Effects of bile salts on hepatic lipoprotein production

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Summary

SUMMARY

Increased VLDL production is a common characteristic of several wide-spread human disorders like diabetes type II and obesity. Insight into the regulatory mechanism of VLDL production could lead to development of new treatment and prevention strategies for these disorders. Over the years several research groups have focussed on a possible regulatory role for bile salts in VLDL metabolism. In clinical studies patients with familial hypertriglyceridemia (FHT) were shown to have intestinal bile salt malabsorption, which could be explained by recently reported reduced expression levels of the intestinal bile salt uptake transporter ASBT. Inhibition of ASBT in guinea pigs was demonstrated to significantly increase plasma VLDL-triglyceride (TG) concentration and the formation of larger VLDL particles. In agreement with a regulatory role for bile salts in VLDL metabolism bile salts were demonstrated to dose-dependently inhibit secretion of VLDL-triglyceride by primary rat and human hepatocytes *in vitro*. Studies described in this dissertation were aimed to determine the effects of bile salts on VLDL production *in vivo* and to investigate the mechanism(s) by which bile salts mediate their effects on hepatic VLDL production.

Chapter 1 provides an overview of the current knowledge of lipoprotein metabolism with special focus on hepatic VLDL assembly, of bile salt metabolism and the relationship between bile salt metabolism and hepatic VLDL production.

In **chapter 2** the effects of acute and chronic manipulation of the transhepatic bile salt flux on hepatic VLDL-TG production were studied in two animal models. First, VLDL-TG production was studied in bile-diverted rats in which the enterohepatic circulation was interrupted for a week followed by intraduodenal infusion of bile salts for several hours. The results from these experiments showed that *acute* manipulation of the bile salt flux (bile diversion followed by infusion of taurocholate) did not affect plasma TG concentration, hepatic TG production rate or VLDL lipid composition compared to control rats. However, when the transhepatic bile salt flux was manipulated in mice by a 3-week dietary supplementation with the bile salt taurocholate or the bile salt sequestrant cholestyramine, an inverse relationship between transhepatic bile salt flux and either plasma TG concentration (R^2=0.89) or VLDL-TG production rate (R^2=0.87) was observed. These experiments showed that more *chronic* manipulation of bile salt flux could reduce plasma VLDL-TG concentration and inhibit hepatic VLDL-TG production

in vivo in mice. However, the effects on VLDL-TG production were relatively small and were only observed at supraphysiological transhepatic bile salt fluxes in mice.

Earlier studies in our lab had demonstrated an inverse relationship between intracellular bile salt concentration and VLDL-TG secretion in hepatocytes *in vitro*. Intracellular bile salt concentration is largely determined by the expression and activity of bile salt transporters at the basolateral and canalicular membrane of the hepatocytes. On the other hand, bile salts have been demonstrated to influence their own uptake and secretion by affecting expression of hepatic bile salt transporters. In **chapter 3**, the effects of manipulation of bile salt flux on hepatic bile salt transporter expression *in vivo* in mice were described, using the same mouse model as described in chapter 2. In addition, hepatic transporter expression was assessed in *Cyp7A*$^{-/-}$ mice (mice deficient in cholesterol 7α-hydroxylase) that have a strongly reduced bile salt pool. The data presented in this chapter show that large variations in hepatic bile salt flux have relatively minor effects on expression of hepatic bile salt transporters Ntcp (basolateral) and Bsep (canalicular) *in vivo* in mice. The results suggest that the protein expression of these transporters is not rate-limiting for transhepatic bile salt flux under normal physiological conditions. The liver appears to be able to handle adequately large differences in bile salt loads. The fact that Bsep and Ntcp are homogeneously distributed along the liver acinus, while bile salt transport is predominantly localized to the periportal zone, even at high bile salt concentrations, further supports the concept of excess transport capacity for bile salts.

The role of the nuclear hormone receptor Liver X Receptor (LXR) on hepatic VLDL production was studied in **chapter 4**. Oxysterols, cholesterol metabolites which can also be intermediates in bile salt biosynthesis, can bind and activate LXR. During the recent years LXR has been demonstrated to affect the expression of several genes in bile salt and in lipid metabolism. We hypothesized that oxysterol-activated LXR may also affect hepatic VLDL production. To induce LXR activation a synthetic LXR agonist was administered to wild type male C57BL/6J mice for 5 days. The data presented in this chapter demonstrate that an increase in HDL levels in LXR-agonist treated mice was accompanied by potentially adverse effects on triglyceride metabolism. Pharmacological LXR activation in mice led to development of hepatic steatosis and to secretion of large TG-enriched VLDL particles**.** In wild type mice, a 2-fold increased VLDL-TG production was compensated for by efficient metabolism and clearance, resulting in unaffected fasting TG levels. In mice with impaired particle clearance (apoE-/- LDLr-/-

double knockouts, APOE*3-Leiden transgenics) treatment with the LXR agonist resulted in a marked hypertriglyceridemia.

