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Postprandial bile acid levels in intestine and plasma reveal altered biliary circulation in chronic pancreatitis patients

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Abbreviations: BA, bile acid ; CA, Cholic acid ; CA-3S, Cholic acid 3-sulfate; CDCA, Chenodeoxycholic acid; CDCA-3S, Chenodeoxycholic acid 3-sulfate; CP, chronic pancreatitis; DA, discriminative analysis ; DCA, Deoxycholic acid; DCA-3S, Deoxycholic acid 3-sulfate; EPI, exocrine pancreatic insufficiency; GCA, Glycocholic acid; GCDCA, Glycochenodeoxycholic acid; GDCA, Glycodeoxycholic acid; GHCA, Glycohyocholic acid; GHDCa, Glycohyodeoxycholic acid; GLCA, Glycolithocholic acid; GLCA-3S, Glycolithocholic acid 3-sulfate; GUDCA, Glycoursodeoxycholic acid; GUDCA-3S, Glycoursodeoxycholic acid 3-sulfate; HCA, Hyocholic acid; HDCA, Hyodeoxycholic acid; HPL, human pancreatic lipase; HV, healthy volunteers ; LC, liquid chromatography; LCA, Lithocholic acid; LCA-3S, Lithocholic acid 3-sulfate; MCA, Muricholic acid; MS, mass spectrometry; NaTDC; sodium taurodeoxycholate; O-PLS, Orthogonal-Partial Least Square statistical model ; PCA, principal component analysis ; PEG, polyethyleneglycol; TCA, Taurocholic acid; TCDCA, Taurochenodeoxycholic acid; TDCA, Taurodeoxycholic acid; THCA, Taurohyocholic acid; THDCA, Taurohyodeoxycholic acid; TLCA, Taurolithocholic acid; TLCA-3S, Taurolithocholic acid 3-sulfate; TUDCA, Tauroursodeoxycholic acid; TUDCA-3S, Tauroursodeoxycholic acid 3-sulfate; UDCA, Ursodeoxycholic acid; UDCA-3S, Ursodeoxycholic acid 3-sulfate;

ABSTRACT

Bile acid (BA) secretion and circulation in chronic pancreatitis (CP) patients with exocrine pancreatic insufficiency (EPI) were investigated by measuring simultaneously postprandial levels of individual BAs in duodenal contents and blood plasma using LC-MS/MS. CP patients and healthy volunteers (HV) were intubated with gastric and duodenal tubes prior to the administration of a test meal and continuous aspiration of duodenal contents.

Pancreatic lipase outputs in CP patients were very low (0.7 ± 0.2 mg) vs. HV (116.7 ± 68.1 mg; $P < 0.005$), thus confirming the severity of EPI. Duodenal BA outputs were reduced in CP patients (1.00 ± 0.89 mmoles; 0.47 ± 0.42 g) vs. HV (5.52 ± 4.53 mmoles; 2.62 ± 2.14 g; $P < 0.15$). Primary to secondary BA ratio was considerably higher in CP patients (38.09 ± 48.1) than HV (4.15 ± 2.37 ; $P < 0.15$), indicating an impaired transformation of BAs by gut microbiota. BA concentrations were found below the critical micellar concentration in CP patients, while a high BA concentration peak corresponding to gallbladder emptying was evidenced in HV. Conversely, BA plasma concentration was increased in CP patients vs. HV suggesting a cholangiohepatic shunt of BA secretion.

Alterations of BA circulation and levels may result from the main biliary duct stenosis observed in these CP patients. They may aggravate the consequences of EPI on lipid malabsorption.

Keywords : Bile acids; Cholangiohepatic shunt; Chronic pancreatitis; Exocrine pancreatic insufficiency; Lipidomics;

INTRODUCTION

Chronic pancreatitis (CP) is an ongoing inflammatory disorder associated with the loss of exocrine and endocrine pancreatic parenchyma and its replacement by fibrotic tissue. It results in various nutritional deficit linked to maldigestion subsequent to exocrine pancreatic insufficiency (EPI) and diabetes mellitus subsequent to pancreatic endocrine insufficiency. CP is mainly induced by alcohol abuse in western countries (1). Other toxic and/or metabolic agents, gene mutations regulating the pancreatic enzyme-activating cascades and their inhibitors are also factors associated with a hereditary form of the disease (2, 3). The first symptom is commonly the abdominal pain. Over the first five years of course, complications as pseudocysts or bile duct stenosis can occur. Occurrence of pancreatic calcifications is a lately observed diagnostic key. Because the clinical symptoms remain vague and non-specific in many CP patients, biological tests are required (4) for the diagnostic as well as the follow up.

In CP patients, fat digestion is more severely impaired than carbohydrate and protein digestion, and steatorrhea is usually a major symptom. The decrease in fat absorption is explained by a decrease in human pancreatic lipase (HPL) secretion and duodenal pH due to the reduced pancreatic bicarbonate secretion (5, 6). The decrease in duodenal pH impairs the activity and stability of residual HPL. It also promotes the precipitation of bile acids and thus a further deterioration of intestinal lipid absorption (7, 8). It was shown that human gastric lipase (HGL) secretion and contribution to lipolysis are increased in CP patients, but not sufficiently to compensate for the loss of the prominent pancreatic lipase (5).

The current treatment of EPI is enzyme replacement with porcine pancreatic extracts. Fat absorption can be improved with enteric-coated formulations of these exogenous pancreatic enzymes, associated also with proton pump inhibitors to increase the duodenal pH

and enhance enzyme stability and activity (9-11). In some patients with severe EPI, pancreatic enzyme replacement therapy (PERT) may not lead to the reduction of steatorrhea suggesting other mechanisms to interplay with the reduced lipase secretion. Although exogenous lipases act correctly in the small intestine, it can be suspected that other factors affecting the absorption of lipolysis products are limitant. Bile acid (BA) levels for instance were rarely addressed in CP patients (12). The BAs are critical for the digestion and absorption of fats (13, 14). Due to their amphiphilic properties, they are found adsorbed at the surface of oil-in-water emulsions where they regulate the lipolytic activity of the pancreatic lipase-colipase complex. BAs are also found in the form of mixed micelles with other bile lipids (cholesterol, phosphatidylcholine) and lipolysis products (free fatty acids, 2-monoglycerides). The micellar co-solubilization of lipolysis products by BA is required for removing these products from the lipid-water interface and for ensuring their diffusion in the aqueous milieu of the gut towards the enterocytes. In the absence of bile secretion, fat absorption is impaired (15).

Apart from their function in fat absorption and regulation of cholesterol homeostasis, BAs are increasingly being appreciated as complex metabolic integrators and signaling factors. They have become attractive therapeutic targets for metabolic disorders and useful markers of hepatobiliary and intestinal diseases (16). Individual BA molecular species from various biological samples (blood plasma, feces, urine) can now be separated and quantified in the clinical laboratory using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The BA profiles are used for a sensitive diagnosis of cholestasis (17). BA levels and profiles in patients with EPI have not been fully investigated with the LC-MS/MS profiling method whereas BA malabsorption in patients with CP was fully evidenced by the radiolabeled synthetic bile acid standard test (18). In the present study we have detailed the post-prandial individual BA level variations in both the duodenum and plasma of CP patients and healthy volunteers (HV) after a standard test meal.

MATERIALS AND METHODS

Ethical aproval

The duodenal content and blood samples used in this study were collected in the framework of two clinical studies (mrtm02-01 for HV and mrtm03-01 for CP patients; Principal Investigator: Prof René Laugier, MD, PhD) that were not initially designed for the analysis reported here. The clinical study protocols were accepted on January 17, 2003 by the Institutional board of the ethics committee CCPPRB (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Hôpital d'Adultes de la Timone, Marseille). Test meal experiments and sample collection were performed from March to July 2003 at “Centre de Pharmacologie Clinique et d'Etudes Thérapeutiques (CPCET, Hôpital d'adultes de la Timone, Marseille, France)” after written informed consents were obtained from all CP patients and HV. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

CP patients and heathy volunteers

Two groups of 6 HV (mean age: 30 ± 3 years) with no history of pancreatic disease and 6 patients with severe CP (mean age: 53 ± 8 years) were selected for this study. The severity of each patient's pancreatic insufficiency was estimated from the medical record and steatorrhea level in the absence of treatment by pancreatic extracts (see Table S1 in Supplemental Data). All selected CP patients had high levels of steatorrhea (8 to 45 g/day). All were documented with pancreatic calcification. Patients with common biliary duct stenosis had been treated by endoscopy within the 5 years preceeding the study. Some patients were also treated for stenosis after the present study. Only one patient (G.R.) had highly elevated liver enzymes, presumably due to severe hepatic disease.

Test meal

The mixed solid/liquid meal used for the *in vivo* experiments contained 80 g string beans, 90 g beef meat, 70 g fried potatoes, 10 g butter, 15 mL olive oil and water for a total volume of 700 mL. The string beans, beef meat and fried potatoes were passed individually into a mincer with 2-mm holes before they were mixed. The total amount of neutral fat given as triglycerides (TG) in the meal was measured to 31.8 ± 2.9 g after extraction of total lipids with chloroform-methanol and TG quantification using thin-layer chromatography coupled to flame ionization detection (19). A nonabsorbable marker was added to the meal (5 g PEG 4000) to measure the gastric emptying rate and correct the duodenal volumes based on the PEG recovery rates (20).

Experimental device for collecting samples

After fasting overnight, the patients and volunteers were intubated with a double-lumen duodenal tube (outside diameter 5 mm) and a separate single-lumen gastric tube (outside diameter, 3 mm). The tubes were placed under fluoroscopy and their positions were checked by analyzing the pH of their contents. The distal end of the duodenal tube with the occluding balloon was located at the ligament of Treitz (see Figure S1 in Supplemental Data). During the sampling period, the subjects stayed in bed with continuous duodenal aspiration by connection of the duodenal tube to vacuum (around -10 mbar). Aspiration was started a few minutes before the meal and the occluding balloon was inflated (10 mL air). After collecting basal gastric and duodenal samples, the test meal was introduced into the stomach via the gastric tube using a 50 mL syringe, during a period of 5 minutes.

The duodenal fluid was collected continuously for 15-minute periods by aspiration upstream of the occluding balloon. The parameters measured in each sample were: i) volume

and pH (to the nearest 0.5 mL and 0.1 pH unit respectively), ii) PEG 4000 concentration, iii) pancreatic lipase activity and iv) individual BA concentrations. In order to prevent the proteolytic inactivation of lipase before the assay, 1 mL glycerol and 40 μ L protease inhibitors were added immediately to 1 mL duodenal sample before storage at -20°C. The solution of protease inhibitors was prepared by dissolving a pellet of Complete[™] protease inhibitor mix (Roche) in 2 mL distilled water. All samples were kept at -80°C before they were analyzed.

Blood sample collection.

Blood samples were collected before and after the meal for a total duration of 240 minutes. All samples were kept at -80°C before they were analyzed.

Measurement of PEG 4000 in gastric and duodenal samples.

The PEG 4000 concentration was measured using the turbidimetric method developed by Hyden (21) as modified by Malawer and Powell (22).

Assay of pancreatic lipase activity

The enzyme activity of human pancreatic lipase (HPL) in duodenal contents was measured by the pHstat technique as previously reported (20, 23). Lipase levels were expressed in international units per ml (1 international unit = 1 μ mole of fatty acid released per minute from the standard substrate, tributyrin). Lipase outputs were expressed either in total units or mg of active lipase based on the known specific activity (8000 U/mg) of a pure HPL acting on tributyrin under similar condition.

Use of PEG for correcting the volume of duodenal aspirates

A correction factor accounting for the partial recovery in duodenal aspirates of the nonabsorbable marker PEG 4000 was calculated for the whole period of measurement by dividing the total amount of marker initially added in the meal by the recovered amount at the end of the experiment.

Estimation of HPL duodenal outputs

The duodenal output of HPL was estimated from the assay of enzyme activity in duodenal samples, the volume of duodenal aspirates and the correction factor based on the recovery of PEG.

Preparation of bile acid standards for LC-MS/MS analysis

Concentrated BA calibration solutions were prepared in methanol (1mg/ml) and stored in a sealed container at -20°C. These stock solutions were diluted to obtain calibration solutions ranging from 31.3 ng/ml to 31.3 µg/ml. Cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA), hyocholic acid (HCA) and the corresponding glyco- and tauro-conjugates were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). 3-sulfate derivatives of the BAs were a generous gift from Dr J. Goto (Niigita University of Pharmacy and Applied Life Science, Japan). 23-nor-5β-cholanoic acid-3α,12β diol, muricholic acid derivatives and glyco- and tauro-derivatives were purchased from Steraloids Inc. (Newport, USA).

Bile acid analysis by LC-MS/MS

An internal standard solution (2 µL of 23-nor-5β-cholanoic acid-3α,12β diol at 1mg/ml) was added to plasma samples (500 -1000 µL) and duodenal aspirates (100-200 µL). Proteins were precipitated by addition of ammonium carbonate 0.4 M for 30 min at 60°C

(17). The clean-up procedure was achieved by centrifugation (4000 g for 10 min) followed by a solid-phase extraction (SPE). Reverse phase Chromabond C₁₈ cartridges (100 mg; Macherey-Nagel, Düren, Germany) were pre-washed by 5 ml methanol and 5 ml water, successively, before the sample was loaded onto the cartridge. The subsequent steps were processed on a vacuum manifold designed for SPE. The cartridge was rinsed successively with 20 ml water, 10 ml hexane to discard neutral lipids and again with 20 ml water. BAs were finally eluted by methanol. The eluates were dried under a nitrogen stream at 50 °C and the residue dissolved in 150 µl methanol. Five microliters were injected into the HPLC–MS/MS equipment.

The chromatographic separation of BAs (see Figure S2 in Supplemental Data) was carried out on a reverse phase column (Restek C18 Pinnacle II (250mm x 3.2mm, 5µm), Restek, Lisses, France) thermostated at 35°C (Agilent 1100 HPLC, Agilent Massy, France). The column was initially equilibrated with a 65/35 (v/v) mixture of solvent A (15 mM aqueous ammonium acetate, pH 5.3) and B (methanol). BA elution was achieved by increasing proportion of solvent B from 65/35 to 95/5 (v/v). Simultaneously, the flow rate was increased from 0.3 to 0.5 mL/min for 30 min.

The HPLC column eluates were infused into the ESI source of a triple quadrupole mass spectrometer (QTRAP 2000, Applied Biosystems-SCIEX, Concord, Ontario, Canada). Electro-spray ionisation was set in the negative mode. Nebulizer, curtain and heater nitrogen gas were set at 40, 20 and 40 (arbitrary units), respectively. The temperature for the evaporation gas (nitrogen) was set at 400°C. The ion spray, declustering and entrance potentials were set at -4500V, -60 V and -10 V, respectively. The MS/MS detection was operated with a unit resolution in the multiple reaction monitoring (MRM) mode. The dwell time for each transition was set at 70 ms. MRM was performed by examination of the

transition reactions from precursor conjugated BA to product fragment-ions after collision induced dissociation (CID): the sulfite ($m/z=80$, SO_3 fragment-anion cleaved from taurine) and glycine moieties ($m/z=74$) respectively, for tauroconjugated and glycoconjugated BAs. Sulfo-conjugates were identified by the sulfuric anion ($m/z = 97$, HSO_4). No specific fragment-ions are observed for unconjugated BAs, except those corresponding to loss of water and these fragments are not reliable for quantification. Therefore, we used selected ion monitoring (SIM) mode for quantifying unconjugated BAs, with mono-, di- and tri-hydroxylated BAs scanned at m/z of 375, 391 and 407, respectively. 23-nor-5 β -cholanoic acid-3 α ,12 β diol ($m/z = 377$) was used as the internal standard (IS) for normalization. The method for BA analysis was validated according to Humbert et al. (17), who previously checked that similar values were obtained with 3 different IS (23-nor-5 β -cholanoic acid-3 α ,12 α diol, ursodeoxycholic-2,2,4,4-d $_4$ acid and lithocholic-2,2,4,4-d $_4$ acid) with distinct hydrophobicity and retention times.

