A Gender-related Defect in Lipid Metabolism and Glucose Homeostasis in Peroxisome Proliferator–activated Receptor α–deficient Mice

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Abstract

The peroxisome proliferator–activated receptor α (PPARα) is a nuclear receptor implicated in the control of cellular lipid utilization. To test the hypothesis that PPARα is activated as a component of the cellular lipid homeostatic response, the expression of PPARα target genes was characterized in response to a perturbation in cellular lipid oxidative flux caused by pharmacologic inhibition of mitochondrial fatty acid import. Inhibition of fatty acid oxidative flux caused a feedback induction of PPARα target genes encoding fatty acid oxidation enzymes in liver and heart. In mice lacking PPARα (PPARα−/−), inhibition of cellular fatty acid flux caused massive hepatic and cardiac lipid accumulation, hypoglycemia, and death in 100% of male, but only 25% of female PPARα−/− mice. The metabolic phenotype of male PPARα−/− mice was rescued by a 2-wk pretreatment with β-estradiol. These results demonstrate a pivotal role for PPARα in lipid and glucose homeostasis in vivo and implicate estrogen signaling pathways in the regulation of cardiac and hepatic lipid metabolism. (*J. Clin. Invest.* 1998. 102:1083–1091.) Key words: fatty acids • estrogens • myocardial diseases • hypoglycemia • cytoplasmic and nuclear receptors

Introduction

Cellular fatty acid uptake and utilization rates are tightly controlled to meet energy demands and maintain lipid balance. The importance of fatty acid utilization pathways in the maintenance of cellular lipid homeostasis is underscored by the dramatic clinical consequences of human inborn errors in mitochondrial fatty acid β-oxidation enzymes (1). Children afflicted with enzymatic defects in β-oxidation pathway enzymes are usually asymptomatic until clinical manifestations of hypoglycemia, liver dysfunction, and cardiomyopathy are precipitated by conditions that dictate increased cellular fatty acid oxidation to meet energy demands (1–3). Postmortem studies of β-oxidation enzyme–deficient children have demonstrated massive intracellular lipid accumulation in liver and myocardium (1). Therefore, the inborn errors in fatty acid β-oxidation exhibit an extreme example of a perturbation in energy production and cellular lipid balance due to limited capacity for fatty acid oxidation under conditions that require increased catabolism of fats for energy production, a metabolic stress-induced phenotype.

Little is known about the molecular regulatory mechanisms involved in the maintenance of cellular lipid balance under normal physiologic conditions or in the context of disease states such as the inborn errors in fatty acid oxidation. In tissues with high fatty acid utilization rates such as heart and liver, the capacity for peroxisomal and mitochondrial fatty acid β-oxidation, the major lipid catabolic pathways, is regulated at the level of gene expression during development and in response to diverse physiologic stimuli (4–7). Several lines of evidence suggest that the peroxisome proliferator–activated receptor α (PPARα),[1] a member of the nuclear receptor superfamily of transcription factors (8), is involved in the metabolic control of fatty acid oxidation enzyme gene expression. First, PPARα is necessary for the induction of peroxisomal biogenesis in response to peroxisome proliferators (8, 9). The peroxisomal β-oxidation pathway partially oxidizes long-chain fatty acids (LCFAs) derived from the bloodstream and lipid intermediates generated by microsomal oxidation pathways. Second, most known PPARα target genes encode enzymes involved in cellular fatty acid oxidation including the peroxisomal (10, 11), mitochondrial (12, 13), and cytochrome P450 (CYP) pathways (14, 15). Third, PPARα is activated by fatty acids or inhibitors of mitochondrial LCFA import (16–18). These facts suggest that PPARα serves as a cellular "lipostat," transducing changes in cellular lipid levels to the transcriptional regulation of target genes involved in fatty acid utilization.

In this study, we investigated the role of PPARα in the control of cellular lipid homeostasis in vivo. We show that in response to a major perturbation in cellular fatty acid flux caused by pharmacologic inhibition of the mitochondrial import of LCFAs, PPARα activates the expression of target genes involved in cellular fatty acid oxidation, a metabolic feedback response. In mice lacking PPARα, the response of PPARα target genes to a perturbation in fatty acid flux is abolished, resulting in massive accumulation of intracellular lipid in liver and heart and hypoglycemia characterized by depletion of gly-

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1. *Abbreviations used in this paper:* ACO, acyl-CoA oxidase; CPT I, carnitine palmitoyltransferase I; CYP, cytochrome P450; LCFA, long-chain fatty acid; MCAD, medium-chain acyl-CoA dehydrogenase; NEFA, nonesterified fatty acids; PPAR, peroxisome proliferator–activated receptor.

cogen stores and an inadequate counterregulatory response. Remarkably, the defect in cellular lipid and glucose balance in PPARα null mice is more severe in males compared with females, a gender influence that is reversed by estradiol treatment. These results identify an important role for PPARα in a hepatic and cardiac lipid homeostatic stress response and implicate estrogen signaling pathways in the control of lipid and glucose metabolism.

Methods

RNA blot analysis. Isolation of total RNA and RNA blot analyses were performed as described (4). RNA blots were hybridized with ^{32}P-labeled probes derived from the following cDNAs: a 559-bp acyl-CoA oxidase (ACO) cDNA fragment amplified from total mouse liver RNA by RT-PCR (oligonucleotide primers: sense, 5′-CAATCACGCAATAGTTCTGGCTC-3′ and antisense, 5′-AAGCTCAGGCAGTTCACTCAGG-3′); a CYP 4A1 cDNA generated by RT-PCR (oligonucleotide primers: sense, 5′-ACCCTAGACACTGTCATGAAGTGT-3 and antisense, 5′-AGATGTGCTGAGTTCTCTGACAAT-3′); a PCR-generated rat P450 4A3 cDNA (oligonucleotide primers: sense, 5′-CTCTCTACTGTTCTGTATCAGAAT-3′ and antisense, 5′-CCTCCAGACTCCATCCCAGT-3′); and a mouse medium-chain acyl-CoA dehydrogenase (MCAD) cDNA fragment provided by Dr. Philip Wood (University of Alabama, Birmingham, AL) (19).

