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Commentary

# Cytochrome P450 omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases

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ABSTRACT

The cytochrome P450 gene 4 family (CYP4) consists of a group of over 63 members that ω-hydroxylate the terminal carbon of fatty acids. In mammals, six subfamilies have been identified and three of these subfamily members show a preference in the metabolism of short ($C_7$–$C_{10}$)-CYP4B, medium ($C_{10}$–$C_{16}$)-CYP4A, and long ($C_{16}$–$C_{26}$)-CYP4F, saturated, unsaturated and branched chain fatty acids. These ω-hydroxylated fatty acids are converted to dicarboxylic acids, which are preferentially metabolized by the peroxisome β-oxidation system to shorter chain fatty acids that are transported to the mitochondria for complete oxidation or used either to supply energy for peripheral tissues during starvation or in lipid synthesis. The differential regulation of the *CYP4A* and *CYP4F* genes during fasting, by peroxisome proliferators and in non-alcoholic fatty liver disease (NAFLD) suggests different roles in lipid metabolism. The ω-hydroxylation and inactivation of pro-inflammatory eicosanoids by members of the CYP4F subfamily and the association of the *CYP4F2* and *CYP4F3* genes with inflammatory celiac disease indicate an important role in the resolution of inflammation. Several human diseases have been genetically linked to the expression CYP4 gene polymorphic variants, which may link human susceptibility to diseases of lipid metabolism and the activation and resolution phases of inflammation. Understanding how the *CYP4* genes are regulated during the fasting and feeding cycles and by endogenous lipids will provide therapeutic avenues in the treatment of metabolic disorders of lipid metabolism and inflammation.



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*Abbreviations:* SCFA, short chain fatty acid ($C_7$–$C_9$); MCFA, medium chain fatty acid($C_{10}$–$C_{16}$); LCFA, long chain fatty acid ($C_{18}$–$C_{22}$); VLCFA, very long chain fatty acid (>$C_{22}$); ACC1, acetyl-CoA carboxylase; ACSI, acetate CoA synthetase; ACOX, acyl-CoA oxidase; ACOT, acyl-CoA thioesterase; $b_5$, cytochrome $b_5$; SCD-1, stearoyl CoA desarturase 1; LBP, L-Bifunctional protein; DBP, D-Bifunctional protein; TG, triglyceride; PL, phospholipid; CE, cholesterol ester; WE, wax ester; VLDL, very low density lipoprotein; steatosis, fatty liver; steatohepatitis, fatty liver with inflammation; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; 20-HETE, 20-hydroxyeicosatetraenoic acid; ωHEET, 20-hydroxyeicosatrenoic acid; PP, peroxisome proliferators; HNF4α, hepatocyte nuclear factor 4; PPARα, peroxisome proliferator activated receptor α; SREBP, sterol regulatory element binding protein; ChREBP, carbohydrate responsive element binding protein; $LTB_4$, leukotriene $B_4$; APR, acute phase response; PUFA, polyunsaturated fatty acids; LPS, lipopolysaccharide; IL-1, interleukin-1; IL-8, interleukin-8; L-PK, liver pyruvate kinase; CPT1, carnitine palmitoyltransferase1; ROS, reactive oxygen species.

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## 1. Introduction

The CYP4 family of cytochrome P450s catalyzes the ω-hydroxylation of saturated, branched chain, and unsaturated fatty acids, which include the physiological important eicosanoids, prostaglandins, leukotrienes and arachidonic acid. These ω-hydroxylated fatty acids are further metabolized by cytosolic alcohol and aldehyde dehydrogenase to dicarboxylic acids (DCA), which are metabolized by the peroxisome β-oxidation system (Fig. 1). Peroxisomes produce shorter chain fatty acids by several cycles of the β-oxidation system [1–3]. Shorter chain fatty acids can be excreted in the urine as sebacic, adipic, or suberic acid, completely oxidized by the mitochondria β-oxidation system or metabolized by the peroxisome β-oxidation system to succinate and acetyl CoA. Succinate is a gluconeogenic precursor while acetyl CoA is converted to acetate, which can serve either as an energy source for peripheral tissues or possibly lipogenic precursors for cholesterol or fatty acid synthesis in the liver. Although, ω-hydroxylation pathway is a minor pathway in the metabolism of fatty acids (4–15%) [4], its importance is dramatically increased during starvation, by ethanol, hypolipidemic drugs, peroxisome proliferators, and in different metabolic diseases.

The recent identification of CYP4 polymorphic variants associated with different diseases suggests that these fatty acid ω-hydroxylase enzymes may play important roles in human susceptibility to genetic, environmental, and metabolic diseases associated with lipid metabolism [5–7]. The functional role and importance of the different CYP4 subfamily members in lipid metabolism and in different metabolic states are largely unknown.

There are several excellent reviews on the function of the CYP4 members in inflammation, drug metabolism, and eicosanoid metabolism [5–8]. Therefore, this commentary will focus only on identifying, defining and suggesting the multiple roles and fate of ω-hydroxylated fatty acids in lipid metabolism catalyzed by the peroxisome β-oxidation system to anabolic or catabolic substrates in the fed and fasting states. Finally, I will discuss the possible role of CYP4 polymorphic variants in lipid metabolism and inflammatory disorders, and how CYP4 gene regulation might provide new treatments in the management of human diseases associated with abnormal lipid metabolism by CYP4 members.

## 2. Synthesis, fate and function of omega hydroxylated fatty acids

In mammals six CYP4 gene subfamilies have been identified CYP4A, CYP4B, CYP4F, CYP4V, CYP4X, and CYP4Z [5–8]. Three of these subfamily (CYP4A, CYP4B, and CYP4F) members have been shown to ω-hydroxylate saturated, branched, and unsaturated fatty acids, and eicosanoids. Members of the CYP4B subfamily preferentially metabolize short chain fatty acids (SCFA, $C_7$–$C_9$) while members of the CYP4A subfamily members prefer to metabolize medium chain fatty acids (MCFA, $C_{10}$–$C_{16}$) while members of the CYP4F subfamily metabolize long chain fatty acids (LCFA) and very long chain fatty acids (VLCFA, $C_{18}$–$C_{26}$) fatty acids. In contrast, the fatty

