Original article

Fecal bile acid profile after Roux-en-Y gastric bypass and its association with the remission of type 2 diabetes in obese women: A preliminary study

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A R T I C L E   I N F O

S U M M A R Y

Objective: To assess the influence of Roux-en-Y gastric by-pass (RYGB) on fecal bile acid (BA) profile and its relationship with postoperative remission of type 2 diabetes (T2D).
Methods: Fecal samples were collected 3 and 12 months after RYGB from diabetic obese women who were responsive (n = 12) and non-responsive (n = 8) to postoperative remission of T2D. Fecal BA profile was accessed by liquid chromatography coupled to tandem mass spectrometry in a targeted approach.
Results: Relative to pre-operative levels, a total of 10 fecal BA profiles decreased after RYGB (ANOVA, $p \leq 0.05$) with significant fold-changes for glycochenodeoxycholic, glycocholic, taurocholic, and taurochenodeoxycholic acids at 3-months postoperatively, and for glycochenodeoxycholic, glycocholic and taurocholic acids at 12 months postoperatively (Benjamini—Hochberg, $p \leq 0.05$). Postoperative changes in fecal BA were different between responsive and non-responsive women, with a significant reduction in more sub-fractions of BA in responsive women than in non-responsive women, and a marked difference in the temporal behavior of cholic acid (CA) and chenodeoxycholic acid (CDCA), thus reflecting changes in CA/CDCA ratio, and tauroursodeoxycolic (TUDCA) levels between these responsiveness groups (ANOVA, $p \leq 0.05$).
Conclusion: RYGB induces a marked reduction in the concentration of fecal BA, which is heterogeneous according to T2D responsiveness.

1. Introduction

Bile acids (BAs) are a diverse group of amphipathic steroid molecules that enable micelle formation and facilitate intestinal absorption, emulsification, and transport of nutrients, lipids, and lipophilic vitamins [1]. Primary BAs are derived from catabolism of cholesterol in hepatocytes and can be bio-transformed into secondary BA in the intestine by its resident bacteria [2—4].

Recently, BAs were also recognized as a potent signaling molecules implicating pleiotropic physiological responses which includes glucose and energy metabolism by binding to specific receptors [5]. BAs have been shown to regulate glucose homeostasis by activating Farnesoid X receptor (FXR)-mediated pathways and by binding to membrane G-protein coupled bile acid receptor 1 (TGR5) in the intestine [6]. The experimental induction of FXR activation significantly reduced blood glucose levels in diabetic db/db and wild-type mice, while FXR-null mice exhibited both glucose intolerance and insulin insensitivity [7]. Experimental data also suggest that the activation of TGR5 can improve pancreatic function and glucose tolerance by stimulate intestinal glucagon-like peptide-1 (GLP-1) secretion [8,9].

The gastric bypass procedure modifies the anatomical location at which bile enters the upper gastrointestinal tract via the biliopancreatic limb of the Roux-en-Y (RYGB) construction and increases primary and secondary serum BAs levels in both human

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and animal models [10–12]. Specifically in animal models, a targeted ultra-performance liquid-chromatography (UPLC)-MS method of fecal samples detected decreased faecal bile acids after RYGB. This modulation of BAs may trigger the improved glucose regulation, suggesting a potential metabolic role for BA after bariatric surgery, including the remission of type 2 diabetes (T2D) [2–4]. In this scenario, it would be expected that the fecal BAs profile would reflect both healthy and unhealthy conditions [13]. Our present preliminary study aimed to investigate whether fecal BAs profile after RYGB is associated with the post-operative remission of T2D in obese women.

2. Methods

2.1. Ethical issues

This preliminary study is part of the SURmetaGIT trial [14], registered at www.ClinicalTrials.gov (NCT01251016) and its specific protocol was approved by the local ethics Committee (Reference: CAPPesq 1011/09). All protocol interventions were performed according to the ethical standards of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from each patient prior to trial participation.

2.2. Subjects

Obese T2D women (n = 20), who were candidates for RYGB, were recruited from the Surgical Gastroenterology Department of the Hospital das Clínicas of the University of São Paulo School of Medicine between February 2011 and December 2014. Our inclusion criteria were as follows: adults (18–60 years); body mass index (BMI) $\geq$ 35 kg/m^2; proven T2D diagnosis (fasting glucose $\geq$ 126 mg/dL and glycated hemoglobin $\geq$6.5%) and/or use of oral antidiabetic drugs. Our exclusion criteria were as follows: *Helicobacter pylori* infection, diagnosis of thyroid or hepatic diseases; antibiotic use in the month preceding fecal sample collection; probiotic and prebiotic use, current or recent participation in another interventional study protocol.

2.3. Preliminary study design

All patients were submitted to standardized open RYGB without silicon rings with biliary-pancreatic loops (50–60 cm) and feed handles (100–120 cm). The fecal metabolome profile was then determined in all patients 3 months and 12 months after RYGB. Data on fecal BA were then correlated with post-operative T2D remission.

2.4. Fecal sample collection and processing

All patients collected their own fecal samples at home using a specific specimen collection system (Commode Specimen; Fisher Scientific, Ottawa, ON, Canada). The fecal samples were then frozen at −20 °C and transported to our laboratory by a motorcycle courier service, specialized in the transport of biological samples under controlled temperature. At our laboratory, fecal samples were aliquoted (100 mg) in cryogenic vials (Kasvi, Canada) without thawing and stored at −80 °C to await metabolomic evaluations. The fecal aliquots were sent to Biocrates (Innsbruck, Austria) under controlled temperature, under the care of the carrier World Courier (São Paulo, Brazil). There, fecal BAs were obtained using 340 ml ethanol (HPLC grade, Merck, Germany) and 60 ml phosphate buffer (20 mM, Merck, Germany) in a seven step procedure: 1. Ethanol addition and 3 min vortex mixing; 2. Buffer addition and manual homogenization; 3. Centrifugation (200 rpm, 30 min, 0 °C); 4.

