Cholesterol and phospholipid transporters in atherosclerotic lesion development

Pennings, M.

ABCA1/SR-BI DOUBLE DEFICIENT MICE DEMONSTRATE THE INDEPENDENT ROLES FOR ABCA1 AND SR-BI IN REVERSE CHOLESTEROL TRANSPORT

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Abstract

The key regulators of reverse cholesterol transport (RCT) ATP-binding cassette transporter 1 (ABCA1) and scavenger receptor BI (SR-BI) mediate cellular cholesterol efflux and the delivery of HDL cholesterol to the liver, respectively. Since these proteins play an essential role at either end of the RCT pathway, it is conceivable that ABCA1 and SR-BI might act synergistically in this process.

To study the RCT process under conditions in which both of these key mediators are absent, ABCA1/SR-BI double knockout (dKO) mice were generated by cross-breeding. Combined ABCA1/SR-BI deficiency resulted in a decrease in serum HDL cholesterol levels. The dKO mice thus resemble the single ABCA1 KOs with respect to their lipid phenotype, indicating that the virtual absence of HDL cholesterol levels in absence of ABCA1 is not the result of efficient SR-BI-mediated removal but solely of impaired HDL production. Despite the dramatic reduction in serum HDL cholesterol levels, no effect of combined ABCA1/SR-BI deficiency was observed on the hepatic cholesterol content of these mice. In addition, no effect of combined ABCA1xSR-BI deficiency was observed on the bile salt and phospholipid content of the bile. The cholesterol content of the bile, however, was reduced in the double knockout mice. Similar results have previously been shown for the single SR-BI KO mice, indicating that in this respect the ABCA1/SR-BI dKO mice resemble the SR-BI single KOs. Finally, no effect of combined ABCA1xSR-BI deficiency was observed on the fecal excretion of neutral and acidic sterols as previously observed in the single KOs.

In conclusion, SR-BI and ABCA1 appear to function independently in RCT: ABCA1 is the primary determinant for cellular cholesterol efflux and the formation of HDL, while SR-BI is mainly involved in the delivery of HDL cholesterol to the liver and the secretion of cholesterol into the bile.

Introduction

High levels of low-density lipoprotein (LDL) cholesterol are an important risk factor for the development of atherosclerosis and lowering the serum levels of LDL has been a target in the prevention of heart disease for many years (1). However, low levels of high-density lipoprotein (HDL) are an equally important predictor for coronary heart disease (2,3). Reverse cholesterol transport, the transport of cholesterol from the periphery to the liver and eventually into the bile, is thought to play a key role in the protective effect of HDL (4-6).
Two important genes recognized in reverse cholesterol transport are the ATP binding cassette transporter A1 (ABCA1) and the scavenger receptor class B type I (SR-BI) (7-10) The transport of cholesterol from peripheral cells to lipid poor apolipoprotein AI (ApoAI), to form HDL particles, is the controlling first step in reverse cholesterol transport and is mediated by ABCA1 (7). ABCA1 is identified as the gene mutated in patients suffering from Tangier disease (8-10). Humans with this disease display very low levels of HDL cholesterol (12). The virtual absence of serum HDL is a direct effect of the impaired formation of HDL by the liver and the intestine.
Targeted deletion of hepatic ABCA1 in liver specific ABCA1 KO mice (13) or through RNA interference (14) revealed the critical role of hepatic ABCA1 in the formation of plasma HDL. HDL-cholesterol levels in these mice resembled the HDL-cholesterol levels found in total body ABCA1 KO mice. ABCA1 mediates cholesterol and phospholipid efflux to ApoAI (15). As a consequence of the impaired efflux in the absence of ABCA1 the intima-media thickness in patients with Tangier disease is enlarged, and the risk for an early onset of atherogenesis is increased (16). Although ABCA1 deficiency in mice results in severe xanthomatosis, ABCA1 deficiency in ApoE or LDLr KO mice did not promote atherosclerotic lesion development (17). Bone marrow transplantations revealed that macrophage ABCA1 has a minimal contribution in the determination of cholesterol levels, however macrophage ABCA1 does influence atherosclerotic lesion formation (18).The second gene that is important in reverse cholesterol transport is SR-BI (19). SR-BI transfers the cholesteryl esters from the HDL particle to the cytoplasm of the hepatocyte where the cholesterol can be processed and secreted into the bile directly or as bile acids (20). The selective uptake of cholesteryl esters and other lipids from HDL by SR-BI does not require endocytosis, this in contrast to classical receptor-mediated endocytosis. The lipoprotein particle is depleted from lipids without being internalized and degraded (21). Disruption of the SR-BI gene in mice results in an impaired metabolization of HDL, hampered depletion of cholesteryl esters from HDL, and the

accumulation of large HDL particles in the circulation (22,23). The increased HDL particle size and number leads to an enhanced susceptibility to atherosclerosis in mice lacking SR-BI (23, 24). In humans, the physiological role of SR-BI is largely unknown, although the expression patterns and tissue distribution closely resemble those observed in mice (25). Several studies on common polymorphisms of human SR-BI have shown that variants of the SR-BI gene interfere with the metabolism of apoB lipoproteins. However, the effects differ in men and women and are affected by age (26). Human SR-BI deficiency has thus far not been identified. Heterozygosity for a single nucleotide (proline to serine at position 297) was recently demonstrated to result in markedly increased HDL cholesterol levels, suggesting that in humans SR-BI may be equally important in controlling HDL cholesterol levels (27). Interestingly both ABCA1 and SR-BI are expressed by macrophages as well as parenchymal liver cells (28). It is therefore conceivable that ABCA1 and SR-BI might act synergistically in the process of reverse cholesterol transport. To study the RCT process under conditions in which both of these key mediators are absent, ABCA1/SR-BI double knockout mice were generated. In this article we describe the generation and characterization of these ABCA1/SR-BI double knockout mice.

