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ORIGINAL CONTRIBUTIONS

Serum Bile Acid Levels Before and After Sleeve Gastrectomy and Their Correlation with Obesity-Related Comorbidities

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Abstract

Background and Aims The rising prevalence of morbid obesity is increasing the demand for bariatric surgery. The benefits observed after bariatric surgery seems to be not fully explained by surgery-induced weight loss or traditional cardiovascular risk factors regression or improvement. Some evidences suggest that bile acid (BA) levels change after bariatric surgery, thus suggesting that BA concentrations could influence some of the metabolic improvement induced by bariatric surgery. In this report, we have characterized circulating BA patterns and compared them to metabolic and vascular parameters before and after sleeve gastrectomy (SG).

Patients and Methods Seventy-nine subjects (27 males, 52 females, aged 45 ± 12 years, mean BMI 45 ± 7 kg/m^2) SG candidates were included in the study. Before and about 12 months after SG, all subjects underwent a clinical examination, blood tests (including lipid profile, plasma glucose and insulin, both used for calculating HOMA-IR, and glycated hemoglobin), ultrasound visceral fat area estimation, ultrasound flow-mediated dilation evaluation, and determination of plasma BA concentrations.

Results Before SG, both primary and secondary BA levels were higher in insulin-resistant obese subjects than in non-insulin resistant obese, and BA were positively associated with the markers of insulin-resistance. After SG, total (conjugated and unconjugated) cholic acids significantly decreased (p 0.007), and total lithocholic acids significantly increased (p 0.017). SG-induced total cholic and chenodeoxycholic acid changes were directly associated with surgery-induced glycemia (p 0.011 and 0.033 respectively) and HOMA-IR (p 0.016 and 0.012 respectively) changes.

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Conclusions Serum BA are associated with glucose metabolism and particularly with markers of insulin-resistance. SG modifies circulating BA pool size and composition. SG-induced BA changes are associated with insulin-resistance amelioration. In conclusion, an interplay between glucose metabolism and circulating BA exists but further studies are needed.

Keywords Bile acids · Sleeve gastrectomy · Morbid obesity · Bariatric surgery · Metabolism

Abbreviations

BA	bile acids
FXR	farnesoid X receptor
TGR5	Takeda G protein-coupled receptor-5
SG	sleeve gastrectomy
BMI	body mass index
WC	waist circumference
TC	total cholesterol
TG	triglycerides
LDL	low-density lipoprotein
HDL	high-density lipoprotein
HbA1c	glycated hemoglobin
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
tCA	taurocholic acid
tCDCA	taurochenodeoxycholic acid
tDCA	taurodeoxycholic acid
tLCA	taurolithocholic acid
gCA	glycocholic acid
gCDCA	glycochenodeoxycholic acid
gCA	glycolithocholic acid
CA	cholic acid
CDCA	chenodeoxycholic acid
DCA	deoxycholic acid
LCA	lithocholic acid
VFA	visceral fat area
FMD	flow-mediated dilation
FGF-19	fibroblast growth factor 19
SBP	systolic blood pressure
DBP	diastolic blood pressure
CA	cholic acid
CDCA	chenodeoxycholic acid
DCA	deoxycholic acid
LCA	lithocholic acid
tCA	taurocholic acid
tCDCA	taurochenodeoxycholic acid
tDCA	taurodeoxycholic acid
tLCA	taurolithocholic acid
gCA	glycocholic acid
gCDCA	glycochenodeoxycholic acid
gCA	glycolithocholic acid
HOMA	homeostasis model assessment

Introduction

Obesity, associated with an increased mortality, is occurring at epidemic rates worldwide [1]. The obesity-related mortality is explained by the many comorbidities which can develop as a consequence of obesity. Among them, cardiovascular diseases are the main cause of death [2].

The rising prevalence of morbid obesity is increasing the demand for bariatric or "metabolic" surgery [3], which currently is the most effective treatment for severe obesity and its associated disorders [4].

It is noteworthy that the association between cardiovascular risk and obesity is not fully explained by the increased prevalence of the traditional cardiovascular risk factors (i.e., hypertension, type-2 diabetes, dyslipidemia) seen in obese subjects [5]. Similarly, the mortality and cardiovascular risk reductions observed after bariatric surgery seem to be not fully explained by surgery-induced weight loss or traditional cardiovascular risk factors regression or improvement [6]. Thus, additional putative mechanisms explaining the link between obesity, metabolic comorbidities, and cardiovascular diseases have been proposed, including visceral fat depots accumulation [7] and sub-clinical chronic inflammatory [8] or oxidative stress [9].

