Upregulation of hepatic bile acid synthesis via fibroblast growth factor 19 is defective in gallstone disease but functional in overweight individuals

Olga Renner¹, Simone Harsch¹, Silke Matysik², Dieter Lütjohann³, Gerd Schmitz² and Eduard F Stange⁴

United European Gastroenterology Journal
2014, Vol. 2(3) 216–225
© Author(s) 2014
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/2050640614527938
ueg.sagepub.com

Abstract

Background: Fibroblast growth factor 19 (FGF19) is an enteric hormone regulating bile acid *de novo* synthesis by sensing ileal bile acid flux. However, the role of FGF19 in cholelithiasis has not yet been elucidated and therefore is investigated in the present study.

Methods: Total mRNA and protein were isolated from ileal biopsies and used for tissue expression analysis. FGF19, 7 α -hydroxycholesterol (7 α -OH-Chol), 27-hydroxycholesterol (27-OH-Chol), and different bile acids were determined in the blood samples.

Results: FGF19 serum levels did not differ between gallstone carriers and controls but were significantly decreased in the overweight individuals (-32% , $p = 0.0002$), irrespective of gallstone status (normalweight to overweight controls -29% , $p = 0.0017$; normalweight to overweight gallstone carriers -44% , $p = 0.0338$), and correlated inversely with bodyweight ($p < 0.0001$, $\rho = -0.3317$). Compared to non-overweight controls, apical sodium-dependent bile acid transporter expression was significantly diminished in the non-overweight gallstone carriers (-42% , $P_{\text{mRNA}} = 0.0393$; -52% , $p_{\text{protein}} = 0.0169$) as well as in the overweight controls (-24% , $P_{\text{mRNA}} = 0.0148$; -43% , $p_{\text{protein}} = 0.0017$). FGF19 expression varied widely and was similar in all groups. A significant negative correlation was noted between 7 α -OH-Chol, 27-OH-Chol, and FGF19 serum levels ($p < 0.01$; $\rho_{7\alpha\text{-OH-Chol}} = -0.2155$; $\rho_{27\text{-OH-Chol}} = -0.2144$) in obesity.

Conclusion: Upregulation of hepatic bile acid synthesis via FGF 19 is defective in gallstone disease but functional in overweight individuals.

Keywords

Bile acid transport/absorption, bodyweight, enteric hormone, expression, intestine

Received: 14 November 2013; accepted: 12 February 2014

Introduction

Fibroblast growth factor 19 (FGF19) is a humoral factor with several regulatory functions including metabolic rate,¹ bile acid homeostasis,² and gallbladder filling.³ FGF19 transcripts are found in the small intestine, brain, cartilage, skin, retina, and gall bladder but very low in the normal liver.^{4,5} Initial interest in FGF19 as a metabolic regulator was prompted by the phenotype of FGF15 (the mouse homologue of FGF19) of transgenic mice where decreased obesity, increased energy expenditure, reduced liver

¹Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and University of Tuebingen, Stuttgart, Germany

²University Hospital Regensburg, Regensburg, Germany

³Institute for Clinical Chemistry and Clinical Pharmacology, Laboratory for Special Lipid Diagnostics, University Hospital Bonn, Bonn, Germany

⁴Department of Gastroenterology, Robert Bosch Hospital, Stuttgart, Germany

Corresponding author:

Olga Renner, Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and University of Tuebingen, Auerbachstr. 112, 70376 Stuttgart, Germany.

Email: olga.renner@ikp-stuttgart.de

triglycerides, elevated fatty acid oxidation, and improved insulin sensitivity were observed.¹ Administration of human recombinant FGF19 increased the metabolic rate and reversed dietary and leptin-deficient diabetes in mice.⁶ Besides controlling gall bladder refilling by relaxation of gall bladder smooth muscle,³ FGF19 is also a key player involved in the regulation of bile acid homeostasis.⁷ Using genetically modified mice it was shown that intestinal farnesoid X receptor (FXR)/FGF15 has a predominant role in the inhibition of bile acid biosynthesis.⁸ Intestinal FGF19 expression is stimulated by bile acid mediated activation of FXR and the enterokine is secreted into the enterohepatic circulation. In addition to FXR, intestinal expression of FGF19 is regulated by several nuclear receptors such as pregnane X receptor (PXR) together with its heterodimer partner retinoid X receptor (RXR).^{9,10} In the liver, a stabilizing cofactor β Klotho is necessary for binding of FGF19 to its hepatic receptor fibroblast growth factor receptor 4 (FGFR4), creating a complex signalling through the c-Jun kinase cascade,⁴ ultimately leading to suppression of cholesterol 7 α -hydroxylase/cytochrome P450 7A1 (CYP7A1) expression.² Circulating FGF19 exhibits a diurnal rhythm controlled by transintestinal bile acid flux.¹¹ Diminished serum FGF19 and increased bile acid synthesis were found in patients characterized by intestinal bile acid malabsorption (primary (idiopathic) bile acid malabsorption, inflammatory bowel disease, or ileal resection).^{12,13} Fasting FGF19 levels were shown to exhibit differences between cases and controls in patients with cholestasis,¹⁴ obesity,¹⁵ and bile acid diarrhoea.¹⁶

