# Apical sodium bile acid transporter and ileal lipid binding protein in gallstone carriers[S]

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**Abstract** **Although a cholesterol supersaturation of gallbladder bile has been identified as the underlying pathophysiologic defect, the molecular pathomechanism of gallstone formation in humans remains poorly understood. A deficiency of the apical sodium bile acid transporter (ASBT) and ileal lipid binding protein (ILBP) in the small intestine may result in bile acid loss into the colon and might promote gallstone formation by reducing the bile acid pool and increasing the amount of hydrophobic bile salts. To test this hypothesis, protein levels and mRNA expression of ASBT and ILBP were assessed in ileal mucosa biopsies of female gallstone carriers and controls. Neither ASBT nor ILBP levels differed significantly between gallstone carriers and controls. However, when study participants were subgrouped by body weight, ASBT and ILBP protein were 48% and 67% lower in normal weight gallstone carriers than in controls ($P < 0.05$); similar differences were found for mRNA expression levels. The loss of bile transporters in female normal weight gallstone carriers was coupled with a reduction of protein levels of hepatic nuclear factor 1α and farnesoid X receptor. In conclusion, in normal weight female gallstone carriers, the decreased expression of ileal bile acid transporters may form a molecular basis for gallstone formation.**—Bergheim, I., S. Harsch, O. Mueller, S. Schimmel, P. Fritz, and E. F. Stange. **Apical sodium bile acid transporter and ileal lipid binding protein in gallstone carriers.** ***J. Lipid Res.*** **2006.** 47: **42–50.**

**Supplementary key words** gallstone • intestine • nuclear receptor

Despite decades of research, gallstone disease remains a significant health problem worldwide, particularly in the female adult population. In the United States and European countries, 10–20% of adults develop gallstones, mostly cholesterol-rich stones (1). Even though cholesterol supersaturation of gallbladder bile has been identified as the underlying pathophysiologic defect (2), the molecular pathogenesis of cholesterol gallstone formation remains poorly understood. Disorders contributing to the cholesterol supersaturation of bile could result from *a*) uncoupling of phospholipid and/or cholesterol secretion from bile acid secretion or *b*) augmentation of hepatic cholesterol synthesis or uptake. The source of the excess cholesterol is unclear, but it is probably derived from lipoprotein (3) rather than from synthesis (4). Furthermore, evidence is available that *c*) alterations of intestinal bile acid recycling (5), *d*) prolonged intestinal transit (5), *e*) altered bile salt synthesis, and *f*) gallbladder motility defects are important in human gallstone formation and biliary pain (6). Accordingly, the pools of cholic and chenodeoxycholic acid have been found to be reduced in most normal weight gallstone patients, whereas that of deoxycholic acid is often increased (7). Cholic acid is almost completely 7-α-dehydroxylated to deoxycholic acid by anaerobic bacteria in the colon (8), and ~30–40% of this deoxycholic acid is absorbed from the intestinal lumen (7). The expansion of the deoxycholic acid pool observed in gallstone patients (7) could possibly result from increased cholic acid synthesis, small intestinal spill of cholic acid into the colon, bacterial overgrowth, or a change in bacterial flora favoring cholic acid deconjugation.

Bile acids synthesized in the liver undergo a very efficient cycling between the liver and the intestine. A key component of the enterohepatic circulation of bile salts is the intestinal reclamation of bile acids (9). Both conjugated and unconjugated bile acids are passively recovered along the entire axis of the intestine. In the terminal ileum, the bulk of conjugated bile acids are reabsorbed by an active sodium-dependent dimeric transport system [the ileal apical sodium bile acid transporter (ASBT)] (10). Bile acids escaping active reabsorption in the distal ileum are 7-α-dehydroxylated and deconjugated by colonic bacteria; these secondary bile acids may then reach the portal circulation by passive diffusion along the colon. Decreased bile acid uptake as a result of genetic disruption of ASBT activity (11, 12), ileal diseases, ileal resection, or congenital

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Abbreviations: ASBT, apical sodium bile acid transporter; FXR, farnesoid X receptor; HNF1α, hepatic nuclear factor 1α; ILBP, ileal lipid binding protein; PPARα, peroxisome proliferator-activated receptor α.

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[S] This article contains supplemental data in the form of 3 figures.

primary bile acid malabsorption may lead to bile acid pool depletion and the subsequent development of cholesterol gallstones (13–15).

After being actively reabsorbed from the ileal lumen by the integral brush border membrane glycoprotein ASBT, bile acids are presumed to be associated with the 14 kDa ileal lipid binding protein (ILBP) for cytosolic transport (16, 17). The secretion of bile salts from the basolateral surface of enterocytes into the splanchnic circulation has not yet been fully clarified. Recently, it was shown that besides an alternatively spliced truncated form of ASBT (18) and/or multidrug resistance protein 3 (19), the organic solute transporter α-β (20) seems to be involved in the efflux of bile acids in the intestine.

