Bile acids enterohepatic circulation
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CHAPTER 1

GENERAL INTRODUCTION

It is well established that cholesterol often causes hypercholesterolemia when fed. However, some individuals are not sensitive to the intake of dietary cholesterol and, thus, might consume more in the diet without risk \(^{(1)}\) \(^{(2)}\). The different plasma responses to dietary cholesterol have been also observed in various animal species. For example, rats fed a high cholesterol diet are resistant and do not develop hypercholesterolemia because cholesterol 7\(\alpha\)-hydroxylase, the rate-controlling enzyme for the classic bile acid synthesis pathway, is upregulated \(^{(3-6)}\) to utilize the extra cholesterol to make more bile acids. In contrast, New Zealand White rabbits usually are sensitive to dietary cholesterol and accumulate large amounts in plasma \(^{(7)}\). Xu et al. has described marked inhibition of cholesterol 7\(\alpha\)-hydroxylase after 10 days of cholesterol feeding to New Zealand White rabbits \(^{(8)}\). It is believed that the inhibition of cholesterol 7\(\alpha\)-hydroxylase and classic bile acid synthesis contributed to hypercholesterolemia in the cholesterol-fed rabbit model. It is suggested that cholesterol 7\(\alpha\)-hydroxylase was a key factor in regulating cholesterol homeostasis.
There is a fundamental difference in regulation of cholesterol 7α-hydroxylase and classic bile acid synthesis between rats and rabbits is responsible for the difference in susceptibility to develop plasma hypercholesterolemia.

In order to understand the bile acids synthesis and its re-absorption, then to explore the mechanism about these differences, the following brief introduction of bile acids enterohepatic circulation is given.

**Enterohepatic circulation of Bile acids:**

Bile acids are synthesized from cholesterol exclusively by the liver. The biosynthetic steps that collectively accomplish the conversion of water-insoluble cholesterol molecules into more water-soluble compounds also confer detergent properties to the bile acids that are crucial for their physiological functions in bile formation and intestinal fat absorption. On conjugation with glycine or taurine, bile acids are actively secreted by the hepatocytes into the bile canaliculi that drain into intrahepatic bile ducts, stored in the gallbladder, and expelled into the intestinal lumen in response to a fatty meal. In the small intestine, bile acids act as detergents to emulsify and facilitate the absorption of dietary fats and lipid-soluble vitamins. Subsequently, they are reabsorbed from the terminal ileum
by specific transporter proteins: ~95% return to the liver to be secreted again into the bile, completing the so-called enterohepatic circulation, whereas 5% escape reabsorption and are lost via the feces.\textsuperscript{9} Bile acid synthesis in adult humans amounts up to ~500 mg, which accounts for ~50% of total cholesterol turnover.\textsuperscript{10} Bile acids are conjugated to taurine or glycine, by sequential actions of the enzymes bile acid coenzyme A (CoA) synthetase (BACS) and the bile acid-CoA amino acid N-acetyltransferase (BAT), to increase their hydrophilicity in a process regulated by FXR.\textsuperscript{12} Conjugated bile acids require a transporter network to cycle between liver and intestine, which is to a certain extent also under FXR control. Bile acids are secreted by hepatocytes into the bile canaliculi by the bile salt export pump (BSEP or ABCB11) via an ATP-dependent process.\textsuperscript{13-15} BSEP mutations underlie progressive familial intrahepatic cholestasis type II (PFIC II), an inborn cholestatic liver disease. BSEP expression is induced by FXR at the transcriptional level.\textsuperscript{16,17} Because relatively hydrophobic bile acids are potentially toxic, protective mechanisms such as oxidation by CYP3A4,\textsuperscript{18} sulfation by dehydroepiandrosterone-sulfotransferase SULT2A1,\textsuperscript{19} or glucuronidation catalyzed by uridine glucuronosyltransferase 2B4\textsuperscript{20,21} have evolved (Figure 1). SULT2A1,\textsuperscript{22} UGT2B4,\textsuperscript{23} and CYP3A4\textsuperscript{24} all are positively regulated by FXR by means of a nonclassical inverted
**Figure 1.** Overview of bile circulation between the liver and the intestine.

