Unsaturated fatty acid regulation of cytochrome P450 expression via a CAR-dependent pathway

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The liver is responsible for key metabolic functions, including control of normal homoeostasis in response to diet and xenobiotic metabolism/detoxification. We have shown previously that inactivation of the hepatic cytochrome P450 system through conditional deletion of POR (P450 oxidoreductase) induces hepatic steatosis, liver growth and P450 expression. We have exploited a new conditional model of POR deletion to investigate the mechanism underlying these changes. We demonstrate that P450 induction, liver growth and hepatic triacylglycerol (triglyceride) homoeostasis are intimately linked and provide evidence that the observed phenotypes result from hepatic accumulation of unsaturated fatty acids, which mediate these phenotypes by activation of the nuclear receptor CAR (constitutive androstane receptor) and, to a lesser degree, PXR (pregnan X receptor). To our knowledge this is the first direct evidence that P450s play a major role in controlling unsaturated fatty acid homoeostasis via CAR. The regulation of P450s involved in xenobiotic metabolism by this mechanism has potentially significant implications for individual responses to drugs and environmental chemicals.

Key words: constitutive androstane receptor (CAR), cytochrome P450, linoleic acid, P450 oxidoreductase (POR), pregnane X receptor (PXR), steatosis.

INTRODUCTION

Cytochrome P450 enzymes (P450s) play a major role in the metabolism and disposition of chemicals and their activities are critical in determining the efficacy or side effects of drugs. These enzymes also have additional functions in adrenal steroid hormone biosynthesis and the hepatic synthesis of cholesterol and its degradation to bile acids [1]. The P450s involved in these different pathways are distinct and their regulation has conventionally been considered to be by different mechanisms. This is manifest in that many of the hepatic enzymes involved in foreign compound metabolism are expressed constitutively at low levels, but become highly induced on exogenous chemical exposure, resulting in an increased rate of excretion of the compound(s). Significant advances have been made in our understanding of this adaptive response through the identification of transcription factors, such as AhR (aryl hydrocarbon receptor), PXR (pregnan X receptor) and CAR (constitutive androstane receptor), which have the capacity to bind drugs and exogenous chemicals, resulting in transcriptional activation of genes that mediate their detoxification [2–5]. Although distinct classes of compounds bind to each receptor, owing to the diversity of these compounds’ chemical structures, promiscuity exists in their specificity, particularly in the case of PXR and CAR [6,7].

We, and the group of Ding, have reported a mouse model where hepatic P450 activity has been reduced by > 95% by the conditional deletion of the Por (P450 oxidoreductase) gene [8,9]. This was achieved by crossing mice carrying the Por gene flanked by loxP sites with mice expressing Cre recombinase under the control of the rat albumin promoter. This resulted in the specific hepatic deletion of POR neonatally with a concomitant reduction in P450 activity [8,9]. Although HRN (hepatic reductase-null) mice developed normally and were fertile, there were a number of intriguing phenotypic changes, including an enlarged liver and hepatic steatosis. Also, there was a profound increase in the expression of a range of P450s, with the most marked changes being in the expression of CYP2B10 (CYP is cytochrome P450) and CYP3A11 [10]. These enzymes, which are expressed at low levels in untreated animals, are highly inducible by exogenous chemicals such as barbiturates and synthetic glucocorticoids [11,12]. These data show that the P450 system is a key regulator of hepatic lipid homoeostasis and liver growth and that this metabolic homoeostasis can have a profound effect on the control of P450 gene expression and, as a consequence, on the metabolism/deposition of drugs. The HRN mouse therefore provides a model for investigating the endogenous factors involved in regulating P450 genes and the role of the P450 system in controlling hepatic steatosis and liver growth.

Studies of the temporal events that lead to the hepatic phenotype are, however, not possible in the HRN mice because the deletion of the Por gene occurs neonatally. To overcome this, we have created a model where the HRN genotype is regulated conditionally and in an inducible manner. This was achieved by crossing Por-floxed mice with mice where Cre recombinase is conditionally regulated through the AhR, and administration of a single dose of 3MC (3-methylcholanthrene) at 40 mg/kg results in a specific, time-dependent, hepatic deletion of POR and a phenotype identical with that observed in HRN mice [13].

In the present study, we have used the new model to study the temporal biochemical changes that control lipid accumulation and P450 overexpression. We provide evidence that dietary unsaturated fatty acids play a key role in both lipid accumulation and P450 induction. Also, using nuclear receptor/HRN

Abbreviations used: AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; Cpt1a, carnitine palmitoyltransferase 1a; CYP, cytochrome P450; FAS, fatty acid synthase; HRN, hepatic reductase-null; i.p., intraperitoneal; 3MC, 3-methylcholanthrene; P450, cytochrome P450; PB, phenobarbitral; POR, P450 oxidoreductase; PPAR, peroxisome-proliferator-activated receptor; PUFA, polyunsaturated fatty acid; PXR, pregnane X receptor; TCPOBOP, 1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene.

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double- and triple-knockout mice, we show that P450 overexpression occurs predominantly through the activation of CAR.

**EXPERIMENTAL**

**Reagents**

All chemicals were purchased from Sigma–Aldrich, except where indicated.