Materials and Methods

Generation of Mice Homozygous deficient for ABCA1 and SR-BI

SR-BI deficient (18) and ABCA1 deficient mice (30) were kindly provided by Dr M. Krieger and Dr. G. Chimini, respectively. A SR-BI-deficient mouse was crossed with an ABCA1-deficient mouse to generate double heterozygous offspring. These double heterozygous SR-BI/ABCA1 mice were intercrossed to generate SR-BI/ABCA1 double knockout (dKO) mice, SR-BI knockout (SR-BI KO), ABCA1 knockout (ABCA1 KO) and wildtype (WT) littermates. The genotypes were analyzed using the primers shown in table 1. Mice were housed in 12h:12h light:dark cycle, and had food and drinking water ad libitum available. All animal procedures were approved by the Committee for Animal Ethics of the Leiden University.

	Forward Primer	Backward Primer	Resulting products (in bp)
ABCA1 KO	’5-TTTCTCA TAGGGTTGGTCA-3’	‘5-TGCAATCCA TCTTGTTCAAT-3’	600
ABCA1 WT	‘5-TGGGAAC TCCTGCTAAAAT-3’	‘5-CCATGTGGTG TGTAGACA-3’	800
SR-BI	‘5-GATGGGACATGG GACACGAAGCCATTCT-3’	‘5-TCTGTCTCCGTC TCCTTCAGGTCCTGA-3’	1000 for the wildtype gene and 1200 for the disrupted gene

Table 1: Primers used for the detection of both the wild type and disrupted ABCA1 and SR-BI gene.

Serum Lipid Analyses

After an overnight fasting-period, 100 µl of blood was drawn from each individual mouse (n=10). The concentrations of cholesterol in serum were determined by enzymatic colorimetric assays with 0.025 U/mL cholesterol oxidase (Sigma) and 0.065 U/mL peroxidase and 15 µg/mL cholesteryl esterase (Roche Diagnostics, Mannheim, Germany) in reaction buffer (1.0 KPi buffer, pH=7.7 containing 0.01 M phenol, 1 mM 4-amino-antipyrine, 1% polyoxyethylene-9-laurylether, and 7.5% methanol). Absorbance was read at 490 nm. The distribution of cholesterol over the different lipoproteins in serum was determined by fractionation of 30 µL serum of each mouse using a Superose 6 column (3.2 x 300 mm, Smart-system; Pharmacia, Uppsala, Sweden). Cholesterol content of the effluent was determined as indicated. The serum triglyceride concentration was determined using enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany). The concentration of serum phospholipid was analyzed with an enzymatic colorimetric assay from Wako Chemicals GmbH (Neuss, Germany).

VLDL secretion

VLDL secretion was determined by intravenous injection of 500 mg Triton WR1339 (Sigma, Zwijndrecht, The Netherlands) per kg bodyweight to inhibit lipolysis and lipoprotein clearance from the circulation. Blood samples were collected before and at hourly intervals for four hours after triton injection. Serum triglyceride, free and total cholesterol concentrations were determined as described above.

Food intake and fecal sterol secretion

Food intake was determined by placing the mice in a metabolic cage with food and water available ad libitum for 24 hours. The food intake was determined, and feaces were collected. Faeces were weighed, lyophilized, and homogenized. Neutral sterols and bile salts were analyzed according to Arca et al. (31) and Setchell et al. (32), respectively.

Lipid content of the liver

Mice were anaesthetized, a whole body perfusion was performed using phosphate buffered saline (100 mm Hg), the liver was excised, frozen in liquid nitrogen and stored at -80ºC until further analysis. Lipids were extracted from the livers according to Bligh and Dyer (33) and cholesterol content was measured as described above.

Bile sampling and biliary lipid content determination

Cannulation of the gallbladder and bile collection were performed as described previously (34). Directly after cannulation, bile was collected at 10 minute intervals. To determine maximal rates of biliary lipid secretion, bile fractions were collected for 90 min to deplete the endogenous bile salt pool. Subsequently, Tauroursodeoxycholate (TUDC, 45 mM stock solution from Sigma), dissolved in phosphate buffered saline, pH 7.4, was infused into the jugular vein and bile collection continued for another 150 minutes. All collected bile samples were examined for bile salt and lipid content as described previously (35).

Statistics

Statistical analysis of the data were performed using GraphPad Instat software. Probability values of ≤ 0.05 were considered significant.

Results

To analyze the potential synergistic role of ABCA1 and SR-BI in the RCT process we generated ABCA1/SR-BI double knockout (dKO) mice. The ABCA1/SR-BI dKO pups were born from double heterozygous crossings at a frequency of 5%, which is close to the of expected Mendelian inheritance rate of 6.25%. Interestingly, upon weaning at the age of 4 weeks the ABCA1/SR-BI dKO pups had a significantly ($p>0.001$) lower body weight (11±0.8 g and 9.4±0.5 g for males and females, respectively) as compared to their WT littermates (18.0±2.1 g and 15±1.0 g for males and females, respectively).