Animal studies. All experiments were performed with mice ranging in age from 3 to 6 mo (21–33 g). Adult C57BL/6 × SJL/J mice were used for the experiments shown in Fig. 1. The PPARα−/− and PPARα+/+ mice, which were used for the experiments shown in Figs. 2–7, have been described (9). For the metabolic inhibitor studies, etomoxir (50 μg/g body wt) or vehicle (sterile water) was given as a daily intraperitoneal injection at 0900 for 5 d. At the time of harvest, animals were killed by CO_2 inhalation and liver and cardiac ventricle were rapidly dissected free, snap-frozen in liquid nitrogen, and stored at −80°C until processed for isolation of RNA or lipid extraction. For histologic analyses, the tissue was processed at the time of harvest as described below.

Mouse tail vein blood glucose levels were determined by use of a standard clinical blood glucometer (One Touch II; Johnson and Johnson, New Brunswick, NJ). This method allowed for serial glucose measurements without a significant drop in blood volume because reproducible measurements are possible using 5-μl samples.

Plasma insulin levels were determined by the Diabetes Research and Training Center Core at Washington University School of Medicine. After the animals were killed, blood was withdrawn from the axial artery for preparation of plasma. Insulin levels were determined in 100-μl plasma aliquots by radioimmunoassay (Linco Research, St. Charles, MO).

Plasma nonesterified fatty acid (NEFA) levels were determined using the NEFA C WAKO kit (Wako Chemicals, Richmond, VA).

For estradiol treatment studies, a 3-wk release 17 β-estradiol pellet was inserted into a subcutaneous pocket (posterior neck region) of adult male PPARα−/− mice under anesthesia induced by ketamine/xylazine. Each pellet delivered 0.5 mg of active 17 β-estradiol over 3 wk via continuous release. 2 wk after implantation of the pellet, the male PPARα−/− mice were subjected to the etomoxir regimen described above. Sham-operated controls were also included. At the end of the 5-d etomoxir protocol the mice were killed, blood was drawn from the axial artery for preparation of plasma, and tissues were harvested. Estradiol levels were determined in 100-μl plasma samples by a solid phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Mean estradiol levels in male PPARα−/− mice (n = 8) that received the estradiol pellets were 807±61 pg/ml, which is within the normal range for pregnant female mice. Estradiol levels in male PPARα−/− mice not treated with estradiol were < 10 pg/ml.

All animal study protocols were approved by the Washington University School of Medicine Animal Studies Committee.

Tissue histology studies. For histologic analysis, organs were removed from the mice and quickly sliced into small pieces weighing < 2 mg. The tissue was snap frozen in a cryomold and stored at −80°C until it was prepared for cutting and oil red O staining.

Tissue lipid analysis. Tissue lipid extraction and TLC were performed using a modification of a previously described protocol (20). In brief, a piece of liver was baked to a dry weight of ~ 100 mg. The desiccated tissue was extracted with chloroform/methanol (2:1) ×2. The lipid layer was removed, dried, and weighed. Percent lipid was calculated by dividing the extracted fat dry weight by the tissue sample dry weight (×100). For TLC separation, the lipid extract was resuspended in chloroform/methanol and separated in a two-step chromatograph in $CHCl_3$/methanol/acetic acid: 98:2:1 followed by hexane/ethyl ether/HCl: 94:6:0.2. The sample was run adjacent to a panel of standards (triglyceride, cholesterol, 1-2 diacylglyceride, and monoglyceride). After separation, the plate was stained with 3% copper acetate/8% phosphoric acid, followed by baking. The migration and quantity of sample species was compared with that of the standards.

Liver glycogen analysis. Liver glycogen content was determined according to the method of Roehrig and Allred (21). In brief, homogenates of liver were treated with amyloglucosidase, which degrades glycogen to glucose. Glucose concentration was measured by an enzymatic assay using the Peridochrom Glucose kit (Boehringer Mannheim, Mannheim, Germany).

Statistical methods. Statistical comparisons were made using Student's t test or ANOVA coupled to the Fisher's test ("Statview" software) as described in the figure legends. A statistically significant difference was defined as a P value < 0.05.

Results

The expression of PPARα target genes encoding cellular fatty acid oxidation enzymes is induced by inhibition of mitochondrial LCFA import. To explore the role of PPARα in cellular lipid homeostasis, PPARα target gene expression was characterized in response to a perturbation in fatty acid oxidative flux. We hypothesized that in tissues with a high capacity for mitochondrial fatty acid β-oxidation such as heart and liver, inhibition of mitochondrial LCFA import would cause an accumulation of intracellular lipids, inducing a PPARα-mediated feedback activation of target genes involved in alternate oxidation pathways. The expression of the gene encoding peroxisomal ACO, a known PPARα target, was induced in heart and liver of mice receiving a 5-d course of etomoxir (22), an irreversible inhibitor of carnitine palmitoyltransferase I (CPT I), a pivotal enzyme in the mitochondrial LCFA import pathway (Fig. 1, *A* and *B*). Etomoxir also markedly induced the expression of PPARα target genes encoding hepatic CYP oxidation enzymes (CYP 4A1 and CYP 4A3) and MCAD, an enzyme catalyzing a rate-limiting step in the mitochondrial oxidation of medium-chain fatty acyl-thioesters produced by peroxisomal β-oxidation of LCFAs (Fig. 1, *A* and *B*). All of the mice tolerated the metabolic perturbation induced by etomoxir. The magnitude of the etomoxir-mediated induction of PPARα target gene expression was not significantly different in male compared with female mice (data not shown).

