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Specific Gene Expression of ATP-binding Cassette Transporters and Nuclear Hormone Receptors in Rat Liver Parenchymal, Endothelial, and Kupffer Cells*

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Hepatic cholesterol(ester) uptake from serum coupled to intracellular processing and biliary excretion are important features in the removal of excess cholesterol from the body. ATP-binding cassette (ABC) transporters play an important role in hepatic cholesterol transport. The liver consists of different cell types, and ABC transporters may exert different physiological functions dependent on the individual cell type. Therefore, in the current study, using real time PCR we compared the mRNA expression of ABC transporters and genes involved in the regulation of cholesterol metabolism in liver parenchymal, endothelial, and Kupffer cells. It appears that liver parenchymal cells contain high expression levels compared with endothelial and Kupffer cells of scavenger receptor class BI (~3-fold), peroxisome proliferator-activated receptor (PPAR)α and PPARγ (8–20-fold), cholesterol 7α-hydroxylase A1 (>100-fold), and ABCG5/G8 (~5-fold). Liver endothelial cells show a high expression of cholesterol 27-hydroxylase, liver X receptor (LXR)β, PPARδ, and ABCG1, suggesting a novel specific role for these genes in endothelial cells. In Kupffer cells, the expression level of LXRα, ABCA1, and in particular ABCG1 is high, leading to an ABCG1 mRNA expression level that is 70-fold higher than in parenchymal cells. It can be calculated that 51% of the total liver ABCG1 expression resides in Kupffer cells and 24% in endothelial cells, suggesting an intrahepatic-specific role for ABCG1 in Kupffer and endothelial cells. Because of a specific stimulation of ABCG1 in parenchymal cells by a high cholesterol diet, the contribution of parenchymal cells to the total liver increased from 25 to 60%. Our data indicate that for studies of the role of ABC transporters and their regulation in liver, their cellular localization should be taken into account, allowing proper interpretation of metabolic changes, which are directly related to their (intra)cellular expression level.

Transport of cholesterol through the body is an important process in the maintenance of total body cholesterol homeostasis. One specific component is the transport of excessive cholesterol from the periphery to the liver by the high density lipoprotein (HDL).[1] This process is called reverse cholesterol transport and consists of three different stages: the efflux of peripheral cellular cholesterol to HDL, the transport of cholesterol esters through the blood to the liver, and the uptake of cholesterol esters by the liver (1). Cholesterol esters are taken up into the liver through selective uptake by the HDL receptor, scavenger receptor class BI (SR-BI) (2), where they are primarily (~50%) catabolized to bile acids, through conversion by cholesterol 7α-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27) (3) for biliary excretion via the bile salt export protein (4). A second major catabolic route (~40%) is the direct efflux of cholesterol from the liver into the bile via the ABC half-transporters ABCG5 and ABCG8, which together function as a biliary sterol efflux regulator (5). Additionally, cholesterol also effluxes from the liver to produce very low density lipoproteins, which are converted to remnants that function as precursors for low density lipoproteins. Native HDL might also be formed by the ABC transporter A1 (ABCA1)-mediated cholesterol efflux (6). Recently, another member of the ABC transporter family, ABCG1, has been proposed to play a role in the intracellular trafficking and efflux of cholesterol (7). However, the exact role of ABCG1 in the liver, especially in relation to ABCG5 and ABCG8, remains to be clarified.

The identification of SR-BI and novel members of the ABC transporter family, including ABCA1, ABCG1, ABCG5, and ABCG8, has allowed the molecular characterization of the individual transporters responsible for the intracellular trafficking and excretion of cholesterol (derivatives). In the liver, it has been shown that SR-BI is responsible primarily for the selective uptake of cholesterol esters from HDL (8), whereas ABCG5/G8 and ABCG1, and ABCA1, are proposed mediators of efflux to the bile and to HDL, respectively (6, 9). However, the liver is a complex tissue and contains, in addition to the parenchymal cells, which are localized around the bile canaliculi, endothelial cells, and tissue macrophages (Kupffer cells). To assess the individual function of the ABC transporters and their regulation by nuclear hormone receptors it is therefore essential to establish their cellular localization in the liver.