Binding sites of several nuclear receptors [e.g., hepatic nuclear factor 1α (HNF1α) and peroxisome proliferator-activated receptor α (PPARα)] have been identified in the promoter of ASBT (21–23). HNF1α seems to be of particular importance. For example, the minimal ASBT promoter construct that confers full transcriptional activity contains three functional HNF1α recognition sites (22). Furthermore, site-directed mutagenesis of HNF1α binding sites in the ASBT promoter abrogates transcription activity, and HNF1α knockout mice are characterized by the absence of ileal ASBT expression along with marked fecal bile acid wasting (21). In addition, results of in vitro studies suggest a PPARα-dependant induction of human ASBT gene expression (22). A transcriptional activation of ILBP gene expression has been assigned to the direct effect of a complex of bile acids and the farnesoid X-receptor (FXR) (24). In addition, binding sites of other nuclear transcription factors, such as the liver X-receptor and the sterol element regulator protein 1 (24, 25), have been identified in the ILBP promoter.

Because little is known about the expression of these bile acid transporters in human cholelithiasis, the main objective of this study was to determine the expression of ASBT and ILBP in the ileum of patients with gallstones and controls. In addition, protein levels of the nuclear transcription factors HNF1α, FXR, and PPARα were measured.

## MATERIALS AND METHODS

### Subjects

The study protocol was approved by the Ethics Committee of the University of Tuebingen (Tuebingen, Germany). Informed consent was obtained from all subjects. Subjects included had *a*) no history of taking lipid-lowering drugs or drugs interfering with bile acid uptake, *b*) no known medical conditions affecting lipid metabolism (e.g., diabetes), *c*) normal liver function tests and absence of signs of hemolysis or other conditions associated with pigment stones, *d*) no medical records indicating findings affecting bile acid uptake (e.g., inflammatory bowel diseases) or colonic surgery, and *e*) no clinical indication of impaired nutritional status. A total of 41 female subjects, all of whom were undergoing colonoscopy for medical reasons, were included in the study. Seventeen subjects had gallstones, and 24 gallstone-free subjects served as controls. None of the subjects had symptomatic gallstone disease. Six of the 17 gallstone carriers had a known history of gallstone disease that was first diagnosed by routinely performed abdominal ultrasound; however, in addition, the presence or absence of gallstones was confirmed in all patients and controls by ultrasound on the day of colonoscopy. None of the gallstone carriers suffered from a disease associated with pigment gallstones (e.g., hemolytic syndromes), so most likely patients has cholesterol gallstones in this cohort. The characteristics of patients and controls did not differ between groups and are summarized in **Table 1**. None of the patients or controls displayed any histological signs of inflammation in the ileum. Using standard pinch forceps, eight biopsies were obtained from the ileum within 10 cm of the ileocecal valve and either placed immediately in liquid nitrogen and stored at −80°C until use or fixed in 10% buffered formalin.

### Isolation of RNA and protein

Total RNA and protein were isolated using Trizol (Invitrogen), based on the single-step method described by Chomczynski and Sacchi (26).

### Real-time reverse transcription-polymerase chain reaction

The integrity, quality, and quantity of RNA were analyzed by subjecting samples to gel electrophoresis (1.2% agarose gel) and measuring absorption at 260 and 280 nm. First-strand cDNA was synthesized from 400 ng of total RNA by the random primer method using an avian myeloblastosis virus (AMV)-reverse transcriptase system (Promega). Using real-time PCR, amplification of ASBT (sense primer, 5′-ATGCAGAACACGCAGCTATG-3′; antisense primer, 5′-GCTCCGTTCCATTTTCTTG-3′) and ILBP (sense primer, 5′-CCTCAGCAACTGGGAGAGTTTAT-3′; antisense primer, 5′-TTTTATTGGTGGGTTTGTAGCTC-3′) was performed with the LightCycler sequence detection system (Roche Molecular Biochemicals). Because SYBR Green was used for measurements of amplification-associated fluorescence, RT-PCR products were also analyzed on ethidium bromide-stained agarose gels to ensure that a single amplicon of the expected size was obtained. Villin amplification was used to account for variability in the initial quantities of cDNA and to account for the varying numbers of enterocytes in biopsy specimens (sense

TABLE 1. Characteristics of study participants

| Characteristic | All women | | Overweight women | | Normal weight women | |
|---|---|---|---|---|---|---|
| | Control | Gallstone carriers | Control | Gallstone carriers | Control | Gallstone carriers |
| Number | 24 | 17 | 8 | 9 | 16 | 8 |
| Age (years) | 57 ± 2 | 64 ± 3 | 55 ± 4 | 62 ± 5 | 59 ± 2 | 66 ± 4 |
| Body mass index (kg/m$^2$) | 24.0 ± 0.8 | 25.6 ± 0.8 | 27.5 ± 0.8 | 28.6 ± 0.7 | 22.0 ± 0.7 | 22.8 ± 0.4 |
| Triglyceride (mg/dl) (1–200)[a] | 151 ± 14 | 122 ± 13 | 167 ± 21 | 122 ± 19 | 128 ± 10 | 130 ± 18 |
| Cholesterol (mg/dl) (140–240)[a] | 187 ± 12 | 197 ± 7 | 187 ± 18 | 204 ± 12 | 190 ± 15 | 197 ± 9 |
| Bilirubin (mg/dl) (0.2–1.4)[a] | 0.53 ± 0.05 | 0.73 ± 0.11 | 0.49 ± 0.05 | 0.68 ± 0.15 | 0.59 ± 0.06 | 0.73 ± 0.11 |

Values are given as means ± SEM.
[a] Normal range.

primer, 5′-AGCCAGATCACTGCTGAGGT-3′; antisense primer, 5′-TGGACAGGTGTTCCTCCTTC-3′). The relative quantity for any given transcript was calculated using the second derivative maximum method (LightCycler software 3.5) according to the manufacturer's instructions. Individual quantities of each sample were determined in triplicate.