CPT I inhibition causes a gender-related metabolic phenotype in PPARα null mice. The CPT I inhibition experiments were repeated with mice homozygous for a targeted disruption of the PPARα gene (PPARα−/− mice). These mice do not express PPARα protein and exhibit an impairment in the induction of peroxisomal biogenesis and activation of genes en-

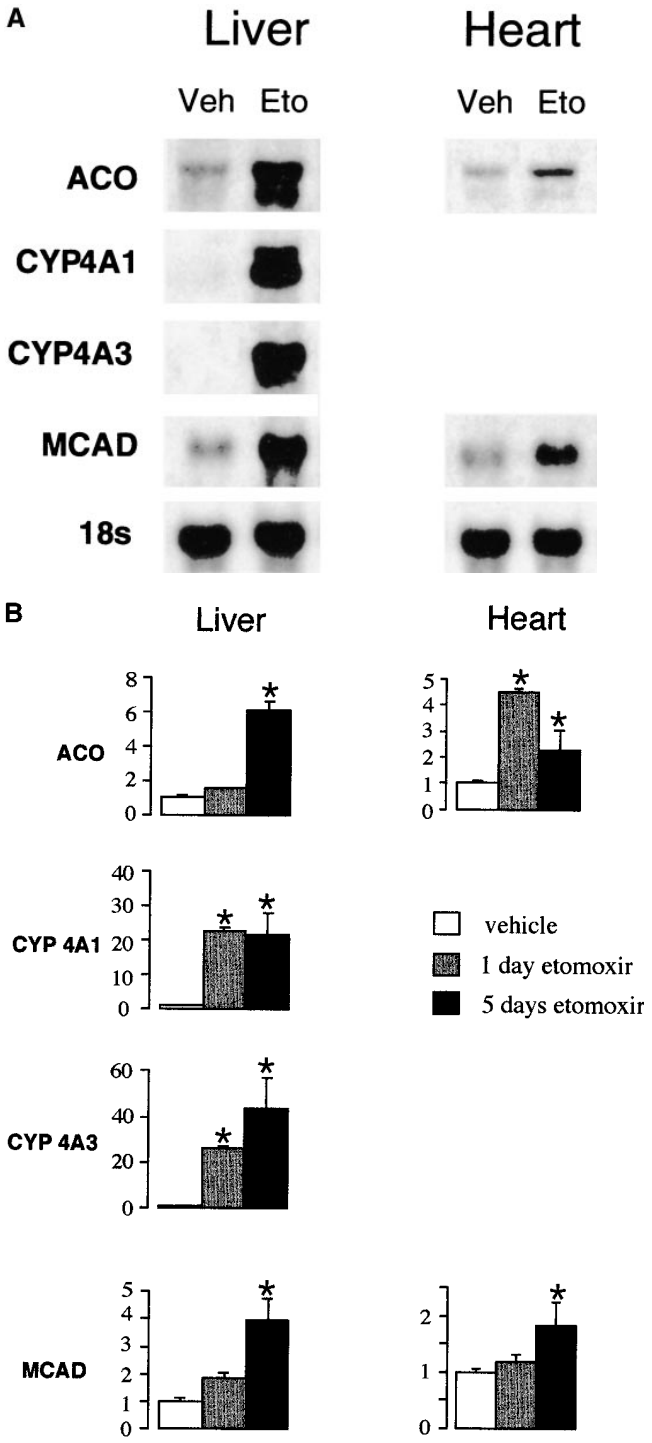

Figure 1. PPARα mediates a lipid metabolic regulatory response by inducing the expression of target genes involved in hepatic and cardiac fatty acid utilization. (*A*) Adult C57BL/6 × SJL/J mice were given daily intraperitoneal injections of vehicle (*Veh*) or etomoxir (*Eto*; 50 μg/g body wt) for 5 d (see Methods). Total cellular RNA (15 μg/lane) was isolated from the liver and heart samples and used to perform the Northern blot analyses. A representative autoradiogram is shown. ^{32}P-labeled cDNA probes used were ACO, CYP 4A1, CYP 4A3, MCAD, and 18s (18s ribosomal RNA). CYP 4A1 and CYP 4A3 signals were only detected in liver. Results shown were obtained with male mice. No significant difference was observed between male and female mice (data not shown). (*B*) Induction of PPARα gene target mRNA levels after 1 and 5 d of etomoxir. The signals on the Northern blot autoradiograms described in *A* were quantified by laser densitometric analysis within the linear range of film sensitivity. Values were normalized to the signals obtained with the 18s ribosomal cDNA probe. The bars represent the mean±SE from at least six animals in each group normalized (= 1.0) to the results obtained with 5 d of vehicle administration. The asterisks denote a statistically significant difference ($P < 0.05$) compared with the value obtained with vehicle alone using ANOVA and Fisher's test.

coding peroxisomal and mitochondrial enzymes in response to peroxisome proliferators, but they are viable (9). Etomoxir was given daily for 5 consecutive days. All male PPARα−/− mice died (9/9) after receiving etomoxir, the majority within 24 h of the first dose (Fig. 2). In contrast, only 25% of the female PPARα−/− mice died (2/8) after etomoxir administration, and none died within the first 24 h. All male and female age- and strain-matched PPARα+/+ control mice tolerated the 5-d course of etomoxir (Fig. 2). Etomoxir did not induce the expression of PPARα target genes in the PPARα−/− female survivors, further supporting a role for this nuclear receptor in the lipid homeostatic response (Fig. 3).

