Postprandial gut hormone responses and glucose metabolism in cholecystectomized patients

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Sonne DP, Hare KJ, Martens P, Rehfeld JF, Holst JJ, Vilsbøll T, Knop FK. Postprandial gut hormone responses and glucose metabolism in cholecystectomized patients. Am J Physiol Gastrointest Liver Physiol 304: G413–G419, 2013. First published December 28, 2012; doi:10.1152/ajpgi.00435.2012.—Preclinical studies suggest that gallbladder emptying, via bile acid-induced activation of the G protein-coupled receptor TGR5 in intestinal L cells, may play a significant role in the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1) and, hence, postprandial glucose homeostasis. We examined the secretion of gut hormones in cholecystectomized subjects to test the hypothesis that gallbladder emptying potentiates postprandial release of GLP-1. Ten cholecystectomized subjects and 10 healthy, age-, gender-, and body mass index-matched control subjects received a standardized fat-rich liquid meal (2,200 kJ). Basal and postprandial plasma concentrations of glucose, insulin, C-peptide, glucagon, GLP-1, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-2 (GLP-2), cholecystokinin (CCK), and gastrin were measured. Furthermore, gastric emptying and duodenal and serum bile acids were measured. We found similar basal glucose concentrations in the two groups, whereas cholecystectomized subjects had elevated postprandial glucose excursions. Cholecystectomized subjects had reduced postprandial concentrations of duodenal bile acids, but preserved postprandial plasma GLP-1 responses, compared with control subjects. Also, cholecystectomized patients exhibited augmented fasting glucagon. Basal plasma CCK concentrations were lower and peak concentrations were higher in cholecystectomized patients. The concentrations of GIP, GLP-2, and gastrin were similar in the two groups. In conclusion, cholecystectomized patients demonstrated a slight deterioration of postprandial glycemic control, probably because of metabolic changes unrelated to incretin secretion.

besides their established roles in dietary lipid absorption and cholesterol homeostasis, bile acids are now being recognized as metabolic regulators. Preclinical studies suggest that gallbladder emptying, via bile acid-induced activation of the G protein-coupled receptor TGR5 in intestinal L cells, plays a significant role in the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1) and postprandial glucose homeostasis (40). GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) are the main incretin hormones and act in concert to generate the so-called incretin effect (12). The incretin effect has been defined as the augmentation of insulin secretion after oral glucose compared with insulin secretion after isoglycemic intravenous glucose. The incretin effect accounts for up to 70% of the insulin secreted after ingestion of glucose, depending on the amount of glucose (12). The mechanisms underlying the release of incretin hormones are poorly understood. GLP-1 concentrations in plasma rise sharply after ingestion of a meal (7, 12, 23, 42). Carbohydrates, proteins, and lipids are effective stimuli for GLP-1 secretion from the gut (2, 8, 11, 32, 42, 44). In recent years, it has become clear that bile acids are candidate agents in newly identified pathways through which carbohydrate and lipid metabolism are regulated. Bile acids are ligands of the nuclear farnesoid X receptor (FXR), a receptor (expressed in high concentrations in the liver and intestines) that is implicated in glucose control (3), and they also signal through the cell surface G protein-coupled receptor TGR5, which is known to modulate energy expenditure in brown fat and muscle cells (16, 25, 40, 46). TGR5, present on the GLP-1-secreting L cell (15, 40), is not sensing nutrients but becomes activated by bile acids, resulting in the release of GLP-1 (15, 37, 40). Along this line, flow of bile acids into the intestine, subsequent to gallbladder emptying, has been suggested to play an important role in the regulation of postprandial glucose homeostasis as well as overall human metabolism (19, 40). In this context the unexplained high prevalence of gluco-metabolic disturbances in cholecystectomized patients (5) combined with the lack of data on gluco-metabolic hormones in these patients has attracted new interest (19).

The present study was designed based on the concept that postprandial gallbladder contraction and flow of bile to the intestine potentiate nutrient-induced GLP-1 secretion via bile acid-induced activation of TGR5 in L cells. Consequently, we hypothesized that cholecystectomized individuals might have impaired postprandial GLP-1 release. Furthermore, we aimed to characterize the previously uncharted endocrine “landscape” of cholecystectomized patients, with emphasis on gut hormone profiles and postprandial glucose homeostasis.