Statistics

All the data were expressed as means $\pm$ SD unless stated otherwise. The data obtained in the various series of experiments were compared using paired and unpaired Student's *t*-tests. The data obtained at time 30 min after meal administration were also analyzed using principal component analysis (PCA) and discriminant analysis (DA) using an Orthogonal-Partial Least Square (O-PLS) statistical model (SIMCA14 software package (Umetrics, Umeå, Sweden)).

RESULTS

Variations in the duodenal pH

In HV, the duodenal pH values remained in the 4.1-7.4 pH range during the meal digestion, with a mean value of 6.10 ± 0.8 (Figure 1A). In CP patients, the duodenal pH decreased rapidly after 30 min, and then remained very low with mean pH values ranging between pH 2 and pH 3.5 (Figure 1A). The pH differences between CP patients and HV were significant ($P < 0.05$) from 30 to 180 min.

HPL duodenal concentration and outputs

Pancreatic lipase outputs during the whole digestion period were estimated from the recovery of duodenal contents. The HPL activities recorded in HV were in the 320-5250 U/mL range (i.e. 40-656 $\mu\text{g/mL}$ of active HPL), whereas HPL activity in CP patients was extremely low and never greater than 12 U/mL (1.5 $\mu\text{g/mL}$; Figure 2B). From the cumulated outputs of HPL, it was observed that pancreatic lipase secretion was almost abolished in CP patients (0.7 ± 0.2 mg vs. 116.7 ± 68.1 mg in HV; $P < 0.005$).

Total bile acid concentrations in duodenal contents

In HV, the BA concentration in duodenal contents was increased immediately after the meal intake and reached a maximum value (14.8 ± 12.7 mM) after 30 min (Figure 2A). It then decreased and leveled off around 3-4 mM after 120 minutes. Except during the 75-90 min period, mean BA concentration found in human duodenal contents was above the average critical micellar concentration (CMC) of the main BAs found in human bile (24) (around 4 mM; see Table S2 in Supplemental Data). In CP patients, the BA concentration in duodenum was much lower than in HV. A maximum value of 3.9 ± 5.6 mM was observed at 45 min

(Figure 2A). Except at 30 and 45 minutes, the total BA concentration remained below the CMC.

The ratio of primary BA (7α hydroxylated in the liver) over secondary BA (produced from primary BA by the intestinal microbiote after 7α -dehydroxylation) was considerably higher in CP patients (38.09 ± 48.1) than in HV (4.15 ± 2.37 ; $P < 0.15$) (Figure 2B). The ratio ranged from 19.3 to 72.8 in CP patients as compared with 4.1 to 7.1 in HV .

Bile acid duodenal outputs

Total BA outputs in duodenal contents over the post-prandial period (Table 1) were much lower in CP patients (1.00 ± 0.89 mmol; 0.47 ± 0.42 g) vs. HV (5.52 ± 4.53 mmol; 2.62 ± 2.14 g). The ratio of primary to secondary BA was also much higher in CP patients (38.1 ± 39.4) than in HV (4.2 ± 1.9). The examination of the detailed BA composition (expressed as total BA mole %) revealed a significant decrease in TCDCA ($P < 0.015$), TDCA ($P < 0.025$), GLCA-3-sulfate ($P < 0.02$), TLCA-3-sulfate ($P < 0.01$), UDCA-3-sulfate ($P < 0.035$), LCA-3-sulfate ($P < 0.05$), GUDCA-3-sulfate ($P < 0.03$), GUDCA ($P < 0.035$), GHDCA ($P < 0.005$), GCA ($P < 0.015$) and TLCA ($P < 0.05$) in CP vs. HV (Table 1). Only traces of glucuronated conjugates ($< 0.01\%$ of total BAs) were detected and were not taken into account for comparison of BA profiles between CP and HV. For comparison, total sulfated BAs accounted for around 0.04% (CP) to 0.3% (HV) of total BAs in duodenal contents.

Bile acid concentrations in blood plasma

The BA concentration in blood plasma was in the μM range, that is three orders of magnitude lower than duodenal BA concentration (mM range; Figure 2). After meal intake, the total BA concentration in plasma of CP patients showed a peak (3.88 ± 2.32 μM ; Figure 3C) at 30 min. The peak contrasted with the decreased BA concentration in HV (1.92 ± 1.56

μM ; Figure 2C). In HV plasma BA concentration increased only lately to $2.32 \pm 1.55 \mu\text{M}$ at 60 min (Figure 2C) before decreasing. BA concentrations in HV and CP patients reached similar values at 120-180 min.

The analysis of individual circulating BAs revealed a higher primary to secondary BA ratio in CP patients. The largest difference in the ratio was observed at 90 min (Figure 2D). The comparison of the area under the plasma concentration curve (AUC, $\mu\text{moles.L}^{-1}.\text{min}$) for individual BAs was not statistically different between the HV and CP groups (Table 2), except for the conjugated ursodeoxycholic acid (UDCA and GUDCA) significantly lower in CP patients ($P < 0.05$). The decreased ursodeoxycholic conjugates were unexpected regarding a commonly found increase in prolonged cholestasis (25). Analysis of AUCs showed a significantly higher ($P < 0.05$) proportion of primary BA in CP patients ($87.1 \pm 6.5 \text{ mol } \%$ of total BAs) as compared with HV ($67.1 \pm 17.0 \text{ mol } \%$ of total BAs). Only traces of glucuronated conjugates ($< 0.01\%$ of total BAs) were detected in plasma of CP and HV, while total sulfated BAs accounted for around 0.7% (HV) to 1.5% (CP) of total BAs in plasma.

Simultaneous changes in individual bile acid concentrations in duodenal contents and blood plasma

The variations with time in individual BA duodenal concentrations were similar to those observed with total BAs (Figure 2A), as illustrated by the kinetics of GCA (Figure 3A), a major BAs comprised in the human bile. Variations in other BAs are reported in Supplemental Data (Figure S3). The main deviation between HV and CP patients was observed 30 min after meal with mean GCA duodenal concentrations of $2,012 \mu\text{M}$ and $616 \mu\text{M}$, respectively (Figure 3A). Then, mean GCA duodenal concentration in HV decreased to values similar to CP patients after 60 min. In CP patients, only weak changes in the GCA duodenal concentration were observed (Figure 3A).

Interestingly, opposite variations in GCA concentration were observed in blood plasma (Figure 3B). The plasma concentration of GCA increased clearly in CP patients and peaked at 30 min (mean concentration of 0.72 μ M) but in HV the mean GCA concentration remained low (mean 0.15 μ M). Plasma GCA concentration of CP patients returned to low HV values after 60 min.

Since the main differences between CP patients and HV were observed at 30 min, we focused on duodenal (Figure 3C) and plasma (Figure 3D) concentrations of individual BAs in duodenal and blood samples at 30 min (TCDCA, TDCA, TCA, GUDCA, GCDCA, GDCA, GCA). For each individual BA, the duodenal concentration measured in CP patients was significantly lower ($P < 0.05$) than in HV (Figure 3C). Conversely, plasma concentrations in CP patients were higher with significant differences ($P < 0.05$) for the abundant BAs, GCDCA and GCA (Figure 3D).

Conjugated BA levels

Both glyco- and tauro-conjugated BA levels were much lower in CP patients (0.6 ± 0.7 and 0.4 ± 0.5 mmol, respectively) than in HV patients (3.3 ± 3.7 and 2.2 ± 2.7 mmol, respectively). The glyco- to tauro-conjugated BA ratio in CP (2.5 ± 1.7) and HV (1.5 ± 0.7) were however not significantly different.

Unconjugated BA levels

Unconjugated BA levels (UDCA, CDCA, DCA, CA) were measured at a low level ($< 25 \mu$ M) in the duodenal contents of both HV and CP patients (Table 1; 0.01 to 1.5 % of total BA) except for a CP patient (C.K.) with particularly high concentrations of CDCA, DCA and CA (646, 293 and 124 μ M, respectively at 30 min). This outlier patient has an impact on the significance of the difference with the control HV group (Table 1). Unconjugated BA

showed higher contributions in the plasma than in the bile for both HV ($28,5 \pm 24,2$ % of total BAs) and CP patients ($18,2 \pm 15,4$ % of total BAs). However no significant difference between the two groups was found including for the CP patient showing the particularly high duodenal concentrations of unconjugated BAs.

BA levels in patient with alcoholic liver disease

Only one CP patient at out six had highly elevated liver enzymes, presumably due to alcoholic liver disease (patient 4 (G.R.); see Table S1 in Supplemental data). Total bile acid outputs (0.66 mmoles) in this patient were not significantly different from those found in the five other patients with severe chronic pancreatitis and were lower than those observed in HV. Primary to secondary BA molar ratio in this patient was the highest found among all CP patients, in both duodenal contents (55 to 190) and blood plasma (10 to 110), but this finding did not change the fact that all CP patients showed a higher primary to secondary BA ratio than the healthy volunteers group.

DISCUSSION

The availability of LC-MS/MS analytical methods for BA in the clinical laboratory allows the investigation of BA secretion and circulation. The comparison of healthy volunteers (HV) and chronic pancreatic (CP) patients, using samples of duodenal contents and blood plasma collected during test meal experiments contributes to a detailed understanding of the condition. We selected CP patients with a severe pancreatic exocrine insufficiency authenticated by a low duodenal pH value (Figure 1A) resulting from weak bicarbonate secretion, and extremely low pancreatic lipase (HPL) levels (Figure 1B). On average, HPL secretion in CP patients (0.7 ± 0.2 mg) represented only 0.6 % of control outputs (116.7 ± 68.1 mg in HV). The HPL outputs were found in the range previously reported for severe CP patients (5) and healthy subjects (5, 26), respectively.

Bile acids in healthy volunteers

The data on BA analysis with HV confirmed concentrations and outputs previously reported in healthy humans under fed conditions. Total output (2.62 g; 5.52 mmoles) during the duration of the meal (3 h) was close to the 3 g BA amount estimated for the pool of BAs circulating 4 to 12 times per day in the human GI tract (27). Furthermore, the peak in duodenal concentration of BAs observed 30 min after meal ingestion was typical of a normal gallbladder emptying (27, 28). While highly variable intestinal concentrations of BAs under fed conditions have been reported in literature, ranging from 0.5 mM (29) to 37 mM (30), there is an agreement for duodenal concentrations recorded 30-60 min after meal and following the gallbladder emptying: 14.5 ± 8.8 mM (mean $\pm$ SD, n =5) reported by Hernell et al.(30), 14.7 ± 8.0 mM (mean $\pm$ SD, n = 16) by Tangerman et al. (31), 15.8 ± 5.6 mM (mean $\pm$ SD, n = 5) by Ladas et al. (29), 16.2 ± 1.5 mM (mean $\pm$ SE, n = 13) by Rautureau et al. (32), 14.5 ± 9.4 (mean $\pm$ SD, n = 12) by Fausa et al. (33), and 8.3 mM (normal meal) to 11.9 mM

(high-fat meal) reported by Clarysse et al. (28). The mean duodenal BA concentration measured presently after 30 min in HV (14.8 ± 12.7 mM, $n=6$; Figure 2A) was therefore consistent with previous data. BA profiles were also in the normal range with conjugated BAs >90% but low levels of unconjugated and sulfated BAs (Tables 1 and 2). The primary to secondary BA ratio in duodenal contents of HV (*c.a* 5.5) was close to previously reported values for healthy subjects under fasting (5.5) and fed (8.2) conditions (28).

BAs were found at a low concentration (μ M range) in the peripheral blood circulation, as resulting from the normal highly efficient uptake by the liver (34). Plasma BA composition was also similar to previous report with healthy subjects (17, 35). It is noticeable that the contribution of unconjugated BA to total BAs found in plasma is much higher ($28.5 \pm 24.2\%$; Table 2) than in duodenal contents (around 0.06 %; Table 1). These higher plasma concentrations of unconjugated BAs can be explained by their slower first-pass hepatic clearance, which disproportionately increases their concentration in plasma versus conjugated BAs. Therefore, the measurement of the relative amounts of conjugated versus unconjugated BAs in plasma is not a good indicator of their relative proportions in the whole-body BA pool or in other compartments.

Bile acids in chronic pancreatitis patients

In addition to the deficit in pancreatic bicarbonate and enzyme secretions, total BA concentration in duodenal contents was decreased *c.a* 5-fold in CP patients as compared to HV (Figure 2A). No peak of BA concentration in duodenal contents, that could reflect gallbladder emptying, was observed at 30 min. The maximum concentration was observed with a delay of 15 min compared to HV. Overall, the total BA output in the duodenum was severely reduced in CP patients (0.47 g; 1.00 mmoles). It amounted only 18% of BA output in HV (2.62 g; 5.52 mmoles; Table 1). As a results, BA duodenal concentration was often found

below the average CMC of the main bile salts found in human bile and duodenal contents (Table 3). It is worth noticing that we analyzed complete duodenal samples and therefore, low BA levels in CP could not be explained by some BA precipitation at low pH as reported in previous studies (12, 36). This point will be further discussed.

As in healthy subjects, conjugated BAs represented >90% of total bile BAs and very low levels of unconjugated BAs and sulfated BAs were measured (Table 1). The glyco- to tauro-conjugated BA ratio was not changed compared to HV, indicating that no selective precipitation of glyco-conjugated BA at low pH occurred in CP patients compared to previous reports (12, 36). We therefore re-analyzed these previous studies by comparing BA concentrations and pH levels with those we measured here. In the paper by Go et al. (36), which reports BA precipitation at low jejunal pH in one patient with gastrin-producing islet cell tumor, gastric hypersecretion and steatorrhea, BA precipitation occurred at very low pH values (≤ 2.5), what could be reproduced *in vitro*. Only 48% of BA were present in the micellar phase. This finding is probably the first one that led to the explanation of low BA levels in CP patients by acid precipitation. However, the sum of BA levels in all phases was only 23% of that recorded in normal subjects, which shows a reduced secretion of BAs independently from a loss by acid precipitation and is in agreement with our own observations. It was shown in this study that the precipitated BAs were largely the glycine dihydroxy conjugates (around 70%). In the paper by Regan et al. on patients with advanced acquired EPI (12), the lowest pH values (between 3 and 4) and BA concentrations were not as low as in Go's study and in our work. Indeed, at the BA secretion peak, the concentration in the micellar phase was 8.1 ± 0.6 mM in EPI patients versus 9.3 ± 0.4 mM in normal subjects, which indicates that BA secretion was not drastically reduced in these patients compared to the CP patients with severe EPI enrolled in our study. Although a pH-dependent lowering of BA concentration in the micellar phase was observed, its impact on BA levels

was limited and the patients involved in this study had clearly much higher BA levels than those we included in our study. Nevertheless, this publication and its conclusions have certainly led to an overestimation of the impact of acid precipitation on BA levels in EPI patients, and have delayed the recognition that low BA in these patients could be also explained by an altered BA circulation.

On a relative basis, primary BAs (CA and CDCA conjugates) were increased but secondary BAs (DCA derivatives) decreased in CP patients, which is consistent with a reduced bacterial conversion of primary to secondary BA in the intestine or fecal loss of BA due to intestinal bile acid malabsorption. In any case, this finding suggests changes in the enterohepatic circulation of bile acids (27) and this is supported by the fact that the increased primary to secondary BA ratio observed in duodenal contents (Figure 2B) was also observed in the peripheral blood circulation (Figure 2D). Intestinal BA malabsorption is one hypothesis to explain these findings: it would lead to the fecal loss of total BA pool, depletion of secondary BA formed in the intestine and would derepress hepatic production of newly synthesized primary BAs. We have no information however on the fecal loss of BA in these CP patients and cannot confirm whether it could account for the 5-fold depletion in BA pool observed CP patients versus HV.