Histologic characterization of tissue from the etomoxir-treated male PPARα−/− mice revealed marked lipid accumulation in heart and liver, organs with high fatty acid oxidative flux (Fig. 4). The sections were stained with oil red O to detect neutral lipid. The livers of PPARα−/− male and female vehicle-treated control mice contained patchy areas of small oil red O–positive droplets but their hearts did not, consistent with the existence of a mild baseline defect in hepatic fatty acid oxidation. After a single dose of CPT I inhibitor, PPARα−/− male mice developed massive micro- and macrovesicular hepatic steatosis and diffuse myocardial lipid accumulation (Fig. 4). The histologic appearance of the livers and hearts of the two female PPARα−/− mice that died in response to CPT I inhibition was similar to that of organs from etomoxir-treated PPARα−/− males (data not shown). In striking contrast, the hearts of the PPARα−/− females that survived had no detectable myocardial lipid accumulation, although mild hepatic steatosis was present (Fig. 4). TLC analysis of lipids extracted from liver of the etomoxir-treated male PPARα−/− mice confirmed that > 99% of the accumulated lipid was triglyceride (data not shown). The liver lipid content paralleled the degree of triglyceride accumulation detected on histologic examination in the PPARα+/+ and PPARα−/− mice at baseline and in response to CPT I inhibition (Fig. 5). Thus, lipid accumulation in heart and liver correlated with the gender-related sensitivity of PPARα−/− mice to CPT I inhibition.

Plasma NEFA levels were measured in the PPARα−/− mice to determine whether gender differences in lipolysis after CPT I inhibition accounted for the tissue lipid accumulation pattern shown above. Mean (±SE) plasma NEFA concentrations were similar in male and female PPARα−/− mice at baseline (males, 0.43±0.13 mmol/liter; females, 0.66±0.08 mmol/liter; P = NS). After a single dose of etomoxir, mean plasma NEFA levels increased similarly in male and female PPARα−/− mice (males, 1.42±0.34 mmol/liter; females, 1.11±0.09 mmol/liter; P = NS) demonstrating that gender-related differences in circulating free fatty acid levels do not account for the greater severity of tissue lipid accumulation in male mice treated with etomoxir.

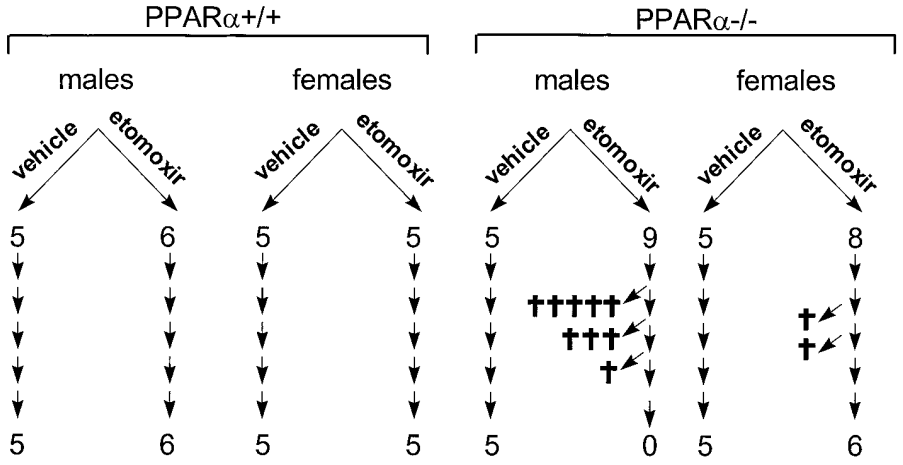

Figure 2. CPT I inhibition results in gender-influenced death in PPARα−/− mice. The numbers refer to number of mice in each group at the onset (*top*) and conclusion (*bottom*) of the experimental protocol. Each arrow denotes a single daily intraperitoneal injection of vehicle (sterile water) or etomoxir (50 μg/g) for 5 consecutive days. The single dagger indicates death.

Male PPARα−/− mice develop severe hypoglycemia in response to CPT I inhibition. In the context of diminished capacity for fatty acid oxidation, the chief energy substrate switches from fatty acids to glucose in tissues such as heart and liver. The importance of this energy metabolic switch is exemplified by the observation that children afflicted with inborn errors in mitochondrial fatty acid oxidation frequently develop life-threatening hypoglycemia (1–3), presumably because glycogen stores are depleted and the capacity for gluconeogenesis is limited due to a reduction in the cellular acetyl-CoA/long-chain acyl-CoA ratio (23, 24). Therefore, we examined blood glucose levels in PPARα−/− and PPARα+/+ mice after CPT I inhibition to determine whether hypoglycemia contributed to the death of the PPARα−/− mice. In male PPARα−/− mice, mean blood glucose levels dropped dramatically from 80 to 26 mg/dl within 20 h after the initial dose of etomoxir (Table I). In contrast, the 20-h blood glucose levels of all etomoxir-treated male PPARα+/+ animals and the female PPARα−/− survivors were within normal limits (Table I). However, in the two etomoxir-treated female PPARα−/− mice that died, hypoglycemia was noted at the time of death (data not shown). Mean plasma insulin levels were appropriately suppressed (to 0.78 ng/ml; normal range 0.2–10.0 ng/ml) in the PPARα−/− males during the hypoglycemic period, excluding the possibility that hyperinsulinemia contributed to the fall in blood glucose levels.

To determine whether depletion of glucose stores contributed to the development of hypoglycemia in PPARα−/− mice treated with CPT I inhibitor, liver glycogen content was determined before and after administration of a single dose of etomoxir. At baseline (vehicle-treated), hepatic glycogen content was not significantly different in female compared with male PPARα−/− mice (Table II). However, 24 h after a single dose of etomoxir, mean hepatic glycogen content was markedly lower in male compared with female PPARα−/− mice (Table II). Thus, glycogen depletion contributes to the development of hypoglycemia in male PPARα−/− mice treated with CPT I inhibitor.