In spite of its important role in energy and bile acid metabolism, the function of FGF19 in gallstone disease has not been studied. In non-overweight gallstone disease bile acid synthesis was paradoxically unchanged despite a reduction of the bile acid pool size and an increased fractional turnover.^{17,18} The latter may be related to the impaired absorption of bile acids in gallstone disease.¹⁹ Most likely, the molecular basis of bile acid malabsorption in gallstone disease is the diminished ileal expression of apical sodium-dependent bile acid transporter (ASBT), ileal lipid binding protein (ILBP), and organic solute transporter α /beta (OST α / β).^{20,21}

The lack of induction of hepatic bile acid synthesis regardless of ileal loss may be a consequence of defective FGF19 response in the ileum. In the present study, we therefore analysed serum levels and ileal expression of FGF19 as well as bile acid metabolite levels, ileal bile acid transporters, and relevant transcription factors in a cohort of gallstone carriers and controls.

Patients and methods

Ethics statement

Informed consent was obtained from each patient included in the study and the study protocol confirms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee (ethics committee of the University Hospital of Tuebingen and University of Tuebingen).

Subjects and materials

The selection as well as including and excluding criteria of study subjects were applied as previously described²² and characteristics of the analysed cohort are summarized in Supplementary Table S1 (available online). Ileal mucosal forceps biopsies and fasting blood samples were collected during routine colonoscopy from a total of 168 individuals, comprising 134 healthy controls and 34 individuals with asymptomatic gallstones. As weight also depends on age^{23–27} and the mean age was over 50 years in the analysed cohort, the normal-weight group was defined as body mass index (BMI) $\leq 25.4 \text{ kg/m}^2$ (as a mathematical value of 25) and individuals with BMI $> 25.4 \text{ kg/m}^2$ were regarded as overweight.

For tissue gene expression analysis in an ileal mucosal biopsy, total mRNA and protein were isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. The quality and quantity controls of isolated material were assessed as reported.²² Blood samples (3–5 ml) were used for determination of total serum cholesterol and triglyceride levels as well as for bile acid metabolism profiling (measurements of bile acid synthesis markers, serum bile acid, and FGF19 concentrations). Serum triglycerides and cholesterol levels were analysed by standard clinical tests.

Determination of serum FGF19

FGF19 levels were determined using a sandwich ELISA kit (BioVendor, Czech Republic). All cooled serum samples were diluted 1:3 and analysed following the manufacturer's instructions.

Real-time quantitative reverse-transcription PCR

RT-PCR was performed with LightCycler sequence detection system (Roche Diagnostics) as reported previously.^{21,22,28} Primer sequences used for amplifications and specific PCR conditions are listed in Supplementary Table S2. At the end of the cycling program, a dissociation curve was calculated. The quantity for any given transcript was calculated using the second

derivative maximum method. Gene-specific plasmid constructs were generated as reported previously^{21,29} and served as positive control templates. All measurements were carried out in duplicate.

Western blot analysis

The antibody used for the detection of human ASBT protein was a kind gift of Prof P Dawson (Wake Forest University, Winston-Salem, USA). Protein determination was performed as previously described.²⁸

Measurement of bile acid synthesis markers

Sample preparation for determination of 7 α -hydroxycholesterol (7 α -OH-Chol) and 27-hydroxycholesterol (27-OH-Chol) as well as consecutive measurement by GC-MS was performed as previously described³⁰ with slight modifications. Details are described in the supplementary file.

Measurement of bile acids in plasma

Sample preparation and methodology for bile acid LC-MS/MS analysis is described elsewhere.³¹ Fifteen bile acid species were quantified: cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), and their glycine (G) and taurine (T) conjugated derivatives.