Here we report that key mediators in liver cholesterol homeostasis, in particular PPARα, PPARγ, and ABCG1, are expressed differentially in specific cell types of the rat liver. Our data stress that it is necessary to focus on the regulation of genes involved in cholesterol homeostasis in the different cell

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[1] The abbreviations used are: HDL, high density lipoprotein; ABC, ATP-binding cassette; C_t, threshold cycle number; CYP7A1, cholesterol 7α-hydroxylase; CYP27, cholesterol 27-hydroxylase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; SR-BI, scavenger receptor class BI.

TABLE I
Primers for quantitative real time PCR analysis

Gene	GenBank accession	Forward primer	Reverse primer	Amplicon size
ABCA1[a]		ATCTCATAGTATGGAAGAATGTGAAGCT	CGTACAACTATTGTATAACCATCTCCAAA	132
ABCG1	NM053502	AGGTCTCAGCCTTCTAAAGTTCCTC	TCTCTCGAAGTGAATGAAATTTATCG	85
ABCG5	NM053754	CGCAGGAACCGCATTGTAA	TGTCGAAGTGGTGGAAGAGCT	67
ABCG8	NM130414	GATGCTGGCTATCATAGGGAGC	TCTCTGCCTGTGATAACGTCGA	69
CYP27	M38566	GTGTCCCGGGATCCCAGTGT	CTTCCTCAGCCATCGGTGA	66
CYP7A1	NM012942	CTGTCATACCACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC	75
HPRT	X62085	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAG	91
LXRα	NM031627	TCAGCATCTTCTCTGCAGACCGG	TCATTAGCATCCGTGGGAACA	144
LXRβ	NM031626	AAGCTGGTGAGCCTGCGC	CGGCAGCTTCTTGTCCTG	81
PPARα	NM013196	TGAACAAAGACGGGATG	TCAAACTTGGGTTCCATGAT	106
PPARγ	NM013124	CATGCTTGTGAAGGATGCAAG	TTCTGAAACCGACAGTACTGACAT	131
PPARδ	NM012141	GAGGGGTGCAAGGGCTTCTT	CACTTGTTGCGGTTCTTCTTCTG	97
SR-BI	U76205	GTTGGTCACCATGGGCCA	CGTAGCCCCACAGGATCTCA	65

[a] Primer sequences based on an alignment of human (NM005502) and murine (XM109397) ABCA1.

types of the liver to get molecular insight in their mechanism of regulation and the consequences for liver cholesterol transport.

EXPERIMENTAL PROCEDURES

Animals—In the study, three male Wistar WU rats (200–250 g) were fed a chow diet containing 4.3% (w/w) fat and no cholesterol, and three rats were fed a high cholesterol diet containing 2% (w/w) cholesterol, 5% olive oil (w/w), and 0.5% (w/w) cholic acid for 2 weeks. Rats were anesthetized and the vena porta was cannulated. Subsequently, the liver was perfused for 10 min with oxygenated Hanks' buffer, pH 7.4, containing HEPES (1.6 g/liter). The perfusion was continued for 10 min with Hanks'/HEPES buffer containing 0.05% (w/v) collagenase (type IV, Sigma) and 1 mM $CaCl_2$. Parenchymal cells were isolated after mincing the liver in Hanks' buffer containing 0.3% bovine serum albumin, filtering through nylon gauze and centrifugation for three times 10 min at 50 $\times$ *g*. The pellets consisted of pure (>99%) parenchymal cells as judged by light microscopy. The supernatants were centrifuged for 10 min at 500 $\times$ *g* to harvest the non-parenchymal cells. By means of centrifugal elutriation the endothelial cells and Kupffer cells were separated (10). The purity of each cell fraction (>95% for both) was checked by light microscopy, after staining for peroxidase activity with 3,3-diaminobenzidine (Sigma).

