Ele Ferrannini,[1] Stefania Camastra,[2] Brenno Astiarraga,[2] Monica Nannipieri,[2] Jose Castro-Perez,[3] Dan Xie,[3] Liangsu Wang,[3] Manu Chakravarthy,[3] and Rebecca A. Haeusler[4]

Increased Bile Acid Synthesis and Deconjugation After Biliopancreatic Diversion

Diabetes 2015;64:3377–3385 | DOI: 10.2337/db15-0214

Biliopancreatic diversion (BPD) improves insulin sensitivity and decreases serum cholesterol out of proportion with weight loss. Mechanisms of these effects are unknown. One set of proposed contributors to metabolic improvements after bariatric surgeries is bile acids (BAs). We investigated the early and late effects of BPD on plasma BA levels, composition, and markers of BA synthesis in 15 patients with type 2 diabetes (T2D). We compared these to the early and late effects of Roux-en-Y gastric bypass (RYGB) in 22 patients with T2D and 16 with normal glucose tolerance. Seven weeks after BPD, insulin sensitivity had doubled and serum cholesterol had halved. At this time, BA synthesis markers and total plasma BAs, particularly unconjugated BAs, had markedly risen; this effect could not be entirely explained by low FGF19. In contrast, after RYGB, insulin sensitivity improved gradually with weight loss and cholesterol levels declined marginally; BA synthesis markers were decreased at an early time point (2 weeks) after surgery and returned to the normal range 1 year later. These findings indicate that BA synthesis contributes to the decreased serum cholesterol after BPD. Moreover, they suggest a potential role for altered enterohepatic circulation of BAs in improving insulin sensitivity and cholesterol metabolism after BPD.

Among bariatric operations, biliopancreatic diversion (BPD) stands out as having the strongest effect on weight loss and the highest frequency of type 2 diabetes (T2D) resolution (1,2). It also significantly reduces cardiovascular risk (3,4). Many of the beneficial metabolic effects of BPD might be expected to be due to weight loss, as with other bariatric surgeries. However, the improvements in insulin sensitivity after BPD exceed predictions based on weight loss (5). Moreover, even in the absence of excessive weight loss, BPD can resolve or improve T2D and reduce serum cholesterol (6,7). The former effects have been attributed to improved insulin sensitivity and enhanced β-cell function, but the mechanisms of these improvements, and the reduced serum cholesterol, remain unknown (6).

Bile acids (BAs), which are synthesized from cholesterol in the liver, potentially play a role in the metabolic effects of bariatric surgery. Several studies have shown that plasma BAs increase after Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (8–11). Because BAs can act in an endocrine fashion to regulate a variety of glucose and lipid metabolic pathways (12–15), it has been speculated that increased BAs may contribute to at least some of the metabolic improvements after surgery (16,17). Indeed, it was recently shown that the BA receptor farnesoid X receptor (FXR) is required for metabolic improvements in mice subjected to sleeve gastrectomy (18). However, despite the abundant evidence for increases in plasma BAs after bariatric surgery, the cause is unknown. Moreover, effects of BPD on BAs have not yet been reported, and BPD may have distinct effects on BAs compared with RYGB.

Plasma BA levels mainly reflect two major aspects of BA transport: *1*) absorption of these molecules from the intestine and *2*) uptake from the plasma, which occurs primarily in hepatocytes (19,20). Under physiologic conditions, the rate of BA synthesis is not a major contributor to plasma BA levels. However, increasing BA synthesis in mice by threefold overexpression of the rate-limiting enzyme of BA synthesis (cytochrome P450, family 7,

[1]Institute of Clinical Physiology, National Research Council, Pisa, Italy
[2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[3]Cardiometabolic Disease, Merck Research Laboratories, Kenilworth, NJ
[4]Department of Pathology and Cell Biology, Columbia University, New York, NY
Corresponding author: Rebecca A. Haeusler, rah2130@columbia.edu.

Received 13 February 2015 and accepted 21 May 2015.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0214/-/DC1.

© 2015 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

subfamily A, polypeptide 1 [*Cyp7a1*]) leads to approximately twofold higher levels of plasma BAs (21). BA synthesis occurs primarily in hepatocytes and is tightly regulated through two negative feedback loops. The first feedback loop is initiated in ileum, where FXR activation induces fibroblast growth factor 19 (FGF19), a secreted protein that binds to FGF receptors on hepatocytes. This pathway suppresses the expression of *CYP7A1*, encoding the first and rate-limiting enzyme of BA synthesis, and cytochrome P450, family 8, subfamily B, polypeptide 1 (*CYP8B1*), encoding the enzyme responsible for BA 12α-hydroxylation. The second feedback loop takes place in hepatocytes, where FXR activation induces the small heterodimer partner (SHP), which also suppresses the expression of *CYP8B1* and, potentially to a lesser extent, *CYP7A1* (22,23).

After synthesis of BAs in the liver, these molecules are conjugated to an amino acid and secreted into the canaliculi. After reaching the distal small intestine, most BAs are actively transported across enterocytes into the portal circulation. A portion of BAs enters the colon, where these molecules are deconjugated and dehydroxylated by gut microbes. Unconjugated BAs can undergo passive diffusion, allowing them to be reabsorbed. Once in the portal vein, the majority of BAs are taken up into hepatocytes, thus completing the enterohepatic circulation, but a fraction enters the systemic circulation. Thus, increases in plasma BAs after bariatric surgery may be due to alterations in BA transport or a combination of altered BA transport and increased BA synthesis.

One might expect BPD to alter both BA transport and synthesis. For example, *1*) there may be reduced absorption of BAs in ileum, because the surgery reduces the surface area of ileum that is exposed to BAs, and *2*) this may result in poor activation of the FXR-FGF19 pathway in enterocytes, thereby releasing feedback inhibition of BA synthesis. The latter effect has been speculated as the cause of decreased plasma cholesterol after BPD (24), but this possibility has not yet been investigated.

The goal of this study was to investigate BA levels, composition, and markers of BA synthesis after BPD, in comparison with RYGB. We examined 15 patients with T2D undergoing BPD and 38 patients undergoing RYGB, 22 with T2D and 16 that displayed normal glucose tolerance (NGT). We measured fasting plasma BAs, markers of BA synthesis, and FGF19 at baseline and at two follow-up visits.

