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# Pretranslational upregulation of microsomal CYP4A in rat liver by intake of a high-sucrose, lipid-devoid diet containing orotic acid

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**Abstract**

In rodents, high-fat diets promote hepatic lipid accumulation in rodents, activation of peroxisome proliferator activated receptor-α (PPARα) and upregulation of cytochrome P450 (CYP) 4A gene expression. Lipid-devoid diets containing sucrose and orotic acid (S/OA-diet) also cause lipid infiltration by stimulating intrahepatic lipid synthesis and preventing lipoprotein transport through the Golgi apparatus. This study evaluated the impact of the lipid-deficient S/OA-diet on CYP4A expression and PPARα activation in rodent liver. CYP4A protein and laurate ω-hydroxylation activity were increased in rat liver after S/OA-feeding for 21 days. CYP4A1 and CYP4A2 mRNAs were induced to 2.1- and 2.6-fold of control, but mRNAs corresponding to CYP4A3 and the peroxisomal acyl-CoA oxidase (AOX) were unchanged. Coadministration of clofibric acid and the S/OA-diet prevented hepatic lipid accumulation and upregulated CYP4A protein to levels comparable with clofibric acid alone (five-fold of control). Clofibric acid, alone and in combination with the S/OA-diet, upregulated CYP4A1-3 and AOX mRNAs. Hepatic PPARα protein was decreased by the S/OA-diet but was increased to 5.7-fold of control by clofibric acid; retinoid X-receptor-α (RXRα) protein was decreased to 26–41% of control by all treatments. In further studies, administration of the S/OA-diet to control and PPARα-null mice promoted hepatic lipid deposition; microsomal CYP4A protein was induced in wild-type but not PPARα-null mice. These findings implicate PPARα in the induction of CYP4A in rodent liver by the lipid-devoid S/OA-diet. Decreased availability of hepatic PPARα and RXRα after intake of the diet may contribute to the selective upregulation of hepatic CYP4A1 and CYP4A2 in this model.
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*Keywords:* Cytochrome P450; Gene regulation; Orotic acid; Peroxisome proliferator-activated receptor-α; Retinoid X-receptor-α; Hepatic lipid synthesis

## 1. Introduction

It is well established that diet has a major influence on hepatic microsomal CYP-mediated drug and xenobiotic biotransformation [1], but detailed mechanistic information is currently unavailable for many nutritional factors. Constitutive xenobiotic and steroid hydroxylating CYPs from the 2C and 3A subfamilies are down-regulated in several species following ingestion of high-fat diets. Thus, CYP suppression was proportional to the extent of hepatic lipid deposition in rats [2] and force-fed geese [3]. Down-regulation of CYPs 2C11 and 3A has also been reported in rat liver following intake of the S/OA-diet [4]. The S/OA-diet does not contain fat [5], but the high carbohydrate content stimulates hepatic acetyl-CoA and L-α-glycerophosphate production, which enhances long chain fatty acyl-CoA formation and lipid synthesis [6]. OA then impairs the transport of lipoproteins through the Golgi apparatus [7], so that the S/OA combination results in extensive deposition of lipid in liver. These findings suggest that increased intrahepatic lipid synthesis and accumulation exerts a similar suppressive effect on CYP drug oxidation to that produced by direct intake of dietary fat.

In addition to down-regulation of drug oxidising CYPs, high fat-containing diets have been found to upregulate

*Abbreviations:* AOX, acyl-CoA oxidase; CYP, cytochrome P450; S/OA-diet, sucrose/orotic acid-containing diet; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TBAR, thiobarbituric acid-reactive substance

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CYP4A fatty acid ω-hydroxylases in liver of rats and mice [8,9]. CYPs 4A may contribute to ongoing injury following lipid deposition. The ω-hydroxylated metabolites of arachidonic acid are vasoactive and may contribute to local decreases in blood pressure, which has been reported in fatty liver of obesity [10]. In addition, studies in experimental animals have shown that CYP4A increases free radical production during uncoupled oxidation of NADPH and substrate; these free radicals may contribute to long term injury [8]. Lipids are established ligands of PPARα, which forms heterodimers with RXR to activate target gene transcription [11]. In the present study, the relationship between ingestion of the lipid-devoid S/OA-diet, expression of PPARα-inducible CYPs 4A and activation of PPARα was explored in rodents. In comparative experiments clofibric acid, an established chemical PP agent, was administered to animals in control and S/OA-containing diets and evaluated for its impact on CYP4A expression and PPARα activation.

