SUMMARY

The work described in this thesis deals with the role of nuclear receptors in transcriptional control and function of transporters involved in the generation of bile. Bile formation is one of the major functions of the liver. Membrane transport proteins that primarily determine bile formation are located at the basolateral (uptake) and canalicular (secretion) membrane domains of hepatocytes. The last step in hepatobiliary transport, i.e., the secretion of the major bile constituents such as bile salts, phospholipids and cholesterol into the canalicular lumen, is mediated by ABC transporter proteins which couple ATP hydrolysis to transport and thereby enable uphill transport. Cholestasis refers to the condition in which the formation of bile is impaired. The causes underlying the development of cholestasis in patients can be extremely varied. It may occur through interference with canalicular secretion from the hepatocyte by drugs or toxins or due to genetic defects in specific transporters. Recently, it has become clear that nuclear receptors play a prominent role in the transcriptional control of transporter genes, but also of genes involved in bile salt, lipid and cholesterol metabolism. Dietary compounds, certain endogenous metabolites and drugs may alter hepatic transporter activity, and thus bile formation, through activation of these nuclear receptors. The aim of this thesis was to define the role of a number of such nuclear receptors in control of hepatobiliary transport function. Most data published thus far are from in vitro studies and their relevance for the in vivo situation has remained largely unknown. Therefore, we have studied the in vivo effects of different nuclear receptors, specifically of the peroxisome proliferator-activated receptor α (PPARα), the liver X receptor (LXR) and the farnesoid X receptor (FXR), on the expression of hepatic transporters and their physiological consequences on bile formation. Furthermore, the overall effects on the enterohepatic circulation of bile salts and on cholesterol transport have been evaluated.

Chapter 1 provides an overview of the hepatic and intestinal transport systems that are of relevance for the conducted studies. This chapter also describes the basics of bile formation, nuclear receptors as well as cholesterol and bile salt metabolism in relation to hepatic and intestinal transport systems.

In the work described in chapter 2, the role of the peroxisome proliferator-activated receptor α (PPARα) in control of bile formation and hepatic transporter expression was studied using PPARα-deficient mice. PPARα is involved in control of the fatty acid oxidation machinery in the liver, but also in that of hepatic bile salt metabolism. PPARα can be activated by its natural ligands, i.e., fatty acids, but also by fibrates. Fibrates consist of a group of lipid-lowering drugs, that also may affect bile composition. The results of this study showed that the absence of PPARα does not affect the expression of important transport systems in the liver or bile formation in mice under normal chow-fed conditions. Stimulation of PPARα activity with ciprofibrate, however, resulted in a specific induction of expression and function of the canalicular phospholipid translocator Mdr2. This effect was independent from the rodent-specific PPARα-mediated induction of liver enlargement. In contrast, increased expression of other members of the ABCB subfamily upon ciprofibrate treatment in vivo, i.e., of Mdr1a and Mdr1b, appeared to be related to the peroxisome proliferating capacities or to the metabolic actions of the drug rather than to direct control of PPARα on expression of these genes.

A physiological condition in which PPARα becomes activated is during fasting. Fatty acids that are liberated from adipose tissue during fasting are, in part, taken up by the liver, where they may serve as ligands for PPARα. Chapter 3 evaluates the effects on expres-
sion of hepatobiliary transporters related to parameters of bile formation in wild-type and PPARα-deficient mice subjected to a fasting-refeeding schedule. This study demonstrates that the induction of \( Mdr2 \) gene expression and of \( Mdr2 \) Pgp function is part of the PPARα-controlled fasting response in the liver of the mouse. The PPARα-dependent increase in expression of the putative hepatobiliary cholesterol transport genes \( Abcg5 \) and \( Abcg8 \) upon fasting was not associated with increased biliary cholesterol excretion. In contrast, maximal cholesterol excretion appeared to be impaired in fasted mice. This indicates that hepatobiliary cholesterol excretion is, in quantitative terms, not strictly controlled by these ABC transporters during fasting.

In chapter 4 we have tried to elucidate the potential role of PPARα in the regulation of the human \( MDR3 \) gene, which is the homologue of the murine \( Mdr2 \) gene. Human hepatocytes treated with different PPARα agonists showed increased \( MDR3 \) mRNA expression levels. A peroxisome proliferator response element (PPRE) was found ~4.9 kb upstream of the major transcription initiation site. This site was tested by electrophoretic mobility shift assays and luciferase studies \textit{in vitro} and was shown to be functional and to bind the PPARα/RXR complex. We propose that \( MDR3 \) regulation occurs at the transcriptional level by binding of PPARα/RXR to a DR1 response element located at -4926 to -4913 in the promoter region of the \( MDR3 \) gene. Five and half kb of the human \( MDR3 \) promoter was cloned and further studies have to be conducted to elucidate whether the human \( MDR3 \) gene is a ‘real’ PPARα target gene.