Glossary

| AUC | Area under the curve |
| BMI | Body mass index |
| DI | Disposition index |
| DPP-4 | Dipeptidyl peptidase 4 |
| FPG | Fasting plasma glucose |
| FXR | Farnesoid X receptor |
**G414 SECRETION OF GUT HORMONES AFTER CHOLECYSTECTOMY**

**GIP** Glucose-dependent insulinoergic polypeptide

**GAD-65** Glutamate decarboxylase-65

**GLP-1** Glucagon-like peptide-1

**HbA1C** Glycated hemoglobin A1c

**HOMA** Homeostatic model assessment

**HOMA2-IR** Insulin resistance according to the homeostatic model assessment

**IGT** Impaired glucose tolerance

**ISR** Insulin secretion rate

**NGT** Normal glucose tolerance

**OGTT** Oral glucose tolerance test

**PG** Plasma glucose

**T2DM** Type 2 diabetes mellitus

**MATERIALS AND METHODS**

The protocol was approved by the scientific-ethical committee of the capital region of Denmark (registration no. H-1-2011-008) and registered with the Danish Data Protection Agency (registration no. 2010-41-4243) and at ClinicalTrials.gov (clinical trial reg. no. NCT01251510). The study was conducted according to the principles of the Helsinki Declaration II. Written informed consent was obtained from all participants.

**Study population.** Following screening according to prespecified inclusion and exclusion criteria (see below), 10 cholecystectomized subjects (uncomplicated surgery performed 3–6 mo before inclusion) and 10 healthy age-, gender-, and body mass index (BMI)-matched control subjects were included in the study. Subject characteristics are presented in Table 1. At the screening visit, all potential subjects (n = 24) underwent a physical examination and had standard hematomatologic and clinical biochemistry parameters measured. Urine was sampled to determine the albumin-to-creatinine ratio. The subjects were without family history of diabetes and had normal glucose tolerance according to a 75-g oral glucose tolerance test (OGTT) performed before inclusion in the study (1–2 wk before the first study day). Exclusion criteria included acute or chronic illnesses, taking ongoing medication, having first-degree relatives with diabetes, and showing repeated abnormalities in hemoglobin, plasma liver enzymes (alanine or aspartate aminotransferases), creatinine concentration, or urinary albumin-to-creatinine ratio.

**Study design.** Subjects arrived at the laboratory in the morning after an overnight (10-h) fast having avoided strenuous physical exercise for 12 h. After local anesthesia of the pharynx (lidocain spray), a double-lumen nasogastric tube (Salem Sump Tube 12 Ch, external diameter 4.7 mm; Coviden, Copenhagen, Denmark) was placed under fluoroscopic control at the ligament of Treitz for aspiration of duodenal content (for evaluation of intraduodenal bile acid concentration). To avoid overstressing the participants, the procedure was allowed to take a maximum of 30 min. Hereafter, the subjects were placed in a semirecumbent position, laying on their backs with the upper part of the body 45° upright, had a cannula inserted in a cubital vein for collection of arterialized blood samples [cumulated forearm was placed in a heating box (50°C) throughout the experiment], and rested for 30 min. After baseline sampling, subjects ingested a 2,200-kJ liquid mixed meal (58 g carbohydrate, 28 g fat, 10 g protein, with 1.5 g acetyaminophen for evaluation of gastric emptying) over 10 min, blood samples were drawn, and duodenal aspirate was collected regularly for 4 h.

**Data collection.** Blood samples were drawn 20, 10, and 0 min before and 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min after ingestion of the mixed liquid meal. Blood was distributed into chilled tubes containing EDTA and a specific dipeptidyl peptidase 4 (DPP-4) inhibitor (3 mmol/l valine-pyrrolidide, 10 μmol/l blood; a gift from Novo Nordisk, Bagsværd, Denmark) for plasma analyses of gut hormones. For analyses of insulin and C-peptide, blood was distributed into chilled tubes containing heparin. EDTA and heparin tubes were immediately cooled and kept on ice until centrifugation. Blood for analysis of acetyaminophen was distributed into dry tubes for coagulation (20 min at room temperature). All samples were centrifuged for 20 min at 1,200 g and 4°C. Plasma samples for GLP-1, GIP, cholecystokin (CCK), and gastrin analyses and serum samples for acetyaminophen and bile acids analyses were stored at −20°C and plasma samples for insulin and C-peptide analyses were stored at −80°C until analysis. For bedside measurement of plasma glucose (PG), blood was distributed into fluoride tubes and centrifuged immediately at 7,400 g for 2 min at room temperature. Duodenal bile samples of 2 ml were aspirated every 10 min during the first hour of the meal test; these samples were stored at −20°C until analysis.

