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# Review

# Peroxisome proliferator-activated receptor α target genes

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**Abstract.** Peroxisome proliferator-activated receptors (PPARs) are nuclear proteins that belong to the superfamily of nuclear hormone receptors. They mediate the effects of small lipophilic compounds such as long-chain fatty acids and their derivatives on transcription of genes commonly called PPAR target genes. Here we review the involvement of PPARα in peroxisomal and mitochondrial fatty acid oxidation, microsomal fatty acid hydroxylation, lipoprotein, bile and amino acid metabolism, glucose homeostasis, biotransformation, inflammation control, hepato-carcinogenesis and other pathways, through a detailed analysis of the different known or putative PPARα target genes.

**Key words.** PPARα; transcriptional regulation; target genes; lipid metabolism; PPAR response element.

## Introduction

Since they were discovered in the early 1990s, peroxisome proliferator-activated receptors (PPARs) have evolved from another member of the nuclear hormone receptor family to an extremely important set of targets for drug discovery. PPARs are ligand-induced transcription factors that regulate the transcription of target genes in response to specific ligands, both synthetic and endogenous. They recognize a well-defined sequence within the promoter region of target genes and bind to this sequence as a heterodimer with the retinoid X receptor. Three different PPAR genes have been identified in many species, including mouse, rat, guinea pig, human, chicken, fish and amphibian [1–10]. The *PPARα* (NR1C1), *PPARβ/δ* (NR1C2) and *PPARγ* (NR1C3) genes encode proteins that share a highly conserved structure and molecular mode of action. Nevertheless, the array of genes regulated by each PPAR isotype are divergent and may also differ from one species to another, leading to regulation of distinct biological processes [11]. The mechanism behind the specificity of regulation by each PPAR isotype is not exactly clear but reflects spatiotemporal expression levels between the different PPARs, as well as differential affinity for promoter elements [4, 12]. Of the three PPAR isotypes known to date, PPARα has been the best characterized. Inasmuch as most of our knowledge about the biological function of PPARα is directly coupled to the function of its target genes, the present review will disentangle the various biological effects of PPARα through a detailed analysis of its target genes (fig. 1).

Similar to other PPARs, transcriptional regulation by PPARα is achieved by its direct binding to specific nucleotidic sequences known as peroxisome proliferator response elements (PPREs), present in the promoter region of target genes, or sometimes in an intronic sequence of a gene [13–15]. PPREs share a common core sequence consisting of a direct repeat of the consensus sequence AGGTCA spaced by a single nucleotide, which allows the proper binding of PPARα and its obligate partner the retinoid X receptor α RXRα (NR2B1). The presence of a functional PPRE within a promoter, which means that the PPRE is able to mediate PPARα-induced

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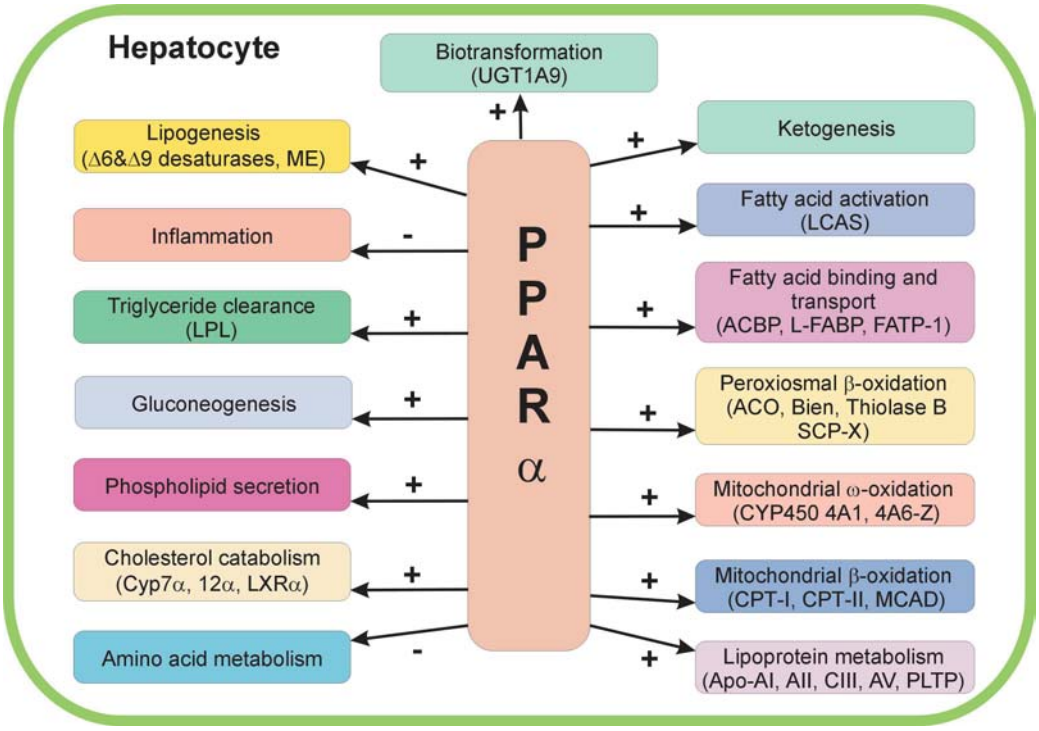


Figure 1. Schematic representation of a hepatocyte with the diverse functions of PPARα illustrated. Known target genes of PPARα are shown within brackets.

transactivation, appears to be the best criterion to classify a gene as a PPARα target gene (table 1). In addition to upregulating gene expression, PPARα is also capable of negatively modulating gene expression. We will discuss the diverse molecular mechanisms responsible for this trans-repression.

## PPARα expression profile

To regulate a target gene, an obligatory coexpression pattern of PPARα and its target gene is required. In rodents, PPARα is highly expressed in liver, where it is present mainly in parenchymal cells, while endothelial, Kupffer and stellate cells do not seem to express any PPARα messenger RNA (mRNA) [16–18]. In addition, PPARα is relatively well expressed in heart, kidney, intestinal mucosa, skeletal muscle and brown fat, which are all tissues characterized by a relatively high rate of fatty acid catabolism [4, 12, 19–21]. Moreover, different groups have reported the presence of PPARα mRNA in immune cells such as monocytes/macrophages, as well as T and B cells [22–30]. Finally, PPARα expression was detected in human vascular wall cells such as smooth muscle and endothelial cells as well as in atherosclerotic lesions [23, 31–34].

## Regulation of PPARα activity by natural and synthetic agonists

PPARα can be activated by both endogenous and synthetic ligands. Briefly, long-chain polyunsaturated fatty acids and eicosanoids such as the inflammatory mediator leukotriene $B_4$, the hydroxyeicosatetraenoic acids and the prostaglandins D1 and D2 are among the best natural PPARα ligands [35–38]. Endogenous ligand-activation of PPARα is believed to occur primarily during fasting as large amounts of free fatty acids enter the blood plasma [39, 40]. However, dietary intake of specific fatty acids can also lead to a potent activation of PPARα [41]. In addition to a host of endogenous ligands, PPARα is also the molecular target for fibrates, which are synthetic PPARα agonists that include gemfibrozil, bezafibrate, clofibrate and fenofibrate. These molecules are used to treat dyslipidemia and cardiovascular disease and are becoming increasingly popular as a combination treatment with statins [13, 14, 42].

## PPARα and cellular proliferation

As indicated by their name, peroxisome proliferators, which are synthetic PPARα agonists, cause peroxisome as well as hepatocyte proliferation in rodent but not human liver. Rather than reflecting the physiological func-

Table 1. List of PPAR$\alpha$ regulated genes with at least one functional PPRE identified within the promoter sequence.

| Gene | Reference PPRE | Species | Function of the gene | Sv129 or C57BL/6 or NZB mice | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Fed WT/KO | Fasted WT/KO | Fibrates WT | KO | |
| **Liver** | | | | | | | | |
| Acyl-CoA binding protein (ACBP) | [59] | rat/human | fatty acyl-CoA ester transport | ? | > | ? | ? | [58, 59] |
| Acyl-CoA oxidase (ACO) | [72] | rat | peroxisomal $\beta$-oxidation | ≈ | > | > | ≈ | [38, 45, 71, 135, 156] |
| Apolipoprotein-AI | [104] | human | plasma HDL metabolism | < | > | <(rat) | ≈ | [105–109] |
| Apolipoprotein-AII | [111] | human | plasma HDL metabolism | ≈ | ? | > | ≈ | [111] |
| Apolipoprotein-AV | [121, 122] | human | plasma TG metabolism | ? | ? | > | ? | [121, 122] |
| Apolipoprotein-C III (apo-CIII) | [118] | rat | plasma HDL metabolism | ≈ | ≈ | < | ≈ | [116–118] |
| Bifunctional enzyme (BIEN or BE) | [73–76, 78, 79] | rat | fatty acid $\beta$-oxidation | ≈ | > | > | ≈ | [46, 52, 62, 73–76, 78, 79, 90] |
| Carnitine palmitoyl-transferase I (CPT-I) | [60, 61] | human | fatty acid oxidation | ≈ | ≈ | > | ≈ | [39, 40] |
| Carnitine palmitoyl-transferase II (CPT-II) | [68] | human | fatty acid oxidation | ≈ | ? | > | ≈ | [63] |
| Cytochrome P450 4A6-Z | [90, 91] | rabbit | fatty acid $\omega$-hydroxylation | ? | ? | > | ? | [90, 91] |
| Cytochrome P450 4A1 | [88] | rat | fatty acid $\omega$-hydroxylation | ≈ | > | > | ≈ | [46, 64, 89, 90, 190] |
| Cytochrome P450 7A1 (CYP7A1) | [135] | mouse/rat | bile acid metabolism | ≈ | > | (∉) | ≈ | [133, 135] |
| Cytochrome P450 12A1 (CYP12A1) | [136] | rat | cholesterol metabolism | ≈ | > | > | ≈ | [133, 135] |
| delta-6-Desaturase | [101] | human | desaturation of fatty-acyl CoA | ≈ | ? | > | ≈ | [100] |
| Cytosolic fatty acid binding protein (L-FABP) | [57] | mouse | fatty acid binding/ transport | ≈ | > | > | ≈ | [38, 45, 51, 57, 64] |
| Fatty acid transport protein-1 (FATP-1) | [49, 50] | mouse | fatty acid transport | ≈ | ? | > | ≈ | [49–52, 191] |
| G0/G1 switch gene 2 (GOS-2) | [♣] | mouse | cell cycle regulation | < | > | > | ? | [♣] |
| Ileal bile-acid transporter (I-BAT) | [140] | human | bile salt intestinal transport | ? | ? | ? | ? | |
| Lipoprotein lipase (LPL) | [119] | rat | triglyceride clearance | ? | ? | > | ? | [52, 191] |
| Liver X receptor $\alpha$ (LXR$\alpha$) | [137] | mouse | cholesterol metabolism | ≈ | > | > | ? | [137, 139, ♣] |
| Long-chain acyl-CoA synthetase (LCAS) | [54] | rat | fatty acid activation | > | ? | > | ≈ | [49, 54, 63] |
| Malic enzyme (ME) | [98] | rat | fatty acid synthesis | > | ? | > | ≈ | [64] |
| Mitochondrial HMG-CoA synthase | [93] | rat | ketogenesis | ≈ | > | > | ? | [94, 138] |
| Medium-chain acyl-CoA dehydrogenase | [69] | mouse | fatty acid oxidation | ≈ | > | > | ≈ | [40, 63] |
| Peroxisome proliferator activated receptor $\alpha$ | [183] | human | lipid metabolism | ≈(ϑ) | > | ≈ | ≈ | [46, 139, 183] |
| Phospholipid transfer protein (PLTP) | [113] | human | plasma HDL metabolism | ≈ | ? | > | ≈ | [111, 112, 191] |
| Rev-erb$\alpha$ | [110] | human | repressor of gene transcription | ? | ? | >(rat) | ? | [104] |
| Stearoyl-CoA desaturase 1 (SCD-1) | [99] | mouse | desaturation of fatty-acyl CoA | ≈ | ? | > | ≈ | [99, 100] |
| Sterol carrier protein-X (SCP-X) | [82] | rat | fatty acid oxidation | ≈ | ? | ≈ | ≈ | [61] |
| Superoxide dismutase (SOD1) | [87] | rat | oxygen-free radical metabolism | ? | ? | >(rat) | ? | [87] |

Table 1 (continued)

| Gene | Reference PPRE | Species | Function of the gene | Sv129 or C57BL/6 or NZB mice | | | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Fed WT/KO | Fasted WT/KO | Fibrates WT | KO | |
| Transferrin | [179] | human | iron transport | ? | ? | < | ? | [179] |
| Thiolase B | [79] | rat | fatty acid oxidation | ≈ | > | > | ≈ | [45, 89] |
| UDP-glucuronosyl-transferase 1A9 (UGT1A9) | [168] | human | biotransformation | ≈ | ? | > | ≈ | [168] |
| **Heart** | | | | | | | | |
| Acyl-CoA oxidase (ACO-A) | [72] | mouse | peroxisomal β-oxidation | ≈ | > | ? | ? | [40] |
| Carnitine palmitoyl-transferase-II (CPT-II) | [68] | human | fatty acid transport | > | ? | ? | ? | [194] |
| Long-chain acyl-CoA synthetase (LCAS) | [54] | rat | fatty acid activation | > | ? | ? | ? | [194] |
| Medium-chain acyl-CoA dehydrogenase | [69] | mouse | fatty acid β-oxidation | > | > | ? | ? | [40, 194] |
| Carnitine palmitoyl-transferase-I (CPT-I) | [60, 61] | human | fatty acid transport | > | > | > | ? | [40, 60, 126] |
| **Muscle** | | | | | | | | |
| Carnitine palmitoyl-transferase-I (soleus) | [61] | human | fatty acid transport | ? | ? | > | ? | [126] |
| **Bown adipose tissue** | | | | | | | | |
| Uncoupling protein-1 | [124] | mouse | Thermogenesis | ≈ | ≈ | > | ? | [39, 124] |
| **White adipose tissue** | | | | | | | | |
| Lipoprotein lipase (LPL) | [119] | rat | triglyceride clearance | < | ? | > | ≈ | [193] |
| Acyl-CoA oxidase (ACO) | [72] | rat | peroxisomal β-oxidation | < | ? | > | > | [193] |
| **Small intestine** | | | | | | | | |
| Apolipoprotein C-III (apo-CIII) | [118] | rat | plasma HDL metabolism | < | ? | < | ≈ | [193] |
| Cytosolic fatty acid binding protein (L-FABP) | [57] | mouse | fatty acid binding/transport | > | ? | > | > | [193] |

PPRE, peroxisome proliferator response element; WT, wild-type mouse; KO, PPARα null mice; ≈, similar gene expression between the two genotypes; >, higher gene expression in wild-type mice; <, lower gene expression in wild-type mice; ?, no information available; (ϑ), a mutant-inactive form of PPARα mRNA remains detectable in the mutant PPARα null mice [46]; ♣, our unpublished data or Zandbergen et al., unpublished; (Φ), Kersten et al. and Aoyama et al. reported different results [39, 63]; ∉, several groups reported on different conclusions [133, our own unpublished data].

tion of PPARα, induction of hepatocyte proliferation may be a toxicological and/or a pharmacological effect of PPARα activation. Hepatocellular proliferation induced by a partial hepatectomy is an effective alternative way to study the role of PPARα in liver cell proliferation. Anderson et al. reported that following partial hepatectomy, PPARα null mice display an impairment in liver regeneration, together with a delay of maximal expression of *cyclin-D1* and *c-myc* genes [43]. Feeding wild-type mice a PPARα agonist increased cyclin-D1, cyclin-dependent-kinase 1 (CDK-1), CDK-4 and *c-myc* expression, suggesting their direct regulation by PPARα. In contrast, Rao et al. found no difference in hepatic levels of mRNAs encoding CDK1, CDK2, CDK4, PCNA, cyclin D1 and B1 between the two genotypes as well as no significant difference in replicative DNA synthesis [44]. Thus, the precise role of PPARα in liver cell proliferation following hepatectomy remains ambiguous.