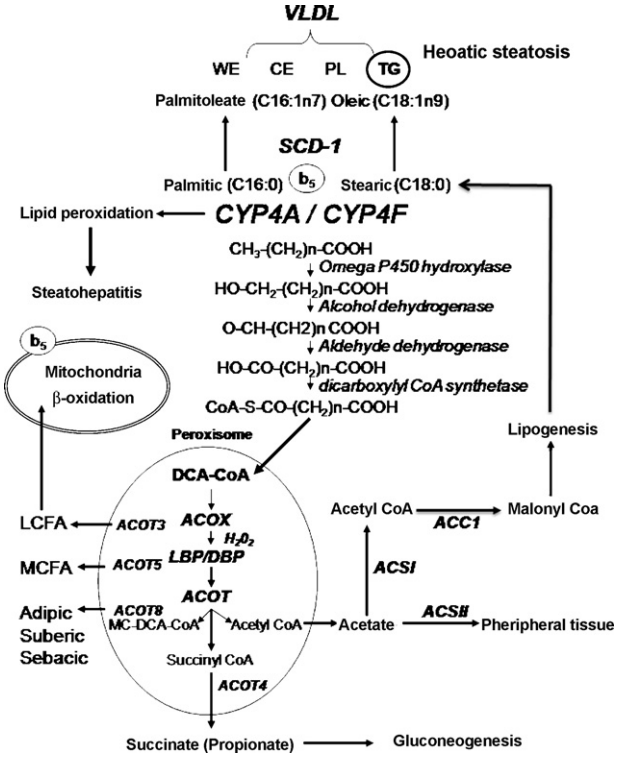


**Fig. 1 – CYP4A and CYP4F function in liver fatty acid metabolism. Fatty acids are delivered to hepatocytes for catabolism by the mitochondria or peroxisome β-oxidation systems or be used for the synthesis and export of triglycerides (TG), phospholipid (PL), cholesterol esters (CE), as very low density lipoprotein (VLDL) particles. Medium and short chain length fatty are transported as acylcarnitine derivatives into mitochondria for complete β-oxidation to $CO_2$. Long and very long chain fatty acid CoA esters are transported into the peroxisome where they undergo chain shorting and as acylcarnitine derivatives to the mitochondria for complete oxidation or as free fatty acids by thioesterase 3, or 5 (ACOT3, 5). Under normal conditions (5–10%) fatty acids are converted to dicarboxylic acids CoA (DCA-CoA) by CYP4A or CYP4F ω-hydroxylation. During starvation the ω-oxidation of fatty increases dramatically (~40%) by through PPARα induction of CYP4A genes. The dicarboxylic acids, adipyl-CoA ($C_6$), sebacyl-CoA ($C_8$) or suberyl-CoA ($C_{10}$) are exported from peroxisome by the action of acyl CoA thioesterase 8 (ACOT8) or when ACOT8 is inhibited by free CoASH, the fatty acid-CoA is converted to succinyl-CoA, which is exported as succinate after removal of CoA by ACOT4. Succinate can function as anaplerotic intermediate in the mitochondria for gluconeogenesis during starvation and excess acetate produced from acetyl-CoA can be used by peripheral tissues after conversion to acetyl-CoA by acetate CoA synthetase (ACSII). In the fed state long chain (LCFA) and very long chain (VLCFA) fatty acids are metabolized by peroxisome β-oxidation to shorter chain products, which can be incorporated into phospholipids, cholesterol esters or triglycerides for export as Very low density lipoprotein (VLDS) and storage in peripheral tissues. Excessive acetate in the cytosol is converted to**

acid specificity of the CYP4AV, CYP4X, and CYP4Z subfamily members has not been determined.

### 2.1. CYP4B

Members of the CYP4B subfamily have been shown to efficiently ω-hydroxylate SCFA and short chain hydrocarbons with a specificity of ω/ω-1 hydroxylation of 7.4 for octanoic acid ($C_{8:0}$) and 1.1 for decanoic acid ($C_{10:0}$) [9]. CYP4B1 is highly expressed in lung, peripheral tissues and liver where it has been reported to function in the activation of pro-carcinogens [5]. Interestingly, CYP4B1 functions in the cornea in the metabolism of arachidonic acid to 12-HETrE (12-hydroxy-5,8,14-eicosatrienoic acid), which has both inflammatory and angiogenic activity [10]. The induction of the *CYP4B1* gene by hypoxia may explain its elevated expression in bladder and lung cancer as well as during injury of the ocular surface [10–12]. It is likely that the short chain fatty acid hydroxylation products catalyzed by CYP4B are converted to dicarboxylic acids, which are directed to the mitochondria for complete β-oxidation. Complete oxidation of short chain fatty acids would increase oxidative phosphorylation and provide necessary energy for repair and proliferation.

### 2.2. CYP4A

Members of the CYP4A family are by far the best understood ω-fatty acid hydroxylase in regard to their induction by peroxisome proliferators and regulation by fasting, high fat diet, ethanol consumption and in diabetes in rodents. The human CYPA11 P450 efficiently and selectively ω-hydroxylates lauric acid ($C_{12:0}$) but shows less selectivity in the metabolism of palmitic acid ($C_{16:0}$) where both ω and ω-1 products are produced (Fig. 2) [13,14]. Although CYP4A11 is able to ω-hydroxylate arachidonic acid to 20-HETE when compared to the metabolism of lauric acid the rate of metabolism is 10–100-fold less, suggesting that the primary function of *CYP4A* genes, at least in the liver, is to metabolize MCFAs. It is known that

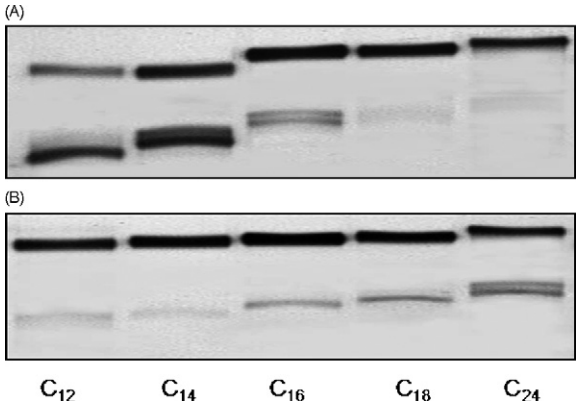


**Fig. 2 – Metabolism of saturated fatty acids by Baculovirus expressed human CYP4A11 and CYP4F2 P450. Saturated fatty acid ω- and ω-1 hydroxylation was measured in a reaction mixture of 20 μg/ml dilauroylphosphatidylcholine, 100 pmol/ml CYP4A11 (A) or CYP4F2 (B), 1000 pmol/ml of cytochrome P450 reductase in 100 mM potassium phosphate buffer pH 7.4, 5 mM $MgCl_2$, 10 mM sodium isocitrate, 1 IU of isocitrate dehydrogenase and 1 mg/ml methyl-β-cyclodextrin containing 50,000 dpm of $C^{14}$ labeled fatty acids (50 mCi/mmol) at final concentration of 200 μM. Reactions were allowed to proceed for 30 min at 37 °C then terminated and fatty acid metabolites isolated and spotted on Silica linear K pre-adsorbent plates that were developed in hexane/ethyl acetate/isooctane/acetic acid/water (50/50/48.5/18.5/40) then exposed to X-ray film. The separation of ω- (lower band) and ω-1 (upper band) hydroxylation metabolites of lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and lignoceric ($C_{24:0}$) fatty acids are indicated.**

production of 20-HETE from arachidonic acid by CYP4 members may be more important in peripheral tissues where 20-HETE cell signaling effects include modulation of ion channels and transporters, mitogenesis, activation of tyrosine kinase and phosphatidylinositol 3-kinase pathways [15]. The importance of CYP4A P450s in the metabolism of MCFAs is evident during starvation, caloric restriction, and in animals fed a high fat diet, which mimics starvation-induced lipolysis and excessive fatty acid transport to the liver. In these situations, there is a dramatic induction of the *CYP4A* genes, which may function to not only prevent lipid toxicity, but also serve as a source of nutrients for peripheral tissue during starvation. MCFAs in hepatocytes are transported into the peroxisome as free fatty acids or as dicarboxylic acid after CYP4A ω-hydroxylation and esterified by peroxisomal acyl-CoA synthetase. MCFA acyl-CoAs undergo two to three rounds of peroxisome β-oxidation producing succinyl-CoA and acetyl-CoA (Fig. 1) [3,16]. These products are converted by several acyl-CoA thioesterases (ACOT), which can catalyze the hydrolysis of CoA esters of different chain length fatty acids including succinate. Succinate can be directly used as an anaplerotic intermediate for gluconeogenesis while released acetate can be taken up and oxidized by other tissues in the same way as ketone bodies are used by extrahepatic tissues for energy production. During starvation or administration of