Sonication (70 W, 5 min, 0 °C); 5. Centrifugation (800G, 10 min, 0 °C); 5. Transfer of supernatant into a reaction tube (Eppendorf, Germany); 6. centrifugation (19,000 G, 10 min, 4 °C) and 7. transfer of supernatant into a second reaction tube and freezing (−80 °C).

2.5. Assessment of fecal BA

Fecal BAs were assessed by electrospray (ESI) mass spectrometry coupled to liquid chromatography (LC) with a tandem mass spectrometry (MS/MS) instrument (AB SCIEX, ThermoScientific, Waters) using a kit customized by Biocrates® (Biocrates® Bile Acids Kit), which enabled the absolute quantification of up to 20 different BA. A highly selective reverse phase analysis method, mass spectrometry coupled with liquid chromatography (CL-MS/MS), in negative detection mode (multiple reaction monitoring, MRM), was then applied to determine the concentrations of BA. For high precision quantification, we ensured that internal standards and external calibration were applied.

2.6. Assessment of T2D remission

One year after the surgical procedure, patients were divided into two sub-groups, according to the presence (the responsive group) or absence (the non-responsive group) of T2D remission. The remission of T2D was assessed by applying the following criteria from the American Diabetes Association (ADA): complete remission = fasting glycemia <100 mg/dL, HBA1c < 42.1 mmol/mol, under the absence of oral antidiabetic medication or other antidiabetic procedures for at least one year [15].

2.7. Statistical analysis

Biocrates MetIDQ™ software (Innsbruck, Austria) was applied to export the fecal BA data, along with the online platform MetaboAnalyst 3.0 (www.metaboanalyst.ca) [16,17]. Metabolites which were >20% of values below the limit of detection (LOD) were removed from our analysis. The concentrations of BAs were normalized to fecal sample weight and given in pmol/mg feces. Selected metabolite sums and ratios were calculated and tested in addition to single analytes. Using R software (version 3.2.2; R Core Team, Vienna, Austria) [18], measures of central tendency and of dispersion were tested by analysis of variance (ANOVA) and by the Benjamini–Hochberg method for multiple testing [19] and data were compared by ANOVA between responsive and non-responsive women.

3. Results

3.1. Sample

From the 20 patients studied, 12 (60%) were responsive and 8 were non-responsive for one year T2D remission, although one responsive patient moved out of town and did not complete the follow-up protocol to the 12-month time-point (Fig. 1). Descriptive data for the responsive and non-responsive patients were previously reported and here are described in supplementary table 1. A main point to highlight from these reported data is that circulating levels of glucose, insulin, C-peptide, and HbA1c did not differ significantly between responsive and non-responsive patients at baseline (Supplementary table 1, p > 0.05). Furthermore, there was no significant difference in the amount of weight lost at 3 months and 12 months post-operatively when compared between groups (Supplementary table 1, p > 0.05).
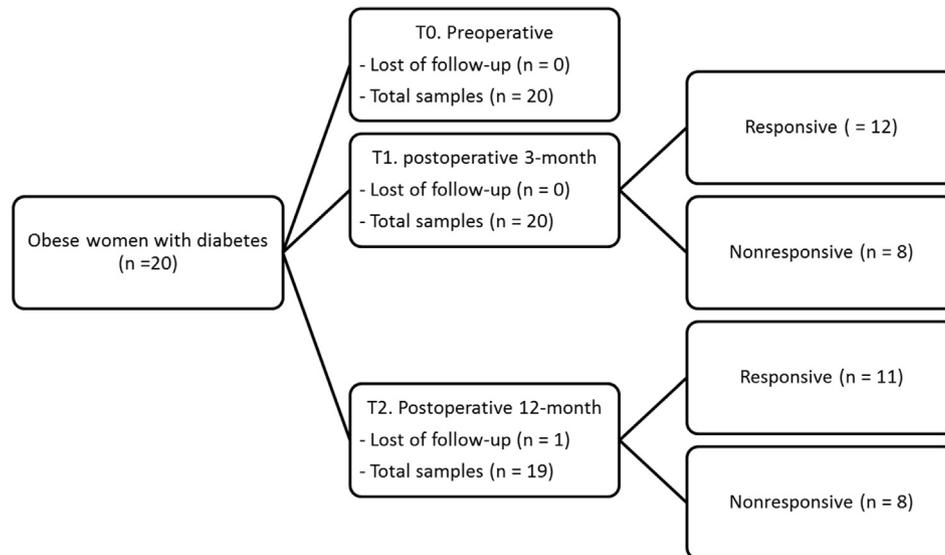
Fig. 1. Flowchart of patient distribution among pre- and post-operative periods, and groups, in relation to the postoperative remission of type 2 diabetes. Fecal samples were collected from the same obese patients prior to and after bariatric surgery.