Whereas rodents show dramatic peroxisome proliferation and develop liver tumors upon exposure to PPARα agonists, the same is not true in humans and monkeys, and is less pronounced in hamsters [45, 46]. By using PPARα null mice, it was shown that peroxisome proliferation and the appearance of tumors were PPARα dependent [46]. The differential response to peroxisome proliferators between primates and rodents may be due to a difference in basal expression of PPARα between the two species [47]. Hepatic PPARα expression is much lower in humans than in rodents. However, using human hepatoma HepG2 cells that were engineered to express human PPARα at con-

centrations similar to mouse liver, known mouse hepatic PPAR$\alpha$ targets did not change expression [48, 49]. Thus, the difference in hepatic PPAR$\alpha$ level between humans and rodents may not explain per se why humans are refractory to peroxisome and hepatocyte proliferation. The mechanism behind the hepatoproliferative effect of PPAR$\alpha$ agonists may involve Kupffer cells, possibly via mechanisms involving an increase in superoxide, activation of nuclear factor-kappa B (NF-$\kappa$B) and production of tumor necrosis factor-alpha (TNF$\alpha$). However, Kupffer cells do not express PPAR$\alpha$, whereas hepatocytes do, consistent with the hypothesis that hepatocytes respond to Kupffer cell-derived TNF$\alpha$ via mechanisms dependent on hepatocyte PPAR$\alpha$ [16–18]. Besides stimulating cellular proliferation, hepatic PPAR$\alpha$ also mediates the peroxisome proliferator-induced suppression of apoptosis. Hepatocytes that were transfected with a dominant-negative form of PPAR$\alpha$, as well as hepatocytes from PPAR$\alpha$ null mice, did not display any decrease in apoptosis by peroxisome proliferators, in contrast to wild-type hepatocytes. Thus, PPAR$\alpha$ combines positive regulation of cellular hepatocyte proliferation with suppression of apoptosis.

## PPAR$\alpha$ and fatty acid oxidation

A functional link between fatty acid oxidation and PPAR$\alpha$ was first established in 1992, when it was shown that the peroxisomal acyl-coenzyme A (CoA) oxidase gene, which is involved in peroxisomal fatty acid $\beta$-oxidation, is regulated by PPAR$\alpha$ [2]. Since then, numerous genes involved in hepatic fatty acid catabolism were shown to be induced by peroxisome proliferators and PPAR$\alpha$.

Hepatic fatty acid catabolism consists of several distinct pathways. First, free fatty acids that are generated by adipose tissue lipolysis are taken up from the blood plasma by the liver, where they are activated into their fatty acyl-CoA derivatives. The activated fatty acyl-CoAs are subsequently taken up into the mitochondria or peroxisomes for degradation to acetyl-CoA via $\beta$-oxidation. Major differences exist between peroxisomal and mitochondrial fatty acid $\beta$-oxidation. Whereas mitochondria oxidize short-, medium- and mostly long-chain fatty acids, peroxisomes oxidize some long-chain but mostly very long chain fatty acids. Both pathways are regulated by PPAR$\alpha$.

## PPAR$\alpha$ and cellular fatty acid uptake

The first step required for oxidation of fatty acids is their transport across the cell membrane. The fatty-acid transport protein-1 gene, which is involved in uptake of long-chain fatty acids and oxidized low-density lipoproteins, was identified as a direct PPAR$\alpha$ target gene with a functional PPRE in its promoter [50, 51]. A functional PPRE was also initially identified in the proximal promoter of the fatty acid translocase (FAT/CD36) gene [52]. Nevertheless, further studies performed by the same group failed to confirm the direct induction of FAT via this PPRE [53]. It was argued that induction by PPAR$\alpha$ ligands probably goes through the distal promoter region of the gene even though no PPRE could be identified. Thus, the mechanism by which PPAR$\alpha$ regulates FAT transcription remains unclear.

Fatty acids per se are inactive molecules that cannot be further metabolized without an obligatory activation step into their fatty acyl-CoA derivatives. The activation step, which is carried out at several intracellular localizations, is under the control of the long-chain fatty acyl-CoA synthetases, whose transcriptional rate is controlled by PPAR$\alpha$ in the liver and by PPAR$\gamma$ in fat tissue, through a functional PPRE [50, 54]. Thus, PPAR$\alpha$ promotes both fatty acid transport across the cell membrane as well as their activation into a metabolic active form that serves as the substrate for further metabolism.

## PPAR$\alpha$ and intracellular fatty acid transport

Different lipid-binding proteins involved in the process of intracellular lipid trafficking have been identified, including keratinocyte (FABP-5), cardiac (FABP-3), hepatic (FABP-1), muscular (FABP-3), brain (FABP-7) and adipocyte fatty acid binding protein (also known as A-FABP, aP2 or FABP-4). One of the functions proposed for these proteins is to serve as a shuttle for fatty acids between the plasma membrane and the nucleus, and selectively enhance the activities of specific PPARs [55, 56]. Interestingly, liver fatty acid binding protein was shown to be upregulated by bezafibrate and fatty acids in a PPAR$\alpha$-dependent fashion, through a functional PPRE located in the proximal promoter [57]. In contrast, in the small intestine the PPAR$\beta$ isotype seems to be the relevant PPAR involved in the transcriptional regulation of liver fatty acid binding protein (FABP-2) [57]. This differential regulation might be due to a different relative abundance of PPAR$\alpha$ between the two tissues [4, 12].

A functional intronic PPRE was also localized in both mouse and human versions of the gene for acyl-CoA binding protein, while another PPRE present in the promoter of acyl-CoA binding protein appears to be nonfunctional [58, 59]. Acyl-CoA binding protein specifically binds acyl-CoA esters with high affinity and is believed to efficiently prevent acyl-CoA esters hydrolysis by thioesterases. Therefore, by lowering the concentration of free acyl-CoA ester, upregulation of acyl-CoA binding protein by PPAR$\alpha$ may relieve acyl-CoA inhibition of long-chain fatty acid synthetases.

### PPARα and mitochondrial fatty acid uptake

The mitochondrial oxidation of fatty acids starts with the translocation of activated long-chain fatty acids into the matrix of mitochondria. The transport across the outer mitochondrial membrane is performed by two isotypes of the carnitine palmitoyltransferase (CPT) enzyme I, CPT-Iα and CPT-Iβ. CPTI-α and -β are encoded by two different genes and are considered to catalyze the rate-limiting step in mitochondrial fatty acid β-oxidation [60–62]. The highest expression level of CPT-Iα is found in heart, liver and pancreatic β cells, whereas CPT-Iβ (also called muscle CPT-I) is predominantly expressed in adipose tissue, testis, heart and skeletal muscle. Both were identified as direct positive PPARα-regulated genes through a functional PPRE present in their respective promoters [60–64]. Whereas long-chain fatty acids but not long-chain fatty acyl-CoA esters induce CPT-Iα expression in a PPARα-independent manner via elements localized in the first intron of the gene, induction of CPT-1α by clofibrate is highly PPARα dependent [65]. This finding suggests that dietary fatty acids and hypolipidemic drugs modulate CPT-Iα expression via different pathways.

Malonyl-CoA is considered as the major physiological allosteric inhibitor of CPT-I. Interestingly, cardiac and likely skeletal malonyl-CoA decarboxylase, which degrade malonyl-CoA to acetyl-CoA, are also PPARα regulated [66, 67]. In contrast, in the same study expression of acetyl-CoA carboxylase, which synthesizes malonyl-CoA from acetyl-CoA, was not changed between wild-type and PPARα mutant mice [67]. Thus, aside from up-regulating transcription of CPT-I, PPARα may increase CPT-1 activity by inducing the degradation of natural inhibitors of CPT-1 via regulation of malonyl-CoA decarboxylase.

While CPT-I is localized in the outer mitochondrial membrane, CPT-II, which catalyzes the conversion of acyl-carnitine to acyl-CoA, is restricted to the inner mitochondria membrane. Expression of CPT-II was found to be up-regulated significantly in response to the PPARα agonist WY14643 in a PPARα-dependent manner in heart, liver and kidney. In accordance with these observations, a partially conserved PPRE was found within the CPT-II promoter that confers responsiveness to PPARα ligands [68].

### PPARα and mitochondrial fatty acid β-oxidation

Besides regulating the translocation of fatty acids into the mitochondria, PPARα also regulates the expression of genes involved in mitochondrial β-oxidation. The short-, medium-, long- and very long chain acyl-CoA dehydrogenase genes encode four different chain-length-specific enzymes that catalyze the first step in mitochondrial fatty acid β-oxidation. A basal PPARα-dependent regulation was found for all of these genes. Nevertheless, so far a functional PPRE has only been identified for the medium-chain acyl-CoA dehydrogenase gene, yet such a PPRE is most likely present in the promoter region of the other dehydrogenase genes as well [69, table 2]. Using microarray, expression of dodecenoly-CoA δ-isomerase (also known as 3-*cis*-2-*trans*-enoyl-CoA isomerase) and 3-hydroxyacyl-CoA dehydrogenase, which are also involved in the mitochondrial β-oxidation of unsaturated and saturated fatty acids, was found to be decreased in fasted PPARα null mice, but no PPRE has yet been identified [F. Zandbergen et al., unpublished].

### PPARα and peroxisomal fatty acid uptake

The adrenoleukodystrophy-related genes ABCD2 (ALDR) and ABCD3 (PMP70) encode for ATP-binding cassette half-transporters located in the peroxisomal membrane, whose precise functions are unknown [70, 71]. Expression of both genes was found to be PPARα dependent and highly sensitive to PPARα agonists [70]. However, no functional PPRE that may trigger this induction has been identified in 2 kb of the rat ABCD2 proximal promoter, suggesting the possible existence of such a sequence far upstream from the initiation start site of the gene or a more complex mechanism for PPARα-dependent ABCD2 gene regulation. No promoter study for the ABCD3 gene has been reported yet.

### PPARα and peroxisomal fatty acid β-oxidation

Peroxisomes carry out the oxidation of very long chain polyunsaturated fatty acids. The enzyme that catalyzes the first and rate-limiting step in this process is a marker of peroxisome proliferator action, e. g. acyl-CoA oxidase, which was the first direct PPARα target identified [72]. Acyl-CoA oxidase is a flavoenzyme that introduces a double bond between the α and β carbons of acyl-CoA, generating enoyl-CoA. Acyl-CoA oxidase also transfers electrons to oxygen-producing hydrogen peroxide, $H_2O_2$, a product that will be further converted to water and oxygen by catalase. Next, enoyl-CoA hydratase adds water to the double bond of enoyl-CoA, producing hydroxyacyl-CoA. Hydroxyacyl-CoA dehydrogenase subsequently oxidizes the hydroxyacyl-CoA to ketoacyl-CoA and passes the hydrogen to NAD+. These two enzymatic activities exist on a single protein, known as the bifunctional enzyme (BIEN) or enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase. Three different PPREs have been identified in the promoter sequence of the BIEN gene, yet only one allows for efficient transactivation of the BIEN gene by PPARα [73–78]. In the next step, thiolase cleaves the ketoacyl-CoA to form acetyl-CoA and

Table 2. List of PPAR$\alpha$-dependent gene regulation (no functional PPRE reported yet).

| Gene | Species | Function | Sv129 mice | | | | References |
|---|---|---|---|---|---|---|---|
| | | | Fed WT/KO | Fasted WT/KO | Fibrates WT | KO | |
| **Liver** | | | | | | | |
| ABCA-1 (via LXR$\alpha$) | Mouse | Cholesterol transport | ≈ | > | ≈ | ≈ | [139] |
| ABCG-5 (via LXR$\alpha$) | Mouse | Cholesterol transport | ≈ | > | ≈ | ≈ | [139] |
| ABCG-8 (via LXR$\alpha$) | Mouse | Cholesterol transport | > | > | ≈ | ≈ | [139] |
| Aldehyde dehydrogenase type 3 | Mouse | Biotransformation | ≈ | > | > | ≈ | [♣] |
| Alanine aminotransferase (AlaAT or GPT) | Mouse | Amino acid metabolism | < | ? | < | ≈ | [152] |
| Alanine glyoxylate transaminase | Mouse | Amino acid metabolism | ≈ | ≈ | < | ≈ | [153] |
| Aldehyde dehydrogenase type 3 | Mouse | Biotransformation | ≈ | > | > | ≈ | [♣] |
| alpha-1 Acid glycoprotein (AGP) | Mouse | Acute phase response | ? | ? | < | ≈ | [157] |
| alpha-2 Urinary-globulin ($\alpha$2u)/ MUP-1 | Mouse† | Pheromone carrier | ? | ? | < | ≈ | [52, 157] |
| Apolipoprotein-Al (via rev-erb$\alpha$) | Rat | Plasma HDL metabolism | < | ? | < | ≈ | [106] |
| Apolipoprotein-A IV | Rat | Plasma HDL metabolism | ? | ? | < | ? | [191] |
| Arginase | Mouse | Amino acid metabolism | < | < | > | ≈ | [153] |
| Arginino-succinate lyase | Mouse | Amino acid metabolism | ≈ | < | < | ≈ | [153] |
| Arginino-succinate synthase | Mouse | Amino acid metabolism | ≈ | < | < | ≈ | [153] |
| ATP binding cassette half-transporter type 2 (ABCD2) | Mouse | Peroxisomal $\beta$-oxidation | ≈ | ? | > | ≈ | [70] |
| ATP binding cassette half-transporter type 3 (ABCD3) | Mouse | Peroxisomal $\beta$-oxidation | ≈ | ? | > | ≈ | [70] |
| 11-beta-Hydroxysteroid dehydrogenase I (11-$\beta$-HSD I) | Mouse | Steroid metabolism | ≈ | ? | < | ≈ | [155, 191] |
| 17-beta-Hydroxysteroid dehydrogenase IV (17-$\beta$-HSD IV) | Rat/Mouse | Steroid metabolism | < | ? | > | ≈ | [157] |
| Carbamoyl-phosphate synthase-I (CPS-I) | Mouse | Amino acid metabolism | < | < | < | ≈ | [153] |
| c-myc (protooncogene) | Mouse | Cell cycle | ≈ | ? | > | ≈ | [43] |
| Coproporphyrinogen oxidase | Mouse | Heme biosynthesis | > | > | > | ? | [♣] |
| Cyclin D1 | Mouse | Cell cycle | > | ? | > | ≈ | [43] |
| Cytochrome P450 2A5 (CYP2A5) | Mouse | Biotransformation | ≈ | ? | < | ≈ | [159] |
| Cytochrome P450 2C11 (CYP2C11) | Mouse† | Steroid hydroxylase | ? | ? | <(rat) | ? | [157] |
| Cytochrome P450 2C12 (CYP2C12) | Mouse‡ | Steroid hydroxylase | ? | ? | <(rat) | ? | [157] |
| Cytochrome P450 2C29 (CYP2C29) | Mouse | Biotransformation | > | ? | < | ≈ | [159] |
| Cytochrome P450 3A11 (CYP3A11) | Mouse | Biotransformation | > | ? | < | ≈ | [159] |
| Cytochrome P450 4A3 (CYP4A3) | Mouse | Fatty acid hydroxylation | ≈ | > | > | ≈ | [40, 46, 62, 64, 151, 188] |
| Cytochrome P450 4A14 (CYP4A14) | Mouse | Fatty acid hydroxylaiton | ≈ | ? | > | ≈ | [43, 159] |
| Cytochrome P450 4A10 (CYP4A10) | Mouse | Fatty acid $\omega$-hydroxylation | ≈ | > | > | ≈ | [159, ♣] |
| cytochrome P450 27A1 (CYP27A1) | Mouse | Cholesterol Metabolism | ≈ | ≈ | < | ≈ | [134, 139] |
| Cytosolic acyl-CoA thioesterase-I (CTE-I) | Mouse | Hydrolysis of Acyl-CoA | ≈ | ≈ | > | ≈ | [80] |
| Cytosolic acyl-CoA thioesterase-II (CTE-II) | Mouse | Hydrolysis of Acyl-CoA | ≈ | ≈ | > | ≈ | [63] |
| Cytosolic aspartate aminotransferase (AspAT or GOT) | Mouse | Amino acid metabolism | < | < | < | ≈ | [152, 153] |
| Cytosolic epoxide hydrolase | Mouse | Biotransformation | ≈ | > | > | ≈ | [167, ♣] |