**Figure 2.** Overview of the mechanisms of bile acid synthesis repression by FXR. After binding by bile acids, FXR induces the expression of SHP, which in turn interacts with LRH-1 or HNF4--to decrease the transcription of CYP7A1 and CYP8B1, respectively. Simultaneously, FXR induces the expression of FGF-19. FGF-19 interacts with its cognate receptor FGFR-4 to negatively regulate bile acid production by repressing CYP7A1 and CYP8B1 gene expression by interfering with the JNK pathway.
repeat 0(IR-0), a monomeric site, and 2 response elements (an ER-8 and another IR-1/DR-3 site), respectively. Interestingly, FXR also induces the expression of the multidrug resistance-associated protein 2(MRP2 or ABCC2), a multispecific ABC transporter able to excrete sulfated and glucuronidated bile acids into the bile, via an everted repeat-8 (ER-8) site25 (Figure 1).

Cholesterol catabolism.

Cholesterol homeostasis in mammals represents a delicate balance between pathways of supply and catabolism. In the liver, the chief organ of cholesterol metabolism (26), supply is accomplished by a receptor-mediated pathway (27) and by the de novo synthesis of cholesterol from acetate precursors (28). A single pathway of catabolism involves the conversion of cholesterol into hydrophilic bile acids that are subsequently excreted from the body via the bile and intestine (29). Although the regulatory mechanisms underlying the expression of key enzymes in the cholesterol supply pathways are beginning to be understood.

The study of bile acids is carried out by at least 10 enzymes in the liver which hydroxylate the four-ring structure of cholesterol and shorten and oxidize the side chain (30). In the human and rat, the major products thus formed are the primary bile acids, cholic acid and chenodeoxycholic acid. The rate limiting step in this pathway
involves the introduction of a hydroxyl moiety at the 7 position of cholesterol\(^\text{(16)}\) and is catalyzed by CYP7A1, a microsomal enzyme that is a member of the cytochrome P-450 family. Once formed, 7\(\alpha\)-hydroxycholesterol is rapidly converted into a primary bile acid, as few of the subsequent enzymatic steps appear to be regulated\(^\text{(29)}\).

The activity of 7\(\alpha\)-hydroxylase is subject to end product repression by bile acids in the enterohepatic circulation\(^\text{(30)}\). Thus, in animals maintained on a diet containing bile acids, the level of 7\(\alpha\)-hydroxylase activity is reduced\(^\text{(30)}\). Conversely, animals fed drugs such as cholestyramine, which enhance the excretion of bile acids\(^\text{(31)}\), increase their hepatic levels of this enzyme and hence the production of bile acids\(^\text{30}\). Because of the complexity of the enzyme assay and the lack of an independent measure of 7\(\alpha\)-hydroxylase protein, the changes in enzyme activity have not yet been shown unequivocally to result from changes in the amount of enzyme as opposed to changes in its activity.

**Bile acids synthesis and its regulation**

Two major pathways, generally referred to as the neutral and the acidic pathway, are involved in bile acid synthesis\(^3\text{2}\). CYP7A1 is the first and rate-controlling enzyme of the neutral pathway and partly controlled by a negative bile acid feedback loop, whereas CYP27A1,
the main enzyme of the acidic pathway, is not regulated by bile acids (Figure 1). It is identified that areas of the CYP7A1 promoter region are hepatocyte-specific. The transcriptional factor HNF3 is an activator of CYP7A1 activity.

Nitta et al. identified a liver-specific regulatory element within the CYP7A1 promoter and isolated a transcription factor, CPF (also called LRH1 or FTF), that binds to the promoter of the human CYP7A gene (33). Studies suggested that CPF is a key regulator of human CYP7A1 gene expression in the liver.

