Bile acids enterohepatic circulation
Li, H.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Summary

The enterohepatic circulation (EHC) is an in vivo ecologic system for the conservation of bile salts, allowing them to be used over and over for the absorption of fat. A minimum of 6 molecules (3 in the ileal mucosal cells and 3 in the hepatocytes) constitutes the active players in the EHC. However Cholesterol 7 α hydroxylase (CYP7A1) and apical sodium dependant bile acids co-transporter (ASBT) are two most important elements among them. CYP7A1 is a microsomal cytochrome P450 that catalyzes the first step in bile acid synthesis. ASBT is a receptor that binds bile salts on the brush boarders of the ileum and translocates them into the ileal mucosal cells. Main focus of this thesis was on studying how cholesterol feeding and bile acids flux regulate the gene expression of CYP7A1 and ASBT.

In Chapter 2 we studied the role of hydrophobic and hydrophilic bile acids in the regulation of CYP7A1 under in vivo model. Studies were carried out in four groups including rabbits with bile fistula drainage, rabbits with bile fistula drainage and replacement with either hydrophobic deoxycholic acid (DCA) or hydrophilic ursocholic acid (UCA), and intact rabbits fed hydrophobic cholic
acid (CA). Signal pathway FXR-SHP-FTF-CYP7A1 was examined by Northern blot and Gel shift. After bile fistula drainage (removal of the endogenous bile acid pool), FXR mRNA and nuclear protein levels declined, FXR mediated transcription was decreased, and CYP7A1 mRNA levels increased. Replacing the enterohepatic bile acid pool with DCA restored FXR mRNA and nuclear protein levels and activated FXR-mediated transcription. CYP7A1 mRNA level and activity substantially decreased. Replacing the bile acid pool with UCA did not activate FXR-mediated transcription, CYP7A1 mRNA level and activity were unchanged. Feeding CA to intact rabbits expanded the bile acid pool enriched with the FXR high affinity ligand, DCA. FXR-mediated transcription became activated and decreased CYP7A1 mRNA level and activity. Thus, both hydrophobic and hydrophilic bile acids are effective in maintaining FXR mRNA and nuclear protein levels. However, the activating ligand (DCA) in the enterohepatic flux is necessary for FXR-mediated transcriptional regulation, which leads to down-regulation of CYP7A1.

In Chapter 3 we investigated how cholesterol feeding regulates cholesterol 7-hydroxylase (CYP7A1) via the nuclear receptors farnesoid X receptor (FXR) and liver X receptor (LXR) in New Zealand white rabbits. After 1 day of 2% cholesterol feeding, when
the bile acid pool size had not expanded, mRNA levels of the FXR target genes short-heterodimer partner (SHP) and sterol 12-hydroxylase (CYP8B) were unchanged, indicating that FXR activation remained constant. In contrast, the mRNA levels of the LXR target genes ATP binding cassette transporter A1 (ABCA1) and cholesteryl ester transfer protein (CETP) increased 5-fold and 2.3-fold, respectively, associated with significant increases in hepatic concentrations of oxysterols. Activity and mRNA levels of CYP7A1 increased 2.4 times and 2.2 times, respectively. After 10 days of cholesterol feeding, the bile acid pool size increased nearly 2-fold. SHP mRNA levels increased 4.1-fold while CYP8B declined 64%. ABCA1 mRNA rose 8-fold and CETP mRNA remained elevated. Activity and mRNA of CYP7A1 decreased 60% and 90%, respectively. Feeding cholesterol for 1 day did not enlarge the ligand pool size or change FXR activation, while LXR was activated highly secondary to increased hepatic oxysterols. As a result, CYP7A1 was up-regulated. After 10 days of cholesterol feeding, the bile acid (FXR ligand) pool size increased, which activated FXR and inhibited CYP7A1 despite continued activation of LXR. Thus, in rabbits, when FXR and LXR are activated simultaneously, the inhibitory effect of FXR overrides the stimulatory effect of LXR to suppress CYP7A1 mRNA expression.
Cholesterol feeding downregulates CYP7A1 in rabbits but upregulates CYP7A1 in rats. In Chapter 4 we clarified the mechanism responsible for the upregulation of CYP7A1 in cholesterol-fed rats. The effects of dietary cholesterol (Ch) and cholic acid (CA) on the activation of the nuclear receptors, liver X-receptor (LXR-) and farsenoid X-receptor (FXR), which positively and negatively regulate CYP7A1, were investigated in rats. Studies were carried out in four groups (n = 12/group) of male Sprague-Dawley rats fed regular chow (control), 2% Ch, 2% Ch + 1% CA, and 1% CA alone for 1 wk. Changes in mRNA expression of short heterodimer partner (SHP) and bile salt export pump (BSEP), target genes for FXR, were determined to indicate FXR activation, whereas the expression of ABCA1 and lipoprotein lipase (LPL), target genes for LXR, reflected activation. CYP7A1 mRNA and activity increased twofold and 70%, respectively, in rats fed Ch alone when the bile acid pool size was stable. But CYP7A1 mRNA and activity decreased 43 and 49%, respectively, after CA was added to the Ch diet, which expanded the bile acid pool 3.4-fold. SHP and BSEP mRNA levels did not change after feeding Ch but increased 88 and 37% in rats fed Ch + CA. This indicated that FXR was activated by the expanded bile acid pool. When Ch or Ch + CA were fed, hepatic concentrations of oxysterols, ligands for LXR
increased to activate LXR, as evidenced by increased mRNA levels of ABCA1 and LPL. Feeding CA alone enlarged the bile acid pool threefold and increased the expression of both SHP and BSEP. These results suggest that LXR was activated in rats fed both Ch or Ch + CA, whereas CYP7A1 mRNA and activity were induced only in Ch-fed rats where the bile acid pool was not enlarged such that FXR was not activated. In rats fed Ch + CA, the bile acid pool expanded, which activated FXR to offset the stimulatory effects of LXR on CYP7A1.

