Diabetologia (2004) 47:104–112
DOI 10.1007/s00125-003-1261-y

Diabetologia

# Down-regulation of hepatic and intestinal *Abcg5* and *Abcg8* expression associated with altered sterol fluxes in rats with streptozotocin-induced diabetes

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## Abstract

*Aim/hypothesis.* Type I diabetes is associated with altered hepatic bile formation and increased intestinal cholesterol absorption. The aim of this study was to evaluate whether altered expression of the ATP-Binding Cassette half-transporters Abcg5 and Abcg8, recently implicated in control of both hepatobiliary cholesterol secretion and intestinal cholesterol absorption, contributes to changed cholesterol metabolism in experimental diabetes.
*Methods.* mRNA and protein expression of *Abcg5* and *Abcg8* were determined in the liver and intestine of rats with streptozotozin-induced diabetes and related to relevant metabolic parameters in plasma, liver and bile.
*Results.* Hepatic mRNA expression of both *Abcg5* (−76%) and *Abcg8* (−71%) was reduced in diabetic rats when compared to control rats. In spite of increased HDL cholesterol, considered a major source of biliary cholesterol, secretion of the sterol into bile relative to that of bile salts was reduced by 65% in diabetic animals. Intestinal mRNA expression of *Abcg5* (−47%) and *Abcg8* (−43%) as well as Abcg5 protein contents were also reduced in insulin-deficient animals. This was accompanied by a three- to four-fold increase in plasma β-sitosterol and campesterol concentrations and by a doubling of the calculated apparent cholesterol absorption. These effects partially normalized upon insulin supplementation.
*Conclusion/interpretation.* Our data indicate that effects of insulin-deficiency on bile composition and cholesterol absorption in rats are, at least partly, attributable to changes in hepatic and intestinal *Abcg5* and *Abcg8* expression. [Diabetologia (2004) 47:104–112]

**Keywords** Cholesterol absorption · hepatobiliary transport · ABC transporters · hypercholesterolemia · intestine · liver · bile · bile salts · phospholipids · plant sterols · streptozotocin

Type I diabetes mellitus is associated with specific changes in cholesterol metabolism in humans [1] and in experimental animals [2, 3], including increased concentrations of plasma cholesterol, enhanced conversion of cholesterol into bile salts and an enhanced intestinal cholesterol absorption. The hepatobiliary pathway is of crucial importance for the maintenance of cholesterol homeostasis [4]. Bile salts that are secreted by the liver into the intestinal lumen are required for intestinal absorption of dietary cholesterol. The majority of bile salts is subsequently reabsorbed from the intestine and returns to the liver for re-secretion into the bile. The relatively small fraction of bile salts that escapes intestinal absorption is compensated for by de novo synthesis from cholesterol in the liver. Secondly, bile contains considerable amounts of free cholesterol. Since only a part of biliary cholesterol is reabsorbed from the intestine [5], the biliary pathway contributes to a major extent to cholesterol turnover. It is well-established that

Received: 9 April 2003 / Revised: 28 July 2003
Published online: 14 November 2003


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*Abbreviations:* ABC, ATP-Binding Cassette; Bsep, bile salt export pump; ECL, enhanced chemoluminesence; FPLC, fast protein liquid chromatography; Mdr, multidrug resistance gene; PCR, polymerase chain reaction; STZ, streptozotocin.

secretion of cholesterol into bile is coupled to that of phospholipids in a process that is, in part, controlled by bile salt secretion [6]. Recent studies, however, indicate that specific ABC transporters, i.e., Abcg5 and Abcg8, are involved in biliary cholesterol secretion [7, 8]. The genes encoding these transporters are highly expressed in the liver [9]. Mutations in the human genes encoding ABCG5 and ABCG8 have been shown to cause sitosterolaemia [10, 11, 12] with a reduced biliary secretion as well as a strongly enhanced intestinal absorption of plant sterols (sitosterol, campesterol). Indeed, ABCG5 and ABCG8 are also highly expressed in the intestine [9] and supposedly involved in efflux of plant sterols taken up by enterocytes back into the intestinal lumen, thereby preventing absorption. Based on the fact that the efficiency of dietary cholesterol absorption is high in sitosterolaemia patients, a role of ABCG5 and ABCG8 in the control of cholesterol absorption efficiency has been proposed [10, 11, 12]. Accordingly, cholesterol absorption was reduced in mice over-expressing both transporters [7] and in mice in which expression of the transporters was induced by pharmacological means [13].

Type I diabetes is associated with altered expression of several ABC transporters in the liver. In a recent study [14], we showed that streptozotocin (STZ)-induced diabetes in rats differentially affects the expression of hepatic ABC transporters which, at least in part, underlie reported effects on bile composition. Specifically, we found a very strong up-regulation of *Abcb4* (multidrug resistance P-glycoprotein subtype 2 or Mdr2) mRNA and Abcb4 protein, in accordance with a strong induction of biliary phospholipid secretion. In spite of the characteristic increase in biliary bile salt output rates, we found no effects on Abcb11 (bile salt export pump or Bsep) protein content in livers of STZ-diabetic rats.