**Laboratory methods.** PG was measured by the glucose oxidase method, using a glucose analyzer (Yellow Springs Instrument model 2300 STAT plus analyzer; YSI, Yellow Springs, OH). Plasma insulin and C-peptide concentrations were measured using a two-sided electrochemiluminescence immunoassay (Roche/Hitachi Modular analytics; Roche Diagnostic, Mannheim, Germany). Plasma concentrations of total GLP-1, GIP, and glucagon-like peptide-2 (GLP-2), amidated gastrin, and CCK were measured by radioimmunoassays as described elsewhere (22, 33, 36, 39). The glucagon assay was directed against the COOH-terminal of the glucagon molecule and, therefore, measures glaucan of pancreatic origin (32). Serum acetyaminophen was measured by the Vitros ACET slide method based on an aryl acetylamide reaction linked to a color shift reaction using liquid chromatography for quantification as described elsewhere (29, 30). Total serum bile acids were measured using the enzymatic colorimetric method in which the enzyme 3-α-hydroxysteroid-dehydrogenase in the presence of nicotinamide adenine dinucleotide transforms bile acids to 3-ketosteroids and NADH. NADH is oxidized by coupling with a tetrazolium salt/diaphorase system to form formazan, a highly colored compound (26).

**Statistical analyses.** Results are reported as means ± SE unless otherwise stated. Area under the curve (AUC) values were calculated using the trapezoidal rule and are presented as incremental AUC values if nothing else is stated. Statistical analyses were carried out using GraphPad Prism version 5.00 for Windows/Mac (GraphPad Software, San Diego, CA). Insulin secretion rate (ISR) was calculated by deconvolution of measured C-peptide concentrations as described previously (17, 18, 42a). Insulin sensitivity was based on the Matsuda sensitivity index, which accounts for mean insulin and mean glucose concentrations during an oral glucose load (27). The homeostatic model assessment (HOMA2, available from www.ocdem.ox.ac.uk) was used to obtain quantitative assessment of mainly hepatic insulin resistance (HOMA2-IR) (24, 45). Insulinogenic index based on ISR and PG [(ISRr − 30 − ISR, r = 0)/(PGF − 30 − PGr − 30), where r is time in min] (34) and disposition index (insulinogenic index × HOMA2-IR−1) were calculated (14). For analysis of intergroup variations and

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**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
<th>Controls</th>
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<tr>
<td>Age, yr</td>
<td>47.5 (29–67)</td>
<td>46.0 (29–66)</td>
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<tr>
<td>Sex (M/F)</td>
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<td>6/4</td>
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<tr>
<td>BMI, kg/m²</td>
<td>25.0 (23.1–28.2)</td>
<td>24.0 (21.2–27.1)</td>
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<td>FPG, mmol/l</td>
<td>5.3 (5.0–5.8)</td>
<td>5.2 (4.8–5.8)</td>
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<tr>
<td>HbA1C, %</td>
<td>5.9 (5.7–6.3)</td>
<td>5.7 (5.2–6.1)</td>
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<tr>
<td>HOMA2-R</td>
<td>1.1 (0.6–1.8)</td>
<td>0.9 (0.5–2.5)</td>
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<tr>
<td>Diabetes in family</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ilet cell or GAD-65 antibodies</td>
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</table>

Data are median values with ranges in parentheses. Cholecystectomized patients and age-, gender-, and body mass index (BMI)-matched control subjects. FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HOMA2-IR, insulin resistance according to the homeostatic model assessment; GAD-65, glutamate decarboxylase-65.
differences between time courses, repeated-measurement ANOVA was used. This analysis provides P values for the comparison between the two groups, for changes over time, and for the interaction between the subject and time course. If an interaction between subject and time course was present, values at individual time points were compared using Student’s t-tests. A two-sided P < 0.05 was used to indicate significant differences. When single P values were available, they were expressed instead of stating nonsignificance.