In an elegant series of experiments designed to understand the effect of retinoid X receptor (RXR) activation on cholesterol balance, Repa et al. (34) treated animals with the r Knoxid LG268. Animals treated with r Knoxid exhibited marked changes in cholesterol balance, including inhibition of cholesterol absorption and repressed bile acid synthesis. Studies with receptor-selective agonists revealed that oxysterol receptors (LXRs) and the bile acid receptor, FXR, are the RXR heterodimeric partners that mediate these effects by regulating expression of the reverse-cholesterol transporter, ABC1, and the rate-limiting enzyme of bile acid synthesis, CYP7A1, respectively. These RXR heterodimers serve as key regulators in cholesterol homeostasis by governing reverse cholesterol transport from peripheral tissues, bile acid synthesis in
liver, and cholesterol absorption in intestine. Activation of RXR/LXR heterodimers inhibits cholesterol absorption by upregulation of ABC1 expression in the small intestine. Activation of RXR/FXR heterodimers represses CYP7A1 expression and bile acid production, leading to a failure to solubilize and absorb cholesterol. Studies have shown that RXR/FXR-mediated repression of CYP7A1 is dominant over RXR/LXR-mediated induction of CYP7A1, which explains why the rexinoid represses rather than activates CYP7A1 (35). It appeared that FXR itself does not bind the putative bile acid response elements (BARE) in its promoter. Two groups independently demonstrated that FXR activation induces expression of the atypical nuclear receptor small heterodimer partner (SHP or NR0B2). SHP, in turn, interacts with 2 other nuclear receptors that transactivate CYP7A1 expression via the BARE region, ie, the hepatic nuclear factor 4 (HNF4 or NR2A1) and the liver receptor homolog-1 (LRH-1 ,FTF or NR5A2) (Figure 2). SHP repression of CYP7A1 gene transcription occurs by promoting the dissociation of coactivators linked to HNF4 and FTF, as well as by histone deacetylation of the promoter.40

FXR also modulates CYP7A1 expression by induction of fibroblast growth factor-19 (FGF-19) expression.41 On its secretion, FGF-19 activates the hepatic FGF receptor-4, which, in turn, downregulates
CYP7A1 through c-Jun N-terminal kinase activation (Figure 2). Activation of the LXR signaling pathway results in the upregulation of ABC1 in peripheral cells, including macrophages, to efflux free cholesterol for transport back to the liver through high density lipoprotein, where it is converted to bile acids by the LXR-mediated increase in CYP7A1 expression. Secretion of biliary cholesterol in the presence of increased bile acid pools normally results in enhanced reabsorption of cholesterol; however, with the increased expression of ABC1 and efflux of cholesterol back into the lumen, there is a reduction in cholesterol absorption and net excretion of cholesterol and bile acid. Rexinoids therefore offer a novel class of agents for treating elevated cholesterol.

The catabolism of cholesterol into bile acids is regulated by oxysterols and bile acids, which induce or repress transcription of the pathway's rate-limiting enzyme, CYP7A1. The nuclear receptor LXR-alpha (LXRA, or NR1H3) binds oxysterols and mediates feed-forward induction. Feedback repression of CYP7A1 is accomplished by the binding of bile acids to FXR, which leads to transcription of SHP. Elevated SHP protein then inactivates LRH1(FTF) by forming a heterodimeric complex that leads to promoter-specific repression of both CYP7A1 and SHP. These results revealed an elaborate autoregulatory cascade mediated by
nuclear receptors for the maintenance of hepatic cholesterol catabolism.

**Bile acids re-absorption:**

In the ileum, bile acids are efficiently taken up by enterocytes via the ileal apical sodium-dependent bile acid transporter (ASBT, also called intestinal bile acid transporter [IBAT] or SLC10A2) protein.\(^{43-45}\) ASBT gene encodes a 348-amino acid polypeptide with 7 predicted transmembrane domains and a predicted molecular mass of 38 kD. The native human protein has a relative molecular mass of 40 kD on SDS gel electrophoresis due to N-linked glycosylation. FXR indirectly influences the expression of ASBT,\(^{46}\) but directly induces the expression of the intestinal bile acid binding protein (IBAB-P or FABP6)\(^{47}\) (Figure 1). It is generally assumed that IBAB-P provides a shuttle allowing bile acids to traffic from the apical to the basolateral side of the enterocytes during their absorption.\(^{48}\) However, the fact that FXR-deficient mice were found to display enhanced intestinal bile acid absorption despite an extremely low IBAB-P expression\(^{49}\) demonstrates that the real physiological function of IBAB-P remains unresolved. At the basolateral side, bile acids are believed to be secreted into the portal vein either by a truncated form of ASBT (tASBT),\(^{50}\) or by the multidrug resistance-associated protein
3(MRP3 or ABCC3) or by the newly described Ost-/- transporters (Figure 1).