Analysis of Gene Expression by Real Time Quantitative PCR—Total RNA was isolated from rat liver parenchymal, endothelial, and Kupffer cells using TriZol reagent (Invitrogen) according to the manufacturer's instructions. Purified RNA was DNase treated (DNase I, 10 units/μg of total RNA) and reverse transcribed (RevertAid M-MuLV reverse transcriptase) according to the protocols supplied by the manufacturer.

Quantitative gene expression analysis was performed on an ABI PRISM 7700 machine (Applied Biosystems, Foster City, CA) using SYBR Green technology. PCR primers (Table I) were designed using Primer Express 1.7 software with the manufacturer's default settings (Applied Biosystems) and validated for identical efficiencies (slope = −3.3 for a plot of the threshold cycle number (C_t) *versus* log ng cDNA). In 96-wells optical plates, 12.5 μl of SYBR Green master mix was added to 12.5 μl of cDNA (corresponding to 50 ng of total RNA input) and 300 nM forward and reverse primers in water. Plates were heated for 2 min at 50 °C and 10 min at 95 °C. Subsequently 40 PCR cycles consisting of 15 s at 95 °C and 60 s at 60 °C were applied. At the end of the run, samples were heated to 95 °C with a ramp time of 20 min to construct dissociation curves to check that single PCR products were obtained. The absence of genomic DNA contamination in the RNA preparations was confirmed by using total RNA samples that had not been subjected to reverse transcription. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used as the standard housekeeping gene. Ratios of target gene and HPRT expression levels (relative gene expression numbers) were calculated by subtracting the C_t of the target gene from the C_t of HPRT and raising 2 to the power of this difference. C_t values are defined as the number of PCR cycles at which the fluorescent signal during the PCR reaches a fixed threshold. Target gene mRNA expressions are thus expressed relative to HPRT expression.

Data Analysis—The significance of differences in relative gene expression numbers among the different liver cell types, from three different cell isolations, measured by real time quantitative PCR was calculated using a two-tailed Student's *t* test on the differences in C_t ($C_{t(\text{HPRT})} - C_{t(\text{target gene})}$). Probability values less than 0.05 were considered significant.

RESULTS

The last step in reverse cholesterol transport is the hepatic uptake and cellular processing of cholesterol esters from HDL by SR-BI (1). The mRNA expression of SR-BI in the different cell types of the liver was investigated with quantitative real time PCR to determine which cell type is mainly expressing SR-BI. A significantly higher SR-BI expression was observed in parenchymal cells compared with endothelial ($p < 0.05$) and Kupffer cells ($p < 0.01$) (Fig. 1).

In the liver, cholesterol is used for bile acid synthesis. Therefore, the mRNA expression patterns of two key enzymes in bile acid synthesis, CYP7A1 and CYP27, in the different hepatic cell types were examined. A relatively high level of CYP7A1 expression was observed in parenchymal cells, which was more than 200-fold ($p < 0.001$) higher than the expression levels found in endothelial and Kupffer cells (Fig. 2*a*). Accordingly, CYP27 expression (Fig. 2*b*) was observed in parenchymal cells and also in endothelial cells.

Because a second route of cholesterol disposal from the liver is through direct excretion of cholesterol into the bile via the half-transporters ABCG5 and ABCG8, we investigated whether the expression of ABCG5/8 is also higher in parenchymal cells than in non-parenchymal cells. Fig. 3 clearly indicates that ABCG5 (*a*) and ABCG8 (*b*) expression was indeed 5–10-fold higher in parenchymal cells compared with endothelial and Kupffer cells.