RESULTS

Glucose. Mean postprandial PG concentrations are displayed in Fig. 1. Similar basal PG concentrations were observed in the two groups (5.4 ± 0.1 vs. 5.2 ± 0.1 mmol/l, P = 0.2), whereas postprandial PG excursions (total AUC) were greater in the cholecystectomized group compared with control subjects (1,431 ± 31 vs. 1,313 ± 36 mmol·l⁻¹·min⁻¹, P = 0.023). A significant interaction between subject and time was found (P = 0.007), and subsequent post hoc tests revealed significant differences in postprandial PG excursions from t = 60 to t = 180 min (Fig. 1). Accordingly, HbA₁c (Table 1) tended to be higher in the cholecystectomized subjects compared with control subjects (5.9 ± 0.1 vs. 5.7 ± 0.1%, P = 0.051).

Insulin, C-peptide, and ISR. Time courses for plasma concentrations of insulin and C-peptide are shown in Fig. 1. There were no significant differences between basal concentrations of either peptides, and no significant AUC differences were observed between patients and healthy controls (AUCinsulin: 20.9 ± 2.5 vs. 16.9 ± 1.6 nmol·l⁻¹·min⁻¹, P = 0.19; AUCC-peptide: 165 ± 17.0 vs. 133 ± 17.6 nmol·l⁻¹·min⁻¹, P = 0.21). Accordingly, the dynamic profiles of the average ISR did not differ in patients compared with healthy control subjects (total AUCISR: 919 ± 76 vs. 1,061 ± 92 nmol·l⁻¹·kg⁻¹·min⁻¹, P = 0.25).

Insulinogenic index and disposition index. During 2-h OGTT no differences in insulinogenic index were seen in patients compared with controls (3.4 ± 0.8 vs. 3.8 ± 0.7, P = 0.7), and, likewise, during meal test, no difference was demonstrated (5.6 ± 0.4 vs. 6.7 ± 1.4, P = 0.45). In addition, differences in disposition index during 2-h OGTT did not reach statistical significance comparing patients and controls (3.1 ± 0.5 vs. 4.6 ± 0.8, P = 0.12), and, during meal test, no differences in disposition index were demonstrated in patients compared with controls (6.4 ± 0.7 vs. 9.3 ± 1.8, P = 0.14).

Glucagon. Time courses for plasma concentrations of glucagon are displayed in Fig. 1. The concentrations of basal glucagon tended to be higher in cholecystectomized patients compared with controls (6.6 ± 0.1 vs. 4.4 ± 0.1 pmol/l, P = 0.11). Postprandial excursions were nonsignificantly higher in cholecystectomized patients compared with controls (1,752 ± 234 vs. 1,552 ± 227 pmol·l⁻¹·min⁻¹, P = 0.55).

GLP-1. Time courses for plasma concentrations of total GLP-1 are shown in Fig. 2. Similar basal GLP-1 concentrations were observed in the two groups, and cholecystectomized subjects exhibited preserved postprandial responses of GLP-1 compared with

A

B

C

D

Fig. 1. Postprandial plasma concentrations of glucose (A), insulin (B), C-peptide (C), and glucagon (D) in healthy control subjects (closed symbols) and cholecystectomized patients (open symbols) during a 2,200-kJ mixed liquid meal. Data points represent mean values ± SE. *Differences at specific time points.
the matched healthy control subjects (1,726 ± 210 vs. 1,665 ± 301 pmol·l⁻¹·min⁻¹, P = 0.87).

**GIP and GLP-2.** Time courses for plasma concentrations of total GIP and GLP-2 are shown in Fig. 2. Similar basal plasma concentrations were observed in the two groups, and cholecystectomized subjects exhibited preserved postprandial responses of both GIP (3,010 ± 442 vs. 3,269 ± 565 pmol·l⁻¹·min⁻¹, P = 0.72) and GLP-2 (4,283 ± 380 vs. 3,820 ± 608 pmol·l⁻¹·min⁻¹, P = 0.52).