## 2. Materials and methods

### 2.1. Chemicals

[α-$^{32}$P]-dCTP (specific activity 3000 Ci/mmol), Hyperfilm-MP Hyperfilm-ECL, ACS II, Hybond-N$^{+}$ filters, [1-$^{14}$C]-lauric acid (specific activity ca. 58 mCi/mmol) and enhanced chemiluminescence reagents were from Amersham Pharmacia Biotech. Terminal deoxynucleotidyl transferase was from Promega and clofibric acid and biochemicals were from Sigma Aldrich or Roche. Reagents for electrophoresis were from Bio-Rad. HPLC solvents were from Rhone-Poulenc and analytical reagents were from Ajax.

### 2.2. Animal treatments

Experimental studies followed National Health and Medical Research Council guidelines as approved by Institutional Animal Ethics Committees; animals received humane care. The diet consisted of sucrose (600 g/kg), casein (200 g/kg), cellulose (110 g/kg), corn oil (40 g/kg), salt mixture (#4179 ICN Biochemicals; 40 g/kg), ICN vitamin fortification mixture (10 g/kg), α-tocopherol (20 mg/kg) and retinyl acetate (8.7 mg/kg). Inbred male Wistar rats (ca 200 g) were obtained from the institutional animal facility and received the diet with or without 1% OA for 21 days [4]. Pair-fed animals were allowed free access to water. In another experiment animals were placed on diets that also contained clofibric acid (10 mg/kg) over a 21 day period. In a further experimental protocol, rats were placed on diets that were fortified with additional vitamin E (200 mg/kg) over a 21 day period.

Female C57BL/6N and PPARα null mice (8–10 weeks of age), originally provided by Dr. F.J. Gonzalez, NCI, Bethesda, MD, USA [12], were bred in the transgenic facility of the Westmead Milennium Institute and were administered the S/OA- or control diets for 21 days.

### 2.3. Hepatic subcellular fractions, lipid extraction and peroxidation

Animals were killed under enflurane anaesthesia, which was found in preliminary studies not to affect CYP expression or function. Livers were removed, perfused with cold saline and then taken for histology. The remainder was snap frozen in liquid nitrogen or used in the preparation of hepatic microsomal fractions and homogenates [13].

Hepatic lipids were extracted, dried to constant weight in a vacuum dessicator and quantified [14]. Esterified triglycerides (Periodochrom GPO-PAP; Boehringer-Mannheim GmbH) and free fatty acids (Half-micro kit; Roche Diagnostics) were quantified. Lipid peroxides, were assayed in rat hepatic homogenates as TBARs [15]. Kidneys were also harvested but there was no increase in weight or lipid content after ingestion of the experimental diet. Further, there was no evidence of lipid accumulation in other organs, thus indicating the liver-specific effect of the S/OA-diet.

### 2.4. Microsomal laurate hydroxylation assay

Microsomal laurate hydroxylation (50 μM; 0.15 μCi) was measured as described previously [16]. Incubations (0.3 mL, 5 min, 37 °C) contained 200 μg protein in 0.1 M phosphate buffer, pH 7.4 and were initiated with NADPH (1 mM). Reactions were terminated by 10% sulphuric acid (0.1 mL) and were extracted with ethyl acetate (2 × 2.5 mL). The extract was dried (anhydrous sodium sulphate), filtered and evaporated under nitrogen. Samples were dissolved in acetonitrile (50 μL) and applied to an Ultrasphere C18 HPLC column (5 μm, 250 mm × 4.6 mm; Beckman). The column was eluted with 33% acetonitrile in acetic acid (1%) for 17 min, followed by 100% acetonitrile for 10 min before reequilibration with 33% acetonitrile in acetic acid (1%). Fractions were collected (1 min) and subjected to β-counting (ACS II, Amersham Pharmacia Biotech). 11- and 12-Hydroxylauric acids eluted at 13 and 15.5 min and laurate at 23 min.

### 2.5. Sodium dodecylsulphate polyacrylamide gel electrophoresis and immunoblotting

Microsomes (5 μg/lane) were incubated at 100 °C for 5 min with 2% sodium dodecyl sulphate and 5% 2-mercaptoethanol and electrophoresed on 7.5% polyacrylamide gels [17] with minor modifications [18]. Proteins were transferred to nitrocellulose [19] and incubated with anti-CYP IgGs for 120 min (3.7 μg protein/mL). The rabbit anti-CYP4A1 IgG was a gift from Prof. G.G. Gibson, University of Surrey, the rabbit anti-CYP2E1 IgG was

from Prof. M. Ingelman-Sundberg, Karolinska Institute and the rabbit anti-CYP2C11 IgG was prepared previously [20]. For immunodetection of PPARα and RXRα, hepatic homogenates (100 μg) were subjected to electrophoresis on 7.5% polyacrylamide gels and transferred to nitrocellulose, as described above. The anti-PPARα and anti-RXRα antibodies were purchased from Santa Cruz. Immunoreactive proteins were detected by enhanced chemiluminescence (Hyperfilm-ECL film) and analysed by densitometry (BioRad GS-7000).