Statistics

For statistical analysis GraphPad Prism 5 was used (GraphPad Software, San Diego, CA, USA). Clinical characteristics of study participants and all data are presented as mean $\pm$ standard error of the mean. Differences between groups were investigated using the Mann–Whitney U-test. All correlations between variables were analysed with Spearman's rank test. All statistical tests were two-tailed and a p -value <0.05 was considered as statistically significant.

Results

Serum FGF19

In the total population, there was no difference between serum FGF19 concentration in gallstone carriers and controls ($p=0.4000$; Figure 1A). Notably, fasting FGF19 serum levels exhibited a significant reduction by -32% in overweight individuals ($p=0.0002$; Figure 1B). Also after stratification into weight-specific subgroups, FGF19 plasma concentrations were comparable between gallstone subjects and controls

Figure 1. Circulating levels of FGF19 in gallstone patients and controls in overnight fasting individuals according to presence of gallstone (A), weight (B), and presence of gallstone with weight (C).

Values are mean $\pm$ SEM. Mann–Whitney U-test, $p < 0.05$. BMI, body mass index; C, control; GS, gallstone carrier; NW, normal-weight; OW, overweight. (A) C, $n=129$; GS, $n=333$. (B) NW, BMI ≤ 25 kg/m², $n=81$; OW, BMI > 25 kg/m², $n=81$. (C) NW: C, $n=70$, GS, $n=11$; OW: C, $n=59$, GS, $n=22$.

(Figure 1C) both in the normalweight and the overweight groups. However, overweight persons exhibited significantly lower FGF19 serum levels than non-overweight individuals independent of gallstone status (normalweight to overweight controls -29% , $p=0.0017$; normalweight to overweight gallstone carriers -44% , $p=0.0338$). Moreover, FGF19 plasma levels correlated inversely with BMI ($p<0.0001$ $\rho=-0.3317$). FGF19 serum levels were similar between overweight ($\text{BMI}>25.4\text{ kg/m}^2$) and obese ($\text{BMI}>30\text{ kg/m}^2$) individuals (data not shown).

Ileal expression of FGF19, bile acid transporters, and relevant transcription factors

Overall, there was no significant difference in FGF19 expression between gallstone carriers and controls (Figure 2A). As in previous investigations,³² the wide interindividual variations of basal FGF19 mRNA expression rendered statistical comparisons difficult.

Next, the expression of ileal bile acid transporters was quantified. In essence, we confirmed our prior finding of diminished bile acid transporters expression in the intestine of female gallstone patients,^{20,21} now irrespective of gender in a composite cohort of female and male gallstone carriers. The expression of all intestinal bile acid transporters was distinctly reduced in non-overweight gallstone carriers (ASBT -42% , $p=0.0393$, Figure 2B; ILBP -74% , $p=0.0046$, Figure 2C; OST α -34% $p=0.1023$, Figure 2D; OST β -52% $p=0.0378$, Figure 2H). Remarkably, overweight subjects exhibited diminished bile acid transporter levels in comparison with non-overweight controls (ASBT -24% , $p=0.0148$; ILBP -47% , $p=0.0449$; OST α -21% , $p=0.0852$; OST β -22% , $p=0.2342$). At the protein level, ASBT expression was reduced by -52% in non-overweight gallstone carriers compared to controls ($p=0.0169$) and by -43% in overweight control individuals compared to normalweight controls ($p=0.0017$). The intestinal expression of the ASBT transporter on protein levels did not differ between overweight controls and the gallstone group ($p=0.7517$; Supplementary Figure S1).

There were significant positive correlation coefficients between the transporters (ASBT/ILBP $\rho=0.72$, ASBT/OST α $\rho=0.71$, ASBT/OST β $\rho=0.68$, ILBP/OST α $\rho=0.82$, ILBP/OST β $\rho=0.70$, OST α /OST β $\rho=0.77$; $n=117$, $p<0.0001$). In contrast, the correlation coefficients for intestinal FGF19 mRNA expression and bile acid transporters were low (ASBT $\rho=0.22$, $p=0.0153$; ILBP $\rho=0.44$, $p<0.0001$; OST α $\rho=0.41$, $p<0.0001$; OST β $\rho=0.35$, $p=0.0002$). FXR, PXR, and RXR mRNA expression does not differ significantly between gallstone carriers and controls (Figure 2E–G). No correlation was found between

intestinal FXR and FGF19 expression ($\rho=0.03$, ns). There was moderate association between mRNA expression of FGF19, PXR, and RXR (FGF19/PXR $\rho=0.35$, $p<0.0001$; FGF19/RXR $\rho=0.23$, $p=0.0156$).