The liver X receptor (LXR) has been recognized as an important regulator of whole-body cholesterol metabolism and natural ligands for LXR are the so-called oxysterols (oxidized derivatives of cholesterol). Using the synthetic LXR agonist T0901317 in wild-type and in Abca1-deficient mice, we have studied the role of LXR in control of bile formation, specifically focusing on biliary cholesterol excretion (chapter 5). Abca1-deficient mice display a complete absence of HDL, which is thought to be a major source for bile-destined cholesterol. Surprisingly, our results showed that LXR activation by T0901317 increased hepatobiliary cholesterol output in wild-type as well as in Abca1-deficient mice. This increase was associated with increased hepatic and intestinal \( Abcg5 \) and \( Abcg8 \) expression. Fecal sterol loss was increased upon T0901317 treatment in wild-type and in Abca1-deficient mice, independent of elevation of plasma HDL levels and the (putative) role of Abca1 in intestinal cholesterol absorption. These data imply that the proposed role of HDL in reverse cholesterol transport needs substantial revision.

The bile salt-activated farnesoid X receptor (FXR) is involved in control of bile salt and lipid metabolism. Bile salts inhibit their own synthesis via FXR-mediated feedback repression. Furthermore, activated FXR induces the expression of the canalicular bile salt export pump (Bsep). In chapter 6, we have determined the \textit{in vivo} role of FXR on the enterohepatic circulation of bile salts by quantifying bile salt kinetics in FXR-deficient mice using a stable isotope dilution method. The kinetic parameters were related to bile formation and the expression of hepatic and intestinal transporters involved in the enterohepatic circulation of bile salts. These studies were performed in a new FXR knockout model, generated by homologous recombination. In the absence of FXR, the basal expression of \( Bsep \) was downregulated, whereas biliary bile salt output rates were actually increased under normal chow-fed conditions. FXR-deficiency was associated with a clearly defective feedback inhibition of hepatic cholate synthesis, i.e., cholesterol 7α-hydroxylase (\( Cyp7a1 \)) was increased which correlated with an increased cholate synthesis rate. These combined effects resulted in an enlarged circulating cholate pool size in FXR-defi-
cient mice. The ileal bile salt-binding protein (Ibabp), a well-known FXR target gene, was shown to be absent in the terminal ileum of FXR-deficient mice. Despite this, the intestinal cholate reabsorption was markedly increased. The absence of Ibabp does not negatively interfere with the intestinal absorption of cholate in mice.

A similar series of studies were conducted in chapter 7, in which rats were treated with the synthetic FXR agonist GW4046 to evaluate the effects of chronic activation of FXR on the enterohepatic circulation of bile salts. The results of this study showed that chronic activation of FXR does not affect basal bile formation in rats, despite significantly increased Bsep expression levels. As expected, and opposite to the situation observed in the FXR-deficient mice, activation of FXR led to a decreased cholate synthesis rate and a decreased cholate pool size. Upon FXR activation, Ibabp protein levels were increased in the terminal ileum with a shift towards more proximal segments. Yet, our kinetic study revealed that the absolute amount of cholate reabsorbed by the intestine was not increased. Data from chapter 6 and 7 together imply that the presumed role of Ibabp in intestinal bile salt transport needs to be revised.

Chapter 8 gives an overview of the known hepatic ABC transporters involved in bile formation. Available ABC transporter gene knockout mouse models and their phenotypic appearance is compared to characteristic hallmarks of (cholestatic) inherited diseases in humans caused by mutations in homologous transporters. Furthermore, several nuclear receptor knockout mouse models are discussed as far as they affect ABC transporter expression and bile formation.

In conclusion, the nuclear receptors PPARα, LXR and FXR have a function in transcriptional regulation of hepatic (and intestinal) transporter genes. Nuclear receptors act as metabolic sensors, by inducing the appropriate metabolic pathways able to protect the organism from ‘overexposure’. Most data published thus far on the subject are from in vitro studies, but studies described in this thesis demonstrate their relevance for the in vivo situation in rodents. We show that regulation of transporter genes by nuclear receptors in vivo, in most cases, is of physiological relevance for the bile formation process. Nuclear receptors may be potential pharmacological targets for treatment of metabolic and cholestatic liver diseases in the future.