### 2.6. Isolation and analysis of hepatic CYP mRNAs

RNA was extracted from rat liver by the $CsCl_2$ method [21]. Full length CYP4A1 and AOX cDNA probes were gifts from Prof. G.G. Gibson, University of Surrey, UK and Dr T. Pineau, Toulouse, France, respectively. cDNAs were labelled using [α-$^{32}$P] dCTP, Klenow enzyme and the Amersham Megaprime System II.

Oligonucleotides were from Geneworks (Adelaide): CYP4A2 (5′-GCT-GGG-AAG-GTG-TCT-GGA-GT-3′ [22], as the complement of the genomic sequence [23]), CYP4A3 (5′-ACT-GGG-ATG-GAG-TCT-GGA-GG-3′ [22], as the complement of the 3′-untranslated region from 1691–1710 [24]) and 18S rRNA (5′-CGG-CAT-GTA-TTA-GCT-CTA-GAA-TTA-CCA-CAG-3′, as the complement of the coding region 151–180 [25]). Oligonucleotides were labelled with [α-$^{32}$P] dCTP and deoxynucleotidyl transferase. Probes were purified with ProbeQuant G50 microcolumns (Amersham Pharmacia Biotech).

For Northern analysis, total RNA (1–10 μg/lane) from individual livers was electrophoresed on 1% agarose in the presence of 2.2 M formaldehyde and transferred to Hybond-N+ (0.45 μm). Prehybridisation, hybridisation and washing [4,21] was followed by autoradiography on Hyperfilm MP. Slot blotting was used for mRNA quantitation, with the exception of CYP4A3 mRNA, which was quantitated by Northern analysis because of its low abundance. To demonstrate equivalence of RNA loading, filters were stripped and rehybridised to the 18S rRNA [α-$^{32}$P]-labelled probe. Signals were quantified by densitometry.

### 2.7. Statistics

Data are presented as means ± S.D. from measurements in individual animals ($n = 4$–6/group). Differences between means of two groups were detected by Student's $t$-testing and between multiple groups by one-way analysis of variance and Fisher's PLSD test.

## 3. Results

### 3.1. CYP4A expression in rat liver after ingestion of the S/OA diet

Consistent with previous studies [4], 21 days of intake by male rats of the lipid-devoid synthetic S/OA-diet led to extensive hepatic triglyceride deposition (132 ± 29 mg lipid/g liver versus 9 ± 2 mg lipid/g liver in control; $P < 0.01$). Free fatty acids in liver were also increased to 4.3-fold of control by this regimen (34 ± 16 mg/g liver versus 8 ± 4 mg/g in control; $P < 0.01$). Weight gain was not different in rats that received the S/OA or control diets (not shown).

CYP4A immunoreactive protein was increased to ∼3-fold of control in microsomes from rats that received the S/OA-diet ($P < 0.01$; Fig. 1), but not after administration of standard rat chow containing 1% OA (not shown). In contrast to the upregulation of CYP4A by S/OA-dietary conditioning, immunoblot analysis indicated that microsomal CYP2E1 and CYP2C11 proteins were decreased to 68 ± 12% ($P < 0.05$) and 53 ± 14% ($P < 0.01$) of respective control (Fig. 1). Consistent with these findings, CYP4A-mediated laurate 12-hydroxylation was increased in hepatic microsomes from rats that received the S/OA diet to 2.8-fold of control (2.13 ± 0.86 nmol/mg protein/min versus 0.77 ± 0.36 in control liver; Fig. 2; $P < 0.01$). By comparison, laurate 11-hydroxylation, which is mediated by several CYPs, including CYPs 2C11, 2E1 and 4A, was increased to ∼1.6-fold of control by S/OA-intake (1.04 ± 0.34 versus 0.64 ± 0.24 nmol/mg protein/min; $P < 0.01$; Fig. 2). Down-regulation of CYP2E1 was supported by the finding that aniline 4-hydroxylation