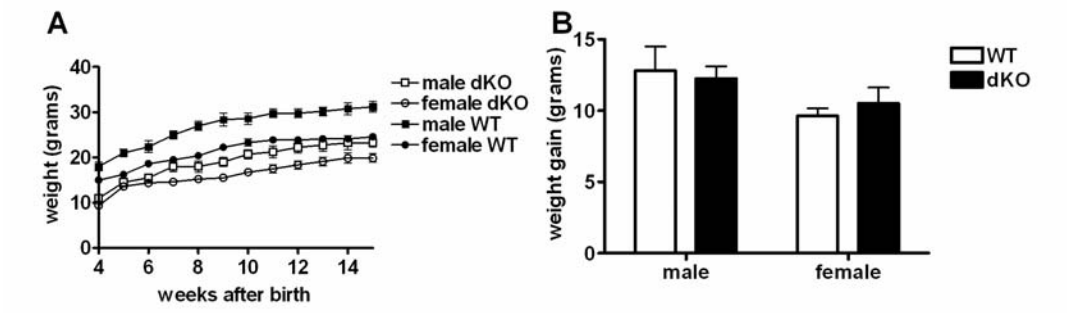

Figure 1: Weight and weight gain in ABCA1/SR-BI dKO mice and their wildtype littermates
(A) From week 4 after birth until 15 weeks of age the ABCA1/SR-BI dKO mice (open symbols)and their wildtype (WT) littermates (closed symbols) were weighed. The average weight of males (squares) and females (circles) were put in the graph separately. (B) The weightgain over 11 weeks in ABCA1/SR-BI dKO (closed bars) and their wildtype (WT) littermates (open bars).

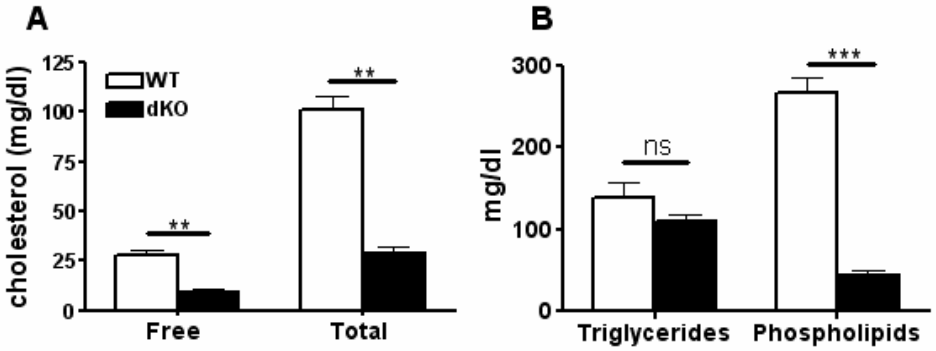

Figure 2:**Serum analysis of ABCA1/SR-BI dKO mice and their wild type littermates**
(A) Serum free and total cholesterol levels in ABCA1/SR-BI dKO mice (closed bars) and their wild type littermates (open bars). (B) Serum triglycerides and phospholipids in ABCA1/SR-BI dKO mice (closed bars) and their wild type littermates (open bars). Values are means ± SEm of n=4. Statistically significant difference ** $p<0.005$, *** $p<0.001$.

To study the effect of combined ABCA1 and SR-BI deficiency on growth development of the animals, a growth curve was made over a period of 11 weeks from the age of 4 weeks to 15 weeks, as shown in Figure 1A. No significant differences between female and male mice could be detected in growth over this 11-week period, although there was the anticipated trend towards an increased weight in the male population compared to the female. The WT littermates had a higher birth weight, and this difference was maintained during at least 11 weeks. However, the dKO mice, despite a lower birth weight, showed a similar weight gain (12.3±1.7 g and 10.5±3.1 g for males and females, respectively) over the 11 week period as compared to the WT mice (12.8±3.8 g and 9.6±1.5 g for males and females, respectively) (Figure 1B).