3.2. RYGB induced a rapid reduction in fecal BAs that persisted until one year postoperatively

Across the preliminary study participants, a total of 10 fecal BAs changed after RYGB (Fig. 2). Levels of all of these 10 BAs were significantly reduced in relation to their pre-operative levels (Fig. 2). Analysis of fold-changes confirmed a reduction in the levels of glycochenodeoxycholic (GCDCA), glycocholic (GCA), taurocholic

acid (TCA), and taurochenodeoxycholic (TCDCA) acids at 3-months post-operatively ($p \leq 0.05$, Fig. 3). Of these, GCDCA, GCA, and TCA remained significantly reduced when tested 12 months after RYGB (Fig. 3). The Fig. 4 illustrate the changes in fecal bile acid levels in obese women after Roux-en-Y gastric bypass, according to the flowchart of its synthesis from cholesterol. We did not observe significant changes in fecal BAs levels between 3- and 12-month postoperative periods (Table 1; $p > 0.05$).
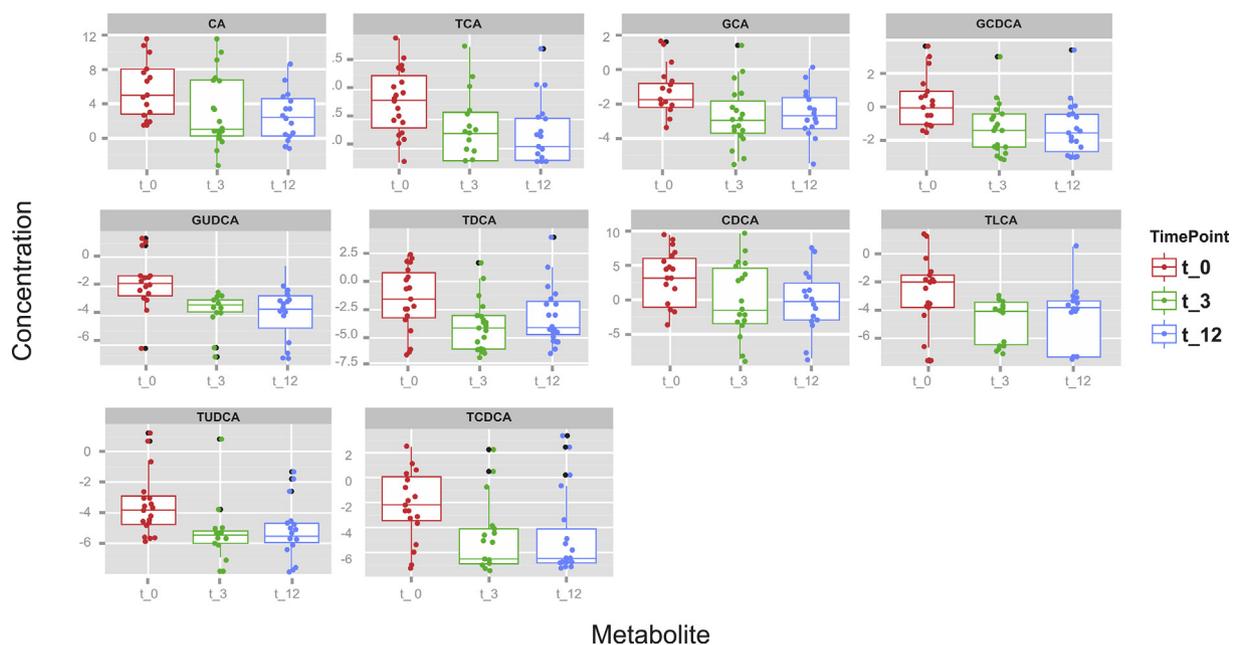

Fig. 2. Box plots of single BAs metabolites, significantly altered ($p < 0.05$) in the 3 time point (before bariatric surgery (t_0), 3 and 12 months after surgery (t_3, t_12)) according to ANOVA using a paired test. Outliers are highlighted by an additional black dot next to the original data point. Data is log2 transformed. Primary bile acids: (CA) Cholic acid; (CDCA) Chenodeoxycholic acid; (GCA) Glycocholic acid; (GCDCA) Glycochenodeoxycholic acid; (TCA) Taurocholic acid; (TCDCA) Taurochenodeoxycholic acid. Secondary bile acids: (GUDCA) Glycoursodeoxycholic acid; (TLCA) Taurolithocholic acid; (TDCA) Taurodeoxycholic acid; (TUDCA) Tauroursodeoxycholic acid.

UNIVARIATE ANALYSIS

Fold Change Analysis (Single metabolites)

Fold change (Before vs 3 months after bariatric surgery)			Fold change (Before vs 12 months after bariatric surgery)		
METABOLITE	FC_t0_t3	qVal_t0_t3	METABOLITE	FC_t0_t12	qVal_t0_t12
GCDCA	-1.454854915	0.022453021	GCDCA	-1.577429593	0.021887366
GCA	-1.682247422	0.022453021	GCA	-1.490994189	0.023347004
TCA	-2.430811252	0.036111541	TCA	-2.722670775	0.030419994
TCDA	-2.695841205	0.036111541			

Fig. 3. Fold changes in single metabolites upon bariatric surgery. Fold changes were calculated for each patient individually, followed by calculating the mean fold change for each metabolite. Negative values represent a decrease in concentration. Only metabolites with significant alterations are listed. Threshold for significance was set at 0.05 after correction for multiple testing using Benjamini–Hochberg. Primary bile acids: (GCDCA) Glycochenodeoxycholic acid; (GCA) Glycocholic acid; (TCA) Taurocholic acid; (TCDCA) Taurochenodeoxycholic acid.
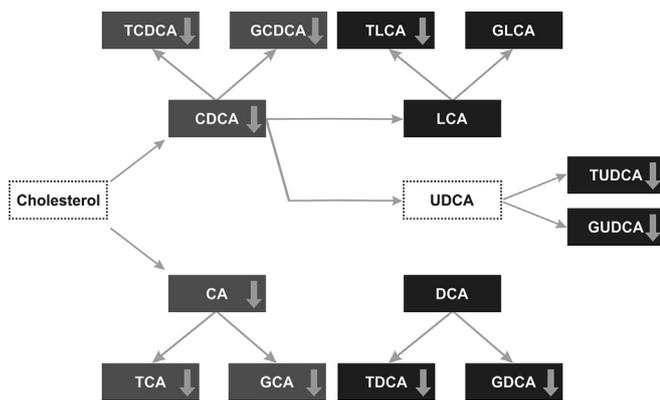