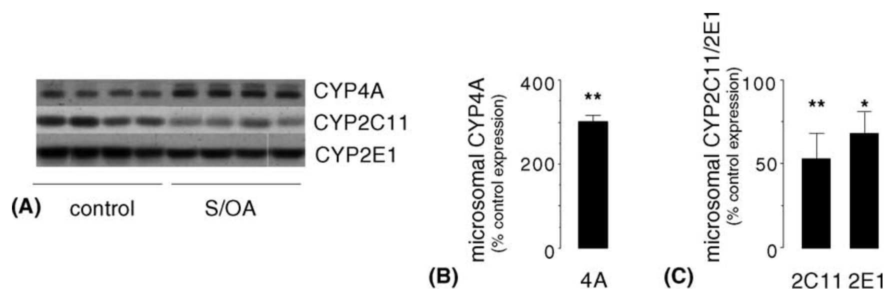


Fig. 1. (A) Representative immunoblots of CYP4A, CYP2C11 and CYP2E1 proteins in hepatic microsomal fractions from rats that received the control and S/OA-diets for 21 days. (B) Microsomal CYP4A expression in S/OA-fed rats relative to control diet ($n = 4$ animals per group). (C) Microsomal CYP2C11/2E1 expression in S/OA-fed rats relative to control diet ($n = 4$ animals per group). $^{*}P < 0.05$, $^{**}P < 0.01$. Note that the scales for CYP4A and CYP2C11/2E1 expression are different, which reflects the upregulation of CYP4A and the down-regulation of CYPs 2C11/2E1.

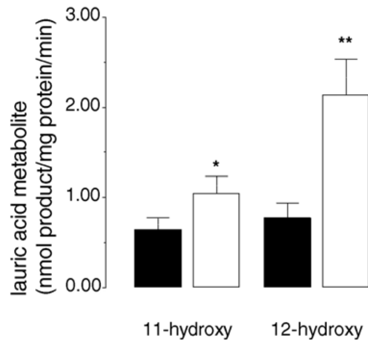


Fig. 2. Microsomal lauric acid 11- and 12-hydroxylation in liver of rats maintained for 21 days on the control diet (closed bars) or the S/OA-diet (open bars); ($n = 4$ animals per group); $^{*}P < 0.05$, $^{**}P < 0.01$.

activity was decreased in microsomes from S/OA-fed rats (not shown). CYP2C11-mediated androstenedione 16α-hydroxylation was shown previously to be decreased in S/OA-rat liver [4].

### 3.2. Clofibric acid modulates hepatic CYP expression and lipid infiltration in rats fed the S/OA-diet

To evaluate whether microsomal CYP4A expression was optimally induced by intrahepatic lipids that accumulated after intake of the S/OA-diet, the PP chemical clofibric acid was coadministered to some animals in diets. The increase ($P < 0.001$) in CYP4A immunoreactive protein expression induced by the S/OA-diet (Fig. 3) was less pronounced than that produced by clofibric acid (to five-fold of control; $P < 0.001$); the induction potency of the S/OA and clofibric acid combination was not different from clofibric acid alone (Fig. 3). Selective induction of CYP4A mRNAs by the S/OA-diet was observed, with CYP4A1 and CYP4A2 mRNA expression increased to 2.1- and 2.6-fold of respective control ($P < 0.05$); CYP4A3 remained at control levels (Table 1). In contrast, clofibric acid administration strongly increased the expression of CYP4A1, 4A2 and 4A3 mRNAs to 4.1-, 2.3- and 2.9-fold of control; the combination of S/OA and clofibric acid was again comparable to clofibric acid alone. The PPARα-inducible gene AOX, which contributes to peroxisomal β-oxidation activity, was also upregulated at the mRNA level by clofibric acid ($P < 0.001$), but not by the S/OA-containing diet. Thus, intake of the S/OA-diet selectively upregulated CYPs 4A1 and 4A2, but not CYP4A3 or AOX, whereas all four genes were strongly induced by clofibric acid (Table 1).

By comparison with these findings, microsomal CYP2C11 protein was decreased by intake of the S/OA-diet to 59 ± 28% of control ($P < 0.02$) and by the S/OA-clofibric acid combination to 27 ± 20% of control