Table 2 (continued)

| Gene | Species | Function | Sv129 mice | | | | References |
|---|---|---|---|---|---|---|---|
| | | | Fed WT/KO | Fasted WT/KO | Fibrates WT | KO | |
| delta-5-Desaturase | Rat/Mouse | Desaturation of fatty acid | ≈ | ? | > | ≈ | [99, 100] |
| Dodecenoyl-CoA delta-isomerase | Mouse | Fatty acid oxidation | ? | > | > | ? | [♣, 191] |
| Farnesoid X receptor (FXR)/ Bile Acid Receptor (BAR) | Mouse | Hepatic sterol metabolism | ≈ | > | > | ? | [139, ♣] |
| Fasting-induced adipose factor (FIAF) | Mouse | Lipid metabolism | ≈ | > | > | ≈ | [170] |
| Fatty acid translocase (FAT/CD36) | Mouse | Fatty acid transport | ≈ | ? | > | ≈ | [52] |
| Fibrinogen | Mouse | Acute phase response | < | ? | < | ≈ | [157, 180] |
| Glucose-regulated protein-94 (GRP-94) | Mouse | Cellular proliferation | > | ? | > | ? | [♣, 84] |
| Glutaminase | Mouse | Amino acid metabolism | ≈ | < | < | ≈ | [153] |
| Glutathione-*S*-transferase alpha | Mouse | Biotransformation | ? | ? | < | ? | [165, ♣] |
| Hydroxypyruvate/gloxylate reductase (HPR/GR) | Mouse | Amino acid metabolism | ≈ | > | > | ≈ | [153] |
| Interleukin-6 (IL-6) | Mouse | Vascular inflammation | ≈ | ? | ≈ | ? | [43] |
| Interleukin-1 receptor-type 1 (IL-1 R) | Mouse | Vascular inflammation | > | ? | > | ? | [43] |
| Interleukin-6 receptor (IL-6 R) | Mouse | Vascular inflammation | > | ? | < | ? | [43] |
| I$\kappa$B$\alpha$ | Human | Inflammation/Apoptosis | ≈ | ? | > | ? | [174, 175] |
| Lactate dehydrogenase A4 | Mouse | Gluconeogenesis | ? | > | ≈ | ? | [♣] |
| Lactoferrin | Mouse | Iron-binding protein | ≈ | ? | < | ≈ | [192] |
| Long-chain acyl-CoA dehydrogenase (LCAD) | Mouse | Fatty acid oxidation | > | ? | > | ≈ | [63, 64] |
| Mitochondrial acyl-CoA thioesterase-I | Mouse | Hydrolysis of acyl-CoA | ≈ | ≈ | > | ? | [80] |
| Mitochondrial aspartate aminotransferase | Mouse | Amino-acid metabolism | ≈ | ≈ | > | ≈ | [52, 152, 153] |
| Mitochondrial HMG CoA synthase (mHMG CoA synthase) | Mouse | Ketogenesis | ≈ | > | > | ≈ | [95, 138] |
| Multidrug resistance 2 (Mdr-2, ABC-B4) | Mouse | Hepatobiliary lipid transport | ≈ | > | > | ≈ | [138, 139] |
| Na+ taurocholate cotransporting polypeptide (Ntcp) | Mouse | Bile salt transport | ≈ | > | ≈ | > | [138, 139] |
| Ornithine transcarbamoylase | Mouse | Amino acid metabolism | ≈ | ≈ | < | ≈ | [84, 153] |
| Peroxisomal acyl-CoA thioesterase 1A | Mouse | Hydrolysis of acyl-CoA | ≈ | > | > | ? | [80, 191] |
| Peroxisomal acyl-CoA thioesterase 1B | Mouse | Hydrolysis of acyl-CoA | ≈ | > | > | ? | [80, 191] |
| Peroxisomal acyl-CoA thioesterase 2 | Mouse | Hydrolysis of acyl-CoA | ≈ | > | > | ≈ | [81] |
| Pyruvate dehydrogenase kinase isoform 4 (PDK-4) | Mouse | Glucose oxidation | ≈ | > | ? | ? | [145] |
| Scavenger receptor B-type I | Mouse | Plasma HDL metabolism | ≈ | ? | < | ≈ | [115] |
| Short-chain-specific 3-ketoacyl-CoA thiolase (T1) | Mouse | Fatty acid oxidation | > | ? | > | ≈ | [63] |
| Short-chain acyl-CoA dehydrogenase (SCAD) | Mouse | Fatty acid oxidation | ≈ ( $\sum$ ) | ≈ | > | ≈ | [39, 63] |
| Uncoupling protein-2 (UCP-2) | Mouse | ROS production | ≈ | ≈ | > | ? | [39, 127, 128] |
| Very long chain acyl-CoA dehydrogenase (VLCAD) | Mouse | Fatty acid oxidation | > | ? | > | ≈ | [63] |
| **Heart** | | | | | | | |
| Cytoplasmic acyl-CoA thioesterase | Mouse | Hydrolysis of Acyl-CoA | > | > | ? | ? | [80] |
| Malonyl-CoA decarboxylase | Mouse | Fatty acid oxidation | > | ? | > | ? | [66, 67] |
| Mitochondrial acyl-CoA thioesterase | Mouse | Hydrolysis of Acyl-CoA | > | > | ? | ? | [80] |
| Uncoupling protein-2 (UCP-2) | Mouse | ROS production | ≈ | ? | ≈ (rat) | ? | [126] |
| Uncoupling protein-3 (UCP-3) | Mouse | Body energy metabolism | > | > | > (rat) | ? | [126, 144] |
| Pyruvate dehydrogenase kinase isoform-4 | Mouse | Glucose oxidation | ≈ | > | > | ≈ | [144, 147] |

Table 2 (continued)

| Gene | Species | Function | Sv129 mice | | | | References |
|---|---|---|---|---|---|---|---|
| | | | Fed WT/KO | Fasted WT/KO | Fibrates WT | KO | |
| **Aortas** | | | | | | | |
| Interleukin 6 (IL-6) | Mouse | Vascular Inflammation | < | ? | < | ≈ | [173] |
| **White adipose tissue** | | | | | | | |
| Fasting-induced adipose factor | Mouse | Lipid metabolism | < | ≈ | ? | ? | [170] |
| Resistin | Mouse | Insulin resistance | > | ? | ≈ | ? | [169] |
| Glucose transporter 4 | Mouse | Glucose metabolism | ? | > | ? | ≈ | [♣] |
| Fatty acid translocase (FAT/CD36) | Mouse | Fatty acid transport | > | ? | > | > | [193] |
| Uncoupling protein-2 (UCP-2) | Mouse | ROS production | < | ? | < | ≈ | [193] |
| **Brown adipose tissue** | | | | | | | |
| Uncoupling protein-2 (UCP-2) | Mouse | ROS production | ≈ | ≈ | ? | ? | [39] |
| **Kidney** | | | | | | | |
| 17-beta-Hydroxysteroid dehydrogenase IV (17-$\beta$-HSD IV) | Mouse | Steroid metabolism | < | ? | > | ≈ | [158] |
| Pyruvate dehydrogenase kinase isoform-4 (PDK-4) | Mouse | Glucose oxidation | ≈ | > | > | ≈ | [147] |
| **Monocytes/macrophages** | | | | | | | |
| Scavenger receptor type B class I (SR-BI) | Human | HDL metabolism | ? | ? | > | ? | [23] |
| **Muscle** | | | | | | | |
| pyruvate dehydrogenase kinase isoform-4 (anterior tibialis) | Mouse | Glucose oxidation | ≈ | ≈ | ? | ? | [150] |
| Pyruvate dehydrogenase kinase isoform-4 (soleus) | Mouse | Glucose oxidation | ≈ | ≈ | > | ? | [126, 150] |
| Pyruvate dehydrogenase kinase isoform-4 (quadriceps) | Mouse | Glucose oxidation | ≈ | ≈ | ? | ? | [144] |
| Uncoupling protein-2 (UCP-2-soleus) | Mouse | ROS production | ? | ? | > (rat) | ? | [126] |
| Uncoupling protein-3 (UCP-3-soleus) | Mouse | Body energy metabolism | ? | ? | > (rat) | ? | [126] |
| Uncoupling protein-3 (UCP-3- quadriceps) | Mouse | Body energy metabolism | ≈ | < | ? | ? | [144] |

>, higher gene expression in wild-type mice; <, lower expression in wild-type mice; ≈, similar gene expression between the two genotypes; WT, wild-type mouse; KO, knockout PPAR$\alpha$ mouse; †, expressed only in males; ‡, expressed only in females; (∑), Aoyama et al., and Kersten et al. reported conflicting results [39, 63].

an acyl-CoA shortened by two carbons. The gene encoding 3-ketoacyl-CoA thiolase (thiolase B) was found to be directly regulated by PPAR$\alpha$ [79]. However, it is unclear whether the DR-1 sequence identified within the thiolase B promoter mediates PPAR$\alpha$ transactivation [79]. Other putative targets of PPAR$\alpha$ are the long-chain acyl-CoA thioesterases, which catalyze the hydrolysis of acyl-CoAs into free fatty acids and CoA [80, 81]. Cytosolic, mitochondrial and to a lesser extend peroxisomal acyl-CoA thioestereases were upregulated during fasting in a PPAR$\alpha$-dependent manner. In addition, PPAR$\alpha$ ligands strongly increased cytosolic and mitochondrial acyl-CoA thioesterase expression, suggesting the direct involvement of PPAR$\alpha$. Finally, Lopez et al. reported that the sterol carrier protein X gene encoding a thiolase involved in the peroxisomal $\beta$-oxidation of branched-chain fatty acids was also directly regulated by PPAR$\alpha$ through two functional PPREs in rat, but whether the same is true in mice remains questionable [64, 82].

Hydrogen peroxide, a compound highly toxic for the cell, is generated by the reaction catalyzed by acyl-CoA oxidase. The enzyme catalase neutralizes hydrogen peroxide by causing its degradation into $H_2O$ and $O_2$. Interestingly, in vascular endothelial cells, expression of catalase was shown to be upregulated directly by the PPAR$\gamma$/RXR$\alpha$ heterodimer through a functional PPRE [83]. As a follow-up to these data, the possible regulation of catalase gene expression by PPAR$\alpha$ was also investigated. Neither primary mouse hepatocytes nor livers from rats treated with PPAR$\alpha$ activators displayed significant changes in the hepatic levels of catalase mRNA, whereas both catalase protein level and activity were increased [84–86]. In addition, no change in liver catalase mRNA was found between fed or fasted wild-type and PPAR$\alpha$ mutant mice, and ciprofibrate failed to induce a significant upregulation in catalase gene expression [64, 77]. This discrepancy between mRNA and protein is indicative of a post-transcriptional event. Besides, catalase, rat Cu/Zn super-

oxide dismutase, which protects the cell against oxidative stress by neutralizing superoxide radicals, was also identified as a direct PPARα target [87]. Based on these data, a protective role of PPARα against oxidative stress can be envisioned.

## PPARα and microsomal ω-hydroxylation of fatty acids

ω-Hydroxylation is a process that occurs almost exclusively in smooth endoplasmic reticulum and represents a minor pathway of hepatic fatty acid catabolism under basal conditions. However, in pathophysiological conditions such as diabetes mellitus or fasting, ω-hydroxylation is enhanced. Fatty acid ω-hydroxylation is governed by a subfamily of cytochrome P450 4A (CYP4A) enzymes (CYP4A, 4B and 4F) expressed constitutively in liver and kidney [88]. PPARα was shown to upregulate the expression of hepatic cytochrome P450 4A1 in rodents through a functional PPRE [40, 46, 77, 89, 90]. CYP4A3 was also found to be induced by PPARα, but no PPRE has yet been identified [77, 90]. Furthermore, three functional PPRE were localized in the rabbit promoter of the cytochrome P450 4A6-Z gene [91, 92]. Using microarray, we observed a dramatic fasting-induced PPARα-dependent increase in liver Cyp4A10 expression [F. Zandbergen et al., unpublished].
In conclusion, from the above discussion it is clear that by regulating peroxisomal, mitochondrial and microsomal fatty acid oxidation in a coordinated manner, PPARα serves as a master regulator of fatty acid catabolism.

## PPARα and ketogenesis

During prolonged fasting, synthesis of ketone bodies in liver is enhanced, in concert with an increase in hepatic fatty acid oxidation. These include beta-hydroxybutyrate, acetoacetate and acetone. The first and rate-limiting step in ketogenesis is catalyzed by mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-Co AS), which causes condensation of acetyl-CoA and acetoacetyl-CoA in the mitochondria to generate HMG-CoA and free CoA. In accordance with an important role of PPARα in ketogenesis, a functional PPRE was identified within the promoter of the rat and pig mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene [93–96]. Interestingly, another novel and unexpected mechanism of PPARα-mHMG-CoA synthase gene regulation was discovered [97]. In addition to regulating transcriptional activity, PPARα also interacts physically with mHMG-CoA synthase protein, potentiating the PPARα-dependent transcription of mHMG-CoA synthase. Thus, mHMG-CoA synthase, which is believed to be mainly localized within the mitochondria, specifically stimulates its own transcription rate by behaving as a coactivator of PPARα. The second and final step in ketone body synthesis is catalyzed by HMG-CoA lyase, which according to microarray is upregulated during fasting in a PPARα-dependent manner [F. Zandbergen et al., unpublished]. No PPRE has yet been identified in the promoter of this gene.

## PPARα and hepatic lipogenesis

Whereas most of the PPARα target genes are involved in fatty acid catabolism, some genes involved in lipogenesis were identified as PPARα targets too. Malic enzyme, also known as malate NADP oxidoreductase, catalyzes the oxidative decarboxylation of malate into pyruvate, generating NADPH required for fatty acid biosynthesis. Expression of malic enzyme gene is decreased in livers of PPARα mutant null mice, and a functional PPRE that confers fibrate responsiveness was found in the promoter of the gene [98]. Other then malic enzyme, stearoyl-CoA desaturase 1, also known as Δ9-desaturase, which catalyzes the rate-limiting step in the biosynthesis of n-9 unsaturated fatty acids from saturated fatty acids, was identified as a direct PPARα target gene with a functional PPRE in its promoter [99]. Moreover, expression of the related genes Δ5 desaturase and Δ6 desaturase was found to be upregulated by PPARα ligands, and in a PPARα-dependent fashion for Δ5 desaturase [100, 101]. Although no PPRE has yet been identified in the –Δ5 desaturase promoter, it is likely a direct target of PPARα, similar to what was found for the Δ6 desaturase promoter [101]. Thus, several genes involved in fatty acid biosynthesis appear to be regulated by PPARα. The question arises why the same transcription factor would enhance both fatty acid oxidation and fatty acid biosynthesis? Guillou et al. proposed that positive Δ5, Δ6 and Δ9 gene regulation by PPARα may serve to counteract the excessive fatty acid breakdown caused by the induction of peroxisomal-β-oxidation. Alternatively, as unsaturated fatty acids seem to be the better PPARα ligand, it is possible that induction of desaturases serves to guarantee an ample supply of endogenous ligands for PPARs. Matsuzaka et al. reported on the dual regulation of Δ5, Δ6 by both SREBP1, which is induced during feeding, and PPARα, which is induced during fasting, placing Δ5 and Δ6 in a distinct situation from regular lipogenic enzymes [102]. A similar regulation was also observed for Δ9 [100, 103]. It was argued that this dual regulation of both desaturases contributes to a stable production of polyunsaturated fatty acids (PUFA) that are essential for cellular function regardless of energy state. However, it is hard to imagine why such a complex mechanism would be necessary to maintain stable expression levels.