Fig. 4. Changes in fecal bile acid levels in obese women after Roux-en-Y gastric bypass, according to the flowchart of its synthesis from cholesterol. Primary bile acids are highlighted in the grey box and secondary bile acids in the dark box. Metabolites in dashed lines were not considered for statistical analysis as they were not measured (cholesterol) or did not meet quality criteria (UDCA). Significant reductions in metabolite levels in fecal samples after bariatric surgery according to multiple testing corrected ANOVA are indicated by the black arrows. No significant elevations were observed. Primary bile acids: cholic acid (CA) and chenodeoxycholic acid (CDCA)/Primary bile acids conjugated: taurocholic acid (TCA), glicocholic acid (GCA), TCDCA, taurochenodeoxycholic acid (TCDCA) and glicochenodeoxycholic acid (GCDCA)/Secondary bile acids: deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA)/Secondary bile acids conjugated: taurolithocholic acid (TLCA), glicodeoxycholic acid (GLCA), tauroursodeoxycholic acid (TUDCA), glicoursodeoxycholic acid (GUDCA), glycodeoxycholic acid (GDCA) and taurodeoxycholic acid (TDCA).

Table 1

Fold changes of fecal bile acids in obese women 3 months and 12 months after Roux-en-Y gastric bypass in comparison to preoperative levels.

Bile acid sub-fraction	Postoperative time-point			
	3-month		12-month	
	FC	p value	FC	p value
Glycochenodeoxycholic acid (GCDCA)	−1.45	0.022	−1.57	0.021
Glycocholic acid (GCA)	−1.68	0.022	−1.49	0.023
Taurocholic acid (TCA)	−2.43	0.036	−2.73	0.030
Taurochenodeoxycholic acid (TCDCA)	−2.69	0.036	−	−

Fold changes (FC) in single metabolites upon bariatric surgery. Fold changes were calculated for each patient individually, followed were calculated for each patient individually, followed by calculating the mean for each metabolite. Negative values represent a decrease in concentration. Only metabolites with significant alterations are listed. Threshold for significance was set at 0.05 after correction for multiple testing using Benjamini-Hochberg. Primary bile acids: (GCDCA) Glycochenodeoxycholic acid; (GCA) Glycocholic acid (TCA), Taurocholic acid (TCDCA) Taurochenodeoxycholic acid.

3.3. Distinct fecal BAs sub-fractions decreased after RYGB in responsive and non-responsive patients

We observed reduced fecal levels of deoxycholic acid (DCA), glicodeoxycholic acid (GDCA), glicolithocholic acid (GLCA), glicoursodeoxycholic acid (GUDCA) lithocholic acid (LCA) taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), taurolithocholic acid (TLCA), and changes in cholic acid (CA), and chenodeoxycholic acid (CDCA), expressed by an increase in the CA/CDCA ratio, at 3-months post-operatively in responsive patients ($p \leq 0.05$; Table 2). Of these BAs, only GUDCA remained at reduced levels when tested 12 months postoperatively and compared to preoperative levels. Only GLCA and LCA showed significant increases when compared between postoperative timepoints (3-months vs. 12-months, $p \leq 0.05$). Furthermore, 12-months postoperatively, responsive patients showed a reduction in GCDCA and TUDCA when compared to preoperative levels ($p \leq 0.05$).

In contrast to the responsive patients, few changes were observed in the fecal BA levels of non-responsive patients after RYGB (Table 3). In non-responsive patients, GCDCA and TCA were respectively reduced at 3 and 12 months postoperatively when compared to basal levels. As with the responsive patients, CA/CDCA ratio also increased in non-responsive patients after RYGB, but this change occurred latter and was observed at 12 months postoperatively compared to 3 months postoperatively, but not with the preoperative period ($p \leq 0.05$).

3.4. Surgically-induced changes in the behavior of fecal CA/CDCA ratio and TUDCA levels were associated with post-operative T2D remission

By comparing the variation (Δ) of BA levels among the studied time-points, it was evident that ΔCA/CDCA from the preoperative stage to 3 months postoperatively was significantly different when compared between the responsiveness groups ($p = 0.0001$, Fig. 5A) due to an increase in responsive patients and a reduction in non-responsive patients; the opposite trend occurred when comparing ΔCA/CDCA from 3 to 12 month time-points ($p = 0.0001$, Fig. 5B). Furthermore, the ΔTUDCA from the preoperative stage to 12 months postoperatively was also significantly different between the responsiveness groups ($p = 0.0327$, Fig. 5C) due to a reduction only in responsive patients. ANOVA showed that these postoperative changes in the behavior of fecal CA/CDCA ratio and TUDCA levels were able to distinguish between responsive from non-responsive patients ($p \leq 0.05$).

Table 2
Changes in BAs concentration among obese women responsive for the remission of type 2 diabetes 3 and 12 months after Roux-en-Y gastric bypass.