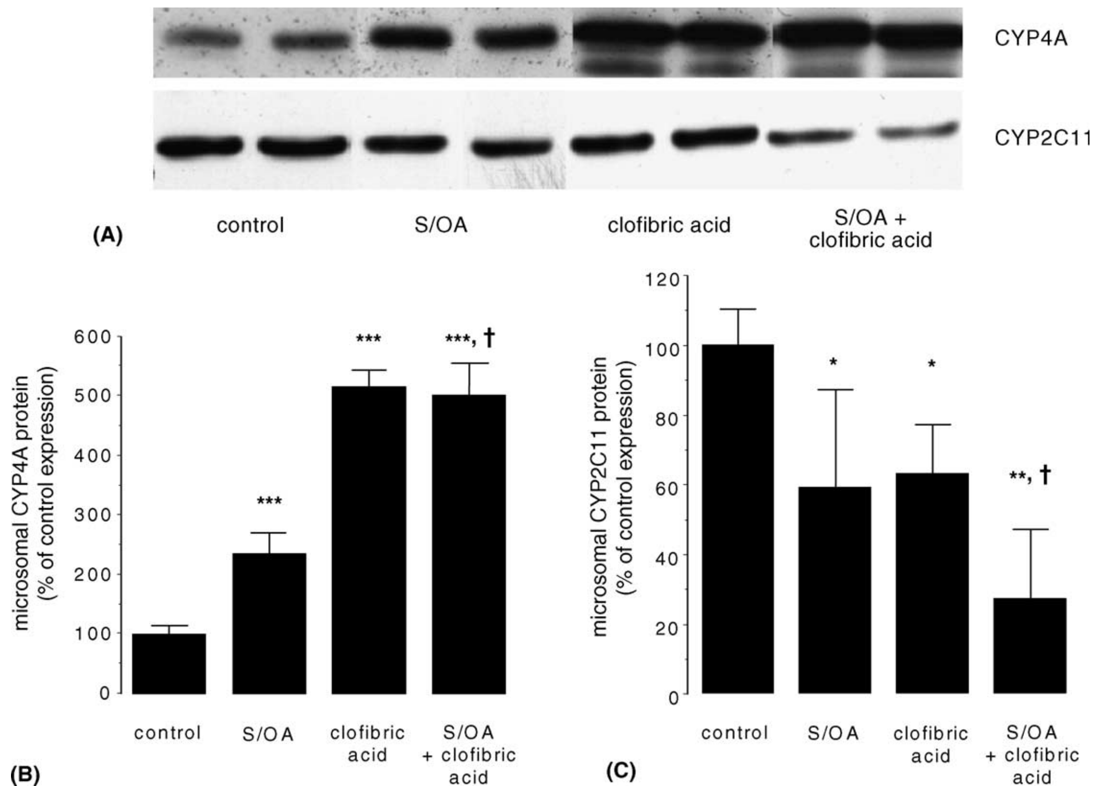


Fig. 3. (A) CYP4A and CYP2C11 proteins in hepatic microsomal fractions from rats that received control and S/OA-diets, with and without supplementation with clofibric acid (10 mg/kg diet) for 21 days as described in Materials and Methods. (B) Microsomal CYP4A expression in rat liver relative to the control diet ($n = 4$ animals per group). (C) Microsomal CYP2C11 expression in rat liver relative to the control diet ($n = 4$ animals per group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (relative to control); $^{\dagger}P < 0.001$ (relative to S/OA-diet). Note that the scales for CYP4A and CYP2C11 expression are different, which reflects the upregulation of CYP4A and the down-regulation of CYPs 2C11.

Table 1
Effects of the S/OA diet and clofibric acid on rat hepatic mRNAs encoding CYPs 4A and AOX

| Treatment | CYP4A1 | CYP4A2 | CYP4A3 | AOX |
|---|---|---|---|---|
| Control diet | $100 \pm 12$ | $100 \pm 34$ | $100 \pm 44$ | $100 \pm 27$ |
| S/OA diet | $204 \pm 45^{*}$ | $264 \pm 127^{*}$ | $158 \pm 72$ | $115 \pm 72$ |
| Clofibric acid | $407 \pm 68^{***}$ | $467 \pm 138^{**}$ | $874 \pm 320^{***}$ | $789 \pm 230^{***}$ |
| S/OA + clofibric acid | $445 \pm 108^{***,\dagger}$ | $948 \pm 162^{***,\dagger}$ | $1520 \pm 330^{***,\dagger}$ | $705 \pm 48^{***,\dagger}$ |

mRNAs were quantified by slot blotting relative to the 18S signal in livers except CYP4A3 mRNA, which was quantified by Northern analysis. Data are mean ± S.D. of 4–6 individual livers/group, with values expressed as percentages of control.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$, relative to control.

† $P < 0.001$, relative to S/OA diet alone.

($P < 0.001$); clofibric acid alone also strongly decreased expression (Fig. 3).

Hepatic lipid infiltration produced by the combination of the S/OA diet and clofibric acid (Fig. 4) was less than that produced by the S/OA diet alone. Consistent with these findings, triglycerides (11 ± 4 mg/g in control liver) were increased by the S/OA-diet to 110 ± 38 mg/g liver ($P < 0.001$), but were returned by clofibric acid coadministration to control levels (27 ± 22 mg/g liver). This effect of clofibric acid coadministration on lipid deposition was also evident from a comparison of the light micrographs in Fig. 4 (panels B and D).

### 3.3. Antioxidant supplementation does not alter CYP4A upregulation by the S/OA-feeding regimen

TBARs were readily detected in liver homogenates from S/OA-fed rats (5 ± 2 μmol malondialdehyde equivalents/mg protein), but not in control fractions. Thus, lipid hydroperoxide formation was increased in rat liver after ingestion of the S/OA-diet and this study tested whether these pro-oxidant species may contribute to CYP4A upregulation in liver. Inclusion of vitamin E (200 mg/kg) in diet over the 21 day period of dietary conditioning prevented the increase in lipid peroxides. However, dietary vitamin E supplementation did not significantly alter CYP4A expression induced by the S/OA-diet or basal CYP4A expression in control rat liver (not shown). These findings suggest that lipid peroxides do not contribute to the induction of CYP4A by the S/OA-diet.