Another hypothesis could be a bypassed BA circulation via the cholehepatic shunt, a pathway which is minored under the normal physiological conditions (37) by the extensive enterohepatic recycling of BAs from intestine. Indeed, a remarkable particularity in CP patients was a sharp increase in plasma BA concentration 30 min after meal ingestion (Figure 2C) and the absence of BA concentration peak in duodenal contents corresponding to gallbladder emptying, which is consistent with a shunt of BA circulation. The cholehepatic shunt represents the fraction of BAs absorbed by cholangiocytes lining the biliary ducts and gallbladder, which is recycled back to hepatocytes before reaching the intestinal lumen (38).

BA absorption by cholangiocytes involves specific BA transporters like the Apical Sodium Bile Acid Transporter (ASBT) and heteromeric organic solute transporter (OST) (38-40). The cholehepatic shunt pathway might be favored in severe CP patients due to the drastic acidification of intestinal contents. Indeed, using a mouse model of cystic fibrosis (*Cftr*^{-/-}), Debray et al. have shown that increased duodenal acidity results in attenuation of gallbladder contraction through the overexpression of vasoactive intestinal peptide (VIP), which leads to an increase in cholecystohepatic shunting of BAs and reduced levels of secondary BAs (41). Cholecystectomy reversed those changes which provided evidence that the gallbladder rather than the bile duct epithelia may be the major site of BA re-absorption in the cholehepatic shunt. In our study, the rise in plasma BAs observed at the 30-minute time point in the CP patient group suggests that the gallbladder contraction after meal intake occurs properly but BAs may be re-absorbed before reaching the duodenum. The cholehepatic shunt has also been proposed as a protection mechanism for the liver (41, 42), particularly in response to bile duct obstruction. Indeed, it has been shown that this pathway provides an alternative route for continuation of hepato-cholangiocyte flux of BAs in bile duct-ligated rats (43, 44).

Based on these findings, we hypothesized that the low BA levels observed in the duodenal contents of CP patients (Figure 2A) might be explained by bile duct obstruction, which could eventually lead to a cholehepatic shunt of BA secretion, rather than by BA precipitation at low pH or intestinal BA malabsorption. We checked the medical history of the CP patients selected for this study (Table S1). They all had alcohol abuse-related chronic pancreatitis. Four of them were diagnosed for stenosis of the main bile duct before they were involved in this study and underwent an endoscopic treatment in the years preceeding the study (1998-2002). The two other CP patients were diagnosed for main bile duct stenosis after the study (2005-2006). None of them had cholecystectomy. A cholehepatic shunt of bile

secretion in these patients could therefore be explained by the main bile duct stenosis encountered in chronic pancreatitis (45).

The cholehepatic shunt being usually considered a minor pathway, it might not be sufficient to explain the extensive duodenal depletion in BAs in CP patients. As indicated before, there is no evidence that the precipitation of BAs at low pH (46) could explain this depletion. Alternatively, BA secretion by the liver might be reduced in CP patients by a feedback control of the liver BA synthesis and transport (47). Measurements of the plasma levels of 7 α -hydroxy-4-cholesten-3-one (C4) and fibroblast growth factor 19 (FGF19) would have helped distinguishing between cholehepatic shunting of BAs and intestinal BA malabsorption (elevated C4, reduced FGF19 (48)) as an explanation for the BA phenotype in CP patients. Unfortunately, we did not have enough plasma samples to perform these assays.

Test meal and plasma bile acid analysis as a potential diagnosis tool for the follow up of EPI and biliairy duct stenosis

Using a test meal and BA analysis in both duodenal contents and blood plasma, we found a clear anti-correlation between duodenal and plasma BA concentrations in the time frame (30 min after meal ingestion) corresponding to gallbladder emptying (well seen in HV). In HV, high BA concentrations in duodenal contents corresponded to low BA concentrations in plasma, while in CP patients, low BA concentrations in duodenal contents corresponded to high BA concentrations in plasma (Figure 4A). In CP patients, the primary to secondary BA ratio also increased in duodenal contents and blood plasma. A discriminant analysis performed with the Orthogonal-PLS statistical model has establish a S-plot for the circulating blood plasma BAs assayed at 30 minutes after meal intake. The S-plot revealed the most discriminative biomarkers for CP patients (DCA, UDCA, CDCA) out of 17 plasma BA (Figure 4B; Additional analysis can be found in Figures S4 to S8 in Supplemental Data).

Therefore these three plasma BA assay may be used as a quantitative appreciation of EPI complications related to bile acids. BA profiling could also be used in the follow up after endoscopic treatment of main bile duct stenosis in parallel with the assay of hepatic enzyme levels in cholestatic plasma. It should be noted that the plasma bile acid biomarkers in this study (Figure 4, Supplemental Figures S4 to S8) were identified following a test meal in patients that were intubated with a duodenal occluding balloon near the ligament of Treitz (Figure S1). Additional studies are required to determine if these plasma bile acid biomarkers would be predictive in patients without the duodenal occluding balloon since this latter is restricting the movement of luminal contents towards the distal intestine and may distort the pattern (post-prandial rise) in BAs present in plasma.

In conclusion, the data presented here show that duodenal BA levels can be drastically reduced in patients with severe CP. Low levels of BAs in intestinal contents have already been described in cases of pancreatic insufficiency. This was usually attributed to BA precipitation at low pH, particularly that of glyco-conjugated BAs (7, 8, 12, 46), what we did not observed here. Our results strongly suggest that low duodenal BA levels in the CP patients involved in our clinical study results from the bile duct stenosis, that may lead to a cholehepatic shunt of BA. Enzyme replacement therapy based on the oral administration of pancreatic extracts might not be sufficient to restore fat absorption in these patients, since BAs are essential for the micellar solubilization of lipolysis products and absorption by enterocytes (49). BA duodenal concentrations were often below CMC (Figure 2A) and formation of mixed BA-lipolysis product micelles could be seriously impaired. Also, since BAs have antimicrobial effects, their deficiency in the small intestine of CP patients can promote bacterial growth and changes of intestinal microbiota (50).

The analytical method describe here for measuring BAs in plasma samples can be used easily to obtain additional information on EPI patients and identify whether their exocrine pancreatic insufficiency is coupled with changes in BA secretion and enterohepatic circulation. The “cholehepatic shunt” signature obtained from plasma BA analysis might also be useful for orientating the diagnosis towards intra-hepatic or extra-hepatic cholestasis, with more specific markers than those currently used in clinics such as levels in hepatic enzymes (γ -glutamyl transpeptidase, alkaline phosphatase, transaminases). The increase in primary to secondary BA ratio could also be used as a secondary marker. In connection with this potential diagnosis tool, it is now essential to develop new treatments for improving fat absorption in EPI patients and one can think about a co-administration of pancreatic enzymes and BAs. BA replacement therapy has already been used for improving fat absorption in patients using dessicated ox bile or the synthetic conjugated BA cholylsarcosine (51-53). Other approaches are using the formulation of pancreatin with a semi-solid self-micro-emulsifying excipient (Gelucire[®] 44/14) to improve pancreatic lipase activity and intestinal absorption(54, 55). These studies could serve as a basis for further improvement of EPI treatment.

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REFERENCES

1. Samokhvalov, A. V., J. Rehm, and M. Roerecke. 2015. Alcohol Consumption as a Risk Factor for Acute and Chronic Pancreatitis: A Systematic Review and a Series of Meta-analyses. *EBioMedicine* **2**: 1996-2002.
2. Büchler, M. W., H. Friess, W. Uhl, and P. Malfertheiner. 2002. Chronic pancreatitis: novel concepts in biology and therapy. Blackwell Wissenschafts-Verlag, Berlin.
3. Etemad, B., and D. Whitcomb. 2001. Chronic Pancreatitis: Diagnosis Classification, and New Genetic Developments. *Gastroenterology* **120**: 682-707.
4. Duggan, S. N., H. M. Ni Chonchubhair, O. Lawal, D. B. O'Connor, and K. C. Conlon. 2016. Chronic pancreatitis: A diagnostic dilemma. *World J Gastroenterol* **22**: 2304-2313.
5. Carrière, F., P. Grandval, C. Renou, A. Palomba, F. Prieri, J. Giallo, F. Henniges, S. Sander-Struckmeier, and R. Laugier. 2005. Quantitative study of digestive enzyme secretion and gastrointestinal lipolysis in chronic pancreatitis. *Clin Gastroenterol Hepatol* **3**: 28-38.
6. Youngberg, C. A., R. R. Berardi, W. F. Howatt, M. L. Hyneck, G. L. Amidon, J. H. Meyer, and J. B. Dressman. 1987. Comparison of gastrointestinal pH in cystic fibrosis and healthy subjects. *Dig. Dis. Sci.* **32**: 472-480.
7. Zentler-Munro, P. L., D. R. Fine, W. J. Fitzpatrick, and T. C. Northfield. 1984. Effect of intrajejunal acidity on lipid digestion and aqueous solubilisation of bile acids and lipids in health, using a new simple method of lipase inactivation. *Gut* **25**: 491-499.
8. Zentler-Munro, P. L., W. J. Fitzpatrick, J. C. Batten, and T. C. Northfield. 1984. Effect of intrajejunal acidity on aqueous phase bile acid and lipid concentrations in pancreatic steatorrhoea due to cystic fibrosis. *Gut* **25**: 500-507.
9. Sander-Struckmeier, S., K. Beckmann, G. Janssen-van Solingen, and P. Pollack. 2013. Retrospective analysis to investigate the effect of concomitant use of gastric acid-suppressing drugs on the efficacy and safety of pancrelipase/pancreatin (CREON(R)) in patients with pancreatic exocrine insufficiency. *Pancreas* **42**: 983-989.
10. Vecht, J., T. Symersky, C. B. Lamers, and A. A. Masclee. 2006. Efficacy of lower than standard doses of pancreatic enzyme supplementation therapy during acid inhibition in patients with pancreatic exocrine insufficiency. *J Clin Gastroenterol* **40**: 721-725.

11. DiMagno, E. P. 2001. Gastric acid suppression and treatment of severe exocrine pancreatic insufficiency. *Best Pract Res Clin Gastroenterol* **15**: 477-486.
12. Regan, P. T., J. R. Malagelada, E. P. Dimagno, and V. L. Go. 1979. Reduced intraluminal bile acid concentrations and fat maldigestion in pancreatic insufficiency: correction by treatment. *Gastroenterology* **77**: 285-289.
13. Hofmann, A. F. 1963. The Function of Bile Salts in Fat Absorption. The Solvent Properties of Dilute Micellar Solutions of Conjugated Bile Salts. *Biochem J* **89**: 57-68.
14. Hofmann, A. F., and B. Borgström. 1964. The Intraluminal Phase of Fat Digestion in Man: The Lipid Content of the Micellar and Oil Phases of Intestinal Content Obtained During Fat Digestion and Absorption. *J Clin Invest* **43**: 247-257.
15. Hofmann, A. F. 1999. Bile Acids: The Good, the Bad, and the Ugly. *News Physiol Sci* **14**: 24-29.
16. Thomas, C., R. Pellicciari, M. Pruzanski, J. Auwerx, and K. Schoonjans. 2008. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* **7**: 678-693.
17. Humbert, L., M. A. Maubert, C. Wolf, H. Duboc, M. Mahe, D. Farabos, P. Seksik, J. M. Mallet, G. Trugnan, J. Masliah, and D. Rainteau. 2012. Bile acid profiling in human biological samples: comparison of extraction procedures and application to normal and cholestatic patients. *J Chromatogr B Analyt Technol Biomed Life Sci* **899**: 135-145.
18. Reid, F., J. Peacock, B. Coker, V. McMillan, C. Lewis, S. Keevil, R. Sherwood, G. Vivian, R. Logan, and J. Summers. 2016. A Multicenter Prospective Study to Investigate the Diagnostic Accuracy of the SeHCAT Test in Measuring Bile Acid Malabsorption: Research Protocol. *JMIR Res Protoc* **5**: e13.
19. Cavalier, J. F., D. Lafont, P. Boullanger, D. Houisse, J. Giallo, J. M. Ballester, and F. Carriere. 2009. Validation of lipolysis product extraction from aqueous/biological samples, separation and quantification by thin-layer chromatography with flame ionization detection analysis using O-cholesteryl ethylene glycol as a new internal standard. *Journal of Chromatography A* **1216**: 6543-6548.
20. Carrière, F., J. A. Barrowman, R. Verger, and R. Laugier. 1993. Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterology* **105**: 876-888.

21. Hydén, S. 1956. A turbidimetric method for determination of higher polyethyleneglycols in biological materials. *Annals of the Royal Agricultural Coll.* **22**: 139-145.
22. Malawer, S. J., and D. W. Powell. 1967. An improved turbidimetric analysis of polyethylene glycol utilizing an emulsifier. *Gastroenterology* **53**: 250-256.
23. Erlanson, C., and B. Borgström. 1970. Tributyrin as a substrate for determination of lipase activity of pancreatic juice and small intestinal content. *Scand. J. Gastroenterol.* **5**: 293-295.
24. Roda, A., A. F. Hofmann, and K. J. Mysels. 1983. The influence of bile salt structure on self-association in aqueous solutions. *J Biol Chem* **258**: 6362-6370.
25. Smith, J. L., P. J. Lewindon, A. C. Hoskins, T. N. Pereira, K. D. Setchell, N. C. O'Connell, R. W. Shepherd, and G. A. Ramm. 2004. Endogenous ursodeoxycholic acid and cholic acid in liver disease due to cystic fibrosis. *Hepatology* **39**: 1673-1682.
26. Lengsfeld, H., G. Beaumier-Gallon, H. Chahinian, A. De Caro, R. Verger, R. Laugier, and F. Carrière. 2004. Physiology of gastrointestinal lipolysis and therapeutical use of lipases and digestive lipase inhibitors. *In* Lipases and phospholipases in drug development. G. Müller and S. Petry, editors. Wiley-VCH, Weinheim. 195-229.
27. Carey, M. C. 1982. The enterohepatic circulation. *In* The liver: biology and pathobiology. I. Arias, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 429-465.
28. Clarysse, S., J. Tack, F. Lammert, G. Duchateau, C. Reppas, and P. Augustijns. 2009. Postprandial evolution in composition and characteristics of human duodenal fluids in different nutritional states. *J Pharm Sci* **98**: 1177-1192.
29. Ladas, S. D., P. E. Isaacs, G. M. Murphy, and G. E. Sladen. 1984. Comparison of the effects of medium and long chain triglyceride containing liquid meals on gall bladder and small intestinal function in normal man. *Gut* **25**: 405-411.
30. Hernell, O., J. E. Staggars, and M. C. Carey. 1990. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry* **29**: 2041-2056.