Variable	T0	T1	T2	T0 vs. T1	T0 vs. T2	T1 vs. T2
	Mean ± SD	Mean ± SD	Mean ± SD	p value	p value	p value
CA/CDCA	1.51 ± 0.84	3.06 ± 2.04	2,04 ± 4,01	0.010	0.206	0.102
DCA	2791.67 ± 2469.78	1393.2 ± 1923.81	1773.09 ± 1371.79	0.042	0.173	0.093
GDCA	3.34 ± 2.68	1.57 ± 1.95	1.89 ± 0.82	0.007	0.102	0.067
GLCA	0.53 ± 0.49	0.26 ± 0.27	0.37 ± 0.19	0.012	0.320	0.032
GUDCA	0.32 ± 0.42	0.07 ± 0.03	0.06 ± 0.05	0.016	0.007	0.634
LCA	1154.53 ± 1168.66	515.95 ± 565.97	760.36 ± 463.9	0.027	0.413	0.014
TCDCA	1.05 ± 1.68	0.16 ± 0.42	0.51 ± 1.65	0.042	0.083	0.700
TDCA	1.66 ± 1.87	0.20 ± 0.37	1.76 ± 5.28	0.007	0.102	0.175
TLCA	0.41 ± 0.50	0.08 ± 0.07	0.17 ± 0.36	0.003	0.054	0.765
GCDCA	2.27 ± 3.51	0,56 ± 0,61	0.38 ± 0.29	0.092	0.005	0.831
TUDCA	0,33 ± 0,48	0,03 ± 0,01	0.05 ± 0.05	0.001	0.001	0.520

Key: T0, preoperative; T1, postoperative 3-month; T2, postoperative 12-month; SD, standard deviation; CA/CDCA ratio of cholic and chenodeoxycholic acids; DCA, deoxycholic acid, GDCA, glicodeoxycholic acid; GLCA, glicolithocholic acid; GUDCA, glicoursodeoxycholic acid; LCA, lithocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA; taurodeoxycholic acid; TLCA, taurolithocholic acid; GCDCA, glycochenodeoxycholic acid and tauroursodeoxycholic acid (TUDCA).

Table 3
Changes in bile acid concentration among obese women who were non-responsive for the remission of type 2 diabetes 3 and 12 months after Roux-en-Y gastric bypass.

Variable	T0	T1	T2	T0 vs. T1	T0 vs. T2	T1 vs. T2
	Mean ± SD	Mean ± SD	Mean ± SD	p value	p value	p value
CA/CDCA	1.59 ± 1.14	0.171 ± 0.47	3.54 ± 6.01	0.055	0.945	0.039
TCA	1.00 ± 1.45	1.62 ± 4.18	0.37 ± 0.54	0.641	0.016	1000
GCDCA	1.99 ± 2.44	1.44 ± 2.52	1.42 ± 2.52	0.008	0.250	0.742

Key: T0, preoperative; T1, postoperative 3-month; T2, postoperative 12-month; SD, standard deviation; CA/CDCA ratio of cholic and chenodeoxycholic acids; TCA, taurocholic acid; GCDCA, glycochenodeoxycholic acid.

4. Discussion

This preliminary study showed, for the first time, changes in fecal BAs profiles in obese women with T2D in response to RYGB. These changes were marked by an early reduction in primary and secondary BAs concentrations that persisted until one-year after surgery. The postoperative profile of BAs differed between patients with or without T2D remission, suggesting a potential link between BAs metabolism and glucose homeostasis [20–23].

A reduction in total fecal BAs and their sub-fractions CDCA, CA, LCA, DCA and ursodeoxycholic acid (UDCA), has been reported in animal models after RYGB [12,24–27]. In our preliminary study of diabetic women, significant reduction in the levels of fecal BAs also occurred after RYGB, mainly in those BAs conjugated to glycine and taurine. Conjugated BAs can be deconjugated by the intestinal microbiota, specifically by bile salt hydrolases (BSH) synthesized mainly by *Lactobacillus* and *Bifidobacteria* [2]. The deconjugation of BAs by BSH activity accelerates cholesterol catabolism by increasing the elimination of fecal BA [5]. Therefore, our data suggest that the loss of fecal BAs may be decreased by a reduction of its deconjugation by gut bacteria after RYGB and/or may reflect the increased hepatic conjugation of primary BA following the surgical procedure.

The observed reduction in the elimination of fecal BAs following RYGB may reflect its increased absorption and recirculation through the enterohepatic pathway. This agrees with the increased levels of circulating BAs reported after the procedure in human and animal models, which have been considered to represent a metabolic mechanism that influences post-operative glycemic homeostasis [10–12]. Activation of the LXR/RXR pathway in gut has been associated with increased BAs absorption and enterohepatic recirculation [28]. An ongoing study from our group demonstrated that RYGB increased the expression of genes that promote activation of the LXR/RXR pathway in the jejunum of responding patients. These data suggested that the decrease in fecal AB may have occurred due

to the increase in its recirculation and that this metabolic pathway may have been important for glycemic homeostasis.

Therefore, it could be expected that changes in the levels of fecal BAs would affect post-operative remission of T2D in our patients. In our preliminary study, more BAs sub-fractions were reduced in responsive than non-responsive patients. Furthermore, changes in the CA/CDCA ratio and levels of TUDCA were quite distinct between these groups of patients.

Enhanced plasma CA/CDCA ratios are commonly observed during cholestasis, a clinical condition characterized by a reduction or cessation of bile formation or flow. For example, cholestatic hamsters exhibit a significant increase in serum CA/CDCA ratio, and a reduced proportion of unconjugated BA to total BA, via a mechanism associated with the inhibition of the mitochondrial sterol 27-hydroxylase pathway (CYP27A1) [29]. These changes in the pool of BA may therefore be considered as negative predictors of liver function and BA synthesis.

