Peroxisome proliferator–activated receptor α mediates the adaptive response to fasting

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Prolonged deprivation of food induces dramatic changes in mammalian metabolism, including the release of large amounts of fatty acids from the adipose tissue, followed by their oxidation in the liver. The nuclear receptor known as peroxisome proliferator–activated receptor α (PPARα) was found to play a role in regulating mitochondrial and peroxisomal fatty acid oxidation, suggesting that PPARα may be involved in the transcriptional response to fasting. To investigate this possibility, PPARα-null mice were subjected to a high fat diet or to fasting, and their responses were compared with those of wild-type mice. PPARα-null mice chronically fed a high fat diet showed a massive accumulation of lipid in their livers. A similar phenotype was noted in PPARα-null mice fasted for 24 hours, who also displayed severe hypoglycemia, hypoketonemia, hypothermia, and elevated plasma free fatty acid levels, indicating a dramatic inhibition of fatty acid uptake and oxidation. It is shown that to accommodate the increased requirement for hepatic fatty acid oxidation, PPARα mRNA is induced during fasting in wild-type mice. The data indicate that PPARα plays a pivotal role in the management of energy stores during fasting. By modulating gene expression, PPARα stimulates hepatic fatty acid oxidation to supply substrates that can be metabolized by other tissues.

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Introduction

Mammals have evolved a metabolic response system that allows them to survive long periods of energy deprivation. The overall metabolic response to fasting operates at numerous levels and has been relatively well characterized (1). One prominent feature is the gradual shift in whole-body fuel utilization from carbohydrates and fat in the fed state to almost exclusively fat after a day of fasting. This adaptation is particularly striking in the brain, an obligate glucose utilizer in the fed state, which is able to acquire energy predominantly from ketone bodies after prolonged fasting. Most of the actual interconversions in energy substrates occur in the liver, which plays a central role in the adaptive response to fasting.

Prolonged fasting is characterized by low insulin concentrations and high glucagon, glucocorticoids, and (nor)epinephrine concentrations in plasma. This hormonal profile promotes the hydrolysis of triacylglycerols in adipose tissue, thereby increasing the concentration of free fatty acids (FFAs) in plasma. The fatty acids are taken up by the liver, where they are either reesterified to triacylglycerol and secreted as VLDL or oxidized in the mitochondria via β-oxidation. The majority of fatty acids are only partially oxidized to acetyl-coenzyme A (acetyl-CoA), which then condenses with itself to form ketone bodies, an important fuel for the brain. The energy released in the process of β-oxidation is used by the liver to carry out gluconeogenesis from substrates such as glycerol, lactate, and amino acids. Thus, efficient hepatic fatty acid oxidation is obligatory to the metabolic response to fasting.

Studies over the past few years have shown that a group of nuclear hormone receptors, the peroxisome proliferator–activated receptors (PPARs), have an important role in fatty acid metabolism (2). Nuclear hormone receptors are ligand-activated transcription factors that control gene transcription in response to small lipophilic compounds such as retinoic acid, thyroid hormone, vitamin D, and fatty acids (3). They activate transcription by binding to the promoter region of target genes, where they recognize a specific sequence of nucleotides called hormone response elements. Two lines of evidence have linked PPARs to lipid metabolism. First, all genes whose transcription is controlled by PPAR are in one way or another involved in lipid metabolism and energy homeostasis. These include lipoprotein lipase, fatty acid binding protein (FABP), carnitine palmitoyltransferase (CPT), acyl-CoA oxidase, and many others (4). Second, PPARs bind and are activated by unsaturated long-chain fatty acids, as well as by their eicosanoid derivatives (5–9).

The PPAR family contains 3 distinct isotypes: α, β, and γ (2). The γ isotype is mainly expressed in adipose tissue (10), where it has been shown to be an essential component of the adipocyte differentiation program (11); and in macrophages, where it modulates differentiation and cytokine production (12–15). PPARβ is expressed in all tissues examined (10), but its function remains an enigma. The third isotype, PPARα, is primarily expressed in brown

adipose tissue (BAT) and the liver, and to a lesser extent in the kidneys, skeletal muscle, and heart (10). Of the 3 isotypes, PPARα has been the best characterized, a fortunate consequence of the availability of PPARα-null mice (16). Studies with these mice have demonstrated that PPARα controls the expression of numerous genes related to lipid metabolism in the liver, including genes involved in mitochondrial β-oxidation, peroxisomal β-oxidation, fatty acid uptake and/or binding, and lipoprotein assembly and transport (17–19). Several functional consequences of lowered gene expression levels were observed: PPARα-null mice are refractory to peroxisome proliferators, and male mice appeared to be overly sensitive to etomoxir, an inhibitor of carnitine palmitoyltransferase I (CPTI) (16, 20). A striking metabolic defect was observed in aged (8-month-old) PPARα-null mice, characterized by a sexually dimorphic dyslipidemia with pronounced adiposity in females and steatosis in males (21). Despite this great expansion of our understanding of the function of PPARα, what remains unclear is when and how, in an intact organism, the PPARα signaling pathways are triggered, and how this specifically affects lipid and carbohydrate metabolism.

One physiological condition during which PPARα-dependent signaling should become challenged is fasting, because (a) huge amounts of fatty acids are delivered to the liver to be oxidized; (b) once taken up, fatty acids have to be delivered to the mitochondria for oxidation; and (c) β-oxidation is accelerated in conjunction with increased synthesis of ketone bodies.

A second physiological stimulus that may challenge the PPARα-dependent signaling system is a high fat diet. With a high-fat diet, the rate of delivery of fatty acids to the liver is lower than in the fasting state, and the requirement for fatty acid oxidation is concomitantly less. However, in contrast to fasting, a high-fat diet represents a chronic stimulus.

In this study, PPARα-null mice were either fasted or fed a high fat diet to investigate the role of PPARα under each physiological condition. Fasted PPARα-null mice show enhanced accumulation of lipid in the liver, suffer from severe hypoglycemia and hypothermia, and reveal a plasma metabolite profile that suggests a dramatic impairment of fatty acid uptake and oxidation. Furthermore, it is demonstrated that to accommodate the increased requirement for hepatic fatty acid oxidation during fasting, PPARα mRNA is induced in wild-type mice. The data indicate that PPARα is a key part of a complex network of signaling pathways in the liver that operate during fasting and stimulate fatty acid oxidation to form substrates that can be metabolized by other tissues.

Methods

Animals. Mice were housed in a temperature-controlled room (23°C) on a 10-hour dark/14-hour light cycle. Unless otherwise indicated in the figure legends, male mice were used and were provided unrestricted amounts of food and water. Purebred wild-type or PPARα-null mice on an SV129 background were used (16). All experiments were performed with mice 8–12 weeks of age (except for the high fat diet, for which the mice were 6–10 weeks older at the time of sacrifice). Animal experiments were approved by the animal authorization commission of the canton of Vaud (Switzerland).

Diet. Under normal conditions, mice were fed a standard lab chow containing about 12 energy percent (en%) fat. Two types of high fat diets (39 en% fat) were used: a high saturated fat diet (coconut oil based) and a high unsaturated fat diet (safflower oil based) (ICN Research Diets, Costa Mesa, California, USA).