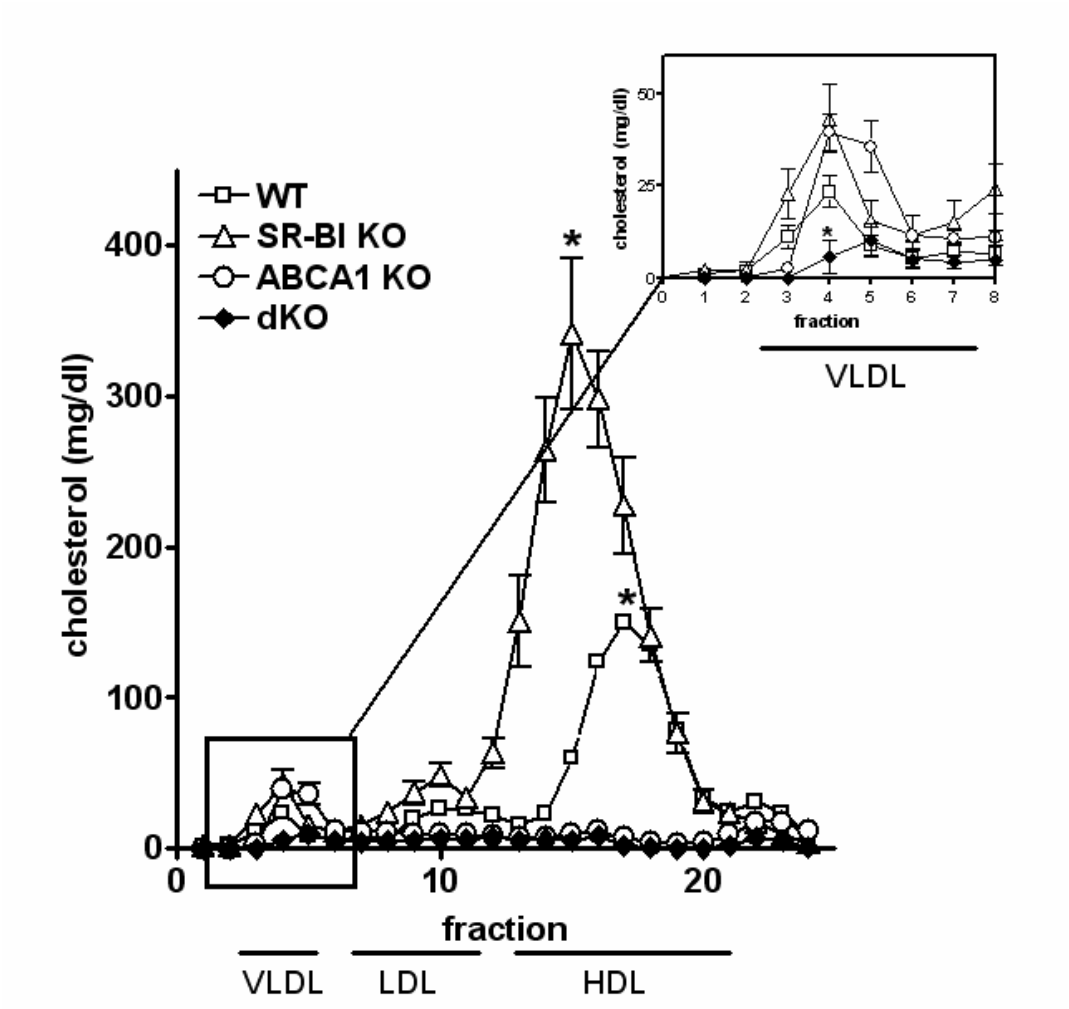

Figure 3: Serum lipoprotein profile in wild type, SR-BI deficient, ABCA1 deficient and ABCA1/SR-BI double deficient mice.
Serum lipoprotein profile in wild type (WT, open squares), SR-BI deficient (SR-BI KO open triangles), ABCA1 deficient (ABCA1 KO, open circles) and ABCA1/SR-BI double deficient (dKO, closed squares) mice. The insert is an enlargement of fractions representing the serum VLDL.
Values are means ± SEM of n=4. Statistically significant difference * p ? 0.05.

Serum analysis of the dKO mice showed dramatically lower serum cholesterol levels compared to WT littermates, as shown in Figure 2A. Both free and total cholesterol were decreased 3-fold ($p<0.005$) and 4-fold ($p<0.005$) in the dKO mice compared to the WT littermates. Disruption of both ABCA1 and SR-BI also led to a decrease of 80% ($p<0.001$) in serum phospholipid levels (Figure 2B). Serum triglyceride levels, however were not affected (Figure 2B). The lower serum cholesterol concentration observed in the dKO mice was the result of largely decreased VLDL and HDL cholesterol levels (Figure 3). While single SR-BI KO littermates have larger HDL particles compared to WT mice, both single ABCA1 KO and dKO mice displayed decreased HDL levels compared to the WT littermates. With respect to their HDL phenotyping the dKO animals thus resemble the single ABCA1 KO mice. No significant differences were observed in VLDL cholesterol levels between SR-BI KO, ABCA1 KO, and WT littermates. However, in the dKO mice VLDL were found to be very low (50.97 versus 20.6 mg/dl for wild type and dKO, respectively). To determine if this decrease in VLDL cholesterol is due to an impaired VLDL secretion, VLDL production was studied after intravenous injection of the mice with Triton WR1339 to block endogenous VLDL processing.

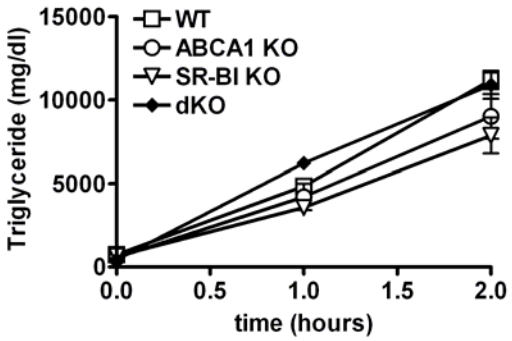

Figure 4: VLDL secretion in wild type, SR-BI deficient, ABCA1 deficient and ABCA1/SR-BI double deficient mice.
VLDL secretion in wild type (WT, open squares), SR-BI deficient (SR-BI KO open triangles), ABCA1 deficient (ABCA1 KO, open circles) and ABCA1/SR-BI double deficient (dKO, closed squares) mice. Values are means ± SEM of n=4.

Compared to SR-BI KO and ABCA1 KO mice the VLDL secretion in the dKO mice was even slightly, although not significantly increased (Figure 4). VLDL production was 11194±1108 in wild type, 7856±2153 in SR-BI KO, 9000±1899 in ABCA1 KO and 10888±1667 mg/dl measured two hours after intravenous Triton injection in dKO mice, respectively. The observed decrease in VLDL cholesterol levels in mice with combined deletion of ABCA1 and SR-BI was thus not the result of an impaired VLDL secretion from the liver.