31. Tagerman, A., A. van Schaik, and E. W. van der Hoek. 1986. Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects. *Clin Chim Acta* **159**: 123-132.
32. Rautureau, M., A. Bisalli, and J. C. Rambaud. 1981. [Bile salts and lipids in aqueous intraluminal phase during the digestion of a standard meal in normal man (author's transl)]. *Gastroenterol Clin Biol* **5**: 417-425.
33. Fausa, O. 1974. Duodenal bile acids after a test meal. *Scand. J. Gastroenterol.* **9**: 567-570.
34. Monte, M. J., J. J. Marin, A. Antelo, and J. Vazquez-Tato. 2009. Bile acids: chemistry, physiology, and pathophysiology. *World J Gastroenterol* **15**: 804-816.
35. Schmid, A., H. Neumann, T. Karrasch, G. Liebisch, and A. Schaffler. 2016. Bile Acid Metabolome after an Oral Lipid Tolerance Test by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). *PLoS One* **11**: e0148869.
36. Go, V. L., J. R. Poley, A. F. Hofmann, and W. H. Summerskill. 1970. Disturbances in fat digestion induced by acidic jejunal pH due to gastric hypersecretion in man. *Gastroenterology* **58**: 638-646.
37. Yoon, Y. B., L. R. Hagey, A. F. Hofmann, D. Gurantz, E. L. Michelotti, and J. H. Steinbach. 1986. Effect of side-chain shortening on the physiologic properties of bile acids: hepatic transport and effect on biliary secretion of 23-nor-ursodeoxycholate in rodents. *Gastroenterology* **90**: 837-852.
38. Xia, X., H. Francis, S. Glaser, G. Alpini, and G. LeSage. 2006. Bile acid interactions with cholangiocytes. *World J Gastroenterol* **12**: 3553-3563.
39. Lazaridis, K. N., L. Pham, P. Tietz, R. A. Marinelli, P. C. deGroen, S. Levine, P. A. Dawson, and N. F. LaRusso. 1997. Rat cholangiocytes absorb bile acids at their apical domain via the ileal sodium-dependent bile acid transporter. *J Clin Invest* **100**: 2714-2721.
40. Ballatori, N., W. V. Christian, J. Y. Lee, P. A. Dawson, C. J. Soroka, J. L. Boyer, M. S. Madejczyk, and N. Li. 2005. OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. *Hepatology* **42**: 1270-1279.
41. Debray, D., D. Rainteau, V. Barbu, M. Rouahi, H. El Mourabit, S. Lerondel, C. Rey, L. Humbert, D. Wendum, C. H. Cottart, P. Dawson, N. Chignard, and C. Housset. 2012.

Defects in gallbladder emptying and bile Acid homeostasis in mice with cystic fibrosis transmembrane conductance regulator deficiencies. *Gastroenterology* **142**: 1581-1591 e1586.

42. Boyer, J. L., and C. J. Soroka. 2012. A cholecystohepatic shunt pathway: does the gallbladder protect the liver? *Gastroenterology* **142**: 1416-1419.

43. Lamri, Y., S. Erlinger, M. Dumont, A. Roda, and G. Feldmann. 1992. Immunoperoxidase localization of ursodeoxycholic acid in rat biliary epithelial cells. Evidence for a cholehepatic circulation. *Liver* **12**: 351-354.

44. Alpini, G., S. Glaser, L. Baiocchi, H. Francis, X. Xia, and G. Lesage. 2005. Secretin activation of the apical Na⁺-dependent bile acid transporter is associated with cholehepatic shunting in rats. *Hepatology* **41**: 1037-1045.

45. Cahen, D. L., A. M. van Berkel, D. Oskam, E. A. Rauws, G. J. Weverling, K. Huibregtse, and M. J. Bruno. 2005. Long-term results of endoscopic drainage of common bile duct strictures in chronic pancreatitis. *Eur J Gastroenterol Hepatol* **17**: 103-108.

46. Hofmann, A. F., and K. J. Mysels. 1992. Bile acid solubility and precipitation in vitro and in vivo: the role of conjugation, pH, and Ca²⁺ ions. *J Lipid Res* **33**: 617-626.

47. Telbisz, A., and L. Homolya. 2016. Recent advances in the exploration of the bile salt export pump (BSEP/ABCB11) function. *Expert Opin Ther Targets* **20**: 501-514.

48. Vijayvargiya, P., M. Camilleri, P. Carlson, A. Lueke, J. O'Neill, D. Burton, I. Busciglio, and L. Donato. 2017. Performance characteristics of serum C4 and FGF19 measurements to exclude the diagnosis of bile acid diarrhoea in IBS-diarrhoea and functional diarrhoea. *Aliment Pharmacol Ther* **46**: 581-588.

49. Hofmann, A. F., and L. R. Hagey. 2008. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci* **65**: 2461-2483.

50. Hofmann, A. F., and L. Eckmann. 2006. How bile acids confer gut mucosal protection against bacteria. *Proc Natl Acad Sci U S A* **103**: 4333-4334.

51. Lillienau, J., C. D. Schteingart, and A. F. Hofmann. 1992. Physicochemical and physiological properties of choly sarcosine. A potential replacement detergent for bile acid deficiency states in the small intestine. *J Clin Invest* **89**: 420-431.

52. Schmassmann, A., H. F. Fehr, J. Locher, J. Lillienau, C. D. Schteingart, S. S. Rossi, and A. F. Hofmann. 1993. Cholylsarcosine, a new bile acid analogue: metabolism and effect on biliary secretion in humans. *Gastroenterology* **104**: 1171-1181.
53. Gruy-Kapral, C., K. H. Little, J. S. Fordtran, T. L. Meziere, L. R. Hagey, and A. F. Hofmann. 1999. Conjugated bile acid replacement therapy for short-bowel syndrome. *Gastroenterology* **116**: 15-21.
54. Shlieout, G., B. Boedecker, S. Schaefer, B. Thumbeck, and P.-C. Gregory. 2005. Oral pharmaceutical compositions of lipase-containing products, in particular of pancreatin, containing surfactants. In: S. P. Gmbh, editor, Germany.
55. Lukovac, S., K. E. Gooijert, P. C. Gregory, G. Shlieout, F. Stellaard, E. H. Rings, and H. J. Verkade. 2010. Gelucire 44/14 improves fat absorption in rats with impaired lipolysis. *Biochim Biophys Acta* **1801**: 665-673.

TABLES

Table 1. *Bile acid outputs (μ moles) and distribution (expressed as mole % of total BA) in duodenal contents of healthy volunteers (n=6) and patients with severe chronic pancreatitis (n=6) after intake of a liquid-solid test meal. Abbreviations: CA, Cholic acid ; CA-3S, Cholic acid 3-sulfate; CDCA, Chenodeoxycholic acid; CDCA-3S, Chenodeoxycholic acid 3-sulfate; DCA, Deoxycholic acid; DCA-3S, Deoxycholic acid 3-sulfate; GCA, Glycocholic acid; GCDCA, Glycochenodeoxycholic acid; GDCA, Glycodeoxycholic acid; GHCA, Glycohyocholic acid; GHDCA, Glycohyodeoxycholic acid; GLCA, Glycolithocholic acid; GLCA-3S, Glycolithocholic acid 3-sulfate; GUDCA, Glycoursodeoxycholic acid; GUDCA-3S, Glycoursodeoxycholic acid 3-sulfate; HCA, Hyocholic acid; HDCA, Hyodeoxycholic acid; LCA, Lithocholic acid; LCA-3S, Lithocholic acid 3-sulfate; MCA, Muricholic acid; TCA, Taurocholic acid; TCDCA, Taurochenodeoxycholic acid; TDCA, Taurodeoxycholic acid; THCA, Taurohyocholic acid; THDCA, Taurohyodeoxycholic acid; TLCA, Tauroolithocholic acid; TLCA-3S, Tauroolithocholic acid 3-sulfate; TUDCA, Tauroursodeoxycholic acid; TUDCA-3S, Tauroursodeoxycholic acid 3-sulfate; UDCA, Ursodeoxycholic acid; UDCA-3S, Ursodeoxycholic acid 3-sulfate;*

Bile acids	Healthy volunteers (controls)		Chronic Pancreatitis patients		
	Output (μ moles)	Total BA % (mole %)	Output (μ moles)	Total BA % (mole %)	% of controls
TUDCA	53.02 $\pm$ 58.93	0.78 $\pm$ 0.79	0.67 $\pm$ 0.53	0.07 $\pm$ 0.03	1.27
THDCA	10.04 $\pm$ 14.32	0.08 $\pm$ 0.12	0.54 $\pm$ 0.75	0.02 $\pm$ 0.04	5.42
TCDCA	777.22 $\pm$ 618.97	14.56 $\pm$ 2.99	101.68 $\pm$ 94.82	8.78 $\pm$ 3.15	13.08

(continued)

Bile acids	Healthy volunteers (controls)		Chronic Pancreatitis patients		
	Output (μ moles)	Total BA % (mole %)	Output (μ moles)	Total BA % (mole %)	% of controls
TDCA	522.95 $\pm$ 549.81	7.80 $\pm$ 4.93	27.33 $\pm$ 32.81	1.71 $\pm$ 1.37	5.23
THCA	2.30 $\pm$ 1.79	0.05 $\pm$ 0.02	1.36 $\pm$ 1.37	0.16 $\pm$ 0.21	59.35
TCA	827.88 $\pm$ 570.98	19.13 $\pm$ 8.82	229.80 $\pm$ 214.30	22.76 $\pm$ 12.92	27.76
GLCA-3S	4.43 $\pm$ 3.01	0.11 $\pm$ 0.06	0.43 $\pm$ 0.56	0.02 $\pm$ 0.03	9.62
TLCA-3S	5.81 $\pm$ 4.63	0.14 $\pm$ 0.08	0.27 $\pm$ 0.37	0.01 $\pm$ 0.02	4.62
UDCA-3S	0.03 $\pm$ 0.03	<0.01	0.00	0.00	0.00
CDCA-3S	0.05 $\pm$ 0.04	<0.01	0.01 $\pm$ 0.01	<0.01	15.33
DCA-3S	0.23 $\pm$ 0.24	<0.01	0.02 $\pm$ 0.02	<0.01	10.16
LCA-3S	0.21 $\pm$ 0.10	<0.01	0.02 $\pm$ 0.02	<0.01	8.12
CA-3S	0.00	-	0.00	-	-
TUDCA-3S	0.42 $\pm$ 0.49	0.01 $\pm$ 0.01	0.01 $\pm$ 0.02	<0.01	3.22
GUDCA-3S	1.92 $\pm$ 1.65	0.03 $\pm$ 0.02	0.09 $\pm$ 0.12	0.01 $\pm$ 0.01	4.86
GLCA	20.37 $\pm$ 13.31	0.43 $\pm$ 0.30	2.39 $\pm$ 3.06	0.15 $\pm$ 0.18	11.76
GUDCA	181.35 $\pm$ 193.46	2.33 $\pm$ 1.52	5.38 $\pm$ 5.51	0.61 $\pm$ 0.45	2.97
GHDCA	29.32 $\pm$ 28.11	0.42 $\pm$ 0.15	1.40 $\pm$ 1.74	0.10 $\pm$ 0.08	4.77
GCDCA	1438.15 $\pm$ 1126.45	26.19 $\pm$ 8.74	278.11 $\pm$ 277.77	29.35 $\pm$ 11.58	19.34
GDCA	889.17 $\pm$ 867.84	12.55 $\pm$ 5.13	79.39 $\pm$ 99.12	5.71 $\pm$ 4.87	8.93
GHCA	1.74 $\pm$ 1.41	0.03 $\pm$ 0.02	0.90 $\pm$ 0.73	0.13 $\pm$ 0.16	51.63
GCA	736.51 $\pm$ 559.03	15.02 $\pm$ 4.34	242.89 $\pm$ 184.29	28.23 $\pm$ 8.82	32.98
TLCA	13.83 $\pm$ 11.07	0.29 $\pm$ 0.22	1.02 $\pm$ 1.22	0.06 $\pm$ 0.05	7.40

(continued)

Bile acids	Healthy volunteers (controls)		Chronic Pancreatitis patients		
	Output (μ moles)	Total BA % (mole %)	Output (μ moles)	Total BA % (mole %)	% of controls
LCA	0.02 $\pm$ 0.03	<0.01	0.06 $\pm$ 0.10	<0.01	315.21
UDCA	0.11 $\pm$ 0.10	<0.01	0.04 $\pm$ 0.07	<0.01	36.24
HDCA	0.00	-	0.00	-	-
CDCA	0.84 $\pm$ 0.66	0.02 $\pm$ 0.01	5.71 $\pm$ 9.10	0.41 $\pm$ 0.77	680.48
DCA	0.28 $\pm$ 0.21	<0.01	2.27 $\pm$ 3.72	0.15 $\pm$ 0.32	804.00
MCA	0.01 $\pm$ 0.01	<0.01	0.03 $\pm$ 0.05	<0.01	312.19
HCA	0.00	-	0.00	-	-
CA	1.77 $\pm$ 1.50	0.04 $\pm$ 0.03	16.62 $\pm$ 22.53	1.54 $\pm$ 1.87	937.62
Total BA	5519.97 $\pm$ 4534.78	100.00	998.46 $\pm$ 889.62	100.00	18.09

Table 2. Kinetics (AUC, $\mu\text{moles.L}^{-1}.\text{min}$) and composition (mole %) of bile acids in serum of healthy volunteers (n=6) and patients with severe chronic pancreatitis (n=6) after intake of a liquid-solid test meal.

Bile acids	Healthy volunteers (controls)		Chronic Pancreatitis patients		
	AUC ($\mu\text{moles.L}^{-1}.\text{min}$)	Total BA % (mole %)	AUC ($\mu\text{moles.L}^{-1}.\text{min}$)	Total BA % (mole %)	% of controls
TUDCA	0.00	-	0.00	-	-
THDCA	0.00	-	0.00	-	-
TCDCa	17.29 $\pm$ 12.50	5.93 $\pm$ 1.56	18.99 $\pm$ 12.38	6.70 $\pm$ 4.47	109.81
TDCA	4.19 $\pm$ 3.66	1.54 $\pm$ 0.67	3.57 $\pm$ 3.30	1.11 $\pm$ 1.05	85.19
THCA	0.00	-	0.00	-	-
TCA	6.51 $\pm$ 5.84	1.98 $\pm$ 0.78	12.92 $\pm$ 12.44	5.27 $\pm$ 5.63	198.41
GLCA-3S	0.73 $\pm$ 1.34	0.24 $\pm$ 0.34	1.42 $\pm$ 0.96	0.44 $\pm$ 0.26	193.32
TLCA-3S	0.09 $\pm$ 0.13	0.03 $\pm$ 0.04	0.80 $\pm$ 1.05	0.27 $\pm$ 0.32	891.11
UDCA-3S	0.00	-	0.00	-	-
CDCA-3S	0.00	-	0.00	-	-
DCA-3S	0.10 $\pm$ 0.19	0.03 $\pm$ 0.04	0.18 $\pm$ 0.27	0.05 $\pm$ 0.07	182.25
LCA-3S	0.00	-	0.00	-	-
CA-3S	0.02 $\pm$ 0.04	0.01 $\pm$ 0.02	0.00	-	0.00
TUDCA-3S	0.00	-	0.05 $\pm$ 0.08	0.03 $\pm$ 0.04	-
GUDCA-3S	0.72 $\pm$ 1.22	0.36 $\pm$ 0.47	1.58 $\pm$ 1.89	0.73 $\pm$ 1.02	218.94
GLCA	0.36 $\pm$ 0.72	0.10 $\pm$ 0.19	0.02 $\pm$ 0.06	0.01 $\pm$ 0.01	6.66
GUDCA	8.81 $\pm$ 8.30	3.77 $\pm$ 2.21	0.82 $\pm$ 1.37	0.21 $\pm$ 0.34	9.27
GHDCA	0.00	-	0.00	-	-

(continued)

Bile acids	Healthy volunteers (controls)		Chronic Pancreatitis patients		
	AUC ($\mu\text{moles.L}^{-1}.\text{min}$)	Total BA % (mole %)	AUC ($\mu\text{moles.L}^{-1}.\text{min}$)	Total BA % (mole %)	% of controls
GCDCA	109.08 $\pm$ 72.62	38.93 $\pm$ 9.02	140.99 $\pm$ 62.57	45.15 $\pm$ 6.32	129.26
GDCA	24.82 $\pm$ 23.00	8.58 $\pm$ 5.18	19.39 $\pm$ 13.71	5.64 $\pm$ 4.01	78.12
GHCA	0.00		0.00		-
GCA	27.79 $\pm$ 21.80	9.51 $\pm$ 3.52	45.60 $\pm$ 17.61	16.20 $\pm$ 6.61	164.09
TLCA	0.00	-	0.00	-	-
LCA	0.05 $\pm$ 0.12	0.02 $\pm$ 0.05	0.00	-	0.00
UDCA	8.14 $\pm$ 9.76	3.52 $\pm$ 3.68	0.26 $\pm$ 0.49	0.07 $\pm$ 0.14	3.21
HDCA	0.27 $\pm$ 0.4	0.08 $\pm$ 0.10	0.00	-	-
CDCA	20.03 $\pm$ 30.15	9.67 $\pm$ 11.80	21.69 $\pm$ 26.06	7.04 $\pm$ 7.32	108.26
DCA	21.82 $\pm$ 18.98	7.85 $\pm$ 2.09	14.85 $\pm$ 15.71	4.35 $\pm$ 4.06	68.05
MCA	0.56 $\pm$ 1.24	0.22 $\pm$ 0.50	0.00	-	0.00
HCA	0.41 $\pm$ 0.91	0.11 $\pm$ 0.25	0.00	-	-
CA	19.89 $\pm$ 32.67	7.48 $\pm$ 6.68	21.04 $\pm$ 16.13	6.75 $\pm$ 3.91	105.79
Total BA	271.69 $\pm$ 185.62	100.00	304.18 $\pm$ 104.84	100.00	111.96

Table 3. *Critical micellar concentrations of the main bile acids found in duodenal contents.*

CMC values are from Roda et al. (24) and were determined by surface tension measurements, using 0.15 M Na⁺ solution for bile salt dispersion. Occurrences in duodenal contents (mol % of total BAs) are based on data obtained with healthy subjects (HV).