### Western blot analysis

Antibodies used for the detection of human ASBT and human ILBP were kind gifts of P. Dawson and W. Kramer, respectively. Primary antibodies for the detection of FXR, HNF1α, and PPARα were purchased from Santa Cruz Biotechnology. Protein concentration was determined using a commercial kit (Bio-Rad). Protein extracted from biopsies obtained from 10 different controls was pooled and used as a standard on each blot (see supplementary Fig. 1). Samples of 6–12 μg of total protein and serial dilutions of standard protein (5, 10, and 20 μg) were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked in 5% nonfat milk in TBS-Tween 20 and probed with dilutions of primary antibody in 5% nonfat milk, TBS-Tween 20. After being washed three times, immunoblots were incubated with peroxidase-conjugated anti-rabbit IgG and anti-chicken IgG (both Dianova) and exposed to a chemiluminescent reagent (SuperSignal*T* West Dura; Pierce). Bands were photographed (Camera LAS 1000; Fuji), and immunoquantitation was accomplished by densitometric analysis using the software AIDA (Raytest). Furthermore, to account for variability in the amounts of enterocytes in biopsy specimens, villin contents of all samples were determined using a primary antibody against human villin (Sigma) and a secondary peroxidase-conjugated anti-mouse IgG (Oncogene). All measurements were carried out in duplicate.

### Immunhistochemistry

Formalin-fixed biopsies were embedded in paraffin and cut into 3 μm sections. The immunohistochemical localization the ASBT was performed using the EnVision technique (EnVision™ Detection Kit; DAKO), according to the manufacturer's instructions. Briefly, tissue sections were deparaffinized in xylene and rehydrated in a series of ethanol solutions (100–70%). Endogenous peroxidase activity was blocked by the addition of 0.9% $H_2O_2$ in methanol for 30 min, and sections were washed in Tris saline (pH 7.6), microwaved, and again washed with Tris saline (0.15 M). Slides were then incubated with the polyclonal rabbit anti-ASBT primary antibody (overnight), followed by a 30 min incubation with a horseradish peroxidase-labeled polymer secondary antibody (dextran backbone coupled with peroxidase and a multifunctional secondary antibody; EnVision™). To stop the reaction, sections were rinsed with tap water. Slides were then counterstained with hemalaun for 15 s.

For the immunochemical detection of ILBP, the EnVision technique described above was modified slightly, as the primary antibody used for the detection of ILBP was raised in chicken. Therefore, before incubating with EnVision solution and the secondary antibody, dextran-enzyme complex sections were incubated with an unconjungated secondary rabbit anti-chicken antibody.

To ensure the specificity of staining of ASBT, the following controls were used: *1*) omission of primary antibody; *2*) omission of horseradish peroxidase-labeled secondary antibody; *3*) omission of EnVision complex; *4*) omission of primary and secondary antibody as well as EnVision complex; *5*) omission of primary and secondary antibody; *6*) omission of secondary antibody and EnVision complex; and *7*) positive controls using tissue sections of the terminal ileum and antibodies against CK20. All of these controls were used to ensure the specificity of staining of ILBP as well.

### Statistical analysis

Results are presented as means ± SEM. The statistical comparison between groups was performed using the Mann-Whitney *U*-test. Correlation was tested by calculating Spearman's rank-order correlation coefficient. $P < 0.05$ was considered statistically significant.

## RESULTS

### ASBT: protein levels and mRNA expression in terminal ileum

**Figure 1** and supplementary Fig. 2 summarize the results of protein and mRNA measurements performed in biopsy specimens obtained from gallstone carriers and controls. Western blot analyses demonstrated that ASBT was present as a major immunoreactive band representing the 48 kDa monomer (Fig. 1B, lane 1 depicts a representative Western blot for the control). Infrequently, a 93 kDa band representing the dimeric form of the protein was visible but too faint to be included in the densitometic estimations. ASBT protein levels of women with gallstones and controls did not differ; however, ASBT differed considerably among subjects. Therefore, study participants were further subgrouped by weight into normal weight (body mass index $< 25$) and overweight (body mass index $> 25$). Protein levels of ASBT did not differ between overweight gallstone carriers and controls. However, when ASBT protein levels of normal weight female gallstone carriers (n = 8) and controls (n = 16) were compared, ASBT levels were found to be significantly lower in patients with gallstones than in the controls. Specifically, mean ASBT protein levels of normal weight gallstone carriers were ∼48% lower than those of controls (Fig. 1A, B). In addition, ASBT was analyzed by immunohistochemical methods in four normal weight gallstone carriers and five controls. Staining was restricted to the apical membrane of enterocytes. Figure 1C shows representative photomicrographs of ASBT protein staining in paraffin-embedded tissue of normal weight gallstone carriers and controls.