The first step in the cholesterol metabolism is the dietary intake of cholesterol. A reduced food intake or impaired intestinal diet processing could result in diminished circulating cholesterol levels. Therefore the food intake was measured in the dKO mice. The food intake did not significantly differ between the double deficient mice and their wild type controls; all mice consumed about 4.5 grams per 24 hours. The reduced serum cholesterol levels are thus not a result of diminished food intake.

A disturbed hepatic cholesterol homeostasis could also result in differences in serum cholesterol levels. To determine if the hepatic cholesterol content was altered livers of both wild type and ABCA1/SR-BI double knock out mice were excised, lipids were extracted and the cholesterol content measured. There were no differences found in cholesterol content of the liver of wild type (0.19±0.07 µg cholesterol/mg protein) and double knock mice (0.27±0.12 µg cholesterol/mg protein).

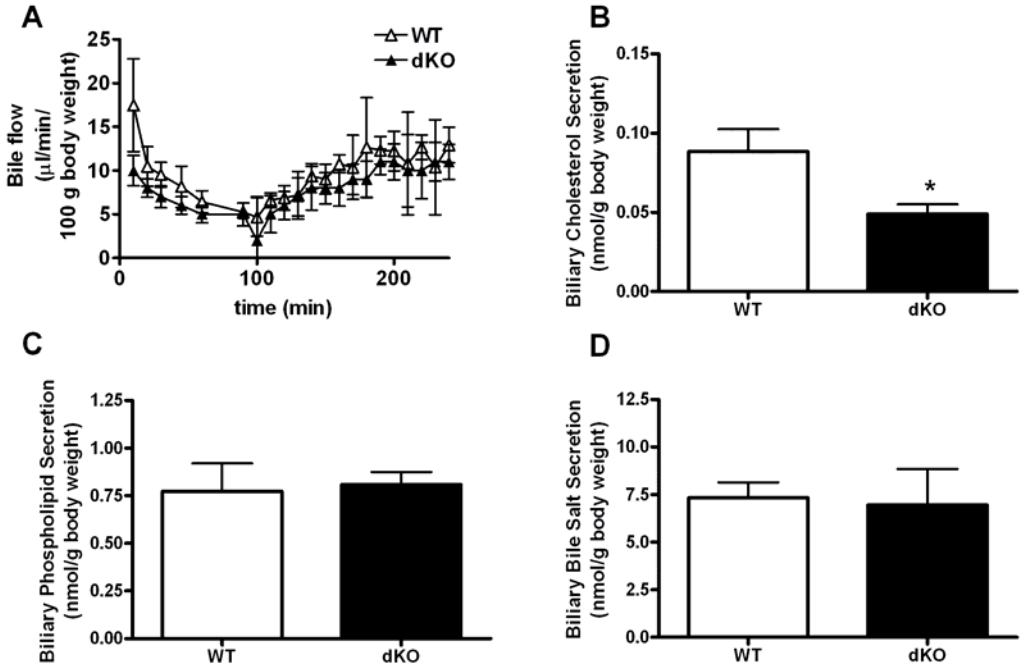

Figure 5: Bile flow and bile excretion in wild type and ABCA1/SR-BI double deficient mice.
(A) Bile flow in wild type (WT, open triangles) and ABCA1/SR-BI double deficient (dKO, closed triangles) mice. Biliary cholesterol secretion (B), phospholipid secretion (C) and bile salt production (D) in wild type (WT, open triangles) and ABCA1/SR-BI double deficient (dKO, closed triangles) mice. Values are means ± SEM of n=4. . Statistically significant difference * p ? 0.05.

Subsequently, the next step in the reverse cholesterol transport, the excretion of cholesterol and bile acids into bile, was analyzed in the dKO mice (Figure 5). Phospholipid secretion was not altered in ABCA1/SR-BI dKO mice as compared to wild type littermates. In addition, no effect of combined ABCA1/SR-BI deficiency was observed on bile flow and bile salt production. However, a significant decrease ($p<0.05$) in the secretion of cholesterol into the bile was observed (0.088±0.028 nmol cholesterol/g body weight and 0.049±0.012 nmol cholesterol/g body weight for the wild type and the dKO, respectively).

This difference in biliary cholesterol secretion, however did not result in a difference in fecal cholesterol content(Figure 6A). Furthermore, the bile salt content of the faeces did not differ significantly between the wild type mice and the dKO mice (Figure 6B). The wild type mice produced 1.13±0.08 g/day of faeces, and the double knock out mice 1.26±0.35 g/day.

Thus combined ABCA1 and SR-BI deletion did not affect fecal secretion.