**Production of transgenic mice**

Porlox<sup>lox</sup>/?Cre<sup>AB</sup> (HRN) and Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> mice on a C57/BL6 genetic background were generated as described previously [9,13]. Littermates with the Porlox<sup>lox</sup> genotype were used as controls. PXR-null (Pxr<sup>−/−</sup>) and CAR-null (Car<sup>−/−</sup>) mice on a C57/BL6 genetic background were crossed on to the HRN line to generate Pxr<sup>−/−</sup>/Porlox<sup>lox</sup>/?Cre<sup>AB</sup> (Pxr<sup>−/−</sup>/HRN) and Car<sup>−/−</sup>/Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> (Car<sup>−/−</sup>/HRN) double-knockout mice respectively. Pxr<sup>−/−</sup>/HRN and Car<sup>−/−</sup>/HRN were crossed to generate Car<sup>−/−</sup>/Pxr<sup>−/−</sup>/Porlox<sup>lox</sup>/?Cre<sup>AB</sup> (Car<sup>−/−</sup>/Pxr<sup>−/−</sup>/HRN) triple-knockout mice. Pxr<sup>−/−</sup>/Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> and Car<sup>−/−</sup>/Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> littermates were used as controls. Pxr<sup>−/−</sup> and Car<sup>−/−</sup> mice were identified by PCR as described previously [14,15]. All mice were maintained under standard animal house conditions with a 12 h light/12 h dark cycle and free access to water and RM1 (control) diet (Special Diets Services). All studies were carried out on 12-week-old male mice, except where indicated, in accordance with the Animal Scientific Procedures Act (1986) and after local ethical review.

**Drug treatments**

3MC in corn oil was administered to Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> and Porlox<sup>lox</sup>/?Cre<sup>AB</sup> mice by i.p. (intraperitoneal) injection at 40 mg/kg of body weight, and animals were killed at the indicated times.

**Dietary restrictions**

Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> and Porlox<sup>lox</sup>/?Cre<sup>AB</sup> mice were weaned and maintained on a control diet until 8 weeks of age before being transferred to either a fat-deficient diet (Special Diets Services, 821459) or a fatty-acid-supplemented fat-deficient diet (Supplementary Table S1 at http://www.BiochemJ.org/bj/417/bj4170043add.htm), for an additional 6 weeks. As controls, both Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> and Porlox<sup>lox</sup>/?Cre<sup>AB</sup> mice were weaned and maintained on the control diet for the duration of the experiment. All diets were isocaloric and free from cholesterol. At 12 weeks of age, mice were administered either a single i.p. dose of 3MC (40 mg/kg of body weight) or corn oil, and killed 14 days later.

For linoleic acid treatment, Porlox<sup>lox</sup>/?Cre<sup>CYP3A11</sup> mice were maintained on the fat-deficient diet as described above. At 12 weeks of age, mice were administered a single i.p. dose of 3MC (40 mg/kg of body weight) followed by an oral dose of linoleic acid of 65 mg/day from day 5 after 3MC administration until being killed 9 days later.

**Immunoblotting and biochemical analysis**

Microsomal fractions were prepared by differential centrifugation [12], and protein concentrations were determined using the Bio-Rad Protein Assay Reagent. Western blot analysis was carried out as described previously [16] using 5 μg of microsomal protein per lane and polyclonal antisera raised against human POR [17], murine CYP7A1 [13] or rat P450s [16]. Purified human POR, rat CYP4A1 and the following murine P450s, CYP2B10, His<sub>−</sub>–CYP3A11 and His<sub>−</sub>–CYP7A1, were used as standards. Immunoreactive proteins were detected using horseradish-peroxidase-conjugated donkey anti-(rabbit IgG) as a secondary antibody (Dako) and visualized by ECL® Plus (enhanced chemiluminescence (GE Healthcare)). The relative protein content of individual P450s in microsomal fractions was determined by direct scanning of ECL® membranes with Bio-Rad Quantity One densitometry software. POR activity was determined by NADPH-dependent reduction of cytochrome c as described previously [17].

**RNA isolation and real-time quantitative PCR analysis**

Total RNA was isolated from snap-frozen liver samples using TRIzol® (Invitrogen). Genomic DNA was removed using RQ1 DNase (Promega), and RNA (600 ng) was reverse-transcribed using random hexamers and Superscript II RNase H reverse transcriptase polymerase (Invitrogen). Real-time quantitative PCR analysis was carried out with gene-specific primers for CYP2B10, CYP3A11 and CAR as described previously [10] and CD36 (Mm01135198_m1), FAS (fatty acid synthase) (Mm01253292_m1) and Cpt1a (carnitine palmitoyltransferase 1a) (Mm00550438_m1) were supplied by Applied Biosystems. Reactions were carried out in triplicate and monitored by measuring fluorescence at 518 nm with excitation at 494 nm. mRNA levels were quantified using the Prism 7700 associated software and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as an internal standard.

**Blood chemistry**

Blood was collected by cardiac puncture into heparinized tubes, and plasma was analysed for non-fasting total cholesterol and non-fasting triacylglycerols (triglycerides) using assay kits (Thermo Trace) on a Cobas Fara II centrifugal analyser (Roche).

**Histopathology**

Tissue samples were snap-frozen in Cryo-M-Bed (Bright Instrument Co.), cryo-sectioned (10 μm) and stained with Oil Red O (lipid content) or haematoxylin and eosin (morphological analysis).

**Lipid isolation and analysis**

A small piece of liver was homogenized in PBS containing 0.1 % Triton X-100, and hepatic triacylglycerol and cholesterol levels were determined using assay kits on a Cobas Fara II centrifugal analyser [18]. Detailed fatty acid analysis of the various diets was carried out by Special Diets Services (Supplementary Tables S2 and S3 at http://www.BiochemJ.org/bj/417/bj4170043add.htm).

**Plasmid construction**

The plasmids pCMX Gal4-hPXR-LBD and p4xGal4-UAS-luciferase were a gift from Luisella Vignati (Department of Pharmacokinetics, Dynamics and Metabolism, Gruppo Pfizer Inc., Italy) [19] and the former was used as a source of the pCMX Gal4 vector to facilitate the generation of further reporter constructs. The mouse CAR ligand-binding domain from amino acids 118–358 (mCAR-LBD) was amplified using 5′-GGCAGATTCGGTACTAAAGCATTCTCTTGGCAACTGAA-3′ and 5′-GGCGATCTTCACTGCAAATCTCCCCGAGC-3′ as forward

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and reverse primers respectively, incorporating 5’ EcoRI and 3’ BamHI sites to facilitate cloning. The resulting 738 bp PCR product was cloned as an EcoRI/BamHI fragment into pCMX Gal4-hPXR-LBD replacing the hPXR-LDB section and generating the vector pCMX Gal4-mCAR-LBD.