Real-time RT-PCR measurements of ASBT expression also revealed significant differences between groups (Fig. 1D). Specifically, ASBT mRNA levels of gallstone carriers were ∼45% lower than those of controls when comparing all patients and controls, regardless of body weight. Subjects were again subgrouped by weight as described above. ASBT mRNA levels did not differ significantly between overweight gallstone carriers and controls. However, mRNA expression of ASBT was ∼65% lower in normal weight gallstone carriers than in controls.

Furthermore, ASBT protein and mRNA levels were correlated. Regardless of body weight, ASBT protein and mRNA levels were correlated significantly in a positive manner. Specifically, when correlating ASBT mRNA and protein levels of all study participants, $R = 0.41$, with a level of statistical significance of $P < 0.01$.

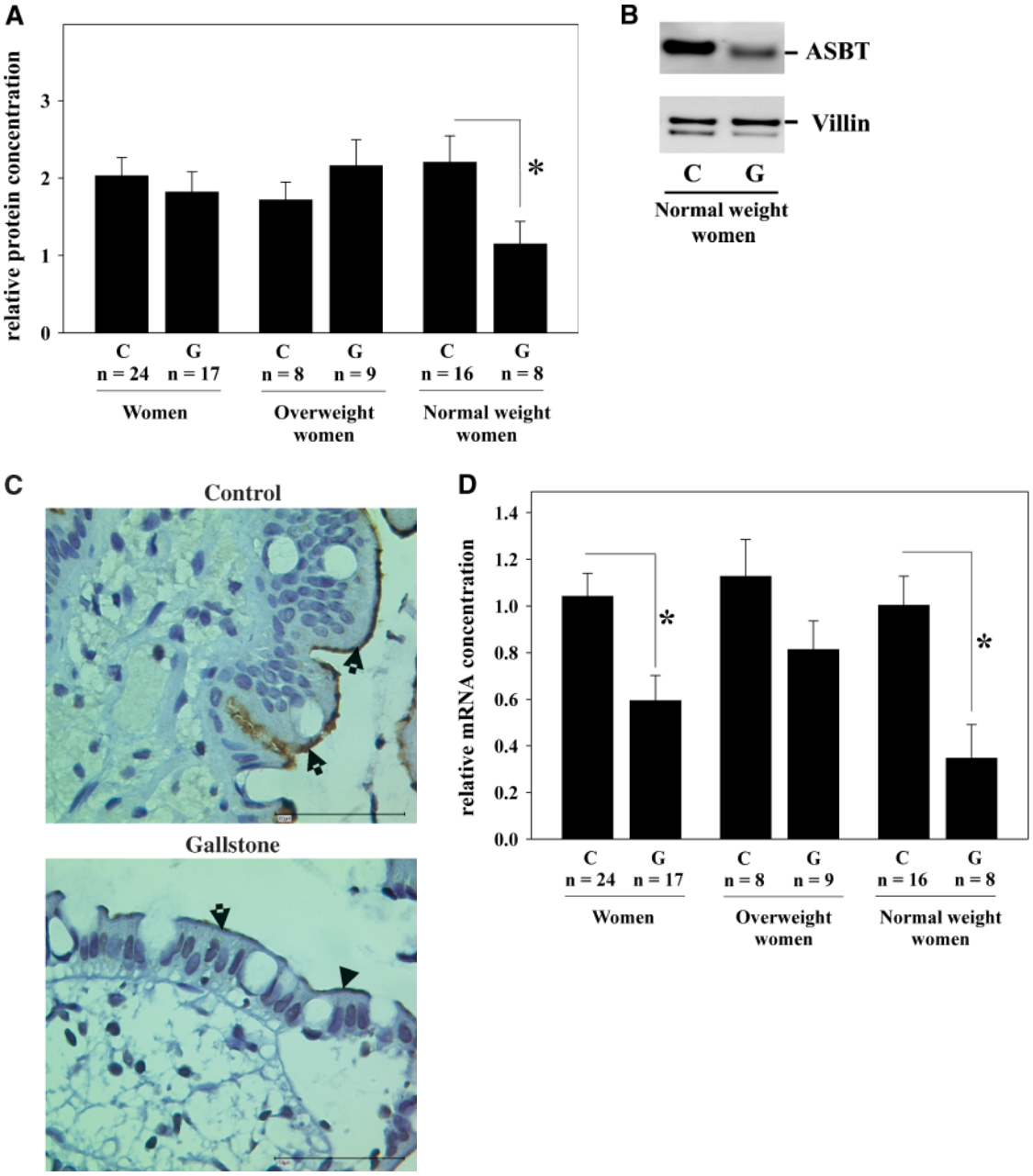


**Fig. 1.** Apical sodium bile acid transporter (ASBT) protein levels and mRNA expression in ileal mucosa biopsies of women. A: Quantitative analysis of ASBT protein levels of gallstone carriers (G) and controls (C). Protein levels of villin were determined to normalize to the amount of enterocytes in biopsies. Data are means ± SEM. * $P < 0.05$ compared with controls. B, C: Representative Western blot (B) and immunostaining (C) of ASBT in terminal ileum biopsy specimens of female normal weight gallstone carriers and controls. D: Relative ASBT mRNA expression, normalized to villin. Expression of ASBT and villin was measured by real-time RT-PCR. Data are means ± SEM. * $P <$ 0.05 compared with controls.