To investigate whether changes in *Abcg5/Abcg8* expression contribute to the established effects of insulin deficiency on cholesterol metabolism, we have determined their mRNA abundances and protein contents in the liver and intestine of rats with STZ-diabetes and related these to the actual sterol fluxes. Our data indicate that the suppressive effects of insulin-deficiency on biliary cholesterol secretion and its stimulatory effects on cholesterol absorption in rats are, at least in part, attributable to changes in hepatic and intestinal *Abcg5* and *Abcg8* expression.

## Materials and methods

*Animals.* Male Wistar rats (260–300 g) were purchased from Harlan (Zeist, The Netherlands) and housed in a temperature-controlled environment with alternating 12-h light and dark periods. The rats received standard laboratory chow (RMH-B; Hope Farms BV, Woerden, The Netherlands) and had free access to food. Experimental procedures were approved by the local Ethics Committee for Animal Experimentation.

*Experimental procedures.* Diabetes was induced by a single intraperitoneal injection (60 mg/kg body weight) of STZ (Pharmacia & Upjohn, Kalamazoo, Mich., USA). Control animals received an injection of the solvent (sodium citrate, 3% w/v). Induction of diabetes was perceived by development of hyperphagia, polydipsia and polyuria and confirmed by determination of the degree of hyperglycaemia. Three weeks after STZ injection, one half of the diabetic group was treated with subcutaneously administered insulin (long acting insulin, Humuline NPH, Eli Lilly, Nieuwegein, The Netherlands, 1 IU in the morning and 2 IU in the evening). Experiments were carried out at 4 weeks after STZ injection. Food intake was monitored by weighing of food containers and faeces was collected quantitatively during the last 3 days prior to death of the animals. At that time, six control, six diabetic and six diabetic insulin-treated rats were anaesthetized with pentobarbital (60 mg/kg body weight) and bile was collected for 30 min upon cannulation of the bile duct. Blood samples were collected by heart puncture, transferred to EDTA-containing tubes and centrifuged immediately (10 000 g). Plasma was stored at –20°C until analyses and the livers were rapidly excised and weighed. Parts of the liver were snap-frozen in liquid nitrogen for RNA isolation, isolation of plasma membrane fractions or determination of lipid concentrations. The small intestines were flushed with a buffered salt solution; representative parts of the intestine were snap-frozen in liquid nitrogen for RNA isolation or isolation of brush border membranes.

*Analytical procedures.* Plasma concentrations of total cholesterol, triglycerides and free fatty acids were measured with commercially available kits (Roche, Mannheim, Germany, or Wako, Neuss, Germany) [13, 15]. Pooled plasma samples of the three groups of rats were used for lipoprotein separation by fast protein liquid chromatography (FPLC) [15]. Plasma plant sterol and cholesterol concentrations were determined by gas chromatography [16]. Biliary bile salt concentrations were measured enzymatically [13, 15]. Hepatic and biliary phospholipids and cholesterol lipid contents were measured after extraction [17, 18, 19]. Faecal and chow contents of neutral sterols were measured as described [15].

*Western blotting.* Plasma lipoprotein fractions separated by FPLC were taken for semi-quantitative assessment of apoA-I contents by Western blotting [15].

Hepatic plasma membrane fractions were prepared and characterized as described [20]. Proteins from liver homogenates (75 µg protein / lane) or liver plasma membranes (10 µg protein/lane) were separated on 4–15% Ready Gels (Bio-Rad Laboratories, Hercules, Calif., USA) and blotted onto nitrocellulose membranes by tank blotting. Membranes were blocked overnight in a 5% skimmed milk powder solution in Tris-buffered saline containing 0.1% Tween 20 (TTBS) and subsequently incubated with the primary antibody (rabbit polyclonal anti-SR-BI, Novus Biologicals, Littleton, Colo., USA, NB400–101) diluted 1:20 000 in TTBS for 1 h at room temperature. After washing, anti-rabbit IgG linked to horse radish peroxidase, diluted 1:1000 in TTBS, was added for 1 h. Detection was carried out using ECL, according to the manufacturer's instructions (Amersham, Roosendaal, the Netherlands).

Proteins from intestinal brush border membranes, isolated as described previously [20], were separated on 8% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Membranes were blocked in TTBS and subsequently incubated for 1 h at room temperature with primary antibodies, raised in rabbits against amino acids 256–392 of murine Abcg5 [21], diluted 1:1000 in blocking buffer. Membranes were washed thrice in TTBS and incubated with horseradish peroxidase-conjugated

goat anti-rabbit antibodies (Bio-Rad) diluted 1:2000 in blocking buffer. Membranes were washed four times in TTBS and bands were visualized using Lumi-light$^{plus}$ Western blotting substrate in a Lumi-Imager F1 workstation (Roche).