Recently, bile salts were demonstrated to be natural ligands for the nuclear hormone Farnesoid X Receptor (FXR). Bile salts were shown to inhibit their own biosynthesis via bile salt-activated FXR, but also to affect genes involved in lipid metabolism. **Chapter 5** describes the effects of FXR-deficiency on hepatic and intestinal production of TG-rich lipoproteins *in vivo* in mice and *in vitro* in primary hepatocytes of FXR-deficient mice. Under basal conditions, FXR-deficient ($Fxr^{(-/-)}$) mice showed a ~2-fold increased plasma TG content in the VLDL-sized fractions. The increased plasma TG concentration was not caused by enhanced hepatic TG production, since similar VLDL-TG production rates were measured in control and $Fxr^{(-/-)}$ mice. Interestingly, the nascent VLDL particles were increased in size in $Fxr^{(-/-)}$ mice. In contrast, intestinal production of TG-rich chylomicrons was impaired by ~50% in $Fxr^{(-/-)}$ mice and chylomicron particle size was reduced by 50%. Measurement of VLDL-TG production *in vitro* in primary hepatocytes from $Fxr^{(-/-)}$ mice revealed that, under control conditions, $Fxr^{(-/-)}$ hepatocytes secreted 30% less TG than control hepatocytes. Incubation with physiological concentrations of the bile salt taurocholate decreased VLDL-TG secretion similarly in control and $Fxr^{(-/-)}$ cells, showing that the inhibitory action of bile salts on VLDL-TG secretion *in vitro* is FXR-independent. This study demonstrates that disruption of FXR differentially affects formation of TG-rich lipoproteins in liver and intestine *in vivo*. Hypertriglyceridemia in $Fxr^{(-/-)}$ mice does not result from increased production of TG-rich lipoproteins by liver and/or intestine, but probably from impaired processing of differently structured TG-rich lipoproteins.

Chapter 6 further explored the mechanism by which bile salts inhibit VLDL secretion *in vitro*. We investigated whether bile salts inhibit VLDL secretion by affecting assembly of apolipoprotein B into a TG-rich buoyant VLDL particle or via a mechanism independent of intracellular lipoprotein assembly. Effects of the bile salt taurocholate on secretion of lipid-free, truncated apoB peptide or of apoB assembled into a buoyant TG-rich lipoprotein particle, were studied in doubly transfected McA-RH7777 hepatoma cells, expressing either carboxyl-truncated human apoB18 (secreted in lipid-free form) or full-length human apoB100 (secreted as a buoyant lipoprotein) and the rat bile salt transporter Ntcp. The study shows that taurocholate does not only inhibit the secretion of human apoB100 assembled in lipoproteins but also that of the truncated, lipid-free apoB18 peptide. Thus, the inhibitory action of bile salts on VLDL secretion *in vitro* is not

based on interference with intracellular assembly of apoB into TG-rich VLDL. Inhibition of intracellular protein degradation via the proteasomal degradation route did neither revert decreased apoB secretion nor completely abolish the increased degradation of apoB in TC-treated cells. This indicates that enhanced proteasomal degradation of apoB was not the primary cause of inhibited secretion of apoB. The results in this chapter provide evidence for the existence of a novel mechanism by which bile salts interfere with the secretion of apolipoprotein B via a currently unknown mechanism that most likely involves the N-terminal part of the apoB protein.

The studies described in this dissertation demonstrate that bile salts can exert an effect on VLDL production via a series of pathways mainly independent of transcriptional regulation of genes involved in VLDL metabolism. Under physiological conditions, bile-salt mediated effects on hepatic VLDL production *in vivo* are limited. LXR and FXR are certainly involved in hepatic VLDL production. Yet, they are not the primary target of the bile salt-mediated effect on hepatic VLDL production. The experiments described in this dissertation provide new information on the effects of bile salts and their metabolites on formation and production of TG-rich lipoproteins, but at the same time implicate that more research has to be conducted to further characterize the relationships between bile salts and lipoprotein metabolism.