Bile acids	Occurrence in duodenal contents (mol % of total BAs)	CMC (mM)
GCA	15.02	10
GDCA	12.55	2
GCDCA	26.19	1.8
GUDCA	2.33	4
TCA	19.13	6
TDCA	7.8	2.4
TCDCa	14.56	3
TUDCA	0.78	2.2
Weighted average		4.1

Figure 1: Variations with time in duodenal pH (A) and HPL concentration (B). Values are means $\pm$ SD (severe CP patients: n=6; HV: n=6). In panel A, stars indicate significant differences ($P<0.05$) between severe CP patients and HV. In panel B, lipase concentration is expressed in U/mL and values for CP patients were always significantly different ($P<0.005$) from those obtained with HV.

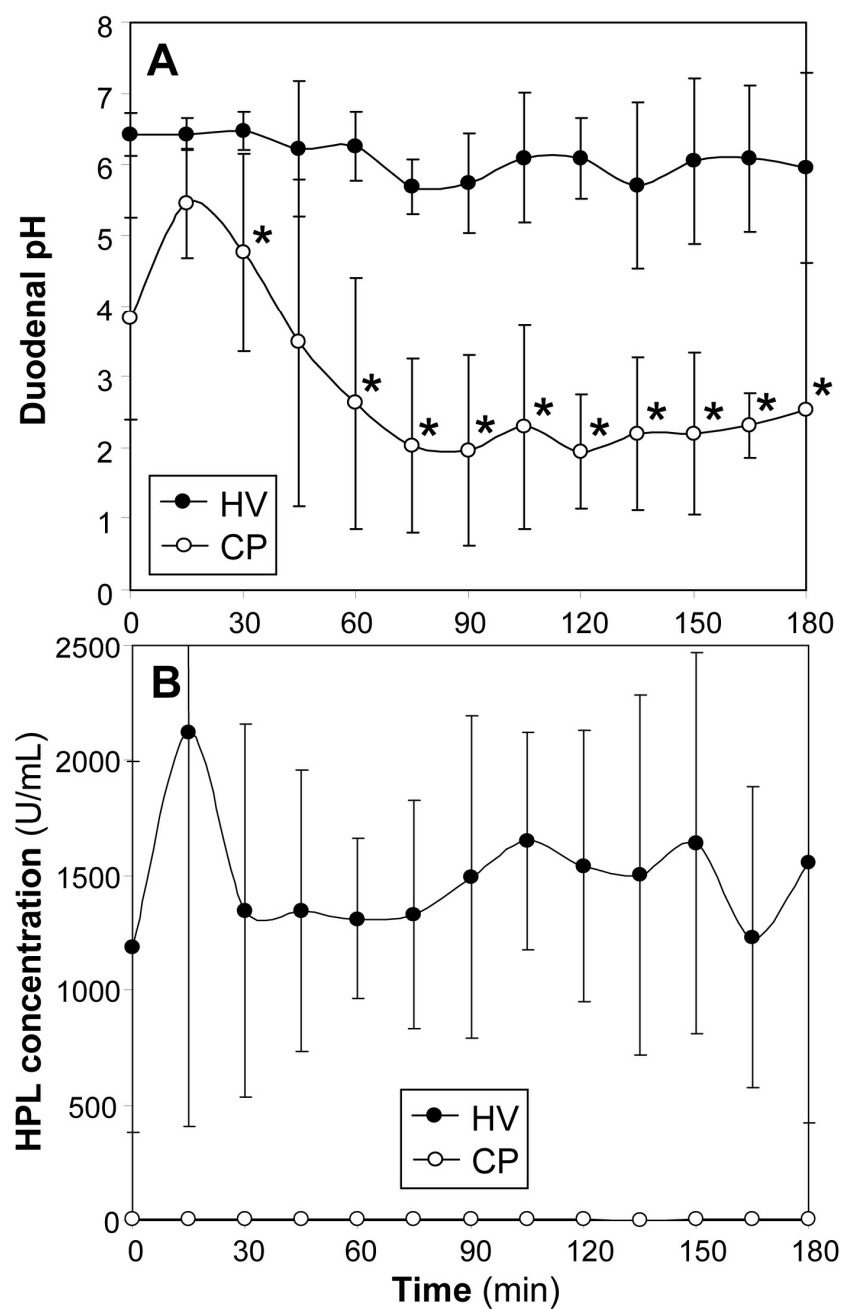


Figure 2: Bile acid (BA) levels in duodenal contents and blood plasma. (A) Variations with time in total BA concentration in duodenal contents. The horizontal dotted line indicates the mean CMC of main BAs finds in human bile (around 4 mM according to (24)). (B) Primary to secondary BA ratio in duodenal contents as a function of time. (C) Variations with time in total BA concentration in blood plasma. (D) Primary to secondary BA ratio in blood plasma as a function of time. The vertical dotted line in panels C and D indicates the time at which the test meal was given. Values are means $\pm$ SD (n=6 for CP patients and n=6 for HV).

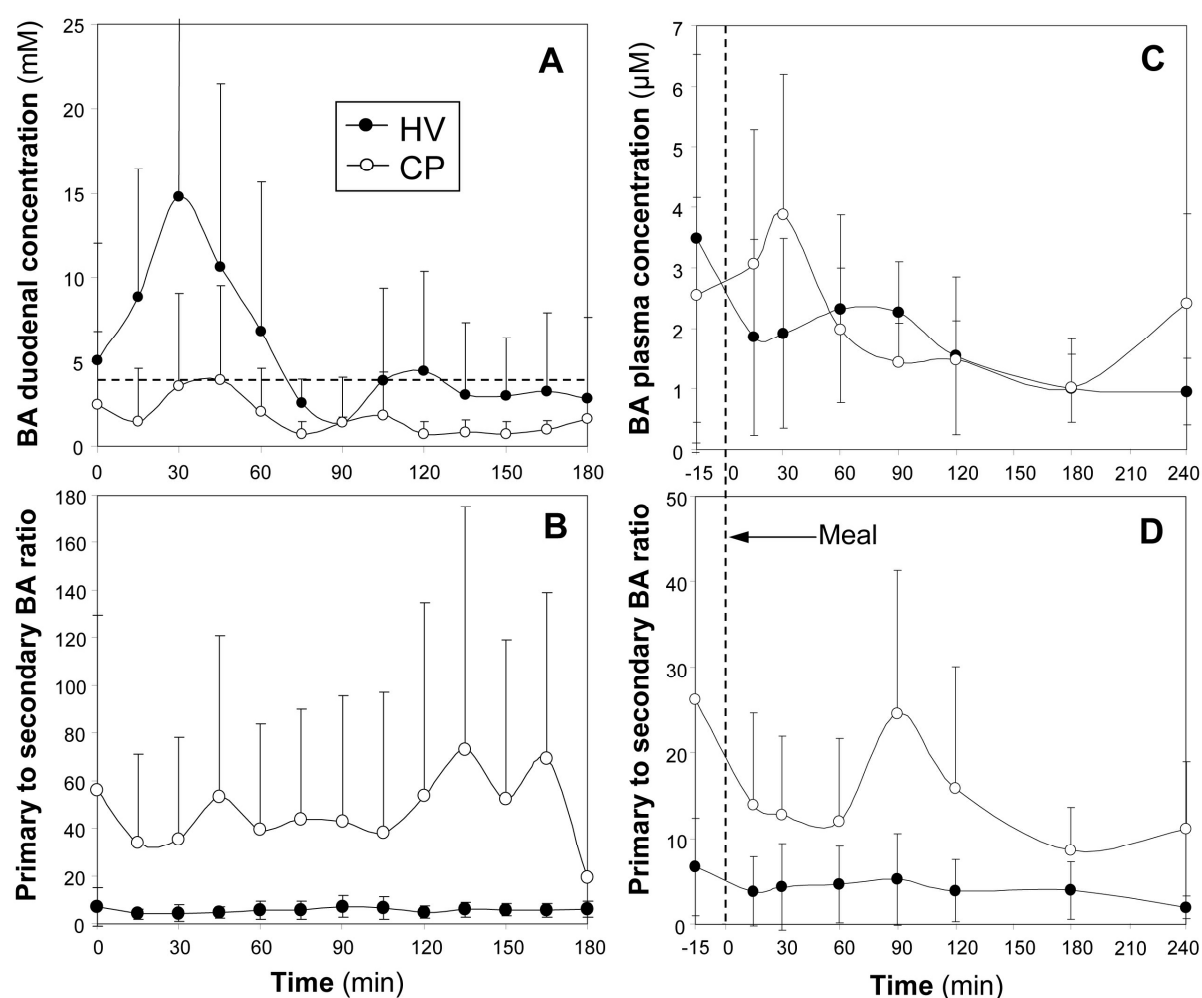


Figure 3: Comparison of main bile acid (BA) concentrations in duodenal contents and blood plasma of healthy volunteers (HV) and severe chronic pancreatitis (CP) patients. Panels A and B show the variations with time of the concentration (μM) of a representative bile acid (glycocholic acid; GCA) in duodenal contents and blood plasma, respectively. Individual data points for CP patients (n=6) and HV (n=6) are displayed at times for which both duodenal and blood plasma sample were collected (15, 30, 60, 90, 120 and 180 min). The solid and dotted lines represent the variations of mean values for HV and CP patients, respectively. Panels C and D show the concentrations (μM) in main bile acids measured 30 min after meal ingestion, in duodenal contents and blood plasma, respectively. All individual data point are displayed and the horizontal bars indicate median values for each group.

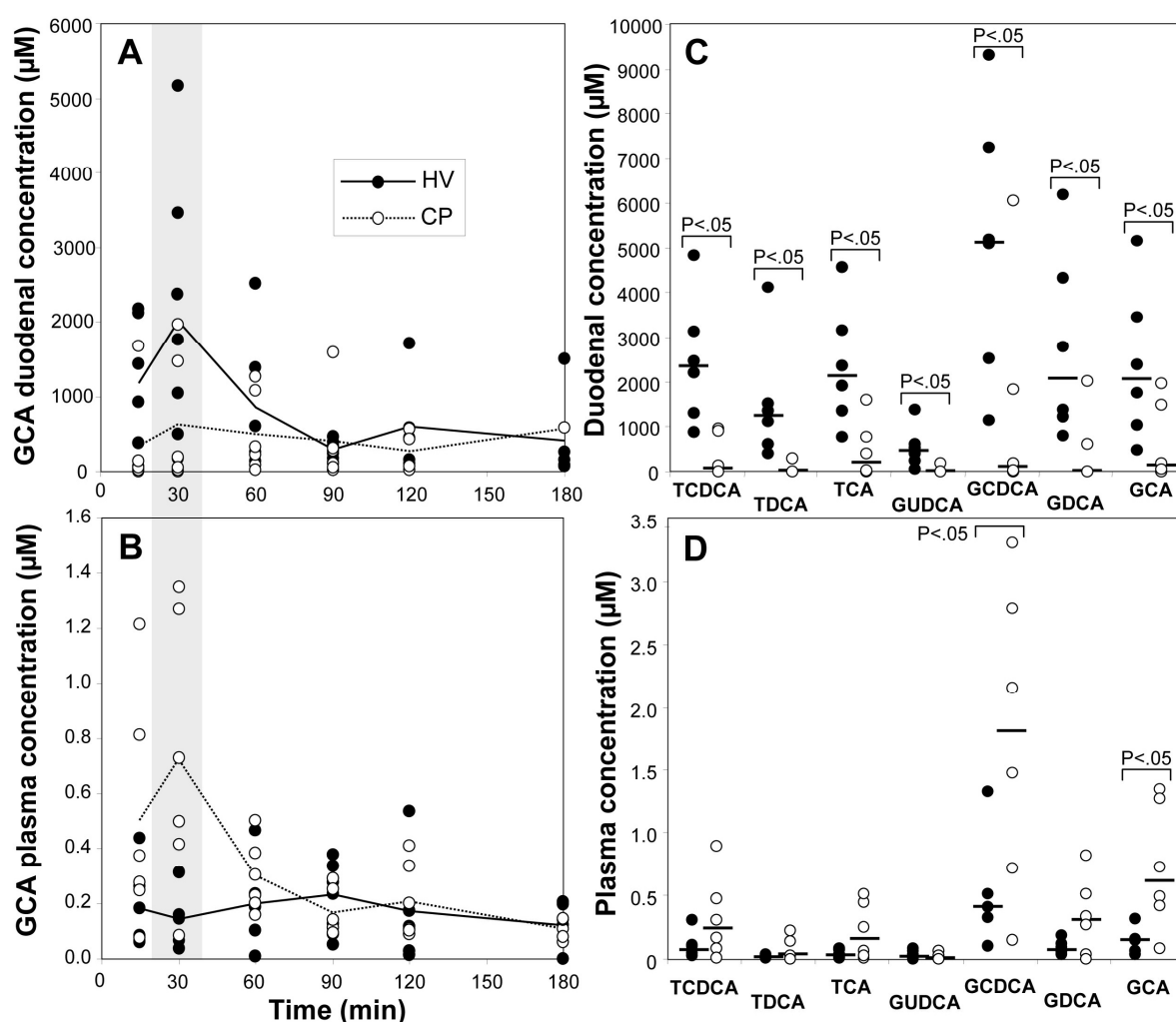
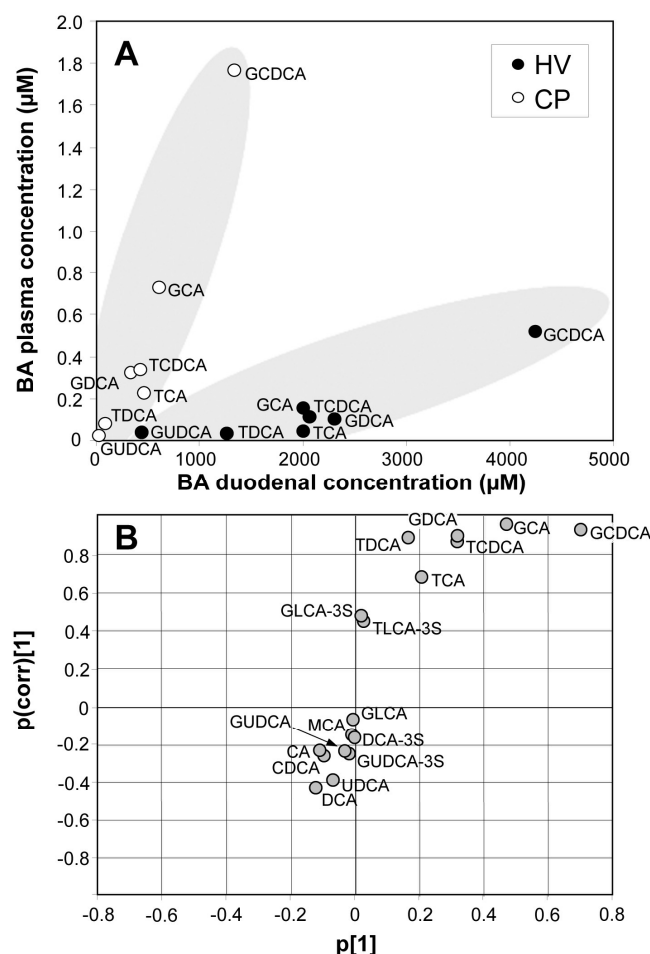


Figure 4: Discrimination of severe chronic pancreatitis (CP) patients and healthy volunteers (HV) based on bile acid (BA) analysis. (A) Correlation between BA duodenal and blood plasma concentrations measured 30 min after meal ingestion. Only main conjugated BA found in both duodenal contents and blood plasma are displayed. Values are means (n=6 for CP patients and n=6 for HV). (B) S-plot of the circulating blood plasma BAs 30 minutes after ingestion of the test meal. The plot illustrates the selection of the most significant biomarkers among of the various plasma BA (n=17) in an O-PLS model calculated to discriminate CP patients and HV. The BA levels measured in plasma by LC-MS/MS are “Pareto” scaled. The vertical axis in the S-plot (p(corr)) represents the correlation between BAs and the score of CP patients and HV. The horizontal axis (p(1)) is the contribution of the distinct BAs estimated by the O-PLS-DA model. The S-plots identifies BAs with the best prediction capability and the largest variation between the CP group (high unconjugated DCA, UDCA, CDCA) and the control group (high GDCA, GCA, TCDCA). $R^2X[1] = 0.617$, i.e. 61.7 % of the BA total variability.