Recently, several authors focused on the possible role of bile acids (BA) (Fig. 1). In addition to their role in nutrient absorption, primary and secondary BA regulate liver and adipose tissues metabolism by activating a family of transcription factors, collectively known as the bile acid-activated receptors. This family include both nuclear receptors, such as the farnesoid X receptor (FXR), and the G protein-coupled receptor GPBAR1 also known as Takeda G protein-coupled receptor-5 (TGR5) [10], with both receptors highly expressed in the vascular system [11].

Some evidences suggest that BA levels increase after bariatric surgery, thus suggesting that changes in circulating BA concentrations could influence some of the metabolic improvement induced by bariatric surgery [12]. However, the current available studies, mainly carried on small population sample size, are inconsistent [12]; moreover, different bariatric surgery procedures could have different effects on circulating BA concentrations [12].

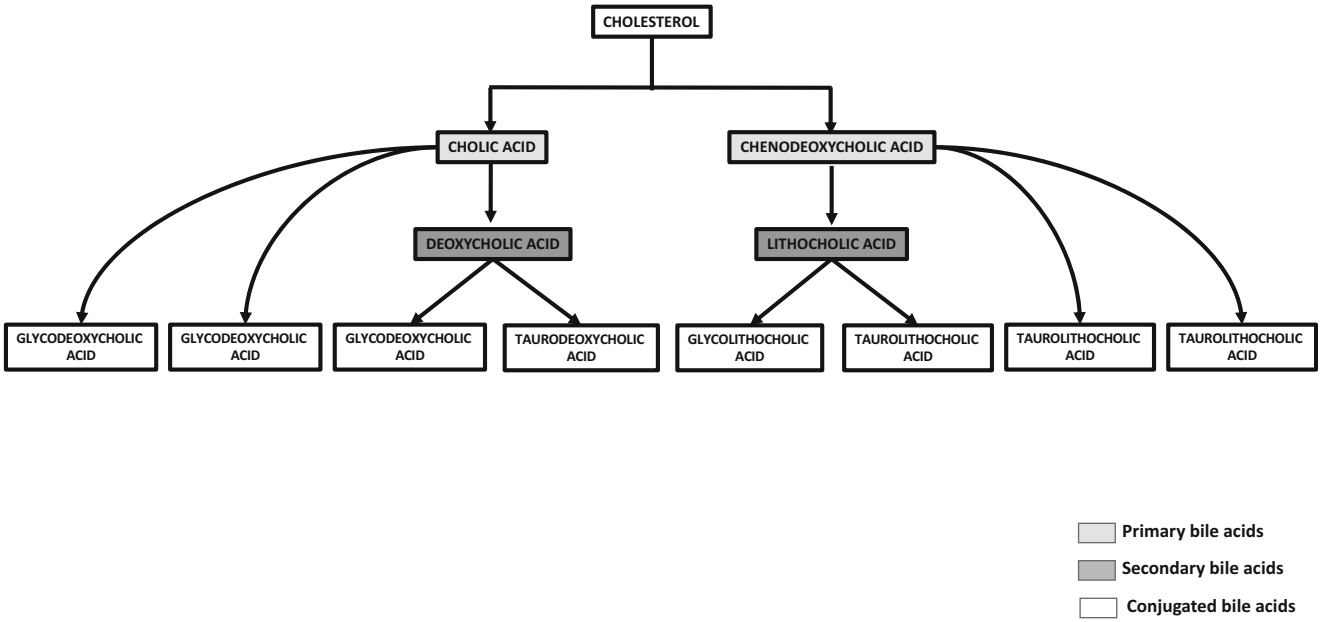

Fig. 1 Graphic representation of primary, secondary, and conjugated bile acids

In this report, we have characterized circulating BA patterns and compared them to metabolic and vascular parameters before and after sleeve gastrectomy (SG), which is a relatively new introduced bariatric surgery procedure. Particularly, the aims were to investigate the possible associations between serum BA and metabolic and vascular parameters before surgery; to study both primary and secondary circulating BA concentrations' changes before and after SG, which is a relatively new introduced bariatric surgery procedure; and to investigate if serum BA changes following bariatric surgery could influence metabolic and vascular improvement.