The uptake of bile salts that return to the liver after intestinal absorption is mainly mediated by the Na~ taurocholate cotransporting polypeptide (NTCP or SLC10A1). Bile acids downregulate NTCP expression via a FXR-dependent mechanism, but NTCP expression is not changed in FXR-deficient mice compared with wild-type controls. A potential mechanism involves SHP activation that inhibits RXR/RAR (retinoic acid receptor or NR1B1) transactivation of the promoter (Figure 1). Approximately 75% of uptake occurs by this Na~ dependent process; yet, another family of transporters is also involved, the organic anion transporter polypeptides (OATPs). OATP-C (or SLCO1B1) is the most ubiquitously expressed OATP transporter in human hepatocytes, but it is currently unknown whether FXR is involved in its regulation (Figure 1). In mice, it has been reported that bile acids regulate OATP-1 (or Slco1a1) expression, but the basal expression of OATP-1 was not modified in FXR-deficient mice.

Outline of the thesis
The farnesoid X receptor (FXR) is an orphan nuclear transcription factor that has recently been identified as a negative regulator of CYP7A1, a gene encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme in the classic bile acid synthesis pathway (56-58). Based on in vitro studies in cell culture, the most effective (high affinity) ligands for FXR activation are chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and lithocholic acid (LA), which are all hydrophobic bile acids. In contrast, hydrophilic bile acids such as ursodeoxycholic acid and muricholic acid are not effective. Recently, other target genes for FXR have been identified that are positively regulated, including the bile salt export pump (BSEP), which is responsible for the canalicular transport of bile acids (59), and short heterodimer partner (SHP), which plays an important role in the feedback regulation of CYP7A1 by bile acids (60,61). FXR/RXR does not bind directly to the bile acid response element in the promoter region of human CYP7A1 (62), thus, CYP7A1 transcriptional down-regulation by bile acids via FXR is indirect via SHP (60,61). The increased SHP is believed to inactivate liver receptor homolog-1 (LRH-1), an essential transcription factor for CYP7A1 expression (63). Recently, Chen et al. (64) suggested that α-fetoprotein transcription factor (FTF), a human homolog of mouse LRH-1, was an inhibitor rather than a transcription factor for
CYP7A1. More recently, studies in SHP knock-out mice (65,66) suggested that CYP7A1 could also be regulated by bile acids through SHP-independent pathways, because cholic acid feeding to the SHP-I- mice also repressed CYP7A1 expression. Therefore, the mechanism by which activated FXR down-regulates CYP7A1 has not been completely elucidated.

To examine the proposed theories of the role of bile acids in FXR activation and the regulation of CYP7A1 under in vivo conditions, this study was carried out in rabbits with depleted bile acid pool/flux, which was replaced with either hydrophobic DCA or hydrophilic ursodeoxycholic acid (UCA), and intact rabbits fed cholic acid. In addition, the effect of these bile acids on FXR transcription was also evaluated.

It is well established that feeding cholesterol to rats up-regulates cholesterol 7-hydroxylase (CYP7A1), the rate-limiting enzyme for classic bile acid synthesis (67-70). As a result, hepatic cholesterol destined for circulating LDL is diverted to bile acid synthesis, and the accumulation of cholesterol in the plasma and liver is prevented. Unlike in rats, feeding cholesterol to New Zealand white (NZW) rabbits was associated with repressed CYP7A1 (71) and a tremendous increase in plasma cholesterol levels. Further studies
suggested that expansion of the bile acid pool size was responsible for inhibition of CYP7A1 in the cholesterol-fed rabbits \(^{(72)}\), and that the inhibition was not directly related to the accumulated cholesterol \(^{(73)}\). However, the molecular mechanism(s) for these findings remains unknown.