## PPARα and lipoprotein metabolism

Fibrates are hypolipidemic drugs that are used clinically for their ability to efficiently decrease plasma triglyceride and increase plasma high-density lipoprotein (HDL) levels in humans. Epidemiological studies have shown an inverse correlation between high plasma HDL levels and cardiovascular diseases, thereby generating interest in the study of the mechanism behind the fibrate-induced increase in plasma HDL. The plasma HDL level is determined by numerous factors including production of apolipoproteins, plasma abundance of phospholipid and cholesteryl-ester transfer proteins, and cellular expression of cell surface receptors, lipases and plasma membrane lipid transporters. Several of these factors have been shown to be under the control of fibrates and PPARα. At the level of production of apolipoproteins, fibrates upregulate the expression level of the human apolipoprotein A-I (ApoA-1) gene. Indeed, a functional PPRE has been identified in the human apo A-I promoter [104–106]. Intriguingly, in rodents fibrates decrease ApoA-I mRNA [107–109]. While the apparent lack of induction of rat ApoA-I expression by fibrates is due to a slight difference of three nucleotides between the rat and human apo A-I promoter, the PPARα agonist-induced decrease in rat ApoA-I expression is believed to be mediated through the repressor Rev-erb α, a reported but unconfirmed positive target gene of PPARα [110]. Expression of the other apoA protein, ApoA-II, is also upregulated by fibrates in a PPARα-dependent manner [111]. Thus, production of the two major apolipoproteins that are part of HDL is under the influence of PPARα.

In the circulation, HDL undergoes significant remodeling carried out by two important transfer proteins: cholesteryl-ester transfer protein and phospholipid transfer protein. Phospholipid transfer protein gene expression was found to be stimulated by PPARα, and a functional PPRE has been identified in the mouse promoter [112–114]. Via this mechanism, PPARα would favor the surface transfer of phospholipids between lipoproteins and promote the remodeling of HDL particles in blood plasma.

HDL is removed from the circulation via the scavenger receptor-class B type I (SR-BI) in liver, perhaps assisted by a putative SR-BI-stabilizing protein called CLAMP (also called PDZK1, Diphor-1, CAP70 or NaPi-Cap1). Interestingly, hepatic protein expression of SR-BI and CLAMP has been reported to be suppressed by fibrates without any change in their respective steady state mRNA, suggesting a posttranscriptional event [115]. The observed effect was absent in PPARα null mice, indicating the involvement of PPARα. The PPARα-induced breakdown of SR-BI protein may represent another mechanism by which PPARα modulates HDL metabolism.

Besides elevating plasma HDL levels, fibrates are known for their ability to diminish plasma triglyceride levels. Several mechanisms have been proposed to contribute to this effect, including a reduction of VLDL synthesis and increased catabolism of triglyceride-containing lipoproteins. At the level of lipoprotein catabolism, fibrates inhibit hepatic apolipoprotein C-III gene expression in humans and rats [116–118]. Apolipoprotein C-III is a potent inhibitor of lipoprotein lipase and thereby raises plasma triglyceride levels. Transcriptional suppression of the apolipoprotein C-III gene by fibrates involves the hepatic nuclear factor-4α. Displacement of hepatic nuclear factor-4α from the apolipoprotein C-III promoter by activated PPARα leads to the suppression of hepatic nuclear factor-4α-enhanced apolipoprotein C-III gene transactivation. Accordingly, fibrates may exert their hypolipidemic effect by modulating apolipoprotein C-III gene expression.

Although it has been argued that fibrates may lower plasma triglycerides by increasing lipoprotein lipase expression, there is no evidence that PPARα agonists upregulate lipoprotein lipase mRNA in the tissues where it is mostly expressed, that is muscle and adipose tissue [119]. In fact, it has been reported that PPARα ligands inhibit lipoprotein lipase activity in cardiomyocytes, which would be expected to raise plasma triglyceride levels [120]. Recently, a novel mechanism for the triglyceride lowering effect of fibrates was proposed. It was observed that the apolipoprotein A5 gene, which is critical for maintaining normal plasma triglyceride levels, is regulated by PPARα with two functional PPREs located in its promoter [121, 122]. Finally, fibrates and PPARα may lower plasma triglyceride levels by stimulating hepatic fatty acid oxidation and thereby diminishing their incorporation into triglycerides and their secretion as VLDL.

In addition to the genes mentioned above, several other genes involved in lipoprotein metabolism have been reported to be regulated by PPARα, yet the impact of their regulation is not completely clear. Lipoprotein (a) is a highly atherogenic particle whose main protein constituent is apolipoprotein (a). Puckey et al. purported to have identified a direct DR-1 sequence in the core enhancer of apolipoprotein (a) [apo(a)] gene that binds PPARα and triggers upregulation of apo(a) mRNA [123]. Using microarray, our group discovered differential expression of the apolipoprotein C-I and LDL receptor-related protein between wild-type and PPARα null mice, yet the details of this regulation remain to be investigated [F. Zandbergen et al., unpublished]. Regulation of these genes by PPARα may contribute to the effect of fibrates on plasma HDL and triglyceride concentrations.

## PPARα and uncoupling proteins

Uncoupling describes the movement of protons into the mitochondrial matrix without the concomitant pro-

duction of ATP. Several channel proteins (called uncoupling proteins, UCPs) that can mediate this proton transport have been identified, i.e. UCP-1, UCP-2 , UCP-3 and UCP-4. UCP-1 is exclusively expressed in brown adipose tissue and is responsible for the generation of heat by this tissue. It was recently identified as a direct PPARα target gene in brown adipose tissue, suggesting that PPARα is involved in thermogenesis [124]. However, expression of UCP-1 was unchanged in PPARα null mice, either in fed or fasted state, casting doubt on the physiological relevance of this regulation [39]. In contrast to UCP-1, UCP-2 is expressed in a wide range of tissues and may have a role in the production of reactive oxygen species, the production of insulin by pancreatic β cells and in the control of the ratio of [adenosine triphosphate]/[adenosine diphosphate]. UCP-3 is expressed primarily in skeletal muscle and may influence whole-body energy metabolism. Interestingly, it was found that cardiac and skeletal muscle *Ucp-3* expression was modulated by PPARα agonists in a PPARα-dependent manner, whereas the same was not true for UCP-2 [125, 126]. However, in neonatal cardiomyocytes, in primary cultures of hepatocytes, and in an insulinoma cell line PPARα agonists were able to induce UCP-2 gene expression [127–130]. Taken together, these observations point to a role of PPARα in UCP-2 and UCP-3 gene regulation. Inasmuch as the functions of UCP-2 and UCP-3 are presently not very clear, the importance and consequences of this regulation remain to be determined.

## PPARα and hepatic bile acid metabolism

Over the past few years, different studies have established a functional role for PPARα in bile acid homeostasis. De novo hepatic biosynthesis of bile acids is regulated by the key enzyme cholesterol 7α-hydroxylase (Cyp7A1), a microsomal cytochrome P450 isoenzyme. Although several studies have reported on regulation of Cyp7A1 gene expression by PPARα, conflicting observations were made [131]. It was observed that in human hepatoma HepG2 cells, cotransfection of expression vectors for both PPARα and RXRα in the presence of PPARα agonist caused a decrease in human and rat Cyp7A1 promoter activities [132, 133]. Despite the presence of a DR-1 in the Cyp7A1 promoters of both species, no direct binding of PPARα was detected. It was concluded that PPARα and its agonists decrease Cyp7A1 expression and activity by reducing the availability of hepatic nuclear factor 4α for binding to this DR-1. The physiological consequences of such regulation were studied in vivo in rats and mice. As in humans, PPARα-dependent downregulation of Cyp7A1 by PPARα agonists was found in both species [134]. In agreement with these observations, fibrate treatment led to a decrease in bile acid synthesis.

In contrast to the above data, Cheema et al. showed that both human Cyp7A1 and murine Cyp7A1 genes are upregulated by PPARα/RXRα [135]. A conserved and functional PPRE was identified in the promoter of both species, plus an additional PPRE in the mouse version. This latter element may possibly explain the better responsiveness to fibrates of the mouse Cyp7A1 gene compared with the human gene. Furthermore, Hunt et al. observed that hepatic expression of Cyp7A1 was diminished in PPARα null mice, particularly under fasting conditions, providing compelling evidence that PPARα upregulates Cyp7A1 expression [136]. The reasons for the discrepancy with the other study reporting negative regulation of Cyp7A1 by PPARα are unclear. Other genes implicated in bile acid synthesis that are regulated by PPARα include sterol-27-hydroxylase (Cyp27), whose activity was downregulated by PPARα agonists in a PPARα-dependent manner, and sterol-12α-hydroxylase (rat Cyp8B1), which was found to be directly upregulated by PPARα through a functional PPRE [134, 136]. Overall, regulation of Cyp7A1 by PPARα may help to explain the fibrate-induced increase and decrease in the relative biliary concentration of cholic acid and chenodeoxycholic acid, respectively.

Liver X receptor α is a nuclear receptor that plays an important role in the regulation of Cyp7A1 transcription. Expression of LXRα was found to be stimulated by PPARα agonist in macrophages, and decreased in livers of fasted PPARα null mice compared with fasted wild-type mice [137]. Five potential PPRE-like sequences were identified throughout the LXRα-5′ flanking region. Some of these PPREs, if not all, may support PPARα transactivation. By stimulating LXRα expression, PPARα was suggested to upregulate ABCA1 expression, which is a direct LXRα target gene. ABCA1 promotes efflux of cholesterol from macrophages and other peripheral tissues to HDL, yet its role in the liver is not very clear. Although these data point towards interesting cross-talk between the PPARα and LXRα signaling pathways, the importance and consequences of this regulation remain to be demonstrated.

Finally, Kok et al. found a fasting- and PPARα-dependent induction of the canalicular phospholipid translocator (Mdr2/Abcb4) and a complementary increase in phospholipid secretion [138, 139]. Taken together, numerous genes involved in bile metabolism appear to be regulated by PPARα. Despite this regulation, basal bile formation is unaffected by PPARα deletion, suggesting that the role of PPARα in bile metabolism may be more subtle and probably only important under conditions of increased free fatty acids levels, as is the case during fasting [138, 139].

### PPARα and intestinal absorption

Following their synthesis and excretion by the liver into the bile, bile salts end up in the intestine where they will undergo intestinal reabsorption via the transporter ASBT/SLC10A2, which is mainly responsible for the efficient enterohepatic circulation of bile salts [140]. Interestingly, human ASBT was shown to be directly regulated by PPARα, through a functional DR-1 motif located in its promoter [140]. Thus, PPARα may be implicated in regulation of the reabsorption of bile salts in the intestine. Bile salts are necessary for efficient biliary phospholipid secretion by Mdr2, which, as mentioned above, is upregulated during fasting in a PPARα-dependent manner, pointing to a possible relationship between both regulatory processes. Recent evidence also suggests that PPARα may regulate expression of the cellular retinol-binding protein type-II gene, indicating a role of PPARα in intestinal metabolism of retinoids [141, 142].

### PPARα and glucose metabolism

Maintenance of plasma glucose levels is essential, as very low blood glucose concentrations can lead to brain dysfunction, coma and death. A role of PPARα in glucose metabolism emerged from several studies showing that PPARα null mice suffer from pronounced hypoglycemia after fasting [39, 40, 143–145]. Inasmuch as plasma glucose is maintained during fasting by a combination of de novo glucose synthesis (gluconeogenesis) and glycogen breakdown (glycogenolysis), fasting-induced hypoglycemia could be caused by inhibition of either of these two pathways. Alternatively, it is possible that glucose utilization is increased in PPARα null mice.

### PPARα and β-cell function

By influencing secretion of pancreatic hormones, PPARα deletion could have a major effect on plasma glucose levels. Whereas Xu et al. failed to find a significant difference in plasma insulin levels between fed or fasted wild-type compared with PPARα mutant mice, unpublished data from Burcelin indicate that insulin is modestly but significantly elevated in fasted PPARα null mice [143]. However, plasma glucagon levels were increased as well, and consequently fasting-induced hypoglycemia cannot be ascribed to an altered insulin/glucagon ratio. The mechanism by which PPARα may alter insulin and glucagon production and/or release remains ambiguous. Pyruvate dehydrogenase kinase 4 was identified as a probable PPARα target in islets, but the effect of this regulation on glucose-stimulated insulin secretion is not very clear.

### PPARα and fatty acid oxidation

Hepatic fatty acid β-oxidation is associated with the production of certain cofactors (ATP, NADH, acetyl CoA) that have a stimulatory effect on gluconeogenesis. As PPARα is critical for fatty acid oxidation, it is conceivable that the hypoglycemia in fasted PPARα mutant mice may be secondary to a defect in fatty acid oxidation [39]. At the level of peripheral tissues, defective fatty acid oxidation as a result of PPARα deficiency may cause a corresponding increase in the rate of utilization of glucose.

### PPARα and glycogenolysis

Early during the course of fasting, blood glucose is mainly maintained by hepatic glycogen breakdown (glycogenolysis). It was reported by some groups that under basal conditions, hepatic glycogen stores are strongly decreased in PPARα mutant compared with wild-type mice, providing a potential explanation for fasting hypoglycemia [39, 143]. Decreased hepatic glycogen content is suggestive of a defect in glycogen synthesis or storage, or increased utilization. So far, no specific gene targets of PPARα in any of these pathways have yet been identified. In contrast, other studies failed to find a difference in basal liver glycogen content between the two sets of mice [77, 145]. The reason for this discrepancy is presently unclear.

### PPARα and gluconeogenesis

Another pathway that may be impaired in PPARα mutant mice is gluconeogenesis. The major substrates for gluconeogenesis are alanine, lactate and glycerol. Two rate-limiting enzymes are believed to regulate the whole process of gluconeogenesis, phosphoenol-pyruvate carboxykinase and pyruvate carboxylase. Hepatic expression of phosphoenol-pyruvate carboxykinase does not appear to be different between wild-type and PPARα mutant mice, regardless of the nutritional status [39, 143]. This is surprising considering that the PEPCK promoter contains a functional PPRE, which was shown to be functional in adipocytes [146]. Interestingly, DNA microarrays revealed that fasted PPARα mutant mice display a reduced hepatic expression of pyruvate carboxylase, as well as lactate dehydrogenase A4 [S. Kersten, unpublished]. This suggests that the hypoglycemia might be due to impaired gluconeogenesis caused by decreased expression of these genes. In line with this, hepatic glucose production from lactate was decreased in both fed and fasted PPARα mutant mice [143]. However, despite decreased expression of these genes, overall hepatic glucose production was increased in the PPARα null mice after a 17-h fast. These data are hard to reconcile with the notion that the hypoglycemia in fasted PPARα null mice may (partially) be attributed to defective gluconeogenesis. No

PPRE has yet been identified in the promoter of either pyruvate carboxylase or lactate dehydrogenase A4.

### PPARα and glycolysis

As mentioned above, hypoglycemia in fasted PPARa null mice may also be caused by increased glucose utilization. The only gene involved in glucose utilization that has been identified as a PPARα target is pyruvate dehydrogenase kinase isoform 4 (PDK4). PDK4 phosphorylates and inactivates pyruvate dehydrogenase, thereby limiting the flux through glycolysis. It was found that the fasting-induced increase of PDK4 in heart, kidney and skeletal muscle is PPARα dependent and, furthermore, that direct PPARα activation upregulates PDK4 expression [147–151]. In PPARα null mice, the lack of PPARα-induced PDK4 expression during fasting may lead to a dephosphorylated active form of the pyruvate dehydrogenase complex that, via increased glycolysis, could give rise to increased glucose utilization. So far, no PPRE has yet been identified in the promoter of the PDK4 gene.

### PPARα and urea cycle

Not only does PPARα govern lipid and glucose metabolism, recent studies have also associated PPARα with regulation of hepatic amino acid metabolism [152, 153]. By comparing wild-type and PPARα null mice using oligonucleotide microarray and subtractive hybridization, PPARα-dependent downregulation of genes involved in major pathways of amino acid metabolism was found, i.e. transamination, deamination, urea cycle, oxidation of alpha keto acids and synthesis of amino acid-derived-products, leading to increased plasma urea levels in PPARα null mice. The precise molecular mechanism behind this regulation is still unknown and requires further investigation. While PPARα downregulates expression of genes involved in the urea cycle, HNF-4α and C/EBPα have the opposite effect. It was shown that C/EBPα null mice display lower levels of plasma urea, a consequence of the lack of induction of urea cycle enzymes by C/EBPα. The same was true for liver-specific HNF4-α null mice, which display a higher plasma ammonia concentration together with a lower plasma urea concentration [154]. This phenotype may be explained by the lack of HNF-4α-dependent induction of ornithine transcarbamoylase gene expression. Indeed, two different functional binding sites for HNF-4α, one being more important than the other, were found. As HNF-4α and PPARα both recognize DR-1 sequences, it is possible that PPARα may interfere negatively with regulation of ornithine transcarbamoylase by HNF-4α, leading to downregulation of ornithine transcarbamoylase gene expression.