### ILBP: protein levels and mRNA expression in terminal ileum

Because the results of in vitro and in vivo studies indicate that the intracellular transport of bile acids in the intestine is mediated by ILBP, ILBP protein levels and mRNA expression were determined. Results are summarized in **Fig. 2** and in supplementary **Fig. 3**. Similar to ASBT, protein levels did not differ between gallstone carriers compared with controls when all gallstone carriers and controls were compared. Again, women were further subgrouped by weight. No differences were found between overweight gallstone carriers and controls. However, when ILBP protein levels of normal weight female controls and gallstone carriers were compared, ILPB protein levels of the latter were found to be significantly lower compared with controls. Specifically, ILPB protein concentration was ~67% lower in normal weight women with gallstones than in controls. To further verify these results, ILBP protein was localized in paraffin-embedded biopsy specimens obtained from four of these normal weight gallstone

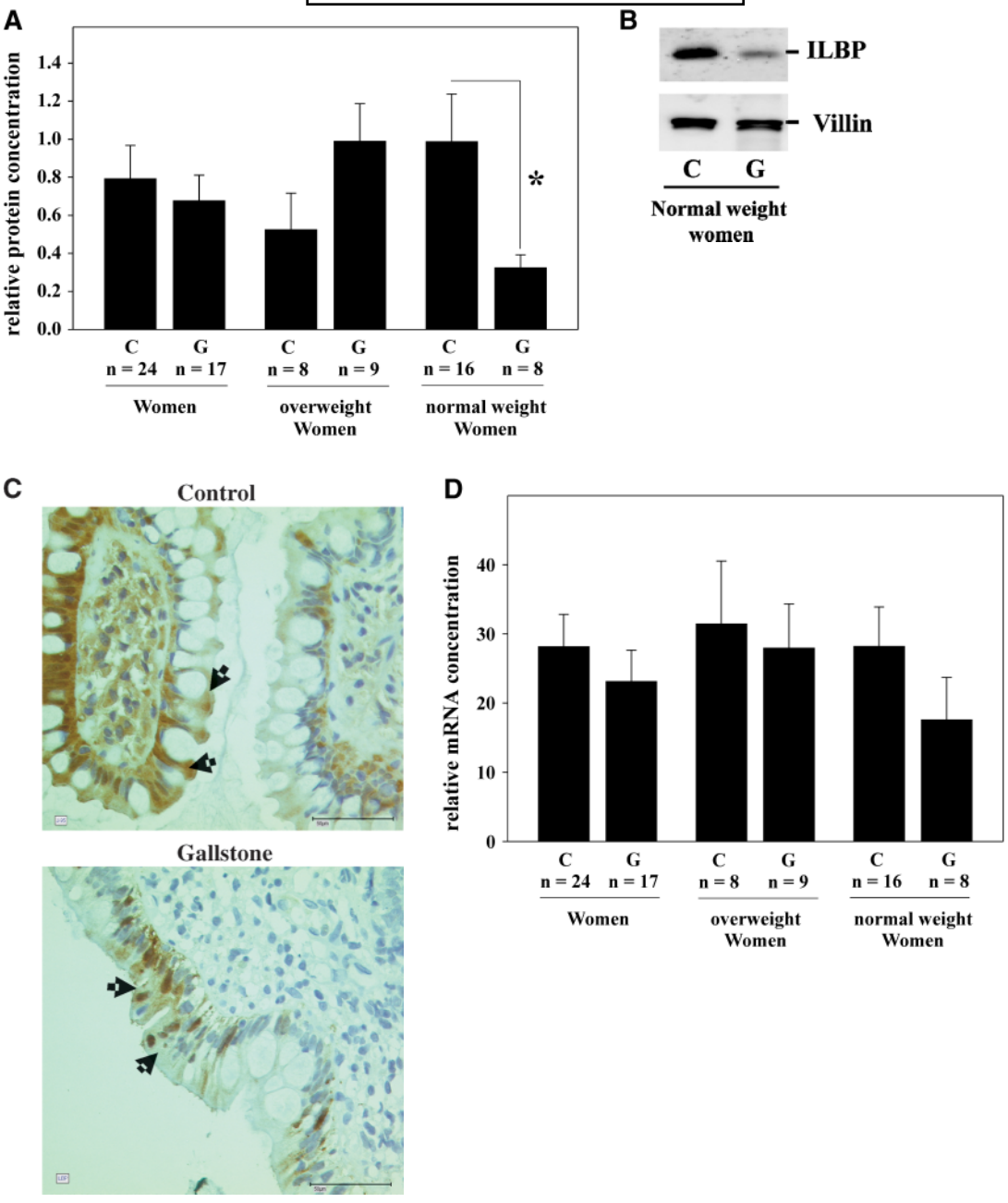


**Fig. 2.** Ileal lipid binding protein (ILBP) protein levels and mRNA expression in ileal mucosa biopsies of women. A: Quantitative analysis of ILBP protein levels of gallstone carriers (G) and controls (C). Protein levels of villin were determined to normalize to the amount of enterocytes in biopsies. Data are means ± SEM. * $P < 0.05$ compared with controls. B, C: Representative Western blot (B) and immunostaining (C) of ILBP in female normal weight gallstone carriers and controls. D: Relative mRNA expression of ILBP in gallstone carriers and controls. Expression of ILBP was measured by real-time RT-PCR and normalized to villin. Data are means ± SEM. * $P < 0.05$ compared with controls.

carriers and five controls. The bile acid-cotransporting protein was restricted to the cytoplasm of ileal enterocytes. Figure 2C shows representative photomicrographs of a gallstone carrier and a control.

