Physiological functions of biliary lipid secretion
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CHAPTER 10

GENERAL DISCUSSION
This thesis deals with the physiological role of biliary lipid secretion in the maintenance of cholesterol homeostasis in the body. The liver plays a central role in cholesterol metabolism, participating in synthesis, redistribution and catabolism of the sterol. Biliary secretion of bile salts, cholesterol and phospholipids reflects one of the distinct functions of the liver that is crucial for several of these aspects. Under normal physiological circumstances, biliary bile salt secretion drives biliary cholesterol/phospholipid secretion (chapter 1). The generation of mdr2 P-glycoprotein-deficient (Mdr2(-/-)) mice made it possible to evaluate the physiological functions of biliary lipids in the in vivo situation, since these mice show absence of biliary phospholipid secretion, a strongly impaired biliary cholesterol secretion, but unaffected biliary bile salt secretion. The results of studies described in this thesis show that the hepatobiliary flux of cholesterol (and phospholipids) into the intestine is of key importance in the regulation of whole body cholesterol homeostasis; its relevance goes far beyond the long-known function of bile in removal of excess cholesterol from the body.

1. The absence of biliary lipid secretion in Mdr2(-/-) mice is associated with an increased fecal cholesterol secretion (chapter 2). This is paradoxical in view of the fact that, under normal conditions, bile delivers far more cholesterol to the intestine than dietary intake does (chapter 1), particularly in mice fed standard low-cholesterol chow. The increased fecal cholesterol content in Mdr2(-/-) mice must be derived from the diet or from the intestine itself. Similar observations were made in our laboratory in rats with a permanent bile fistula, that also showed an increased fecal cholesterol excretion [1], i.e., by a factor of 2 compared to a factor of 4 in Mdr2(-/-) mice. Although intestinal cholesterol absorption is decreased in Mdr2(-/-) mice by ~50% (chapter 2, 9), the contribution of dietary cholesterol could not account for the strong increase in fecal sterols observed. It is most likely that intestinal cholesterol biosynthesis is derepressed due to the absence of biliary cholesterol delivery to the intestine [2]. The finding that intestinal mRNA levels of HMG-CoA reductase are markedly increased in rats with a permanent bile fistula (chapter 9) confirms this hypothesis. An accelerated desquamation of enterocytes due to the exposure to lipid-free bile in Mdr2(-/-) mice may also contribute to the increase in fecal cholesterol. Changes in intestinal cholesterol synthesis do not necessarily reflect in plasma cholesterol kinetics, as shown by compartmental analysis of the decay of labeled cholesterol (Chapter 2).

2. Plasma HDL-cholesterol levels are strongly decreased in Mdr2(-/-) mice (chapter 2). Low HDL levels could be due to reduced formation of nascent HDL particles, to impaired conversion of nascent HDL into mature particles or to increased clearance from the circulation. Steady state mRNA levels of apolipoprotein A-I, the major apolipoprotein constituent of HDL, in both liver (chapter 2) and intestine (relative ratio apoA-I mRNA to 28S mRNA 1.0 ± 0.5 versus 1.0 ± 0.6, for control and Mdr2(-/-) mice, respectively) did not differ between Mdr2(-/-) and control mice. Reduced intestinal cholesterol absorption (chapter 2) and/or the impaired chylomicron formation (chapter 7) might contribute to low HDL, because chylomicrons provide surface material needed for formation of mature HDL particles from nascent HDL particles [3]. The absence of mdr2 Pgp-dependent biliary secretion of cholesterol/phospholipids in itself, and not the presence of these lipids in the intestine is crucial for the observed decrease of plasma HDL in Mdr2(-/-) mice, since dietary supplementation of excess bile-type
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phospholipids and/or cholesterol did not lead to increased plasma cholesterol or HDL levels (chapter 3). Recently, mutations in the gene encoding the ATP-binding Cassette transporter ABC-1 are shown to be involved in the phenotype of Tangier disease, characterized by absence of HDL, and familial HDL-deficiency [4-6]. It may be that alterations in ABC-1 expression contribute to the low HDL levels found in Mdr2(-/-) mice. Preliminary studies showed no differences in ABC-1 expression in the intestine of Mdr2(-/-) mice (P.J. Voshol, personal observations, 1999), but expression in other organs may well be affected. Furthermore, a novel gene of the ABC superfamily, ABC-8, was found to be involved in regulation of macrophage cholesterol and phospholipid transport [7], demonstrating the possibility of more novel genes to be involved in HDL metabolism. In addition, altered kinetic behavior of HDL due to alterations in expression of the HDL receptor, SR-BI [8], could contribute to the low HDL levels found in Mdr2(-/-) mice [9]. Data showed that hepatic protein expression of SR-BI indeed is increased in these mice (chapter 3). Also hepatic lipase activity is increased in Mdr2(-/-) mice (chapter 4). Hepatic lipase has been shown to facilitate HDL-cholesterol ester uptake via SR-BI in the liver and to reduce plasma HDL levels [10]. These (preliminary) data suggest that the HDL kinetics might be altered in Mdr2(-/-) mice and further investigations are needed to resolve this issue. Hepatic VLDL, apoB100 and apoB48 production are increased in vivo in the Mdr2(-/-) mice when compared to controls (chapter 4), while mRNA levels of apoB, apoB mRNA editing activity and microsomal triglyceride transfer protein (MTP) did not differ between the two groups. The capacity of phosphatidylcholine synthesis, essential for VLDL production [11] did not seem to differ between Mdr2(-/-) and control mice, since activities of CDP-choline transferase and phosphatidylethanolamine N-methyltransferase, two key-enzymes in phosphatidylcholine biosynthesis [12], were similar in both groups. Increased activities of HMG-CoA reductase and ACAT (chapter 2) may contribute to the observed 4-fold increase in hepatic VLDL-cholesterol production (chapter 4) in Mdr2(-/-) mice and, in fact, to increased VLDL formation per se [13,14]. The de-repressed activity of both enzymes is probably due to the absence of chylomicron cholesterol-mediated down-regulation of these enzymes (chapter 7) [15]. The fractional turnover rate of apoB100 did not differ while the fractional turnover rate of apoB48 was increased in Mdr2(-/-) mice, suggesting altered handling of apoB100- and apoB48-containing VLDL particles resulting in the observed increased fasting plasma apoB100 levels in Mdr2(-/-) mice whereas fasting apoB48 and triglycerides levels were not different from those in control mice.