**acetyl-CoA by hepatocyte acetate CoA synthetase I (ACSI) and then converted to malonyl CoA for fatty acid and cholesterol synthesis by acetyl CoA carboxylase (ACC1). *CYP4F* gene induction by insulin is mediated by sterol regulatory element binding protein (SREBP) and saturated fatty acid CoA activation of hepatocyte nuclear factor 4α (HNF4α). In the presence of excessive fatty acids insulin will activate SREBP-1c increasing the synthesis of stearoyl CoA desaturase 1 (SCD-1) and conversion of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids to palmitoleate ($C_{16:1}$) and oleic ($C_{18:1}$), respectively, which are stored as triglycerides. Both CYP4 P450 and SCD-1 use cytochrome $b_5$ and cytochrome $b_5$ reductase ($b_5$) in their catalytic cycle. The induction of *CYP4A* genes by high fat diet with and an increase in SCD-1 and suppression of *CYP4F* genes may prevent the liver from lipotoxicity at the expense of steatosis and development of steatohepatitis. ACOX (acyl-CoA oxidase), LBP (L-Bifunctional protein), DBP (D-Bifunctional protein), TG (triglycerides), PL (phospholipid), CE (cholesterol ester), WE (wax ester).**

hypolipidemic drugs there is a rapid proliferation of peroxisomes in rodents but not humans. The proliferation of peroxisomes requires expression of CYP4A genes suggesting that the ω-hydroxylation of the CYP2C arachidonic acid metabolite epoxyeicosatrienonic acid (EET) to ω-hydroxylated eicosatrienoic acid (ωHEET) [17] can induce peroxisome proliferation in rodents [18]. In humans, the *CYP4A11* and *CYP4F2* genes are not induced by peroxisome proliferators (PP), and therefore the absence of peroxisome proliferation may be due to decreased levels of ωHEET, which is a high affinity ligand in the activation of PPARα. In humans the hypolipidemic effect of peroxisome proliferators is not mediated through PPARα activation but through the suppression of HNF4α by PPs-CoAs. HNF4α controls genes involved in the production of lipoproteins [19]. Thus the activation of PPARα in rodents by ωHEET and suppression of HNF4α in humans by PP-CoA may explain the absence of peroxisome proliferation in humans. It is not known whether the human CYP4A11 or CYP4F2 P450s can ω-hydroxylate EET to ωHEET, even though CYP4F2 P450 ($K_m$ 24 μM) has a 10-fold greater affinity for arachidonic acid than CYP4A11 ($K_m$ 228 μM) [20]. Because peroxisome proliferators repress the expression of the *CYP4F2* gene [21], it is likely that the absence of CYP4A11 induction by PPs with reduced expression of CYP4F2 results in the reduced formation of ωHEET.

### 2.3. *CYP4F*

The CYP4F P450s ω-hydroxylate a variety of long chain (LCFA) and very long chain saturated (VLCFA) (Fig. 2), unsaturated and branched chain fatty acids, vitamins with long alkyl side chains, and the physiologically important leukotrienes (LT), prostaglandins (PG) and hydroeicosatetraenoic acids (HETE) [13,14,20,22,23]. The human CYP4F P450s metabolize and inactivate the pro-inflammatory leukotriene $B_4$ ($LTB_4$) [22], with the myeloid expressed CYP4F3A having a 2-fold greater affinity for $LTB_4$ than CYP4F2 expressed in liver, kidney, and skin, but not myeloid cells. A splice variant of the CYP4F3 gene produces CYP4F3B expressed in liver and has a similar affinity as CYP4F2 for $LTB_4$ [24]. Both the CYP4F3B ($K_m/V_{max}$ 0.6) and CYP4F2 P450 ($K_m/V_{max}$ 0.3) can metabolize arachidonic acid to 20-HETE while CYP4F3A ($K_m/V_{max}$ 0.06) has little activity towards arachidonic acid. Both CYP4F3B ($K_m$ 22 μM) and CYP4F2 ($K_m$ 24 μM) have greater affinity for arachidonic acid than CYP4A11 ($K_m$ 228 μM) indicating that these CYP4F P450s are quantitatively more important in the metabolism of 20-HETE in humans. Besides ω-hydroxylating other pro-inflammatory eicosanoids, 5-HETE, 12-HETE and 8-HETE, CYP4F P450s can metabolize the anti-inflammatory lipoxins, $LXA_4$, and $LXB_4$. The ability of CYP4F3 and CYP4F2 to ω-hydroxylate both pro- and anti-inflammatory leukotrienes indicate they may function both in the activation and resolution phases of the inflammatory response. Furthermore, the ability of CYP4F2, 4F3A and 4F3B to ω-hydroxylate $C_{18}$ fatty acid epoxides and EETs suggests that they may be important in the synthesis of ωHEET [25]. CYP4F2 and CYP4F3 are also able to omega hydroxylate the vitamin E, tocopherol phytyl side chain metabolized by the peroxisome β-oxidation system to 3′-carboxychromanol metabolite. The metabolically inactive γ-tocopherol is preferentially ω-hydroxylated instead of the physiologically active vitamin E α-tocopherol analogue, suggesting a novel mechanism for the retention of the biologically active vitamin E [23]. It is probable that CYP4F P450s are also able to metabolize vitamin K and Coenzyme Q that have long isoprenoid side chains with accessible ω-terminal carbon.

In contrast to liver expressed *CYP4F3B*, *CYP4F2*, *CYP4F11* genes, *CYP4F8* is expressed in prostate, seminal vesicles, and several extrahepatic tissues where it metabolizes prostaglandin endoperoxides, $PGH_1$ and $PGH_2$, to 19-OH $PGE_1$ and 19-OH-$PGE_2$ by PGE synthase mediated isomerization of $PGH_1$ and $PGH_2$ [26]. CYP4F8 has little activity towards $LTB_4$, $PGD_2$, $PGE_1$, $PGE_2$ or $PGF_2$, but can ω-1 hydroxylate arachidonic acid. The reason for the large amounts of PGE compounds in the human seminal fluid compared to other tissues is unknown as well as the reason why there is a large inter-individual variation in the metabolism of PGE to 19-OH-PGE [27]. PGs have a local effect on the pregnant uterus and are used to induce cervical ripening and abortion. PGEs activate PGE receptors and 19R-OH-$PGE_2$ is an agonist of the $PGE_2$ receptor, but less potent than $PGE_2$. PGE has a powerful effect on cytokine production in antigen presenting cells, can induce tolerance in the cervix to antigen, and plays a significant role in the resolution phase of inflammation [28]. $PGE_2$ and hydroxylated $PGE_1$ are known to stimulate synthesis of angiogenic factors, promote vascular sprouting, migration, tube formation and enhance endothelial cell survival. It will be of interest to determine if the *CYP4F8* gene is expressed in placenta tissue and liver as well in the fetal aorta during ductus arteriolous closure [27].

### 2.4. *Other CYP4Fs*

Unlike most CYP4F P450s, CYP4F11 does not catalyze the metabolism of eicosanoids, but is active in drug metabolism of many drug substrates that include benzphetamine, ethylmorphine, chlorpromazine, imipramine, and erythromycin [29]. CYP4F11 can ω-hydroxylate 3-OH-stearic and palmitic acid similar to CYP4F2 [13]. CYP4F11 is expressed in liver, kidney, heart, brain and skeletal muscle. Of note was the increased expression of the *CYP4F11* gene in ovarian and colon tumor tissues [30,31] and the finding that the *CYP4F11* promoter has an estrogen receptor α responsive site [32].

CYP4F12 is rather unique with respect to other human CYP4Fs in that it has activity towards eicosanoids, drugs and xenobiotics. CYP4F12 is expressed the liver, kidney, the placenta at term, seminal vesicles, prostate, and epidermis [33]. CYP4F12 is prominently expressed in the epithelial cell of the gastrointestinal tract. CYP4F12 metabolizes the novel antihistamine prodrug ebastine to the active drug carebastine via hydroxylation in the intestine. CYP4F12 may also be the major CYP4F P450 catalyzing the initial oxidative O-demethylation of the antiparasitic prodrug pafuranidine (DB289). Similar to CYP4F8 hydroxylation of $PGH_2$ at the ω-1 position, CYP4F12 hydroxylates $PGH_2$ at the ω-2 and ω-3 positions and arachidonic acid at the ω-3 position. The preference of CYP4F8 and CYP4F12 in the ω-1 to ω-3 hydroxylation of eicosanoid substrates is due to a glycine replacing glutamate Glu328Gly at the heme-binding region, which influences the placement of substrates in the CYP4 catalytic pocket in determining positional oxygenation and formation of docosahexanenoic

and eicospentanenoic acid epoxides by these P450s [34]. Both CYP4F8 and CYP4F12 display little activity in the hydroxylation of $LTB_4$, which distinguishes them from other human CYP4F members.