RNA preparation and Northern blots. Total RNA was prepared from frozen livers, BAT, and gastrocnemius by RNeasy Midi Kit (QIAGEN , Basel, Switzerland) or Trizol reagent (GIBCO BRL, Basel, Switzerland). Twenty micrograms of total RNA (10 µg for BAT, 15 µg for gastrocnemius) was loaded per lane. Electrophoresis, blotting, and hybridization were according to standard protocols. Radioactive DNA probes were prepared by High
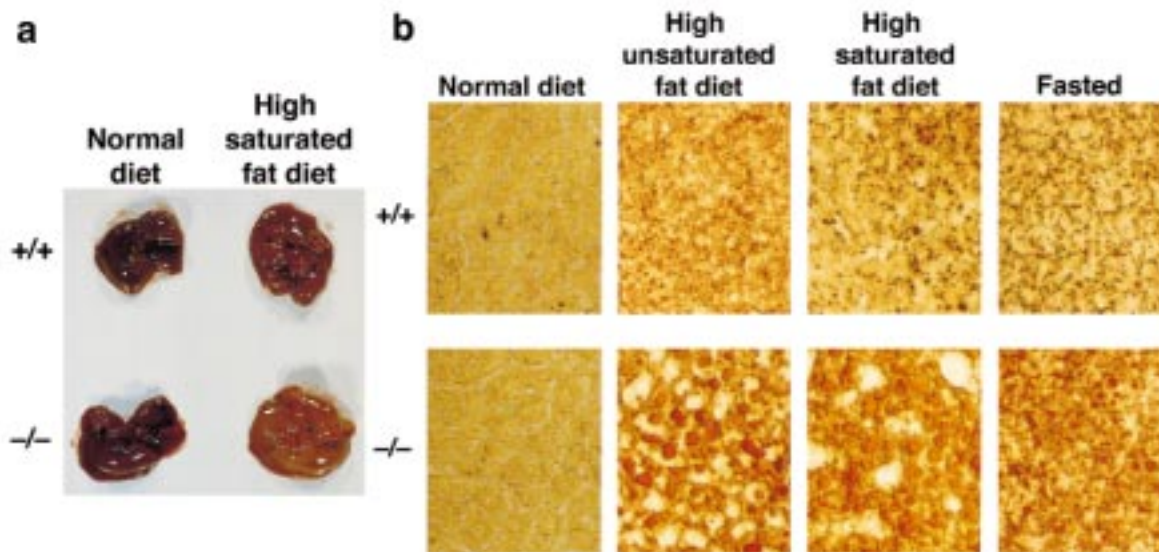

Figure 1
PPARα-null mice fed a high fat diet or subjected to fasting develop a fatty liver. Wild-type SV129 and PPARα-null mice were fed a high saturated fat diet for 10 weeks or a high unsaturated fat diet for 7 weeks. Fasted mice were deprived of food for 24 hours. (**a**) Gross morphology and color of livers of mice fed the high saturated fat diet or the normal diet. (**b**) Oil red O staining of liver sections of mice fed the normal diet, a high unsaturated fat diet, a high saturated fat diet (sections taken during the light cycle), or fasted for 24 hours.

Figure 2

Fasting-induced gross disturbances in the levels of several plasma metabolite levels in PPARα-null mice. SV129 wild-type or PPARα-null mice were sacrificed at the end of the dark cycle (fed state) or after a 24-hour fast that was started at the beginning of the light cycle (fasted state). (**a**) Plasma FFA concentrations. (**b**) Plasma β-hydroxybutyrate concentrations. (**c**) Plasma lactate concentrations. (**d**) Glycogen concentrations in liver. Error bars represent SEM. For the data in **a–c**, ANOVA yielded a significant effect for fasting vs. feeding ($P <$ 0.01). For the data in **a** and **b**, the same was true for genotype and for the interaction between fasting/feeding and genotype ($P <$ 0.01). §Significantly different from fed wild-type mice ($P <$ 0.05). *Significantly different from all other values ($P <$ 0.01). †Significantly different from fed mice ($P <$ 0.01). All analyses by post hoc t test.


Prime Kit (Boehringer Mannheim Biochemicals, Rotkreuz, Switzerland). Probes were obtained from various sources: the rat liver fatty acid binding protein (L-FABP) was from J.I. Gordon (Washington University School of Medicine, St. Louis, Missouri, USA), rL-CPTI from J.O. McGarry (University of Texas Southwestern Medical Center, Dallas, Texas, USA), and mPEPCK from E.G. Beale (Texas Technological University Health Sciences Center, Lubbock, Texas, USA). Mouse apo E, apo B, and microsomal triglyceride transfer protein (MTP) were obtained by PCR amplification of a mouse cDNA library (either self-made or purchased from CLONTECH Laboratories Inc., Palo Alto, California, USA). Probes were cloned into Bluescript plasmid, and the sequence was verified by DNA sequencing. mSCAD (SCAD=short chain acyl-COA dehydrogenase) was obtained by subtractive hybridization of mRNA of livers from wild-type and PPARα-null mice (22). Uncoupling protein (UCP) probes were obtained by RT-PCR of total RNA from BAT (UCP1) or skeletal muscle (UCP2 and UCP3), using gene-specific primers. The cDNA of the ribosomal protein L27 was used as a control probe.

Intraperitoneal glucose tolerance test. Wild-type and PPARα-null mice were fasted for 6 hours, starting at the beginning of the light cycle. No anesthesia was performed. At time 0, blood glucose was measured, and immediately thereafter, 2 g glucose/kg body weight was injected intraperitoneally by means of a 20% sterile glucose solution. Blood glucose was subsequently measured at several time points.

Metabolite assays. Blood for glucose measurements was taken from the tail vein. Otherwise, blood was drawn by retro-orbital puncture. Blood was collected into heparinized tubes, kept on ice, and spun for 10 minutes to collect plasma. Plasma was kept at –70°C. Blood glucose was measured using an Accutrend DM glucose analyzer (Boehringer Mannheim Biochemicals). Plasma FFAs and β-hydroxybutyrate were determined using kits from Boehringer Mannheim. Liver glycogen was determined by the amyloglucosidase method using Trinder reagent (Sigma Chemical Co., St. Louis, Missouri, USA) to measure released glucose. Plasma lactate was measured with a kit from Sigma Chemical Co.

Rectal temperature. Rectal temperature was recorded with a thermoprobe (Ellab, Copenhagen, Denmark) inserted 3 cm into the colon.

Metabolic rate. Metabolic rate was measured by indirect calorimetry during a 24-hour period beginning at the start of the light cycle. An open-circuit calorimeter, equipped with a sensitive mass flowmeter (model 5875; Brooks Instruments, Veenendaal, the Netherlands), was used. The mice were placed individually in a 3.5-L Plexiglas chamber through which air was flowing at 0.6 L/min. Except when fasting, food and water were freely available. When fasted for 24 hours, the mice had free access to water containing 4.5 g/L of NaCl. Changes in oxygen and carbon dioxide content of the air passed through the chamber were measured with oxygen and carbon dioxide
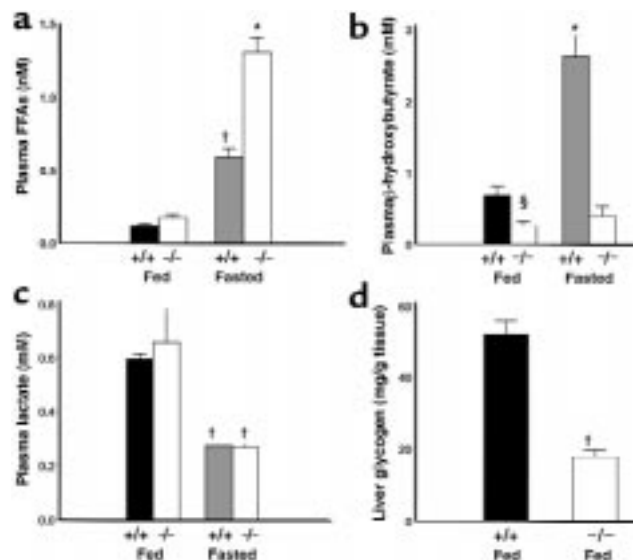
analyzers (Magnos 4G and Uras4; Hartmann and Braun, Frankfurt am Main, Germany). The ambient temperature was set at 22°C. The data were recorded every 5 seconds by an on-line computerized data acquisition system (Service Instrumental of the Centre Médicale Universitaire, Geneva, Switzerland). The metabolic rate was calculated using Weir's equation (23) and expressed in terms of watts per kilogram of body weight to 0.75 power.