3. The absence of biliary lipid secretion leads to bile duct proliferation [16] and is associated with decreased Ntcp expression (chapter 5) in the liver and increased abst expression in liver (cholangiocytes) and intestine (chapter 6) of Mdr2(-/-) mice. Abst expression in cholangiocytes of the liver is hypothesized to be involved in intrahepatic shunting of bile salts, especially in cholestatic conditions [17]. The increased expression of abst and decreased expression of Ntcp probably reflect compensatory reactions of bile salt transport functions in reaction to the formation of lipid-free bile. Although the total bile salt pool is increased in Mdr2(-/-) mice, the pool size and fractional turnover rate of the primary, relative hydrophobic, bile salt cholate did not differ between Mdr2(-/-) and control mice. It seems that in Mdr2(-/-) mice the relative hydrophilic bile salts are
more efficiently maintained in the enterohepatic circulation. Substrate specificity analysis showed that muricholates, the major bile salts in \textit{Mdr2}\(^{(-/-)}\) mice, do not have a higher affinity for abst than more hydrophobic bile salts like taurocholate [18], implying that other factors must be involved. Despite the increased total bile salt pool size, the synthesis of the primary bile salt cholate appeared not to be affected in \textit{Mdr2}\(^{(-/-)}\) mice. The more hydrophilic bile salt pool of \textit{Mdr2}\(^{(-/-)}\) mice (chapter 6) is apparently less potent in activating the newly identified bile salt receptor FXR [19] and thereby less effective in repressing cholesterol 7\(\alpha\)-hydroxylase.

4. Absence of biliary lipids is associated with changes in the kinetics of fat absorption. Chylomicron formation is strongly impaired in the \textit{Mdr2}\(^{(-/-)}\) mice (chapter 7). Bile type phospholipids have been shown to stimulate secretion of apoB-containing particles in rats with a permanent bile fistula [20] and in CaCo-2 cells [21]. Surprisingly, total fat (chapter 7) and essential fatty acid (chapter 8) absorption appeared to be normal while cholesterol absorption was strongly impaired (chapter 2) in \textit{Mdr2}\(^{(-/-)}\) mice, indicating that separate mechanisms are involved in both absorption processes. The increased bile salt pool size (chapter 6) could contribute to the relatively efficient fat absorption in \textit{Mdr2}\(^{(-/-)}\) mice [22]. Alternatively, absorption may shift from proximal to more distal parts of the intestine: both \(^{3}\text{H}\)-triolein kinetic studies and histochemistry data showed accumulation of fat in more distal parts of intestine of \textit{Mdr2}\(^{(-/-)}\) mice (chapter 7).

In conclusion: bile forms the physiological connection between liver and intestine and serves several important functions in these organs that control plasma lipoprotein concentrations and thus the risk of cardiovascular disease.
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References