RESEARCH DESIGN AND METHODS

Subjects

The study subjects were 38 morbidly obese patients (16 NGT and 22 T2D) undergoing RYGB and 15 patients with T2D undergoing BPD. Subjects were studied before surgery, early after surgery (2–10 weeks), and 1–2 years later. Lengths of time between surgeries and follow-up visits are listed in Table 1. At each of three time points, we performed a euglycemic-hyperinsulinemic clamp (respectively, in 12, 9,

Table 1—Study population and metabolic characteristics

	RYGB-NGT			RYGB-T2D			BPD-T2D		
	Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
n (female/male)	16 (15/1)	9 (8/1)	16 (15/1)	22 (11/11)	12 (7/5)	20 (11/9)	15 (6/9)	15 (6/9)	15 (6/9)
Age (years)	44 ± 8	—	—	49 ± 7	—	—	56 ± 5	—	—
Weeks since surgery	—	2.3 [0.3]	56 [19]	—	2.4 [0.4]	54 [6]	—	7.0 [4.1]	53 [14]
BMI (kg·m^2)	53.5 ± 6.2	51.1 ± 6.4#	38.3 ± 5.4#	48.8 ± 8.6#	45.2 ± 7.1#	38.2 ± 6.0	28.2 ± 2.6	25.1 ± 2.4#	23.9 ± 3.2#
FPG (mmol/L)	5.3 ± 0.3	5.1 ± 0.2	4.9 ± 0.3#	7.6 ± 2.3	6.0 ± 0.6#	5.1 ± 0.4#	12.1 ± 1.7	9.0 ± 3.1#	8.1 ± 2.7#
FPI (pmol/L)	122 [64]	104 [31]#	49 [15]#	136 [116]	87 [45]#	48 [29]#	64 [44]	42 [33]#	35 [10]#
LDL-C (mg/dL)	109 ± 24	85 ± 15	102 ± 24	104 ± 42	101 ± 27	93 ± 22	127 ± 58	59 ± 15#	70 ± 12#
HDL-C (mg/dL)	47 ± 14	27 ± 9#	48 ± 18	38 ± 10	26 ± 7#	50 ± 14#	46 ± 10	38 ± 7#	41 ± 11#
TG (mg/dL)	111 ± 16	115 ± 13	88 ± 11#	176 ± 148	144 ± 61	98 ± 24#	172 ± 54	170 ± 65	169 ± 84
M§	24 [13]	31 [18]	39 [13]#	17 [24]	26 [14]	42 [13]#	19 [3]	36 [10]#	34 [10]#

Entries are mean ± SD or median [interquartile range] for the presurgery (visit 1), early postsurgery (visit 2), and late postsurgery (visit 3) study. FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglycerides. §In μmol · min^{-1} · kg_{FFM}^{-1}. #$P \leq 0.05$ or less vs. baseline by Wilcoxon signed rank test.

and 12 RYGB-NGT patients; in 14, 12, and 13 RYGB-T2D patients; and in 15, 15, and 15 BPD-T2D patients). In the BPD group, the patients were treated by oral hypoglycemic agents and/or insulin (metformin in 6, metformin plus sulfonylurea in 3, and metformin plus insulin in 6), and in the RYGB group, 5 patients were treated by diet alone and 17 by oral hypoglycemic agents and/or insulin (metformin in 13 and metformin plus sulfonylurea and insulin in 2 each). The patients with diabetes on oral antidiabetic agents were asked to stop them 48–72 h before surgery; in those on insulin, injections were discontinued 16 h before the metabolic study (patients on bedtime glargine had been switched to NPH 2 days before the study). Glucose flux data from the 15 BPD-T2D patients (7) and from 12 RYGB-NGT and 13 RYGB-T2D patients (25) have been previously reported.

For comparison purposes, subjects without diabetes from a previous study (26) were selected to match the BMI of subjects at the second follow-up for each surgery. Subjects were matched to post-RYGB: n = 23, BMI = 39.7 ± 1.4 kg · m^{-2}, and P = 0.56 compared with post–RYGB-NGT and P = 0.49 compared with post–RYGB-T2D. Subjects were matched to post-BPD: n = 10, BMI = 23.5 ± 0.7 kg · m^{-2}, and P = 0.98 compared with post BPD-T2D.

Euglycemic-Hyperinsulinemic Clamp

After an overnight (12-h) fast, two catheters were inserted into an antecubital vein for infusion of all test substances and retrogradely into a vein on the dorsum of the hand for blood drawing. The hand was heated at 55°C to achieve arterialization of venous blood. At 9:00 A.M., baseline blood samples were drawn. At time −20, −10, and 0 min, blood samples were obtained from the arterialized vein for the measurement of glucose and insulin. At time 0, a primed-continuous insulin (Humulin R; Eli Lilly and Company, Indianapolis, IN) infusion (at a rate of 240 pmol · min^{-1} · m^{-2}) was started and continued for 180 min; plasma glucose levels were measured every 5 min throughout the clamp. Plasma insulin concentrations were measured every 20 min between 120 and 180 min after the start of insulin infusion. Fat-free mass (FFM) was estimated by electrical bioimpedance (TBF 300; Tanita, Tokyo, Japan) as previously described (25).

Analytical Procedures

Plasma glucose was measured by the glucose oxidase technique on a Beckman Glucose Analyzer (Beckman, Fullerton, CA). Plasma insulin was assayed by a specific radioimmunoassay (Linco Research, St. Charles, MO, and MYRIA, Technogenetics, Milan, Italy, respectively). BAs were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Waters Quattro Micro with Waters 2795 Alliance HPLC) as previously described (26). FGF19 was measured by ELISA (R&D Systems) in the three surgical groups (n at each of three time points, respectively): RYGB-NGT (n = 12, 6, and 11), RYGB-T2D (n = 13, 10, and 14), and BPD-T2D (n = 13, 10, and 6).