**CCK and gastrin.** Time courses for plasma concentrations of CCK and gastrin are shown in Fig. 3. Basal concentrations of CCK were lower in cholecystectomized patients compared with healthy control subjects (0.2 ± 0.0 vs. 0.7 ± 0.1 pmol/l, P < 0.0001). In cholecystectomized patients, however, the postprandial incremental values of CCK were augmented compared with the control subjects (629 ± 73 vs. 423 ± 48 pmol·l⁻¹·min⁻¹, P = 0.03). No significant differences in total AUC were observed. The postprandial CCK response in cholecystectomized patients demonstrated two peaks (at 30 and 90 min), whereas a plateau phase between 30 and 120 min was observed in healthy control subjects. In cholecystectomized patients, the maximum peak concentrations of CCK (at 30 min) tended to be higher compared with the peak concentration in control subjects (at 20 min), but the difference did not yield statistical significance (7.3 ± 1.4 vs. 5.8 ± 0.5 pmol/l, P = 0.32). Cholecystectomized subjects did not exhibit significant differences in fasting plasma concentrations of gastrin compared with control subjects (9.7 ± 0.9 vs. 8.8 ± 0.9 pmol·l⁻¹·min⁻¹, P = 0.47) and exhibited preserved postprandial responses of gastrin (860 ± 155 vs. 986 ± 161 pmol·l⁻¹·min⁻¹, P = 0.59).

**Gastric emptying.** Time courses for the rate of gastric emptying (assessed by serum acetaminophen excursions) are shown Fig. 2. No interaction between subject and time courses was found (P = 0.92), and, likewise, in cholecystectomized patients no significant differences in AUC values were demonstrated compared with healthy control subjects (13.0 ± 0.7 vs. 14.1 ± 1.0 mmol·l⁻¹·min⁻¹, P = 0.38).

**Intraduodenal and serum total bile acids.** Time courses for postprandial total bile acid concentrations (measured intraduodenally and in serum) are shown in Fig. 3. Basal concentrations of total intraduodenal bile acids were higher in cholecystectomized patients compared with healthy controls (8.1 ± 1.4 vs. 3.8 ± 3.6 mmol/l, P = 0.03). In addition, healthy controls were able to increase the intraduodenal concentration of bile acids by a factor 10 at 10 min after the meal, resulting in a total bile acid concentration of 28.0 ± 7.8 mmol/l (vs. 7.7 ± 1.3 mmol/l in the cholecystectomized subjects, P = 0.04). Cholecystectomized subjects exhibited a flat curve, suggesting that these patients were not sufficiently capable of concentrating and ejecting bile into the duodenal lumen in response to meal intake. Postprandial AUC responses of total serum bile acid concentrations showed no significant differences in cholecystec-
tomized patients compared with healthy subjects (1,134 ± 261 vs. 1,164 ± 121 μmol·l⁻¹·min⁻¹, P = 0.92). However, comparing time courses, a significant interaction between time and subject was found (P = 0.02), and subsequent post hoc tests revealed significant differences in concentration at t = 20 min (10.6 ± 1.5 vs. 6.7 ± 0.9 μmol/l, P = 0.04) and at t = 30 min (13.5 ± 2.2 vs. 7.7 ± 0.8 μmol/l, P = 0.02). Indeed, the postprandial increase of serum bile acids in cholecystectomized patients tended to be more brisk during 0–30 min (Fig. 3).

DISCUSSION

The present study shows that cholecystectomized patients have preserved postprandial GLP-1 responses, suggesting that gallbladder contraction is not a prerequisite for peripheral plasma GLP-1 responses after meal intake.

Our findings also reveal a disturbed postprandial glucose homeostasis in cholecystectomized patients that gives rise to some pathophysiological considerations. Glucose responses in cholecystectomized patients were slightly exaggerated after the mixed meal, but within the normal range, indicating that these patients, albeit their normal OGTT and preserved postprandial GLP-1 and GIP secretion, may have a deteriorated postprandial glycemic control. The patients were recruited 3–6 mo after laparoscopic cholecystectomy. Bearing this in mind, it is noteworthy that HbA₁c values in these patients tended to be higher (5.9 ± 0.1 vs. 5.7 ± 0.1, P = 0.051) compared with carefully age-, gender-, and BMI-matched control subjects (matched 1:1). Our findings of slightly exaggerated postprandial PG excursions and marginally increased basal concentrations of glucagon indicate the presence of an early prediabetic state in cholecystectomized patients. However, with no dissimilarities in postprandial secretion of glucagon, virtually always present in subjects with type 2 diabetes compared with nondiabetic individuals, and differences in fasting concentrations of glucagon not reaching statistical significance, our results regarding glucagon should be interpreted with caution.