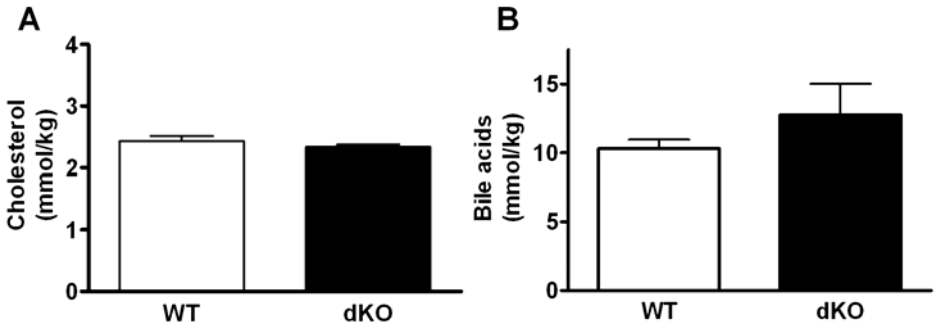

Figure 6: Fecal Cholesterol and bile acid content.
(A) Fecal cholesterol content of wild type (WT, open bars) and ABCA1/SR-BI double deficient (dKO, closed bars) mice. Fecal bile acid content (B) of wild type (WT, open bars) and ABCA1/SR-BI double deficient (dKO, closed bars) mice.
Values are means + SEM of n=4. .

Discussion

HDL plays a key role in reverse cholesterol transport, the transport of cholesterol form the periphery to the liver and eventually the bile (4-6). Both ABCA1 and SR-BI are of physiological importance for HDL metabolism. Where ABCA1 facilitates the efflux of cholesterol and phospholipid to lipid-poor apoA1 (15), SR-BI is important for HDL metabolism, by mediating the depletion of cholesteryl esters from the HDL particle (20). ABCA1 and SR-BI might thus act synergistically in the process of reverse cholesterol transport. To investigate the combined action of these two important cholesterol transporters we generated ABCA1/SR-BI double knockout mice in which both key mediators of the RCT pathway were absent.

Interestingly, the ABCA1/SR-BI dKO mice displayed a dramatically reduced bodyweight upon birth compared to wildtype littermates. Rigotti *et al* showed that the single SR-BI KO mice do not differ from their wildtype littermates regarding their birth weight (22). Furthermore, no differences in the birth weight of single ABCA1 KO have been described, suggesting that the reduced birth weight observed in the dKO mice might be the result of the combined deficiency of ABCA1 and SR-BI: ABCA1/SR-BI dKO mice displayed similarly low serum HDL cholesterol levels as single ABCA1 KO mice. It is thus unlikely that the large decrease in cholesterol levels in the dKO mice could have resulted in a pre-natal growth retardation, resulting in a lower birth weight. However, the low serum cholesterol levels did not affect the post-natal development as evidenced by normal weight gain of the dKO mice. In addition, there were no indication of premature death, since no difference in mendelian inheritance expectance were found. This in contrast to both single ABCA1 and single SR-BI knock out mice (36, 23). Breeding of heterozygous ABCA1 knock out mice was associated with a distortion in mendelian inheritance, probably as the result of peri-natal lethality of the ABCA1 knockout pups, due to malformation of the placenta (36). The reproduction of single SR-BI KO mice is also disturbed. Homozygous SR-BI KO male mice are fertile, in contrast to the homozygous SR-BI KO female mice, which exhibit abnormal fertility (23).. The infertility of female SR-BI KO mice can, however, be reversed by administration of the cholesterol lowering drug probucol (37). Breeding of heterozygous ABCA1/SR-BI mice, in absence of additional dietary probucol, resulted in a Mendelian inheritance of 5% for ABCA1/SR-BI dKO offspring, which is close to the expected rate of 6.25%. Thus breeding of heterozygous ABCA1/SR-BI dKO mice does not seem to be associated with premature death. Both male and female homozygous ABCA1/SR-BI dKO mice were fertile when bred after administration of probucol in the diet. No comprehensive data on (in)fertility of homozygous ABCA1/SR-BI dKO mice without the administration of this drug are available yet. It is currently thus still unknown if the infertility of the female SR-BI KO mice is a direct result of SR-BI deficiency or the result of the accumulation of the large, dysfunctional HDL particles.

The generated ABCA1/SR-BI dKO mice have little to no circulating cholesterol. Deletion of only ABCA1 in mice results in a virtual absence of serum HDL-cholesterol (11, 36). This reduction in HDL is thought to be primarily attributable to the absence of hepatic ABCA1(38). Macrophage ABCA1 was found to have a minimal effect on serum cholesterol levels (39). However, recently it was shown that also intestinal ABCA1 contributes to the plasma HDL pool for approximately 30% (40). ABCA1 mediates the efflux of phospholipid to lipid-poor apoA1 to form preβ1-HDL. This formed preβ1-HDL particle is a good acceptor for

cholesterol, and is thought to be essential for the first step in the reverse cholesterol transport process. Preβ1-HDL also stimulates ABCA1-mediated cholesterol efflux and is thus not only a product, but also a substrate for ABCA1 (15). ABCA1/SR-BI dKO mice display similarly low serum HDL cholesterol levels as single ABCA1 KO mice and thus resemble the ABCA1 single KO mice with respect to their HDL phenotype. The virtual absence of HDL cholesterol in ABCA1 KO mice is thus not the results of efficient SR-BI mediated removal but solely of impaired HDL production.

In single ABCA1 KO mice the lowered serum cholesterol levels as compared to wildtype mice are primarily associated with a large reduction in HDL cholesterol, while VLDL cholesterol is unaltered on chow diet (36).

