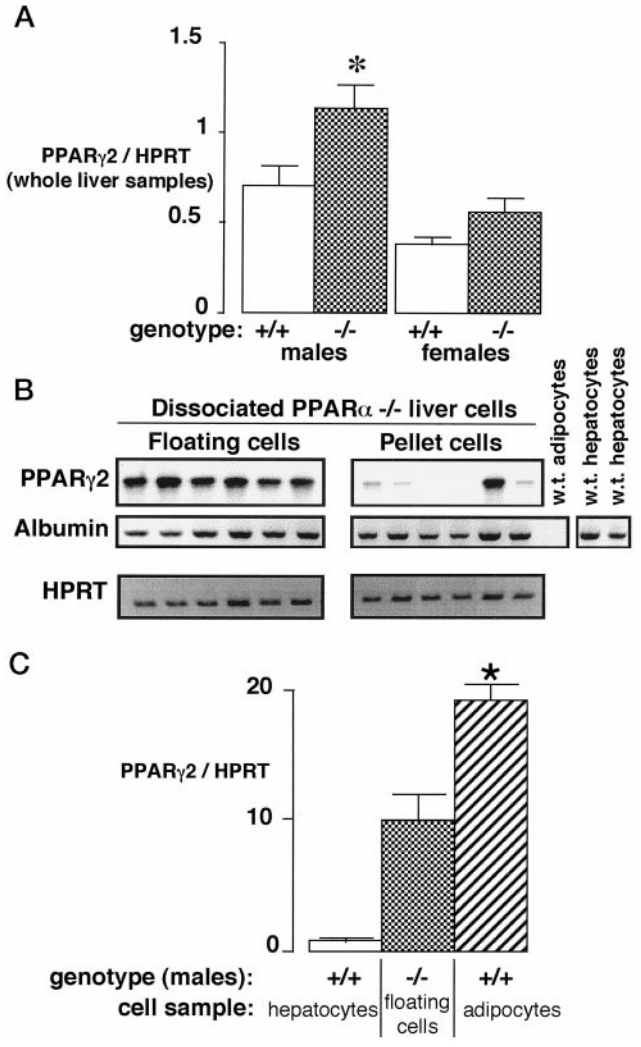

FIG. 6. *A*, RT-PCR analysis for PPARγ2 in whole liver samples. 250 ng of reverse-transcribed liver RNAs from 240-day-old wild type and deficient mice of both sexes were subject to PCR amplification using PPARγ2-specific or HPRT-specific sets of primers. Following electrophoresis, the agarose gel was analyzed, and digital data were statistically treated. $n = 9$ for each group. Statistical relevance of discrepancies between groups (*asterisks*) was assessed by Student's *t* test. *B*, RT-PCR analysis for PPARγ2, albumin, and HPRT on isolated subpopulations of liver cells from 240-day-old PPARα −/− male mice. A single-cell suspension was obtained following collagenase perfusion of livers. Fat-loaded floating cells were separated from pellet cells by centrifugation. Specific PCR analyses were performed on reverse-transcribed RNAs from these cells, using a mouse white adipose tissue sample (*w.t. adipocytes*) or liver C57BL/6 dissociated hepatocytes (*w.t. hepatocytes*) as controls. HPRT-specific amplification was performed to assess equivalent amouts of PCR substrate. Following electrophoresis of PCR products and Southern blotting, hybridization with specific radiolabeled probes was performed to generate the autoradiograms shown. *C*, RT-PCR analysis for PPARγ2 in 240-day-old wild type hepatocytes, wild type adipocytes, or PPARα −/− fat-loaded hepatic floating cells. Results are presented as a PPARγ2/HPRT ratio. Two wild type male hepatocytes samples were used to assess the basal level of expression in the liver. Six animals were used in each group for wild type adipocytes or deficient floating cell samples. Values from scanned Southern blots of amplification products were statistically interpreted with Student's *t* test.