Similar to protein levels, mRNA expression of ILBP did not differ significantly between groups comprising all individuals with and without gallstones. However, ILBP mRNA expression varied considerably among individuals (Fig. 2D). Subjects were again subgrouped according to their body weight. However, regardless of their body weight, no significant differences were found between groups when mRNA expression was compared. A trend toward a difference was found between ILBP mRNA expression levels of controls and normal weight gallstone carriers: mRNA levels of the latter were ~37% lower than those of controls ($P = 0.065$).

Similar to ASBT, ILPB protein and mRNA levels were significantly positively correlated. Specifically, when correlating ILBP mRNA and protein levels of all study participants regardless of gender and body weight, R = 0.53, with the level of statistical significance being $P < 0.001$.

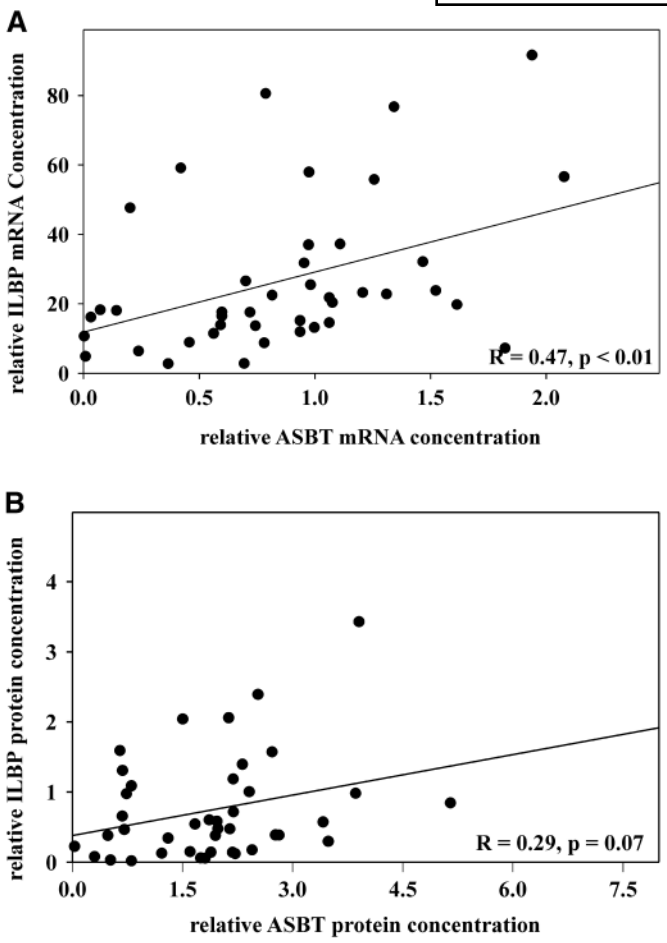


**Fig. 3.** Correlation of ASBT and ILBP mRNA expression and protein levels in ileal biopsy specimens of female gallstone carriers and controls. RNA expression (A) and protein levels (B) were normalized to villin.

### Correlation of ASBT and ILBP

To determine whether the expression of the apical membrane-bound ASBT and the cytosolic ILBP were linked, results of Western blot analysis and real-time RT-PCR for all subjects were correlated (Fig. 3). This analysis revealed a positive correlation of mRNA levels of ASBT and ILBP ($r = 0.47$, $P < 0.01$). Similar results were found when protein expression levels of the two transporters were correlated; however, the correlation did not quite reach significance ($R = 0.29$, $P = 0.07$).

### Ileal levels of nuclear receptors in female normal weight gallstone carriers and controls

Because the results of several studies with knockout animals and promoter constructs of bile acid transporters suggest that several nuclear receptors (e.g., HNF1α, FXR, and PPARα) are crucial for the maintenance of ASBT and ILBP expression in the intestine (21, 22, 24), protein levels of HNF1α, FXR, and PPARα were evaluated in biopsies of normal weight female gallstone carriers and controls. **Figure 4** depicts the quantitative analysis and representative Western blots.

HNF1α protein levels of normal weight gallstone carriers were significantly lower than those of controls. Specifically, HNF1α levels were ~70% lower in gallstone carriers compared with controls. Similar results were also found when comparing FXR protein levels between groups. FXR levels of female normal weight gallstone carriers were ~68% lower compared with those of controls. No differences were found when comparing PPARα protein levels between groups.