Statistical analysis. The effects of fasting/feeding and genotype on plasma metabolite levels, rectal temperature, and metabolic rate were tested by two-way ANOVA. If F was found to be significant, the Student's t test was used to test individual differences (protected least significant difference; one-sided).

Results

A high fat diet induces a fatty liver in PPARα-null mice. The high fat diet used in the experiments was based on either coconut oil (saturated fat diet) or safflower oil (unsaturated fat diet), and contained about 40 en% fat, which is more than 3-fold higher than the normal diet. In our studies, feeding mice a high fat diet visually increased fat deposition in the adipose tissue of both wild-type and PPARα-null mice. Elevated adipose tissue stores promote the development of a fatty liver, as has been observed in obese human patients (24). Feeding a high fat (saturated or unsaturated) diet for several weeks resulted in an accumulation of triacylglycerols in the livers of wild-type mice, as indicated by a slight discoloration of the liver (Figure 1a). This was confirmed by oil red O staining of liver sections, which revealed numerous little lipid droplets that were not observed in mice fed the normal diet (Figure 1b). Strikingly, the appearance of lipid droplets was much more pronounced in PPARα-null mice fed the high fat diet; as a result, the liver had a pale appearance (Figure 1a). Oil red O staining also revealed large empty white spaces, which were probably large fat droplets that had their contents washed away during the staining procedure. Liver weights of PPARα-null mice fed the high saturated fat diet were 22% higher than the liver weights of wild-type mice fed the same diet (6.0% of total body weight for PPARα-null mice vs. 4.9% for wild-type mice;

P < 0.01). These differences were not observed in PPARα-null and wild-type mice fed a normal diet.

Combined, these results show that PPARα-null mice are particularly sensitive to a high fat diet and develop a fatty liver in response. However, because the metabolic responses to a high fat diet are relatively ill-defined, and because different mouse strains display a marked disparity in their reactions to a high fat diet (25, 26), we decided to focus our efforts on better characterizing the response to fasting.

Fasting induces a fatty liver in PPARα-null mice. Paradoxically, not only obesity but also chronic undernutrition is associated with a fatty liver (27). In mice and other animals, an overnight fast leads to significant lipid accumulation in the liver (28, 29, and see below). This can be explained by the high rate of fatty acid uptake by the liver, which under fasting conditions can exceed the capacity of the liver to secrete triacylglycerols (29). To test the effect of deletion of PPARα on lipid accumulation, PPARα-null and wild-type mice were subjected to 24 hours of fasting. Visual inspection of livers of fasting PPARα-null mice showed that they were distinctly paler than the livers of fasting wild-type mice. Staining for lipids with oil red O demonstrated that the pale color is due to the presence of numerous lipid droplets (Figure 1b), which were observed only to a small extent in fasted wild-type mice. Control staining by hematoxylin and eosin revealed these lipid droplets as small unstained vacuoles in liver sections of fasted PPARα-null mice but not wild-type mice (data not shown). These findings show that deletion of PPARα leads to a marked increase in lipid accumulation in the liver during fasting.

Diminished hepatic uptake of fatty acids in fasted PPARα-null mice. Once inside the liver cell, fatty acids can enter the mitochondria for subsequent oxidation, or they can be re-esterified to triacylglycerols, followed by secretion of the triacylglycerols in the form of VLDL. The capacity for secretion of VLDL is limited, which may lead to accumulation of triacylglycerols under conditions of an enlarged intracellular pool of fatty acids. Theoretically, the greater increase in lipid accumulation in fasted PPARα-null mice compared with fasted wild-type mice may be due to 3 potential, nonexclusive mechanisms: increased uptake of fatty acids, decreased secretion of VLDL, and/or decreased oxidation of fatty acids. To determine whether fatty acid uptake is increased in fasted PPARα-null mice, the concentration of circulating FFAs was measured. Plasma FFAs were significantly elevated in fasted mice compared with fed mice (Figure 2a), consistent with the increased rate of lipolysis occurring in the adipose tissue. Interestingly, plasma FFAs were more than 2-fold higher in the fasted PPARα-null mice compared with fasted wild-type mice. Because the liver represents the major sink of fatty acids under fasting conditions, this result suggests that uptake of fatty acids into the liver is decreased rather than increased. This is in agreement with the known stimulatory effect of PPARα on transcription of fatty acid transporter genes (19, 30).

Expression of genes involved in VLDL secretion is not altered in PPARα-null mice. Another possible explanation for the increased triacylglycerol accumulation in livers of fasted PPARα-null mice is that VLDL secretion is defective. To test whether some of the genes involved in VLDL secretion or that encode constituents of VLDL are controlled by PPARα, the expression of 3 genes coding for apo E, apo B, and MTP were determined in PPARα-null and wild-type mice. The expression of all 3 genes was identical between the 2 sets of mice, in both the fed and fasted states (Figure 3, left). Expression of apo C-III, another constituent of VLDL, has been reported to be identical between PPARα-null and wild-type mice (18). Although this does not completely rule out the possibility that secretion of triacylglycerols is altered in PPARα-null mice, these results indicate that VLDL secretion is unlikely to be a PPARα target.

Hepatic fatty acid oxidation is dramatically impaired in fasted PPARα-null mice. The third possibility that may explain the accumulation of lipid in the livers of fasted PPARα-null mice is impairment of fatty acid oxidation. To examine whether fatty acid oxidation is affected in PPARα-null mice, the concentration of plasma β-hydroxybutyrate, a
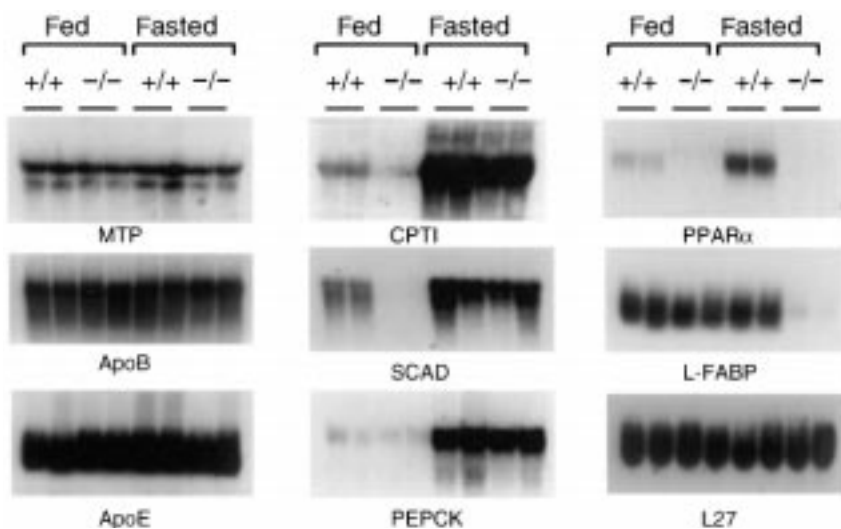