Markers of bile acid synthesis

Furthermore, markers of hepatic neutral/classic (7 α -OH-Chol) and acidic/alternative (27-OH-Chol) bile acid synthesis pathways were analysed and displayed as ratio of oxysterol to plasma total cholesterol level (Figure 3). In line with observations from serum FGF19 levels, both markers of bile acid synthesis were comparable between controls and gallstone carriers in the total group (Figure 3A and B), but significantly increased in overweight individuals irrespective of gallstones (7 α -OH-Chol $+20\%$, $p=0.0055$; 27-OH-Chol $+12\%$, $p=0.0403$; Figure 3C and D). Moreover, levels of the bile acid precursors were not significantly different between gallstone carriers and controls in the weight-specific subgroups (Figure 3E and F). Therefore, 7 α -OH-Chol- and 27-OH-Chol-mediated bile acid synthesis ratios were higher in overweight individuals than in normalweight persons ($+19\%$ $p=0.0093$; $+12\%$ $p=0.0806$). Besides, intermediates of bile acid synthesis correlated inversely with serum FGF19 levels (7 α -OH-Chol $\rho=-0.2155$, $p<0.01$; 27-OH-Chol $\rho=-0.2144$, $p<0.01$). Finally, ileal bile acid transporter as well as FGF19 expression and the markers of hepatic *de novo* bile acid synthesis revealed no significant association (data not shown).

Plasma bile acid concentrations

To investigate the influence of reduced bile acid reabsorption in the intestine on serum levels, the concentrations of different bile acids were measured. As shown in Figure 4, the total amount of bile acids in plasma is significantly diminished (-74% , $p=0.0374$) in non-overweight gallstone carriers compared to relevant controls. This reduction was most pronounced for primary bile acids in plasma (-75% , $p=0.0334$), secondary bile acids in serum of normalweight gallstone carriers were not significantly reduced. In overweight gallstone carriers, the total bile acid concentration was lower by about -33% compared to overweight controls, but this effect also did not reach statistical significance ($p=0.6215$). Notably, bile acid concentration in overweight persons was reduced up to -21% compared to non-overweight healthy individuals ($p=0.3235$).

Table 1 shows the data of all analysed bile acid species including their free form as well as glycine or taurine conjugates. The calculated data exhibit the uniform pattern of reduction for every bile acid type in the

Figure 2. Ileal expression of FGF19 (A), apical sodium-dependent bile acid transporter (ASBT), (B), ileal lipid-binding protein (C), organic solute transporter α (D), FXR (E), PXR (F), RXR (G), and organic solute transporter β (H) in gallstone patients and controls. Quantification of mRNA was performed in human ileal mucosal biopsies of gallstone carriers and controls and is given as copy number. All experiments were carried in duplicate. Values are mean $\pm$ SEM. Mann-Whitney U-test, $p < 0.05$. BMI, body mass index; C, control; GS, gallstone carrier; NW, normalweight; OW, overweight. NW: C, $n = 57$; GS, $n = 9$; OW: C, $n = 50$, GS, $n = 21$.

Figure 3. Circulating markers of the hepatic *de novo* bile acid synthesis in gallstone patients and controls according to presence of gallstone (A, B), weight (C, D), and presence of gallstone with weight (E, F): 7α-hydroxycholesterol (A, C, E) and 27-hydroxycholesterol (B, D, F).

Serum concentrations are ratio oxysterol (ng/ml)/cholesterol (mg/dl)*100. Values are mean $\pm$ SEM, Mann-Whitney U-test, $p < 0.05$. BMI, body mass index; C, control; GS, gallstone carrier; NW, normalweight; OW, overweight. (A, B) C, $n = 90$; GS, $n = 29$. (C, D) NW, BMI ≤ 25 kg/m², $n = 60$; OW, BMI > 25 kg/m², $n = 59$. (E, F) NW: C, $n = 51$, GS, $n = 9$; OW: C, $n = 39$, GS, $n = 20$.

Figure 4. Comparison of plasma bile acid concentrations in gallstone patients and controls according to presence of gallstone with weight: total bile acids (A), primary bile acids (B), and secondary bile acids (C).