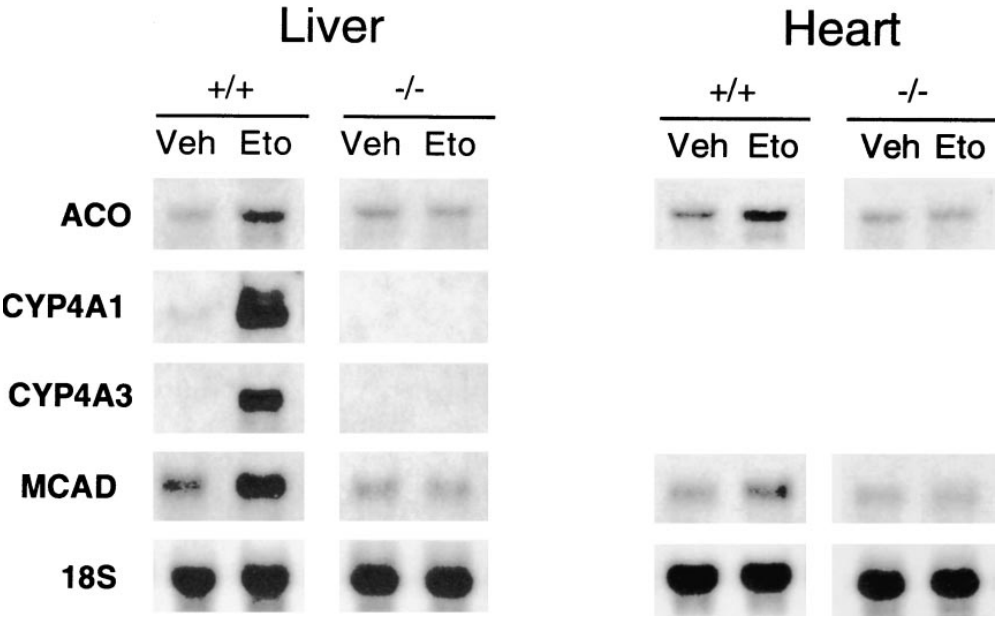

Figure 3. The feedback upregulation of PPARα target genes in response to CPT I inhibition is absent in female PPARα−/− mice. Northern blots performed with total RNA isolated from heart and liver of vehicle- or etomoxir-treated (5 d) PPARα+/+ (+/+) or PPARα−/− (−/−) female mice. The autoradiographs shown are representative of at least five female PPARα−/− survivors and age-matched PPARα+/+ female mice.

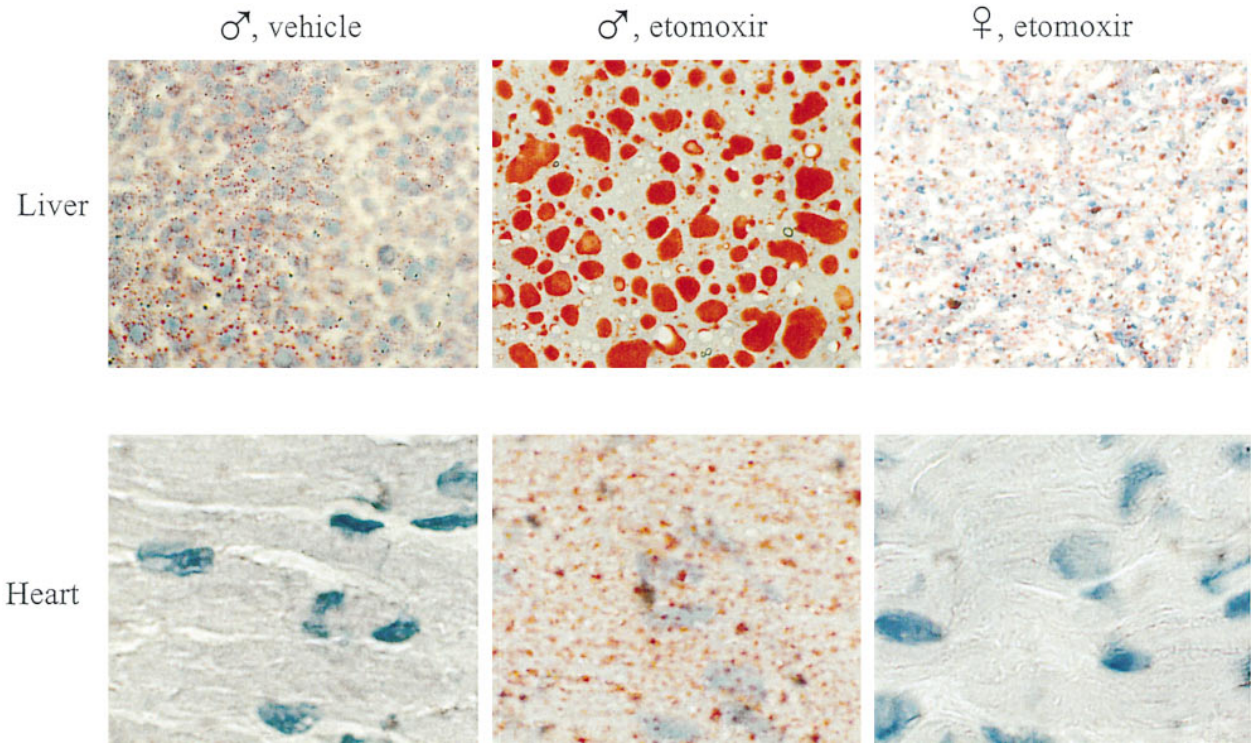

Figure 4. Hepatic and myocardial lipid accumulation in etomoxir-treated PPARα−/− mice parallels gender-related lethality. Representative photomicrographs of oil red O–stained sections of liver and heart prepared from the organs of a male PPARα−/− mouse that received vehicle (*left*), a male PPARα−/− mouse that died within 24 h of the first dose of etomoxir (*middle*), and a female PPARα−/− mouse that survived three injections of etomoxir (*right*; representative of the hearts and livers of all female PPARα−/− survivors at 1, 3, or 5 d of the etomoxir protocol). Sections of liver are displayed at ×300, heart at ×750.

To further explore the gender-related hypoglycemic response to CPT I inhibition, blood glucose levels of PPARα−/− mice were determined 5, 10, and 24 h after administration of etomoxir. Surprisingly, the majority of female PPARα−/− mice (10/13) developed hypoglycemia (blood glucose < 50 mg/dl) within 5 h after receiving the first dose of CPT I inhibitor. The blood glucose curves after a single dose of etomoxir for male PPARα−/− mice and the subgroup of female PPARα−/− mice that developed hypoglycemia are shown in Fig. 6. The blood glucose dropped significantly in both groups within 5 h after etomoxir administration. After the 5-h time point, blood glucose levels of the male PPARα−/− mice remained low until death (Fig. 6). In contrast, the blood glucose levels of female PPARα−/− mice exhibited a rebound increase between 10 and 24 h after receiving etomoxir, returning to the normal range by 24 h (Fig. 6). These results indicate that in contrast to the male PPARα−/− mice, most female PPARα−/− mice have an adequate compensatory response to CPT I inhibitor–induced hypoglycemia.