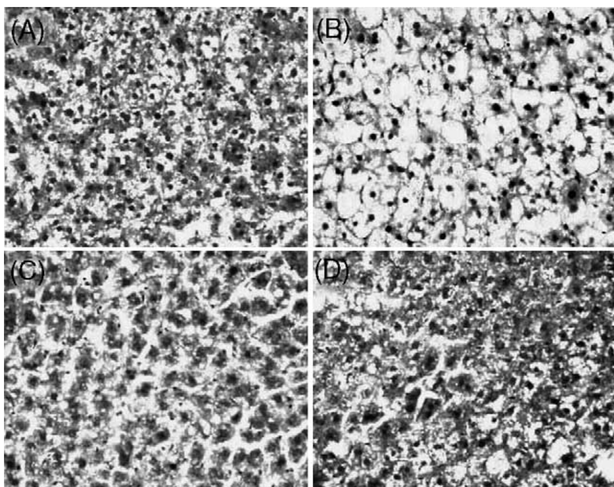


Fig. 4. Light micrographs of hepatic sections from rats that received (A) control diet, (B) the S/OA-diet, (C) the control diet supplemented with clofibric acid (10 mg/kg diet) or (D) the S/OA-diet supplemented with clofibric acid (10 mg/kg diet) for 21 days. Hematoxylin and eosin stain; original magnification400×.

### 3.4. PPAR status in rodent liver after feeding of the S/OA-diet

Ingestion of the S/OA-diet led to the hepatic accumulation of fatty acids and other lipids that may act as PPARα ligands and activate gene transcription in liver. As part of the present study the expression of PPARα and its heterodimerisation partner RXRα was measured in rat liver after dietary conditioning. Thus, ingestion of the S/OA diet decreased PPARα expression in hepatic homogenates to 49 ± 14% of control ($P < 0.05$; Fig. 5). In contrast, clofibric acid strongly increased immunoreactive PPARα protein expression to 574 ± 56% of control ($P < 0.001$; Fig. 5). The combination of clofibric acid and the S/OA diet also increased PPARα protein, but this effect was less pronounced than that produced by clofibric acid alone ($P < 0.001$; Fig. 5). In contrast with these findings, RXRα was decreased in liver by all treatments (Fig. 5). Thus, it emerges that the intrahepatic availability of PPARα was markedly decreased after ingestion of the S/OA diet but not by clofibric acid administration, whereas RXRα availability was uniformly decreased. Decreased PPARα expression may contribute to the observed selective activation of some PPARα responsive genes in rat liver after dietary S/OA feeding.

To further evaluate the participation of PPARα in CYP4A induction after feeding of the S/OA-diet, PPARα-null and C57BL/6N wild-type mice were administered the diet for 21 days. Consistent with findings in rat liver, lipid accumulation was also evident in liver of mice placed on the S/OA-dietary regimen. Most notably, however, Cyp4a immunoreactive protein was increased to 1.8-fold of control in microsomes from wild-type, but not PPARα null, mice after ingestion of the S/OA diet (Fig. 6).