Values are mean $\pm$ SEM, Mann-Whitney U-test, $p < 0.05$. BMI, body mass index; C, control; GS, gallstone carrier; NW, normal-weight; OW, overweight. NW: C, $n = 74$, GS, $n = 11$; OW: C, $n = 59$, GS, $n = 22$.

gallstone group. As reported by Matysik et al.,³³ the glycine-conjugated primary bile acid GCDCA represented the highest plasma level, followed by TCDCA and CDCA. Remarkably, only TUDCA and CA were increased (TUDCA +20%, $p = 0.4200$; CA +180%, $p = 0.5500$) in overweight gallstone patients compared with respective controls, but without significance.

Discussion

In the present study, we examined the role of FGF19 in gallstone disease and excess weight, determining its circulating levels as well as ileal expression of FGF19, relevant transcription factors, bile acid transporters, and bile acid metabolite serum levels. The major novel finding suggests that FGF19 expression as well as concentrations in the blood do not adequately respond to the diminished ileal bile acid reabsorption in gallstone carriers and therefore fail to stimulate the bile acid synthesis in the liver. The additional non-gallstone control group of overweight individuals, where ileal bile acid reabsorption is also reduced and FGF19 concentrations are declined as expected (unlike gallstone patients), exactly showed the FGF19 response which would be expected in the case of adequate upregulation of bile acid synthesis. Accordingly, FGF19 levels are reduced and bile acid synthesis is enhanced in the overweight but not in the gallstone subjects.

The pathogenesis of gallstones is complex and is related to a large number of risk factors such as obesity, age, female gender, nutrition, and genetics. Most data point to a diminished intestinal bile acid absorption as well as pool size in gallstone patients,^{17,19} which should prompt a compensatory increase in synthesis. However, bile acid synthesis remains unaltered in non-overweight gallstone patients.^{17,18} Also, we found unchanged bile acid synthesis markers and remarkably a significant decrease of bile acids in plasma of normalweight gallstone carriers. Such a metabolic imbalance would explain the enhanced cholesterol lithogenicity due to a relative lack of bile acids in gallstone bile. This process apparently depends on ethnicity, as paradoxically in Hispanic gallstone carriers with unaltered ileal transporter expression and faecal excretion of bile acids, the level of CYP7A1 is increased.³⁴ To further clarify the factors involved in this dysregulation, we studied the recently identified major regulator of bile acid synthesis, FGF19.

Animal models provide evidence that stimulation of FXR-mediated intestinal FGF15 expression suppresses bile acid synthesis in the liver in a dominant way.⁷ Moreover, circulating intestinal FGF19 has a pronounced diurnal variation and modulates bile acid synthesis in men.¹¹ Also in several diseases FGF19 is an

Table 1. Serum bile acid profile in gallstone patients and controls

Bile acid type	Normalweight				Overweight			
	Controls (n = 74)	Gallstone carriers (n = 11)	Difference (%)	p-value	Controls (n = 59)	Gallstone carriers (n = 23)	Difference (%)	p-value
Primary								
TCA	0.200 ± 0.044	0.021 ± 0.006	90	0.017	0.174 ± 0.038	0.101 ± 0.033	42	0.47
GCA	0.444 ± 0.080	0.073 ± 0.021	84	0.017	0.375 ± 0.074	0.258 ± 0.077	31	0.47
CA	0.018 ± 0.007	0.005 ± 0.002	72	0.128	0.015 ± 0.004	0.041 ± 0.025	180	0.55
TCDC	0.809 ± 0.113	0.327 ± 0.049	60	0.068	0.562 ± 0.095	0.375 ± 0.070	33	0.49
GDC	1.894 ± 0.302	0.438 ± 0.138	77	0.034	1.387 ± 0.271	0.959 ± 0.224	31	1.00
CDCA	0.243 ± 0.050	0.042 ± 0.008	83	0.010	0.214 ± 0.040	0.162 ± 0.040	24	0.42
Secondary								
TDCA	0.122 ± 0.019	0.023 ± 0.009	81	0.042	0.127 ± 0.035	0.069 ± 0.030	46	0.66
GDCA	0.628 ± 0.110	0.170 ± 0.046	73	0.122	0.642 ± 0.171	0.348 ± 0.105	46	0.60
DCA	0.164 ± 0.061	0.039 ± 0.012	76	0.249	0.167 ± 0.044	0.112 ± 0.024	33	0.97
TLCA	0.010 ± 0.001	0.008 ± 0.004	20	0.400	0.011 ± 0.003	0.009 ± 0.003	16	0.80
GLCA	0.045 ± 0.007	0.032 ± 0.011	29	0.675	0.052 ± 0.009	0.038 ± 0.010	27	0.87
LCA	0.008 ± 0.002	0.003 ± 0.001	61	0.208	0.013 ± 0.003	0.008 ± 0.002	36	0.61
Tertiary								
TUDCA	0.027 ± 0.011	0.005 ± 0.003	81	0.061	0.010 ± 0.002	0.013 ± 0.004	20	0.42
GUDCA	0.296 ± 0.120	0.095 ± 0.050	68	0.074	0.136 ± 0.022	0.114 ± 0.025	16	0.59
UDCA	0.037 ± 0.006	0.005 ± 0.003	87	0.001	0.038 ± 0.007	0.025 ± 0.006	36	0.32

