Nuclear receptors in control of cholesterol transport
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CHAPTER 8

General discussion
GENERAL DISCUSSION

Cholesterol is essential for various physiological functions in mammals. It is a structural component of membranes and is thus important for normal cell functioning. Moreover, cholesterol is a precursor of biologically important compounds such as steroid hormones and bile acids. Because of its crucial importance, virtually all cells have the capacity for de novo synthesis of cholesterol. However, excess cholesterol can have deleterious effects for individual cells and for the organism as a whole. Therefore, extensive control of cholesterol synthesis, catabolism, transport and excretion is required. Elevated plasma cholesterol levels constitute an important risk factor for development of cardiovascular disease (CVD), which is still a major cause of mortality in the Western society. High plasma levels of HDL are associated with protection against the development of atherosclerosis. This protective effect is ascribed, in part, to its capacity to transport cholesterol from peripheral tissues and vascular macrophages towards the liver for subsequent elimination via the feces, a process referred to as reverse cholesterol transport. It is generally assumed that HDL is the obligate transport vehicle for RCT and that plasma HDL levels reflect the capacity to accommodate this flux. The classical concept of reverse cholesterol transport, however, may require modification. The liver is often referred to as being the only organ capable to eliminate cholesterol via excretion into bile, however, recent studies have indicated that the intestine is an important secretory organ for cholesterol as well. During the last decade, it has become clear that a number of nuclear receptors control expression of transporters crucial for cellular excretion of cholesterol. Therefore, pharmacological interference with nuclear receptor activities is considered to provide a basis for promising new strategies for prevention and treatment of atherosclerotic cardiovascular diseases. The research described in this thesis revealed interference of different nuclear receptors with several components of reverse cholesterol transport, which are all summarized in figure 1.

Reducing Intestinal Cholesterol Absorption

Cholesterol in the body originates from both endogenous synthesis and intestinal absorption of dietary cholesterol. Inhibition of intestinal cholesterol absorption can be an attractive approach to reduce plasma cholesterol levels and the risk for development of atherosclerosis. Recently, proteins that are involved in cholesterol absorption have been identified, providing insight in the mechanism of this process. The newly identified Niemann-Pick C1-like 1 protein (NPC1L1), expressed at the apical membrane of enterocytes, is crucial for the uptake of sterols from the intestinal lumen into the enterocyte. NPC1L1 has been identified as the target for the cholesterol absorption inhibitor ezetimibe, a drug now widely used to reduce plasma cholesterol levels in hypercholesterolemic subjects. In sitosterolemia patients, ezetimibe strongly reduces intestinal plant sterol absorption, indicating that NPC1L1 does not discriminate between cholesterol and plant sterols. The heterodimeric ABC transporter ABCG5/ABCG8, localized at the apical membrane of enterocytes, is known to efflux sterols from the enterocytes back into the intestinal lumen and thereby limit net cholesterol absorption. Identification of these proteins provided evidence that overall intestinal cholesterol absorption is dependent on both rates of uptake and efflux by enterocytes. Several studies described in this thesis indicate that nuclear receptors can influence expression levels of these proteins and affect fractional cholesterol absorption.
absorption. Experiments described in chapter 2 revealed that activation of PPARδ reduces fractional intestinal cholesterol absorption by ~43% in mice, coinciding with reduced intestinal expression of *Npc1l1*. Treatment of Caco-2 cells with a PPARδ agonist reduced mRNA expression of *NPC1L1*, whereas neither PPARα nor PPARγ were effective in this respect, indicating that the effect is PPARδ-specific. Similar effects were observed in ApoE2-KI mice treated with the RXR agonist bexarotene, as described in chapter 3. Activation of RXR significantly reduced expression of *Npc1l1* in duodenum and jejunum which was associated with a 64% reduction of fractional intestinal cholesterol absorption in apoE2-KI mice. Both PPARδ and RXR did not affect intestinal expression of *Abcg5/Abcg8*. General or intestine-specific activation of LXR induces expression of *Abcg5/Abcg8* in the enterocytes and several studies have revealed that this results in reduced fractional intestinal cholesterol absorption5,13,14. Recent studies have shown that *Npc1l1* expression is also responsive to LXR activation. Both ApoE2-KI15 and *Mdr2*−/− mice, but not their FVB wild-type controls5, exhibited reduced intestinal mRNA levels of *Npc1l1* upon treatment with an LXR-agonist. These studies indicate that (intestine-specific) activation of certain nuclear
receptor systems by pharmacological agonists may be a promising approach for treatment of atherosclerotic cardiovascular diseases through their inhibitory effect on fractional intestinal cholesterol absorption.