Figure 3
Fasting/feeding has dramatic effects on the expression of several PPAR target genes in a PPARα-dependent manner. Northern blot analysis of RNA from livers of fed and fasted SV129 wild-type or PPARα-null mice. Total RNA was isolated from livers of SV129 wild-type or PPARα-null mice sacrificed at the end of the dark cycle (fed state) or after a 24-hour fast started at the beginning of the light cycle (fasted state). Probes used were as indicated.
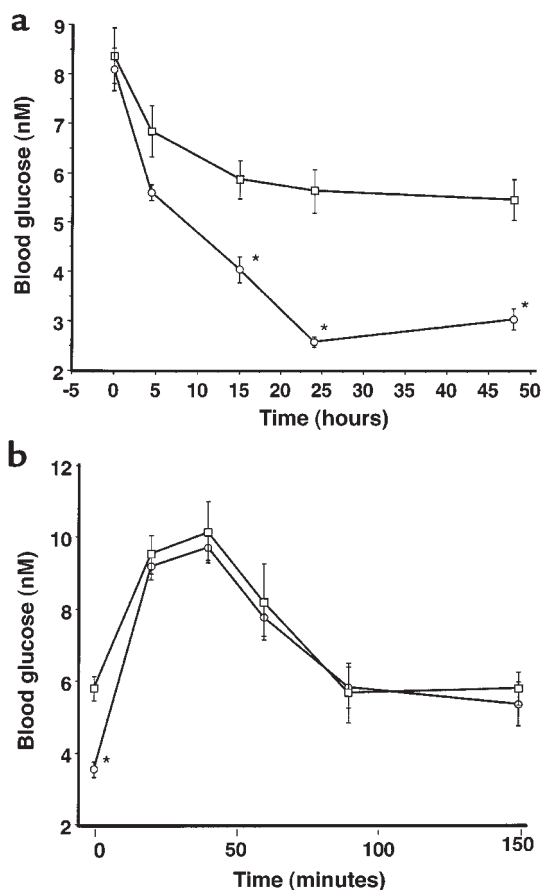
Figure 4

PPARα-null mice subjected to fasting become severely hypoglycemic. (**a**) Time course of blood glucose after removal of food. Blood glucose was measured at the end of the dark cycle when the animals were in the fully fed state. Food was subsequently withdrawn and blood glucose measured at several time points. Values at different time points are not necessarily from the same group of animals. Open squares, SV129 wild-type mice; open circles, PPARα-null mice. Error bars represent SEM. ANOVA showed a significant effect for genotype ($P < 0.01$), for time after removal of food ($P < 0.01$), and for interaction between these 2 parameters ($P < 0.01$). *Significantly different from wild-type mice ($P < 0.01$ by post hoc t test). (**b**) Intraperitoneal glucose tolerance test. Food was withdrawn for 6 hours starting at the beginning of the light cycle. At time 0, blood glucose was measured. Immediately thereafter, 2 g glucose/kg body weight was injected intraperitoneally by means of a sterile 20% glucose solution. Blood glucose was subsequently measured at several time points. Open squares, SV129 wild-type mice; open circles, PPARα-null mice. Error bars represent SEM. *Significantly different from wild-type mice ($P < 0.01$ by t test).

ketone body that represents an important intermediate in the fatty acid oxidation pathway, was assessed. As expected, β-hydroxybutyrate levels were greatly increased in fasted wild-type mice (Figure 2b) but only slightly increased in fasted PPARα-null mice, resulting in a concentration that was about 7-fold less than in fasted wild-type mice. This low β-hydroxybutyrate level most likely reflects a markedly reduced rate of fatty acid oxidation, as well as a potential diminution in the rate of the reaction catalyzed by HMG-CoA synthase, a PPARα target gene in the liver (4).

An increased rate of hepatic fatty acid oxidation during fasting is not only responsible for the enhanced production of ketone bodies; it also assures efficient operation of gluconeogenesis. Indeed, gluconeogenesis is driven by fatty acid oxidation, which supplies ATP and reducing equivalents and directs pyruvate and lactate away from oxidation toward glucose synthesis. To examine whether deletion of PPARα may have an effect on gluconeogenesis, we measured the concentration of glucose in the blood during fasting. Remarkably, it was observed that PPARα-null mice suffer from hypoglycemia just several hours after food has been withdrawn (Figure 4a). Because blood glucose at this time is mostly maintained by glycogenolysis, the steeper drop in blood glucose in PPARα-null mice probably reflects the reduced concentration of glycogen in the livers of fed PPARα-null mice (Figure 2d). After prolonged fasting, when blood glucose is exclusively maintained by gluconeogenesis, the glucose concentration continued to drop more sharply in PPARα-null mice, being less than half the value of that in the wild-type animals. These data suggest that gluconeogenesis is impaired in PPARα-null mice.

Intraperitoneal injection of glucose into PPARα-null mice fasted for 6 hours resulted in rapid normalization of their blood glucose concentration (Figure 4b). This intraperitoneal glucose tolerance test also showed that PPARα-null mice do not suffer from oversensitivity (or insensitivity) to insulin, because the glucose response curves were similar in PPARα-null and wild-type mice. This important result indicates that deletion of PPARα has no effect on the sensitivity of peripheral tissues to insulin.

Finally, plasma lactate, which reflects the rate of glycolysis in muscle, decreased during fasting, but no differences in plasma lactate levels were observed between wild-type and PPARα-null mice (Figure 2c). Taken together, these data demonstrate that hepatic fatty acid oxidation becomes severely impaired during fasting in the absence of PPARα, resulting in hypoglycemia, hypoketonemia, elevated plasma levels of FFAs, and a fatty liver.

Fasted PPARα-null mice are hypothermic and have a lower metabolic rate. When PPARα-null mice were subjected to fasting, we noticed that they appeared to be very cold. Rectal temperature measurements revealed that the mice suffered from severe hypothermia (Figure 5a). In wild-type SV129 mice, a 24-hour fast caused a drop in rectal temperature of 1.6°C, whereas in the PPARα-null mice, mean rectal temperature fell by 9.1°C, indicating that PPARα plays a major role in temperature homeostasis during fasting. This was also evident by measurement of the whole-body metabolic rate by indirect calorimetry. The data show that in fed mice of both genotypes, the metabolic rate was similar and fasting caused a significant rate decrease (Figure 5b). However, consistent with the lowered body temperature, the metabolic rate in fasted PPARα-null mice was significantly (about 30%) lower than in fasted wild-type mice.

The lowered body temperature and associated lowered metabolic rate in fasted PPARα-null mice indicate that their temperature regulation was adjusted so that their metabolic needs would match the low, fasting-induced energy supply. In fact, when mice were exposed to the cold (5°C), no differences in rectal temperature were observed

between PPARα-null and wild-type mice after 3 hours (Figure 6a), suggesting that cold-induced thermogenesis is not affected in PPARα-null mice. Similarly, expression levels of UCPs, which are postulated to be involved in thermogenesis and are probable target genes of PPAR, were similar in PPARα-null and wild-type mice in several tissues (Figure 6b). This was true despite a fasting-induced increase in expression levels of UCP2 in liver, gastrocnemius, and BAT, and of UCP3 in gastrocnemius. These data indicate that the hypothermia in fasted PPARα-null mice is not due to a defect in overall thermogenic capacity caused by reduced expression levels of UCPs.