*RNA isolation and RT-PCR procedures.* RNA isolation and cDNA synthesis were carried out as described [22]. Real-time quantitative PCR was done as described [23] and modified in our laboratory [13]. Primer and probe sequences (Invitrogen, Carlsbad, USA) and detection probes (Eurogentec, Seraing, Belgium) for the genes of interest, labelled with the 5′ linked fluorescent reporter dye 6-carboxy-fluorescein (FAM) and the 3′ linked fluorescent quenching dye 6-carboxy-tetramethyl-rhodamine (TAMRA) [13]. Measurements were done using an ABI Prism 7700 Sequence Detector with 1.6.3 software (Perkin-Elmer Corp., Foster City, Calif., USA).

*Statistics.* Results are presented as mean values±SD. Statistic analyses were carried out using one-way ANOVA with Bonferroni correction or, when two groups were compared, by the Mann-Whitney U test. A $p$ value of less than 0.05 was considered statistically significant.

## Results

*Animal characteristics.* STZ rats had lower body weights than control rats at the end of the experiment (–29%, $p<0.05$), which did not normalize upon treatment with insulin (–25%). The ratio liver-to-body weight was increased by 37% ($p<0.05$) upon STZ treatment and this effect did not disappear after insulin treatment. Blood glucose concentrations were higher in diabetic rats than in controls, i.e., 23.2±3.5 compared with 5.8±0.3 mmol/l ($p<0.05$), whereas those in insulin-treated diabetic rats were intermediate (11.9±5.4 mmol/l).

Plasma cholesterol, triglyceride, and free fatty acid concentrations were all increased in diabetic rats and showed a tendency towards normalization in insulin-treated diabetic rats (Table 1). FPLC separation of plasma lipoproteins showed that the increase in plasma cholesterol in diabetic animals was due to increases in VLDL-, LDL- as well as HDL-sized fractions (Fig. 1A), whereas the increase in triglycerides was exclusively in VLDL-sized fractions (Fig. 1B). Western blot analysis of FPLC fractions revealed a higher apolipoprotein (apo) A-I content in HDL fractions of diabetic rats than in those of control rats (Fig. 1C). Hepatic mRNA abundance of *Apoa-I* was more than two-fold induced in diabetic rats and normalized upon insulin treatment (data not shown).

No differences in hepatic free cholesterol contents were noted between control, diabetic and insulin-treated diabetic animals, i.e., 25.5±1.4, 24.0±3.6 and 25.5±4.5 nmol/mg protein, respectively. As expected, expression of sterol regulatory element binding protein 1c (*Srebp1c*) was strongly reduced in diabetic animals whereas that of *Srebp2*, the transcription factor that is primarily responsible for control of genes involved in maintenance of hepatocytic cholesterol homeostasis, remained unaffected (Fig. 2). Expression of genes encoding proteins involved in cholesterol synthesis (HMG CoA reductase, *Hmgr*) and lipoprotein uptake (LDL receptor, *Ldlr*) were down-regulated in