In our preliminary study, increased fecal CA/CDCA ratio observed in responsive patients at early postoperative stage can reflect lower CA and higher CDCA absorption in the intestine, potentially resulting in a lower circulating CA/CDCA ratio following RYGB. Furthermore, responsive patients showed a reduction in high sub-fraction types of conjugated fecal BA than non-responsive patients, suggesting a higher absorption of conjugated BA in the intestine [30].

The higher fecal CA/CDCA ratio observed in the early postoperative (3-month) stage in responsive patients may also reflect an increased CA and/or decreased CDCA production in the liver, but this is less likely to be consistent with the control of glycemic homeostasis. An increased circulating CA/CDCA ratio is also observed in pregnant women with intrahepatic cholestasis, and is associated with increased fasting triglycerides and reduced levels of HDL cholesterol (similar to changes observed in metabolic syndrome), and increased blood glucose, compared to healthy pregnant women [29–32]. Furthermore, Kimura et al. (1991) found an abnormally low CA/CDCA ratio in pediatric patients with cholestasis presenting with severe hypoglycemia [33]. Taken together, these findings suggest that a high circulating CA/CDCA ratio favors hyperglycemia, while patients with a low circulating CA/CDCA ratio are prone to hypoglycemia.

In our present preliminary study, decreased levels of fecal TUDCA were also a hallmark of responsiveness to T2D remission during the late postoperative stage (12-month). TUDCA is a taurine-conjugated form of UDCA, a BA approved by the Food and Drug Administration (FDA) for the treatment of cholestatic liver disease [34]. Improved insulin sensitivity and pancreatic beta cell islets restoration via the AMP/PKA pathway, have been reported after treatment with oral TUDCA [35,36]. Both increased synthesis in the
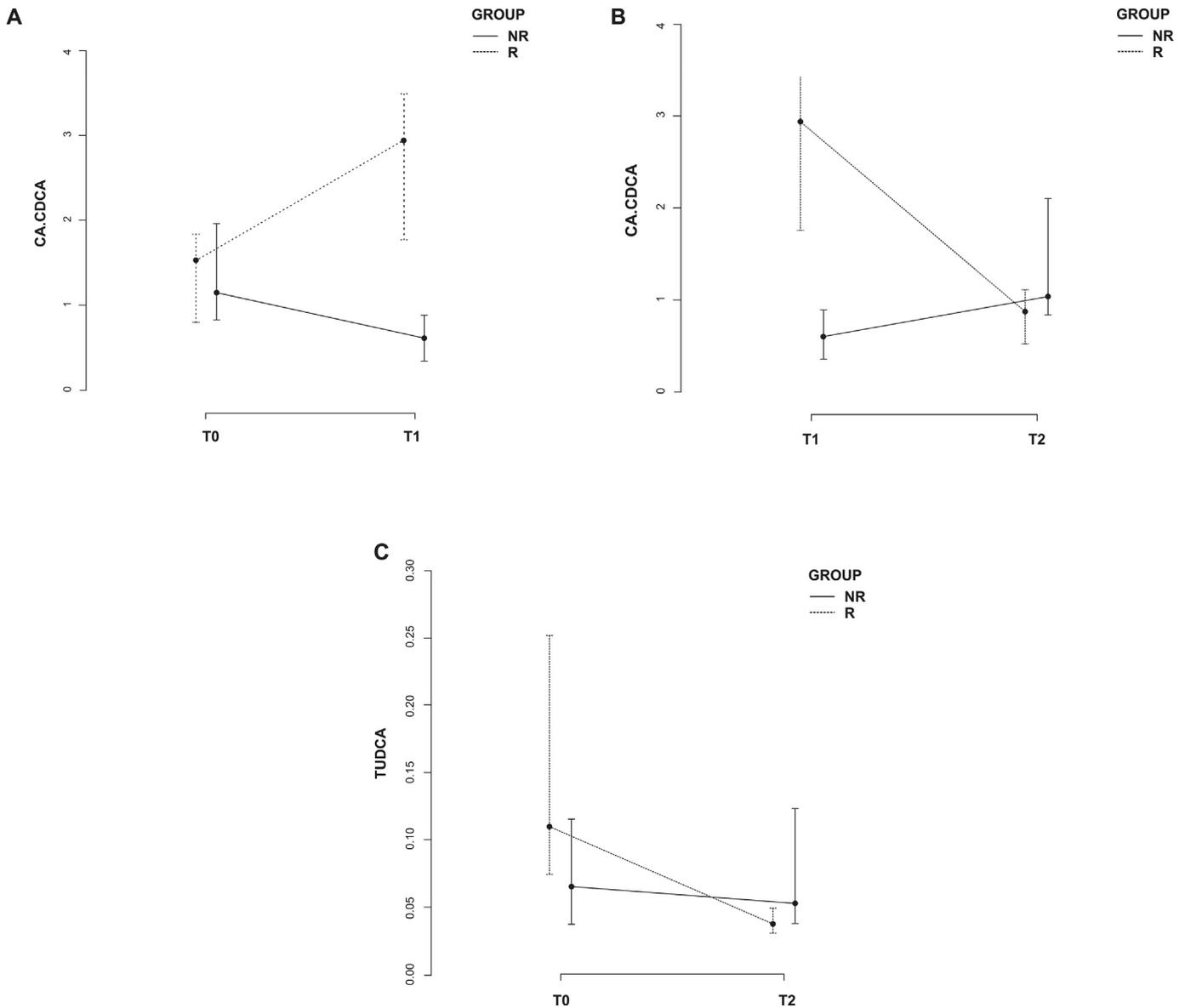
Fig. 5. A. Changes in CA/CDCA ratio behavior among obese women responsive (R) and non-responsive (NR) for the remission of type 2 diabetes 3 months after Roux-en-Y gastric bypass. Responsive patients presented with increased, and non-responsive patients presented with decreased, cholic acid (CA)/chenodeoxycholic acid (CDCA) ratio 3 months postoperatively (ANOVA p = 0.0001). T0, preoperative; T1, postoperative 3-month; **B**. Changes in CA/CDCA ratio behavior among obese women responsive (R) and non-responsive (NR) for the remission of type 2 diabetes 12 months after Roux-en-Y gastric bypass. The opposite effect was found for cholic acid (CA)/chenodeoxycholic acid (CDCA) ratio at 12 months postoperatively compared with the period at 3 months postoperatively (ANOVA, p = 0.0001). Responsive patients presented decreased, and non-responsive patients presented increased a at 12 months postoperatively. T0, postoperative 3-month; T2, postoperative 12-month. **C**. Changes in TUDCA levels among obese women responsive (R) and non-responsive (NR) for the remission of type 2 diabetes 12 months after Roux-en-Y gastric bypass. Only responsive patients presented with a statistically significant reduction in the levels of tauroursodeoxycholic acid (TUDCA) at 12 months postoperatively (ANOVA, p = 0.0327). T0, preoperative; T2, postoperative 12-month.