### PPARα and biotransformation

Toxins, drugs, carcinogens and other xenobiotics are eliminated from the body via a series of reactions described as biotransformation. Biotransformation usually consists of three separate steps: First, in phase I, a polar group is introduced into the xenobiotic molecule, making it more reactive. Subsequently, in phase II, the modified xenobiotic is conjugated to a polar molecule such as glucuronic acid or glutathione. Finally, conjugated xenobiotics are excreted into the bile or urine via specific transporters (phase III), mainly by members of the superfamily of ATP-binding cassette transporter proteins [71]. Studies with synthetic PPARα agonists and PPARα null mice have indicated that PPARα is able to both up- and downregulate expression of xenobiotic metabolizing enzymes. Genes that were found to be upregulated by PPARα agonists in liver include phase I cytosolic epoxide hydrolase and aldehyde dehydrogenase type 3. Comparison of gene expression between wild-type and PPARα null mice using microarray revealed that the effect of peroxisome proliferators on the expression of these enzymes is clearly mediated by PPARα [unpublished data]. So far no PPRE has been identified in the promoter of either of these genes.

Numerous genes involved in phase I biotransformation appear to be downregulated by PPARα. This includes several phase I enzymes involved in the hydroxylation (Cyp2A4, Cyp2A1, Cyp3A11,Cyp17) as well as the dehydrogenation (3α, 11β and 17β hydroxysteroid dehydrogenase) of steroids [155–158]. Furthermore, downregulation of hepatic Cyp2A5, Cyp2C29 and Cyp3A11 by lipopolysaccharide is attenuated or blocked in PPAR α null mice [159]. In contrast, expression of 17-β-hydroxysteroid-dehydrogenase type IV is upregulated by PPARα ligands. Thus, PPARα seems to govern diverse aspects of steroid metabolism. Interestingly, at the functional level, it was shown that rats fed peroxisome proliferators suffered from alterations in reproductive organs together with impaired estrogen metabolism. Female rats displayed a decrease in serum estradiol levels together with suppression of ovulation, a possible consequence of modulation of the fatty acyl-CoA:estradiol acetyl-transferase protein level by PPARα [160]. Males suffered from a decrease in testicle size and Sertoli cell vacuolation, but whether this phenotype is connected with the PPARα-mediated induction of fatty acyl-CoA:testosterone acetyltransferase remains to be investigated. Furthermore, peroxisome proliferators were found to markedly influence the profile of site- and stereospecific microsomal metabolites of testosterone. This is likely caused by altered expression of steroid-metabolizing enzyme. Indeed, fibrates decreased CYP2A4 levels both at the protein and mRNA levels [161]. In addition, expression of the male-specific genes α2 urinary-globulin (α pheromone car-

rier) and Cyp2C11, (a steroid 2 and 16$\alpha$ hydroxylase) are repressed by peroxisome proliferators, as well as the female-specific Cyp2C12 gene [157]. Overall, it is clear that PPAR$\alpha$ potently downregulates expression of genes involved in steroid metabolism, yet the mechanisms involved still need to be elucidated.

The ability of peroxisome proliferators to influence phase II biotransformation has been extensively documented. However, unlike phase I biotransformation, direct involvement of PPAR$\alpha$ in phase II biotransformation has yet to be demonstrated. A wide array of different peroxisome proliferators are able to decrease the activity of various glutathione-*S*-transferases via changes at both protein and mRNA level [162]. This can result in decreased biliary excretion of glutathione conjugates such as sulphobromophthalein [163]. It also may increase exposure to carcinogenic xenobiotics by retarding their conjugation to glutathione and thereby their detoxification [164]. Fibrates have been shown to decrease glutathione-*S*-transferase alpha and mu protein concentrations in rat liver, an effect which is probably mediated by PPAR$\alpha$ [our unpublished results; 165]. In addition to glutathione conjugation, peroxisome proliferators also affect phase II glucuronidation. Whereas bilirubin UDP-glucuronosyltransferase mRNA levels are stimulated by peroxisome proliferators, resulting in increased bilirubin glucuronidation, testosterone glucuronidation is diminished by peroxisome proliferators [166, 167]. Recently, the UDP-glucuronosyltransferase 1A9 gene was identified as a direct positive PPAR$\alpha$ target gene with a functional PPRE in its promoter [168]. In conclusion, exposure to peroxisome proliferators may profoundly influence steroid biotransformation, which is probably mediated by PPAR$\alpha$, possibly leading to altered metabolism of oral contraceptive and other steroids.

## PPAR$\alpha$ and white adipose tissue

It is well established that PPAR$\gamma$ is the major PPAR isotype expressed in white adipose tissue and that PPAR$\alpha$ is barely detectable [4]. Nevertheless, different lines of evidence suggest a functional role of PPAR$\alpha$ in this tissue. The basal expression of resistin in epididymal fat was significantly higher in wild-type than in PPAR$\alpha$ null mice, yet PPAR$\alpha$ agonist had no effect on resistin expression [169]. Furthermore, expression of the fasting-induced adipose factor in white adipose tissue was higher in PPAR$\alpha$ null mice, regardless of the nutritional state [170]. While PPAR$\alpha$ activation may modulate expression of the fasting-induced adipose factor and resistin in fat, it is also possible that the altered expression of these genes in PPAR$\alpha$ null mice are an indirect consequence of metabolic perturbations in the PPAR$\alpha$ null mice. Thus, in spite of its weak expression it is not inconceivable that PPAR$\alpha$ may play a regulatory role in the biology of white adipose tissue.

## Trans-repression by PPAR$\alpha$: implications in acute phase response and inflammation

A critical role of PPAR$\alpha$ in inflammation was first illustrated by Devchand et al., who provided evidence of a prolonged duration of inflammation in PPAR$\alpha$ null mice [38]. Many studies aimed at defining the molecular pathways behind this phenotype have since been performed and have shown interference of PPAR$\alpha$ with several other transcription factors pathways. PPAR$\alpha$ commonly operates as a positive regulator of gene expression, but effects of PPAR$\alpha$ on inflammatory pathways are mostly carried out by trans-repression. In T lymphocytes PPAR$\alpha$ is expressed at a relatively high level in resting T cells, and diminishes upon activation with a corresponding upregulation of PPAR$\gamma$ [27, 28]. Interestingly, treatment of activated T cells with different PPAR$\alpha$ agonists results in decreased interleukin-2 and interferon-$\gamma$ gene expression [29]. PPAR$\alpha$ was found to mediate trans-repression on gene expression, a process by which PPAR$\alpha$ antagonizes several signal transduction pathways through various DNA-dependent and independent mechanisms. In line with this, gene transactivation by different transcription factors such as NF-$\kappa$B, NFAT (nuclear factor of activated T cells), C/EBP (CAAT/box enhancer binding protein), STAT (signal transducer and activator of transcription) and c-Jun/c-Fos, were repressed by PPAR$\alpha$ via DNA binding-independent events such as protein-protein interactions and cofactor competition [31, 32]. Moreover, interference between PPAR$\alpha$ and C/EBP$\beta$ signaling pathways through depletion of the coactivator glucocorticoid receptor-interacting protein/transcriptional intermediary factor 2 (GRIP1/TIF2) was identified, leading to reduced C/EBP$\beta$ transactivation of the fibrinogen promoter [171].

Other examples of trans-repression on gene expression by PPAR$\alpha$ are (i) inhibition of cytokine-induced vascular cell adhesion molecule-1 expression by PPAR$\alpha$ activators in human endothelial cells, through interference with NF-$\kappa$B signaling [32]; (ii) repression of thrombin-induced expression of endothelin-1 by PPAR$\alpha$ agonists by modulating c-Jun/c-Fos DNA binding activities on the endothelin-1 promoter [33]; (iii) Interference with the smad4 transcription factor signaling that results in an inhibition of the transforming growth factor-beta (TGF-$\beta$) induced $\beta$5 integrin gene expression [172] and (iv) Inhibition of interleukin (IL)-6 gene expression by PPAR$\alpha$-dependent repression of p65 as well as c-Jun transactivation of the IL-6 promoter [173]. This can be seen as a consequence of protein-protein interactions between different parts of PPAR$\alpha$, c-Jun and p65, one of the five members of the NF-$\kappa$B/Rel family.

Expression of IκBα (for inducer of nuclear factor-κB) was also found to be upregulated by PPARα [174, 175]. This is important because IκBα is an inhibitory protein of NF-κB that maintains NF-κB in a cytoplasmic inactive form, leading to strongly reduced NF-κB DNA binding and thus preventing the NF-κB-dependent inflammatory response. Surprisingly, PPARα-mediated IκBα upregulation did not require any functional PPRE in the IκBα promoter, but rather Sp1 and NF-κB1 sites. Activated PPARα, rather than directly binding on the DNA promoter of IκBα, enhanced the occupancy of the NF-κB response element located in the IκBα promoter. The ligand binding domain of PPARα was identified as the part that potentiates p65 (a subunit of NF-κB)-stimulated IκBα transcription in a DRIP205 (VDR interacting protein)/TRAPP220(TR-associated protein)-dependent manner. Thus, these results provide a new molecular basis for PPARα-induced gene transcription and open new routes of investigations.

Last, cyclooxygenase 2 (COX2) was identified as a direct PPARα target that was strongly upregulated in mammary epithelial cells to a large diversity of fatty acids and PPARα agonists [176]. COX2 is believed to be responsible for much of the prostaglandin production in inflamed tissues and was found to be overexpressed in several human cancers.

## PPARα and the acute phase response

Hepatic acute phase response, which describes the early and immediate set of homeostatic control reactions that are induced during inflammatory processes associated with endotoxemia or liver regeneration, is known to be associated with a decrease in the hepatic expression of genes involved in lipid metabolism. PPARα gene expression is downregulated during the hepatic acute phase response [177, 178]. Treatment of hamsters with endotoxin results in a rapid downregulation of PPARα mRNA levels as well as of other nuclear receptors, such as RXR, PXR, LXR and FXR. This downregulation is associated with reduced RXR:PPAR binding activities in nuclear extracts as well as a decrease of both basal and WY14643-induced expression of a target gene, acyl-CoA synthetase [178]. The tightly regulated signaling associated with the acute phase response plays also an important role during liver regeneration, which occurs when major parts of the liver tissue are destroyed or removed, e.g. after 70% partial hepatectomy. The very early phase of liver regeneration (<4 h) is called the priming phase. Using microarray analysis, it was observed that PPARα mRNA levels were rapidly downregulated during this priming phase of liver regeneration [177]. No mechanisms, however, were presented and a role of proinflammatory cytokines is unlikely, as cytokines such as IL-6, IL-2, IL-3, IL-4, IL-5, TNFs and interferon gamma (IFN-γ) were upregulated in the first 4 h after partial hepatectomy [177]. A possible explanation could be that other liver-enriched transcription factors that are upstream of PPARα are also rapidly downregulated in part by phosphorylation via mitogen-activated protein kinase.

PPARα can be considered a negative acute phase protein, and it represents a key player in the regulation of the acute phase response. One of the acute phase proteins that is regulated by PPARα is transferrin. Transferrin was identified as a negative PPARα target via a mechanism that involves disruption of the HNF4-enhanced expression of transferrin gene, probably by interfering with the ability of HNF4 to transactivate via a PPRE-like element located in the transferrin promoter [157, 179]. Furthermore, the gene expression of two other acute phase proteins, fibrinogen and α-1 acid glycoprotein, was shown to be repressed by PPARα agonists [157, 171, 180]. Thus, by a novel molecular mechanism involving negative gene regulation, hepatic PPARα is able to modulate the acute phase response. The cross-talk between proinflammatory signaling and PPARα-dependent signaling is discussed in more detail elsewhere [181, 182].

## Alternative PPAR response elements?

While a DR-1-like sequence is still believed to be the favorite DNA-binding site for PPARα, different groups have reported on the characterization of new regulatory sequences onto which PPARα can bind and regulate transcriptional activity. For example, a new kind of PPRE was identified in the promoter of the nuclear orphan receptor Rev-erbα [110]. This PPRE is not a DR-1 but rather a DR-2 motif. An alternative DR-1 sequence was also identified in the human promoter of PPARα, suggesting a possible autoregulation of its own transcription rate [183]. Whether the same is also true in rodents remains to be investigated and will require further characterization of the mouse PPARα promoter [184]. Finally, an alternative PPRE was also identified in the proximal promoter of the human CPT-II gene [68]. This PPRE only contains one half-site which is a perfect consensus sequence for RXRα, whereas the second half-site normally occupied by PPARα is not well conserved. Chromatin immunoprecipitation verified the binding of PPARα, and transient transfections experiments confirmed its functionality.

## Some PPARα target genes are also PPARβ- and/or PPARγ-regulated genes

Genes regulated by PPARα may also be targets of PPARβ and PPARγ. This concept has been illustrated by the work

of Yu et al. who overexpressed PPAR$\gamma$1 in the liver of PPAR$\alpha$ null mice [185]. They noticed a strong PPAR$\gamma$1-induced expression of genes such as $\Delta$9 desaturase, malic enzyme and fasting-induced adipose factor, genes that were previously identified as PPAR$\alpha$ targets [98, 99, 170]. In addition, overlapping functions of PPAR$\alpha$ and PPAR$\beta$ in skeletal muscle and cardiac cells were identified [144, 186]. Last, an interesting parallel in PPAR$\alpha$ and PPAR$\beta$ function was recently proposed. Whereas hepatic fatty acid $\beta$-oxidation is controlled by PPAR$\alpha$, PPAR$\beta$ was postulated as a master regulator of fatty acid $\beta$-oxidation in brown adipose tissue [187]. Whether this is also true for PPAR$\alpha$ in this tissue remains to be investigated. Thus, it is possible that PPAR$\alpha$ and PPAR$\beta$ may share partial functional redundancy that renders the analysis of their respective functions difficult. The generation of transgenic mice (double PPAR$\alpha$/$\beta$ mutant mice, tissue selective overexpression and ablation of each isotype) may help to bypass this problem.

## Conclusions and future perspectives

Starting out as a transcription factor involved in the regulation of peroxisomal fatty acid metabolism, in the past decade PPAR$\alpha$ has taken on the identity of a global regulator of metabolism, governing such diverse processes as inflammation and nutrient metabolism, particularly under conditions of fasting (fig. 2). Indeed, it is mostly during fasting that the effects of PPAR$\alpha$ become manifest. Experiments with PPAR$\alpha$ null mice have clearly demonstrated that deletion of PPAR$\alpha$ has minor effects on fed mice, but dramatically disrupts homeostasis in fasted mice [39, 40, 64].