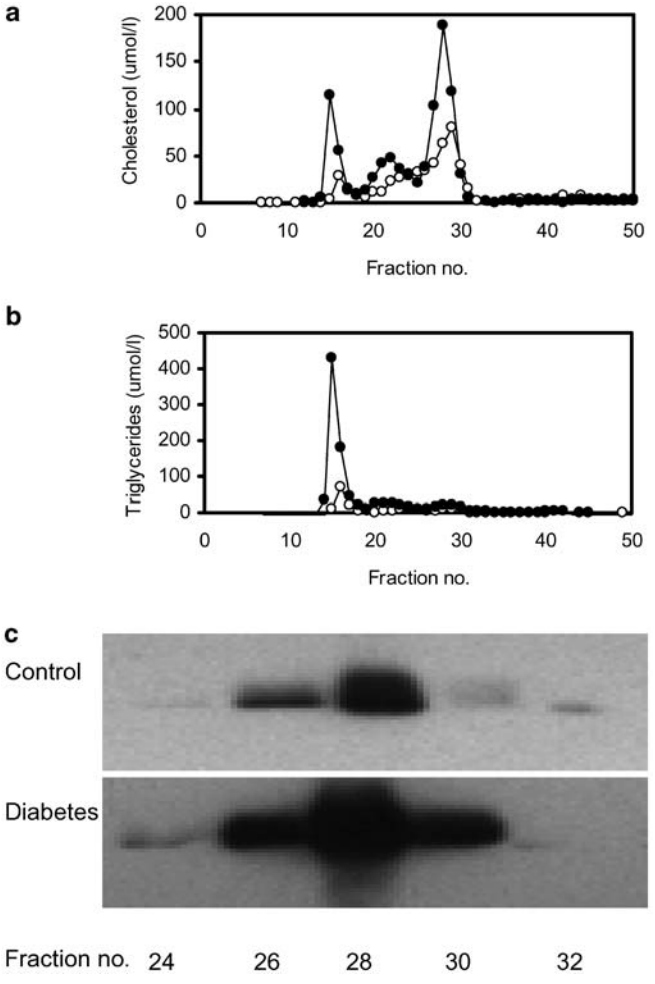


**Fig. 1a–c.** Effects of streptozotocin-induced diabetes on distribution of cholesterol (**a**), triglycerides (**b**) and apolipoprotein A-I (**c**) in plasma lipoprotein fractions *Open symbols*, control rats; *closed symbols*, diabetic rats. Diabetic rats treated with insulin showed concentrations of cholesterol and triglycerides that were intermediate between those of controls and untreated diabetic rats across all lipoprotein fractions: these data are not shown for reasons of clarity. *Top panel c*, control rats; *bottom panel c*, diabetic rats

**Table 1.** Plasma concentrations of cholesterol, triglycerides and free fatty acids in control, diabetic and insulin-treated diabetic rats

| | Control | Diabetes | Diabetes + Insulin |
|---|---|---|---|
| Cholesterol (mmol/l) | 1.62±0.13 | 2.59±0.48* | 2.11±0.56 |
| Triglycerides (mmol/l) | 1.29±0.60 | 5.99±2.29* | 3.61±1.47* |
| Free fatty acids (μmol/l) | 231±32 | 572±184* | 406±107* |

Mean values±SD. are shown for six animals per group. Asterisks indicate significant difference from control values

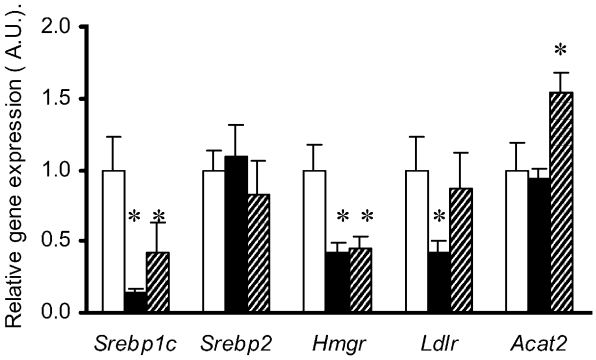


**Fig. 2.** Changes in relative hepatic gene expression of *Srebp1c*, *Srebp2*, *Hmgr*, *Ldlr*, and *Acat* 2 upon induction of streptozotocin-diabetes, determined by realtime PCR *Open bars*, control rats; *closed bars*, diabetic rats; *striped bars*; diabetic rats treated with insulin. Mean values±SD of 4–6 rats per group, asterisks indicate significant difference from control values

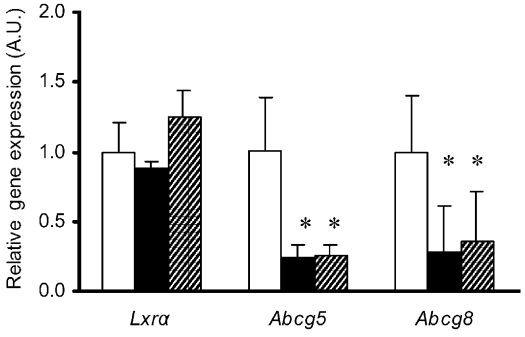


**Fig. 3.** Changes in relative hepatic gene expression of *Lxrα*, *Abcg5* and *Abcg8* upon induction of streptozotocin-diabetes in rats, determined by realtime PCR. *Open bars*, control rats; *closed bars*, diabetic rats; *striped bars*; diabetic rats treated with insulin. Mean values±SD of six rats per group, asterisks indicate significant difference from control values

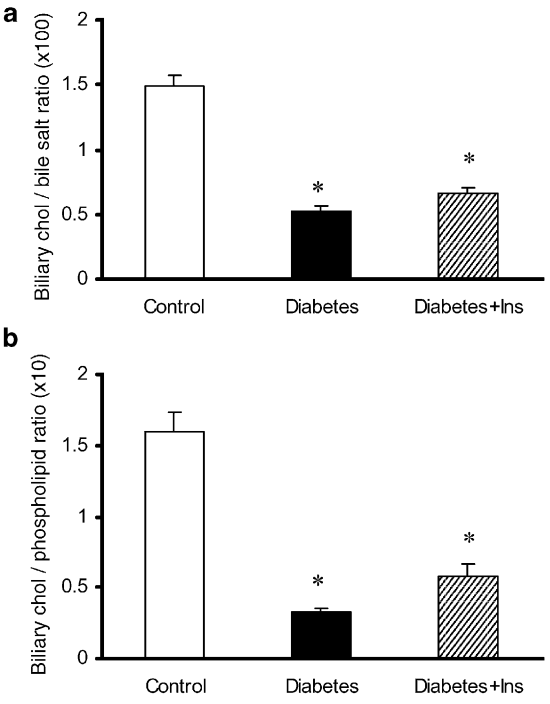


**Fig. 4a, b.** Changes in biliary cholesterol content upon induction of streptozotocin-diabetes in rats. Biliary cholesterol concentration was expressed relative to that of bile salts (**a**) or phospholipids (**b**). *Open bars*, control rats; *closed bars*, diabetic rats; *striped bars*; diabetic rats treated with insulin. Mean values±SD of six rats per group, asterisks indicate significant difference from control values