BA Synthesis Markers

Sample plasma (150 μL) was transferred into a 2-mL, 96-deep well plate; this was followed by the addition of 585 μL ice-cold acetonitrile containing 0.1% formic acid solution and 5 μL 60 ng/mL internal standard mixture made of d6-7α,12α-dihydroxy-4-cholesten-3-one (d6-7,12-diHCO) and d7-7α-hydroxy-4-cholesten-3-one (d7-7-HCO). The plate was sealed and vortexed for 1 min. Then, the plate was centrifuged for 20 min at 4,000 rpm. After this, 600 μL of supernatant was passed under positive pressure through a protein precipitation plate, which retained phospholipids but eluted the BA synthesis markers (Ostro plate; Waters Corp., Milford, MA). The eluent was collected and evaporated under a constant flow of N_2 at 45°C. Samples were then reconstituted in 100 μL of 80% acetonitrile + 0.1% formic acid/20% water. The resultant extract was injected (10 μL) onto an LC-MS/MS system operated in positive ion mode electrospray (UPLC/Waters TQS mass spectrometer). Isotopic dilution quantitation was conducted to obtain concentrations of 7α,12α-dihydroxy-4-cholesten-3-one (7,12-diHCO) and 7α-hydroxy-4-cholesten-3-one (7-HCO).

Data Analysis

Whole-body insulin-stimulated glucose disposal (M, in μmol/min) was calculated as the mean exogenous glucose infusion rate during the last 40 min of the clamp corrected for changes in glucose concentration within a distribution volume of 200 mL per kilogram of body weight. M values were expressed per kilogram of FFM (in μmol · min^{-1} · kg$_{FFM}^{-1}$) (26).

Statistical Analysis

Data are reported as mean ± SD (or median [interquartile range] for variables with a skewed distribution). Group differences were analyzed by Mann-Whitney U test and paired observations by Wilcoxon signed rank test. For selected variables, two-way ANOVA for repeated measures was used to compare surgical operations across time of study. Linear and nonlinear associations were tested by standard methods. A $P \leq 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

We analyzed three groups of patients: *1*) RYGB patients who had NGT at baseline (RYGB-NGT), *2*) RYGB patients who had T2D at baseline (RYGB-T2D), and *3*) BPD patients who had T2D at baseline (BPD-T2D). Patient characteristics are shown in Table 1. At baseline, BPD-T2D subjects were older and had lower BMI, lower insulin, and higher glucose compared with RYGB subjects. Clamp-derived insulin sensitivity (M) showed that all patients were markedly insulin resistant. After surgery, insulin sensitivity improved in all study participants (Table 1). In particular, in RYGB patients (both NGT and T2D), M rose essentially in proportion to the corresponding weight changes, whereas in BPD patients, M improvements

peaked at the earlier time (when weight changes were modest) and were of greater magnitude than in RYGB patients (14 [14] vs. 7 [21] μmol · min^{-1} · $kg_{FFM}{}^{-1}$, P = 0.013) (Fig. 1). In support of this different time course, two-way ANOVA was significant (P = 0.0018) for the time × group interaction; viewed otherwise, the slope of the relationship between M and BMI was significantly steeper ($P < 0.02$) in BPD than RYGB patients (Supplementary Fig. 1).

Serum lipids changed differentially between the two operations. After RYGB, LDL cholesterol levels were only marginally (and not significantly) reduced in both NGT and T2D patients. In contrast, after BPD, LDL cholesterol halved already at 7 weeks, and this drop was maintained longer term. Accordingly, two-way ANOVA was significant ($P < 0.01$) for the time × group interaction.

On the other hand, with RYGB, serum triglycerides decreased late after surgery (by 20 and 45% in NGT and T2D, respectively), whereas they were essentially unchanged with BPD. HDL cholesterol levels were acutely decreased in all groups but recovered (in NGT) or improved (in T2D) after RYGB, whereas they remained slightly, if significantly, lower with BPD (Table 1).

Effects of Surgery on BA Levels and Composition

Fasting plasma BAs increased in all groups after surgery (Fig. 2*A*). Full BA data are provided in Supplementary Table 1. However, the magnitude, timing, and species composition of these changes were different between operations.

RYGB

Effects of RYGB on total BA levels and composition were similar in NGT and T2D subjects. A few weeks after surgery, plasma BAs increased only slightly, whereas levels were higher 1 year later (Fig. 2*A*). Although these changes reached statistical significance only among RYGB-NGT subjects, the trend was the same in RYGB-T2D subjects. When we pooled NGT and T2D subjects together, BAs were significantly higher 13 months after RYGB (7.27 ± 1.28 vs. 2.99 ± 0.32 μmol/L, P = 0.003).

We next compared these post-RYGB measurements to nonsurgical subjects by choosing healthy subjects from our database who were BMI matched to RYGB subjects at the second follow-up visit. Postsurgical patients had three- to fivefold higher fasting plasma BAs than BMI-matched nonsurgical subjects (Fig. 2*A*). This is consistent with prior observations (11) and indicates that increased plasma BAs are due to a body weight–independent feature of bariatric surgery.

The composition of BA species in plasma was also affected by RYGB. Within the first few weeks after surgery, patients showed a preferential increase in conjugated BA species (Fig. 2*B–D*). By the second follow-up visit, unconjugated BAs had also increased. Thus, the feature was partially reversed.

BPD

Plasma BAs increased markedly in the first 2 months after BPD and then partially declined 1 year later (Fig. 2*A*). When we compared 1 year post-BPD subjects to BMI-matched nonsurgical subjects, we found the former had nearly ninefold higher levels compared with the latter. Thus, the magnitude of BA alterations was much greater, and the timing was earlier, compared with RYGB.

After BPD, conjugated BAs were increased, but this effect was eclipsed by greater increments in unconjugated BAs (Fig. 2*B* and *C*). This led to a preferential accumulation of unconjugated BAs in plasma (Fig. 2*D*). Thus, BPD and RYGB have opposite effects on the conjugation status of plasma BAs.

Effects of Surgery on BA Synthesis and FGF19

To investigate whether increases in fasting plasma BAs after bariatric surgery were due to changes in BA synthesis, we measured two indirect markers of BA synthesis: *1*) 7-HCO, a marker of total BA synthesis (27), and *2*) 7,12-diHCO, a marker of the branch of BA synthesis that is 12α-hydroxylated by *CYP8B1* (Supplementary Table 1). Furthermore, we considered the ratio of 7-HCO to 7,12-diHCO to be a marker of the proportion of new BA synthesis that is 12α-hydroxylated. We also measured FGF19, the ileum-secreted peptide that suppresses hepatic BA synthesis.