SUPPLEMENTAL DATA

Postprandial bile acid levels in intestine and plasma reveal altered biliary circulation in chronic pancreatitis patients

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Table S1: *Clinical data on CP patients.* Six patients with severe chronic pancreatitis were enrolled in this study (mrtm03-01), approved on January 17, 2003 by the Institutional board of the ethics committee CCPPRB (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale) from Hôpital d'Adultes de la Timone, Marseille, and performed from March to July 2003 at "Centre de Pharmacologie Clinique et d'Etudes Thérapeutiques (CPCET), Marseille, France. Patients are identified by their initials. Hepatic enzymes: AST, aspartate transaminase (normal (N): 5-40 IU/mL); ALT, alanine transaminase (normal: 7-56 IU/mL); ALP, alkaline phosphatase (normal: 45-115 IU/mL); GGT, γ -glutamyltransferase (normal: 9-48 IU/mL).

Patient	1 (C.K.)	2 (E.H.T.)	3 (G.G.)	4 (G.R.)	5 (B.P.)	6 (J.C.V.)
Sex	Male	Male	Male	Male	Male	Male
Age	64	53	52	46	59	41
Body weight (kg)	81.6	58.0	70.2	74.0	62.0	91.6
Clinical data	Alcoholic chronic pancreatitis, pancreas divisum, main bile duct stenosis treated by endoscopy, type I diabetes, dyslipidemia, obesity, Chronic obstructive pulmonary disease	Alcoholic chronic pancreatitis, main bile duct stenosis treated by endoscopy since 1998, diabetes	Alcoholic chronic pancreatitis, main bile duct stenosis treated by endoscopy since 2000, diabetes	Alcoholic chronic pancreatitis, main bile duct stenosis treated by endoscopy, diabetes, hepatic disease	Alcoholic chronic pancreatitis, pancreas divisum, main bile duct stenosis treated by endoscopy since 2002, diabetes	Alcoholic chronic pancreatitis, treated for mesenteric vein ischemia without digestive resection, polyneuropathy due to alcohol
Pancreatic calcification	Yes	Yes	Yes	Yes	Yes	Yes
Steatorrhea (g/day fecal fat)	>7	45	8	>7	23	>7
AST (IU/mL)	21 (N)	23 (N)	19 (N)	97 (high, >2N)	19 (N)	17 (N)
ALT (IU/mL)	12 (N)	48 (N)	10 (N)	304 (high, 4N)	18 (N)	15 (N)
ALP (IU/mL)	66 (N)	104 (N)	59 (N)	528 (high, >4N)	52 (N)	66 (N)
GGT (IU/mL)	100 (high, 2N)	160 (high, 3N)	11 (N)	577 (high, 12N)	22 (N)	21 (N)

Table S2: *Critical micellar concentrations of various bile acids.* CMC values are from Roda et al. (1) and were determined by surface tension measurements, using pure water or 0.15 M Na⁺ solution for bile salt dispersion. The main BAs found in duodenal contents of healthy subjects (HV) are shown in blue. It is inferred from the table that in HV the arrangement of BA is probably micelles but dispersed monomers in CP patients (see Results).

Bile acids	CMC (mM)		Occurrence in duodenal contents (mol % of total BAs)
	H ₂ O	0.15 M Na ⁺	
CA	13	11	0,04
DCA	10	3	<0,01
CDCA	9	4	0,02
HCA	17	8	
HDCA	14	6	
UCA	60	39	
UDCA	19	7	<0,01
GCA	12	10	15,02
GDCA	6	2	12,55
GCDCA	6	1,8	26,19
GUCA	35	30	
GUDCA	12	4	2,33
TCA	10	6	19,13
TDCA	6	2,4	7,8
TCDCA	7	3	14,56
TUCA	52	40	
TUDCA	8	2,2	0,78

Figure S1: Tubes and experimental device for collecting samples of gastrointestinal contents during the digestion of a test meal. The fluxes of bile acids are indicated.

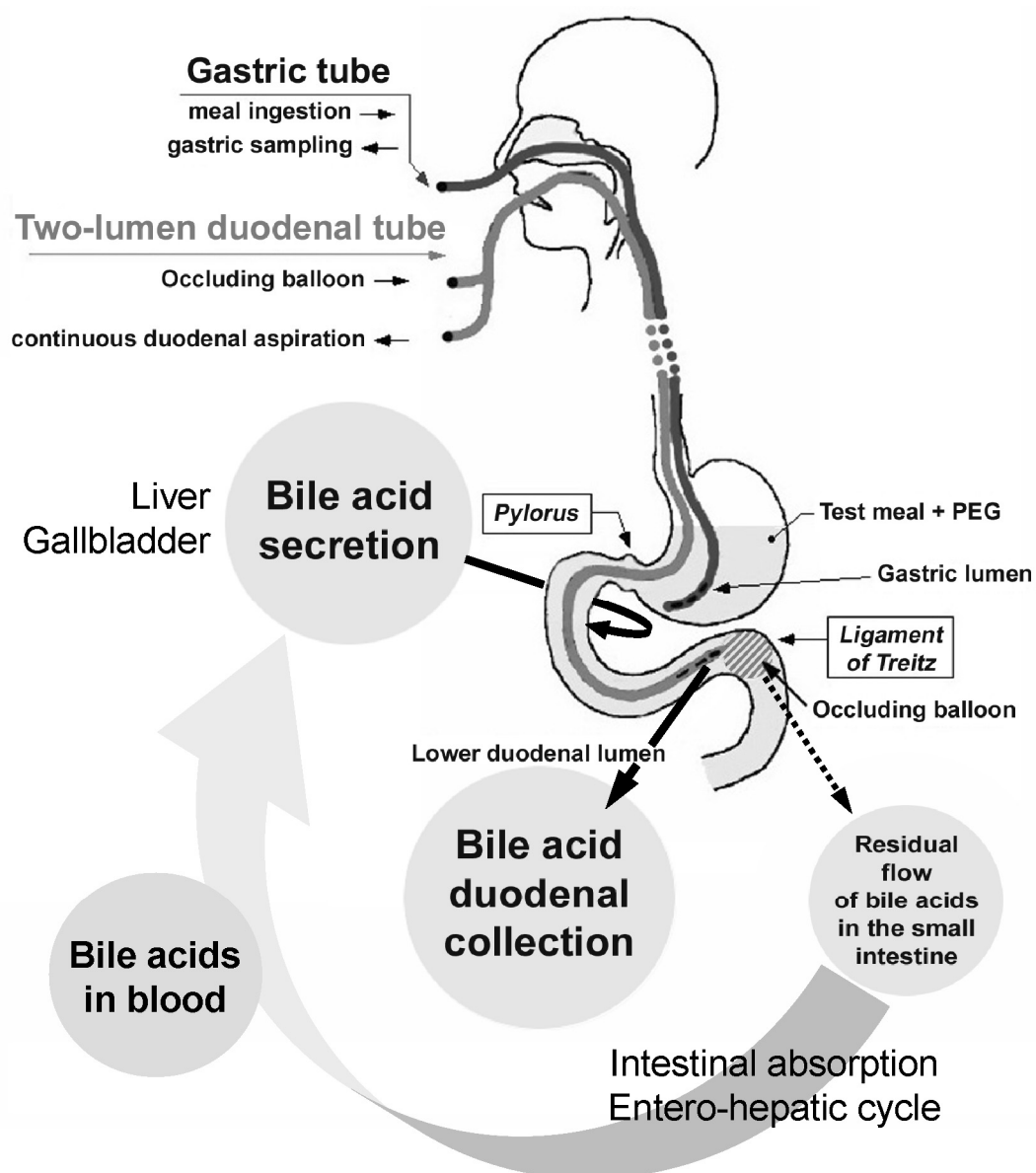


Figure S2: *Separation and identification of bile acid standards by LC-MS.* The chromatogram is obtained by merging the detection by parent ion/fragment ion pairs of the various bile acids (see Materials and Methods in the article).

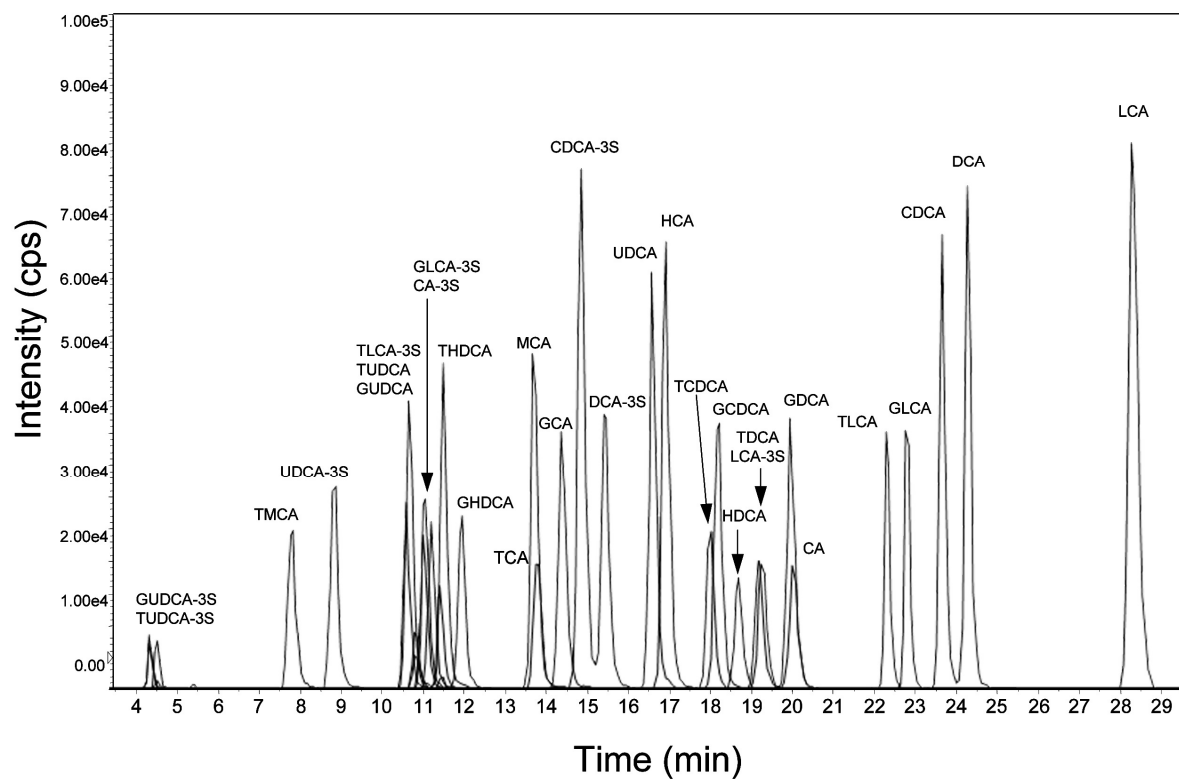


Figure S3: Variations with time of total and individual BA concentrations in duodenal contents and plasma. Open symbols correspond to CP patients while close symbols correspond to healthy volunteers (HV).

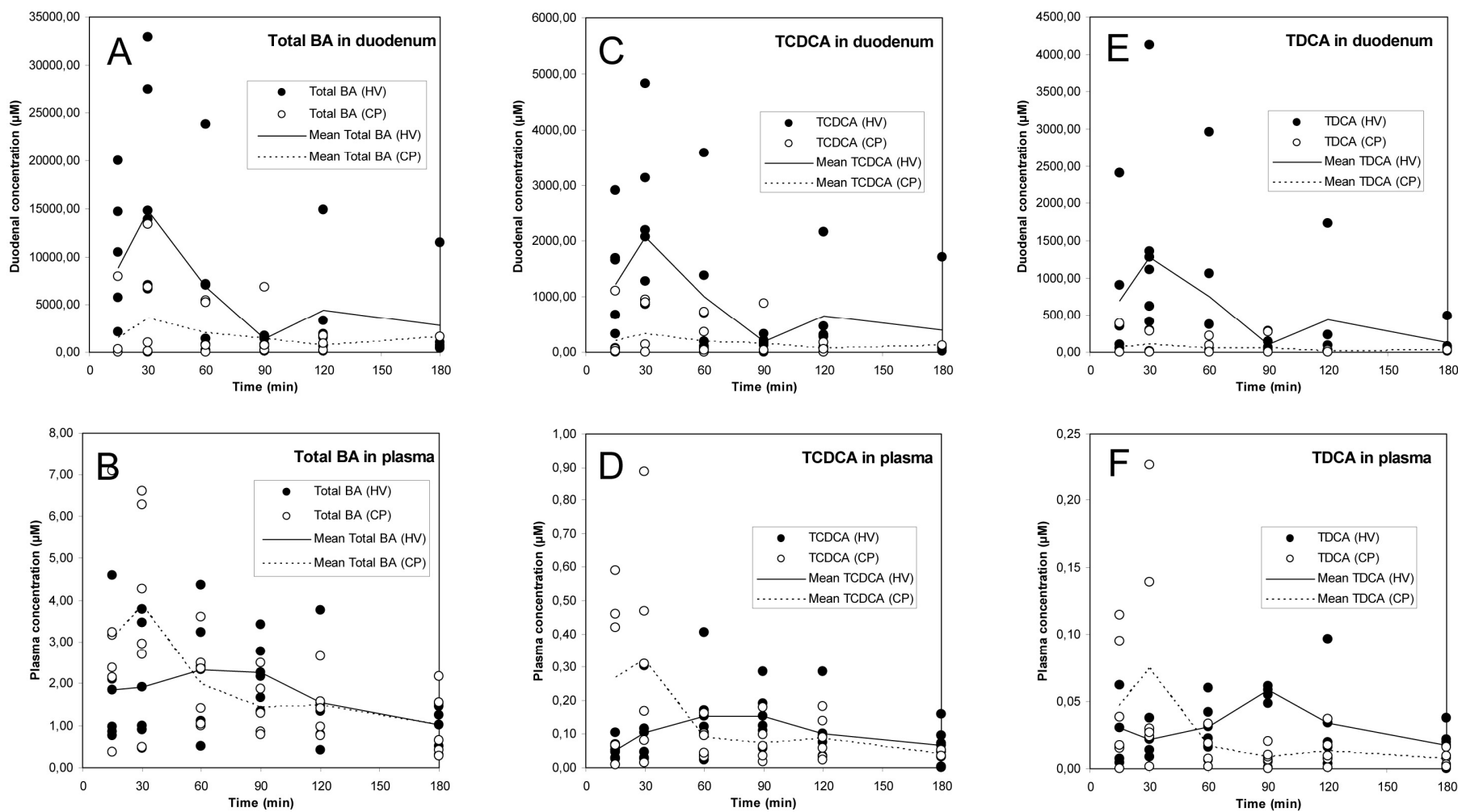


Figure S3 (continued): Variations with time of total and individual BA concentrations in duodenal contents and plasma. Open symbols correspond to CP patients while close symbols correspond to healthy volunteers (HV).