In the liver, ABCA1 was recently suggested to be involved in the efflux of cholesterol for production of HDL (6). Although no conclusive evidence has been shown, in the liver ABCG1, like ABCG5/G8, is proposed to play a role in biliary efflux (11). In macrophages, ABCA1 and ABCG1 expression is induced in response to cholesterol loading, and both proteins are potentially involved in cholesterol efflux to apoA-I (12). Because both ABCA1 and ABCG1 are implicated in the same physiological functions, we determined whether both genes also have a comparable expression distribution profile over the different cell types of the liver. Contrary to the assumption, ABCA1 was expressed mainly in parenchymal and Kupffer cells (Fig. 4*a*), whereas ABCG1 was expressed 76-fold ($p < 0.001$) and 27-fold ($p < 0.01$) higher in Kupffer and endothelial cells than in parenchymal cells, respectively (Fig. 4*b*). In contrast to ABCA1, ABCG1 is thus mainly expressed in non-parenchymal cells, which suggests a limited role of ABCG1 in the excretion of cholesterol directly into the bile under the standard feeding conditions. In addition, ABCG1 expression was analyzed in the different hepatic cells isolated from rats on a high cholesterol diet. Interestingly, hepatic parenchymal cell ABCG1 expression increased ~4-fold ($p < 0.05$) in response to a high cholesterol diet, whereas no significant effect on endothelial and

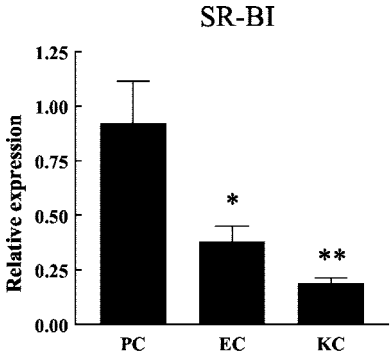

FIG. 1. **Relative SR-BI mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells from rats on a chow diet.** Values are expressed relative to HPRT expression (mean ± S.E.). $p < 0.05$ (*) and $p < 0.01$ (**) compared with PC expression (*t* test, as described under "Experimental Procedures").

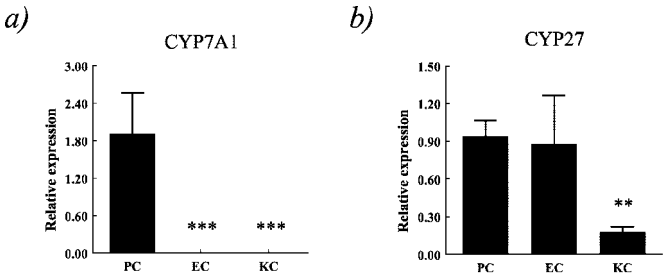

FIG. 2. **Relative CYP7A1 (*a*) and CYP27 (*b*) mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells from rats on a chow diet.** Values are expressed relative to HPRT expression (mean ± S.E.). $p < 0.01$ (**) and $p < 0.001$ (***) compared with PC expression (*t* test, as described under "Experimental Procedures").

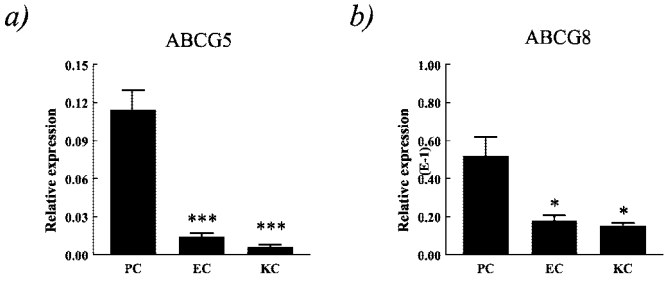

FIG. 3. **Relative ABCG5 (*a*) and ABCG8 (*b*) mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells from rats on a chow diet.** Values are expressed relative to HPRT expression (mean ± S.E.). $p < 0.05$ (*) and $p < 0.001$ (***) compared with PC expression (*t* test, as described under "Experimental Procedures").

Kupffer cell ABCG1 expression was observed (Fig. 5). Although ABCG1 levels were significantly increased in parenchymal cells in response to a high cholesterol diet, ABCG1 expression levels were still, respectively, 10- and 12-fold higher in endothelial and Kupffer cells compared with parenchymal cells.