liver, and/or absorption of TUDCA in the intestine, could be reflected in the feces of our responsive patients and may suggest a potential effect upon T2D remission.

It is worth noting that as RYGB restricts the stomach size, our patients had significantly reduced their fat intake [37], thereby favoring a reduced level of BAs redirection to the intestine for emulsification. However, the specific BA profile found in responsive and nonresponsive patients in relation to T2D remission, highlights the modulation of BAs by RYGB beyond restrictive intake.

Our preliminary study has some limitations which need to be taken into consideration. For example, we did not investigate changes in fecal BAs associated with the gut microbiota profile and systemic BA levels. Nevertheless, changes in the composition and richness of the gut microbiota have been commonly reported after

RYGB and our data is coherent with increased levels of circulating BAs reported previously in patients undergoing this procedure [38–40]. We also evaluated only obese diabetic women and our findings may not be generalized to clinically-matching males. In our Surgical Division women are more prevalent than men among the bariatric patients and by including only one gender we were able to study a more homogeny sample.

Taken together, our data displayed a distinct influence of RYGB on fecal BA pattern between obese women responsive and nonresponsive to T2D remission. Postoperative fecal BA pattern in responsive women seems to reflect an early increase in circulating levels of TUDCA and CA/CDCA ratios, which together can be suggestive of an improvement in glycemic homeostasis. Why nonresponsive women did not follow this protective pattern is

beyond the scope of our present preliminary study. We can conclude that RYGB induces a marked reduction in the concentration of fecal BA concentration, which is heterogeneous according to T2D responsiveness.

Conflict of interest

None.

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CRediT authorship contribution statement

Camila de Siqueira Cardinelli: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Funding acquisition. **Raquel Susana Torrinhas:** Writing - original draft, Writing - review & editing. **Priscila Sala:** Resources, Funding acquisition. **Marcos Albieri Pudenzi:** Methodology. **Célio Fernando F Angolini:** Formal analysis. **Mariane Marques da Silva:** Resources. **Natasha Mendonça Machado:** Resources. **Graziela Ravacci:** Writing - review & editing. **Marcos N. Eberlin:** Supervision. **Dan L. Waitzberg:** Writing - original draft, Writing - review & editing, Supervision, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2018.12.028.