We were not able to show significant differences in insulin resistance and/or β-cell indexes and disposition indexes. In general, the results on β-cell responses did not reach statistical significance, but, overall, cholecystectomized subjects tended to display augmented responses during the entire time course compared with the control subjects (Fig. 1). Combined with the exaggerated glucose responses, fasting hyperglucagonemia (although not statistically significant), and near-significant differences in HbA₁c values, we speculate that glycemic control in cholecystectomized patients progresses to a state of mild insulin resistance, leading to glucose homeostatic disruption, perhaps evolving shortly after the development of gallbladder dysfunction and/or gallbladder removal.

There have been earlier reports suggesting that cholecystectomized patients are at risk of developing type 2 diabetes (1, 5, 35), but no confirmative prospective randomized controlled

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Fig. 3. Postprandial concentrations of plasma cholecystokinin (CCK) (A), plasma gastrin (B), intraduodenal total bile acids (C), and serum total bile acids (D) in healthy control subjects (closed symbols) and cholecystectomized patients (open symbols) during a 2,200-kJ mixed liquid meal. Data points represent mean values ± SE. *Differences at specific time points.
GRANTS
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DISCLOSURES
The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

REFERENCES
11. Gorboulev V, Schirrmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, Veyhl-Wichmann M, Lorenz-Loew M, Balen D, Bredjak D, Rexhepaj R, Parker HE, Griebel FM, Reimann F, Lang F, Wiese S, Sabolic I, Sendtner M, Koepsell H. TGR5 activation may be a possible regulator of GLP-1 secretion after a mixed meal (Fig. 3C). However, no differences in AUC values of postprandial bile acids were found, indicating that the spillover from the enterohepatic circulation of bile acids is not dependent on gallbladder emptying. One explanation could be that cholecystectomized patients exhibit increased uptake of intestinal bile acids (20, 28), resulting in negative feedback regulation on the secretion of CCK (10, 21). Indeed, the basal concentrations of CCK in plasma were very low, suggesting the presence of significant interdigestive bile acid levels in the duodenum, allowing for bile acids to exert persistent negative feedback inhibition of CCK (Fig. 3A). In accordance, we did see a somewhat sharp increase in serum bile acids in cholecystectomized patients compared with controls (Fig. 3A).

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study has examined this risk. Because of the suggestion that TGR5 activation may be a possible regulator of GLP-1 secretion (40), our main hypothesis was that reduced postprandial flow of bile into the duodenum of cholecystectomized subjects would result in reduced postprandial GLP-1 release. Furthermore, the ability of bile acids to activate FXR, a receptor that is implicated in glucose and lipid metabolism, might also be reduced in cholecystectomized patients, possibly affecting their metabolic regulation (3). However, it should be noted that the present experimental setting does not exclude that endogenous GLP-1 secretion in humans is regulated by bile acids (16, 25, 40, 41, 46). One plausible explanation for our negative result could be that any given effect of bile acids on TGR5-mediated GLP-1 release becomes superimposed of the macronutrients from the meal, which themselves constitute a potent stimulus for GLP-1 secretion. Indeed, the activation of FXR and TGR5 depends on the binding affinities for the specific bile acids (25). In this regard, fasting, together with interdigestive concentrations of intraduodenal bile acids, may exceed the half-maximal effective concentration of TGR5 activation (9).

Most bile acids activate TGR5 in the micromolar to nanomolar range, suggesting that a postprandial rise in duodenal bile acids is incapable of increasing TGR5 signaling further.

As expected, cholecystectomized patients were not capable of eliciting a rapid release of bile acids into the duodenum after a mixed meal (Fig. 3C). However, no differences in AUC values of postprandial bile acids were found, indicating that the spillover from the enterohepatic circulation of bile acids is not dependent on gallbladder emptying. One explanation could be that cholecystectomized patients exhibit increased uptake of intestinal bile acids (20, 28), resulting in negative feedback regulation on the secretion of CCK (10, 21). Indeed, the basal concentrations of CCK in plasma were very low, suggesting the presence of significant interdigestive bile acid levels in the duodenum, allowing for bile acids to exert persistent negative feedback inhibition of CCK (Fig. 3A). In accordance, we did see a somewhat sharp increase in serum bile acids in cholecystectomized patients compared with controls (Fig. 3A).

Regarding secretion of the other incretin hormone GIP, and GLP-2, an important regulator of small bowel growth (6), we did not find any differences in basal values or postprandial responses (43) and suggests that gastrointestinal transit time does not explain our results on postprandial glycemia.