diabetic animals, whereas that of acyl-CoA cholesterol acyltransferase 2 (*Acat2*), involved in cholesterol esterification, was not affected (Fig. 2).

*Reduced hepatic expression of Abcg5 and Abcg8 is associated with impaired hepatobiliary cholesterol transport.* mRNA abundances of *Abcg5* and *Abcg8* were strongly reduced in the livers of diabetic rats in comparison to those in control animals. This consequence of long-term insulin-deficiency did not normalize upon treatment of diabetic rats with insulin. Both *Abcg5* and *Abcg8* gene expression are controlled by the liver X-receptor: no differences in expression of the gene encoding the most abundant isoform of this transcription factor in the liver, i.e., *Lxrα*, were noted between the groups (Fig. 3).

To test the functional consequences of reduced hepatic *Abcg5* and *Abcg8* expression, we measured biliary cholesterol output in rats of the three experimental groups. Cholesterol output in the three groups of animals was expressed relative to that of bile salts (Fig. 4A) and to that of phospholipids (Fig. 4B). It is evident that diabetic rats secreted much less cholesterol relative to bile salts and to phospholipids than control rats did: insulin treatment failed to completely restore hepatobiliary cholesterol hyposecretion.

HDL is considered to be an important source of biliary cholesterol and concentrations of plasma HDL cholesterol were clearly elevated in diabetic rats (Fig. 1). To evaluate whether a reduced hepatic uptake capacity of HDL cholesterol (ester) might contribute to biliary cholesterol hyposecretion, we analysed hepatic mRNA and protein expression of the major HDL-receptor, i.e., scavenger receptor class B type 1 (SR-BI) in livers of the three groups of rats. mRNA abundance of *Sr-bI* was slightly higher in diabetic rats than in controls and normalized upon insulin treatment (Fig. 5). SR-BI protein content was clearly increased in liver homogenates of diabetic rats compared to those of controls and insulin-treated diabetic rats (Fig. 5A) while the amounts of the protein in hepatic plasma membrane fractions were rather similar among the three groups (Fig. 5B).

*Reduced intestinal expression of Abcg5 and Abcg8 is associated with enhanced cholesterol absorption.* mRNA expression levels of *Abcg5* and *Abcg8* were

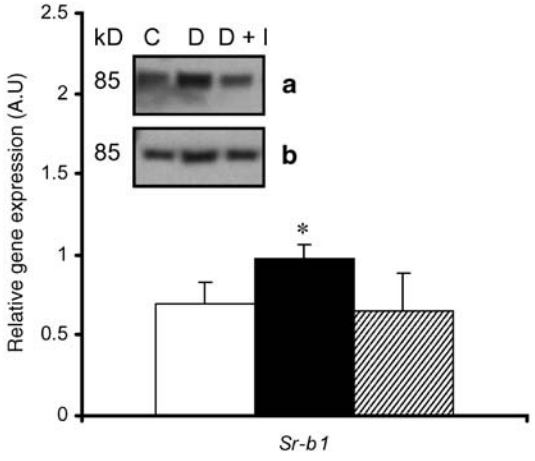


**Fig. 5a, b.** Effects of streptozotocin-diabetes on hepatic mRNA and protein expression of SR-BI. *Open bars*, control rats; *closed bars*, diabetic rats; *striped bars*; diabetic rats treated with insulin. Mean values±SD of six rats per group, asterisks indicate significant difference from control values. Inserts show corresponding levels of SR-BI protein in liver homogenates (**a**) and plasma membrane fractions (**b**). Data shown are representative examples of at least four preparations per group. *C*, control; *D*, diabetics; *D + I*, diabetics + insulin