Analysis of LXR expression in the different cell types was performed to investigate a potential relation with expression patterns of the ABC transporters. LXRα had a distribution pattern comparable to that found for ABCA1, with a relatively high expression in parenchymal ($p < 0.01$) and Kupffer cells compared with endothelial cells (Fig. 6*a*). A significantly higher expression of LXRβ was found in endothelial liver cells compared with parenchymal ($p < 0.05$) and Kupffer cells ($p < 0.05$), respectively (Fig. 6*b*), which suggests that LXRβ may be a more important mediator in endothelial cells.

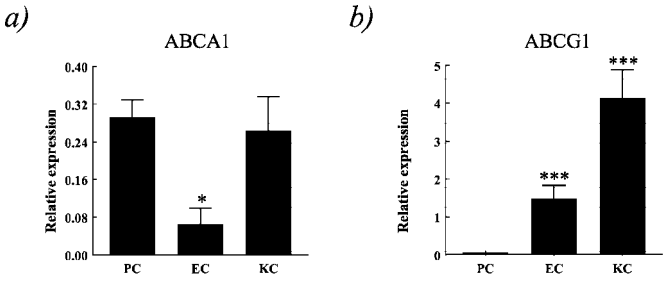

FIG. 4. **Relative ABCA1 (*a*) and ABCG1 (*b*) mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells from rats on a chow diet.** Values are expressed relative to HPRT expression (mean ± S.E.). $p < 0.05$ (*) and $p < 0.001$ (***) compared with PC expression (*t* test, as described under "Experimental Procedures").

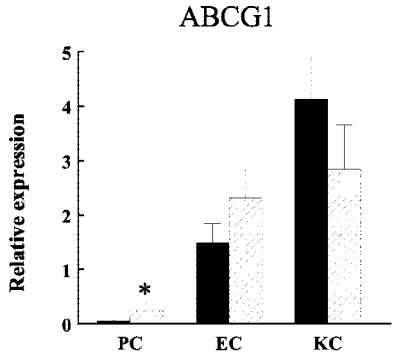

FIG. 5. **Effect of a high cholesterol diet on the relative ABCG1 mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells.** Values from rats on a chow diet (*black bars*) and on a high cholesterol diet (*hatched bars*) are expressed relative to HPRT expression (mean ± S.E.). *, $p < 0.05$ compared with the ABCG1 expression on a chow diet (*t* test, as described under "Experimental Procedures").

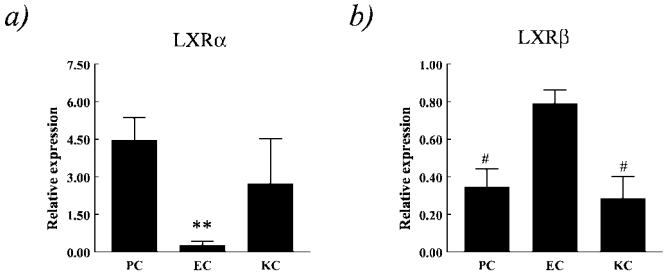

FIG. 6. **Relative LXRα (*a*) and LXRβ (*b*) mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells from rats on a chow diet.** Values are expressed relative to HPRT expression (mean ± S.E.). **, $p < 0.01$ compared with PC expression; #, $p < 0.05$ compared with EC expression (*t* test, as described under "Experimental Procedures").

It has been shown that PPARγ activators are able to regulate LXR expression, and thereby indirectly influence ABCA1 mRNA levels (13), and that ligands for PPARδ directly regulate the expression of ABCA1 via an unknown mechanism (14). Therefore, we also investigated the PPAR gene expression in the different cell types. Fig. 7a clearly indicates that PPARα expression is found mostly in parenchymal cells, with an 82- and 23-fold higher expression ($p < 0.001$ in both cases) in these cells than in endothelial cells and Kupffer cells, respectively. The PPARγ distribution pattern is comparable with that found for PPARα (Fig. 7*b*), suggesting a major function of these genes in parenchymal cells. PPARδ mRNA levels were almost equal in the different cells, although endothelial cell PPARδ expres-