RYGB-NGT

After RYGB, BA synthesis markers were not increased; in fact, they tended to be decreased at the earlier follow-up (Fig. 3*A* and *B*). There was no change in the 12α-hydroxylated proportion of BA synthesis in RYGB-NGT subjects (Fig. 3*C*). There was also no change in FGF19 in these subjects (Fig. 3*D*).

RYGB-T2D

BA synthesis markers tended to decrease after surgery in this cohort (Fig. 3*A* and *B*). This pattern was similar to

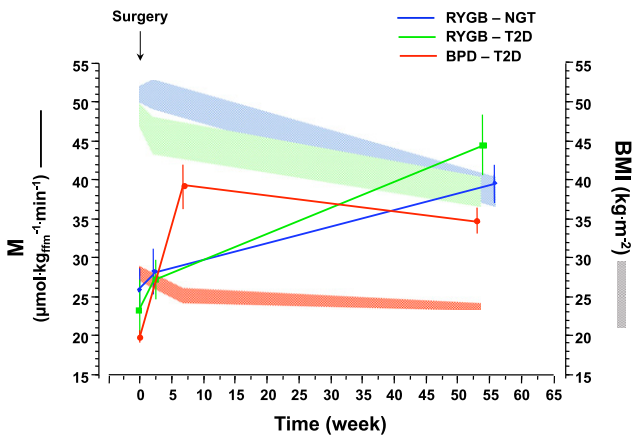

Figure 1—Time course of BMI (shaded areas are mean ± SEM) and M values in the three study groups. Note that M trajectories are mirror images of the corresponding BMI trajectories in RYGB-NGT and RYGB-T2D, whereas in BPD, M values are disjoined from BMI.

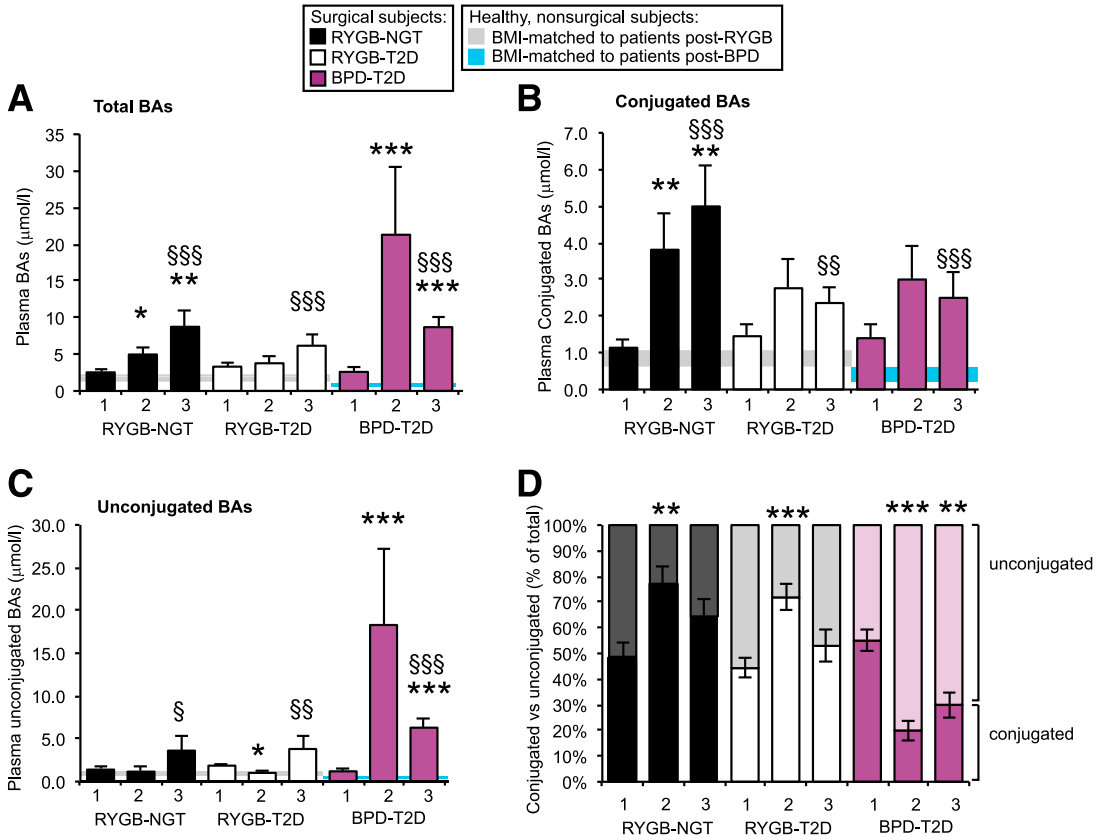

Figure 2—Plasma BA phenotypes after BPD and RYGB. *A*: Total plasma BAs. *B*: Conjugated BAs. *C*: Unconjugated BAs. *D*: The percentage of total BAs that is conjugated vs. unconjugated. Data plotted are mean ± SE. Time points are indicated on the *x*-axis. 1, presurgery; 2, first follow-up visit after surgery; 3, second follow-up visit after surgery. Gray rectangle shows the mean ± SE for nonsurgical patients BMI matched to RYGB subjects at the second follow-up visit (~1 year after surgery). Blue rectangle shows the mean ± SE for nonsurgical patients BMI matched to BPD subjects at the second follow-up visit (~1 year after surgery). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, compared with baseline of each surgery group; §*P* < 0.05, §§*P* < 0.01, and §§§*P* < 0.001, compared with nonsurgical subjects BMI matched to surgical subjects at the second follow-up, by Mann-Whitney *U* test.

what we observed in the RYGB-NGT cohort. When we pooled the RYGB-NGT and RYGB-T2D groups, there was a significant decrease early after RYGB in both BA synthesis markers: 7-HCO (8.97 ± 1.47 vs. 18.17 ± 2.64 ng/mL, $P = 0.02$) and 7,12-diHCO (0.58 ± 0.10 vs. 1.22 ± 0.20, $P = 0.01$). By the second follow-up visit after surgery, these markers rose back up into the normal range and were similar to BMI-matched nonsurgical subjects (Fig. 3*A* and *B*). This demonstrates that the increase of plasma BAs after RYGB cannot be explained by increased BA synthesis.