A more likely explanation for the hypothermia in fasted PPARα-null mice is the lack of fuel available for energy generation, forcing the animals into a state of torpor to reduce energy expenditure (31). Both plasma ketone bodies and glucose levels are dramatically reduced in fasted PPARα-null mice, as a result of inhibition of fatty acid oxidation in the liver. The data thus lead to the argument that the severe hypothermia in fasted PPARα-null mice is essentially caused by a defect in hepatic metabolism.

Lack of obvious phenotype in fed PPARα-null mice is partially due to the low rate of hepatic fatty acid oxidation. An important question is why PPARα-null mice need to be subjected to fasting to elicit a strong phenotype. One possible explanation is that the process regulated by PPARα only becomes important during fasting. Hepatic fatty acid oxidation operates at a relatively modest level in fed mice, whereas it is strongly stimulated during long periods of food deprivation. Hence, it is possible that deletion of PPARα affects gene expression levels in fed mice, but because of the low rate of fatty acid oxidation, the effects do not clearly manifest themselves. Northern blot analysis of liver mRNA revealed that under fed conditions, deletion of PPARα did in fact decrease expression of both CPTI, which catalyzes the rate-limiting step in fatty acid oxidation (32), and SCAD (Figure 3, center, first 4 lanes), both of which are PPARα target genes. This effect was not observed for all PPARα targets, e.g., L-FABP. The expression of L-FABP is dependent upon PPARα during fasting, but not in the fed state. The data indicate that, in the fed state, the marked differences in expression levels of important PPARα target genes between wild-type and PPARα-null mice have little apparent effect at the metabolic level. Thus, the lack of a strong phenotype in fed PPARα-null mice must be partially due to the relatively low rate of hepatic fatty acid oxidation under those conditions.

PPARα mRNA is induced during fasting. Another possible explanation of why PPARα-null mice need to be subjected to fasting to elicit a recognizable phenotype is that PPARα is suppressed under normal conditions and becomes activated during fasting. Accordingly, deletion of the PPARα gene will have a more pronounced effect under fasting conditions. Activation of PPARα during fasting can be effected by induction of PPARα mRNA and a resulting increase in PPARα protein, and/or by an increase in PPARα ligand activation. With respect to the first mechanism, fasting did induce PPARα mRNA expression (Figure 3, right), an effect probably mediated by glucocorticoids (33). Because PPARα protein level is strongly correlated with the expression of its mRNA (33), it can be expected that PPARα protein is similarly induced under fasting conditions. Regarding the second mechanism, it is important to note that fasting is associated with a marked increase in the plasma concentration of FFAs (Figure 2a). Since fatty acids are bona fide ligands for the PPAR receptors, it is conceivable that the elevated levels of FFAs activate PPARα.

Both in vitro and in vivo studies have demonstrated that PPARα preferentially binds polyunsaturated fatty acids (PUFAs) (7–9, 34). Fat tissue rich in PUFAs thus constitutes a source of higher-affinity ligands than does normal fat tissue, possibly resulting in stronger ligand activation of PPARα during fasting. Because the fatty acid composition of adipose tissue directly reflects the long-term diet (35), to enrich fat tissue with PUFAs, wild-type mice were fed a diet high in polyunsaturated fat. L-FABP gene was selected as a marker to assess the effect of PPARα activation, because during fasting, the expression of L-FABP is highly dependent upon PPARα (Figure 3, right) and is increased by fatty acids that act via PPARα (36, 37).
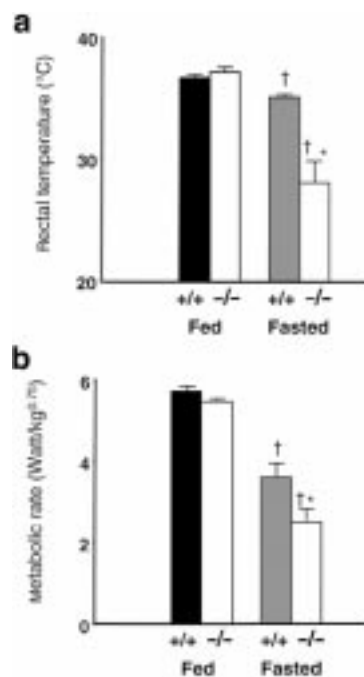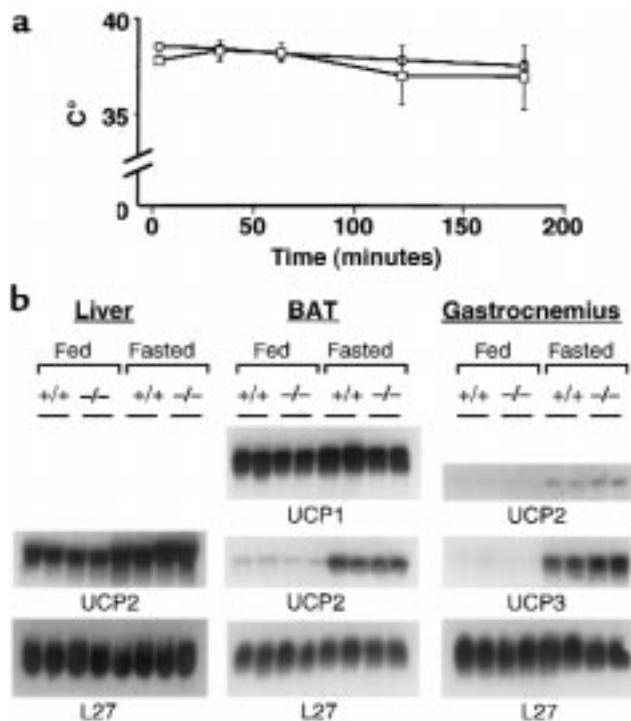
Figure 5

Fasted PPARα-null mice suffer from hypothermia and have a lower metabolic rate than fasted wild-type mice. Only female mice were used for these measurements. (**a**) Rectal temperature. Measurements were taken at the beginning of the light cycle (fed state) or after a 24-hour fast that was started at the beginning of the light cycle (fasted state). Note that the *y* axis starts at 20°C. Error bars represent SEM. ANOVA showed a significant difference between fasting and feeding ($P < 0.05$), genotype ($P < 0.01$), and interaction between fasting/feeding and genotype ($P < 0.01$). *Significantly different from fasted wild-type mice ($P < 0.01$). †Significantly different from fed mice ($P < 0.05$ [+/+] or $P < 0.01$ [–/–]). All analyses by post hoc *t* test. (**b**) Metabolic rate. For each mouse, mean metabolic rate was calculated for a 23-hour period with free access to food and water (fed state), or during the last 3 hours of a 24-hour fast (fasted state). Error bars represent SEM. ANOVA yielded significant effects for fasting vs. feeding ($P < 0.01$) and genotype ($P < 0.05$). *Significantly different from fasted wild-type mice ($P < 0.05$). †Significantly different from fed mice ($P < 0.01$). All analyses by post hoc *t* test.

Figure 6

PPARα-null mice can activate cold-induced thermogenesis. (**a**) Rectal temperature of wild-type and PPARα-null mice exposed to the cold. Mice were placed in individual precooled cages in a cold room maintained at 5°C. Rectal temperature was subsequently measured at various intervals. Open squares, SV129 wild-type mice; open circles, PPARα-null mice. (**b**) Analysis of UCP expression in various tissues by Northern blot. Total RNA was isolated from tissues of SV129 wild-type or PPARα-null mice sacrificed at the end of the dark cycle (fed state) or after a 24-hour fast started at the beginning of the light cycle (fasted state). Probes used were as indicated.