**Cholesterol efflux from macrophages**
The obligate first step in the development of atherosclerotic plaques is accumulation of cholesteryl esters in macrophages in the arterial wall, leading to foam cell formation. Therefore, removal of excess cholesterol from these cells, the initial step of RCT, is of crucial importance for prevention and/or treatment of atherosclerotic cardiovascular diseases. HDL and apoA-I interact with macrophages to promote efflux of excess cholesterol. Therefore, HDL-raising therapies are currently considered as promising strategies for prevention and treatment of these diseases. Elevation of plasma HDL or ApoA-I levels stimulates cholesterol efflux from macrophages. Infusion of HDL particles or apoA-I, as well as overexpression of the human gene for apoA-I, inhibits the development of atherosclerosis in relevant animal models. Also in humans, intravenous infusion of apoA-I(Milano) has been shown to reduce intima-media thickness and to enhance fecal excretion of neutral sterols, consistent with its stimulating role in reverse cholesterol transport.

The ABC-transporters ABCA1 and ABCG1 mediate cholesterol efflux from macrophages towards lipid-poor apoA-I and HDL, respectively. Activation of LXR increases expression of both ABCA1 and ABCG1 and thereby protects the macrophage from cholesterol overload. The studies described in chapter 2 and 3 revealed a role of PPARδ and RXR, respectively, in macrophage cholesterol efflux. Treatment with a synthetic PPARδ agonist increased expression levels of Abca1 and Sr-bi in peritoneal macrophages of DBA/1 mice. Together with the increased plasma HDL-cholesterol levels, these results suggest that pharmacological activation of PPARδ may reduce foam cell formation and thus can be beneficial for the treatment or prevention of atherosclerosis. Activation of RXR with bexarotene reduced lipid-loading of peritoneal macrophages in ApoE2-KI mice fed a Western diet. Cholesterol efflux from macrophages towards apoA-I and HDL was stimulated in treated mice and expression levels of Abca1 and Abcg1 in the aortic sinus were induced. As a result, atherosclerosis development was reduced upon RXR activation, indicating that RXR may also be a promising target for drug development aimed at preventing atherosclerosis.

**Hepatobiliary cholesterol secretion**
Hepatobiliary secretion of cholesterol has been considered to be the main route for elimination of cholesterol from the body. Biliary secretion of cholesterol is closely coupled to that of phospholipids and is stimulated by bile acid secretion. These hepatobiliary secretion processes are mediated by several ABC-transporters localized at the canalicular membrane: the bile salt export pump (BSEP; ABCB11) for bile salts, Mdr2-P-glycoprotein (ABCB4) for phospholipids, and the ABCG5/ABCG8 heterodimer for cholesterol.

Several knock-out and transgenic mouse models have been used to elucidate the process of hepatobiliary cholesterol secretion and its relevance in the concept of RCT. Studies using such animal models have revealed that Abcg5 and Abcg8 are of crucial importance for hepatobiliary cholesterol secretion: hepatobiliary secretion of cholesterol is increased in mice in which Abcg5/Abcg8 expression is induced by overexpression or by pharmacological interventions. Additionally, mice lacking both Abcg5 and Abcg8...
exhibit virtually no hepatobiliary cholesterol secretion (90% reduction in gallbladder bile concentration)\(^{28}\). Mice lacking either one of the genes also show significantly increased plasma and tissue plant sterol levels, consistent with sitosterolemic phenotype that was observed in humans carrying mutations in these genes. Actual biliary cholesterol secretion rates were low but not completely absent in these mice, i.e., reduced by 70% and 82% in \(Abcg8\)^{-/-} and \(Abcg5\)^{-/-} mice, respectively. Cholesterol secretion increased only minimally upon infusion of bile acids in both mouse models. In heterozygous \(Abcg5\)^{-/-} mice, cholesterol secretion rates completely recovered upon bile acid infusion to reach wild-type levels (chapter 5), whereas in heterozygous \(Abcg8\)^{-/-} mice, cholesterol secretion increased to reach a maximal level of about 50% of the wild type values upon infusion\(^{27}\). Kosters \textit{et al.} reported that \(Abcg5/Abcg8\) mRNA expression levels indeed correlated with biliary cholesterol secretion rates in a variety of different mouse strains\(^{32}\). However, they noted some exceptions, e.g. in the diosgenin-fed mouse model, in which hepatic \(Abcg5/Abcg8\) expression levels remained unchanged while biliary cholesterol secretion was ~16-fold increased. Another exception to this rule is described in chapter 5. \(Lxr^{-/-}\) mice that were fed a high cholesterol diet secreted similar amounts of cholesterol into their bile as their wild-type and heterozygous controls did, though these mice were unable to increase hepatic expression of \(Abcg5/Abcg8\) upon cholesterol feeding. Expression levels of these genes were ~4-fold increased in wild-type mice. Together with the biliary cholesterol secretion in \(Abcg5\)^{-/-} mice that reached wild-type levels upon bile acid infusion, these studies provide evidence that, under certain metabolic conditions, a parallel route for biliary cholesterol secretion might be operational that is independent of \(Abcg5/Abcg8\). This notion is supported by recent data obtained in human subjects after liver transplantation, in which no correlation between hepatic ABCG5/ABCG8 mRNA expression and biliary cholesterol secretion was found\(^{33}\).