Recent identification and isolation of a seventh member of the human CYP4F subfamily, CYP4F22 was found to be expressed in epidermis keratinocytes, testes, kidney, brain, placenta, bone marrow, skeletal muscle and liver [35]. Although the catalytic activity of CYP4F22 has not been determined, it is likely to either ω-hydroxylate trioxilin A3 or acylceramides, which are hydrophobic components of the extracellular lamellar membranes that maintain the skins water barrier [36].

Unlike the human CYP4A and CYP4F P450s whose catalytic activity towards a variety of fatty acid and eicosanoid substrates has been determined, there are few studies that have determined the functional role of the CYP4V, CYP4X and CY4X P450s in fatty acid metabolism [8]. CYP4X1 seems to be exclusively expressed in the brain stem, hippocampus, cortex, cerebellum and the vascular endothelial cells where its neurovascular physiological function is unknown [37]. It is not know whether CYP4X1 can produce 20-HETE and act as a vasoconstrictor or metabolize long chain fatty acids such as docosahexanoic acids, which are an abundant fatty acid in the brain that functions in metabolism and cell signaling. Similarly, it is not known if the novel CYP4Z1 P450, exclusively expressed in mammary gland and over-expressed in breast cancer, metabolizes fatty acids and eicosanoids [30,38]. In addition, the CYP4V2 gene was also found to be over expressed in tumors of the colon and in lymph node metastasis [31]. The CYP4V2 is prominently expressed in the retinal, kidney, lung and liver of humans. In patients with corneoretinal dystrophy, a disruption of the *CYP4V2* gene leads to the accumulation of triglycerides and cholesterol with a decrease in the metabolism of n-3 polyunsaturated fatty acids [39]. It is interesting that all these organs have a high content of lipids and fatty acids, and therefore the CYP4V2, CYP4X1 and CYP4Z1 may function to prevent lipotoxicity.

## 3. The different roles for CYP4 genes in hepatic fatty acid metabolism

CYP4F members preferentially metabolize LCFA and VLCFA while members of the CYP4A and CYP4B subfamilies metabolize MCFA and SCFA, respectively. Omega hydroxylated LCFA like the MCFA and SCFA are converted to dicarboxylic acids by the sequential action of cytosolic alcohol and aldehyde dehydrogenase. The roles of different chain length ω-hydroxylated fatty acids in lipid metabolism are indicated by increase ω-hydroxylation of MCFA by CYP4A P450s induced in starvation, and by peroxisome proliferators, while decreased *CYP4F* genes expression by peroxisome proliferator and during starvation results in reduced ω-hydroxylation of LCFA and VLCFA. It has been recently reported that the *CYP4F2* gene is activated by lovastatin through sterol regulatory element binding protein (SREBP-2), which is known to induce lipogenesis [40]. We have recently found that the *CYP4F2* gene is induced by the anabolic hormone insulin in primary human hepatocytes, suggesting a differential regulation of the *CYP4A* and *CYP4F* genes during the starvation and feeding cycles. Unlike the ω-hydroxylated MCFAs, which are β-oxidized to succinyl-CoA an anaplerotic intermediate in gluconeogenesis, and acetate that can be efficiently used by peripheral tissue similar to ketone bodies, the omega hydroxylation of long chain fatty acids would produce only shorter chain fatty acids and acetate. Excessive acetate in the hepatocyte cytosol [41] can be used for the synthesis of cholesterol and fatty acids with malonyl-CoA inhibiting the mitochondria carnitine palmitoyltransferase1 (CPT1) fatty acid uptake and therefore blocks β-oxidation. Dicarboxylic acids are preferentially metabolized by the peroxisome β-oxidation system since the $K_m$ value for dicarboxylic acids by the mitochondria are 15–40-fold higher than those by the peroxisomes [42]. Furthermore, branched and long chain saturated and unsaturated fatty acids are preferentially metabolized by the peroxisome. The transportation of fatty acids into the peroxisome is different for MCFA, LCFA, and VLCFAs. MCFAs are transported as free acids, which are esterified in peroxisomes to CoA derivatives, while LCFA and VLCFAs are transported by ABC transporters as CoA derivatives. In addition β-oxidation in the peroxisome does not completely oxidize fatty acid substrates, but produces shorter chain fatty acids which are transported from the peroxisomes and either completely oxidized by the mitochondria β-oxidation or used for synthesis of other fatty acid as seen in the conversion of C24:6n-3 to docosahexanenoic acid (C22:6n-3) [2,43]. Fatty acids are transported from peroxisomes either by conversion into carnitine esters by peroxisomal carnitine acetyltransferase (cat2p) as is the case for acetyl and propionyl-CoA thioesters or as free fatty acids by several acyl-CoA thioesterases (ACOT). These acyl-CoA thioesterases are capable of catalyzing the hydrolysis of a wide range of substrates, including CoA esters of long-, medium- and short-chain fatty acids, bile acids, branched chain fatty acids, succinate, and acetate [1–3].

Omega hydroxylated fatty acids and eicosanoids have several metabolic fates dependent upon the cell type and *CYP4* gene expressed. In the liver, ω-hydroxylated fatty acids can be metabolized and used for energy production, lipogenesis, the synthesis of structural lipids, and production of fatty acids that function in the regulation of hormone nuclear receptors (HNR) [44]. Omega hydroxylated eicosanoids modulate both the activation and resolution phases of inflammation as well as functioning as vital regulators of cell signaling. Therefore, the tissue specific expression of specific *CYP4A* gene would determine the type of ω-hydroxylated fatty acid or eicosanoid produced.

## 4. The role of CYP4 omega P450 hydroxylase in human diseases

The CYP4 genes have an important yet poorly understood role in human disease. The diverse array of ω-hydroxylated fatty acids produced by different CYP4 family members have different functional roles in metabolism, cell signaling, inflammation and lipid structure. The importance of ω-hydroxylated fatty acids and eicosanoids in human disease is beginning to be understood as evident by the role 20-HETE has in hypertension and vascular disease. In addition, the

association of CYP4F enzymes in the production of ω-hydroxylated leukotrienes and prostaglandins in inflammation, and their association with celiac disease as well as the role of CYP4F ω-hydroxylase in the synthesis of ω-O-acylceramides in ichythyoses skin disorders further implicates *CYP4* genes with these diseases. The strong association of celiac disease with several autoimmune liver disorders, cirrhosis, hemochromatosis, and non-alcoholic fatty liver disease (NAFLD) diseases indicates that *CYP4* genes may have a pivotal role in the ω-hydroxylation of fatty acids in lipid metabolism and pro-inflammatory leukotrienes in inflammation of the liver in fatty liver disease [7,35,45–47].