Cell culture, transient transfection and cell treatments

All cell culture media and supplements were supplied by Invitrogen. HepG2 cells were cultured in DMEM (Dulbecco’s modified Eagle’s medium) containing 10% (v/v) heat-inactivated foetal bovine serum, 1× non-essential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine at 37°C and 5% CO₂.

HepG2 cells were transiently transfected with pCMX Gal4-mCAR-LBD/p4A4xGal4-UAS-luciferase using the method of Vignati et al. [19], with the following modifications: FuGENE™ 6 (Roche) was used instead of Lipofectamine™ Plus (Invitrogen), assays were carried out in serum-containing medium and 0.2 μg of the plasmid pRL-TK was included to allow normalization of luciferase reporter gene activity. Cells were treated with 0.25–0.5 μM TCPOBOP {1,4-bis-[2-(3,5-dichloropyridyloxy)]-benzene} and 1 mM PB (phenobarbital) as positive controls and DMSO as a further control. Linoleic acid, complexed to fatty-acid-free BSA at a molar ratio of 2:1, was tested at various concentrations ranging from 0 to 12.5 μM, with the addition of 20 μM α-tocopheryl succinate to prevent lipid peroxidation. Luciferase reporter activity was determined using the Dual-Luciferase Reporter Assay System (Promega).

RESULTS

Temporal analysis of the phenotypic changes observed in the HRN mice

Porloxlox/Cyp2b10 mice, in the absence of a chemical inducer, were phenotypically normal and indistinguishable from wild-type animals. Treatment with a single dose of 3MC (40 mg/kg) led to a time-dependent complete depletion of the hepatic POR and the development of a phenotype that was identical with that of the original HRN model [13]. We therefore used this model to study the sequential development of the HRN phenotypic traits over a 28 day period following 3MC treatment.

At 6 days after 3MC administration a > 85% reduction in the expression of POR protein, determined both by immunoblotting and cytochrome c activity, had occurred (Figures 1A and 1B, i), increasing to approx. 96% by day 14. These data were confirmed by immunohistochemistry where staining was reduced in all hepatocytes by day 3 and undetectable on day 14 and remained absent for at least 28 days (results not shown). Treatment with 3MC resulted in the anticipated induction of CYP1A1 as a consequence of the activation of the AhR. By days 7–9, CYP1A1 levels, presumably due to the metabolism and elimination of 3MC, were undetectable, indicating that the level of CYP1A1 was not induced by the POR deletion (Figure 1A). The transient changes observed in CYP1A1 expression imply that Cre recombinase expression would follow the same pattern and suggest that the deletion of POR is reversible. Cre recombinase is indeed expressed transiently, similarly to CYP1A1; however, return of POR is dependent on cell turnover, which, in the case of hepatocytes, takes several weeks. In Porloxlox/Cyp2b10 mice, no return of POR was observed for 60 days after 3MC treatment (results not shown). In contrast, very marked increases in the expression of other P450s were detected (Figure 1A). CYP2B, CYP2C, CYP3A and CYP7A1 levels increased profoundly as a function of time and in association with the decrease in POR expression. Particularly marked changes were obtained in P450s identified as CYP2B10 (corresponding to the upper band and CYP2B9 to the lower band according to the findings of Li-Masters and Morgan [20]) and CYP3A11. Interestingly, a stepwise increase in the level of CYP2B proteins occurred between days 9 and 11, whereas the increase in CYP2C and CYP3A proteins appeared to occur more gradually. An induction of CYP7A1 occurred on day 7 and continued to increase until day 21 (Figure 1A). CYP4A levels decreased from day 11 in both control (Porloxlox) as well as HRN mice, after which time they remained slightly higher in HRN mice (results not shown). No changes were observed in the expression levels of CYP2D proteins in any samples. In control Porloxlox animals, 3MC treatment had no effect on any of the P450s measured other than on the induction of CYP1A1 (results not shown).

Associated with the time-dependent reduction in POR were changes in liver size and non-fasting plasma total cholesterol and triacylglycerols levels (Figure 1B, ii, iii and iv). Significant consistent changes in liver/body weight ratio were observed from day 11, suggesting that other phenotypic changes must precede this effect (Figure 1B, ii). A significant decrease in non-fasting plasma triacylglycerol levels also occurred at this time (Figure 1B, iv). A reduction in non-fasting total plasma cholesterol levels was also observed which reduced steadily from day 3, in parallel with POR reduction (Figure 1B, iii), indicating that the reduction in triacylglycerols occurs independently of the changes in cholesterol, but in parallel with liver enlargement.

Loss of POR also led to a profound increase in hepatic lipids (Figure 1C). This accumulation occurred rapidly after 3MC administration and paralleled the loss of POR. At early time points, increases in lipid appeared to be localized to the perivenous region, the major site of POR and P450 expression, and not to the periportal region [21,22]. At later time points, this zonal localization was lost, leading to lipid accumulation throughout the liver. Quantification of hepatic lipid content demonstrated that hepatic steatosis was due to increases in hepatic triacylglycerols by day 11 (Figure 2A). No significant changes in hepatic cholesterol levels were observed over the entire time course of the experiment (Figure 2B). The changes in hepatic triacylglycerol content were closely correlated with changes in both liver/body weight ratio and the reduction in plasma triacylglycerols (Figures 2C and 2D). This suggests that the level of hepatic triacylglycerols determines the circulating triacylglycerol levels and that they are related to liver enlargement.