In fasted wild-type mice that were fed a diet high in linoleic acid for 7 weeks, the expression of L-FABP was about 4-fold higher than in fasted mice fed the normal diet (Figure 7). Surprisingly, the increase in L-FABP mRNA was accompanied by a similar increase in PPARα mRNA (about 3-fold). Thus, although PUFAs were able to increase expression of the PPARα target gene L-FABP, this effect could be almost entirely accounted for by an induction of PPARα mRNA itself. PUFAs seemed to have little or no additional effect on L-FABP mRNA beyond that mediated by increased PPARα expression, indicating that no increased ligand activation of PPARα took place. It cannot be completely ruled out that PUFAs increased L-FABP mRNA via a mechanism not involving PPARα. However, because PPARα has an exclusive role in maintaining L-FABP expression during fasting (Figure 3), we consider this explanation unlikely. Unfortunately, these data do not tell us whether PPARα is saturated with ligand in the fed state, and thus whether the elevation in plasma FFA concentration during the initial stage of fasting might contribute to PPARα activation.

Transcriptional regulation of PPARα target genes during fasting is complicated by other hormonal signaling pathways. Because PPARα activity is enhanced by increased expression, and perhaps by increased ligand activation, it can be expected that some PPARα target genes are upregulated during fasting in a PPARα-dependent manner. Northern blot analysis showed, however, that the situation is considerably more complex. It was found that, in the fasted state, despite a strong increase in expression of CPTI and SCAD, the PPARα dependence was lost, suggesting that other signaling pathways activated during fasting override the effect of PPARα.

The expression of L-FABP displayed a completely different pattern. In the fed state, the expression of L-FABP was identical between wild-type and PPARα-null mice (Figure 3). In sharp contrast to CPTI and SCAD, in the fasted state, the expression of L-FABP was not upregulated in wild-type mice but was reduced more than 30-fold in PPARα-null mice. Although it may be tempting to correlate the emergence of a strong phenotype during fasting with this markedly reduced expression of L-FABP, it should be noted that L-FABP is only one of many genes whose expression is likely to be decreased in fasted PPARα-null mice. These data indicate that although PPARα mRNA is induced during fasting, the effects on PPARα target genes are complicated by the additional influence of other hormonal signaling pathways.

PEPCK is not a PPARα target gene in liver. Lastly, the expression of PEPCK, a PPARγ target gene in adipose tissue that catalyzes the rate-limiting step in gluconeogenesis (38), was unchanged in wild-type and PPARα-null mice in both the fed and fasted states (Figure 3), suggesting that PEPCK is not a PPARα target gene in liver.

Discussion

In this paper, we show that fasted PPARα mice suffer from a severe impairment in hepatic oxidation, resulting in a phenotype characterized by hypoglycemia, hypothermia, hypoketonemia, elevated plasma levels of FFAs, and a fatty liver. Gene expression levels reveal a striking pattern in which some PPARα target genes are affected by PPARα deletion only in the fed state, whereas others are affected only in the fasted state, indicating the convergence of different regulatory pathways toward the expression of a single PPARα target gene. The data clearly demonstrate that PPARα plays a crucial role in the adaptive response to fasting by modulating expression levels of target genes. In addition, the data firmly establish the pivotal role of PPARα in hepatic fatty acid oxidation in vivo. By affecting hepatic fatty acid oxidation, PPARα has a profound effect on other metabolic pathways and overall energy homeostasis.

Over the past 5 years, numerous genes have been identified whose expression is controlled by PPARα in the liver, and most of them are involved in liver fatty acid metabolism. The reduced expression of these genes in PPARα-null mice has been correlated with a decreased capacity for β-oxidation in hepatocytes in culture (17). Despite this wealth of evidence linking PPARα to hepatic lipid oxidation, the phenotype of PPARα-null mice initially appeared to be relatively mild (16, 18). Although this may sound surprising, it should be kept in mind
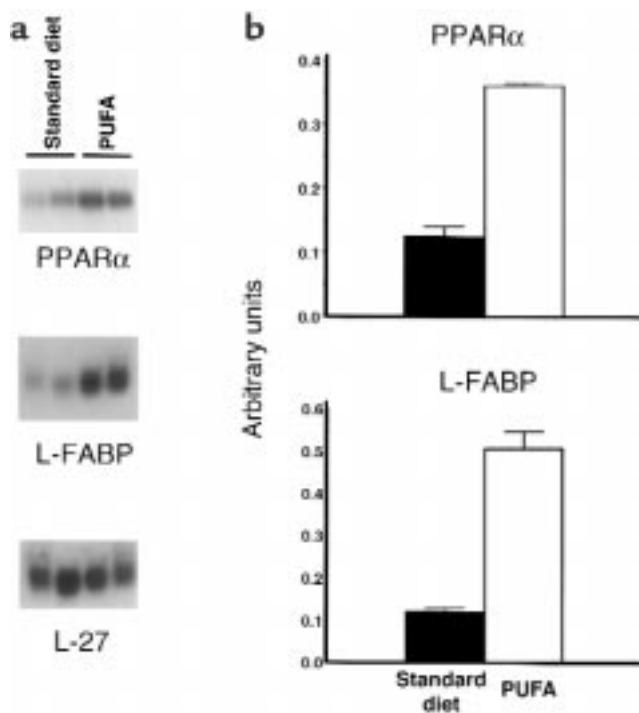
Figure 7

Prolonged feeding of a high unsaturated fat diet more strongly induces expression of PPARα and L-FABP genes after a 24-hour fast than does feeding of a normal diet. (**a**) Northern blot analysis of RNA from livers of fasted SV129 wild-type mice that had been fed a normal diet or a diet high in unsaturated fat (>70% linoleic acid) for 7 weeks. Total RNA was isolated from livers of SV129 wild-type mice sacrificed after a 24-hour fast, started at the beginning of the light cycle. (**b**) Quantitation of the intensity of the autoradiography signal corrected for control probe L27 . Means of 2 identical experiments are shown. Error bars have no statistical meaning but connect the 2 individual values.

that hepatic fatty acid oxidation is suppressed in mice that are fed freely. This situation changes dramatically during fasting, when a complex metabolic response is elicited, aimed at minimizing the use of protein and carbohydrates as fuel and instead relying on the abundant adipose tissue stores (Figure 8). It is under these conditions that a strong upregulation of various fatty acid–metabolizing enzymes is needed, and accordingly, a major role for PPARα can be expected. Indeed, the emergence of a strong phenotype in PPARα-null mice during fasting underscores the pivotal role of PPARα in this process. Our data also indicate that in order to accommodate the increased requirement for hepatic fatty acid oxidation, PPARα is activated during fasting. This is achieved by an increase in expression, and perhaps by an increase in ligand activation, although our data are not conclusive in this respect.

As alluded to above, PPARα can be activated by at least 2 different mechanisms. First, during fasting, the expression of PPARα in liver increases, an effect that is mediated by glucocorticoids (33) and results in an increased amount of PPARα protein. Second, the fatty acids that are liberated from the adipose tissue and travel to the liver may serve as ligands for PPARα. By means of such a

mechanism, the organism assures that the enzymatic system of the liver responds appropriately to the increased demands for fatty acid oxidation; the energy substrate itself may directly activate the expression of enzymes that participate in its metabolism. Unfortunately, this mechanism is extremely difficult to prove in vivo. Our data show that entry of high-affinity ligands (as opposed to low-/moderate-affinity ligands) into the liver has no further effect on the expression of PPARα target genes beyond that already mediated by an increase in expression of PPARα itself. This suggests that PPARα is fully saturated with ligand in the 24-hour fasted state. However, our results can neither confirm nor disprove the idea that an increase in the amount of fatty acids released from adipose tissue contributes to stimulation of PPARα target gene expression during fasting.