Nuclear receptors are involved in the regulation of hepatobiliary cholesterol secretion. Expression of \(Abcg5/Abcg8\) is increased upon LXR activation\(^{6,14}\) and possibly the orphan nuclear receptor LRH-1 also induces expression of these genes\(^{34}\). Besides this control by nuclear receptors, expression of hepatic \(Abcg5/Abcg8\) is also under control of the endogenous clock system in mice. The study described in chapter 6 showed that hepatic \(Abcg5/Abcg8\) expression as well as hepatobiliary cholesterol secretion oscillates during the day. The clock output factor DBP\(^{35}\) is involved in control of the amplitude of this circadian variation, yet the mechanism by which DBP influences \(Abcg5/Abcg8\) expression is not known. Since expression and activities of enzymes governing cholesterol synthesis\(^{36-39}\) and its conversion into bile acids\(^{40-42}\), HMGCoA reductase and cholesterol 7α-hydroxylase, respectively, also show marked diurnal variation, this study underscores the fact that many processes in cholesterol metabolism are tightly attuned during the day.

**Modified Concept of Reverse Cholesterol Transport**

Reverse cholesterol transport (RCT) is classically defined as the flux of excess cholesterol from peripheral tissues, including vascular macrophages, towards the liver followed by biliary secretion and subsequent disposal \textit{via} the feces\(^{3}\). It is generally assumed that HDL is the obligate transport vehicle for RCT and that plasma HDL levels reflect the magnitude of this flux. In this “classical” scenario, the liver has a central role in RCT\(^{4}\). Biliary secretion of free cholesterol, facilitated by the heterodimeric ABC-transporter ABCG5/ABCG8\(^{11}\), and hepatic conversion of cholesterol to bile acids are often referred to as the only routes for
quantitatively important elimination of cholesterol from the body. However, recent studies indicate that the classical concept of RCT may require reconsideration. Studies in apoA-I-deficient mice revealed that the magnitude of the centripetal cholesterol flux from the periphery to the liver is not related to the concentration of HDL-cholesterol or apoA-I in plasma\textsuperscript{43}. Furthermore, Abca1\textsuperscript{−/−} mice that completely lack plasma HDL show unaffected hepatobiliary cholesterol secretion rates and fecal sterol loss\textsuperscript{44,45}. Additionally, mice lacking both Abcg5 and Abcg8 do not show a reduction in fecal neutral sterol excretion to the extent expected on the basis of their strongly reduced hepatobiliary cholesterol secretion\textsuperscript{46}. Plösch \textit{et al.}\textsuperscript{6} showed that the increased fecal neutral sterol loss upon LXR activation cannot be attributable to increased hepatobiliary cholesterol secretion only. Additionally, studies by Kruit \textit{et al.}\textsuperscript{5} revealed that Mdr2\textsuperscript{−/−} mice, which are unable to secrete cholesterol into bile, excrete similar amounts of neutral sterols into the feces as their wild-type control do. Moreover, intravenously administered [\textsuperscript{3}H]-cholesterol could be recovered in the neutral sterol fraction of the feces in these mice. These studies indicated the intestine to be a quantitatively important secretory organ for cholesterol. Moreover, both studies indicated that this direct intestinal secretion pathway could be induced upon treatment with an LXR agonist. In chapter 2 we investigated the effects of pharmacological activation of PPAR\textsubscript{γ} on cholesterol metabolism. Data from this study indicate that activation of PPAR\textsubscript{γ} also stimulates direct intestinal cholesterol secretion, since we observed increased recovery of i.v. administered [\textsuperscript{3}H]-cholesterol in feces upon PPAR\textsubscript{γ} activation, without alterations in biliary cholesterol secretion rates. Furthermore, recent intestinal perfusion studies in mice revealed that, in the presence of mixed micelles as cholesterol acceptors in the lumen, murine enterocytes indeed have a high capacity to secrete cholesterol\textsuperscript{7}. However, the exact quantitative contribution of this pathway in vivo could not be determined in these studies. In chapter 7 we quantified the relative and absolute contributions of several relevant cholesterol fluxes to total fecal neutral sterol loss, including the direct intestinal cholesterol secretion pathway. This study showed that direct intestinal cholesterol secretion is a major route for removal of blood-derived free cholesterol, accounting for 33% of fecal neutral sterol excretion in C57Bl6/J mice. This pathway is highly sensitive to pharmacological activation of LXR, which led to a 6-fold increase in the absolute amount of cholesterol that is secreted by the intestine. Moreover, in Abcg5\textsuperscript{−/−} mice the direct intestinal secretion of cholesterol was impaired, suggesting involvement of Abcg5/Abcg8 in this secretory pathway. However, the exact pathways involved in this excretory process still need to be identified, but it is evident that (intestinal) activation of the nuclear receptors LXR and PPAR\textsubscript{γ} stimulates this quantitatively important route for fecal removal of cholesterol and might thus serve as a potential means to enhance reverse cholesterol transport.