At baseline, RYGB-T2D patients showed an elevation in the proportion of BA synthesis that was 12α-hydroxylated (Fig. 3*C*). This is consistent with the effects of insulin resistance and T2D to increase BA 12α-hydroxylation (26,28). After surgery, this ratio gradually decreased into the range of healthy, nonsurgical subjects (Fig. 3*C*). Thus, this reduction in preferential 12α-hydroxylation may be due to improved insulin sensitivity.

Another potential contributor to the levels of 12α-hydroxylation in T2D subjects is FGF19, as it suppresses both *CYP7A1* and *CYP8B1*. RYGB-T2D patients showed low FGF19 at baseline, in comparison with NGT subjects (Fig. 3*D*). This is consistent with previous observations (8). After surgery, FGF19 gradually increased. Therefore, increased levels of this hormone after RYGB may play a role in the decreases in BA synthesis and 12α-hydroxylation in these patients.

BPD-T2D

The effects of BPD on BA synthesis markers were opposite the effects of RYGB. Within the first 7 weeks after BPD, BA synthesis markers were increased three- and fivefold (Fig. 3*A* and *B*). One year later, these levels rose to be six- and eightfold higher than baseline. When BA synthesis markers were normalized to serum total cholesterol levels (29,30), the pattern was accentuated (Supplementary Table 1). The increase compared with BMI-matched nonsurgical subjects was even greater. There was no significant effect of BPD on the proportion of BA synthesis that was 12α-hydroxylated (Fig. 3*C*). These findings demonstrate that BPD causes a strong and sustained increase in overall BA synthesis.

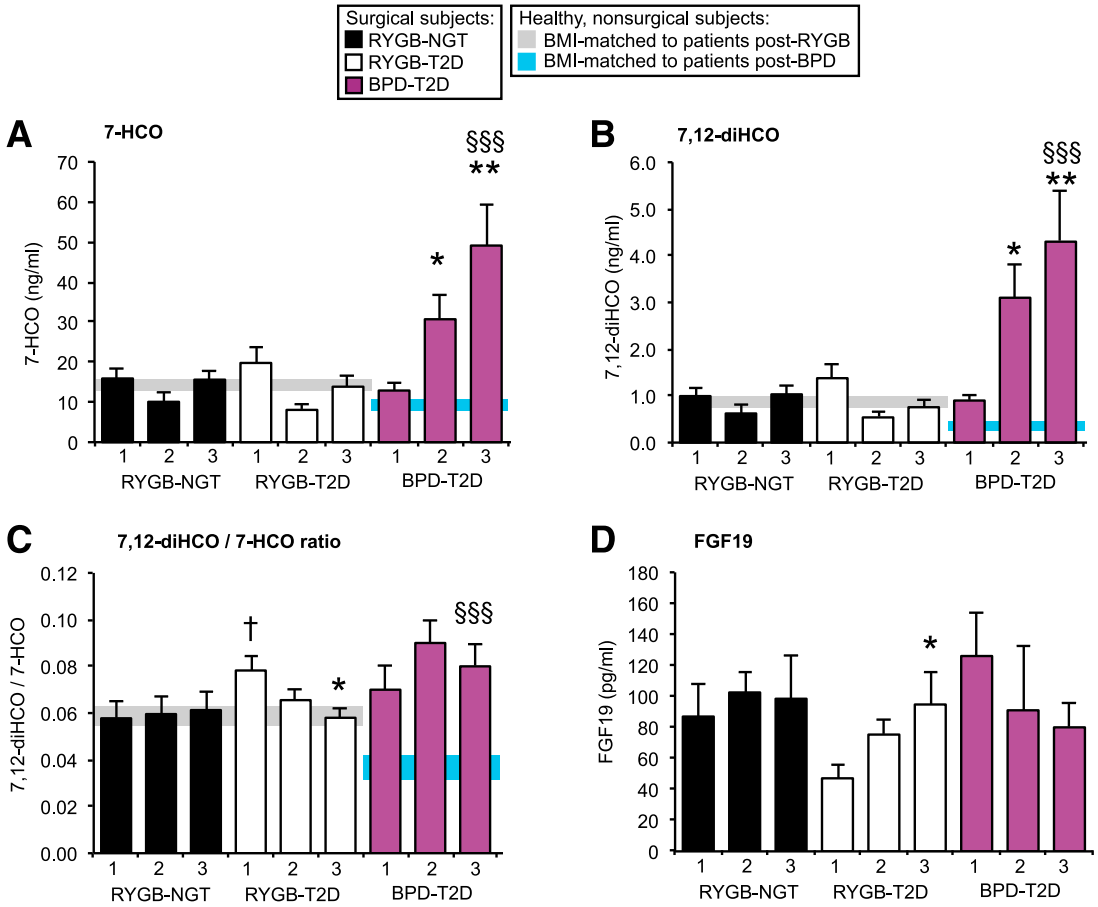

Figure 3—Plasma markers of BA synthesis after BPD and RYGB. *A*: 7-HCO. *B*: 7,12-diHCO. *C*: Ratio of 7,12-diHCO to 7-HCO. *D*: FGF19. Data plotted are mean ± SE. Time points are indicated on the *x*-axis. 1, presurgery; 2, first follow-up visit after surgery; 3, second follow-up visit after surgery. Gray rectangle shows the mean ± SE for nonsurgical patients BMI matched to RYGB subjects at the second follow-up visit (~16 months after surgery). Blue rectangle shows the mean ± SE for nonsurgical patients BMI matched to BPD subjects at the second follow-up visit (~13 months after surgery). **P* < 0.05 and ***P* < 0.01, compared with baseline of each surgery group; §§§*P* < 0.001, compared with nonsurgical subjects BMI matched to surgical subjects at the second follow-up; †*P* < 0.05, RYGB-T2D compared with RYGB-NGT at baseline, by Mann-Whitney *U* test.