Changes in hepatic lipid homeostasis mediate P450 induction

Hepatic deletion of POR led to a profound time-dependent induction of several P450s, including members of the CYP2B and CYP3A families. This appeared to occur at both the protein and the mRNA level [9,10]. Changes in CYP2B10 expression were first seen on day 3, with a very marked increase between days 9 and 11 (Figure 1A). The increases in CYP2B10 protein and mRNA were not correlated with the decrease in POR activity (Supplementary Figure S1A, i and ii, at http://www.BiochemJ.org/bj/417/bj4170043add.htm) and therefore did not appear to be due to changes in the metabolism of an endogenous inducing agent. However, CYP2B10 protein and mRNA levels were correlated with each other, indicating that the induction of the Cyp2b10 gene occurs mainly at a transcriptional level (Supplementary Figure S1A, iii). In addition, Cyp2b10 mRNA levels were correlated with hepatic triacylglycerol concentration (Supplementary Figure S1A, iv). Similar analysis for CYP3A11 indicated that the increased protein expression occurred more rapidly than for
CYP2B10, steadily increasing from day 2, reaching a maximum by day 21, but also did not directly correlate with changes in POR activity (Figure S1B). Although Cyp3a11 mRNA levels were increased in POR mice, this did not correlate with the level of CYP3A11 protein, indicating a more complex mechanism of regulation of this gene (Supplementary Figure S1B, iii), possibly involving both transcriptional and post-translational mechanisms. Unlike Cyp2b10, only a weak correlation between the increases in Cyp3a11 mRNA levels and hepatic triacylglycerol levels was observed (Supplementary Figure S1B, iv). Thus differences observed in the induction kinetics of CYP2B10 and CYP3A11, at both the protein and the mRNA level, suggests that the mechanisms regulating their overexpression are, at least in part, distinguishable.

To determine the contributions of lipid uptake, oxidation and synthesis to the development of the fatty liver phenotype and the induction of P450s, changes in the expression levels of CD36, FAS and Cpt1a were determined in 3MC-treated Porlox/lox/CreCYP1A1 mice compared with Porlox/lox mice (Supplementary Figure S2 at http://www.BiochemJ.org/bj/417/bj4170043add.htm). No significant changes in the expression levels of these genes were observed, except for in Cpt1a on day 28 which showed a minor,
Dietary fatty acids mediate P450 induction in HRN mice

In view of the finding that changes in hepatic fatty acid homoeostasis correlated with the increases in P450 expression, detailed fatty acid analysis was carried out on livers from HRN and Porlox/lox (control) mice (Supplementary Tables S4 and S5 at http://www.BiochemJ.org/bj/417/bj4170043add.htm). Changes in all fatty acid classes occurred; however, the most significant changes were in mono-unsaturated and polyunsaturated fatty acids (Supplementary Table S5), with C16:1, C18:1 and C20:3 increasing 6.78-, 14.06- and 6.19-fold respectively (Supplementary Table S4). These data are in close agreement with the report of Weng et al. [23], who, for their HRN model, reported additional fold changes of 7.3 and 14.9 for C16:1 and C20:1 unsaturated fatty acids respectively. Together, these findings suggest that the accumulated hepatic triacylglycerols preferentially contain unsaturated fatty acid chains and that these fatty acid classes may mediate the P450 induction.

In the light of this possibility, Porlox/lox/Porlox/lox/CYP1A1 mice were placed on a fat-deficient diet for 4 weeks before 3MC administration. This treatment completely prevented the increases in P450 protein and mRNA expression as a consequence of POR deletion (Figure 3A). Maintenance of control Porlox/lox mice on a fat-deficient diet caused slight reductions in the constitutive expression of CYP7A and CYP2C protein, and did not affect the levels of the other P450s (results not shown). In addition to the changes in hepatic P450 expression, maintenance of Porlox/lox/CYP1A1 mice on a fat-deficient diet prevented the accumulation of lipid in the livers of these animals (Figure 3C), resulting in an Oil Red O staining pattern that was indistinguishable from that of Porlox/lox mice on a control diet. Also no significant changes in total plasma cholesterol or triacylglycerol levels were measured on deletion of POR in Porlox/lox/CYP1A1 mice fed a fat-deficient diet (Supplementary Figure S3). These data indicate that hepatic triacylglycerol accumulation in POR mice originates from the diet and that as a consequence of the inactivation of the P450 system an inability to maintain hepatic lipid homoeostasis occurs which triggers increased P450 expression (possibly in an attempt to restore normal hepatic lipid levels). It is worth noting that the fat-deficient diet also prevented the liver enlargement seen on POR deletion, thus providing a metabolic link to all three phenomena.

In order to identify the fatty acids that mediated P450 induction, various fatty acids were added back to the fat-deficient diet as outlined in Supplementary Table S1. Porlox/lox/CYP1A1 and Porlox/lox were maintained on the supplemented diets and analysed 14 days after 3MC administration. In diets containing unsaturated fatty acids, the loss of P450 gene expression observed with the fat-deficient diet was reversed. This was particularly the case for diets containing sunflower oil (which is composed of approx. 88% unsaturated fatty acids) (Figure 3B, Supplementary Table S2).
Western blotting. Hepatic Cyp2b10 (i) and Cyp3a11 (iii) mRNA levels expressed as a fold change with Porlox diets and treated with 3MC. Photomicrographs were taken at 10× magnification, bright-field, and are representative of the results from at least three mice per group. For mRNA analysis in (A) and (B), results are means ± S.E.M. (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001 compared with Porlox/lox mice. Std, standard. Black bars: Porlox/lox/CreCYP1A1; grey bars: Porlox/lox.