Data are mean concentration (μmol/l) ± SEM., p-value obtained from a nonparametric two-tailed Mann-Whitney U-test. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; G, glycine conjugates; LCA, lithocholic acid; T, taurine conjugates; UDCA, ursodeoxycholic acid.

important regulator. For example, to prevent the accumulation of toxic bile acids, the liver FGF19/FGFR4 signalling pathway was induced by the autocrine mechanism of bile acids³⁵ and high FGF19 plasma levels as well as increased FGF19 expression were detected in the liver of patients with extrahepatic cholestasis as a protective mechanism.¹⁴ Otherwise, reduced plasma FGF19 levels were reported for patients with impaired glucose tolerance, Type 2 diabetes mellitus and in bile acid malabsorption, non-alcoholic fatty liver disease, inflammatory bowel disease, or ileal resection and were associated with increased bile acid synthesis.^{12,13,15,36,37} Recently, in patients with idiopathic bile acid-induced diarrhoea, FGF19 and 7α-hydroxy-4-cholesten-3-one were shown to correlate negatively and FGF19 was proposed to serve as diagnostic marker for this disease.¹⁶ Furthermore, in diabetic patients undergoing Roux-en-Y gastric bypass, lower FGF19 levels were significantly correlated with increased hepatic expression of the CYP7A1 gene, modulating bile acid production.³⁸ In the present study, differences in FGF19 levels and bile acid synthesis markers were found between normal weight and overweight individuals but remarkably not between gallstone carriers and non-gallstone controls of similar weight. In the additional control group of overweight

individuals, ileal bile acid reabsorption is reduced and FGF19 concentrations declined as expected (unlike gallstone patients).

As in prior studies,³² ileal FGF19 expression, which is considered to be the source of FGF19 in the circulation, varied widely and did not correlate with serum levels (Supplementary Figure S2). These large inter-individual differences in FGF19 expression are also not explained by the expression of relevant transcription factors (FXR, PXR, and RXR). In addition, FGF19 levels may be affected by sequence variations in the gene, epigenetic as well as posttranslational factors, and variations in protein degradation. The lack of change in bile acid synthesis markers is in accordance with several studies. Moreover, the diminished ileal bile acid transporter expression observed here confirms our previous reports,^{20,21} now in non-overweight male patients (Supplementary Figure S3).

Cholesterol and bile acid synthesis are closely linked but differ among overweight and normalweight individuals. Obesity is characterized by an excessive biliary cholesterol, less increased bile acid secretion, and a high lithogenic index.³⁹ Bile acid synthesis is also elevated⁴⁰ and pool size is normal or enlarged in the obese^{39,41} without evidence of bile acid malabsorption.⁴² There is a loose but positive correlation between

weight and bile acid formation⁴³ and weight reduction is associated with a reduction in biliary bile acid output.³⁹ Therefore, the elevated bile acid synthesis contributes to the higher rates of cholesterol balance⁴¹ but is less than required to dissolve disproportionately elevated cholesterol in bile.⁴⁰ As FGF19 plays a central role in the suppression of bile acid synthesis and secretion,⁷ it was also suggested to participate in the regulation of cholesterol, lipoprotein, triglyceride, and glucose metabolism.^{1,6} In mice, high levels of FGF19 were related to low bodyweight¹ which is in agreement with previous data in humans^{15,44,45} and the observations in the present work.

In conclusion, bile acid transporter expression is low in normalweight gallstone patients and overweight non-gallstone controls, but adaptive upregulation of bile acid synthesis through FGF19 is observed only in the overweight non-gallstone group. This might explain biliary lithogenesis in normalweight gallstone carriers through lack of induction of bile acid synthesis to balance intestinal bile acid loss.