### 4.1. *Role of CYP4 omega hydroxylase in hypertension and vascular system*

Significant progress has been made in understanding the biological function of ω-hydroxylated arachidonic acid (20-HETE) and the molecular mechanism that determine the intracellular levels of this eicosanoid. 20-HETE functions in the kidney to regulate salt and water reabsorption by inhibiting the $Na^+/K^+$-ATPase and $Na^+$–$K^+$–2Cl co-transporter in the proximal tubule and medullary thick ascending limb, respectively [46]. 20-HETE also functions in the kidney to regulate vascular tone by inhibiting the $Ca^{2+}$ activated $K^+$ channel in vascular smooth muscle cells of the renal micro-circulation. Even though most studies suggest that increased levels of 20-HETE in the renal vasculature underlie the development of hypertension, in the salt sensitive rodent deficiency of 20-HETE was associated with the development of hypertension [15]. The renal induction of 20-HETE was shown to lower blood pressure in the salt-sensitive rat, the obesity-induced and angiotensin II-dependent hypertensive rat, and the stroke-prone spontaneously hypertensive rat. Furthermore blockage of 20-HETE production induces hypertension in salt-resistant strains of rats. Although 20-HETE is a vasoconstrictor, which is thought to act as a second messenger for contractile agonist such as angiotension II, norepinephrine and endothelin, an increase in 20-HETE is not always correlated with an increase in blood pressure. 20-HETE has a dual role in the regulation of blood pressure by its ability to induce vasoconstriction and hypertension while also inhibiting sodium re-absorption and therefore blood pressure. Therefore, reduction in 20-HETE levels in the kidney would decrease vascular constriction but enhance sodium re-absorption, while increased 20-HETE levels would increase vascular constriction and hypertension and reduce blood pressure by decreasing sodium re-absorption. In humans there are several CYP4 ω-hydroxylases that can convert arachidonic acid to 20-HETE. The CYP4F2, 4F3b, 4F11, 4F12, and CYP4A11 isoforms are expressed in human kidney and function in 20-HETE production. Both the CYP4A11 and the CYP4F2 are the major kidney CYP4 P450s that metabolize arachidonic acid to 20-HETE. CYP4F2 seems to be quantitatively more important than CYP4A11 in 20-HETE production in the kidney, even though studies have linked a C-to-T mutation in the *CYP4A11* gene with reduced 20-HETE production and elevated blood pressure in three large human population studies [48]. Recently, a polymorphic variant of the *CYP4F2* gene has been identified with an amino acid substitution M433 that shows reduced activity in the ω-hydroxylation of arachidonic acid to 20-HETE [49]. The decrease in 20-HETE excretion in salt-sensitive hypertensive patients may be explained by the expression of the CYP4F2 M433 variant with reduced ω-hydroxylation of arachidonic acid, suggesting that functional polymorphisms may influence reduced 20-HETE production in carriers of low activity alleles for the *CYP4A11* and *CYP4F2* genes. It was of interest that the three *CYP4F2* variants (W12/M433, G12/V433, G12/M433) did not show a reduced ability to convert $LTB_4$ to 20-OH $LTB_4$ compared to the wild type CYP4F2 (W12/V433) [49]. It will be of particular interest to determine the regional expression of the CYP4A11 and CYP4F2 genes in the human kidney and whether increased expression of the CYP4A11 in the preglomerular arteries increase 20-HETE production, vasoconstriction, and hypertension, while increased expression of the CYP4F2 in the thick ascending limb would produce 20-HETE that decrease sodium re-absorption and thus blood pressure. Defining the regional expression of the CYP4A11 and CYP4F2 genes in the human kidney will not only be important in determining the association and function of CYP4 polymorphisms in hypertension, but also provide novel avenues for the treatment of hypertension by modulating the differential expression of these genes.

### 4.2. *CYP4 genes in inflammation*

Systemic and local inflammation in response to infection or injury is characterized by migration of polymorphonuclear cells, PMNs (neutrophils, eosinophils, basophils) to sites of damages to eliminate infected or injurious agents. Pro-inflammatory mediators, eicosanoids, chemokines, cytokines and cell adhesion molecules, activate inflammation by initiating the recruitment of PMNs to sites of injury while catabolism of these mediators initiate the resolution phase of inflammation and return to normal homeostasis. The liver plays a pivotal role in the systemic inflammatory response by the production and elimination of inflammatory mediators, and the clearance of endotoxin and bacteria by endogenous macrophages (Kupffer cells).

The response of the liver to sepsis leads to profound changes in liver metabolism resulting in increased liver weight and hepatic protein content. The hepatocyte modifies its metabolic pathways towards amino acid uptake, ureagenesis, gluconeogenesis and synthesis of acute phase response proteins (APR) that functions as coagulant factors, complement factors, anti-proteolytic enzymes, and modulators of the immune response [50].

The liver plays a central role in the inactivation and elimination of pro-inflammatory $LTB_4$ and the cysteinyl-leukotrienes ($LTC_4$, $LTD_4$, and $LTE_4$) by CYP4-dependent ω-hydroxylation and peroxisome β-oxidation. Enhanced synthesis of $LTB_4$ in the liver by activated Kupffer cells can lead to recruitment and activation of granulocytes, macrophage, and T-cells by $LTB_4$ binding their $LTB_4$ receptors (BLT1) [51]. Elevated hepatic levels of $LTB_4$ can also activate the hepatocyte BLT2 receptors, which possibly initiate synthesis of cysteinyl-leukotrienes through transcellular cooperation between Kupffer or sinusoidal endothelial cells and hepatocytes. The cysteinyl-LTs have potent vasoconstrictor, proliferative, and profibrogenic properties that may participate in

pathophysiologic events associated with both systemic and local inflammation [52]. In humans, the metabolism of $LTB_4$ is quite complex with multiple pathways responsible for the degradation of $LTB_4$ prior to excretion in the urine as 17-, 18-, 19-, and 20-OH $LTB_4$ glucuronide conjugated metabolites [53]. Presently, we have little information on which CYP4F ω-hydroxylate and inactivate which cysteinyl-leukotrienes. The report that $LTC_4$ activates $LTC_4$ receptors and induces the production of the potent neutrophil attaching chemokine IL-8 establishes another important link between pro-inflammatory eicosanoids and the attraction of PMNs [54].

The ω-hydroxylation of leukotrienes by members of the CYP4F subfamily shows considerable differences in substrate specificity and activity. The human neutrophil specific CYP4F3A has a higher affinity for $LTB_4$ (0.64 μM) than the liver specific CYP4F2 (47 μM) and CYP4F3B [55]. The CYP4F11 can ω-hydroxylate $LTB_4$ while CYP4F12 shows no activity towards this eicosanoid. CYP4F3A and CYP4F2 also catalyze the hydroxylation of other pro-inflammatory eicosanoids, including 5-HETE, 12-HETE and 8-HETE as well as the anti-inflammatory leukotrienes, $LTXA_4$ and $LTXB_4$ [6,7]. The ability of human CYP4F P450s to metabolize both pro- and anti-inflammatory leukotrienes and possible cysteinyl-leukotrienes suggest that they have different roles in the activation and resolution phases of the inflammatory response.

There is little information on how the human CYP4F genes are regulated during inflammation and by pro- and anti-inflammatory cytokines, chemokines and eicosanoids. There has been one brief study showing the down-regulation of the *CYP4F2* gene by the pro-inflammatory cytokines TNFα, IL-1 and IL-6, and induction by anti-inflammatory chemokines, IL-10 and IL-13 in human HepG2 cells [56]. Furthermore, there are few studies on how the human *CYP4F* genes are regulated by pro- or anti-inflammatory eicoansoids, even though in human and rat hepatocytes, $LTB_4$ does not induce the *CYP4F* genes. Recently, the induction of the *CYP4F2* gene by retinoic acid in human keratinocytes during hyperproliferative dermatoses suggests a functional role of the *CYP4F2* gene in the resolution phase of inflammation [57]. Indeed the synthesis of anti-inflammatory ReV1 (5S,12R,18R-trihydroxyl eicosapentaenoic acid) by the CYP4F8 and CYP4F12 catalyzed ω-3 hydroxylation of polyunsaturated fatty acid (PUFA) suggests that CYP4F P450s have an important role in the resolution phase of inflammation [34]. Our understandings of how the *CYP4F* genes are regulated during the activation stage of inflammation have largely been through the use of animal models of inflammation. Studies indicate both an induction and repression of selective *CYP4F* genes in rats' administered bacterial endotoxin lipopolysaccharide (LPS) or barium sulfate induced systemic inflammation. In rat hepatocytes, LPS down-regulates the *CYP4F1*, *CYP4F4*, *CYP4F5* and *CYP4F6* genes [7]. Similar results are seen in rat hepatocytes treated with the pro-inflammatory cytokine, interleukin IL-1β. In contrast, incubating rat hepatocytes with pro-inflammatory IL-6 or TNFα cytokines resulted in the induction of CYP4F5 and CYP4F6. Surprisingly, the anti-inflammatory cytokine IL-10, which induces the human *CYP4F2* gene, significantly decreased the rat *CYP4F* genes, suggesting different mechanisms of induction of the human and rodent *CYP4F* genes by pro- and anti-inflammatory cytokines.