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Figure 3 Development of phenotypic changes in conditional HRN mice on restricted fatty acid diets

Porlox/lox/CreCYP1A1 mice were maintained on a control diet until 8 weeks of age, and then either placed on a fat-deficient or fatty-acid-supplemented diet, or continued on the control diet, for a further 6 weeks. At 12 weeks of age, they were treated with a single dose of 3MC (40 mg/kg of body weight) and killed 14 days later. (A) POR and P450 expression levels in Porlox/lox/CreCYP1A1 and Porlox/lox/lox mice maintained on a control or fat-deficient diet treated with 3MC (+) or corn oil (CO) (−). (i) Protein expression determined by Western blotting. Hepatic Cyp2b10 (i) and Cyp3a11 (iii) mRNA levels expressed as a fold change compared with Porlox/lox/lox mice. (B) POR and P450 expression levels in Porlox/lox/CreCYP1A1 mice maintained on fatty-acid-supplemented diets and treated with 3MC. (i) Protein expression determined by Western blotting. Hepatic Cyp2b10 (i) and Cyp3a11 (iii) mRNA levels expressed as a fold change compared with Porlox/lox/lox mice. (C) Hepatic lipid content determined by Oil Red O staining of snap-frozen liver sections of Porlox/lox/CreCYP1A1 mice maintained on fatty-acid-supplemented diets and treated with 3MC. Photomicrographs were taken at 10× magnification, bright-field, and are representative of the results from at least three mice per group. For mRNA analysis in (A) and (B), results are means ± S.E.M. (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001 compared with Porlox/lox/lox mice. Std, standard. Black bars: Porlox/lox/CreCYP1A1; grey bars: Porlox/lox. In all panels: SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; SunFl, 1 % sunflower oil.

Role of nuclear receptors in P450 induction in HRN mice

Nuclear receptors that have been shown to mediate the regulation of many P450 genes in response to exogenous chemicals [24,25] have also been shown to play an important role in controlling liver growth [26,27]. Of particular interest with regards to this study were CAR and PXR, which have been intimately linked to the regulation of CYP2B and CYP3A proteins in response to both exogenous and endogenous inducing agents [28–30], and PXR has been linked to triacylglycerol homoeostasis [27]. In addition, no significant increases in liver/body weight ratio were observed for the diets used, except that containing sunflower oils (Supplementary Figure S3C). These data indicate that lipid accumulation alone was not the cause of liver enlargement, but that unsaturated fatty acids had the capacity to induce the effect. Comparison of the fatty acid composition of the diets (Supplementary Tables S2 and S3), identified C18:2 (linoleic acid), an n-6 PUFA (polyunsaturated fatty acid), as a candidate fatty acid mediator of both P450 induction and liver growth as it was the only fatty acid present in the diets that correlated with the effects observed. This hypothesis suggests that the addition of linoleic acid as the sole dietary fatty acid should be sufficient to induce P450 expression in Porlox/lox/CreCYP1A1 mice maintained on a fat-deficient diet. This was indeed found to be the case, with oral administration of linoleic acid (65 mg/day for 9 days) to Porlox/lox/CreCYP1A1 mice on a fat-deficient diet, resulting in induction of CYP2B10, CYP3A11 and CYP2C proteins (Figure 4A). Analysis of Cyp2b10 and Cyp3a11 mRNA levels showed this induction to occur at the transcriptional level (Figure 4B). In addition, administration of linoleic acid was found to promote hepatic lipid accumulation (Figure 4C). This lipid accumulation was reduced compared with Porlox/lox/CreCYP1A1 mice maintained on a control diet and appeared to be concentrated around the blood vessels, a pattern similar to that observed at early time points following 3MC administration (Figure 1C); however, in this case, it is probably due to differences in the amount of fat intake. These results therefore indicate that linoleic acid, an n-6 dietary essential fatty acid, is a key regulator of P450 expression in HRN models and that members of the CYP2B2, CYP3A and CYP2C, in a normal physiological situation, are involved in its metabolism.
Loss of PXR did not affect POR deletion or the induction of P450 proteins observed in HRN mice (Figure 5A). In agreement with previous findings, Pxr−/− mice exhibited slight increases in CYP3A11 protein [14], and also CYP2B and CYP2C proteins (Figure 5A, i); Cyp3a11 mRNA was also elevated relative to levels in control Porlox/lox mice (Figure 5A, ii). Basal expression of Cyp2b10 mRNA was also very slightly increased (Figure 5A, ii), although this was not reflected in detectable protein expression (Figure 5A, i). The deletion of PXR did not affect the increases in both CYP2B10 and CYP3A11 protein in HRN animals; however, a reduction in both Cyp2b10 and Cyp3a11 mRNA levels was observed, but this was not significant compared with HRN mice (Figure 5A, ii). No changes in plasma and hepatic lipid levels were observed in Pxr−/−/HRN mice compared with HRN mice (Supplementary Table S6 at http://www.BiochemJ.org/bj417/bj4170043add.htm and Figure 5D); however, in Pxr−/− mice, non-fasting plasma total cholesterol and triacylglycerol levels were reduced, the former significantly, in comparison with wild-type mice (Supplementary Table S6).

In relation to HRN mice nulled for CAR, profound changes in the HRN phenotype were observed. The elevated expression of CYP2B, CYP7A and CYP2C was markedly attenuated; however, CYP3A11 expression appeared to be unaffected (Figure 5B). Analysis of mRNA levels also showed that no induction of Cyp2b10 mRNA occurred in Car−/−/HRN mice, indicating that the increase in the expression of this P450 observed in HRN mice was a consequence of CAR activation (Figure 5B, i). Interestingly, deletion of CAR in both control and HRN mice further increased Cyp3a11 mRNA levels (Figure 5B, ii). The reduction in non-fasting plasma total cholesterol and triacylglycerol levels observed in the HRN mice were, however, not reversed on the Car−/− background (Supplementary Table S6); however, a significant reduction in hepatic lipid content for Car−/−/HRN mice compared with HRN mice was observed (Figure 5D). These data suggest that, in addition to mediating P450 induction in HRN mice, CAR plays a role in hepatic lipid accumulation.