The results shown in Table II and Fig. 6 suggested that severe hypoglycemia caused the death of the male PPARα−/− mice. The role of hypoglycemia in the death of PPARα−/− mice treated with CPT I inhibitor was explored further by metabolic rescue experiments. Four etomoxir-treated male PPARα−/− mice with severe hypoglycemia (mean blood glucose 34±4.0 mg/dl) exhibiting signs of impending death includ-

Table I. Male PPARα−/− Mice Develop Hypoglycemia in Response to CPT I Inhibition

	Baseline	10 h	20 h	72 h	5 d
♂PPARα−/−	80±3	41±15*	26±6*	—	—
♂PPARα+/+	90±10	—	91	84	84
♀PPARα−/−	76±7	69±9	71±12	86	81±16

The values represent mean (±SD) blood glucose levels (mg/dl) of mice described in Fig. 2 at baseline and after etomoxir given as a single daily dose for 5 d. The time points indicate time elapsed after the initial drug dose using the protocol shown in Fig. 2. The asterisks denote a statistically significant difference ($P < 0.05$; paired *t* test) compared with baseline levels.

Table II. Depletion of Hepatic Glycogen in Etomoxir-treated Male PPARα−/− Mice

	Vehicle	Etomoxir
♂PPARα−/−	27.6±5.1	4.2±3.6*
♀PPARα−/−	35.1±11.7	25.9±14.8

Values represent mean (±SD) glycogen content (mg/g) in livers of PPARα−/− mice 24 h after receiving vehicle or a single dose of etomoxir. The asterisk denotes a statistically significant difference ($P < 0.03$; unpaired *t* test) compared to the corresponding value in the female group.

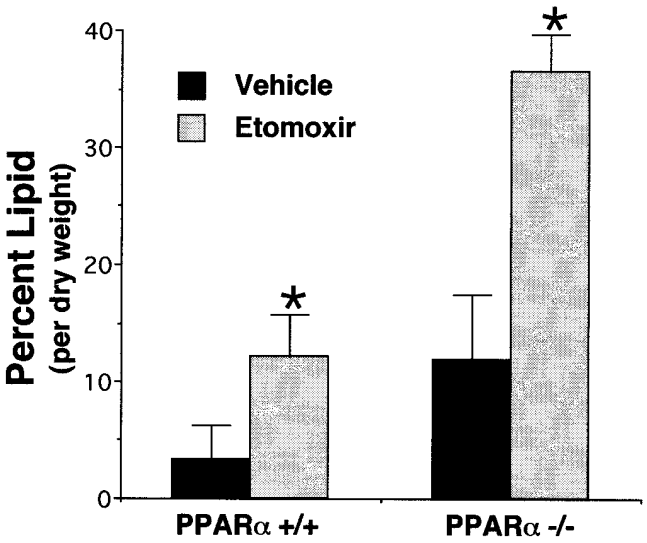

Figure 5. Lipid content of liver from PPARα−/− male mice correlates with histologic evidence of fat accumulation. The bars represent mean (±SD) percent lipid (mg lipid/mg dry wt tissue ×100) from livers of male PPARα+/+ and male PPARα−/− mice given vehicle or a single dose of etomoxir (50 μg/g). Asterisks denote a statistically significant difference between values obtained from vehicle-treated compared with etomoxir-treated mice within the PPARα+/+ and PPARα−/− groups. $*P < 0.05$, Student's *t* test.

ing reduced activity and severe lethargy were given an intraperitoneal injection of a 100-μl solution of 50% dextrose. Immediately after administration of the dextrose-containing solution, each of the animals returned to a normal activity level coincident with restoration of blood glucose levels to the normal range (mean blood glucose levels were 92.5±5.9 mg/dl 6 h after the injection). None of the rescued animals died. These results indicate that hypoglycemia is in large part, if not solely, responsible for the death of PPARα−/− mice treated with CPT I inhibitor.

Estradiol rescues male PPARα−/− mice from the CPT I–induced death and impairment in lipid and glucose homeostasis. To determine whether estrogen signaling pathways are involved in the observed gender-related susceptibility of PPARα−/− mice to CPT I inhibition, the etomoxir studies were repeated with PPARα−/− males after a 2-wk pretreatment with 17 β-estradiol (see Methods). Six of eight estradiol-treated male PPARα−/− mice survived the complete 5-d course of etomoxir without developing hypoglycemia (Fig. 7). The two estradiol-treated PPARα−/− mice that died survived three and four etomoxir injections, respectively. Thus, the survival rate of the estradiol-treated male and female animals converges, in striking contrast to the total lack of survival of the etomoxir-treated male PPARα−/− mice that did not receive estradiol pretreatment.

Histologic studies were performed on the tissues of the etomoxir-treated male PPARα−/− mice pretreated with estradiol. Lipid accumulation was much less severe in the hearts and livers of the estradiol/etomoxir-treated male PPARα−/− mice compared with that in the male PPARα−/− mice that received etomoxir alone. The lipid droplet size was smaller and the pattern of accumulation patchy in the estradiol-treated PPARα−/− male mice compared with the massive lipid droplet accumulation noted in the male PPARα−/− animals that did not receive estradiol (Fig. 8).