Subjects, Materials, and Methods

We retrospectively enrolled 79 obese subjects (27 males, 52 females, aged 45 ± 12 years, mean BMI 45 ± 7 kg/m^2) referred to Perugia University Internal Medicine Unit, from November 2016 to March 2018 for cardiovascular and metabolic assessment, 1 month before and about 12 months after SG. All SG procedures were performed by the same surgeon (M. B.). Exclusion criteria were liver and renal insufficiency, heart failure (NYHA II-IV), alcoholism, secondary causes of obesity, and major psychiatric diseases.

All patients underwent a clinical examination. BMI was defined as weight (kg)/squared height (m^2). Waist circumference (WC) was measured at the level of the umbilicus with a measuring tape, at the end of a normal expiration. Blood pressure was read in triplicate by a physician, with a mercury sphygmomanometer, and a brachial cuff of appropriate size was placed around the non-dominant arm.

After an overnight fast, blood samples were drawn, and the following parameters determined total cholesterol (TC), triglycerides (TG) (enzymatic colorimetric method), HDL-cholesterol (enzymatic colorimetric method after precipitation with polyethylenglycole), LDL-cholesterol (Friedwald formula), plasma glucose (automated analyzer), serum insulin (ELISA), and glycated hemoglobin (HbA1c) (high pressure liquid chromatography).

Using the same blood samples, we determined the following bile acids: cholic acid (CA); chenodeoxycholic acid (CDCA); deoxycholic acid (DCA); lithocholic acid (LCA); taurocholic acid (tCA); taurochenodeoxycholic acid (tCDCA); taurodeoxycholic acid (tDCA); taurolithocholic acid (tLCA); glycocholic acid (gCA); glycochenodeoxycholic acid (gCDCA); and glycolithocholic acid (gLCA). All BA were measured by a liquid chromatography–tandem mass spectrometry (LC-MS/MS) carried out on the UPLC–MS system Q-TRAP 6500 LC-MS/MS System from AB Sciex equipped with Shimadzu LC-20A LC and AutoSampler system (for more details see Supplemental material).

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), as described by Matthews et al. [13], calculated insulin resistance using the following equation: HOMA-IR = fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5.

Written informed consent was obtained from each study participant.

The protocol was approved by the Ethic Committee of our Institution (protocol number 2017-13).

The study was also registered at register.clinicaltrials.gov, number NCT03559842.

Abdominal Ultrasonography

Visceral fat area (VFA) was measured by ultrasonography (MyLab 50, Esaote, Italy), as described by Hirooka et al. [14] as follows: [VFA] = − 9.008 + 1.191 × [distance between the abdominal muscle internal surface and the splenic vein (mm)] + 0.978 [distance between the abdominal muscle internal surface and the posterior aorta wall at the umbilicus (mm)] + 3.644 × [thickness of fat layer in the posterior right renal wall (mm)].

Flow-Mediated Dilation

Brachial artery ultrasonography was used to measure the flow-mediated dilation (FMD), defined as the percentage change of the arterial diameter from the baseline vessel size at the brachial artery following reactive hyperemia, method previously described [15].

Statistical Analysis

We used Student's *t* test and the Mann-Whitney *U* test to compare parametric and non-parametric variables between subjects with obesity and controls. For comparing pre- and post- surgery parametric and non-parametric variables, we used the paired *t* test and Wilcoxon test. Spearman's rank correlation coefficients tested the relation between the variables. Analyses were performed with SPSS software (version 19.0; SPSS, Inc., Chicago, IL), with statistical significance set at $p < 0.05$.

Results

Anthropometric, Clinical, and Metabolic Features

Anthropometric, clinical, and metabolic features of the subjects before surgery are shown in Table 1. There was a prevalence of females in our population; about 20% were smokers; almost 40% of the subjects were affected by hypertension and about 10% were affected by diabetes. Subjects included were affected by morbid obesity (mean BMI 45 kg/m^2) and were on average affected by insulin resistance (mean HOMA-IR 5.6), while blood pressure levels and lipid profile were almost in the normal range.