In contrast, in the generated ABCA1/SR-BI double deficient mice on chow not only dramatically reduced HDL was observed, but also the lowered VLDL cholesterol levels. To investigate the mechanism behind the reduced VLDL cholesterol levels. To investigate the mechanism behind the reduced VLDL cholesterol levels, we analysed several aspects of the cholesterol metabolism including the food intake, VLDL secretion, biliary and fecal cholesterol excretion. No effect of combined ABCA1 and SR-BI deficiency on the intake of food was observed. Thus, the reduction in VLDL cholesterol levels could not be explained by a decreased food intake. In addition, no decrease was observed in the serum VLDL production rates of the ABCA1/SR-BI dKO mice. The VLDL production was even slightly induced in ABCA1/SR-BI dKO mice, although this induction was not significantly different from the other types of mice.

Groen *et al* reported that hepatic cholesterol and phospholipid homeostasis in the total body ABCA1 deficient mice is not impaired and that hepatobiliary processing of cholesterol is independent of ABCA1 (41). The loss of both ABCA1 and SR-BI expression in the liver also did not lead to a difference in hepatic cholesterol level compared to the wildtype controls. Furthermore, the impairment of the SR-BI mediated cholesteryl ester uptake also did not affect the liver cholesterol homeostasis. In agreement, increased SR-BI mediated cholesteryl ester uptake was previously shown to not influence biliary cholesterol secretion (42) The cholesterol content of the bile, however, was reduced in the ABCA1/SR-BI double knockout mice. Similar results have previously been shown for the SR-BI single KO mice (43).

Finally, no effect of combined ABCA1/SR-BI deficiency was observed on the fecal excretion of neutral and acidic sterols which was also previously observed in the single KO mice (43). Strikingly, no alterations in fecal cholesterol were found, although biliary cholesterol excretion was decreased in the dKO mice compared to wild type mice. Van der Velde *et al*

recently reported on the existence of a direct intestinal cholesterol secretion pathway (44). An increased direct excretion of cholesterol from the serum into the intestinal lumen in the dKO mice could be an explanation for the discrepancy found between biliary and fecal cholesterol secretion. Recent preliminary data, however, showed no difference in direct HDL ^{3}H-cholesterol oleate secretion in the intestine between the wild type and dKO mice (Vrins *et al*, personal communication).

In conclusion, SR-BI and ABCA1 appear to function independently in RCT: ABCA1 is the primary determinant for cellular cholesterol efflux and the formation of HDL, while SR-BI is mainly involved in the delivery of HDL cholesterol to the liver and the secretion of cholesterol into the bile.