Finally, owing to the cross-talk between PXR and CAR [31], the effect of deleting both receptors on the development of the HRN phenotype was analysed. In Pxr−/−/Car−/−/HRN mice, consistent with the Car−/− mice, the expression of CYP2B and CYP2C proteins was profoundly reduced, whereas, in contrast, CYP7A expression levels were only slightly affected (Figure 5C). In mice nulled at both the Car and Pxr gene loci, a marked decrease in CYP3A11 protein was also observed (Figure 5C, i). The change in CYP2B protein expression was also reflected in a reduction in Cyp2b10 mRNA levels (Figure 5C, ii). A slight decrease in Cyp3a11 mRNA was observed in Pxr−/−/Car−/−/HRN mice, although this was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii). Deletion of both CAR and PXR on an HRN background resulted in an increase in non-fasting plasma total cholesterol level that was not significantly different from that of HRN or Pxr−/−/Car−/− mice (Figure 5C, ii).
Figure 5  Induction of hepatic P450 expression and other phenotypes in HRN mice on nuclear receptor-null backgrounds

(A) POR and P450 expression levels in Pxr\(^{-/-}\)/HRN mice: (i) protein expression determined by Western blotting; and (ii) hepatic Cyp2b10 (black bars) and Cyp3a11 (grey bars) mRNA levels expressed as a fold change compared with Porlox/lox mice. (B) POR and P450 expression levels in Car\(^{-/-}\)/HRN mice: (i) protein expression determined by Western blotting; and (ii) hepatic Cyp2b10 (black bars) and Cyp3a11 (grey bars) mRNA levels expressed as a fold change compared with Porlox/lox mice. (C) POR and P450 expression levels in Car\(^{-/-}\)/Pxr\(^{-/-}\)/HRN mice: (i) protein expression determined by Western blotting; and (ii) hepatic Cyp2b10 (black bars) and Cyp3a11 (grey bars) mRNA levels expressed as a fold change compared with Porlox/lox mice. (D) Hepatic lipid content determined by Oil Red O staining of snap-frozen liver sections. Photomicrographs were taken at 10× magnification, bright field, and are representative of the results from at least three mice per group. For mRNA analysis in (A), (B) and (C), results are means ± S.E.M. (n = 3). * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001 compared with Porlox/lox mice. WT, wild-type.

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reporter assay in HepG2 cells [32]. In these experiments, both TCPOBOP and linoleic acid could activate CAR directly (Figure 6). PB, which is known to activate CAR by an indirect mechanism, was not active in this assay. In view of reports that the fatty-acid-sensitive transcription factor, PPAR\(\alpha\) (peroxisome-proliferator-activated receptor \(\alpha\)) can effect CAR expression [33,34] and that the effects in the POR mice may be indirect, we measured Car mRNA expression in Por\(^{lox/lox}\)/Cre\(^{Cyp1A1}\) mice. Expression levels of CAR were found to be unchanged (Supplementary Figure S2D).
Figure 6  Activation of CAR by linoleic acid

HepG2 cells were transfected with pCMX Gal4-mCAR-LBD and p4×Gal4-UCAS4-luciferase as outlined in the Experimental section. Transfected cells were subsequently treated with linoleic acid at a concentration ranging from 0 to 12.5 μM, and luciferase activity was analysed 24 h later. Transfected cells were treated with 0.25–0.5 μM TCPD08P or 1 mM PB as positive and negative controls respectively. Results are means ± S.E.M. (n = 6 determinations) and are expressed as a fold change compared with cells treated with either DMSO in the case of TCPD08P and PB or the 0 μM sample in the case of linoleic acid. *P < 0.05, **P < 0.001.

DISCUSSION

The liver is the major organ controlling metabolic homeostasis due to dietary change. It is also the major tissue responsible for drug and dietary chemical detoxification through the actions of phase I and phase II drug metabolism in which the P450 system plays a pivotal role. Although it has been known for some time that alterations in diet and disease states such as diabetes affect the P450 system, there has been little evidence that P450s can play a direct role in metabolic control [35–37]. However, there is a growing body of evidence, as demonstrated in the present paper, that these pathways are intimately linked. This has a wide range of implications not only for how alterations in P450 function may influence the pathogenesis of disease, but also for how diet and disease states may influence the metabolism and disposition of drugs and environmental agents: for example, obesity in rats has been shown to reduce expression levels of CAR and impaired CYP2B induction by PB [38].

The metabolic dialogue between the pathways of detoxification and metabolic homeostasis are manifest in the findings that, when the P450 system is inactivated, profound changes in lipid homeostasis and liver size occur which are paralleled by the marked induction of P450s associated with drug metabolism in the absence of an exogenous inducing agent. We have demonstrated that P450s from a range of gene families are induced and that this induction parallels the accumulation of hepatic triacylglycerols. We have also shown that the regulation of different P450s is, in part, distinguishable, as exemplified by CYP2B10 and CYP3A11. CYP2B10 induction is almost entirely controlled at the transcriptional level and occurs at a time point when there is a stepwise increase in triacylglycerol accumulation, whereas, in the case of CYP3A11, there is only a poor correlation between mRNA induction and protein levels, indicating that both transcriptional and post-translational mechanisms are involved. The latter mechanism could possibly occur via phosphorylation or ubiquitination [39–41]. This may also be the case for CYP2C proteins. Evidence for distinct pathways of regulation is also reflected in the finding that the overexpression of these proteins is, at least in part, mediated by different transcription factors (see below). We have also demonstrated that hepatic steatosis is not sufficient to induce the hepatic P450 expression, but this effect is mediated by dietary unsaturated fatty acids such as linoleic acid, which we have found is sufficient on its own to reverse the fat-deficient phenotype.
Dietary unsaturated fatty acids enter the liver via fatty acid transporters where they are oxidized for energy purposes or detoxified by P450s. Inactivation of the P450 system as a result of Por deletion leads to hepatic accumulation of unsaturated fatty acids and a reduction of plasma lipid levels. Hepatic lipid accumulation triggers the activation of the nuclear receptor CAR (and possibly PXR), leading to P450 induction and liver growth. The overexpression of P450s is aimed at re-establishing hepatic lipid homoeostasis and the prevention of toxicity via increased metabolism of unsaturated fatty acids. Inactivation of POR prevents this increased metabolism and ultimately leads to hepatic steatosis. The development of steatosis establishes a cyclical process that potentiates hepatic lipid accumulation and growth. The adverse effect of P450 induction is altered drug metabolism and environmental chemical detoxification, but, in addition, the increased production of oxidized lipids could result in development of oxidative stress.