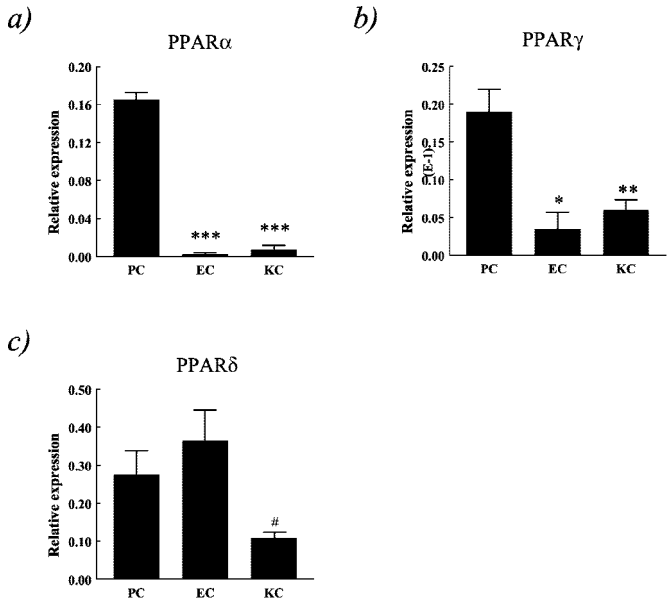

FIG. 7. **Relative PPARα (*a*), PPARγ (*b*), and PPARδ (*c*) mRNA expression levels as determined by real time quantitative PCR in liver parenchymal (*PC*), endothelial (*EC*), and Kupffer (*KC*) cells from rats on a chow diet.** Values are expressed relative to HPRT expression (mean ± S.E.). $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) compared with PC expression; $^{\#}$, $p < 0.05$ compared with EC expression (*t* test, as described under "Experimental Procedures").

sion was somewhat higher as compared with parenchymal and Kupffer cells (Fig. 7*c*). These data indicate that within the various liver cell types PPARδ will have a more general function.

DISCUSSION

Hepatic cholesterol uptake from serum coupled with intracellular processing and bile excretion are important features in the last step of reverse cholesterol transport. It has been shown that disruption of cholesterol homeostasis plays an essential role in the pathology of many diseases such as cholestasis (15) and atherosclerosis (16).

In the liver, SR-BI plays a crucial role in the selective uptake of cholesterol esters from HDL (2). Additionally, studies on ABC transporters suggested that hepatic ABCA1 is involved in HDL production (6), whereas ABCG5/G8 and ABCG1 were indicated to mediate biliary efflux of cholesterol from the liver (7, 9). Repa *et al.* (17) showed that treatment of mice with synthetic ligands of LXR markedly increased liver ABCG5/G8 expression. *In vitro* observations by Malerod *et al.* (18) also indicate that LXR is able to regulate hepatic SR-BI expression through a direct interaction with a newly discovered LXR/retinoid X receptor response element in the SR-BI promoter. It is however still unclear how hepatic ABCA1 and ABCG1 expression is regulated and what the precise consequence of their regulation is on hepatic cholesterol levels and transport.

The liver consists of several different cell types, including parenchymal, endothelial, and Kupffer cells. It is therefore important to study the expression levels of SR-BI and the ABC transporters in the individual hepatic cell types to get a more detailed view of their specific functions and regulatory mechanisms in the liver.

Earlier studies performed by Pieters *et al.* (19) showed that uptake of HDL cholesterol esters into liver parenchymal cells is efficiently coupled to a rapid synthesis of bile acids. Accordingly, Fluiter *et al.* (20) observed that the receptor responsible for the selective uptake of cholesterol esters into the liver, SR-BI, has a relatively high expression in parenchymal cells compared with endothelial and Kupffer cells. These combined observations stressed an important role for parenchymal cells in the last step of the reverse cholesterol transport process.

In the current study, using real time quantitative PCR, we investigated the mRNA expression of genes involved in hepatic cholesterol transport and metabolism in liver parenchymal, endothelial, and Kupffer cells. Real time PCR is a highly sensitive method to quantify mRNA expression levels *in vitro* and *in vivo*. mRNA levels have been shown to correlate strongly with protein expression levels, indicating that a substantial portion of changes in protein levels is a consequence of altered mRNA levels rather than post-transcriptional modifications (21).