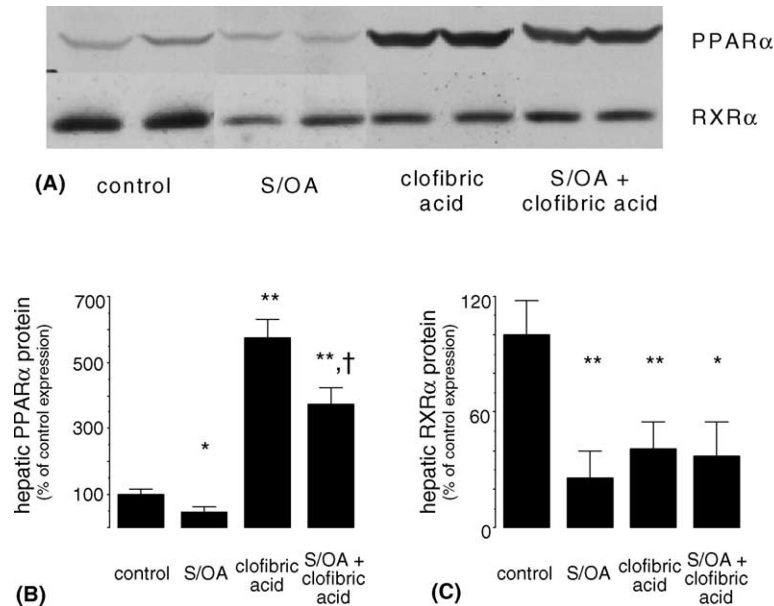


Fig. 5. (A) PPARα- and RXRα immunoreactive proteins in hepatic homogenates from rats that received the control and S/OA-diets for 21 days, with and without supplementation with clofibric acid (10 mg/kg diet). (B) PPARα and (C) RXRα protein in liver after the dietary regimen; $^{*}P < 0.05$, $^{**}P < 0.001$, (relative to control); $^{\dagger}P < 0.001$ (relative to S/OA-diet). Note that the scales for PPARα and RXRα expression are different, which reflects the upregulation of PPARα and the down-regulation of RXRα by certain treatments.

## 4. Discussion

Hepatic lipid accumulation and induction of the PPARα inducible CYP4A has been reported following ingestion of high fat diets by rats and mice [8,9]. The S/OA-diet does not contain fat, but the high carbohydrate content stimulates hepatic acetyl-CoA and L-α-glycerophosphate production, which in turn enhances long chain acyl-CoA formation and increases lipid synthesis [6]. The inclusion of OA in the diet prevents the export of newly synthesised hepatic lipoproteins [7]. Thus, the S/OA combination increases lipid production and also impairs its transport from the liver. In this study, the contents of free fatty acids and esterified triglycerides in liver were increased to 3- and 10-fold of respective control following intake of the S/OA-diet for 21 days. Because exogenous dietary lipid has been shown to activate PPARα and upregulate CYPs 4A, the present study tested whether lipids generated intrahepatically may also influence CYP4A expression in rodent liver. The principal finding to emerge was that microsomal CYP4A protein was increased in livers of rodents fed the lipid-devoid S/OA-diet, but not after ingestion of standard rat chow supplemented with 1% OA. Thus, stimulation of intrahepatic lipid synthesis by carbohydrate intake was required for CYP4A upregulation. In contrast with the upregulation of CYP4A, the S/OA-diet decreased the microsomal expression of the constitutive CYPs 2C11 and 2E1, which contribute to the biotransformation of xenobiotics, steroids and fatty acids in liver. This is in accord with previous studies that have related hepatic lipid deposition to the suppression of CYP-mediated drug oxidation following ingestion of high-fat diets [2,3,8].

The extent of CYP4A induction by the S/OA-diet was less pronounced than that elicited by the PP chemical

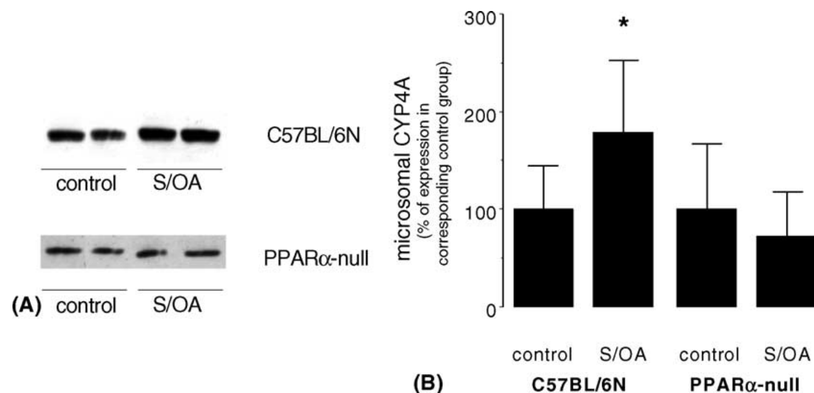


Fig. 6. (A) Representative immunoblots of CYP4A proteins in hepatic microsomal fractions from C57BL/6N wild-type and PPARα null mice that received control and S/OA-diets for 21 days. (Panel B) Microsomal CYP4A expression in mouse liver ($n = 4$ per group); $^{*}P < 0.05$.

clofibric acid, but the diet selectively upregulated hepatic CYP4A1 and CYP4A2 mRNAs. Dietary administration of clofibric acid induced CYP4A protein and CYP4A1/2 mRNAs to a significantly greater extent than that produced by the S/OA-diet, and also induced CYP4A3 and the β-peroxisomal AOX gene. These findings are consistent with the well established potency of clofibric acid and other chemical PPs toward the range of PPARα-inducible genes [26–29]. Combined intake of the S/OA-diet and clofibric acid mobilised hepatic lipid and induced CYPs 4A and AOX to an extent similar to that elicited by clofibric acid alone.