Recent studies have shown that the orphan nuclear receptors farnesoid X receptor (FXR) \(^{(74-76)}\) and liver X receptor (LXR) \(^{(77-79)}\) are negative and positive regulators of CYP7A1 transcription. The potent ligands for FXR activation are the bile acids chenodeoxycholic, deoxycholic, and lithocholic acid \(^{(73)}\), while the ligands for LXR activation are oxysterols \(^{(80,81)}\). It was reported that 24S,25-epoxycholesterol (24S,25E), 24S-hydroxycholesterol (24S-OH), 22R-hydroxycholesterol (22R-OH) \(^{(82,83)}\), and 27-hydroxycholesterol (27-OH) \(^{(84)}\) are potent activating ligands for LXR. Further studies demonstrated that short-heterodimer partner (SHP) \(^{(39-41)}\) and bile salt export pump (BSEP) \(^{(58)}\) are positively regulated and sterol 12-hydroxylase (CYP8B) \(^{(55,23)}\) is a negatively regulated target gene for FXR. ATP binding cassette transporter A1 (ABCA1) \(^{(59)}\) and cholesteryl ester transfer protein (CETP) \(^{(60)}\) are target genes positively regulated by LXR. Chiang et al. found \(^{(61)}\) that FXR/RXR did not directly bind to the promoter region of human CYP7A1 and suggested that bile acid-activated FXR inhibited
CYP7A1 transcription by an indirect mechanism. Recently, two
groups suggested (56, 57) that in mice, activated FXR repressed
CYP7A1 through an FXR-SHP-LRH-1 cascade. More recently, it
has been shown that feeding cholic acid to SHP knock-out mice
could still inhibit CYP7A1 expression (62,63). This report suggests
that other pathways might be involved in negative regulation of
CYP7A1 transcription independent of the FXR-SHP-LRH-1
cascade. For example, induction of cytokines (64) or activation of the
c-Jun N-terminal kinase pathway (65) by bile acids could also result
in repression of CYP7A1 transcription. In addition, the pregnane X
receptor activated by lithocholic acid might play a role in
down-regulation of CYP7A1 (66). However, in FXR knock-out mice,
feeding cholic acid did not suppress Cyp7a1 mRNA levels as seen
in the cholic acid-fed wild-type mice (54). This observation suggested
that in general, FXR-mediated regulation plays the major role in bile
acid-mediated regulation of CYP7A1.

The focus of the present study is on the effects of dietary
cholesterol on FXR and LXR activation and their role in the
regulation of CYP7A1 in NZW rabbits. As there is no method
available to measure directly the activation of FXR and LXR in
whole-animal models, we measured the mRNA expression of
regulated target genes for FXR (SHP, BSEP, and CYP8B) and LXR
(ABCA1 and CETP) to indicate the activation state of the nuclear receptors. We report that after 1 day of cholesterol feeding, LXR but not FXR was activated and thus, CYP7A1 was up-regulated substantially. After 10 days of cholesterol feeding, both FXR and LXR were simultaneously activated, but CYP7A1 was inhibited markedly. These observations suggest that in cholesterol-fed NZW rabbits, the inhibitory effect of activated FXR on CYP7A1 transcription overrides the stimulatory effect of activated LXR.