Table 1 Pre-surgery characteristics of the population

Age, years	45 ± 12
Gender, M/F	27/52
Smoke, %	23
Diabetes, %	9
Hypertension, %	37
BMI, kg/m^2	45 ± 7
Waist circumference, cm	133 ± 18
SBP, mmHg	139 ± 15
DBP, mmHg	87 ± 9
Total cholesterol, mg/dl	193 ± 39
Triglycerides, mg/dl	151 ± 88
HDL-C, mg/dl	51 ± 15
LDL-C, mg/dl	115 ± 31
Glycemia, mg/dl	107 ± 44
Insulinemia, μIU/ml	20 ± 13
HOMA-IR	5.6 ± 5.6
HbA1c, %	6.2 ± 1.3
VFA, cm^2	258 ± 67
Subcutaneous fat, cm	31 ± 11
FMD, %	9.6 ± 6.5

SBP, systolic blood pressure; *DBP*, diastolic blood pressure; *BMI*, body mass index; *HDL-C*, high density lipoprotein-cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *VFA*, visceral fat area; *HOMA-IR*, homeostasis model assessment of insulin resistance; *HbA1c*, glycated hemoglobin; *FMD*, flow-mediated dilation

Serum BA Concentrations and Their Correlations with Other Parameters Before SG

Before SG, we divided our population into two groups on the basis of median HOMA-IR values: above/equal to or below 4.2. We observed that serum concentrations of CA ($p = 0.025$), CDCA ($p = 0.012$), DCA ($p = 0.003$), gCA ($p = 0.001$), gCDCA ($p = 0.010$), and gLCA ($p = 0.021$) were higher in subjects with HOMA-IR above the median value than those with HOMA-IR below the median value (Fig. 2).

As shown in Table 2, before SG, we observed a significant association among both primary and secondary BA and the markers of insulin-resistance. In the same manner, we found a significant association among both total primary and total secondary BA and the markers of insulin-resistance (Table 3).

None of the BA included in the study showed a significant association with FMD.

Moreover, we correlated BA concentrations with the metabolic parameters dividing our population in two groups: above or below the median HOMA index value. For the group of subjects with HOMA index above the median value, we found the following statistically significant associations: tDCA with insulin levels (p 0.035, r 0.343) and HOMA index (p 0.024, r 0.365); gLCA with insulin levels (p 0.029, r 0.354) and HOMA index (p 0.014, r 0.397); and total lithocholic acids with HOMA index (p 0.041, r 0.333). For the group of subjects with HOMA index below the median value, we found the following statistically significant associations: tCA with insulin levels (p 0.009, r 0.418) and HOMA index (p 0.004, r 0.458); gCA with insulin levels (p 0.008, r 0.423) and