We expected that one reason for the increase in BA synthesis would be decreased FGF19, because the area of ileum that is exposed to BAs is shortened by the surgery. In fact, FGF19 levels were highly variable and showed only a trend toward decreased levels after BPD (Fig. 3*D*). Thus, additional mechanisms may be involved in the increased BA synthesis after BPD.

In the pooled data of the BPD-T2D group, M values were positively associated with all BA measures, the strongest one being with the ratio of unconjugated to conjugated BAs, whereas LDL cholesterol levels were reciprocally related to this ratio (Fig. 4). LDL cholesterol was also negatively associated with 7-HCO ($r = -0.34$, $P = 0.039$) and 7,12,-diHCO ($r = -0.39$, $P = 0.015$). No such correlations were found in the RYGB groups.

DISCUSSION

This work shows that BPD causes major alterations in BA homeostasis. Key findings are that after BPD, *1*) BA synthesis markers increase manifold, *2*) plasma BAs are high and preferentially unconjugated, and *3*) these changes are sustained over time. These findings are in contrast to what happens after RYGB: *4*) BA synthesis markers decrease, *5*) plasma BA composition shifts to be preferentially conjugated, and *6*) these features both return to normal after 1 year.

The implication that BA synthesis increases after BPD, but not RYGB, may explain the former's stronger effect to reduce serum cholesterol (Table 1), although reduced cholesterol absorption likely also plays a role (31). In this regard, BPD mimics some of the effects of blocking intestinal BA absorption through the use of BA sequestrants or inhibitors of apical sodium-dependent bile acid transporter (ASBT). Each of these treatments results in increased conversion of cholesterol into BAs (32,33). These effects are predicted to be due to release of feedback inhibition of hepatic BA synthesis, and it has been shown that BA sequestrants cause reductions in fasting and fed levels of FGF19 (28,34). We predicted the same after BPD. However, we saw only a mild reduction in

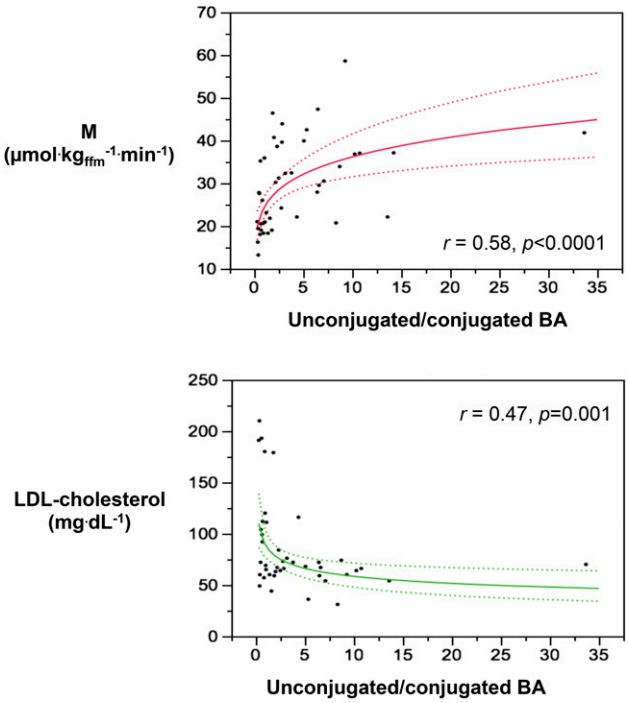

Figure 4—Association between the unconjugated-to-conjugated BA ratio and insulin sensitivity (M) and LDL cholesterol in the BPD group. The power functions fit the pooled data from all three studies.

fasting plasma FGF19 after BPD. Thus, other feedback mechanisms originating in intestine or liver may also be disrupted after BPD.

Studies of another malabsorptive bariatric operation, the biliointestinal bypass, may offer additional insight. This surgery differs from BPD in that the bile is directed into the nonfunctional jejunum, thus permitting normal uptake of BAs throughout the distal small intestine, while still maintaining nutrient malabsorption due to a short common limb. Benetti et al. (35) showed that after biliointestinal bypass, cholesterol absorption was decreased, as expected, and this was compensated by increased hepatic cholesterol synthesis. Interestingly, BA synthesis was also increased, despite the expectation of normal FGF19 (which was not measured). This raises the possibility that additional, non-FGF19, signaling events that regulate BA synthesis become disrupted after this surgery. The authors speculate that the increase in BA synthesis was secondary to increased cholesterol synthesis (35). Perhaps a similar mechanism is invoked after BPD.

How might these changes in BA synthesis and transport relate to other metabolic improvements after surgery? We find it unlikely that increased BA synthesis alone is sufficient to cause these effects, as we and others have found that BA synthesis also increases during conditions of metabolic impairment, including human obesity and T2D (26,36,37). On the other hand, it is possible that the interruption in enterohepatic circulation of BAs after BPD could play a part. In fact, BA sequestrants and ASBT inhibitors are known to affect more than just cholesterol-BA balance; they also cause improvements in glycemia (38,39). The molecular mechanisms of these effects are actively debated but may be related to increased splanchnic glucose uptake or increased glucagon-like peptide 1 (GLP-1) secretion due to increased intestinal signaling by BAs or free fatty acids (38,40). Indeed, GLP-1 is increased after BPD (7,41); thus, it is possible that the reduction in ileal uptake of BAs or nutrients after BPD contributes to the increases in GLP-1. However, GLP-1 is unlikely to explain the improvements in peripheral insulin sensitivity after BPD (5). Could other effects of interrupting the BA enterohepatic circulation be involved in these improvements? Further studies will be required to answer this question.

What of the effect of BPD to preferentially increase unconjugated BAs? This effect may be due to the combination of increased BA flow into the colon and the well-documented expansion of gut microbiota after BPD (42). Deconjugation affects the ability of BA species to pass through cell membranes and to agonize BA receptors (43–45); thus, it is conceivable that increasing the levels of unconjugated species may affect the localization and magnitude of BA receptor activation. The contribution of this aspect of the phenotype may be worth investigating in future studies.