In summary, we have used a novel conditional HRN model to investigate the sequential development of the phenotypic changes observed in mice on deletion of POR. POR deletion and the associated inactivation of hepatic P450 function leads to accumulation of dietary fatty acids in the liver, in the form of triacylglycerols of unsaturated fatty acids, leading to organ enlargement and increased P450 expression, in the case of CYP2B, CYP2C and CYP7A1 via a CAR-dependent signalling pathway. The molecular mechanism underlying CYP3A11 expression appears to involve CAR, PXR and protein stabilization. Induction of the P450 system appears to be mediated by the accumulation of PUFAs, such as linoleic acid (C18:2n-6) and suggests that this may be part of an adaptive response to prevent the deleterious effects of PUFA accumulation. The finding that the major P450s that are inducible by exogenous chemicals are also regulated by changes in fatty acid homoeostasis has potentially significant implications for the variability observed in drug responses in humans.

ACKNOWLEDGEMENTS

We thank Dr Steven Kluwer for his gift of Pxr<sup>−/−</sup> and Car<sup>−/−</sup> mice and Dr Alexander Hill for carrying out the hepatic fatty acid analysis. Dianne Carrie and Catherine Hughes are thanked for excellent technical assistance with the animal work.

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REFERENCES

CAR-dependent fatty acid regulation of cytochromes P450


Weng, Y., Dirusso, C. C., Reily, A. A., Black, P. N. and Ding, X. (2005) Hepatic gene expression changes in mouse models with liver-specific deletion or global suppression of the NADPH-cytochrome P450 reductase gene: mechanistic implications for the regulation of microsomal cytochrome P450 and the fatty liver phenotype. J. Biol. Chem. 280, 31686–31698


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56 Miao, J., Fang, S., Bae, Y. and Kemper, J. K. (2006) Functional inhibitory cross-talk between constitutive androstane receptor and hepatic nuclear factor-4 in hepatic lipid/glucose metabolism is mediated by competition for binding to the DR1 motif and to the common coactivators, GRIP-1 and PGC-1α. J. Biol. Chem. 281, 14537–14546
SUPPLEMENTARY ONLINE DATA

Unsaturated fatty acid regulation of cytochrome P450 expression via a CAR-dependent pathway

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Cancer Research UK Molecular Pharmacology Unit, Biomedical Research Centre, Level 5, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

EXPERIMENTAL

Lipid isolation and analysis

For detailed fatty acid analysis, livers from 12-week-old HRN male mice were collected and lipid-extracted using a modified version of the Folch method [1]. Briefly, internal standard triheptadecanoic acid was added to a weighed amount of wet mouse liver and homogenized in chloroform/methanol (1:1, v/v). An equal volume of saline was added, before being centrifuged, and the organic layer was removed and dried under vacuum. The lipid residue was transesterified with 5% sulfuric acid in methanol, and the methyl fatty acids were extracted into heptane. Separation was achieved by injection into an SGE BPX-70 capillary column in a Unicam series 610 gas chromatograph with temperature programming. Data were collected with a Phillips PU6030 data capture unit and processed using Unicam 4880 data handling software. Fatty acid quantification was by internal standardization with sample concentrations calculated by comparing the sample fatty acid/internal standard ratio against the fatty acid/internal standard ratio of known amounts of a commercial fatty acid standard within a known linear range of standard ratios. The same amount of each fatty acid was added to both standard and sample.

Table S1 Fatty acids added to a fat-deficient base diet

Values are expressed as the amount of fatty acid (in g) in 100 g of food (w/w). The control diet is included for comparison purposes. A fourth supplemented diet was used consisting of the methyl fatty acids were extracted into heptane. Separation was achieved by injection into an SGE BPX-70 capillary column in a Unicam series 610 gas chromatograph with temperature programming. Data were collected with a Phillips PU6030 data capture unit and processed using Unicam 4880 data handling software. Fatty acid quantification was by internal standardization with sample concentrations calculated by comparing the sample fatty acid/internal standard ratio against the fatty acid/internal standard ratio of known amounts of a commercial fatty acid standard within a known linear range of standard ratios. The same amount of internal standard was added to both standard and sample.

<table>
<thead>
<tr>
<th>Table S2 Fatty acid analysis of the diets used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are expressed as a percentage of total fatty acid content on a fresh weight basis. MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.</td>
</tr>
<tr>
<td>Fatty acid group</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
</tr>
</tbody>
</table>

Table S3 Fatty acid analysis of the diets used

Values are expressed on a w/w basis in the diet. MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

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1 To whom correspondence should be addressed (email c.r.wolf@dundee.ac.uk).
Table S4  Fatty acid analysis of total hepatic lipids of control (Porlox/lox) and HRN mice

Results are means ± S.E.M. (n = 3 livers). Fold change is expressed as the ratio of HRN mice to control mice.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fatty acid concentration (μM)</th>
<th>P</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HRN</td>
<td></td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>1.3 ± 0.2</td>
<td>3.5 ± 1.4</td>
<td>0.202</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>24.5 ± 1.0</td>
<td>86.7 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>2.5 ± 0.01</td>
<td>17.1 ± 3.8</td>
<td>0.019</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>10.3 ± 0.2</td>
<td>25.2 ± 2.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>15.2 ± 0.5</td>
<td>233.3 ± 18.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>12.8 ± 0.5</td>
<td>38.8 ± 10.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4)</td>
<td>1.2 ± 0.1</td>
<td>7.5 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4)</td>
<td>1.0 ± 0.6</td>
<td>18.3 ± 1.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Docosahexaenoic acid (C22:6)</td>
<td>4.9 ± 0.2</td>
<td>11.1 ± 0.8</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table S5  Fatty acid analysis of total hepatic lipids of control (Porlox/lox) and HRN mice

Results are means ± S.E.M. (n = 3 livers). Fold change is expressed as the ratio of HRN mice to control mice.