Although ω-hydroxylation of $LTB_4$ is the major pathway for inactivation by human and rodent CYP4F P450s other CYP4F members are able to produce 19-hydroxy-$LTB_4$, 18-hydroxy-$LTB_4$ and 17-hydroxy-$LTB_4$. Further studies are necessary to determine whether the 19-hydroxy-$LTB_4$, 18-hydroxy-$LTB_4$, and 17-hydroxy-$LTB_4$ are inactive and unable to bind and activate the BLT1 or BLT2 receptors or are targeted for elimination by peroxisome β-oxidation. It is possible that these hydroxylated $LTB_4$ metabolites may have a functional role in inflammation that may provide insight into why there is a differential regulation of *CYP4F* genes during the activation and resolution phases of inflammation.

The function of the human *CYP4F* genes in diseases has come from observational studies linking increases in $LTB_4$ and cysteinyl-LTs with diseases having an inflammatory etiology: cholestasis, portal hypertension, hepatorenal syndrome, cystic fibrosis, obstructive jaundice, and hepatocellular carcinoma. Recent data have shown the expression of the *CYP4F2* and *CYP4F3* genes in the colon of patients with celiac disease [58]. Both genes were expressed at various stages of colon remodeling toward normalization in patients on a gluten-free diet. Prior to initiation of the gluten-free diet the *CYP4F3* gene was over expressed during cryptic hyperplasia (Marsh stage III), while in remission stage (Marsh stage 0) there was an 8-fold increase in *CYP4F2* gene expression [59]. These data imply that CYP4F3 was associated with neutrophil infiltration and colon inflammation, while the increased expression of the *CYP4F2* mRNA was associated with the resolution of inflammation. In addition, genetic linkage of 44 haplotype small nucleotide polymorphisms (SNPs) identified familial clustering of the *CYP4F2* and *CYP4F3* as human susceptibility genes in the development of celiac disease.

### 4.3. *CYP4A and CYP4F genes in fatty liver disease*

Non-alcoholic fatty liver disease (NAFLD) encompasses a broad disease spectrum ranging from simple triglyceride accumulation in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis) that progress to fibrosis and cirrhosis. A two hit hypothesis has been proposed to explain the progression of NAFLD with fatty acid accumulation as triglycerides in the cytoplasm of hepatocyte being the first hit, while increased oxidative stress, the second hit, promoting liver inflammation, cell death, and fibrosis in non-alcoholic steatohepatitis (NASH) [45,47]. Both obesity and insulin resistance are strongly associated with NAFLD. Increased insulin resistance in adipocytes results in increased activity of hormone sensitive lipase (HSL) resulting in the hydrolysis of adipose triglycerides and elevation of plasma and liver levels of free fatty acids (FFA). In the liver FFA can be either metabolized by the mitochondria β-oxidation system for energy or esterified to triglycerides and incorporated into VLDL particles with cholesterol esters, and phospholipids for transport and use by peripheral tissues. Through the use of mouse models of fatty liver disease and the generation of knockouts of key regulator enzymes involved in lipid and glucose metabolism, we are beginning to understand the key molecular targets responsible for increase triglyceride accumulation in hepatocytes and how fatty acids induce oxidative stress and the progression of steatosis to steatohepatitis.

As depicted in Fig. 1 key regulatory enzymes control the ability of the liver to provide nutrients to peripheral tissues through gluconeogenesis, ketogenesis, and VLDL secretion. Interestingly, these key regulator enzymes are differentially affected in liver insulin resistance with the major pathway in lipogenesis being activated while the gluconeogenic pathway showing insulin resistance and also being active in the elevation of blood glucose levels. Unfortunately, the mechanisms by which fatty acids induce gluconeogenesis remain poorly understood. Furthermore, the mechanism by which acetyl CoA carboxylase (ACC1) and stearoyl CoA desaturase (SCD-1) are activated in the insulin resistant liver has not been clearly defined. ACC1 functions to convert acetyl CoA to malonyl CoA, which is a precursor for both cholesterol and fatty acid synthesis. Malonyl CoA is a potent inhibitor of mitochondria carnitine palmitoyl transferase (CPT1), which functions in the transport fatty acids to the mitochondria for β-oxidation. SCD-1 functions to desaturate stearic acid ($C_{18:0}$) to oleic acid ($C_{18:1}$) necessary for synthesis of triglycerides. These genes are transcriptionally induced by sterol regulatory element binding protein 1c (SREBP-1c) transcription factor, which is induced by insulin and carbohydrates. Therefore, in the insulin resistant liver excessive glucose may mediate the activation of SREBP-1c leading to the induction of both ACC1 and SCD-1. During hepatic steatosis in both humans and mice there is an excessive accumulation of oleic acid either from increased *de novo* fatty acid synthesis or conversion of imported fatty acids from the adipose tissue. Elevated hepatic glucose production in the presence of hyperinsulinemia is a hallmark of insulin resistance in the liver even with the increased expression of the liver specific pyruvate kinase (L-PK), a key *regulatory* enzyme in converting phosphoenolpyruvate to pyruvate during glycolysis. Increase *L-PK* gene expression is mediated by glucose activation of the carbohydrate responsive element binding protein (ChREBP) transcription factor. ChREBP has also been shown to increase the expression of many of the fatty acid synthesis genes as well as SCD-1throught SREBP thereby facilitating the conversion of glucose to fatty acids. It is presently unknown why hyperinsulinemia is able to activate the lipogenic and glycolytic pathways, but fails to prevent the activation of a gluconeogenic pathway and hyperglycemia observed in insulin resistant diabetes [45,47,60].

The activation of both anabolic and catabolic pathways during insulin resistance in the liver suggest that different fasting and feeding signals are simultaneously controlling the metabolic response of the liver. What are these signals and how they function either directly to activate key regulator enzymes or function as agonist or antagonist to activate transcription factors that control the expression of genes involved in fatty acid catabolism (PPARα, HNF4α) or fatty acid synthesis (SREBP-1c, ChREBP, PPARγ) remains to be determined.

Recent evidence has suggested that activation of the peroxisome β-oxidation pathway exerts a beneficial effect in NAFLD by metabolizing excessive fatty acids to shorter chain fatty acids that can be directly transported and completely oxidized by the mitochondria β-oxidation system. In addition the incomplete peroxisome β-oxidation of fatty acids can supply the anaplerotic mitochondria intermediate, succinate, necessary for gluconeogenesis, while acetate from acetyl-CoA can be used for the anabolic synthesis of cholesterol and fatty acid as seen in NAFLD [1,2,43].

The peroxisomes, unlike mitochondria, metabolize long chain fatty acids, exclusively metabolize branched chain fatty acid, and preferentially metabolize dicarboxylic acid, which are produced by the ω-hydroxylation of fatty acids by members of the CYP4 gene family. The increased expression of the CYP4A omega hydroxylase during steatohepatitis and their induction in animals fed a high fat diet suggest they may play a pivotal role in preventing lipotoxicity [61], and may be responsible for induction of oxidative stress and progression to steatohepatitis. A dramatic induction of both the mouse *CYP4A10* and *CYP4A14* genes is seen in *CYP2E1* knockout mice and account for the increased reactive oxygen species (ROS) that induce lipid peroxidation [62,63]. The increased production of dicarboxylic acids during steatosis by CYP4A members can impair mitochondria function by dissipation of the mitochondria proton gradient and uncoupling of oxidative phosphorylation. In addition, the uncoupling of the P450 catalytic cycle has been known to be a major source of reactive oxygen species, which led to the identification of the ethanol inducible CYP2E1 P450 as a source ROS-induced microsomal lipid peroxidation [64]. CYP2E1 can metabolize fatty acids at the ω-1 position, and CYP4A, which normally ω-hydroxylates lauric acid, hydroxylates longer chain fatty acids at both the ω- and ω-1 positions. It is not known whether different chain length fatty acids assist in the uncoupling of the CYP2E1 and CYP4A catalytic cycle or whether cytochrome $b_5$, which increases P450 catalytic activity and prevents uncoupling, can reduce ROS formation in fatty liver disease [65]. Both cytochrome $b_5$ reductase and cytochrome $b_5$ are also used in the desaturation of stearic and palmitic acid by stearoyl CoA desaturase 1 (SCD-1). It is unknown whether increased conversion of stearic acid to oleic acid observed in NAFLD increases uncoupling of the P450 catalytic cycle resulting in increased ROS formation by SCD-1 sequestering cytochrome $b_5$. Both cytochrome $b_5$ and cytochrome $b_5$ reductase have been identified as susceptibility genes in obesity [66]. While the induction of *CYP4A* genes during fasting provides both gluconeogenic precursors and acetate to supply the needs of peripheral tissues, their induction during steatosis may increase hyperglycemia, shuttle acetate for synthesis of fatty acids and cholesterol, and increase ROS formation by uncoupling of the P450 catalytic cycle.