We are intrigued that RYGB subjects have high plasma BAs but low to normal levels of BA synthesis markers. This indicates that the cause of high plasma BAs is altered plasma BA transport. The two major determinants of BA transport are absorption from the intestine and uptake into the liver. We speculate that after RYGB, uptake of BAs in the small intestine is enhanced. This would be consistent with the increases in FGF19 that we observed in RYGB-T2D patients and that others have also reported (8,46,47) and with the increased postprandial and postglucose BA excursions that occur after RYGB (48,49). This may also explain the preferential increase of conjugated BAs that we observed after RYGB, as these species are the ones that are preferentially taken up by ASBT (19). Others have pointed out that increases in fasting plasma BAs occur relatively late after surgery (~1 year) and therefore cannot explain immediate improvements in glycemia (49,50). However, we would not exclude the possibility that changes in BA transport and tissue-specific signaling may occur earlier than that and may contribute to improvements in glucose homeostasis.

It must be pointed out that the subjects undergoing RYGB were morbidly obese NGT or T2D, whereas those in the BPD group were overweight and T2D. While we set up comparisons with BMI-matched nonsurgical control subjects, the relative influence of BMI and hyperglycemia on the observed BA changes cannot be directly assessed in the surgical groups. Exploratory analyses (not shown) suggest that the surgically induced changes in BAs and their synthetic markers were substantially independent of BMI and presence of diabetes.

Overall, these findings highlight that elevated plasma BAs after different bariatric surgeries can arise through distinct mechanisms. The upregulation of BA synthesis after BPD confirms our prediction and may partially explain the powerful decrease in plasma cholesterol in these subjects. However, the mechanism responsible for the upregulation of BA synthesis is likely to involve signaling other than FGF19, and this warrants further investigation. Finally, we would suggest that further examination of changes in BA transport and BA signaling may shed additional light on the impact of BAs on glucose and lipid metabolism after bariatric surgeries.

Acknowledgments. The authors would like to acknowledge Domenico Accili from Columbia University and Mark Erion, Stephen Previs, Martin Brenner, and David Kelley from Merck Research Laboratories for their scientific discussion, support, and input into this manuscript.

Funding. This study was funded in part by the Italian Ministry of Education, University, and Research (2010329EKE), the National Institutes of Health (NIH) (HL-111206 to R.A.H.), and Columbia Clinical and Translational Science Award grant UL1-TR-000040 (National Center for Advancing Translational Sciences/NIH).

Duality of Interest. This study was funded in part by an unrestricted grant from Merck Research Laboratories. E.F. has been a speaker and consultant for Boehringer Ingelheim, Merck, Sanofi, Eli Lilly and Company, Johnson & Johnson, Astellas, Daiichi Sankyo, Bristol-Myers Squibb/AstraZeneca, and Novartis. J.C.-P., D.X., L.W., and M.C. are employees of Merck Research Laboratories. No other potential conflicts of interest relevant to this article were reported.

Merck Research Laboratories had no input into the design of the study or the analysis of the data.

Author Contributions. E.F. and R.A.H. designed experiments, analyzed data, and wrote the manuscript. S.C., B.A., and M.N. performed in vivo experiments and contributed to discussions. J.C.-P., D.X., L.W., and M.C. measured BA synthesis markers in a blinded fashion and contributed to discussions. All authors edited the manuscript. E.F. and R.A.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Buchwald H, Estok R, Fahrbach K, et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. Am J Med 2009;122:248.e5–256.e5

2. Garrido-Sanchez L, Murri M, Rivas-Becerra J, et al. Bypass of the duodenum improves insulin resistance much more rapidly than sleeve gastrectomy. Surg Obes Relat Dis 2012;8:145–150

3. Piché ME, Martin J, Cianflone K, et al. Changes in predicted cardiovascular disease risk after biliopancreatic diversion surgery in severely obese patients. Metabolism 2014;63:79–86

4. Pontiroli AE, Laneri M, Veronelli A, et al. Biliary pancreatic diversion and laparoscopic adjustable gastric banding in morbid obesity: their long-term effects on metabolic syndrome and on cardiovascular parameters. Cardiovasc Diabetol 2009;8:37

5. Ferrannini E, Mingrone G. Impact of different bariatric surgical procedures on insulin action and beta-cell function in type 2 diabetes. Diabetes Care 2009;32:514–520

6. Scopinaro N, Adami GF, Papadia FS, et al. Effects of biliopanceratic diversion on type 2 diabetes in patients with BMI 25 to 35. Ann Surg 2011;253:699–703

7. Astiarraga B, Gastaldelli A, Muscelli E, et al. Biliopancreatic diversion in nonobese patients with type 2 diabetes: impact and mechanisms. J Clin Endocrinol Metab 2013;98:2765–2773

8. Gerhard GS, Styer AM, Wood GC, et al. A role for fibroblast growth factor 19 and bile acids in diabetes remission after Roux-en-Y gastric bypass. Diabetes Care 2013;36:1859–1864

9. Kohli R, Bradley D, Setchell KD, Eagon JC, Abumrad N, Klein S. Weight loss induced by Roux-en-Y gastric bypass but not laparoscopic adjustable gastric banding increases circulating bile acids. J Clin Endocrinol Metab 2013;98:E708–E712

10. Nakatani H, Kasama K, Oshiro T, Watanabe M, Hirose H, Itoh H. Serum bile acid along with plasma incretins and serum high-molecular weight adiponectin levels are increased after bariatric surgery. Metabolism 2009;58:1400–1407

11. Patti ME, Houten SM, Bianco AC, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. Obesity (Silver Spring) 2009;17:1671–1677

12. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. Cell Metab 2013;17:657–669

13. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009;89:147–191

14. Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap—bile acids in metabolic control. Nat Rev Endocrinol 2014;10:488–498

15. Ma H, Patti ME. Bile acids, obesity, and the metabolic syndrome. Best Pract Res Clin Gastroenterol 2014;28:573–583

16. Kohli R, Seeley RJ. Diabetes: the search for mechanisms underlying bariatric surgery. Nat Rev Endocrinol 2013;9:572–574

17. Pournaras DJ, le Roux CW. Are bile acids the new gut hormones? Lessons from weight loss surgery models. Endocrinology 2013;154:2255–2256

18. Ryan KK, Tremaroli V, Clemmensen C, et al. FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 2014;509:183–188

19. Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. Physiol Rev 2003;83:633–671