receptor RXR (53, 54). Mice deficient in LXR display a fatty liver when fed 2% cholesterol.[2] Unlike the PPARα knockout model, this type of lipid accumulation is not spontaneous; it is restricted to cholesterol accumulation and does not show marked histological heterogeneity. Noticeably, these two genetically engineered models of impaired hepatic lipid processing underline the pivotal role of two nuclear receptors (PPARα and LXR) that both heterodimerize with RXR. These mammalian pathological phenotypes exemplify the crucial involvement of the liver in regulating lipid homeostasis and, in the case of PPARα, the subsequent extent of circulating lipids and fat deposition. These three nuclear receptors can be legitimately considered as putative molecular targets for therapeutic intervention. Further studies should delineate the potential rate-limiting role of RXR in the recruitment of PPARα *versus* LXR metabolic pathways. The combined use of the corresponding deficient mouse lines in pharmacological studies using selective activators and/or agonists of RXR, LXR, or PPARα should be of considerable relevance to evaluate the limits of action of each of the receptors in these interlinked signaling pathways. Indeed, RXR-specific agonists, rexinoids, have been shown to stimulate the RXR-PPARα pathway in wild type rats, eliciting a fibrate-like response *in vivo* (55).

During preliminary investigations with 20 mice of each genotype, we observed that 8–10-week-old PPARα-deficient male mice exhibited elevated cholesterol serum levels (1.57 g/liter) compared with wild type mice (0.87 g/liter), while their triglycerides values remained comparable (0.75 and 0.84 g/liter, respectively). In slightly older (10–16 weeks of age) male deficient mice, investigators have reported an hypercholesterolemia (1.39 g/liter) with elevated high density lipoprotein cholesterol but normal circulating triglycerides (31). These data are in agreement. Since they originate from different laboratories working with two different sublines derived in 1995 from the same initial knockout mouse line, it shows that the PPARα −/− mice exhibit comparable serum lipid levels in different environments, allowing comparisons between studies. When lipids were evaluated at 240 days, when the spontaneous phenotype is clearly established, we observed the association of hypercholesterolemia and hypertriglyceridemia in males as in females. With a value of 1.85 g/liter in males, the serum cholesterol level shows an increase when compared with the published value for younger deficient mice (31) or when compared with our unpublished preliminary results. This suggests a progressive, spontaneous occurrence of hyperlipidemia in PPARα-deficient mice. In aged animals, it affects cholesterol, triglycerides, and phospholipids with ratios of serum increase in the range of 50% in males and females. One noticeable exception is the marked increase of triglycerides in the aged knockout females that express 2.5 times the wild type level. Their coordinate elevation of VLDL, a triglyceride-rich particle, suggests that it could constitute the primary cause for the increase of circulating triglycerides.

We characterized additional sexually dimorphic parameters in this deficient line. Noticeably, statistical analysis revealed a relative fat deposition in females (238%) higher than in males (150%). PPARα −/− females were significantly stably overweight, *versus* controls, 13 weeks before males. Statistical weight analysis, morphological examination, and microscopic observation revealed an original phenotype expressed in the liver in a male-specific manner. Their enlarged pale livers, observed upon necropsy, show a spontaneous progressive intracellular accumulation of fat droplets that evolves from microvesicular to macrovacuolar. Among cells that initially accumulate lipids in PPARα −/− male livers, polynucleated hepatocytes were often seen. From our study, the simultaneous clonal expansion of fat-storing progenitor cells can not be ruled out. Nevertheless, from histological observations, no proof supporting this hypothesis was found. In addition, molecular data also support the initial lipid accumulation into parenchymal cells, since albumin transcripts, a hepatocyte marker, were equally detected in fat-loaded or lean dissociated cells at an advanced stage of steatosis. This mixed hepatocellular accumu-

[2] D. Mangelsdorf, personal communication.

lation of cholesterol and triglycerides defined a striking pattern of centrilobular-restricted steatosis. Even in the older deficient males tested, periportal stripes of morphologically intact heatocytes are preserved. Histologically, this steatosis diverges from that found in fatty acyl-CoA oxidase-deficient mice. Their steatosis is more pronounced, affects cells throughout the lobule, and is not reported as being sexually dimorphic (50, 51). Steatosis in acinar zones, with triglycerides and free fatty acid deposition, is reported by clinicians as initial lesions in alcoholic and nonalcoholic steatohepatitis, while a rat nutritional model for acinar macrovesicular steatosis has been developed (56, 57). In addition, microvesicular steatosis is reported in numerous pathologies related to either spontaneous or drug-induced impaired mitochondrial fatty acid oxidation (discussed in Ref. 58). The selective centrilobular accumulation of lipids that we observed, strongly suggests a primary biochemical discrepancy among hepatocytes within the same hepatic lobule. Indeed, this has been extensively documented for metabolic enzymes such as certain cytochromes P450. Hepatic CYP2E1 exhibits a constitutive centrilobular expression (59), but in the nutritional model of hepatic steatosis, rat livers showed an induced centrilobular distribution with a coordinate superimposed steatosis (57). Sexually specific or zonal CYP expression has been related to growth hormone secretion pattern and hepatic sinusoidal gradient (60). Hepatic zonal expression of cytochromes P450 in the PPARα −/− line has yet to be investigated.