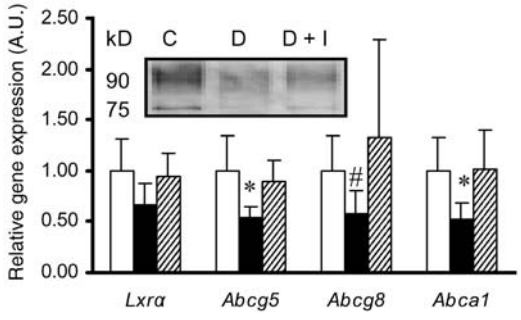


**Fig. 6.** Changes in relative intestinal gene expression of *Lxrα*, *Abcg5*, *Abcg8* and *Abca1*, determined by realtime PCR, and intestinal Abcg5 protein content upon induction of streptozotocin-diabetes in rats. *Open bars*, control rats; *closed bars*, diabetic rats; *striped bars*; diabetic rats treated with insulin. Mean values±SD of six rats per group, asterisks indicate significant difference from control values, #indicates $p$=0.059. Insert shows Abcg5 protein levels in brush border membranes from jejunal sections of rats of the three groups. Bands at 90 and 75 kD reacted to the antibody raised against mouse Abcg5. Competition with the peptide used to raise the antibody strongly decreased these signals. Incubation of the protein fractions with N-glycosidase F to remove all N-linked sugar chains decreased the apparent molecular weight of both bands to a single band of ~64 kD. *C*, control; *D*, diabetics; *D + I*, diabetics + insulin

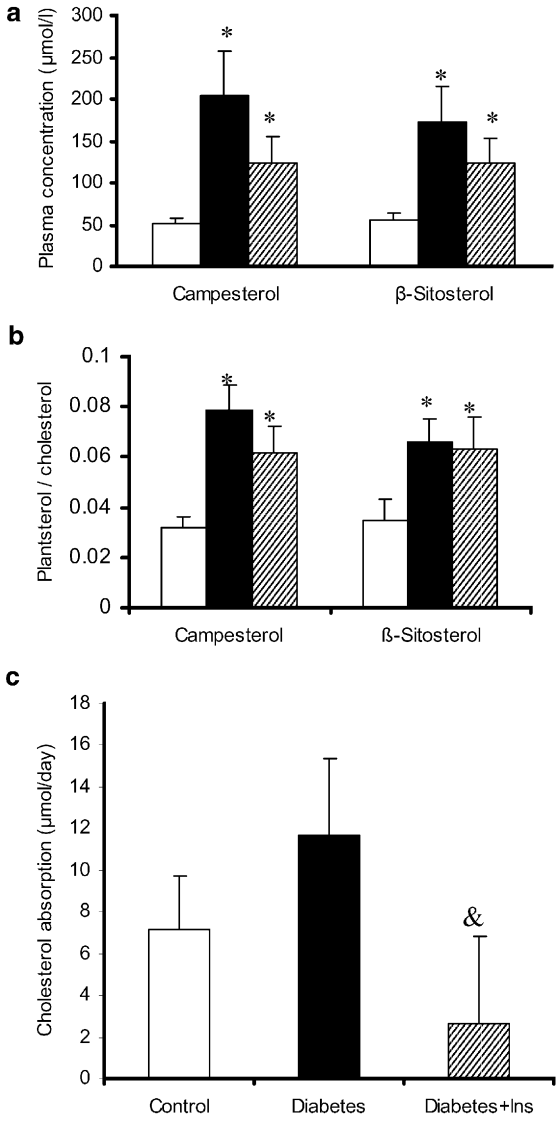


**Fig. 7a–c.** Changes in indices of intestinal cholesterol absorption efficiency upon induction of streptozotocin-diabetes in rats. Plasma concentrations of campesterol and β-sitosterol were measured in control rats, diabetic rats and diabetic rats treated with insulin (**a**) and normalized to that of cholesterol, to exclude that the observed changes in plasma plant sterols were due to a-selective alterations in plasma sterol levels (**b**). Apparent cholesterol absorption was calculated for the three groups by subtraction of the measured daily faecal neutral sterol loss from the estimated daily input (**c**). *Open bars*, control rats; *closed bars*, diabetic rats; *striped bars*; diabetic rats treated with insulin. Mean values±SD of six rats per group, asterisks indicate a significant difference from control values, & indicates significant difference from diabetes group

reduced by 47% and 43% in the jejunum of diabetic rats when compared to controls and normalized upon insulin treatment (Fig. 6). The expression of *Abca1*, also implicated in the control of intestinal cholesterol absorption, showed a similar pattern as those of *Abcg5* and *Abcg8*. Intestinal expression of *Lxrα* tended to be reduced in diabetic rats and normalized upon insulin treatment.

Reduced mRNA abundance was associated with a clearly reduced protein content of Abcg5 in brush border membrane preparations isolated from the small intestines of diabetic rats (Fig. 6).

To assess the functional consequences of reduced *Abcg5* and *Abcg8* expression in the intestines of diabetic rats, plasma concentrations of the plant sterols campesterol and β-sitosterol were determined. These sterols

are considered natural substrates of Abcg5/Abcg8: their concentrations are strongly increased in sitosterolaemia patients and have been advocated as an indirect measure of cholesterol absorption efficiency. The concentration of both sterols was increased in diabetic rats when compared to control values and decreased upon insulin treatment of diabetic animals (Fig. 7A). Plasma plant sterol concentrations remained increased in diabetic rats when normalized to plasma cholesterol concentrations (Fig. 7B). The difference between calculated daily biliary excretion rates of cholesterol and an estimation of dietary cholesterol intake (input) and daily faecal neutral sterol loss (output) were used to calculate the "apparent cholesterol absorption" in the three groups of animals (Fig. 7C). It was found that this calculated value was increased in the diabetic animals in comparison to controls, while it decreased again after treatment of diabetic animals with insulin.