The advent of modern-day technologies such as transcriptomics and proteomics, which permit the monitoring of thousands of genes or proteins, is expected to lead to further breakthroughs in our understanding of PPAR$\alpha$ function. Examples of such a genomics approach towards PPAR$\alpha$ have recently emerged. Utilizing microarray, our group managed to identify novel pathways governed by PPAR$\alpha$ (hepatic amino acid metabolism) as well as novel specific PPAR$\alpha$ target genes such as the G0/G1 switch-gene 2 (GOS-2) [153; Zandbergen et al., unpublished]. DNA arrays also revealed their efficacity in the characterization of new PPAR$\gamma$ targets in fat, liver and skeletal muscle [188]. A comprehensive metabolomic assessment of lipid metabolites was successfully used to study the impact of the PPAR$\gamma$ agonist rosiglitazone on lipid metabolism [189]. Finally, proteomics was employed to better characterize the effect of PPAR$\alpha$ ligands on rat liver [87]. A more comprehensive analysis of these studies will

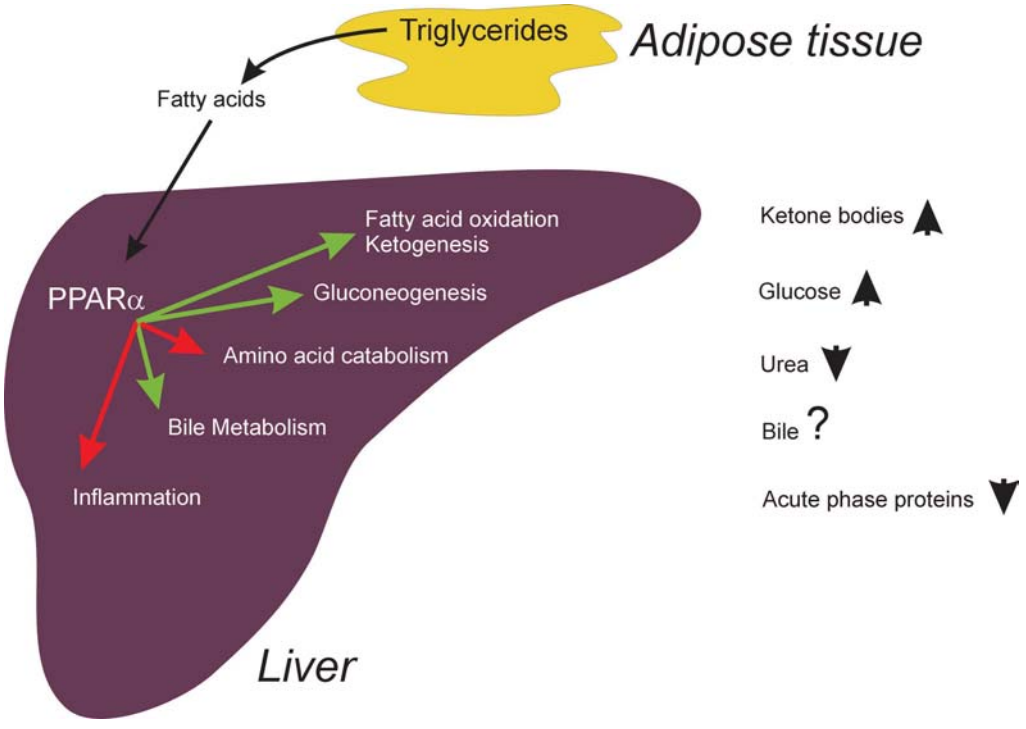


Figure 2. Schematic view of the role of PPAR$\alpha$ in the hepatic reponse to fasting. During fasting, fatty acids are released from adipose tissue and travel to the liver, where they bind and activate PPAR$\alpha$. Activation of PPAR$\alpha$ results in stimulation of a number of pathways, including fatty acid oxidation, ketogenesis and gluconeogenesis, and in suppression of amino acid catabolism and the acute phase response. This is associated with an increase or decrease of specific components in the blood.

be published elsewhere. Large-scale screening of functional PPRE may also be started using chromatin immunoprecipitation. Using a suitable antibody against PPARα, it is possible to pull down and purify genomic DNA fragments to which PPARα is bound. After a cloning step, nucleotide sequencing is performed to determine the identity of each fragment. Chromatin immunoprecipitation appears to be a suitable method for the characterization of a large set of direct PPARα target genes.

In conclusion, while much is already known about the function and targets of PPARα, the use of modern genomics tools will further advance our understanding of PPARα in the next few years. However, although the power of a genomics approach should not be underestimated, the true value of genomics is only uncovered when used in combination with other experimental techniques and approaches.

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1 Issemann I. and Green S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature **347:** 645–650
2 Dreyer C., Krey G., Keller H., Givel F., Helftenbein G. and Wahli W. (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell **68:** 879–887
3 Dreyer C., Keller H., Mahfoudi A., Laudet V., Krey G. and Wahli W. (1993) Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). Biol. Cell. **77:** 67–76
4 Escher P., Braissant O., Basu-Modak S., Michalik L., Wahli W. and Desvergne B. (2001) Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding. Endocrinology **142:** 4195–4202
5 Tugwood J. D., Holden P R., James N. H., Prince R. A. and Roberts R. A. (1998) A peroxisome proliferator activated receptor- alphα (PPARα) cDNA cloned from guinea-pig liver encodes a protein with similar properties to the mouse PPARα: implications for species differences in responses to peroxisome proliferators. Arch. Toxicol. **72:** 169–177
6 Sher T., Yi H. F., McBride O. W. and Gonzalez F. J. (1993) cDNA cloning, chromosomal mapping and functional characterization of the human peroxisome proliferator activated receptor. Biochemistry **32:** 5598–5604
7 Diot C. and Douaire M. (1999) Characterization of a cDNA sequence encoding the peroxisome proliferator activated receptor alpha in the chicken. Poult. Sci. **78:** 1198–1202
8 Escriva H., Safi R., Hanni C., Langlois M. C., Saumitou-Laprade P., Stehelin D. et al. (1997) Ligand binding was acquired during evolution of nuclear receptors. Proc. Natl. Acad. Sci. USA **94:** 6803–6888
9 Ibabe A., Grabenbauer M., Baumgart E., Fahimi H. D. and Cajaraville M. P. (2002) Expression of peroxisome proliferator-activated receptors in zebrafish (*Danio rerio*). Histochem. Cell. Biol. **118:** 231–239
10 Kliewer S. A., Forman B. M., Blumberg B., Ong E. S., Borgmeyer U., Mangelsdorf D. J. et al. (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc. Natl. Acad. Sci. USA **91:** 7355–7359
11 Mukherjee R., Jow L., Noonan D. and McDonnell D. P. (1994) Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. J. Steroid. Biochem. Mol. Biol. **51:** 157–166
12 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
13 Desvergne B. and Wahli W. (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr. Rev. **20:** 649–688
14 Kersten S., Desvergne B. and Wahli W. (2000) Roles of PPARs in health and disease. Nature **405:** 421–424
15 IJpenberg A., Jeannin E., Wahli W. and Desvergne B. (1997) Polarity and specific sequence requirements of peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. J. Biol. Chem. **272:** 20108–20117
16 Hoekstra M., Kruijt J. K., Van Eck M. and Van Berkel T. J. (2003) Specific gene expression of ATP-binding cassette transporters and nuclear hormone receptors in rat liver parenchymal, endothelial and Kupffer cells. J. Biol. Chem. 10.1074/jbc.M301189200
17 Hellemans K., Michalik L., Dittie A., Knorr A., Rombouts K., De Jong J. et al. (2003) Peroxisome proliferator-activated receptor-beta signaling contributes to enhanced proliferation of hepatic stellate cells. Gastroenterology **124:** 184–201
18 Peters J. M., Rusyn I., Rose M. L., Gonzalez F .J. and Thurman R. G. (2000) Peroxisome proliferator-activated receptor alpha is restricted to hepatic parenchymal cells, not Kupffer cells: implications for the mechanism of action of peroxisome proliferators in hepatocarcinogenesis. Carcinogenesis **21:** 823–826
19 Auboeuf D., Rieusset J., Fajas L., Vallier P., Frering V. et al. (1999) Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor α in humans: no alteration in adipose tissue of obese and NIDDM patients. Diabetes **46:** 1319–1327
20 Braissant O. and Wahli W. (1998) Differential expression of peroxisome proliferator-activated receptor-alpha, -beta and -gamma during rat embryonic development. Endocrinology **139:** 2748–2754
21 Loviscach M., Rehman N., Carter L., Mudaliar S., Mohadeen P., Ciaraldi T. P. et al. (2000) Distribution of peroxisome proliferators-activated receptors (PPARs) in human skeletal muscle and adipose tissue: relation to insulin action. Diabetologia **43:** 304–311
22 Chinetti G., Griglio S., Antonucci M., Torra I. P., Delerive P., Majd Z. et al. (1998) Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J. Biol. Chem. **273:** 25573–25580
23 Chinetti G., Gbaguidi F. G., Griglio S., Mallat Z., Antonucci M., Poulain P. et al. (2000) CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. Circulation **101:** 2411–2417
24 Chinetti G., Lestavel S., Bocher V., Remaley A. T., Neve B., Torra I. P. et al. (2001) PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage

foam cells through stimulation of the ABCA1 pathway. Nat. Med. **7:** 53–58
25 Gbaguidi F. G., Chinetti G., Milosavljevic D., Teissier E., Chapman J., Olivecrona G. et al. (2002) Peroxisome proliferator-activated receptor (PPAR) agonists decrease lipoprotein lipase secretion and glycated LDL uptake by human macrophages. FEBS Lett. **512:** 85–90
26 Marx N., Mackman N., Schönbeck U., Yilmaz N., Hombach V., Libby P. et al. (2001) PPAR$\alpha$ activators inhibit tissue factor expression and activity in human monocytes. Circulation **103:** 213–219
27 Neve B. P., Corseaux D., Chinetti G., Zawadzki C., Fruchart J. C., Duriez P. et al. (2001) PPARalpha agonists inhibit tissue factor expression in human monocytes and macrophages. Circulation **103:** 207–212
28 Cunard R., Ricote M., DiCampli D., Archer D. C., Kahn D. A., Glass C. K. et al. (2002) Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors. J. Immunol. **168:** 2795–2802
29 Jones D. C., Ding X. and Daynes R. A. (2002) Nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha) is expressed in resting murine lymphocytes. The PPARalpha in T and B lymphocytes is both transactivation and transrepression competent. J. Biol. Chem. **277:** 6838–6845
30 Marx N., Kehrle B., Kohlhammer K., Grüb M., Koenig W., Hombach V. et al. (2002) PPAR activators as anti-inflammatory mediators in human T Lymphocytes-implications for atherosclerosis and transplantation-associated arteriosclerosis. Circ. Res. **90:** 703–710
31 Staels B., Koenig W., Habib A., Merval R., Lebret M., Pineda-Torra I. et al. (1998) Activation of human aortic smooth-muscle cells is inhibited by PPAR$\alpha$ activators but not by PPAR$\gamma$ activators. Nature **393:** 790–793
32 Marx N., Sukhova G., Collins T., Libby P. and Plutzky J. (1999) PPAR$\alpha$ activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. Circulation **99:** 3125–3131
33 Delerive P., Martin-Nizard F., Chinetti G., Trottein F., Fruchart J. C., Najib J. et al. (1999) Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. Circ. Res. **85:** 394–402
34 Inoue I., Shino K., Noji S., Awata T. and Katayama S. (1998) Expression of peroxisome proliferator-activated receptor $\alpha$ (PPAR$\alpha$) in primary cultures of human vascular endothelial cells. Biochem. Biophys. Res. Commun. **246:** 370–374
35 Keller H., Dreyer C., Medin J., Mahfoudi A., Ozato K. and Wahli W. (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
36 Forman B. M., Chen J. and Evans R. M. (1997) Hypolipidemic drugs, polyunsaturated fatty acids and eicosanoids are ligands for peroxisome proliferator-activated receptor alpha and delta. Proc. Natl. Acad. Sci. USA **94:** 4312–4317
37 Yu K., Bayona W., Kallen C. B., Harding H. P., Ravera C. P., McMahon G. et al. (1995) Differential activation of peroxisome proliferator-activated receptors by eicosanoids. J. Biol. Chem. **270:** 23975–23983
38 Devchand P. R., Keller H., Peters J. M., Vazquez M., Gonzalez F. J. and Wahli W. (1996) The PPARalpha-leukotriene B4 pathway to inflammation control. Nature **384:** 39–43
39 Kersten S., Seydoux J., Peters J. M., Gonzalez F. J., Desvergne B. and Wahli W. (1999) Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J. Clin. Invest. **103:** 1489–1498
40 Leone T. C., Weinheimer C. J. and Kelly D. P. (1999) A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proc. Natl. Acad. Sci. USA **96:** 7473–7478
41 Ren B., Thelen A. and Jump D. B. (1996) Peroxisome proliferator-activated receptor alpha inhibits hepatic S14 gene transcription. Evidence against the peroxisome proliferator-activated receptor alpha as the mediator of polyunsaturated fatty acid regulation of s14 gene transcription. J. Biol. Chem. **271:** 17167–17173
42 Fruchart J. C., Duriez P. and Staels B. (1999) Molecular mechanism of action of the fibrates. J. Soc. Biol. **193:** 67–75
43 Anderson S. P., Yoon L., Richard E. B., Dunn C. S., Cattley R. C. and Corton J. C. (2002) Delayed liver regeneration in peroxisome proliferator-activated receptor-$\alpha$-null mice. Hepatology **36:** 544–554
44 Rao M. S., Peters J. M., Gonzalez F. J. and Reddy J. K. (2002) Hepatic regeneration in peroxisome proliferator-activated receptor alpha-null mice after partial hepatectomy. Hepatol. Res. **22:** 52–57
45 Choudhury A. I., Chahal S., Bell A. R., Tomlinson S. R., Roberts R. A., Salter A. M. et al. (2000) Species differences in peroxisome proliferation; mechanisms and relevance. Mutat. Res. **448:** 201–212
46 Lee S. S., Pineau T., Drago J., Lee E. J., Owens J. W., Kroetz D. L. 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
47 Palmer C. N., Hsu M. H., Griffin K. J, Raucy J. L. and Johnson E. F. (1998) Peroxisome proliferator activated receptor-alpha expression in human liver. Mol. Pharmacol. **53:** 14–22
48 Lawrence J. W., Li Y., Chen S., DeLuca J. G., Berger J. P., Umbenhauer D. R. et al. (2001) Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) alpha. PPAR alpha fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expression. J. Biol. Chem. **276:** 31521–31527
49 Hsu M. H., Savas U., Griffin K. J. and Johnson E. F. (2001) Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor alpha in HepG2 cells. J. Biol. Chem. **276:** 27950–27958
50 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 PPARalpha and PPARgamma activators. J. Biol. Chem. **272:** 28210–28217
51 Frohnert B. I., Hui T. Y. and Bernlohr D. A. (1999) Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene. J. Biol. Chem. **274:** 3970–3977
52 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
53 Sato O., Kuriki C., Fukui Y. and Motojima K. (2002) Dual promoter structure of mouse and human fatty acid translocase/CD36 genes and unique transcriptional activation by peroxisome proliferator-activated receptor alpha and gamma ligands J. Biol. Chem. **277:** 15703–15711
54 Schoonjans K., Watanabe M., Suzuki H., Mahfoudi A., Krey G., Wahli W. et al. (1995) Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. J. Biol. Chem. **270:** 19269–19276
55 Wolfrum C., Borrmann C. M., Borchers T. and Spener F. (2001) Fatty acids and hypolipidemic drugs regulate peroxi-