Peroxisomal β-oxidation mediated by genes such as AOX, appears to modulate the extent of hepatic lipid infiltration. Lipid levels in livers of AOX-null mice are reportedly higher than in control animals and are increased further on fasting (which stimulates hydrolysis of stored lipids in adipose tissues for transport to the liver where they can be utilised as an energy source) [30]. Consistent with this finding, mice fed lipotrope-deficient diets that are high in fat accumulated excessive amounts of hepatic lipid unless they received concurrent treatment with the PP chemical Wy14,643 [27]. In the present study, activation of AOX by the combination of clofibric acid and S/OA-diet prevented the accumulation of lipid in liver associated with intake of the S/OA-diet alone. This finding supports the contention that enhanced lipid β-oxidation capacity after clofibric acid treatment is critical in preventing hepatic lipid infiltration following ingestion of high-fat diets [27].

Evidence of a role for PPARα in CYP4A upregulation was obtained by feeding the S/OA-diet to PPARα null mice, which are unresponsive to PP chemicals [28]. Hepatic lipid deposition was observed in these mice after intake of the S/OA-diet but CYP4A was not increased. By comparison, hepatic CYP4A expression was increased to two-fold of control in wild-type C57BL/6N mice by the S/OA-diet. Taken together, the present studies indicate that upregulation of hepatic CYPs 4A by the S/OA-diet occurred in wild-type rats and mice, but not in PPARα-null mice. Lipid deposition was evident in rodents that received the S/OA-diet.

The present study implicates PPARα in the upregulation of CYPs 4A after intake of the S/OA-diet but also indicates that the diet and the PP chemical clofibric acid exert differential effects on gene expression. Whereas clofibric acid induced AOX and all three rat CYPs 4A, the S/OA-diet selectively induced CYP4A1 and CYP4A2. In view of these findings we measured PPARα protein in rat liver after dietary manipulation. Receptor expression was decreased substantially in rat liver after ingestion of the S/OA diet but was strongly upregulated by clofibric acid; the latter finding is consistent with a previous study in liver cell lines [31]. In contrast, expression of RXRα, the transcription partner of PPARα, was significantly decreased by both clofibric acid and the S/OA-diet. Thus, the intrahepatic availability of PPARα and RXRα was decreased by the S/OA-diet, which may contribute to the preferential induction of CYPs 4A1/2 observed in the present study. It is feasible that the observed effects of clofibric acid may be due in part to greater hepatic PPARα expression after treatment with the chemical.

Early studies suggested that CYP4A and peroxisomal β-oxidation enzymes are coregulated by clofibric acid and other PP chemicals [26,32]. However, the differential responsiveness of PPARα-inducible genes to PPARα ligands has been documented more recently [29,33]. Belury et al. have related the differential induction of hepatic CYP4A1, AOX and fatty-acid binding protein mRNAs to blood concentrations of the PP chemical Wy14,643. CYP4A1 induction was maximal at much lower circulating concentrations of the chemical than those required for AOX induction [29]. Thus, the differential effect of the S/OA-diet on PPARα-inducible gene expression resembles recent findings from detailed studies with PP chemicals. Moreover, the present study indicates that diet may be a significant factor that modulates the expression of PPARα, RXRα and possibly other trans-activating proteins.

TBARs were increased in liver after feeding of the S/OA-diet, which could be due to increased hepatic activity of radical-generating enzymes that utilise lipids as substrates, such as CYPs 4A. Supplementation of the S/OA-diet with vitamin E prevented lipid peroxide accumulation in liver but did not influence the induction of CYP4A protein produced by the diet alone. Thus, lipid hydroperoxides do not appear to have a direct role in the upregulation of CYPs 4A by the diet.

The growth hormone-responsive CYP2C11 was down-regulated in microsomal fractions from S/OA-treated rat liver. Growth hormone signalling is mediated by the JAK2-STAT system [34] and recent studies have demonstrated that JAK2-STAT5b signalling is inhibited by PPARα, which could well account for the impaired hepatic expression of growth hormone-inducible genes in the presence of PPARα ligands [35]. The present findings that CYP2C11 is down-regulated by ingestion of the S/OA-diet may be due to modulation of STAT5b by activated PPARα. Indeed, potent activation of PPARα by clofibric acid suppressed CYP2C11 expression to a greater extent than the S/OA-diet.

Although CYP4A appears to have a minimal role in drug biotransformation, its upregulation may be significant for the pathogenesis of fatty liver, which may progress to more severe injury. CYP4A supports lipid peroxidation [8], which could contribute to liver damage after lipid accumulation, and the formation of ω-hydroxylated eicosanoids [36], which modulate vasoactive tone and may contribute to the increased incidence of hypertension in subjects with fatty liver [10,37]. The present study establishes that lipids may be generated in situ from ingested carbohydrates and, like direct ingestion of lipid from high-fat diet, activate the expression of the PPARα-regulated CYPs 4A in liver.

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