References

[1] de Aguiar Vallim TQ1, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. Cell Metabol 2013;17:657–69.
[2] Li T, Udayan A. Bile acid metabolism and signaling in cholestasis, inflammation, and cancer. Adv Pharmacol 2015;74:263–302.
[3] Wahlström A, Sayin SI, Marschall H, Backhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metabol 2016;24:41–50.
[4] Ridlon JM, Kang DJ, Hylemon P, Bajaj JS. Bile acids and the gut microbiome. Curr Opin Gasterenterol 2014;30:332.
[5] Fiorucci S, Distrutti E. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. Trends Mol Med 2015;21:702–14.
[6] Chiang JYL. Bile acid metabolism and signaling. Comp Physiol 2013;3: 1191–212.
[7] Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzales FJ, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci USA 2006;103:1006–11.
[8] Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metabol 2009;10:167–77.
[9] Kumar DP, Rajagopal S, Mahavadi S, Mirshahi F, Grider JR, Murthy KS, et al. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet á cells to promote glucose homeostasis. J Biol Chem 2016 Mar 25;291(13):6626–40. https://doi.org/10.1074/jbc.M115.699504.
[10] 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 Metabol 2013;98: E708–12.
[11] Steinert RE, Peterli R, Keller S, Meyer-Gerspach AC, Drewe J, Peters T, et al. Bile acids and gut peptide secretion after bariatric surgery: a 1-year prospective randomized pilot trial. Obesity (Silver Spring) 2013;21:E660–8.
[12] Bhutta HY, Rajpal N, White W, Freudenberg JM, Liu Y, Way J, et al. Effect of Roux-en-Y gastric bypass surgery on bile acid metabolism in normal and obese diabetic rats. PLoS One 2015;10, e0122273.
[13] Ocvirk S, O'Keefe Stephen JD. Influence of bile acids on colorectal cancer risk: potential mechanisms mediated by diet-gut microbiota interactions. Curr Nutr Rep 2017;6:315–22.
[14] Sala P, Belarmino G, Machado NM, Cardinelli CS, Al Assal K, Silva MM, et al. The SURMetaGIT study: design and rationale for a prospective pan-omics examination of the gastrointestinal response to Roux-en-Y gastric bypass surgery. J Int Med Res 2016;44:1359–75.
[15] Buse JB, Caprio S, Cefalu WT, Ceriello A, Del Prato S, Inzucchi SE, et al. How do we define cure of diabetes? Diabetes Care 2009;32:2133–5.
[16] Ramsay SL, Stoegg WM, Weinberger KM, Graber A, Guggenbichler W. Apparatus and method for analyzing a metabolite profile. U.S. Patent No. 8,265,877. Washington, DC: U.S. Patent and Trademark Office; 2012.
[17] Xia J, Psychogios N, Young N, Wishart DS. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res 2009;37: W652–60.
[18] Team, R. Core. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015.
[19] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B 1995;57:289–300.
[20] Maggard MA, Shugarman LR, Suttorp M, Maglione M, Sugerman HJ, Livingston EH, et al. Meta-analysis: surgical treatment of obesity. Ann Intern Med 2005;142:547–59.
[21] Schauer PR, Burguera B, Ikramuddin S, Cottam D, Gourash W, Hamad G, et al. Effect of laparoscopic Roux-en Y gastric bypass on type 2 diabetes mellitus. Ann Surg 2003;238:467–84.
[22] Buchwald H, Braunwald AY, Jensen E, Pories W, Fahrbach K, Schoelles K. Bariatric surgery: a systematic review and meta-analysis. JAMA 2004;292: 1724–37.
[23] Pories WJ, Swanson MS, MacDonald KG, Long SB, Morris PG, Brown BM, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. Ann Surg 1995;222:339–52.
[24] Chiang JY. Bile acids: regulation of synthesis. J Lipid Res 2009;50:1955–66.
[25] Nagengast FM, Grubben MJAL, Van Munster IP. Role of bile acids in colorectal carcinogenesis. Eur J Cancer 1995;31:1067–70.
[26] Nie Y, Hu J, Yan X. Cross-talk between bile acids and intestinal microbiota in host metabolism and health. J Zhejiang Univ Sci B 2015;16:436–46.
[27] Lindqvist A, Ekelund M, Garcia-Vaz E, Ståhlman M, Pierzynowski S, Gomez MF, et al. The impact of Roux-en-Y gastric bypass surgery on normal metabolism in a porcine model. PLoS One 2017;12, e0173137.
[28] Halilbasic E, Claudel T, Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. J Hepatol 2013;58(1):155–68.
[29] Matsuzaki Y, Bouscarel B, Ikegami T, Honda A, Doy M, Ceryak S, et al. Selective inhibition of CYP27A1 and of chenodeoxycholic acid synthesis in cholestatic hamster liver. Biochim Biophys Acta 2002;1588:139–48.
[30] Pires MJ, Colaço AO. The role of bile acids in the pathology and therapy of hepatic diseases in dog and cat. RCPV 2004;99:137–43.
[31] Jurate K, Rimantas Z, Jolanta S, Vladas G, Limas K. Ann Hepatol 2017;16: 569–73.
[32] Martineau MG, Raker C, Dixon PH, Chambers J, Machirori M, King NM, et al. The metabolic profile of intrahepatic cholestasis of pregnancy is associated with impaired glucose tolerance, dyslipidemia, and increased fetal growth. Diabetes Care 2015;38:243–8.
[33] Kimura A, Ken Y, Shigenori Y, Masayoshi K, Hiroshi N, Reijiro M, et al. Abnormal low ratio of cholic acid to chenodeoxycholic acid in a cholestatic infant with severe hypoglycemia. J Pediatr Gastroenterol Nutr 1991;12:383–7.
[34] Paridaens A, Raevens S, Devisscher L, Bogaerts E, Verhelst X, Hoorens A, et al. Modulation of the unfolded protein response by tauroursodeoxycholic acid counteracts apoptotic cell death and fibrosis in a mouse model for secondary biliary liver fibrosis. Int J Mol Sci 2017;18:214.
[35] Kars M, Yang L, Gregor MF, Mohammed BS, Pietka TA, Finck BN. Tauroursodeoxycholic Acid may improve liver and muscle but not adipose tissue insulin sensitivity in obese men and women. Diabetes 2010;59:1899–905.
[36] Lee YY, Hong SH, Lee YJ, Chung SS, Jung HS, Park SG, et al. Tauroursodeoxycholate (TUDCA), chemical chaperone, enhances function of islets by reducing ER stress. Biochem Biophys Res Commun 2010;397:735–9.
[37] da Silva MM, Sala PC, Torrinhas RS, Waitzberg DL. The efficiency of 24-hour food recall for assessing nutrient intake before and after Roux-en-Y gastric bypass. Nutr Hosp 2014;30:1240–7.
[38] Risstad H, Kristinsson JA, Fagerland MW, le Roux CW, Birkeland KI, Gulseth HL. Bile acid profiles over 5 years after gastric bypass and duodenal switch: results from a randomized clinical trial. Surg Obes Relat Dis 2017;13:1544–5.
[39] Graessler J, Qin Y, Zhong H, Zhang J, Licinio J, Wong ML, et al. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. Pharmacogenomics J 2013;13:514–22.
[40] Kong LC, Tap J, Aron-Wisnewsky J, Pelloux V, Basdevant A, Bouillot JL. Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes. Am J Clin Nutr 2013;98:16–24.