Because a HNF1α binding site has also been identified in the promoter of FXR (21), suggesting that HNF1α may regulate the expression FXR, protein levels of the two nuclear receptors were correlated. Indeed, protein levels of HNF1α correlated significantly in a positive manner with those of FXR ($R = 0.52$, $P < 0.015$) (Fig. 4C).

### Correlation of bile acid transporters with the nuclear receptors HNF1α and FXR

To further investigate whether HNF1α and FXR might influence the expression of ASBT and ILBP in the terminal ileum and might play a role in the differences found among female normal weight gallstone carriers and controls, protein and RNA levels of the two ileal transporters were correlated with protein levels of HNF1α and FXR. ASBT mRNA expression and HNF1α protein levels were significantly and positively correlated ($R = 0.45$, $P = 0.04$); however, when correlating ASBT and HNF1α protein levels, only a trend toward a positive correlation was found ($R = 0.39$, $P = 0.08$). No correlations were found for HNF1α and ILBP mRNA and protein levels. Similarly, no correlations were found between FXR protein levels and ILBP or between ASBT mRNA and protein concentration.

## DISCUSSION

### Ileal levels of bile acid transporters are diminished in normal weight female gallstone carriers

Cholesterol supersaturation of gallbladder bile has been identified as a key factor in cholelithiasis. Evidence is mounting that the intestine may play a critical role in the development of gallstones. In this study, the hypothesis was tested that diminished levels of the intestinal bile acid transporter ASBT and the bile acid-cotransporting protein ILBP contribute to the development of gallstones. Indeed, levels of both transporters were found to be significantly lower in the terminal ileum of normal weight female gallstone carriers relative to controls. However, diminished levels of ASBT and ILBP were only found in this subpopulation of gallstone carriers, suggesting that the underlying mechanism leading to the development of cholelithiasis may vary depending on the weight of female patients. Thus, in normal weight women with gallstones, the pool of circulating bile acids may be reduced by an impaired ileal transport and an increased loss of primary bile acids into the colon, where they are dehydroxylated and deconjugated by fecal bacteria before partial reabsorption. In support of this hypothesis, Shoda et al. (5) found a diminished uptake of bile acids in gallstone

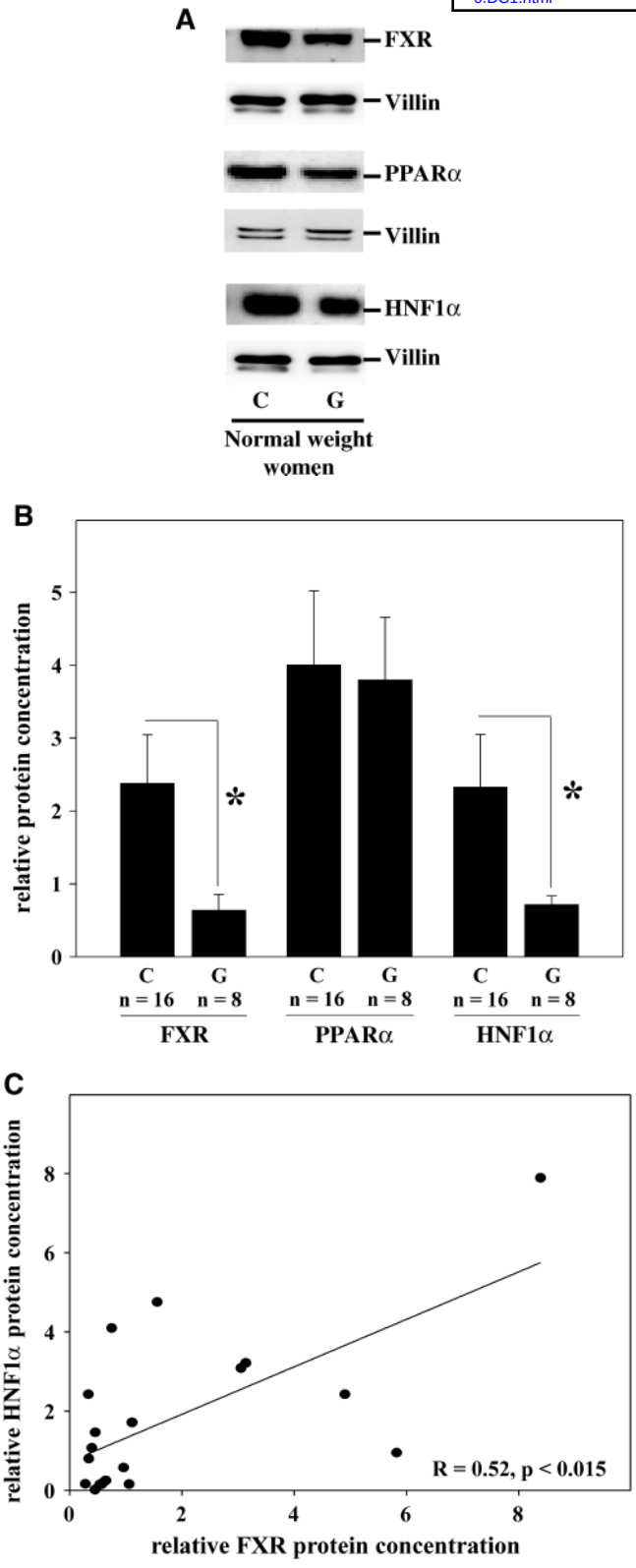


**Fig. 4.** Protein levels of the ileal nuclear receptors hepatic nuclear factor 1α (HNF1α), peroxisome proliferator-activated receptor α (PPARα), and farnesoid X receptor (FXR). A, B: Representative Western blot (A) and quantitative analysis (B) of nuclear protein levels in ileal mucosa of female normal weight gallstone carriers (G) and controls (C). Protein levels of HNF1α, PPARα, and FXR were normalized to villin. Data are means ± SEM. * $P < 0.05$. C: Correlation of HNF1α and FXR protein levels. Protein levels were normalized to villin.