The apical sodium dependant bile acids co-transporter (ASBT) is the primary bile salt uptake protein in the intestine. It is also one of key elements in bile acids enterohepatic circulation. However if ASBT expression is regulated by bile acid flux remains controversial. In Chapter 4 the studies were designed to determine whether rabbits ASBT expression is negatively regulated by bile acids flux. In particular we want to identify the signal pathway about this regulation and whether rabbits ASBT is regulated only by FXR-activating ligands. Our in vivo studying showed that increasing hydrophobic bile acids flux (DCA fed rabbits) could repress ASBT expression, associated with activated FXR. However after blocking bile acids absorption by a competitive ASBT inhibitor(SC-435), rabbits ASBT expression increased associated with decreasing
FXR activation. These results showed rabbits ASBT was negatively regulated by bile acids flux. In order to explore the signal pathway of this regulation, we cloned a 1.9 kb rabbit ASBT 5'-flanking region (promotor) and identified a cis acting element (-1166/-1158) for a fetoprotein transcription factor (FTF). In Caco-2 cells FTF stimulated the rabbit ASBT promotor activity fourfold but not after the FTF binding site was deleted from the promotor. Increasing the SHP protein notably inhibited FTF-dependent trans-activation of rabbit ASBT. Adding hydrophobic bile acids DCA, CDCA and CA, activating ligands for FXR, inhibit rabbit ASBT promoter activity in Caco-2 cells, but this inhibitory effect was abolished after the FTF binding site was deleted. UDCA and UCA did not repress ASBT promoter activity because they are FXR non-activating ligands. Thus the rabbit ASBT promotor is negative feedback regulated by bile acids via a functional FTF binding site. Only FXR activating ligands can down-regulate rabbit ASBT expression through the regulatory cascade FXR-SHP-FTF.

In Chapter 6 we investigated the effect of a competitive inhibitor of ASBT (SC-435) on ileal bile acid absorption and the hepatic nuclear receptor FXR, which regulates cholesterol 7-hydroxylase (CYP7A1) activity and mRNA levels. In rabbits treated with SC-435, fecal bile acid outputs increased by more than 8 times, reflecting substantial
bile acid malabsorption. Plasma cholesterol levels decreased 26%, while bile acid pool sizes and biliary bile acid outputs did not change after treatment. CYP7A1 activity increased 64% and mRNA rose by 4 times after treatment. The expression of FXR target genes in the liver, short heterodimer partner (SHP) and bile salt export pump (BSEP), decreased 11.6 and 2.6 times, respectively, after treatment, which indicates inactivation of hepatic FXR. Rabbits treated with SC-435 developed ileal bile acid malabsorption, which decreased the return of bile acids (FXR ligands) to the liver to inactivate hepatic FXR, which upregulated CYP7A1 and lowered plasma cholesterol levels. Although fecal bile acid malabsorption was substantial, increased bile acid production from hepatic cholesterol kept biliary bile acid outputs intact. Thus, a new balance was reached in the liver, where increased bile acid synthesis compensated for diminished ileal bile acid absorption to maintain the circulating enterohepatic bile acid pool.