References

1 Chapman MJ, Cardiovasc Drugs Ther. 2005; 19:1 35-9
2 Miller, N.E. & Thelle, D.S., The Lancet, 1977; 965-968.
3 Ashen, MD & Blumenthal RS, The new England journal of medicine, 2005;353;1252-1260
4Groen, A.K., Oude Elferink, R.P., Verkade, H.J. & Kuipers, F. Ann Med 2004;**36:** 135-45
5Lewis, G.F. & Rader, D.J. Circ Res 2005;**96:**221-232.
6Van Eck, M., Pennings, M., Hoekstra, M., Out, R. & Van Berkel, T.J Curr Opin Lipidol 2005;**16:**307-315
7 Connely M.A., Williams D.L. Curr Opin Lipidol 2004;15:287-295).
8 Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Drobnik, W., Barlage, S., Buchler, C., Porsch-Ozcurumez, M., Kaminski, W.E., Hahmann, H.W., Oette, K., Rothe, G., Aslanidis, C., Lackner, K.J.& Schmitz, G. Nat Genet 1999;**22:**347-345
9 Brooks-Wilson, A., Marcil, M., Clee, S.M., Zhang, L.H., Roomp, K., van Dam, M., Yu, L., Brewer, C., Collins, J.A., Molhuizen, H.O., Loubser, O., Ouelette, B.F., Fichter, K., Ashbourne-Excoffon, K.J., Sensen, C.W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pimstone, S., Kastelein, J.J., Genest, J.Jr. & Hayden, M.R. Nat Genet 1999;**22:**336-345
10 Rust, S., Rosier, M., Funke, H., Real, J., Amoura, Z., Piette, J.C., Deleuze, J.F., Brewer, H.B., Duverger, N., Denefle, P. & Assmann, G. Nat Genet 1999;**22:**352-355
11Lee, J-Y. & Parks, J.S. *Curr Opin Lipid* 2005;**16:**19-25
12 GK Hovingh, E de Groot, W van der Steeg, SM Boekholdt, BA Hutten, JA Kuivenhoven, JJP Kastelein, Curr Opin Lipid 2005; **16**:139-145
13 Timmins J.M., Lee J.Y., Boudyguina E., Kluvkman K.D., Brunham L.R., Mulya A., Gebre A.K., Coutinho J.M., Colvin P.L., Smith T.L., Hayden M.R., Maeda N. and Parks J.S J Clin Invest. 2005;**115**:1333-1342
14 Ragozin S., Niemeier A., Laatsch A., Loeffler B., Merkel M., Beisiege,U., Heeren J Arterioscler Thromb Vasc Biol 2005**;25**:1-7
15 Duong P.T., Weibel G.L., Lund-Katz S., Rothablat G.H., Phillips M.C., Jour Lipid Res 2008;**49**:1006-1014
16 MJ van Dam, E de Groot, SM Clee, GK Hovingh, R Roelants, A Brooks-Wilson, AH Zwinderman, AJ Smit, AHM Smelt, AK Groen, MR Hayden, JJP Kastelein, The Lancet 2002;**59**:37-41
17 Aiello R.J., Brees D., Bourassa P.A. Royer L., Linsey S., Coskram T., Haghpassand M. and Francone O.L. Arterioscler Thromb Vasc Biol 2002**;22**:630-637
18 Van Eck M., Bos I.S., Kaminski W.E., Orso E., Rothe G., Twisk J., Bottcher A., Van Amersfoort E.S., Christiansen-Weber T.A., Funh-Leung W.P., Van Berkel T.J. and Schmitz G. Proc Natl Acad Sci U S A 2002;**99**:6298-6303)
19 Rigotti A, Miettinen HE, Krieger M, Endocrine reviews, 2003;**24**:357-387
20 Rhainds D, Brissette L, Int Jour Biochem Cell Biol 2004;**39**:39 -77
21 Nieland TJF, Egrlich M, Krieger M, Kirchhausen T, BioChim BioPhys 2005;**1743**:44-51
22 Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M, Proc. Natl. Acad. Sci. 1997;**94**:12610-12615
23 Trigatti B., RayburnH., Viñals M., Braun A., Mietinnen H., Penman M., Hertz M., Schrenzel M,. Amigo L., Rigotti A. and Krieger M. Proc Natl Acad Sci U S A 1999;**96**:9322-9327
24 van Eck M., Twisk J., Hoekstra M., Van der Lans C., BosC., Kruijt J., Kuipers F., Van Berkel T., Jou Biol Chem, 2003;**278**:23699-23705
25 Francone O. Arterio Thromb Vasc Biol 2003;:**23**:1486
26 Rodriguez-Esparragon F., Rodriguez-Perez J.C., Hernandez-Trujiilo Y., Macias-reyes A., Medina A., Caballero A. and Ferraorio C.M.. Arterioscler Thromb Vasc Biol 2005;**25**:854-860
27 Vergeer M., Hovingh K., Visser M.N., Kastelein J.J. and Kuivenhoven J.A. Suppl to Circulation 2006;**114**:II-254
28 Kielar D., Dietmaier W., Langm,ann T., Aslanidis C., Probst M., Naruszewicz M and Schmitz G. Clin Chem 2001;**47**:2089-2097
29 Hoekstra M. Out R., Kruijt J.K., Van Eck M. and Van Berkel T.J. J Hepatol 2005;**42**:400-407
30 Hamon Y, Broccardo C, Chambenoit O, Luciani MF, Toti F, Chaslin S, Freyssinet JM, Devaux PF, McNeish J, Marguet D, Chimini G, Nat Cell Biol. 2000;**2**:399-406
31 Arca, M., Montali, A., Ciocca, S., Angelico, F., and Cantafora, A. *J. Lipid Res.* 1983;**24**:332-335
32) Setchell, K. D., Lawson, A. M., Tanida, N., and Sjovall, J. *J. Lipid Res.* 1983;**24**:1085-1100
33 Bligh, E., and Dyer, W. *Can. J. Biochem. Biophys.* 1959;**37**:911-917
34 Klett E.L., K. Lu, A. Kosters, E. Vink, M.H. Lee, M. Altenburg, S. Shefer, A.K. Batta, H. Yu, J. Chen, R. Klein, N. Looije, R. Oude-Elferink, A.K. Groen, N. Maeda, G. Salen and S. B. Patel. BMC Med. 2004;**2**:5
35 Nibbering C.P., A.K. Groen, R. Ottenhoff, J.F. Brouwers, G.P. van Berge-Henegouwen and K.J. Van Erpecum. J Hepatol. 2001;**35**:164-9.

36 J. McNeish, R.J. Aiello, D Guyot, T. Turi, C. Gabel, C. aldinger, K.L.Hoppe, M.L. Roach, L.J. Royer, J. de Wet, C. Broccardo, G. Chimini, O.L. Francone, PNAS, 2000;**97**:4245 - 4250
37 Miettinen H.E., Rayburn H. and Krieger M.. J Clin Invest 2001;**108**:1717-1722
38 Timmins J.M., Lee J.Y., Boudyguina E., Kluvkman K.D., Brunham L.R., Mulya A., Gebre A.K., Coutinho J.M., Colvin P.L., Smith T.L., Hayden M.R., Maeda N. and Parks J.S. J Clin Invest. 2005;**115**:1333-1342
39 Haghpassand M., Bourassa P.A., Francone O.L., Aiello R.J. J Clin Invest 2001;**108**:13115-1320
40 Brunham L.R., Kruit J.K., Iqbal J., Fievet C., Timmins J.M., Pape T.D., Coburn B.A., Bissada N., Staels B., Groen A.K., Hussain M.M., Parks J.S., Kuipers F., Hayden M.R. J Clin Invest 2006;**116**:1052-1062
41 Groen AK, Bloks VW, Bandsman RH, Ottenhoff R, Chimini G, Kuipers F Jour Clin Invest 2001;**108**:843-850
42 Tietge U.J., Nijstad N., Havinga R., Baller J.F., Van der Sluijs F.H., Bloks V.W., Gautier T. Kuipers F. J Lipid Res 2008;**49**:563-571
43 Mardones P., Quiñones V., Amigo L., Moreno M., Miquel J.F., Schwarz M., Mietinnen H.E., Trigatti B., Krieger M., VanPatten S., Cohen D.E. and Rigotti A. J Lipid Res 2001;**42**:170-180
44 Van der Velde, A.E., Vrins C.L., Van den Oever K., Kunne, C., Oude Elferink R.P., Kuipers F., Groen A.K Gastroemterology, 2007;**133**:967-975