Importantly, Wellington *et al.* (22) showed a high concordance of ABCA1 mRNA and protein levels in the liver. Additionally, we observed in the present study the highest SR-BI mRNA expression in the parenchymal cells compared with endothelial and Kupffer cells, which is in accordance with the high Western blot protein expression data for SR-BI as reported by Fluiter *et al.* (20). We thus suggest that our quantitative mRNA data for the various cell types are indicative for the activity of the particular genes of interest and their metabolic function. For SR-BI, the mRNA and protein expression data in parenchymal cells are consistent with our data that the parenchymal cholesterol ester uptake is reduced dramatically in SR-BI deficient mice as compared with non-transgenic littermates.[2] CYP7A1 and CYP27 are the key enzymes in classical and alternative bile acid synthesis pathways (23). The relatively high expression of these two bile acid synthesis enzymes observed in parenchymal cells is in agreement with the data provided by Pieters *et al.* (19), as it was shown that uptake of HDL cholesterol esters by the parenchymal cells is coupled efficiently to bile acid synthesis. Interestingly, an equally high expression of CYP27 compared with parenchymal cells was observed in liver endothelial cells. In agreement, Reiss *et al.* (24) have detected the same high levels of CYP27 activity in cultured vascular endothelial cells. The observed difference between the CYP7A1 and CYP27 expression patterns suggests differential functions for these bile acid synthesizing enzymes. Interestingly, Babiker *et al.* (25) suggested that CYP27-mediated elimination of cholesterol from macrophages and endothelial cells may be an alternative or complement to HDL-mediated reverse cholesterol transport under low HDL conditions. They observed a high secretion of 3β-hydroxycholestenoic acid, an intermediate of the CYP27-mediated alternative bile acid formation pathway, from endothelial cells and macrophages to albumin containing medium.

Efflux of hepatic cholesterol to the serum compartment by ABCA1 for the production of native HDL is a second important route in maintaining cholesterol homeostasis (6). An equally high relative expression of ABCA1 was observed in parenchymal and Kupffer cells, whereas a ~4-fold lower expression of ABCA1 was seen in liver endothelial cells. In macrophages, ABCA1 is a critical regulator of the specific ATP-dependent cholesterol efflux to apoA-I, leading to an inhibition of foam cell formation (26). Kupffer cells are liver macrophages, which play an important role in the uptake of (modified) lipoproteins (19, 27). The high uptake and an accordingly high efflux of cholesterol from Kupffer cells might be the metabolic mechanism for the relatively high ABCA1 expression level observed in these cells. Haghpassand *et al.* (28) and Van Eck *et al.* (29) have shown that monocyte/macrophage ABCA1 only minimally contributes to the overall plasma HDL levels. The observed high expression levels of ABCA1 in parenchymal cells, combined

[2] J. K. Kruijt and Th. J. C. van Berkel, unpublished data.

with the observation that ABCA1 functions on the basolateral surface of hepatocytes (30), suggest that the liver *does* contribute to HDL production by the efflux of cholesterol from parenchymal cells via ABCA1.

A third catabolic route for hepatic cholesterol is the direct excretion into the bile, which accounts for ~40% of the total liver catabolism. Recently, two members of the ABC transporters, ABCG5 and ABCG8, have been shown to participate coordinately in the hepatic sterol secretion into bile (31). Mutations in either ABCG5 or ABCG8 are sufficient to cause sitosterolemia, a disorder that is characterized by elevated plasma levels of sterols (32). Because parenchymal cells are responsible for bile acid formation, a relatively high expression of the biliary transporters such as ABCG5/G8 in these cells compared with endothelial and Kupffer cells is consistent with their suggested function. The expression pattern of ABCG5 resembled ABCG8, which is in agreement with the statements that these transporters operate as heterodimers to regulate biliary cholesterol efflux (9, 33).