## Discussion

The results show that STZ-induced Type I diabetes in rats is associated with reduced hepatic and intestinal expression of the ABC half-transporters Abcg5 and Abcg8. Reduced hepatic expression of these "sitosterolaemia genes" coincided with a reduction of hepatobiliary cholesterol secretion, whereas their reduced intestinal expression was associated with an increased absorption of cholesterol as deduced from an indirect measure of the process (plasma plant sterol concentrations) and from calculation of the apparent absorption efficiency. Similar changes in cholesterol transport have been reported in sitosterolaemia patients [24] and in Abcg5/Abcg8-deficient mice [8]. Over-expression of the human genes in transgenic mice [7] and pharmacological induction of expression of the endogenous genes in wild-type mice [13] have been reported to have opposite effects, i.e., to stimulate biliary cholesterol excretion and to reduce intestinal cholesterol absorption. Consequently, it is highly likely that effects of insulin-deficiency on cholesterol metabolism are, at least in part, caused by changes in *Abcg5* and *Abcg8* expression.

Recently, two groups have independently identified mutations in either *ABCG5* or *ABCG8* as the cause of the rare, recessively inherited metabolic disease sitosterolaemia [10, 11, 12]. Patients with this disease develop xanthomas and premature atherosclerosis [24, 25]. Affected individuals show high concentrations of plant sterols in plasma due to the fact that, in contrast to healthy subjects, they efficiently absorb these sterols from the intestine and are unable to excrete them into the bile [24, 26]. Sitosterolaemia patients have also been reported to efficiently absorb dietary cholesterol and to show impaired biliary cholesterol excretion [26, 27]. *ABCG5/Abcg5* and *ABCG8/Abcg8* are predominantly expressed in hepatocytes and in small intestinal enterocytes in humans and mice. The two genes are arranged in a head-to-head configuration in the human [28] and mouse [9] genome. Expression of both genes is co-ordinately regulated and highly induced in mice kept on a high-cholesterol diet [29]. LXRα, a nuclear receptor activated by oxysterols that plays a crucial role in regulating genes involved in cholesterol trafficking [30], is required for induction of murine *Abcg5* and *Abcg8* expression upon cholesterol feeding [29]. Treatment of mice with synthetic LXR agonists strongly induces the expression of both genes in liver and intestine [13, 29]. Recent studies [31], in which epitope-tagged mouse Abcg5 and Abcg8 were expressed in cultured cells, have shown that heterodimerization of these half-transporters is required for their transport from the endoplasmic reticulum to the apical plasma membrane. Thus, available data indicate that the Abcg5/Abcg8 heterodimer is present at the canalicular membrane of hepatocytes where it is involved in secretion of cholesterol and plant sterols into the bile. In the intestine, the heterodimer seems to promote the efflux of (dietary) sterols, taken up by the enterocytes by as yet unidentified mechanisms, back into the lumen and thereby reduce the efficiency of their absorption. Overall, the physiological action of the transporter complex limits accumulation of sterols in the body.

Under normal conditions, biliary secretion of cholesterol is tightly coupled to that of phospholipids in a process controlled by bile salt secretion [6]. Accordingly, one would expect biliary cholesterol secretion to be enhanced in diabetic rats. This was evidently not the case: in spite of a strong increase in biliary bile salt and phospholipid secretion [14], diabetic rats displayed a relative hyposecretion of biliary cholesterol. Theoretically, hyposecretion could be explained by a lack of bile-destined cholesterol in the liver. It has been proposed that HDL cholesterol is a primary source of biliary cholesterol after its selective uptake by SR-BI [32]. One study [33] reported biliary cholesterol hyposecretion in SR-BI-deficient mice while another [34] reported hypersecretion in mice with hepatic SR-BI over-expression. We found that plasma HDL cholesterol and apo A-I levels were increased in diabetic rats, as is the case in SR-BI-deficient mice [33]. Yet, hepatic *Sr-bI* mRNA expression was up-regulated and corresponding protein contents were clearly increased in liver homogenates. SR-BI protein remained largely unaffected in plasma membrane fractions isolated from livers of diabetic rats, suggestive for altered sorting. Taken together, our data do not support impaired SR-BI-mediated HDL uptake as a cause of cholesterol hyposecretion in diabetic rats. In fact, our recent observation that cholesterol secretion is unaffected in Abca1 null mice lacking HDL [35] strongly argues against a regulatory role of cholesterol delivery. A concise overview of various models of cholesterol hypo- and hypersecretion indicated that, at least

in mice, biliary cholesterol secretion strongly correlates with hepatic *Abcg5/Abcg8* expression [21]. Accordingly, we propose that impaired hepatic expression of both half-transporters underlies impaired cholesterol secretion in diabetic rats. Insulin treatment of diabetic rats did not normalize hepatic Abcg5/Abcg8 expression and, consequently, failed to normalize cholesterol secretion into bile.