It is quite surprising that expression of CPTI and SCAD is similar in fasted PPARα-null and wild-type mice, despite the fact that expression of PPARα is increased during fasting, and thus, a more pronounced PPARα-dependent effect may be expected. This is probably due to modification of PPARα effects by other hormone signaling pathways, such as those activated by glucocorticoids, glucagon, insulin, and by adrenergic hormones. Additional information concerning the regulation of these genes by various hormones may shed some light on this apparent paradox. Although the activity of CPTI is mostly controlled by allosteric inhibition by malonyl-CoA (32), significant regulation also takes place at the gene expression level. A glucocorticoid-dependent upregulation, combined with negation of the insulin-dependent downregulation, will cause a major increase in CPTI expression during fasting (39, 40). This effect may be so strong as to blunt any further increase mediated by PPARα.

In the case of L-FABP, where a strong PPARα effect is observed only in the fasted state, glucocorticoids decrease expression, while insulin increases expression (41, 42). In the absence of any PPARα signaling, one would thus expect a dramatic reduction in L-FABP expression during fasting. This is indeed what is happening in the PPARα-null mice. In normal mice, however, PPARα is activated and thus able to compensate for the negative signals originating from glucocorticoid and insulin signaling pathways.

Somewhat unexpectedly, the expression of PEPCK was found to be identical between wild-type and PPARα-null mice, in both the fed and fasted states. This is surprising considering that the PEPCK promoter contains an intermediate-affinity PPAR response element (43), which is responsible for the upregulation of PEPCK by PPARγ in adipose tissue (38). The lack of PPARα-dependent regulation in the liver in vivo is probably due to the competition for this site from other nuclear receptors such as HNF4, COUP-TFI, and RXR/RAR (44).

Our data show that expression of UCPs in skeletal muscle and BAT is not regulated by PPARα. These results fit with the lack of effect of the high-affinity PPARα ligand WY14643 on UCP expression in these tissues (45), and with the observed lack of effect of PPARα deletion on thermoregulation during cold exposure. In contrast to a previous report (45), our
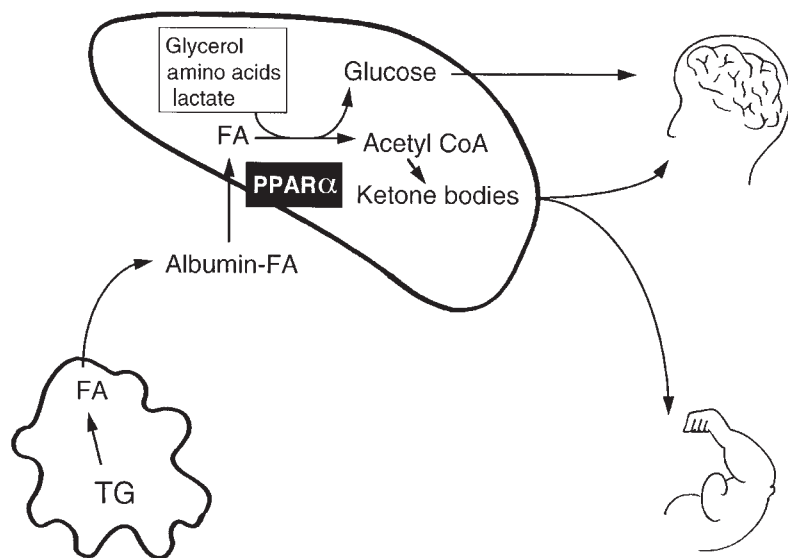
Figure 8
Hepatic fatty acid oxidation is crucial during fasting to ensure an adequate supply of substrates that can be metabolized by other tissues. During fasting, lipolysis of stored triglycerides (TG) in adipose tissue is strongly activated under the influence of changes in hormonal status. The fatty acids (FA) released are delivered to the liver, where they are either re-esterified and secreted (not shown) or oxidized in the mitochondria. Partial oxidation of fatty acids yields acetyl-CoA, which condenses with itself to form ketone bodies. The ketone bodies are subsequently used as an important substrate for energy production by the brain, muscles, and kidney. Oxidation of fatty acids in the liver is also tightly coupled to glucose synthesis. The glucose produced by the liver serves as an important fuel for the brain. The important role of PPARα in these processes is illustrated. Although the figure is drawn with human tissues shown, important differences in lipid metabolism exist between mice and humans, including the function of PPARα. Thus, the validity of this figure with regard to human metabolism remains to be demonstrated.

data do not confirm the role of PPARα in regulating UCP2 expression in the liver. In this context it is important to note that Kupffer cells, and not hepatocytes, are responsible for a high level of expression of UCP2 in the liver (46).

The observation that PPARα does not regulate UCP expression in BAT, and does not influence cold-induced thermogenesis, raises the interesting question of why PPARα is so highly expressed in BAT (expression levels are about 4-fold higher than in the liver; P. Escher and W. Wahli, unpublished observations). Future studies will have to clarify this issue.

In this study, feeding PPARα-null mice a high fat diet for several weeks led to a huge accumulation of lipid in the liver. Since fatty acid uptake and triacylglycerol secretion are not altered in a way that would explain the phenotype, the lipid accumulation must be due to a defect in fatty acid oxidation. Thus, even in the fed state, hepatic fatty acid oxidation is impaired in PPARα-null mice, but the liver needs to be overloaded with fatty acids to elicit a strong phenotype.

Finally, it is remarkable how much the phenotype of PPARα-null mice resembles the phenotype of humans who suffer from a genetic defect of one of the enzymes involved in fatty acid oxidation. These patients suffer from severe fasting-induced hypoglycemia, often have a fatty liver, and show a plasma metabolite profile that is similar to the profile observed for the PPARα-null mice (47). Additionally, inhibition of hepatic fatty acid oxidation by etomoxir, an inhibitor of CPTI, induces a highly similar phenotype, characterized by elevated FFAs, reduced β-hydroxybutyrate levels, and fasting hypoglycemia (48–50). Genetic defects in mitochondrial fatty acid oxidation are also associated with severe metabolic disturbances of the heart and skeletal muscle, tissues where PPARα is also expressed. Thus, it will be of great interest to study further the effects of PPARα deletion on heart and muscle lipid metabolism.

1. van den Berghe, G. 1991. The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. *J. Inherit. Metab. Dis.* **14**:407–420.
2. Desvergne, B., IJpenberg, A., Devchand, P.R., and Wahli, W. 1998. The peroxisome proliferator-activated receptors at the cross-road of diet and hormonal signalling. *J. Steroid Biochem. Mol. Biol.* **65**:65–74.
3. Gronemeyer, H., and Laudet, V. 1995. Transcription factors 3: nuclear receptors. *Protein Profile.* **2**:1173–1308.
4. Schoonjans, K., Staels, B., and Auwerx, J. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37**:907–925.
5. Göttlicher, M., Widmark, E., Li, Q., and Gustafsson, J.A. 1992. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. USA.* **89**:4653–4657.
6. Keller, H., et al. 1993. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA.* **90**:2160–2164.
7. Forman, B.M., Chen, J., and Evans, R.M. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. USA.* **94**:4312–4317.
8. Kliewer, S.A., et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc. Natl. Acad. Sci. USA.* **94**:4318–4323.
9. Krey, G., et al. 1997. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* **11**:779–791.
10. Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology.* **137**:354–366.
11. Tontonoz, P., Hu, E., and Spiegelman, B.M. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* **79**:1147–1156.
12. Jiang, C., Ting, A.T., and Seed, B. 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature.* **391**:79–82.
13. Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., and Glass, C.K. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Cell.* **93**:241–252.
14. Tontonoz, P., Nagy, L., Alvarez, J.G., Thomazy, V.A., and Evans, R.M. 1998. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell.* **93**:241–252.
15. Nagy, L., Tontonoz, P., Alvarez, J.G., Chen, H., and Evans, R.M. Oxidized

LDL regulates macrophage gene expression through ligand activation of PPARgamma. *Cell.* **93**:229–240.