patients, and total fecal bile acid excretion in patients with gallstones was reported to be significantly higher compared with controls (27). Furthermore, it has also been shown that intestinal 7-α-dehydroxylation of cholic acid is enhanced in gallstone carriers (28–30), possibly in relation to the increased number of Gram-positive anaerobic bacteria in feces obtained from gallstone patients (30). It also has been shown that hypersecretion of cholesterol is highly correlated with increased levels of deoxycholic acid in bile (5).

The mechanism of deoxycholic acid-induced cholesterol supersaturation is probably complex. Several studies in normal weight gallstone carriers indicate that bile acid pool size is diminished in patients with cholesterol gallstones (5, 31, 32). However, bile acid synthesis was found to be unchanged or even increased (5, 31, 32), with the activity of the cholesterol 7-α-hydroxylase activity not differing significantly between gallstone patients and controls (33). Based on animal studies, it was postulated that hydrophobic bile acids are particularly strong inhibitors of bile acid synthesis (34–36). Bile acid feedback regulation in humans has also been established (37), although Hillebrant et al. (38) found no effect of deoxycholic acid treatment on hepatic cholesterol 7-α-hydroxylase in patients with cholesterol gallstones. Furthermore, the fractional catabolic rates of cholic and chenodeoxycholic acid were found to be increased in gallstone carriers compared with controls (32). Together, these studies lend further support to the hypothesis that the efficiency of intestinal bile acid absorption, rather than bile acid synthesis, is impaired in female normal weight patients with gallstones. It may be further speculated that in overweight gallstone carriers, different pathomechanisms (e.g., alterations of cholesterol synthesis and/or flux) may predominate (39).

### Do diminished levels of HNF1α and FXR contribute to the loss of ASBT and ILBP in normal weight gallstone carriers?

Information on the molecular regulation of ASBT and ILBP expression in the human intestine is limited. In animal studies with cholesterol 7-α-hydroxylase knockout mice as well as in bile acid feeding experiments in rats, expression of ILBP was found to be opposite to that of ASBT (23, 40). In this human study, levels of ASBT and ILBP mRNA and protein levels of transporters were found to be significantly and positively correlated (Fig. 3). It may be that the differences between the results of others (23) and the present study are attributable to differences in the species studied (rodents vs. humans).

Results of in vitro and in vivo studies indicate that the expression of ASBT is regulated by bile acid (41, 42) but also by several nuclear transcription factors (e.g., PPARα and HNF1α) (21, 22). HNF1α seems to be of major importance for the induction of ASBT expression (21). Furthermore, a HNF1α binding site was also identified in the promotor of FXR (21), which has been shown to be involved in the induction of the expression of the bile acid-cotransporting protein ILBP (24, 43). Hence, HNF1α might also be involved in the regulation of ILBP expression. Furthermore, it was shown recently in a mouse model

of cholelithiasis that FXR-deficient mice display a significantly increased cholesterol supersaturation and bile salt hydrophobicity index compared with wild-type mice (44). Indeed, in this study, protein levels of both HNF1α and FXR were found to be diminished to a similar extent as bile acid transporters in normal weight women. Furthermore, protein levels of HNF1α and FXR were correlated positively in these gallstone carriers and controls, lending further support to the hypothesis that HNF1α may be involved in the regulation of FXR. In addition, HNF1α levels correlated positively with ASBT expression levels, but no correlations were found between FXR and either of the two bile acid transporters. However, it has been suggested that FXR is not regulated solely at the level of transcription but rather by the bioavailability and binding of its ligands (e.g., bile acids) (24, 44). Whether correlations found in this study are correlative or mechanistic remains to be determined. Together, these results suggest that ASBT and ILBP expression may be regulated coordinately and that HNF1α might, at least in part, be a regulator of the enterohepatic circulation of bile acids in humans. Furthermore, alterations of HNF1α and FXR protein levels found in this study might also have implications for the expression of other transporters (e.g., canalicular bile acid transporter).

### Summary and conclusion

Together with the results of others (5, 27), this report provides initial evidence that a diminished level of bile salt transport proteins in the intestine might play a crucial role in the pathogenesis of gallstone formation in normal weight women. Furthermore, our results suggest that the mechanisms underlying the development of cholelithiasis may be gender- and weight-specific. Although future studies will be needed to explore the molecular mechanisms responsible, the results of the present study are compatible with the concept that the two nuclear transcription factors HNF1α and FXR might be important targets.

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