Besides, the role of *CYP4A* genes in initiating hepatocyte cell injury and steatohepatitis, *CYP4F* genes may also play a functional role in lipid accumulation in the liver and recruitment of inflammatory cells during progression from steatosis to steatohepatitis. Unlike *CYP4A* genes that are induced by starvation, hypolipidemic drugs, and peroxisome proliferator through activation of PPARα, the *CYP4F* genes are repressed during starvation and by peroxisome proliferators possibly by PP-CoA or PUFA-CoA inhibition of HNF4α. Furthermore unlike the *CYP4A* genes that are induced in fatty liver, we have evidence that the mouse *CYP4F* genes are repressed in mice fed a high fat diet and in the leptin deficient model of fatty liver disease. The recent report of lipid accumulation in primitive liver cells (oenocytes) of Drosophila that have a mutation in the stearic ω-hydroxylase *CYP4g1* gene

[67] suggest that *CYP4F* genes may play an important role in maintaining lipid homeostasis in the liver. *Drosophila* homozygous for mutant *CYP4g1* manifest a 2-fold increase in the oleic acid:stearic acid ratio ($C_{18:1}/C_{18:0}$) with a notable imbalance in the fatty acid desaturation found in the TAG fraction but not the phospholipid fraction. This suggests that CYP4g1 is important in the metabolic storage of fatty acids and its expression would decrease oleic acid synthesis and storage of fatty acids in TAGs. It is possible that a member of the human CYP4F subfamily may function in a similar fashion as the *Drosophila* CYP4g1 in regulating the oleic acid:stearic acid ratio since both CYP4F11 and CYP4F2 have been shown to ω-hydroxylate 3-hydroxystearic acid [13]. In addition, the human CYP4F2 ω-hydroxylates stearic acid more efficiently than CYP4A11 and therefore may serve the same function as CYP4g1 in competing with SCD-1 in the metabolism of stearic acid (Fig. 2). It is presently unknown whether the human *CYP4F2* gene is repressed by fatty acids in patients with NAFLD. However, the *CYP4F2* gene has been shown to be down-regulated by peroxisome proliferators [21], induced by retinoic acids [68], and its expression increased by lovastatin mediated activation of SREBP-2 [40]. The induction of the *CYP4F2* and *SCD-1* genes by insulin in primary human hepatocytes suggests that their differential regulation in insulin resistance may be a determining factor in the storage or metabolism of fatty acids.

Therefore, the activation of the *CYP4F2* gene in fatty liver disease may decrease the formation of oleic acid and storage of TAG in the liver, and also play a vital role in preventing recruitment of immune cells to the liver during steatohepatitis by metabolism of the pro-inflammatory leukotrienes. Even though there are numerous studies showing that fatty acids induce hepatocytes to produce cytokines and chemokines (IL-8) which attract neutrophils to the liver, the function of leukotrienes in attracting immune cells to the liver during steatohepatitis has not been explored. Whether $LTB_4$ or $LTC_4$ also increase production of the potent neutrophil chemokine IL-8 during hepatic steatosis remains to be determined. The genetic association of *CYP4F2* and the neutrophil specific *CYP4F3* genes in celiac disease establishes a connection between the innate immune response of neutrophil recruitment to the established Th1 adaptive immune response in disease patients [58]. Furthermore, celiac disease has been associated with several inflammatory diseases of the liver; primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, hemochromatosis, and fatty liver disease [69].

It will be of significant importance to understand how the *CYP4F* genes are regulated by fatty acids and in animal models of fatty liver disease. Whether induction of CYP4F long chain fatty acid ω-hydroxylase can prevent steatosis by inhibiting SCD-1 activity and reduce the hepatic levels of pro-inflammatory leukotrienes in steatohepatitis needs further study.

### 4.4. *CYP4F genes in skin barrier function*

The skin epidermis is a stratified layer of cells having the ability of self-renewal from a basal layer of mitotically active cells that replenish the stratum corneum necessary to maintain the barrier function of the cornified envelope. The synthesis of hydrophobic extracellular lipids, consisting of ceramides, cholesterol, and fatty acid, by the stratum corneum provides the barrier to the movement of water and electrolytes. Clinically, genetic disorders of epidermal differentiation or cornification are classified as *ichthyoses* where the skins scaly appearance is due to hyperkeratosis, thickening of stratum corneum, caused by increased cell proliferation or delayed desquamation. Both hepoxilin, an arachidonic acid metabolite of the 12-lipoxygenase/3-lipoxygenase (ALOX12B/ALOXE3) pathways, and long chain fatty acid ω-*O*-acylceramides are necessary for maintenance of the stratum corneum barrier function. Mutations in the *CYP4F22* gene have been identified in patients with autosomal recessive *congential ichthyosis* (ARCI) who have permeability barrier abnormalities characterized by deficiency of very long chain fatty acids in the epidermis and absence of ω-*O*-acylceramides [35]. In contrast, in Gaucher mice with a deficiency of β-glucocerebrosidase there is a 35-fold increase in ω-hydroxylated glucosylceramides [70] and in Sjogren–Larrson syndrome (ichthyosis and spastic paraplegia) mutations in fatty acid aldehyde dehydrogenase (ALDH3A2) leads to an increased cellular accumulation of ω-*O*-acylceramides [36]. We do not know what fatty acids or eicosanoids are metabolized by CYP4F22 and if this P450 ω-hydroxylates hepoxilins that function as pathogen elicited epithelial neutrophil chemoattractants [71] and potent suppressors of cell proliferation or in the ω-hydroxylation of acylceramides [36]. Future studies are necessary to identify the CYP4Fs involved in the ω-hydroxylation of long chain fatty acids and pro-inflammatory hepoxilin in the skin and define their regulation and importance in skin barrier function.

## 5. Genetic regulation of CYP4A and CYP4F gene in lipid metabolism

The differential regulation of the *CYP4A* and *CYP4F* genes by peroxisome proliferators, during starvation and by high fat diets indicates that these P450s have different roles in lipid metabolism during the fasting and feeding cycles, and that different nuclear receptors regulate the expression of these genes in lipid metabolism. It is well established that in rodents the *CYP4A* genes are induced by starvation, by peroxisome proliferators, ethanol, high fat diet, and in diabetes and steatohepatitis. However, little is known about how the human *CYP4A11* gene is controlled by these agents and in these diseases. The modest 2-fold induction of the *CYP4A11* gene by peroxisome proliferator in primary human hepatocytes compared with the 30–70-fold induction of the mouse *CYP4A* genes suggest a species difference in rodent and human regulation of the *CYP4A* genes by peroxisome proliferators. In addition the 60–700-fold increase in mouse CYP4A mRNA during fasting [72] and 2–8-fold decrease in CYP4F mRNA further indicate the differential regulation of these genes. In NAFLD, CYP4A11 mRNA was found to be increased 4-fold [47] while in mice fed a high fat diet the CYP4A mRNAs were increased 2.5–100-fold, while we have found that the CYP4F genes are inhibited by 20–80%, indicating that different fatty acids or metabolites may function to differentially control CYP4 gene expression.