The hepatic microsomal enzyme CYP7A1 (cholesterol 7-hydroxylase) catalyzes the first and rate-controlling reaction in the classic bile acid synthesis pathway. Feeding cholesterol to New Zealand White (NZW) rabbits for 10 days is associated with suppressed CYP7A1 activity and mRNA levels \(^{(67)}\). Furthermore, in cholesterol-fed NZW rabbits, the bile acid pool size increased about twofold, which was responsible for the inhibition of CYP7A1 \(^{(68)}\). This opinion that the expanded bile acid pool caused inhibition of CYP7A1 was further supported by the observation in rabbits fed 2% cholesterol for only 1 day. In those rabbits, the bile acid pool size was not enlarged, and CYP7A1 was stimulated rather than suppressed \(^{(69)}\). Opposite to rabbits, cholesterol feeding to rats stimulated CYP7A1 and classic bile acid synthesis \(^{(70-72)}\). The different response of CYP7A1 to dietary cholesterol (Ch) between
rabbits and rats relates to the enterohepatic bile acid pool size that is increased in rabbits but stable in rats after cholesterol feeding (73). Recent studies (74-76) showed that CYP7A1 transcription is negatively regulated by the nuclear receptor farnesoid X receptor (FXR) and that bile acids such as lithocholic acid, chenodeoxycholic acid (CDCA), and deoxycholic acid (DCA) are ligands that activate FXR. Although studies in CV-1 cells did not show cholic acid (CA) had strong affinity to activate FXR (77-79), it has been demonstrated in mice that CA is a powerful activating ligand for FXR in vivo (77). These findings support the idea that, in rabbits, expansion of the bile acid pool that contains 85% DCA and 15% CA is responsible for inhibition of CYP7A1 by providing additional activating ligands to activate FXR, the negative regulator of CYP7A1. Further studies suggest that FXR does not directly bind to the CYP7A1 promoter region (78) but regulates CYP7A1 transcription indirectly by activating short heterodimer partner (SHP), which interacts with LRH-1, a transcription factor for CYP7A1 (82,83). Liver X receptor (LXR-), another nuclear receptor, has been identified as a positive regulator of CYP7A1 transcription (79,80). The ligands that activate LXR- are oxysterols (81,82), which increase after cholesterol feeding. Recently, we showed that, in rabbits fed cholesterol for 10 days, the inhibitory effects of activated
FXR secondary to the enlarged bile acid pool overrode the stimulatory effects of activated LXR- to downregulate CYP7A1. However, in other experiments, Gupta et al. suggested that LXR- was the dominant regulator of CYP7A1 transcription in rats.

To understand the mechanism why CYP7A1 is stimulated in rats fed cholesterol, this study investigated the role of activation of LXR- and FXR in the regulation of CYP7A1 in rats fed cholesterol and CA. Our results suggest that upregulation of CYP7A1 in rats fed cholesterol is not because rat CYP7A1 is not sensitive to bile acids or activated FXR, but rather because FXR was not activated.

The apical sodium-dependant bile acid co-transporter (ASBT/SLC10A2) is the primary bile salt uptake protein in the intestine. It is mainly located in the terminal ileal apical surface of enterocytes, and is also expressed on renal proximal tubular cells and large cholangiocytes. ASBT is an efficient transporter for conjugated and unconjugated bile salt. Bile salts reabsorption by ASBT in the ileum is sodium dependent and saturable. The human ASBT gene is located on chromosome 13q33 and it encodes a 4.0 kb transcript. The ASBT protein is 348 amino acids in length and has seven or possible nine transmembrane domains.\(^{85,86,87,88,89,90}\)
transmembrane-spanning domains. ASBT has been cloned from human\(^{91}\), rabbit \(^{92}\), rat \(^{90}\), mouse \(^{93}\) and hamster \(^{94}\).

ASBT gene expression is tightly regulated at the transcriptional level. \(^{95-97}\). A recent study \(^{98}\) showed that negative feedback regulation of ASBT by bile acids was species specific. There was a physiologically functional LRH-1(FTF) transcriptional binding site in the mouse ASBT promoter, which was not present in the rat's. Chenodeoxycholic acid (CDCA), a potent FXR ligand, can repress mouse ASBT expression through FXR/SHP/LRH-1 cascade, while this negative feedback regulation did not occur in rats because of a lack of the LRH-1(FTF) binding site.

Bile acids enterohepatic circulation consists of bile acids synthesis (CYP7A1) and bile acids reabsorption (ASBT). In our previous study \(^{99}\) bile acids pool can be regulated by physiological hydrophobic bile acids but not by physiological hydrophilic bile acids. In the study we further identified that hydrophobic bile acids (such as DCA) could act as FXR activating ligands to activate FXR then down-regulating CYP7A1 expression. However hydrophilic bile acids (such UCA) could not activate FXR, so it could not down-regulate CYP7A1 through FXR/SHP/FTF cascade. We also have interests to know if rabbit ASBT could be regulated by
bile acids and whether similarly as CYP7A1, ASBT only be
down-regulated by FXR activating ligands or not.