Blood glucose levels were measured after etomoxir administration in two of the estradiol-treated male PPARα−/− mice. In both mice, blood glucose levels fell (below 50 mg/dl) within 5 h after administration of CPT I inhibitor followed by a rebound increase to normal levels in a pattern identical to that shown in Fig. 6 for etomoxir-treated female PPARα−/− mice

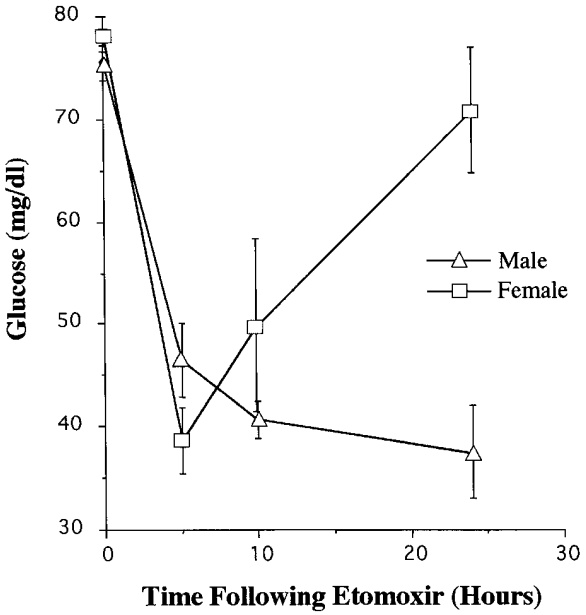

Figure 6. Blood glucose levels in PPARα−/− mice after administration of CPT I inhibitor exhibit gender-specific patterns. Mean (±SD) blood glucose levels (ordinate) 5, 10, and 24 h after a single dose of etomoxir given to male PPARα−/− mice and the subgroup of female PPARα−/− mice that developed hypoglycemia in response to etomoxir. Each point represents a minimum of five mice.

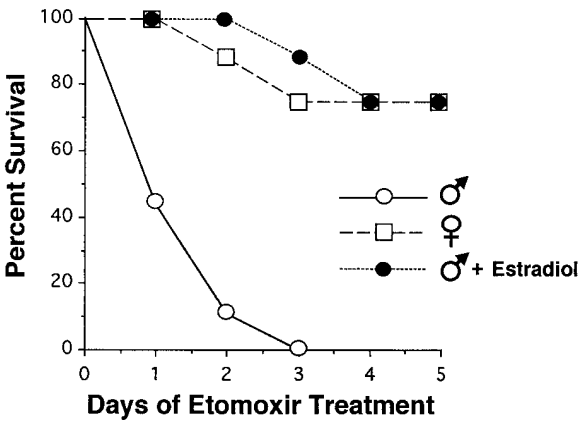

Figure 7. Estradiol pretreatment reduces lethality in male PPARα−/− mice treated with CPT I inhibitor. Adult PPARα−/− mice were treated for 2 wk with a sustained-release 17 β-estradiol–coated pellet (see Methods) before receiving a 5-d course of etomoxir. The graph depicts the survival rate (as a percentage) of etomoxir-treated male PPARα−/− mice ($n = 9$), etomoxir-treated female PPARα−/− mice ($n = 8$), and etomoxir-treated male PPARα−/− mice pretreated with 17 β-estradiol for 2 wk ($n = 8$).

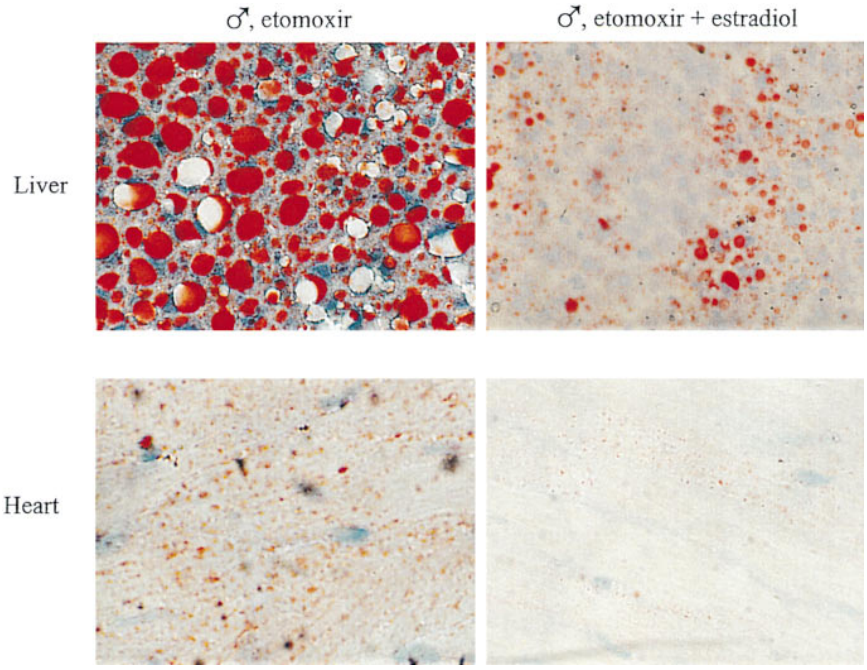

Figure 8. Estradiol pretreatment reduces hepatic and myocardial lipid accumulation in the liver and hearts of male PPARα−/− mice treated with CPT I inhibitor. Representative oil red O–stained sections of liver and heart from a PPARα−/− male after a single dose of etomoxir (50 μg/g) (*left*) compared with sections prepared from an etomoxir-treated PPARα−/− male pretreated with 17 β-estradiol for 2 wk (*right*). The estradiol-treated male mice exhibited significantly less lipid accumulation after one (shown here) or five doses of CPT I inhibitor (data not shown) compared with the PPARα−/− male mice that received etomoxir but not estradiol.

(data not shown). Thus, estradiol pretreatment converted the glucose regulatory response of male PPARα−/− mice receiving CPT I inhibitor to that of the female PPARα−/− mice.