some proliferator-activated receptors alpha- and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. Proc. Natl. Acad. Sci. USA **98:** 2323–2328
56 Tan N. S., Shaw N. S., Vinckenbosch N., Liu P., Yasmin R., Desvergne B. et al. (2002) Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. Mol. Cell. Biol. **22:** 5114–5127
57 Poirier H., Niot I., Monnot M. C., Braissant O., Meunier-Durmort C., Costet P. et al. (2001) Differential involvement of peroxisome-proliferator-activated receptors alpha and delta in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine. Biochem. J. **355:** 481–488
58 Elholm M., Bjerking G., Knudsen J., Kristiansen K. and Mandrup S. (1996) Regulatory elements in the promoter region of the rat gene encoding the acyl-CoA-binding protein. Gene **173:** 233–238
59 Helledie T., Grontved L., Jensen S. S., Kiilerich P., Rietveld L., Albrektsen T. et al. (2002) The gene encoding the acyl-CoA-binding protein is activated by peroxisome proliferator-activated receptor gamma through an intronic response element functionally conserved between humans and rodents. J. Biol. Chem. **277:** 26821–26830
60 Brandt J. M., Djouadi F. and Kelly D. P. (1998) Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. J. Biol. Chem. **273:** 23786–23792
61 Mascaro C., Acosta E., Ortiz J. A., Marrero P. F., Hegardt F. G. and Haro D. (1998) Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J. Biol. Chem. **273:** 8560–8563
62 Yu G. S., Lu Y. C. and Gulick T. (1998) Co-regulation of tissue-specific alternative human carnitine palmitoyltransferase Iβ gene promoters by fatty acid enzyme substrate. J. Biol. Chem. **273:** 32901–32909
63 Aoyama T., Peters J. M., Iritani N., Nakajima T., Furihata K., Hashimoto 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
64 Hashimoto T., Fujita T., Usuda N., Cook W., Qi C., Peters J. M. et al. (1999) Peroxisomal and mitochondrial fatty acid beta-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. J. Biol. Chem. **274:** 19228–19236
65 Louet J. F., Chatelain F., Decaux J. F., Park E. A., Kohl C., Pineau T. et al. (2001) Long-chain fatty acids regulate liver carnitine palmitoyltransferase I gene (L-CPT I) expression through a peroxisome-proliferator-activated receptor alpha (PPARalpha)-independent pathway. Biochem. J. **354:** 189–197
66 Young M. E., Goodwin G. W., Ying J., Guthrie P., Wilson C. R., Laws F. A. et al. (2001) Regulation of cardiac and skeletal muscle malonyl-CoA decarboxylase by fatty acids. Am. J. Physiol. Endocrinol. Metab. **280:** 471–479
67 Campbell F. M., Kozak R., Wagner A., Altarejos J. Y., Dyck J. R., Belke D. D. et al. (2002) A role for peroxisome proliferator-activated receptor alpha (PPARalpha) in the control of cardiac malonyl-CoA levels: reduced fatty acid oxidation rates and increased glucose oxidation rates in the hearts of mice lacking PPARalpha are associated with higher concentrations of malonyl-CoA and reduced expression of malonyl-CoA decarboxylase. J. Biol. Chem. **277:** 4098–4103
68 Barrero M. J., Camarero N., Marrero P. F. and Haro D. (2003) Control of human carnitine palmitoyltransferase II gene transcription by peroxisome proliferator-activated receptor through a partially conserved PPRE. Biochem. J. **369:** 721–729
69 Gulick T., Cresci S., Caira T., Moore D. D. and Kelly D. P. (1994) The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. Proc. Natl. Acad. Sci. USA **91:** 11012–11016
70 Fourcade S,. Savary S., Albet S., Gauthe D., Gondcaille C., Pineau T. et al. (2001) Fibrate induction of the adrenoleukodystrophy-related gene (ABCD2): promoter analysis and role of the peroxisome proliferator-activated receptor PPARalpha. Eur. J. Biochem. **268:** 3490–3500
71 Borst P. and Elferink R. O. (2002) Mammalian ABC transporters in health and disease. Annu. Rev. Biochem. **71:** 537–592
72 Tugwood J. D., Issemann I., Anderson R. G., Bundell K. R., McPheat W. L. and Green S. (1992) The mouse peroxisome proliferator activated receptor recognizes a response element in the 5′ flanking sequence of the rat acyl CoA oxidase gene. EMBO J. **11:** 433–439
73 Zhang B., Marcus S. L., Sajjadi F. G., Alvares K., Reddy J. K., Subramani S. et al. (1992) Identification of a peroxisome proliferator-responsive element upstream of the gene encoding rat peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase. Proc. Natl. Acad. Sci. USA **89:** 7541–7545
74 Bardot O., Aldridge T. C., Latruffe N. and Green S. (1993) PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. Biochem. Biophys. Res. Commun. **192:** 37–45
75 Marcus S. L., Miyata K. S., Zhang B., Subramani S., Rachubinski R. A. and Capone J. P. (1993) Diverse peroxisome proliferator-activated receptors bind to the peroxisome proliferator-responsive elements of the rat hydratase/dehydrogenase and fatty acyl-CoA oxidase genes but differentially induce expression. Proc. Natl. Acad. Sci. USA **90:** 5723–5727
76 Chu R., Lin Y., Rao M. S. and Reddy J. K. (1995) Cooperative formation of higher order peroxisome proliferator-activated receptor and retinoid X receptor complexes on the peroxisome proliferator responsive element of the rat hydratase-dehydrogenase gene. J. Biol. Chem. **270:** 29636–29639
77 Hashimoto T., Cook W. S., Qi C., Yeldandi A. V., Reddy J. K. and Rao M. S. (2000) Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. J. Biol. Chem. **275:** 28918–28928
78 Bardot O., Clemencet M. C., Passilly P. and Latruffe N. (1995) The analysis of modified peroxisome proliferator responsive elements of the peroxisomal bifunctional enzyme in transfected HepG2 cells reveals two regulatory motifs. FEBS Lett. **360:** 183–186
79 Nicolas-Frances V., Dasari V. K., Abruzzi E., Osumi T. and Latruffe N. (2000) The peroxisome proliferator response element (PPRE) present at positions –681/–669 in the rat liver 3-ketoacyl-CoA thiolase B gene functionally interacts differently with PPARalpha and HNF-4. Biochem. Biophys. Res. Commun. **269:** 347–351
80 Hunt M. C., Lindquist P. J., Peters J. M., Gonzalez F. J., Diczfalusy U. and Alexson S. E. (2000) Involvement of the peroxisome proliferator-activated receptor alpha in regulating long-chain acyl-CoA thioesterases. J. Lipid. Res. **41:** 814–823
81 Hunt M. C., Solaas K., Kase B. F. and Alexson S. E. (2002) Characterization of an acyl-coA thioesterase that functions as a major regulator of peroxisomal lipid metabolism. J. Biol. Chem. **277:** 1128–1138
82 Lopez D., Irby R. B. and McLean M. P. (2003) Peroxisome proliferator-activated receptor α induces rat sterol carrier protein X promoter activity through two peroxisome proliferator-response elements. Mol. Cell. Endocrinol. **205:** 169–184
83 Girnun G. D., Domann F. E., Moore S. A. and Robbins M. E. (2002) Identification of a functional peroxisome proliferator-

activated receptor response element in the rat catalase promoter. Mol. Endocrinol. **16:** 2793–2801
84 MacDonald N., Barrow K., Tonge R., Davison M., Roberts R. A. and Chevalier S. (2000) PPAR$\alpha$-dependent alteration of GRP94 expression in mouse hepatocytes. Biochem. Biophys. Research. Commun. **277:** 699–704
85 Meyer K., Völkl A., Endele R., Kühnle H. F. and Pill J. (1999) Species differences in induction of hepatic enzymes by BM 17.0744, an activator of peroxisome proliferator-activated receptor alpha (PPAR$\alpha$). Arch. Toxicol. **73:** 440–450
86 White I. R., Man W. J., Bryant D., Bugelski P., Camilleri P., Cutler P. et al. (2003) Protein expression changes in the Sprague Dawley rat liver proteome following administration of peroxisome proliferator activated receptor alpha and gamma ligands. Proteomics **3:** 505–512
87 Yoo H. Y., Chang M. S. and Rho H. M. (1999) Induction of the rat Cu/Zn superoxide dismutase gene through the peroxisome proliferator-responsive element by arachidonic acid. Gene **234:** 87–91
88 Johnson E. F., Palmer C. N., Griffin K. J. and Hsu M. H. (1996) Role of the peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. FASEB J. **10:** 1241–1248
89 Aldridge T. C., Tugwood J. D., and Green S. (1995) Identification and characterization of DNA elements implicated in the regulation of CYP4A1 transcription. Biochem. J. **306:** 473–479
90 Kroetz D. L., Yook P., Costet P., Bianchi P. and Pineau T. (1998) Peroxisome proliferator-activated receptor alpha controls the hepatic CYP4A induction adaptive response to starvation and diabetes. J. Biol. Chem. **273:** 31581–31589
91 Muerhoff A. S., Griffin K. J. and Johnson E. F. (1992) The peroxisome proliferator activated receptor mediates the induction of CYP4A6, a cytochrome P450 fatty acid omega-hydroxylase, by clofibric acid. J. Biol.Chem. **267:** 19051–19053
92 Palmer C. N., Hsu M. H., Muerhoff A. S., Griffin K. J. and Johnson E. F. (1994) Interaction of the peroxisome proliferator-activated receptor a with the retinoid X receptor a unmasks a cryptic peroxisome proliferator response element that overlaps an ARP-1 binding site in the CYP4A6 promoter. J. Biol. Chem. **269:** 18083–18089
93 Rodriguez J. C., Gil-Gomez G,. Hegardt F. G. and Haro D. (1994) Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J. Biol. Chem. **269:** 18767–18772
94 Hegardt F. G. (1998) Transcriptional regulation of mitochondrial HMG-CoA synthase in the control of ketogenesis. Biochimie **80:** 803–806
95 Le May C., Pineau T., Bigot K., Kohl C., Girard J. and Pegorier J. P. (2000) Reduced hepatic fatty acid oxidation in fasting PPARalpha null mice is due to impaired mitochondrial hydroxymethylglutaryl-CoA synthase gene expression. FEBS Lett. **475:** 163–166
96 Ortiz J. A., Mallolas J., Nicot C., Bofarull J., Rodriguez J. C., Hegardt F. G. et al. (1999) Isolation of pig mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene promoter: characterization of a peroxisome proliferator-responsive element. Biochem J. **337:** 329–335
97 Meertens L. M., Miyata K. S., Cechetto J. D., Rachubinski R. A. and Capone J. P. (1998) A mitochondrial ketogenic enzyme regulates its gene expression by association with the nuclear hormone receptor PPARalpha. EMBO J. **17:** 6972–6978
98 Castelein H., Gulick T., Declercq P. E., Mannaerts G. P., Moore D. D. and Baes M. I. (1994) The peroxisome proliferator activated receptor regulates malic enzyme gene expression. J. Biol. Chem. **269:** 26754–26758
99 Miller C. W. and Ntambi J. M. (1996) Peroxisome proliferators induce mouse liver stearoyl-CoA desaturase 1 gene expression. Proc. Natl. Acad. Sci. USA **93:** 9443–9448
100 Guillou H., Martin P., Jan S., D'Andrea S., Roulet A., Catheline D. et al. (2002) Comparative effect of fenofibrate on hepatic desaturases in wild-type and peroxisome proliferator-activated receptor $\alpha$-deficient mice. Lipids **37:** 981–989
101 Tang C., Cho H. P., Nakamura M. T. and Clarke S. D. (2003) Regulation of human delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. J. Lipid. Res. **44:** 686–695
102 Matsuzaka T., Shimano H., Yahagi N., Amemiya-Kudo M., Yoshikawa T., Hasty A. H. et al. (2002) Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPARalpha. J. Lipid Res. **43:** 107–114
103 Tabor D. E., Kim J. B., Spiegelman B. M. and Edwards P. A. (1999) Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. J. Biol. Chem. **274:** 20603–20610
104 Vu-Dac N., Schoonjans K., Laine B., Fruchart J. C., Auwerx J. and Staels B. (1994) Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. J. Biol. Chem. **269:** 31012–31018
105 Vu-Dac N., Chopin-Delannoy S., Gervois P., Bonnelye E., Martin G., Fruchart J. C. et al. (1998) The nuclear receptors peroxisome proliferator-activated receptor alpha and Rev-erbalpha mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. J. Biol. Chem. **273:** 25713–25720
106 Berthou L., Duverger N., Emmanuel F., Langouet S., Auwerx J., Guillouzo A. et al. (1996) Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. J. Clin. Invest. **97:** 2408–2416
107 Peters J. M., Hennuyer N., Staels B., Fruchart J. C., Fievet C., Gonzalez F. J. et al. (1997) Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. J. Biol. Chem. **272:** 27307–27312
108 Berthou L., Saladin R., Yaqoob P., Branellec D., Calder P., Fruchart J.C. et al. (1995) Regulation of rat liver apolipoprotein A-I, apolipoprotein A-II and acyl-coenzyme A oxidase gene expression by fibrates and dietary fatty acids. Eur. J. Biochem. **232:** 179–187
109 Raspe E., Madsen L., Lefebvre A. M., Leitersdorf I., Gelman L., Peinado-Onsurbe J. et al. (1999) Modulation of rat liver apolipoprotein gene expression and serum lipid levels by tetradecylthioacetic acid (TTA) via PPARalpha activation. J. Lipid. Res. **40:** 2099–2110
110 Gervois P., Chopin-Delannoy S., Fadel A., Dubois G., Kosykh V., Fruchart J. C. et al. (1999) Fibrates increase human REV-ERBalpha expression in liver via a novel peroxisome proliferator-activated receptor response element. Mol. Endocrinol. **13:** 400–409
111 Vu-Dac N., Schoonjans K., Kosykh V., Dallongeville J., Fruchart J. C., Staels B. et al. (1995) Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. J. Clin. Invest. **96:** 741–750
112 Bouly M., Masson D., Gross B., Jiang X. C., Fievet C., Castro G. et al. (2001) Induction of the phospholipid transfer protein gene accounts for the high density lipoprotein enlargement in mice treated with fenofibrate. J. Biol. Chem. **276:** 25841–25847
113 Tu A. Y. and Albers J. J. (2001) Functional analysis of the transcriptional activity of the mouse phospholipid transfer protein gene. Biochem. Biophys. Res. Commun. **287:** 921–926
114 Tu A. Y. and Albers J. J. (2001) Glucose regulates the transcription of human genes relevant to HDL metabolism: responsive elements for peroxisome proliferator-activated re-