\textit{Perspectives}

Atherosclerosis and cardiovascular diseases are major causes of morbidity and mortality in the Western society. Elevated plasma cholesterol levels constitute a major risk for development of these diseases. For many years, inhibition of cholesterol synthesis by statins has been the main therapeutic approach to reduce plasma cholesterol levels. Although LDL-cholesterol levels are significantly decreased, on average two thirds of CVD-events will not be prevented by this treatment\textsuperscript{46-48}. Therefore, other strategies aimed at reducing plasma cholesterol levels are needed. Interference with intestinal cholesterol absorption has become an attractive cholesterol-lowering approach and the identification of
the potent cholesterol absorption inhibitor ezetimibe has been of major importance. Treatment with ezetimibe reduces plasma LDL-cholesterol levels by approximately 20% in mildly hypercholesterolemic subjects\textsuperscript{49,50} and this drug is now widely used either as mono-therapy or in combination with statins\textsuperscript{51} to reduce plasma cholesterol levels.

Additionally, strategies targeting reverse cholesterol transport and plasma HDL levels have gained major interest as potential approaches to increase cholesterol efflux from macrophages/foam cells and hence reduce the development of atherosclerosis. HDL-raising therapies may have high potential in this respect because of the multiple atheroprotective effects of this HDL particle. Beside its stimulatory effect on cholesterol efflux from vascular macrophages, HDL also excerts anti-inflammatory, anti-oxidative and anti-coagulant activities\textsuperscript{52}. These anti-atherogenic properties of HDL are currently extensively investigated.

During the last decade, agonists for certain nuclear receptors have been shown to interfere with several components of reverse cholesterol transport and are therefore considered as promising tools for the development of novel drugs aimed at preventing atherosclerosis. In this perspective, activation of LXR seems to be an attractive approach to enhance reverse cholesterol transport. However, clinical application of synthetic LXR agonists is hampered by the fact that LXR also induces de novo lipogenesis, leading to hepatic steatosis and increased VLDL-TG production in rodents\textsuperscript{53,54}. To circumvent such undesirable side-effects, development of tissue-specific and/or gene-specific LXR modulators is required.

The intestine may be a promising target organ because of its importance in reverse cholesterol transport. Recent studies have shown that, besides the liver, intestinal Abca1 contributes for approximately 30% to the steady-state plasma HDL pool in mice\textsuperscript{55}. Studies described in this thesis revealed that direct intestinal cholesterol secretion represents a quantitatively important route for fecal removal of neutral sterols independent of biliary secretion in mice. This pathway is stimulated by pharmacological activation of PPAR\textsubscript{\alpha} as well as of LXR. In addition, activation of these nuclear receptors, as well as activation of RXR, reduces fractional intestinal cholesterol absorption. Together, these studies indicate that the intestine may be a highly attractive potential target organ for nuclear receptor interference in order to stimulate reverse cholesterol transport. Activation of LXR specifically aimed at the intestine could be an attractive approach to develop novel drugs for treatment or prevention of atherosclerotic cardiovascular diseases.
REFERENCES


4. Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 1993;34:1637-1659.


43. Jolley CD, Woollett LA, Turley SD, Dietschy JM. Centripetal cholesterol flux to the liver is dictated by events in the peripheral organs and not by the plasma high density lipoprotein or apolipoprotein A-I concentration. *J Lipid Res* 1998;39:2143-2149.