The primary aim of this study was to determine if there was a
functional FTF binding site in the rabbit ASBT promoter region and
to investigate whether a regulatory pathway exists that could
account for the bile acid responsiveness of ASBT expression in
vitro and in vivo.

We reported previously\(^{(97)}\) that in hypercholesterolemic Watanabe
rabbits and cholesterol-fed New Zealand White (NZW) rabbits,
cholesterol 7-hydroxylase (CYP7A1), the rate-controlling enzyme of
bile acid synthesis, mRNA and activity was inhibited. We believe
this effect played a major role in the development of
hypercholesterolemia in these rabbits and reported\(^{(98)}\) that
stimulating CYP7A1 by bile fistula drainage (bile acid depletion)
reduced plasma cholesterol levels significantly. Further, we
demonstrated that in cholesterol-fed rabbits, the bile acid pool
actually doubled and hypothesized that the enlarged bile acid pool
was responsible for the feedback inhibition of CYP7A1\(^{(99)}\). In
addition, in these rabbits fed cholesterol where the bile acid pool
size doubled, increased protein levels of ileal apical
sodium-dependent bile acid transporter (ASBT), which is
responsible for active absorption of bile acids from the ileum, was present.\(^{(100)}\) We hypothesized that the increased absorption of bile acids from the ileum together with increased activity of cholesterol 27-hydroxylase (alternative bile acid synthesis) were responsible for maintaining the expanded bile acid pool in the cholesterol-fed rabbits.\(^{(99)}\).

The mechanism by which an enlarged bile acid pool inhibits CYP7A1 is now better understood since recent studies demonstrated that farnesoid X receptor (FXR) serves as a negative regulator of CYP7A1 transcription. FXR is located in the nucleus of hepatocytes. As a bile acids sensor, FXR is activated by the flux of bile acids through the liver.\(^{(102-103)}\) Thus, in cholesterol-fed rabbits, expansion of the circulating bile acid pool, which contains 85% deoxycholic acid—a potent high-affinity ligand, activates FXR, which in turn downregulates CYP7A1. Short heterodimer partner (SHP) is a positively regulated target gene of FXR. Currently, it is thought that activated FXR induces transcription of SHP that in turn inactivates liver receptor homolog-1 (LRH-1), an essential transcriptional factor for CYP7A1 expression\(^{(104,105)}\) to inhibit CYP7A1 transcription. Bile salt export pump (BSEP) is also a target gene positively regulated by FXR.\(^{10}\) BSEP is responsible for canalicular bile acid excretion into the bile.
SC-435 is a competitive inhibitor of ASBT that produces ileal bile acid malabsorption. Plasma low-density lipoprotein (LDL) cholesterol and apolipoprotein B (apoB) levels decreased in miniature pigs treated with SC-435.(27)

In this study, we investigated effect of SC-435 on FXR activation, which regulates CYP7A1 expression and, in turn, reduces plasma cholesterol levels. We showed that the SC-435 treatment increased fecal bile acid outputs that inactivated FXR in the liver and stimulated CYP7A1 activity and mRNA levels. As a result of increased bile acid synthesis, plasma cholesterol concentrations decreased.
AIMS OF THIS STUDY

The aim of this thesis was to study the gene regulation of cholesterol 7α hydroxylase and apical sodium dependant bile acid cotransporter, two key elements in enterohepatic circulation. The study was going to get a deeper understanding of the different plasma responses to dietary cholesterol between rat and rabbit.

Specifically I wanted:

- To determine how FXR regulates CYP7A1 expression
- To understand why rabbit CYP7A1 activity is down-regulating under cholesterol feeding.
- To understand why rat CYP7A1 activity is up-regulating under cholesterol feeding.
- To determine the how bile acids regulate ASBT expression.
- To characterize mechanisms about how ASBT inhibitor lowers plasma cholesterol level.
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