ceptor are involved in the regulation of phospholipid transfer protein. Diabetes **50:** 1851–1856
115 Mardones P., Pilon A., Bouly M., Duran D., Nishimoto T. and Arai H. et al. (2003) Fibrates downregulate hepatic scavenger receptor class B type I protein expression in mice. J. Biol. Chem. **278:** 7884–7890
116 Staels B., Vu-Dac N., Kosykh V. A., Saladin R., Fruchart J. C., Dallongeville J. et al. (1995) Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. J. Clin. Invest. **95:** 705–712
117 Haubenwallner S., Essenburg A. D., Barnett B. C., Pape M. E., DeMattos R. B., Krause B. R. et al. (1995) Hypolipidemic activity of select fibrates correlates to changes in hepatic apolipoprotein C-III expression: a potential physiologic basis for their mode of action. J. Lipid Res. **36:** 2541–2551
118 Hertz R., Bishara-Shieban J. and Bar-Tana J. (1995) Mode of action of peroxisome proliferators as hypolipidemic drugs. Suppression of apolipoprotein C-III. J. Biol. Chem. **270:** 13470–13475
119 Schoonjans K., Peinado-Onsurbe J., Lefebvre A. M., Heyman R. A., Briggs M., Deeb S. et al. (1996) PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J. **15:** 5336–5348
120 Carroll R. and Severson D. L. (2001) Peroxisome proliferator-activated receptor-alpha ligands inhibit cardiac lipoprotein lipase activity. Am. J. Physiol. Heart Circ. Physiol. **281:** 888–894
121 Vu-Dac N., Gervois P., Jakel H., Nowak M., Baugé E., Dehondt H. et al. (2003) Apolipoprotein A5, a crucial determinant of plasma triglyceride levels, is highly responsive to PPAR alpha activators. J. Biol. Chem. **278:** 17982–17985
122 Prieur X., Coste H. and Rodriguez J. C. (2003) The human apolipoprotein AV gene is regulated by PPAR alpha and contains a novel FXR response element. J. Biol. Chem. **278:** 25468–25480
123 Puckey L. H. and Knight B. L. (2002) Interaction of oestrogen and peroxisome proliferator-activated receptors with apolipoprotein(a) gene enhancers. Biochem. J. **366:** 157–163
124 Barbera M. J., Schluter A., Pedraza N., Iglesias R., Villarroya F. and Giralt M. (2001) Peroxisome proliferator-activated receptor alpha activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. J. Biol. Chem. **276:** 1486–1493
125 Brun S., Carmona M. C., Mampel T., Vinas O., Giralt M., Iglesias R. et al. (1999) Activators of peroxisome proliferator-activated receptor-alpha induce the expression of the uncoupling protein-3 gene in skeletal muscle: a potential mechanism for the lipid intake-dependent activation of uncoupling protein-3 gene expression at birth. Diabetes **48:** 1217–1222
126 Young M. E., Patil S., Ying J., Depre C., Ahuja H. S., Shipley G. L. et al. (2001) Uncoupling protein 3 transcription is regulated by peroxisome proliferator-activated receptor (alpha) in the adult rodent heart. FASEB J. **15:** 833–845
127 Armstrong M. B. and Towle H. C. (2001) Polyunsaturated fatty acids stimulate hepatic UCP-2 expression via a PPARalpha-mediated pathway. Am. J. Physiol. Endocrinol. Metab. **281:** 1197–2004
128 Tsuboyama-Kasaoka N., Takahashi M., Kim H. and Ezaki O. (1999) Up-regulation of liver uncoupling protein-2 mRNA by either fish oil feeding or fibrate administration in mice. Biochem. Biophys. Res. Commun. **257:** 879–885
129 Nakatani T., Tsuboyama-Kasaoka N., Takahashi M., Miura S. and Ezaki O. (2002) Mechanism for peroxisome proliferator-activated receptor-alpha activator-induced up-regulation of UCP2 mRNA in rodent hepatocytes. J. Biol. Chem. **277:** 9562–9569
130 Tordjman K. Standley K. N., Bernal-Mizrachi C., Leone T. C., Coleman T., Kelly D. P et al. (2002) PPARalpha suppresses insulin secretion and induces UCP2 in insulinoma cells. J. Lipid. Res. **43:** 936–943
131 Chiang J. Y. (2002) Bile acid regulation of gene expression: roles of nuclear hormone receptors. Endocr. Rev. **23:** 443–463
132 Marrapodi M. and Chiang J. Y. (2000) Peroxisome proliferator-activated receptor alpha (PPARalpha) and agonist inhibit cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription. J. Lipid. Res. **41:** 514–520
133 Patel D. D., Knight B. L., Soutar A. K., Gibbons G. F. and Wade D. P. (2000) The effect of peroxisome-proliferator-activated receptor-alpha on the activity of the cholesterol 7 alpha-hydroxylase gene. Biochem. J. **351:** 747–753
134 Post S. M., Duez H., Gervois P. P., Staels B., Kuipers F. and Princen H. M. (2001) Fibrates suppress bile acid synthesis via peroxisome proliferator-activated receptor-alpha-mediated downregulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase expression. Arterioscler. Thromb. Vasc. Biol. **21:** 1840–1845
135 Cheema S. K. and Agellon L. B. (2000) The murine and human cholesterol 7alpha-hydroxylase gene promoters are differentially responsive to regulation by fatty acids mediated via peroxisome proliferator-activated receptor alpha. J. Biol. Chem. **275:** 12530–12536
136 Hunt M. C., Yang Y. Z., Eggertsen G., Carneheim C. M., Gafvels M., Einarsson C. et al. (2000) The peroxisome proliferator-activated receptor alpha (PPARalpha) regulates bile acid biosynthesis. J. Biol. Chem. **275:** 28947–28953
137 Tobin K. A., Steineger H. H., Alberti S., Spydevold O., Auwerx J., Gustafsson J. A. et al. (2000) Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-alpha. Mol. Endocrinol. **14:** 741–752
138 Kok T., Bloks V. W., Wolters H., Havinga R., Jansen P. L., Staels B. et al. (2003) Peroxisome proliferator-activated receptor alpha (PPARalpha)-mediated regulation of Mdr2 expression and function in mice. Biochem. J. **369:** 539–547
139 Kok T., Wolters H., Bloks V. W., Havinga R., Jansen P. L., Staels B. et al. (2003) Induction of hepatic ABC transporter expression is part of the PPARalpha-mediated fasting response in the mouse. Gastroenterology **124:** 160–171
140 Jung D., Fried M. and Kullak-Ublick G. A. (2002) Human apical sodium-dependent bile salt transporter gene (SLC10A2) is regulated by the peroxisome proliferator-activated receptor alpha. J. Biol. Chem. **277:** 30559–30566
141 Suruga K., Mochizuki K., Suzuki R., Goda T. and Takase S. (1999) Regulation of cellular retinol-binding protein type II gene expression by arachidonic acid analogue and 9-cis retinoic acid in caco-2 cells. Eur. J. Biochem. **262:** 70–78
142 Mochizuki K., Suruga K., Kitagawa M., Takase S. and Goda T. (2001) Modulation of the expression of peroxisome proliferator-activated receptor-dependent genes through disproportional expression of two subtypes in the small intestine. Arch. Biochem. Biophys. **389:** 41–48
143 Xu J., Xiao G., Trujillo C., Chang V., Blanco L., Joseph S. B. et al. (2002) PPAR alpha influences substrate utilization for hepatic glucose production. J. Biol. Chem. **277:** 50237–50244
144 Muoio D. M., MacLean P. S., Lang D. B., Li S., Houmard J. A., Way J. M. et al. (2002) Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. J. Biol. Chem. **277:** 26089–26097
145 Sugden M. C., Bulmer K., Gibbons G. F., Knight B. L. and Holness M. J. (2002) Peroxisome-proliferator-activated recep-

tor-alpha (PPARalpha) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin. Biochem. J. **364:** 361–368
146 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
147 Sugden M. C., Bulmer K., Gibbons G. F. and Holness M. J. (2001) Role of peroxisome proliferator-activated receptor-alpha in the mechanism underlying changes in renal pyruvate dehydrogenase kinase isoform 4 protein expression in starvation and after refeeding. Arch. Biochem. Biophys. **395:** 246–252
148 Wu P., Peters J. M. and Harris R. A. (2001) Adaptive increase in pyruvate dehydrogenase kinase 4 during starvation is mediated by peroxisome proliferator-activated receptor alpha. Biochem. Biophys. Res. Commun. **287:** 391–396
149 Wu P., Inskeep K., Bowker-Kinley M. M., Popov K. M. and Harris R. A. (1999) Mechanism responsible for inactivation of skeletal muscle pyruvate dehydrogenase complex in starvation and diabetes. Diabetes **48:** 1593–1599
150 Holness M. J., Bulmer K., Gibbons G. F. and Sugden M. C. (2002) Up-regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) protein expression in oxidative skeletal muscle does not require the obligatory participation of peroxisome-proliferator-activated receptor alpha (PPARalpha). Biochem. J. **366:** 839–846
151 Holness M. J., Smith N. D., Bulmer K., Hopkins T., Gibbons G. F. and Sugden M. C. (2002) Evaluation of the role of peroxisome-proliferator-activated receptor alpha in the regulation of cardiac pyruvate dehydrogenase kinase 4 protein expression in response to starvation, high-fat feeding and hyperthyroidism. Biochem. J. **364:** 687–694
152 Edgar A. D., Tomkiewicz C., Costet P., Legendre C., Aggerbeck M., Bouguet J. et al. (1998) Fenofibrate modifies transaminase gene expression via a peroxisome proliferator activated receptor alpha-dependent pathway. Toxicol. Lett. **98:** 13–23
153 Kersten S., Mandard S., Escher P., Gonzalez F. J., Tafuri S., Desvergne B. et al. (2001) The peroxisome proliferator-activated receptor alpha regulates amino acid metabolism. FASEB J. **15:** 1971–1978
154 Inoue Y., Hayhurst G. P., Inoue J., Mori M. and Gonzalez F. J. (2002) Defective ureagenesis in mice carrying a liver-specific disruption of hepatocyte nuclear factor 4alpha (HNF4alpha ). HNF4alpha regulates ornithine transcarbamylase in vivo. J. Biol. Chem. **277:** 25257–25265
155 Hermanowski-Vosatka A., Gerhold D., Mundt S. S., Loving V. A., Lu M. and Chen Y. (2000) PPARalpha agonists reduce 11beta-hydroxysteroid dehydrogenase type 1 in the liver. Biochem. Biophys. Res. Commun. **279:** 330–336
156 Corton J. C., Bocos C., Moreno E. S., Merritt A., Cattley R. C. and Gustafsson J. A. (1997) Peroxisome proliferators alter the expression of estrogen-metabolizing enzymes. Biochimie **79:** 151–162
157 Corton J. C., Fan L. Q., Brown S., Anderson S. P., Bocos C., Cattley R. C. et al. (1998) Down-regulation of cytochrome P4502C family members and positive acute-phase response gene expression by peroxisome proliferator chemicals. Mol. Pharmacol. **54:** 463–473
158 Fan L. Q., Cattley R. C.and Corton J. C. (1998) Tissue-specific induction of 17 beta-hydroxysteroid dehydrogenase type IV by peroxisome proliferator chemicals is dependent on the peroxisome proliferator-activated receptor alpha. J. Endocrinol. **158:** 237–246
159 Barclay T. B., Peters J. M., Sewer M. B., Ferrari L., Gonzalez F. J. and Morgan E. T. (1999) Modulation of cytochrome P-450 gene expression in endotoxemic mice is tissue specific and peroxisome proliferator-activated receptor-alpha dependent. J. Pharmacol. Exp. Ther. **290:** 1250–1257
160 Xu S., Zhu B. T., Turan V., Rusyn I., Thurman R., Peters J. M. et al. (2001) PPARalpha-dependent induction of liver microsomal esterification of estradiol and testosterone by a prototypical peroxisome proliferator. Endocrinology **142:** 3554–3557
161 Kojo A., Pellinen P., Juvonen R., Raunio H., Pelkonen O. and Pasanen M. (1996) Distinct responses of mouse hepatic CYP enzymes to corn oil and peroxisome proliferators. Biochem. Pharmacol. **51:** 1137–1143
162 Voskoboinik I., Drew R. and Ahokas J. T. (1996) Differential effect of peroxisome proliferators on rat glutathione S-transferase isoenzymes. Toxicol. Lett. **87:** 147–155
163 James S. I. and Ahokas J. T. (1992) Effect of peroxisome proliferators on glutathione-dependent sulphobromophthalein excretion. Xenobiotica **22:** 1425–1432
164 Voskoboinik I., Ooi S. G., Drew R. and Ahokas J. T. (1997) Peroxisome proliferators increase the formation of BPDE-DNA adducts in isolated rat hepatocytes. Toxicology **122:** 81–91
165 Foliot A. and Beaune P. (1994) Effects of microsomal enzyme inducers on glutathione S-transferase isoenzymes in livers of rats and hamsters. Biochem. Pharmacol. **48:** 293–300
166 Magdalou J., Fournel-Gigleux S., Pritchard M. and Siest G. (1993) Peroxisome proliferators as inducers and substrates of UDP-glucuronosyltransferases. Biol. Cell. **77:** 13–16
167 Arand M., Coughtrie M. W., Burchell B., Oesch F. and Robertson L. W. (1991) Selective induction of bilirubin UDP-glucuronosyl-transferase by perfluorodecanoic acid. Chem. Biol. Interact. **77:** 97–105
168 Barbier O., Villeneuve L., Bocher V., Fontaine C., Pineda-Torra I., Duhem C. et al. (2003) The UDP-glucuronosyl-transferase 1A9 enzyme is a peroxisome proliferator-activated receptor alpha and gamma target gene. J. Biol. Chem. **278:** 13975–13983
169 Fukui Y. and Motojima K. (2002) Expression of resistin in the adipose tissue is modulated by various factors including peroxisome proliferator-activated receptor alpha. Diabetes Obes. Metab. **4:** 342–345
170 Kersten S., Mandard S., Tan N. S., Escher P., Metzger D., Chambon P. et al. (2000) Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. J. Biol. Chem. **275:** 28488–28493
171 Gervois P., Vu-Dac N., Kleemann R., Kockx M., Dubois G., Laine B. et al. (2001) Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor alpha agonists via inhibition of CCAAT box/enhancer-binding protein beta. J. Biol. Chem. **276:** 33471–33477
172 Kintscher U., Lyon C., Wakino S., Bruemmer D., Feng X., Goetze S. et al. (2002) PPARalpha inhibits TGF-beta-induced beta5 integrin transcription in vascular smooth muscle cells by interacting with Smad4. Cir. Res. **91:** 35–44
173 Delerive P., De Bosscher K., Besnard S., Vanden Berghe W., Peters J. M., Gonzalez F. J. et al. (1999) Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. J. Biol. Chem. **274:** 32048–32054
174 Delerive P., Gervois P., Fruchart J. C. and Staels B. (2000) Induction of IkappaBalpha expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-alpha activators. J. Biol. Chem. **275:** 36703–36707
175 Delerive P., De Bosscher K., Vanden Berghe W., Fruchart J. C., Haegeman G. and Staels B. (2002) DNA binding-independent induction of IkappaBalpha gene transcription by PPARalpha. Mol. Endocrinol. **16:** 1029–1039
176 Meade E. A., McIntyre T. M., Zimmerman G. A. and Prescott S. M. (1999) Peroxisome proliferators enhance cyclooxyge-

nase-2 expression in epithelial cells. J. Biol. Chem. **274:** 8328–8334
177 Su A. I., Guidotti L. G. , Pezacki J. P., Chisari F. V. and Schultz P. G. ( 2002) Gene expression during the priming phase of liver regeneration after partial hepatectomy in mice. Proc. Natl. Acad. Sci. USA **99:** 11191–11185
178 Beigneux A. P., Moser A. H., Shigenaga J. K., Grunfeld C. and Feingold K. R. (2000) The acute phase response is associated with retinoid X receptor repression in rodent liver. J. Biol. Chem. **275:** 16390–16399
179 Hertz R., Seckbach M., Zakin M. M. and Bar-Tana J. (1996) Transcriptional suppression of the transferrin gene by hypolipidemic peroxisome proliferators. J. Biol. Chem. **271:** 218–224
180 Kockx M., Gervois P., Poulain P., Derudas B., Peters J. M., Gonzalez F. J. et al. (1999) Fibrates suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferator-activated receptor-alpha. Blood **93:** 2991–2998
181 Müller M. and Kersten S. (2003) Nutrigenomics: goals and strategies. Nat. Rev. Genet. **4:** 315–322
182 Daynes R. A and Dallas C. J. (2002) Emerging roles of PPARs in inflammation and immunity. Nat. Rev. Immunol. **2:** 748–759
183 Pineda-Torra I., Jamshidi Y., Flavell D. M., Fruchart J. C. and Staels B. (2002) Characterization of the human PPARalpha promoter: identification of a functional nuclear receptor response element. Mol. Endocrinol. **16:** 1013–1028
184 Gearing K. L., Crickmore A. and Gustafsson J. A. (1994) Structure of the mouse peroxisome proliferator-activated receptor $\alpha$ gene. Biochem. Biophys. Res. Com. **199:** 255–263
185 Yu S., Matsusue K., Kashireddy P., Cao W. Q., Yeldandi V., Yeldandi A. V. et al. (2003) Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to PPAR$\gamma$1 overexpression. J. Biol. Chem. **278:** 498–505
186 Gilde A. J., van der Lee K. A., Willemsen P. H., Chinetti G., van der Leij F. R., van der Vusse G. J. et al. (2003) Peroxisome proliferator-activated receptor (PPAR) alpha and PPARbeta/delta, but not PPARgamma, modulate the expression of genes involved in cardiac lipid metabolism. Circ. Res. **92:** 518–524
187 Wang Y. X., Lee C. H., Tiep S., Yu R. T., Ham J., Kang H. et al. (2003) Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell **113:** 159–170
188 Way J. M., Harrington W. W., Brown K. K., Gottschalk W. K., Sundseth S. S., Mansfield T. A. et al. (2001) Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. Endocrinology **142:** 1269–1277
189 Watkins S. M., Reifsnyder P. R., Pan H. J., German J. B. and Leiter E. H. (2002) Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. J. Lipid. Res. **43:** 1809–1817
190 Yu S., Cao W. Q., Kashireddy P., Meyer K., Jia Y., Hughes D. E. et al. (2001) Human peroxisome proliferator-activated receptor alpha (PPARalpha) supports the induction of peroxisome proliferation in PPARalpha-deficient mouse liver. J. Biol. Chem. **276:** 42485–42491
191 Yamazaki K., Kuromitsu J. and Tanaka I. (2002) Microarray analysis of gene expression changes in mouse liver induced by peroxisome proliferator- activated receptor alpha agonists. Biochem. Biophys. Res. Commun. **290:** 1114–1122
192 Hasmall S., Orphanides G., James N., Pennie W., Hedley K., Soames A. et al. (2002) Downregulation of lactoferrin by PPARalpha ligands: role in perturbation of hepatocyte proliferation and apoptosis. Toxicol. Sci. **68:** 304–313
193 Peters J. M., Aoyama T., Burns A. M. and Gonzalez F. J. (2003) Bezafibrate is a dual ligand for PPARalpha and PPARbeta: studies using null mice. Biochim. Biophys. Acta **1632:** 80–89
194 Watanabe K., Fujii H., Takahashi T., Kodama M., Aizawa Y., Ohta Y. et al. (2000) Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor alpha associated with age-dependent cardiac toxicity. J. Biol. Chem. **275:** 22293–22299