20. Dawson PA, Lan T, Rao A. Bile acid transporters. J Lipid Res 2009;50:2340–2357

21. Li T, Owsley E, Matozel M, Hsu P, Novak CM, Chiang JY. Transgenic expression of cholesterol 7alpha-hydroxylase in the liver prevents high-fat diet-induced obesity and insulin resistance in mice. Hepatology 2010;52:678–690

22. Kim I, Ahn SH, Inagaki T, et al. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. J Lipid Res 2007;48:2664–2672

23. Kong B, Wang L, Chiang JY, Zhang Y, Klaassen CD, Guo GL. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. Hepatology 2012;56:1034–1043

24. Scopinaro N. Thirty-five years of biliopancreatic diversion: notes on gastrointestinal physiology to complete the published information useful for a better understanding and clinical use of the operation. Obes Surg 2012;22:427–432

25. Camastra S, Gastaldelli A, Mari A, et al. Early and longer term effects of gastric bypass surgery on tissue-specific insulin sensitivity and beta cell function in morbidly obese patients with and without type 2 diabetes. Diabetologia 2011;54:2093–2102

26. Haeusler RA, Astiarraga B, Camastra S, Accili D, Ferrannini E. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. Diabetes 2013;62:4184–4191

27. Gälman C, Arvidsson I, Angelin B, Rudling M. Monitoring hepatic cholesterol 7alpha-hydroxylase activity by assay of the stable bile acid intermediate 7alpha-hydroxy-4-cholesten-3-one in peripheral blood. J Lipid Res 2003;44:859–866

28. Brufau G, Stellaard F, Prado K, et al. Improved glycemic control with colesevelam treatment in patients with type 2 diabetes is not directly associated with changes in bile acid metabolism. Hepatology 2010;52:1455–1464

29. Gälman C, Angelin B, Rudling M. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. Gastroenterology 2005;129:1445–1453

30. Honda A, Yoshida T, Xu G, et al. Significance of plasma 7alpha-hydroxy-4-cholesten-3-one and 27-hydroxycholesterol concentrations as markers for hepatic bile acid synthesis in cholesterol-fed rabbits. Metabolism 2004;53:42–48

31. Buchwald H, Avidor Y, Braunwald E, et al. Bariatric surgery: a systematic review and meta-analysis. JAMA 2004;292:1724–1737

32. Einarsson K, Ericsson S, Ewerth S, et al. Bile acid sequestrants: mechanisms of action on bile acid and cholesterol metabolism. Eur J Clin Pharmacol 1991;40(Suppl. 1):S53–S58
33. Root C, Smith CD, Sundseth SS, Pink HM, Wilson JG, Lewis MC. Ileal bile acid transporter inhibition, CYP7A1 induction, and antilipemic action of 264W94. J Lipid Res 2002;43:1320–1330
34. Beysen C, Murphy EJ, Deines K, et al. Effect of bile acid sequestrants on glucose metabolism, hepatic de novo lipogenesis, and cholesterol and bile acid kinetics in type 2 diabetes: a randomised controlled study. Diabetologia 2012;55: 432–442
35. Benetti A, Del Puppo M, Crosignani A, et al. Cholesterol metabolism after bariatric surgery in grade 3 obesity: differences between malabsorptive and restrictive procedures. Diabetes Care 2013;36:1443–1447
36. Steiner C, Othman A, Saely CH, et al. Bile acid metabolites in serum: intraindividual variation and associations with coronary heart disease, metabolic syndrome and diabetes mellitus. PLoS ONE 2011;6:e25006
37. Ståhlberg D, Rudling M, Angelin B, et al. Hepatic cholesterol metabolism in human obesity. Hepatology 1997;25:1447–1450
38. Prawitt J, Caron S, Staels B. Glucose-lowering effects of intestinal bile acid sequestration through enhancement of splanchnic glucose utilization. Trends Endocrinol Metab 2014;25:235–244
39. Chen L, Yao X, Young A, et al. Inhibition of apical sodium-dependent bile acid transporter as a novel treatment for diabetes. Am J Physiol Endocrinol Metab 2012;302:E68–E76
40. Hofmann AF. Bile acid sequestrants improve glycemic control in type 2 diabetes: a proposed mechanism implicating glucagon-like peptide 1 release. Hepatology 2011;53:1784
41. Valverde I, Puente J, Martín-Duce A, et al. Changes in glucagon-like peptide-1 (GLP-1) secretion after biliopancreatic diversion or vertical banded gastroplasty in obese subjects. Obes Surg 2005;15:387–397
42. Picard M, Frédéric Simon H, Stéfane L, Simon M, Simon B. Complications of combined gastric restrictive and malabsorptive procedures: part 2. Curr Surg 2003;60:274–279; discussion 279–281
43. Makishima M, Okamoto AY, Repa JJ, et al. Identification of a nuclear receptor for bile acids. Science 1999;284:1362–1365
44. Parks DJ, Blanchard SG, Bledsoe RK, et al. Bile acids: natural ligands for an orphan nuclear receptor. Science 1999;284:1365–1368
45. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999;3:543–553
46. Jansen PL, van Werven J, Aarts E, et al. Alterations of hormonally active fibroblast growth factors after Roux-en-Y gastric bypass surgery. Dig Dis 2011; 29:48–51
47. Pournaras DJ, Glicksman C, Vincent RP, et al. The role of bile after Roux-en-Y gastric bypass in promoting weight loss and improving glycaemic control. Endocrinology 2012;153:3613–3619
48. Ahmad NN, Pfalzer A, Kaplan LM. Roux-en-Y gastric bypass normalizes the blunted postprandial bile acid excursion associated with obesity. Int J Obes 2013; 37:1553–1559
49. Dutia R, Embrey M, O'Brien CS, et al. Temporal changes in bile acid levels and 12α-hydroxylation after Roux-en-Y gastric bypass surgery in type 2 diabetes. Int J Obes 2015;39:806–813
50. Steinert RE, Peterli R, Keller S, et al. Bile acids and gut peptide secretion after bariatric surgery: a 1-year prospective randomized pilot trial. Obesity (Silver Spring) 2013;21:E660–E668