The intestine is another site were Abcg5/Abcg8 exert control on cholesterol metabolism, i.e., by regulating the efficiency of cholesterol absorption. It is known that cholesterol absorption is increased in chronically diabetic rats [2, 3], but underlying mechanisms have remained elusive so far. Our study confirms the increased cholesterol absorption in STZ-treated rats by two independent methods. We found that plasma concentrations of β-sitosterol and campesterol were three- to four-fold higher in diabetic rats than in controls and tended to normalize upon treatment of diabetic rats with insulin. Furthermore, we have calculated the apparent absorption by subtracting daily faecal neutral sterol output from the estimated daily input of dietary and biliary cholesterol. Although this calculation is based on a number of assumptions, the results strongly suggest that the apparent absorption is increased in diabetic animals. Intestinal hypertrophy [36] and the enlarged bile salt pool [3] have been proposed as rather unspecific causes of enhanced cholesterol absorption in experimental diabetes. Furthermore, cholesterol esterification by the enzyme acyl-CoA cholesterol acyltransferase, producing cholesteryl esters that can be incorporated into chylomicrons, has been shown to be enhanced in diabetic animals [37]. Our data show that reduced expression of *Abcg5* and *Abcg8* in the intestine of diabetic rats could contribute to the enhanced cholesterol absorption associated with this condition.

The obvious question concerns the cause of *Abcg5/Abcg8* down-regulation in liver and intestine of insulin-deficient rats. As mentioned previously, both genes are under control of LXRα, a sterol-sensing nuclear receptor that, upon dimerization with activated RXR, induces transcription of a large number of genes involved in cholesterol trafficking [30]. We found no changes in mRNA expression of *Lxrα* itself or of *Rxrα*, either in the liver or intestine. *Lxrα* expression was reported to be induced in cultured hepatocytes exposed to insulin and in livers of rats and mice upon acute administration of insulin [38]. This suggests that, in our chronic model of insulin deficiency, alternative modes of regulation maintain hepatic *Lxrα* expression. This obviously does not exclude the possibility of reduced LXRα protein concentrations or reduced amounts of its most potent ligands, i.e., (24S), 25-epoxycholesterol, (24S)-hydroxycholesterol, or (22R)-hydroxycholesterol. Alternatively, it could be that metabolic consequences of diabetes interfere with LXRα signalling. In a recent paper [39], evidence was provided to suggest that reduced expression of another important ABC transporter, i.e., Abca1, in liver and macrophages of diabetic mice is due to the characteristically high concentrations of free fatty acids and ketone bodies (particularly acetoacetate). In this study, we have confirmed down-regulation of *Abca1* expression in the liver of diabetic rats and show that expression of this gene is also strongly reduced in their small intestine. Expression of *Abca1* is also under control of LXRα [30] and unsaturated fatty acids have been shown to antagonize activation of this nuclear receptor by oxysterols so that transcription of target genes is inhibited [40, 41]. The mechanism by which acetoacetate interferes with ABC transporter expression is fully elusive at the moment but, in view of the similar mode of regulation of *Abca1* and *Abcg5/Abcg8* genes [30], it could well be involved in the down-regulation of *Abcg5/Abcg8* observed in this study. Thus, impaired *Abcg5/Abcg8* expression in liver and intestine in diabetes may be related to the accelerated lipolysis and/or increased ketogenesis that is associated with this condition. A direct role of insulin-deficiency per se is not likely, since we found no differences in *Abcg5/Abcg8* expression between rat hepatocytes cultured for up to 24 h in the absence or presence of insulin.

In conclusion, we have provided evidence that suppression of *Abcg5* and *Abcg8* expression in the liver and intestine contributes to altered hepatobiliary and intestinal sterol fluxes that collectively promote accumulation of these sterols in the body in diabetic rats. When similar events occur in human diabetic patients, as suggested by increased plasma levels of plant sterols in subjects with poorly controlled Type I diabetes [42], this could contribute to an enhanced risk for development of atherosclerosis [43].

*Acknowledgements*. This study was supported by grant 2001B043 from the Netherlands Heart Foundation (to A.K.G. and F.K.) and grant 902-23-262 from the Netherlands Organization for Scientific Research. We thank R. Havinga, R. Boverhof, J. Baller and C. van der Ley for their excellent technical assistance, Dr. F.G. Schaap for antibodies and Prof. Dr. P.J.J. Sauer for critical reading of the manuscript and his valuable comments.

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