Interestingly, a novel member of the ABC transporter family, ABCG1, has also been proposed to have a function in the intracellular trafficking and biliary efflux of cholesterol in the liver (11). Contrary to the expectations, ABCG1 expression was observed mainly in non-parenchymal cells of the rat liver. A 76-fold and 27-fold higher ABCG1 expression was observed in Kupffer and endothelial cells than in parenchymal cell under standard feeding conditions. Although Kupffer and endothelial cells only contribute 2.5 and 3.3% to the total liver protein, they do contain 51 and 24% of total liver ABCG1 expression, respectively. Such a high specific ABCG1 expression in Kupffer cells was not expected, although ABCG1 has also been proposed to play a role in the cholesterol efflux from peripheral macrophages (7). Importantly, after putting rats on a high cholesterol diet for 2 weeks, ABCG1 expression increased 4-fold in parenchymal cells, whereas no significant change in ABCG1 expression in endothelial and Kupffer cells was observed. The absence of a similar induction of ABCG1 in endothelial and Kupffer cells in response to diet feeding may well be caused by an already maximal activity of ABCG1 in these cells even on a chow diet. Also, the differences in expression and intracellular localization of direct activators (*e.g.* LXRα) and repressors (*e.g.* ZNF202) of ABCG1 might contribute to the difference in its transcriptional regulation between different cell types as earlier mentioned by Schmitz and Langmann (11). Although endothelial and Kupffer cell ABCG1 expressions were still 10- and 12-fold higher than that in parenchymal cells, the relative contribution of ABCG1 in the parenchymal cells to total liver increased from 25 to 60%. This suggests that under high cholesterol conditions ABCG1 might indeed contribute to the transport of cholesterol in the parenchymal cells.

Recent pharmacological interest is focused upon the regulation of SR-BI and the ABC transporters by newly discovered nuclear hormone receptors, the LXRs and PPARs, respectively (34, 35). Therefore, we also studied their cellular localization in the different cell types of the liver.

Two different types of the LXR have been discovered so far, LXRα and LXRβ. A relatively high expression of LXRα was observed in parenchymal cells. In the liver, LXRα plays an essential role in the regulation of CYP7A1 and thus the formation of bile acids (36). CYP7A1 was found almost exclusively in the parenchymal cells, which coincides with the high expression of LXRα in these cells. Contrarily, equally high LXRα expression levels were observed in Kupffer cells, where CYP7A1 expression was almost absent. In macrophages, however, LXRα plays a crucial role in the regulation of lipid efflux via ABCA1 (37). Kupffer cells also contain high expression levels of ABCA1, which is consistent with a role for LXRα in the regulation of ABCA1 in these cells. Among the different cell types of the liver, LXRβ was ubiquitously expressed, with a somewhat higher expression in endothelial cells *versus* parenchymal and Kupffer cells. The expression distribution of LXRβ thus does not resemble that found for LXRα, which suggests that in the liver, LXRβ may have a function different from that of LXRα.

In the liver, PPARα is suggested to play a role in the formation of bile acids because it is able to bind a PPAR response element in the sterol 12α-hydroxylase promoter, leading to increased levels of cholic acid (38). This might explain the extremely high expression of PPARα found in parenchymal cells compared with endothelial and Kupffer cells. The high expression of PPARγ observed in parenchymal cells suggests that PPARγ, like PPARα, also has a major function in these cells. Contrarily, PPARδ is expressed ubiquitously among the different cell types of the liver, suggesting a more general function for PPARδ in all cell types of the liver.

In conclusion, we have provided data that several ABC transporters and nuclear hormone receptors involved in liver cholesterol homeostasis are expressed differentially in the specific cell types of the liver. To study their intracellular transport function inside the liver it appears to be essential to take into account their cellular localization, as especially evident for ABCG1. This appears specifically true for studies on the regulation of the transporters by nuclear receptors because metabolic changes are coupled directly to the specific (intra)cellular expression level of the cholesterol transporters.

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