Acknowledgements

We are greatly indebted to Professor Dr Klaus von Bergmann for valuable initiation of bile acid precursor measurements of this study. We thank Dr Oliver Müller and the staff of the gastroenterological department and the endoscopy team for recruitment of study participants and collecting tissue biopsies. We also thank Dr Stephan Winter for competent statistical support; André Strohmeyer and Anja Kerksiek for excellent technical assistance.

Funding

This study was supported by the Robert Bosch Foundation (P4-1/03 and Innovationsfond 2011).

Conflict of interest

The authors declare that they have no competing interests.

References

- Tomlinson E, Fu L, John L, et al. Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology* 2002; 143: 1741–1747.
- Holt JA, Luo G, Billin AN, et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev* 2003; 17: 1581–1591.
- Choi M, Moschetta A, Bookout AL, et al. Identification of a hormonal basis for gallbladder filling. *Nat Med* 2006; 12: 1253–1255.
- Xie MH, Holcomb I, Deuel B, et al. FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4. *Cytokine* 1999; 11: 729–735.
- Zweers SJ, Booij KA, Komuta M, et al. The human gallbladder secretes fibroblast growth factor 19 into bile: towards defining the role of fibroblast growth factor 19 in the enterobiliary tract. *Hepatology* 2012; 55: 575–583.
- Fu L, John LM, Adams SH, et al. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology* 2004; 145: 2594–2603.
- Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005; 2: 217–225.
- Kong B, Wang L, Chiang JY, et al. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology* 2012; 56: 1034–1043.
- Kliwer SA and Willson TM. Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor. *J Lipid Res* 2002; 43: 359–364.
- Wistuba W, Gnewuch C, Liebisch G, et al. Lithocholic acid induction of the FGF19 promoter in intestinal cells is mediated by PXR. *World J Gastroenterol* 2007; 13: 4230–4235.
- Lundasen T, Galman C, Angelin B, et al. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J Intern Med* 2006; 260: 530–536.
- Walters JR, Tasleem AM, Omer OS, et al. A new mechanism for bile acid diarrhea: defective feedback inhibition of bile acid biosynthesis. *Clin Gastroenterol Hepatol* 2009; 7: 1189–1194.
- Lenicek M, Duricova D, Komarek V, et al. Bile acid malabsorption in inflammatory bowel disease: assessment by serum markers. *Inflamm Bowel Dis* 2011; 17: 1322–1327.
- Schaap FG, van der Gaag NA, Gouma DJ, et al. High expression of the bile salt-homeostatic hormone fibroblast growth factor 19 in the liver of patients with extrahepatic cholestasis. *Hepatology* 2009; 49: 1228–1235.
- Mraz M, Lacinova Z, Kavalkova P, et al. Serum concentrations of fibroblast growth factor 19 in patients with obesity and type 2 diabetes mellitus: the influence of acute hyperinsulinemia, very-low calorie diet and PPAR-alpha agonist treatment. *Physiol Res* 2011; 60: 627–636.
- Pattni SS, Brydon WG, Dew T, et al. Fibroblast growth factor 19 and 7alpha-hydroxy-4-cholesten-3-one in the diagnosis of patients with possible bile acid diarrhea. *Clin Transl Gastroenterol* 2012; 3: e18.
- Berr F, Pratschke E, Fischer S, et al. Disorders of bile acid metabolism in cholesterol gallstone disease. *J Clin Invest* 1992; 90: 859–868.
- Honda A, Yoshida T, Tanaka N, et al. Hepatic cholesterol and bile acid synthesis in Japanese patients with cholesterol gallstones. *Gastroenterol Jpn* 1993; 28: 406–414.
- Duane WC, Hartich LA, Bartman AE, et al. Diminished gene expression of ileal apical sodium bile acid transporter explains impaired absorption of bile acid in