Discussion

Cellular fatty acid utilization rates are controlled with exquisite accuracy to maintain lipid and energy homeostasis in tissues with high fatty acid oxidative flux such as heart and liver. Our results indicate that the lipid-activated nuclear receptor, PPARα, transduces changes in cellular fatty acid oxidative flux to the transcriptional control of genes involved in cellular fatty acid utilization in vivo. We propose that the expression of PPARα target genes encoding mitochondrial, peroxisomal, and CYP enzymes is orchestrated by PPARα in accordance with intracellular levels of fatty acid intermediates, several of which serve as activating PPARα ligands (16–18).

Our results demonstrate the importance of PPARα in cardiac as well as hepatic metabolism. Several lines of published evidence indicate that PPARα is involved in the regulation of liver metabolism. PPARα is required for the hepatic peroxisomal proliferative response to peroxisome proliferators (8, 9). The expression of enzymes involved in peroxisomal and mitochondrial fatty acid oxidation is reduced in liver of PPARα−/− mice (25). However, the relatively high level of expression of PPARα in heart, kidney, and brown adipose tissue (1, 26) suggests that this nuclear receptor also serves a metabolic regulatory function in extrahepatic tissues. Indeed, most PPARα target genes identified to date are expressed abundantly in heart, a tissue with high capacity for fatty acid oxidation. Our results define a role for PPARα in heart in vivo. The cardiac phenotype of the etomoxir-treated PPARα−/− mice strongly suggests that PPARα is involved in the control of myocardial lipid utilization pathways. In further support of this conclusion, we have shown recently that fatty acids activate the transcription of the muscle-type CPT I gene via PPARα in cardiac myocytes (27). Given that the postnatal mammalian heart relies primarily on fatty acid oxidation for energy production, PPARα likely plays a central role in the control of cardiac energy metabolism.

Children afflicted with inborn errors in mitochondrial fatty acid oxidation are typically asymptomatic until a crisis is precipitated by a dietary or physiologic condition that dictates increased reliance on fatty acid oxidation for energy production such as fasting or prolonged exercise (1–3). The molecular pathogenesis of the hepatic and cardiac dysfunction caused by inherited defects in β-oxidation has not been characterized; potential contributing factors include energy deficiency or accumulation of toxic intracellular lipid intermediates. The latter is supported by the observation of myocardial and hepatic lipid accumulation in postmortem studies of children with β-oxidation defects. The cardiac and liver phenotype of the PPARα−/− mice in response to the metabolic stress imposed by CPT I inhibition is remarkably similar to that of humans with genetic defects in mitochondrial fatty acid oxidation. These results suggest that PPARα may serve as a metabolic stress response factor by increasing the expression of enzymes involved in several fatty acid utilization pathways in response to normal physiologic conditions and, perhaps, in disease states such as the inborn errors in fatty acid oxidation. We propose that the PPARα−/− mouse should prove useful as an animal model of this important group of metabolic diseases.

The development of hypoglycemia in etomoxir-treated male PPARα−/− mice underscores the link between fatty acid oxidation and glucose homeostasis. Flux through fatty acid oxidation pathways generates acetyl-CoA. The intracellular acetyl-CoA/long-chain acyl-CoA ratio is an important determinant of the activity of pyruvate carboxylase, which catalyzes a rate-limiting step in hepatic gluconeogenesis (23, 24). Accordingly, the diminished capacity for β-oxidation in the PPARα−/− mice would be expected to place a constraint on gluconeogenic capacity. Our results indicate that in etomoxir-treated male PPARα−/− mice, depletion of glycogen stores

coupled with a defect in glucose production results in a profound hypoglycemia.

A surprising result of this study was the observation that the metabolic stress-induced phenotype of the PPARα−/− mice is strongly influenced by gender and ameliorated by estradiol. The mortality rate, hypoglycemia, and tissue lipid accumulation in PPARα−/− mice in response to CPT I inhibitor treatment were much greater in male compared with female PPARα−/− mice, a phenotype that is largely reversed by estradiol treatment. Our results do not define the mechanism whereby estradiol influences cellular lipid utilization. It is possible that the capacity for fatty acid oxidation is greater, at baseline, in female compared with male mice although we have not detected a significant sex-specific difference in the expression of several key enzymes involved in cellular fatty acid oxidation including CPT I (muscle or liver-type), MCAD, and ACO in PPARα−/− mice (Djouadi, F., and D.P. Kelly, unpublished results). Accordingly, our data are most consistent with the existence of an as yet unidentified, estradiol-triggered, fatty acid utilization pathway and strongly suggest that in addition to PPARα, estrogen signaling plays a role in cardiac and hepatic lipid homeostasis via regulation of fatty acid utilization pathways. In the context of increased cellular lipid levels, an independent, estrogen-regulated cellular lipid clearance pathway may be induced. It is also possible that estrogen receptors activate PPARα target genes via PPAR or estrogen response elements. It will be of considerable interest to delineate the mechanisms involved in the estradiol-mediated control of cellular lipid metabolic pathways and to determine whether this pathway regulates human metabolism under normal conditions and in disease states known to be influenced by gender, such as atherosclerosis and ischemic heart disease.

Our results also implicate a role for estrogen signaling in glucose homeostasis in the PPARα−/− mice. The metabolic response to a fall in blood glucose caused by CPT I inhibition is impaired in male compared with female PPARα−/− mice leading to severe hypoglycemia and death. Pretreatment with β-estradiol converts the glucose homeostatic response of male PPARα−/− mice to that of the female mice. These results strongly suggest that estrogen signaling influences the capacity for gluconeogenesis or glycogenolysis in the PPARα−/− mice in response to CPT I inhibition, possibly via the action of counterregulatory hormones such as glucagon or glucocorticoid.

In summary, our results define an important role for PPARα in the maintenance of lipid and glucose homeostasis in vivo via the transcriptional control of target genes encoding mitochondrial and extramitochondrial fatty acid oxidation enzymes. We also demonstrate that estrogen signaling pathways are involved in hepatic and cardiac lipid metabolism and in the counterregulatory response to hypoglycemia. Lastly, we propose that the PPARα−/− mouse may prove useful as a model of human diseases due to inborn and acquired alterations in cellular lipid metabolism.

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