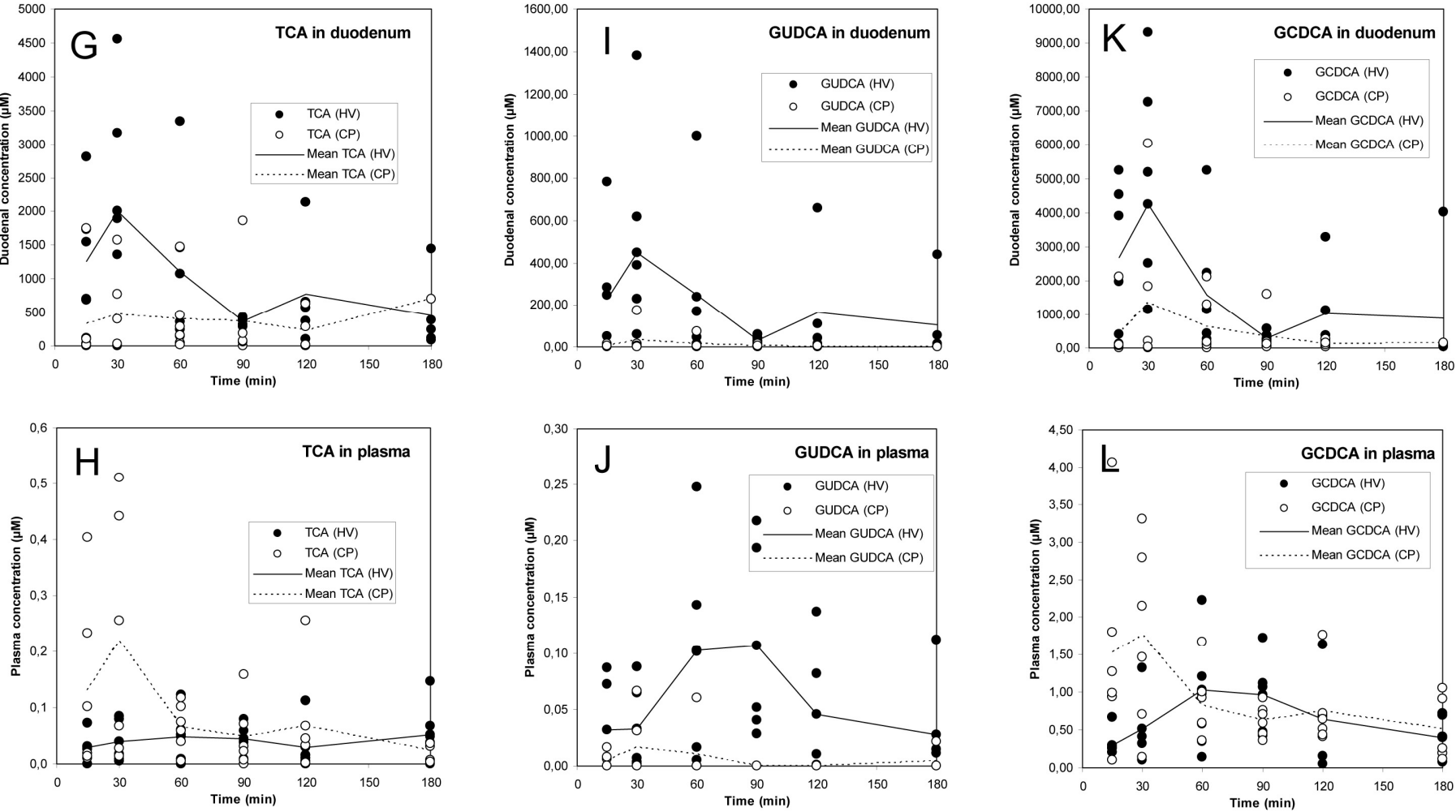


Figure S3 (continued): Variations with time of total and individual BA concentrations in duodenal contents and plasma. Open symbols correspond to CP patients while close symbols correspond to healthy volunteers (HV).

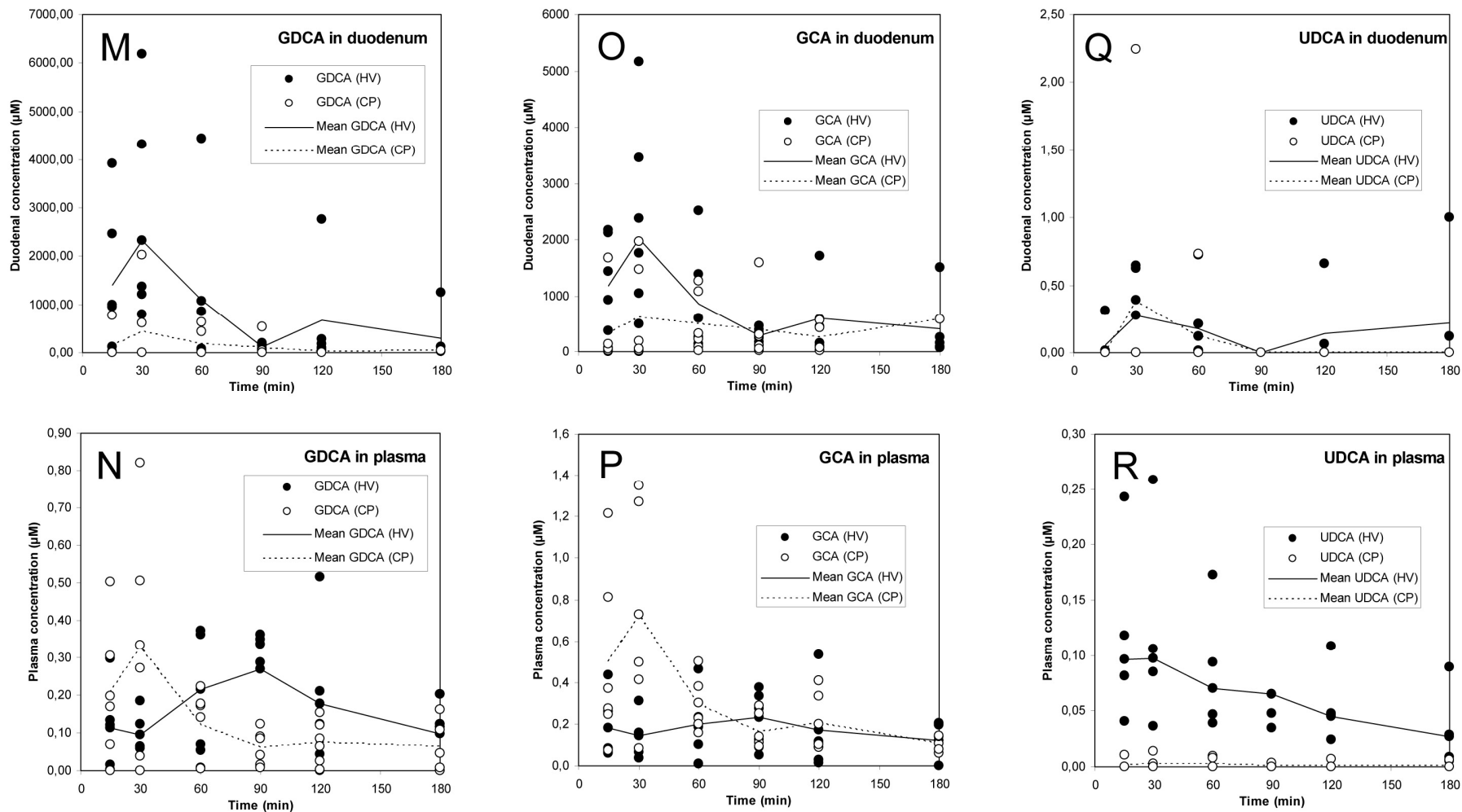


Figure S3 (continued): Variations with time of total and individual BA concentrations in duodenal contents and plasma. Open symbols correspond to CP patients while close symbols correspond to healthy volunteers (HV).

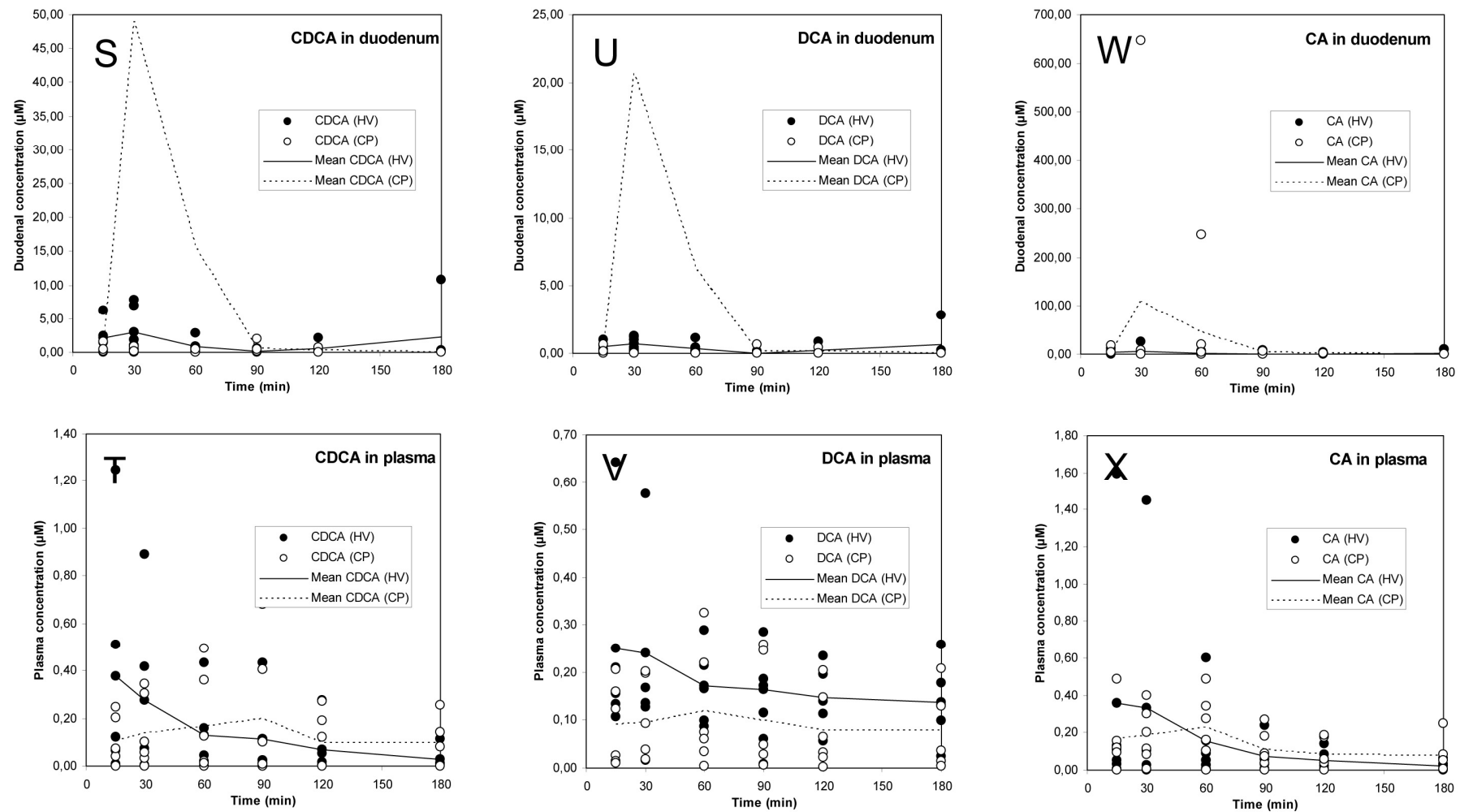


Figure S4: *Principal Components Analysis (PCA) showing the separation of chronic pancreatitis patients based on the plasma BAs assayed 30 minutes after the test meal.*

CP patients are characterized by their low duodenum lipase activity (HPL range 2-12 U) and healthy control (HV) by the high duodenum lipase activity (HPL range 320-3066 U). Scores of CP patients (white hexagonal symbols are numbered from 14 to 20D) and controls (light grey to black hexagonal symbols are numbered 1D0 to 13D) are projected on the factorial plan formed by the 2 first principal components $t(1)$ and $t(2)$. The principal components $t(1)$ and $t(2)$ comprises 39,4% and 22,2%, respectively, of the blood plasma BAs levels variance. Loadings in the PCA are also projected on the same factorial plan for 17 non-zero out of 25 blood plasma BAs detected and assayed individually in the plasma by LC-MS/MS (black filled circle symbols).

The scores of CP patients (white hexagonal symbols) are projected in areas (right) suggesting the enrichment in tauro- and glyco-conjugated BAs (TCA, TCDCA, TDCA, GCA, GDCA, GCDCA) relative to the control scores. Conversely, the scores of control plasmas (grey hexagonal symbols) are projected in the (left) areas indicating a relative enrichment as regard to CP patients in unconjugated BAs (CA, CDCA, DCA, MCA, UDCA). The minor species glycoconjugated UDCA are also enriched in control at 30min after meal.