Long chain fatty acids (LCFA) are endogenous ligands in the activation of hormone nuclear receptors (HNR), PPARα and HNF4α. LCFA and LCFA-CoAs are significant NHR ligands as shown by their presence in the nucleus, their high affinity binding (nM Kds), there ability to induce conformational changes in HNRs, and their ability to induce co-regulator recruitment to nuclear receptors [19,44]. Support for LCFA-CoAs in the hyperactivation of PPARα was evident in peroxisome acyl-CoA oxidase (ACOX) knockout mice with accumulated VLCFA and VLCFA-CoA, and the observation that the thioesterification inhibitor, 2-bromopalmitate inhibits bezafibrate induction of peroxisome proliferation in rodents. In humans, the importance of VLCFA-CoA in PPARα activation was evident in adrenoleukodystrophy where there is accumulation of VLCFA in the cytosol, but no peroxisome formation of VLCFA-CoA and no hyper-activation of PPARα [14]. Serum fatty acids increase dramatically from the normal physiological range of 200 μM to 1 mM under fasting and up to 8 mM in Refsum's disease, adrenoleukodystrophy, Zellwegers syndrome, and fatty liver disease, diabetes, and inflammation. This suggests an important link between peroxisome fatty acid metabolism and conversion of VLCFA to VLCFA-CoAs in the activation of PPARα and control of *CYP4* gene expression. PPARα has a high affinity for polyunsaturated LCFA, LCFA-CoA and VLCFA-CoA, but not saturated LCFA or VLCFA. In contrast, HNF4α has affinity for saturated LCFA and VLCFA acyl-CoA but not polyunsaturated acyl-CoA indicating that fatty acid CoA chain length and degree of unsaturation determines whether HNF4α or PPARα will be activated [73]. The mechanism of LCFA uptake and importation into the nucleus has recently been shown to be mediated by liver fatty acid binding protein (L-FABP), which binds polyunsaturated LCFA with a greater affinity than saturated LCFA and associates with PPARα, while acyl-CoA binding protein (ACBP) preferentially binds saturated LCFA and associates with HNF4α [19,44]. These studies indicate that ACBP selectively cooperates with HNF4α and L-FABP selectively cooperates with PPARα, which is though to elicit downstream alteration in co-activator and co-repressor association with HNRs. Thus, the binding of saturated LCFA-CoA to HNF4α would increase HNF4α activity and inhibit PPARα trans-activation while polyunsaturated LCFA-CoA would decrease HNF4α activation and increase L-FABP PPARα trans-activation. Since PPARα and HNF4α regulate transcription through similar promiscuous DR1 sequences, and compete for the same co-activators and co-repressors, the specificity of receptor activation may be determined by either saturated or polyunsaturated fatty acid ligand while the cross-talk between these receptors would be determined by the FABP/ACBP mediated co-regulator recruitment and repression of the cognate receptor. The differential regulation of the *CYP4A* and *CYP4F* genes may be determined by cross-talk between PPARα and HNFα through the type of fatty acid ligand and method of nuclear import and receptor activation by L-FABP or ACBP. This scenario is highly likely in the regulation of *CYP4A* and *CYP4F* genes since peroxisome proliferators (PP) activate PPARα while PP-CoA inhibits HNF4α trans-activation. It is also possible that MCFA and VLCFA metabolized by the CYP4A and CYP4F may produce fatty acid metabolites that reciprocally regulate the expression of the *CYP4A* and *CYP4F* genes. The induction of CYP4A genes by a high fat diet leads to increased production of dicarboxylic acids that are potent inhibitors of HNF4α trans-activation, which may mediate suppression of the *CYP4F* genes during steatosis.

## 6. Concluding remarks

Although the ω-hydroxylation of saturated and unsaturated fatty acids by CYP4 family members has long been thought to be of minor importance by contributing only 5–10% to fatty acid metabolism, its importance is dramatically elevated during fasting, starvation, and in several human diseases where its contribution to fatty acid metabolism increases dramatically. The close and intimate association of CYP4 ω-hydroxylation of medium chain fatty acids (MCFA) and peroxisome β-oxidation is evident by the conversion of dicarboxylic acids to the succinate, an anaplerotic gluconeogenic precursor, and acetate, which can be used by peripheral tissue like ketone bodies during fasting and starvation when there is an induction of *CYP4A* genes. The increased expression of *CYP4A* genes during fasting, starvation, high fat diet and in steatohepatitis may be a mechanism to prevent lipotoxicity of fatty acids, but at the expense of increased uncoupling of the P450 catalytic cycle and increased production of ROS.

In contrast to the induction of the *CYP4A* genes by starvation, peroxisome proliferators, and high fat diet members of the CYP4F subfamily are down-regulated, suggesting a differential regulation of the *CYP4A* and *CYP4F* genes during the fasting and fed states. CYP4F P450 show a preference in the metabolism of LCFA and VLCFA saturated and unsaturated fatty acids, which are metabolized by peroxisome β-oxidation to shorter chain fatty acids that are incorporated into phospholipids, triglycerides, and cholesterol esters. The excessive acetate produced by peroxisome β-oxidation may be used in hepatocytes to produce malonyl CoA, which can be used in the synthesis of fatty acids and cholesterol. The induction of the *CYP4F2* gene by lovastatin activation of SREBP-2 and insulin activation of SREBP-1c suggest that CYP4F P450s may function in the coordinate regulation of cholesterol and fatty acid synthesis. Furthermore, the ability of stearic ω-hydroxylase CYP4g1 to change the desaturation index ($C_{18:1}$/$C_{18:0}$) by either inhibiting stearoyl CoA desaturase 1 (SCD-1) or competition for stearic acid indicates that the CYP4F P450s may have an important role in triglyceride synthesis and VLDL production. The metabolic role, function, and regulation of *CYP4A* and *CYP4F* genes during sepsis and SIRS are largely unknown even though they may have important functional roles in gluconeogenesis and lipogenesis. Although members of the CYP4F subfamily are able to ω-hydroxylate the pro-inflammatory eicosanoid, $LTB_4$ and thus reduce, delay or inhibit recruitment of myeloid cell with $LTB_4$ receptors (BLT1) to sites of inflammation, their functional role in the activation and resolution phases of inflammation are unknown. The genetic association of the human myeloid *CYP4F3A* and liver *CYP4F2* genes with inflammatory celiac disease and the increased expression of both genes in the inflammatory atrophic stage of the disease with increased CYP4F2 in remission stage suggest that an activated innate immunity in celiac patients may reflect their genetic susceptibility

towards relapsing with the disease. The strong association of celiac disease with autoimmune liver disorders such as autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis suggests that there may be a genetic association of these liver diseases with the *CYP4F* genes. The role and function of CYP4F genes in NAFLD and inflammatory liver disease needs further study to determine if expression of CYP4F polymorphic variants increases disease susceptibility.

As discussed herein, there are many human diseases in which the expression of the *CYP4A* and *CYP4F* genes are altered in both lipid and inflammatory disorders, and therefore they may be therapeutic targets in the control of inflammatory, vascular, and liver diseases, as well as peroxisome disorders of fatty acid metabolism. Induction of the *CYP4F* genes in patients with Adrenoleukodystrophy or Refsum disease may provide a method to degrade VLCFAs and phytanic acid, respectively, which may serve as an alternative treatment for these diseases. The repression of *CYP4* and induction of *CYP4F* genes may also provide an avenue to prevent CYP4A P450 production of reactive oxygen species and progression of steatosis to steatohepatitis, while CYP4F P450 may prevent hepatic triglyceride accumulation by decreasing the desaturation index ($C_{18:1}/C_{18:0}$) and thus steatosis. In addition, the genetic association of the *CYP4F22* gene with skin disorders and other CYP4F members expressed in different tissues needs to be studied to determine their functional role in the metabolism of 5-lipoxygenase, 12-lipoxygenase, and 15-lipoxygenase products. Finally, we need to understand the association of *CYP4A* and *CYP4F* genes with human diseases in relation to CYP4 polymorphic variants that may increase human susceptibility to these diseases. Understanding how the *CYP4A* and *CYP4F* genes are controlled by nuclear hormone receptors may provide novel avenues to prevent disease progression in lipid and inflammatory diseases.

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