16. Lee, S.S., et al. 1995. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15**:3012–3022.

17. Aoyama, T., et al. 1998. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J. Biol. Chem.* **273**:5678–5684.

18. Peters, J.M., et al. 1997. Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. *J. Biol. Chem.* **272**:27307–27312.

19. Motojima, K., Passilly, P., Peters, J.M., Gonzalez, F.J., and Latruffe, N. 1998. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. *J. Biol. Chem.* **273**:16710–16714.

20. Djouadi, F., et al. 1998. A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor alpha-deficient mice. *J. Clin. Invest.* **102**:1083–1091.

21. Costet, P., et al. 1998. Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **273**:29577–29585.

22. Lavery, D.J., Lopez-Molina, L., Fleury-Olela, F., and Schibler, U. 1997. Selective amplification via biotin- and restriction-mediated enrichment (SABRE), a novel selective amplification procedure for detection of differentially expressed mRNAs. *Proc. Natl. Acad. Sci. USA.* **94**:6831–6836.

23. Weir, J.B. 1949. New method for calculating metabolic rate with special reference to protein metabolism. *J. Physiol. (Lond.)* **109**:1–9.

24. Van Steenbergen, W., and Lanckmans, S. 1995. Liver disturbances in obesity and diabetes mellitus. *Int. J. Obes. Relat. Metab. Disord.* **19**:S27–S36.

25. Surwit, R.S., et al. 1995. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism.* **44**:645–651.

26. Rebuffe-Scrive, M., Surwit, R.S, Feinglos, M.N, Kuhn, C.M., and Rodin, J. 1993. Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non-insulin-dependent diabetes mellitus. *Metabolism.* **42**:1405–1409. *Nature* 1998 Jan 1;391(6662):82–86.

27. Doherty, J.F., Adam, E.J., Griffin, G.E., and Golden, M.H. 1992. Ultrasonographic assessment of the extent of hepatic steatosis in severe malnutrition. *Arch. Dis. Child.* **67**:1348–1352.

28. Reif, S., et al. 1993. Perinatal food restriction in rats reduces the content but not concentration of liver extracellular matrix proteins. *J. Nutr.* **123**:811–816.

29. Yasuhara, M., et al. 1991. Induction of fatty liver by fasting in suncus. *J. Lipid. Res.* **32**:887–891.

30. Martin, G., Schoonjans, K., Lefebvre, A.M., Staels, B., and Auwerx, J. 1997. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARα and PPARγ activators. *J. Biol. Chem.* **272**:28210–28217.

31. Webb, G.P., Jagot, S.A., and Jakobson, M.E. 1982. Fasting-induced torpor in *Mus musculus* and its implications in the use of murine models for human obesity studies. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **72**:211–219.

32. McGarry, J.D., and Brown, N.F. 1997. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur. J. Biochem.* **244**:1–14.

33. Lemberger, T., et al. 1996. Expression of the peroxisome proliferator-activated receptor alpha gene is stimulated by stress and follows a diurnal rhythm. *J. Biol. Chem.* **279**:1764–1769.

34. Ren, B., Thelen, A.P., Peters, J.M., Gonzalez, F.J., and Jump, D.B. 1997. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha. *J. Biol. Chem.* **272**:26827–26832.

35. Katan, M.B., van Birgelen, A., Deslypere, J.P., Penders, M., and van Staveren, W.A. 1991. Biological markers of dietary intake, with emphasis on fatty acids. *Ann. Nutr. Metab.* **35**:249–252.

36. Meunier-Durmort, C., Poirier, H., Niot, I., Forest, C., and Besnard, P. 1996. Up-regulation of the expression of the gene for liver fatty acid-binding protein by long-chain fatty acids. *Biochem. J.* **319**:483–487.

37. Poirier, H., Braissant, O., Niot, I, Wahli, W., and Besnard, P. 1997. 9-cis-retinoic acid enhances fatty acid-induced expression of the liver fatty acid-binding protein gene. *FEBS Lett.* **412**:480–484.

38. Tontonoz, P., Hu, E., Devine, J., Beale, E.G., and Spiegelman, B.M. 1995. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol. Cell. Biol.* **15**:351–357.

39. Arias, G., Asins, G., Hegardt, F.G., and Serra, D. 1997. The effect of fasting/refeeding and insulin treatment on the expression of the regulatory genes of ketogenesis in intestine and liver of suckling rats. *Arch. Biochem. Biophys.* **340**:287–298.

40. Arias, G., Asins, G., Hegardt, F.G., and Serra, D. 1998. The effect of dexamethasone treatment on the expression of the regulatory genes of ketogenesis in intestine and liver of suckling rats. *Mol. Cell. Biochem.* **178**:325–333.

41. Klemm, D.J., et al. 1998. Insulin stimulates cAMP-response element binding protein activity in HepG2 and 3T3-L1 cell lines. *J. Biol. Chem.* **273**:917–923.

42. Kaikaus, R.M., Chan, W.K., Ortiz de Montellano, P.R., and Bass, N.M. 1993. Mechanisms of regulation of liver fatty acid-binding protein. *Mol. Cell. Biochem.* **123**:93–100.

43. Juge-Aubry, C., et al. 1997. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5′-flanking region. *J. Biol. Chem.* **272**:25252–25259.

44. Sugiyama, T., Scott, D.K., Wang, J.C., and Granner, D.K. 1998. Structural requirements of the glucocorticoid and retinoic acid response units in the phosphoenolpyruvate carboxykinase gene promoter. *Mol. Endocrinol.* **12**:1487–1498.

45. Kelly, L.J., et al. 1998. Peroxisome proliferator-activated receptors gamma and alpha mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. *Endocrinology.* **139**:4920–4927.

46. Larrouy, D., et al. 1997. Kupffer cells are a dominant site of uncoupling protein 2 expression in rat liver. *Biochem. Biophys. Res. Commun.* **235**:760–764.

47. Stanley, C.A. 1987. New genetic defects in mitochondrial fatty acid oxidation and carnitine deficiency. *Adv. Pediatr.* **34**:59–88.

48. Ratheiser, K., et al. 1991. Inhibition by etomoxir of carnitine palmitoyltransferase I reduces hepatic glucose production and plasma lipids in non-insulin-dependent diabetes mellitus. *Metabolism.* **40**:1185–1190.

49. Reaven, G.M., Chang, H., and Hoffman, B.B. 1988. Additive hypoglycemic effects of drugs that modify free-fatty acid metabolism by different mechanisms in rats with streptozocin-induced diabetes. *Diabetes.* **37**:28–32.

50. Bryson, J.M., Cooney, G.J., Wensley, V.R., Phuyal, J.L., and Caterson, I.D. 1996. The effects of the inhibition of fatty acid oxidation on pyruvate dehydrogenase complex activity in tissues of lean and obese mice. *Int. J. Obes. Relat. Metab. Disord.* **20**:738–744.