In old PPARα-deficient animals, cells with macrovacuolar lipid accumulation evoked adipocytes. Indeed, PPARγ2, an adipocyte marker (61), was found to be overexpressed in male livers due to its specific positive regulation in fat-loaded cells. Although in lean cells we observed in only one sample a clear PPARγ2 signal, this signal may have resulted from incomplete tissue dissociation, cell clustering, and subsequent contamination of lean cells with fat cells. Nevertheless, the failure to detect other adipocyte markers and the coordinate persistent expression of albumin transcripts in these cells imply that their phenotype did not evolve substantially despite morphological changes. *In vivo* PPARγ2 expression, in these cells, does not sustain by itself hepatocyte transdifferentiation as it does *in vitro* for fibroblasts (61), myoblasts (40), or osteoblasts (62). That may specifically result from inappropriate cofactor environment or the lack of a biological activator in hepatocytes. Even more, lower PPARγ2 transcripts levels, in the hepatic fat-loaded cells compared with wild type adipocytes, could also contribute to the lack of advanced phenotypic evolution.

A key feature of the PPARα −/− mouse line appears to be its constitutively impaired lipid oxidation capability (β-oxidation, ω-hydroxylation) and its inability to stimulate it. The observed hyperlipidemia could have been expected from such a disorder, but obesity and the remarkable hypertriglyceridemia in females associated with a more pronouced obesity than in males was not suspected. The male-specific steatosis constitutes also an unexpected consequence of the deficiency that provides additional evidence of discrepancies between the sexes toward lipid metabolism and susceptibility to obesity. The association of triglyceride levels and extent of obesity in females suggests the regulation of circulating lipids as a means to limit fat storage and subsequently suggests PPARα-activating drugs as potentially valuable tools for therapeutic intervention against the metabolic lipid abnormalities present in obesity. Nevertheless, rodents remain original models exhibiting pathological effects of fibrate drugs that do not occur in human livers. The recent finding that human hepatocyte lysate exhibits more than 10-fold lower PPARα DNA binding activity than mouse hepatic lysate, in relation with lower amounts of the receptor in humans, provides evidence for a quantitative origin to the interspecies discrepency toward peroxisome proliferator exposure (63). Nevertheless, this does not imply that therapeutic intervention on the PPARα signaling pathway is irrelevant in humans. Indeed, the efficacy of fibrate drugs in modulating circulating lipids in humans has been documented (19, 64, 65). Moreover, the recent report that the LDL-cholesterol-lowering efficacy of fibrate drugs is positively associated with on-treatment weight loss in humans (66), corroborated by an initial observation in rodents, is consistent with our report of weight gain when the murine PPARα pathway is abolished. This model of monogenic, maturity onset, spontaneous obesity with deficient PPARα transduction, could lead to new insights into the etiology of some form(s) of mammalian obesity and their heritability. Report of a mutation in the mouse gene, with functional consequences (67) and the recent identification of a human nonfunctional splice variant of the receptor (63) suggest that genetic determinants can influence the PPARα signaling pathway. Therefore, it could be of considerable interest to further investigate the genetics of the receptor in human obese populations and in individuals selected for their lack of therapeutic response to fibrate drugs.

Acknowledgments—PPARα-deficient mice were a gift from F. J. Gonzalez. We thank C. Bétoulière, R. Meddour, I. Oswald, and C. Dozois for technical assistance; C. Calleja, P. Barbe, D. Langin, and J-P. Vinel for comments on the manuscript; and D. Mangelsdorf for permission to refer to unpublished results.

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