- patients with hypertriglyceridemia. *J Lipid Res* 2000; 41: 1384–1389.
20. Berghheim I, Harsch S, Mueller O, et al. Apical sodium bile acid transporter and ileal lipid binding protein in gallstone carriers. *J Lipid Res* 2006; 47: 42–50.
 21. Renner O, Harsch S, Strohmeyer A, et al. Reduced ileal expression of OST α -OST β in non-obese gallstone disease. *J Lipid Res* 2008; 49: 2045–2054.
 22. Renner O, Lutjohann D, Richter D, et al. Role of the ABCG8 19H risk allele in cholesterol absorption and gallstone disease. *BMC Gastroenterol* 2013; 13: 30.
 23. Zamboni M, Mazzali G, Zoico E, et al. Health consequences of obesity in the elderly: a review of four unresolved questions. *Int J Obes (Lond)* 2005; 29: 1011–1029.
 24. Lechleitner M. Obesity and the metabolic syndrome in the elderly – a mini-review. *Gerontology* 2008; 54: 253–259.
 25. Kuczmarski RJ and Flegal KM. Criteria for definition of overweight in transition: background and recommendations for the United States. *Am J Clin Nutr* 2000; 72: 1074–1081.
 26. Hemmelmann C, Brose S, Vens M, et al. Percentiles of body mass index of 18–80-year-old German adults based on data from the Second National Nutrition Survey. *Dtsch Med Wochenschr* 2010; 135(17): 848–852.
 27. Babiarczyk B and Turbiarz A. Body Mass Index elderly people - reference ranges matter. *Prog Health Sci* 2012; 2: 58–67.
 28. Renner O, Harsch S, Schaeffeler E, et al. Mutation screening of apical sodium-dependent bile acid transporter (SLC10A2): novel haplotype block including six newly identified variants linked to reduced expression. *Hum Genet* 2009; 125: 381–391.
 29. Geick A, Eichelbaum M and Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 2001; 276: 14581–14587.
 30. Hahn C, Reichel C and von Bergmann K. Serum concentration of 7 α -hydroxycholesterol as an indicator of bile acid synthesis in humans. *J Lipid Res* 1995; 36: 2059–2066.
 31. Scherer M, Gnewuch C, Schmitz G, et al. Rapid quantification of bile acids and their conjugates in serum by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; 877: 3920–3925.
 32. van Schaik FD, Gadaleta RM, Schaap FG, et al. Pharmacological activation of the bile acid nuclear farnesoid X receptor is feasible in patients with quiescent Crohn's colitis. *PLoS One* 2012; 7: e49706.
 33. Matysik S, Martin J, Bala M, et al. Bile acid signaling after an oral glucose tolerance test. *Chem Phys Lipids* 2011; 164: 525–529.
 34. Herrera J, Amigo L, Husche C, et al. Fecal bile acid excretion and messenger RNA expression levels of ileal transporters in high risk gallstone patients. *Lipids Health Dis* 2009; 8: 53.
 35. Song KH, Li T, Owsley E, et al. Bile acids activate fibroblast growth factor 19 signaling in human hepatocytes to inhibit cholesterol 7 α -hydroxylase gene expression. *Hepatology* 2009; 49: 297–305.
 36. Fang Q, Li H, Song Q, et al. Serum fibroblast growth factor 19 levels are decreased in Chinese subjects with impaired fasting glucose and inversely associated with fasting plasma glucose levels. *Diabetes Care* 2013; 36: 2810–2814.
 37. Eren F, Kurt R, Ermis F, et al. Preliminary evidence of a reduced serum level of fibroblast growth factor 19 in patients with biopsy-proven nonalcoholic fatty liver disease. *Clin Biochem* 2012; 45: 655–658.
 38. Gerhard GS, Styer AM, Wood GC, et al. A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. *Diabetes Care* 2013; 36: 1859–1864.
 39. Bennion LJ and Grundy SM. Effects of obesity and caloric intake on biliary lipid metabolism in man. *J Clin Invest* 1975; 56: 996–1011.
 40. Mabee TM, Meyer P, DenBesten L, et al. The mechanism of increased gallstone formation in obese human subjects. *Surgery* 1976; 79: 460–468.
 41. Leijd B. Cholesterol and bile acid metabolism in obesity. *Clin Sci (Lond)* 1980; 59: 203–206.
 42. Mok HY and Grundy SM. Cholesterol and bile acid absorption during bile acid therapy in obese subjects undergoing weight reduction. *Gastroenterology* 1980; 78: 62–67.
 43. Einarsson K, Hellstrom K and Kallner M. Bile acid kinetics in relation to sex, serum lipids, body weights, and gallbladder disease in patients with various types of hyperlipoproteinemia. *J Clin Invest* 1974; 54: 1301–1311.
 44. Dostalova I, Kavalkova P, Haluzikova D, et al. Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. *J Clin Endocrinol Metab* 2008; 93: 3627–3632.
 45. Stejskal D, Karpisek M, Hanulova Z, et al. Fibroblast growth factor-19: development, analytical characterization and clinical evaluation of a new ELISA test. *Scand J Clin Lab Invest* 2008; 68: 501–507.