<table>
<thead>
<tr>
<th>Fatty acid group</th>
<th>Fatty acid concentration (μM)</th>
<th>P</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>82.7 ± 1.1</td>
<td>421.4 ± 32.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C16:0/C18:0 ratio</td>
<td>2.4 ± 0.1</td>
<td>3.5 ± 0.3</td>
<td>0.024</td>
</tr>
<tr>
<td>Saturated</td>
<td>36.1 ± 1.1</td>
<td>115.4 ± 5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mono-unsaturated</td>
<td>17.7 ± 0.5</td>
<td>220.4 ± 20.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>28.9 ± 0.6</td>
<td>75.7 ± 11.9</td>
<td>0.018</td>
</tr>
<tr>
<td>n = 3</td>
<td>4.9 ± 0.2</td>
<td>11.1 ± 0.8</td>
<td>0.002</td>
</tr>
<tr>
<td>n = 6</td>
<td>24.1 ± 0.5</td>
<td>64.6 ± 11.2</td>
<td>0.022</td>
</tr>
<tr>
<td>Triacylglycerol (mM)</td>
<td>0.03 ± 0.0004</td>
<td>0.14 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table S6  The induction of other phenotypes in HRN mice on nuclear receptor-null backgrounds

Por−/−/HRN, Car−/−/HRN and Por−/−/Car−/−/HRN mice were generated as outlined in the Experimental section of the main text and were analysed at 12 weeks of age for non-fasting plasma lipid concentrations and liver/body weight ratio as detailed in the Experimental section of the main text. Results are means ± S.E.M. (n = 3). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001 compared with Porlox/lox (control) mice. †P ≤ 0.05 compared with HRN mice.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plasma cholesterol</th>
<th>Plasma triacylglycerol</th>
<th>Liver/body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Porlox/lox)</td>
<td>3.73 ± 0.24</td>
<td>1.09 ± 0.27</td>
<td>0.051 ± 0.002</td>
</tr>
<tr>
<td>HRN</td>
<td>1.15 ± 0.10***</td>
<td>0.29 ± 0.09*</td>
<td>0.075 ± 0.004**</td>
</tr>
<tr>
<td>Por−/−</td>
<td>2.31 ± 0.16*</td>
<td>0.41 ± 0.13</td>
<td>0.062 ± 0.001*</td>
</tr>
<tr>
<td>Car−/−</td>
<td>2.90 ± 0.05</td>
<td>0.70 ± 0.16</td>
<td>0.045 ± 0.002</td>
</tr>
<tr>
<td>Por−/−/Car−/−</td>
<td>2.60 ± 0.49</td>
<td>0.95 ± 0.01</td>
<td>0.051 ± 0.002</td>
</tr>
<tr>
<td>Por−/−/HRN</td>
<td>1.03 ± 0.32**</td>
<td>0.14 ± 0.01*</td>
<td>0.091 ± 0.002***</td>
</tr>
<tr>
<td>Car−/−/HRN</td>
<td>1.35 ± 0.15**</td>
<td>0.28 ± 0.04*</td>
<td>0.073 ± 0.007*</td>
</tr>
<tr>
<td>Por−/−/Car−/−/HRN</td>
<td>2.63 ± 0.28†</td>
<td>0.61 ± 0.12</td>
<td>0.073 ± 0.004**</td>
</tr>
</tbody>
</table>
Porlox\textsuperscript{lox}/lox and Porlox\textsuperscript{lox}/lox mice were treated with a single dose of 3MC (40 mg/kg of body weight), and harvested over a 28 day period. Hepatic triacylglycerol levels, mRNA and protein levels of CYP2B10 and CYP3A11, and POR activity were determined as detailed in the Experimental section of the main text. (A) Relationship between POR activity and (i) CYP2B10 protein, (ii) Cyp2b10 mRNA content. Correlation of Cyp2b10 mRNA with (iii) CYP2B10 protein and (iv) hepatic triacylglycerol (triglyceride) concentration. (B) Relationship between POR activity and (i) CYP3A11 protein and (ii) Cyp3a11 mRNA content. Correlation of Cyp3a11 mRNA with (iii) CYP3A11 protein and (iv) hepatic triacylglycerol concentration. mRNA expression and POR activity are expressed compared with 3MC-treated Porlox\textsuperscript{lox}/lox mice. Results are means for three animals per experimental time point.

![Figure S1 Relationship of CYP2B10 and CYP3A11 expression with loss of POR activity and hepatic lipid accumulation](image-url)

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R. D. Finn and others

Figure S2 Gene expression changes on loss of POR

Porlox/lox/CreCYP1A1 and Porlox/lox mice treated with a single dose of 3MC (40 mg/kg of body weight) were analysed for changes in the expression levels of various genes involved in controlling hepatic lipid levels. mRNA expression of the fatty-acid transporter CD36 (A), FAS (B), CPT1a (C) and CAR (D) as a function of time post 3MC administration. Results are means ± S.E.M. (n = 3) and are expressed as a fold change compared with Porlox/lox mice at the same time point. *P \leq 0.05.

Reference


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