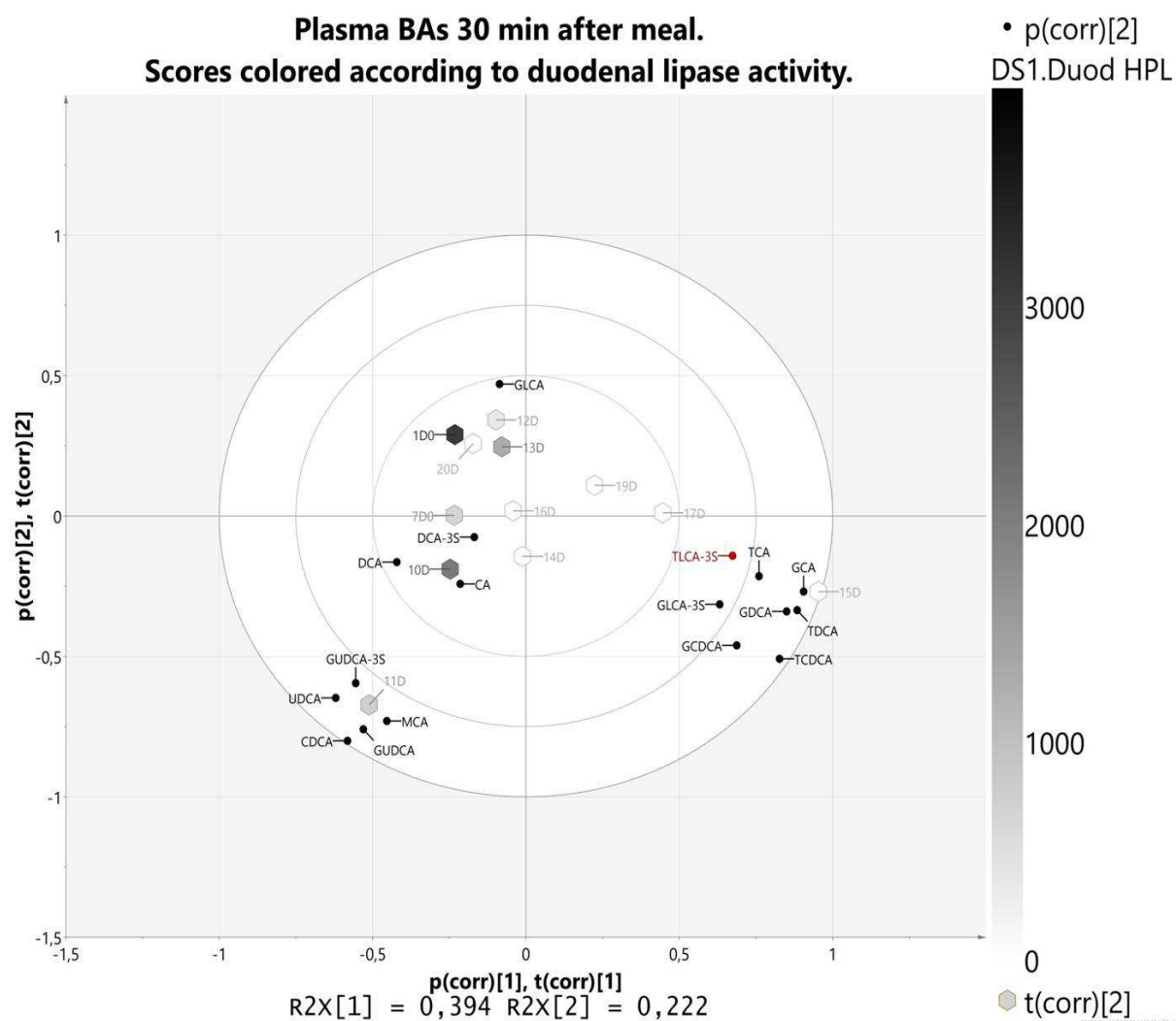


Figure S5: Discriminant analysis of BAs in plasma of CP patients and HV control groups according to a OPLS-DA model. A statistical model (OPLS for Orthogonal-Partial Least Square) was calculated and cross-validated for the variety of blood plasma BAs. The component predictive of either class CP (chronic pancreatitis patients) or class HV (healthy volunteers) is shown. 61,7% of the total variance of plasma bile acids is taken into account by the fitting in the calculated model.

The scores of patient or control and loadings of the individual BAs, which have been adjusted, are projected on the same factorial plan. The figure shows the separation of the 2 classes, patients and healthy volunteers. 5 out of 6 CP patients (empty hexagonal symbols) are projected in the upper part of the plot ($pq(\text{corr}) > 0$) but all 6 control HV (grey to black hexagonal symbols) are projected below the value ($pq(\text{corr}) < 0$) in the lower half of the plot.

In the lower half of the plot a high duodenal lipase activity identifies the control group with light grey to black colored hexagonal symbols.

Unconjugated BAs (UDCA, CDCA, DCA, CA, MCA) and glycoconjugated UDCA (GUDCA, GUDC-3S) and LCA (GLCA) show the highest loadings, i.e the highest proportions associated with the healthy volunteers group.

Conversely, Chronic Pancreatitis (CP) patients plasma BAs (empty symbols are associated to a very low HPL activity) are comprised of high loadings for tauroconjugated (TCDCA, TCA, TDCA, TLCA-3S) and glycoconjugated BAs (GDCA, GCA, GCDCA).

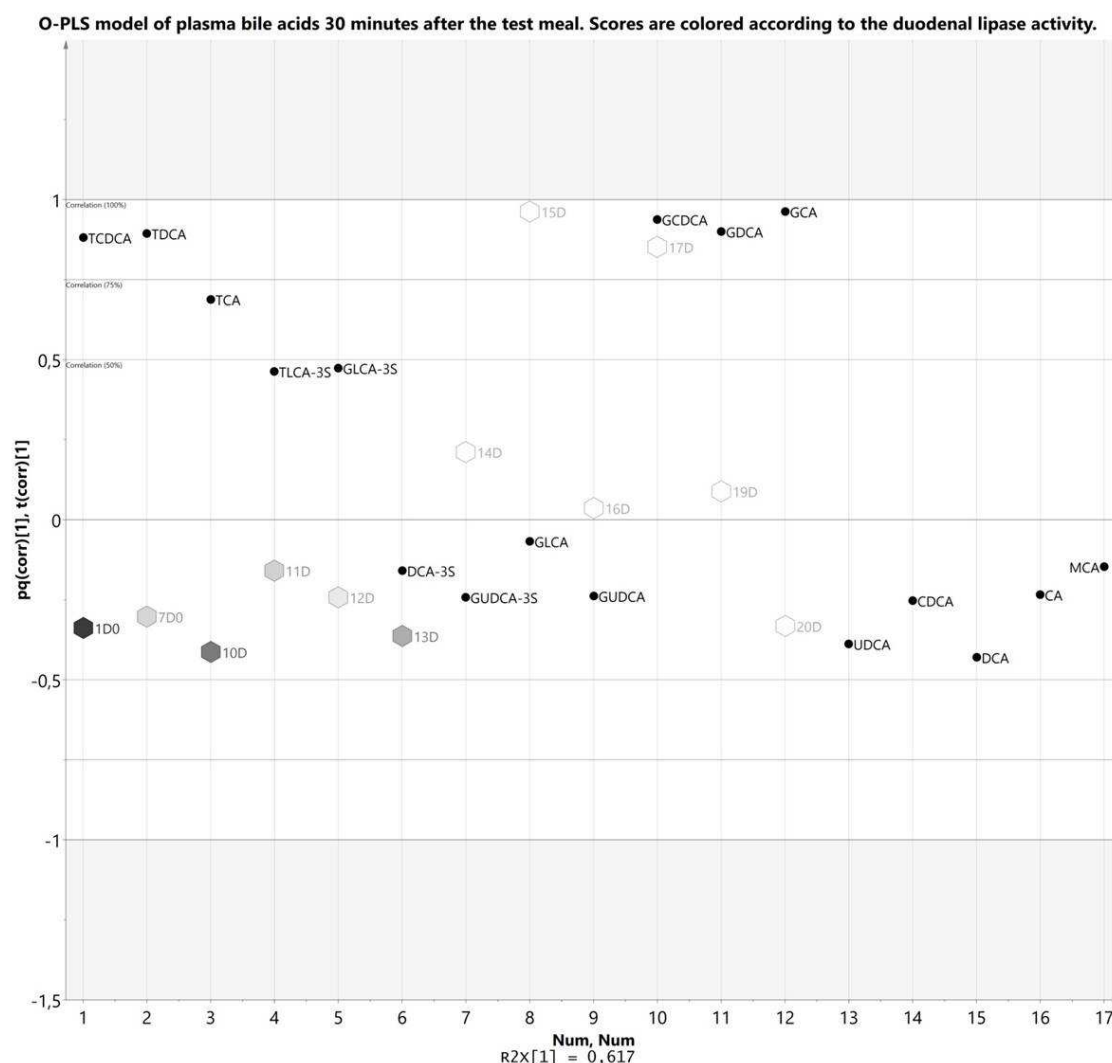


Figure S7: *Principal Component Analysis of BAs in the duodenum fluid 30 minutes after ingestion of the test meal. Scores of CP patients (empty hexagonal symbols) and healthy control (black hexagonal symbols) are calculated after exclusion of an outlying CP patient (16D). The control and patient groups are separated along the principal component t(1) (horizontal axis) but not clearly discriminated for a specific change in the duodenum BA composition.*

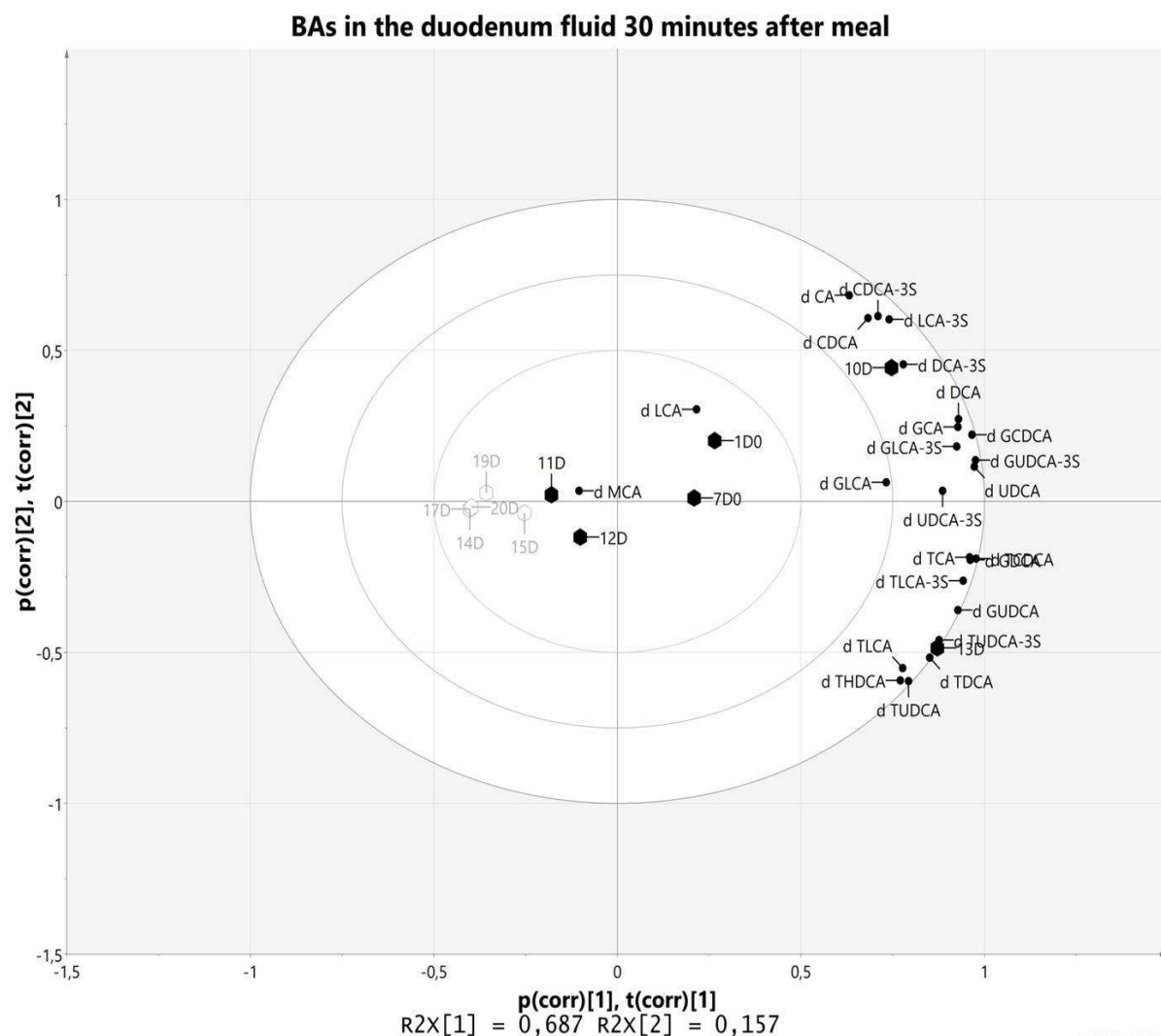
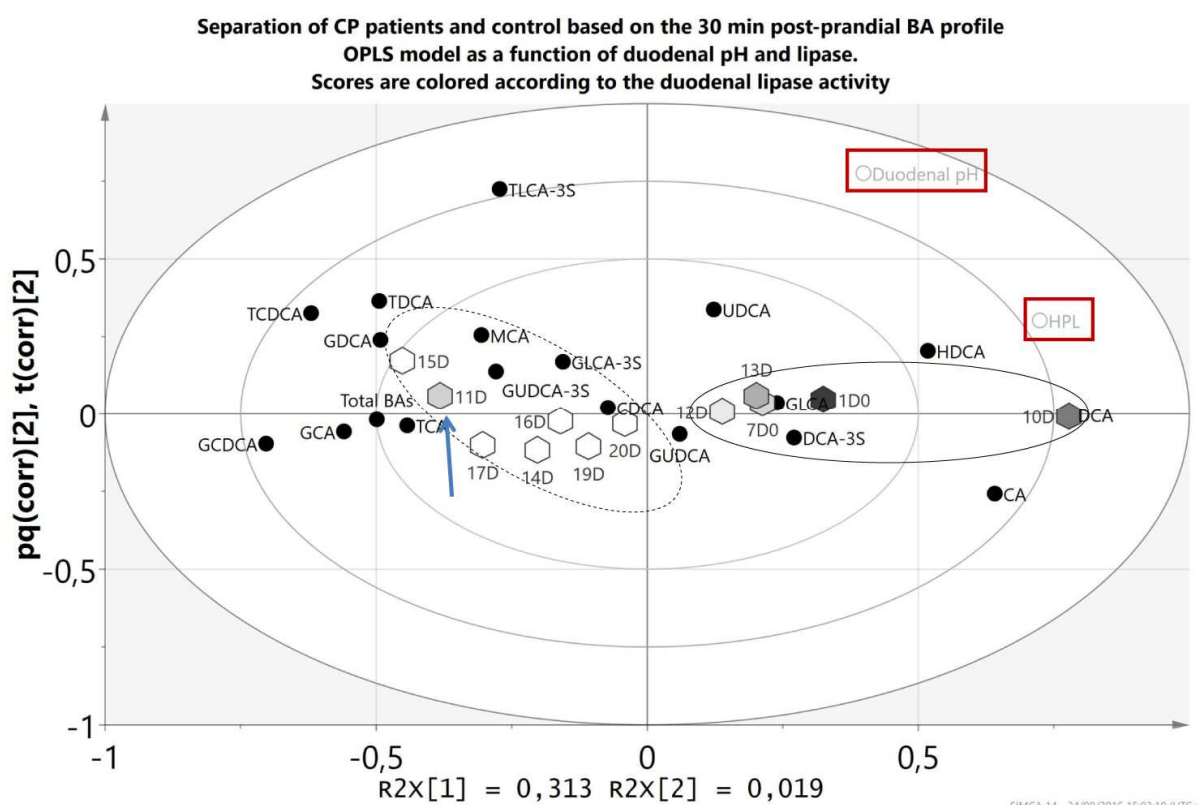


Figure S8: Analysis of 30 min post-prandial plasma BAs in Chronic Pancreatitis patients (white hexagonal symbols) and Healthy Volunteers (light grey to black hexagonal symbols, corresponding to increasing lipase activity). The BAs profile is modeled according to an Orthogonal-Partial Least Square regression as a function of the duodenal lipase activity and duodenal pH (framed labels in the upper right quadrant). The statistical regression identifies a predictive component representing the correlations with duodenum lipase activity and pH (horizontal axis). The orthogonal component for variations not correlated with duodenal lipase and pH (vertical axis) represents a small fraction of the plasma BAs variance (1,9% vs of 31,3% for the predictive component). The separation of CP patients (solid line) and healthy volunteers (dashed line) is shown horizontally but for an outlier control (see arrow). The detected outlier control has a duodenal pH (6,19) and lipase activity (720 U/mL) in agreement with other healthy volunteers. Method: The biplot shows scores of patients and control projected in the same factorial plan. The biplot reveals that unconjugated BAs (UDCA, HDCA, DCA, CA, DCA-3S) are associated with high duodenal pH and lipase in the healthy volunteers group. Conversely, Chronic Pancreatitis (CP) patients identified by empty hexagonal symbols for their low duodenal lipase activity show the higher loadings (i.e., post-prandial levels) of major tauroconjugated (TCDCA, TCA, TDCA) and glycoconjugated BAs (GDCA, GCA, GCDCA), which results in a higher total plasma post-prandial BA concentration.



References

1. Roda A, Hofmann AF, and Mysels KJ. The influence of bile salt structure on self-association in aqueous solutions. *J Biol Chem* 258: 6362-6370, 1983.