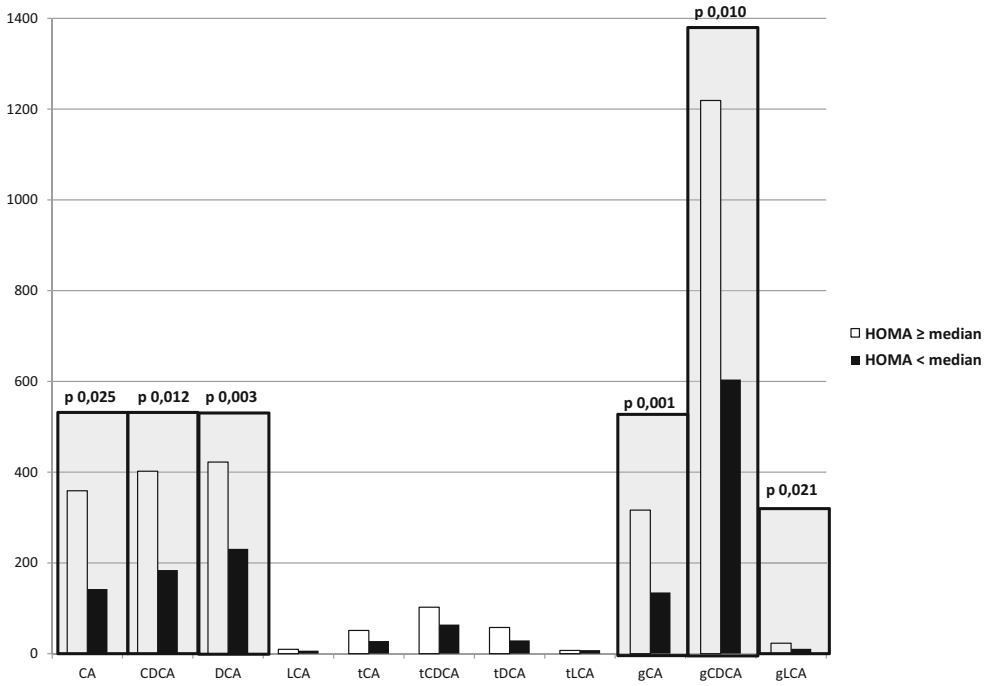

Fig. 2 Differences of serum bile acid concentrations between subjects with HOMA above and below the median value

HOMA index (p 0.010, r 0.415); and total cholic acids with HOMA index (p 0.039, r 0.336).

Effects of SG on Anthropometric, Clinical. and Metabolic Parameters and on Serum BA Concentrations

After SG, we observed a significant decrease of BMI ($p < 0.001$), WC ($p < 0.001$), VFA ($p < 0.001$), subcutaneous fat ($p < 0.001$), SBP ($p < 0.001$), DBP ($p < 0.001$), TG ($p < 0.001$), glycemia ($p < 0.001$), insulinemia ($p < 0.001$), HOMA-IR ($p < 0.001$), and Hb1Ac ($p < 0.001$); HDL levels significantly increased ($p < 0.001$) and FMD significantly improved ($p = 0.001$).

After SG, we observed a significant reduction of serum CA concentrations ($p = 0.028$) and a significant increase of tLCA concentrations ($p = 0.030$); we did not observe any other significant change before and after surgery of the other serum BA included in the study. As shown in Fig. 3, after SG, we observed a significant decrease of total (conjugated and unconjugated) cholic acids ($p = 0.007$) and a significant increase of total lithocholic acids ($p = 0.017$).

Correlations of SG-Induced Circulating BA Variations (Δ) and Other Parameter Variations

In Tables 4 and 5, are shown the correlations among SG-induced variations (calculated as Δ, where "Δ" means "value before surgery − value after surgery") of serum bile acid concentrations and metabolic parameters and FMD. We observed that total primary BA were directly associated with some of the markers of insulin-resistance, while we did not observe any significant correlation between total secondary BA and the markers of insulin-resistance.

Discussion

In this study, we wanted to describe BA concentrations and composition changes after SG and to evaluate their putative involvement in the metabolic ameliorations, and thus on diabetes remission, induced by bariatric surgery.

Firstly, we observed that before SG, both primary and secondary BA levels were higher in insulin-resistant obese subjects than in non-insulin resistant obese and that BA were positively associated with HOMA-index but neither with BMI nor with visceral fat depots. Our results confirm that an association between circulating BA levels and insulin-resistance exists. Some previous studies showed that fasting serum BA concentrations are higher in diabetics than in non-diabetic subjects [16, 17], and others showed BA levels are associated with markers of insulin-resistance [18]. The lack of association between BA and BMI was unexpected. The current available data on the relationship between BA concentrations and BMI in humans are scarce and inconsistent. Cariou B et al. [16] showed significant positive associations between BMI and DCA and between BMI and CDCA, while other studies showed that BA concentrations are lower in obese

Table 2 Correlations among serum bile acids, BMI, metabolic parameters, and FMD

	BMI	VFA	DBP	GLYC	INSUL	HOMA	Hb1AC	FMD
CA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	−0.075	0.054	−0.025	0.125	0.202	*0.241*	0.099	0.091
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.514	0.643	0.830	0.276	0.080	*0.036*	0.453	0.429
CDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.097	0.172	0.099	0.139	*0.336*	*0.334*	0.101	−0.014
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.396	0.135	0.393	0.225	*0.003*	*0.003*	0.423	0.902
DCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.124	*0.228*	−0.007	0.172	*0.399*	*0.404*	0.211	−0.065
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.273	*0.046*	0.953	0.132	*< 0.001*	*< 0.001*	0.091	0.573
LCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.022	0.090	−0.072	0.104	0.169	0.158	0.097	−0.051
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.849	0.438	0.533	0.365	0.144	0.174	0.441	0.660
tCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.002	−0.062	*−0.250*	0.168	*0.362*	*0.318*	−0.036	−0.009
	p	*P*	*p*	*p*	*p*	*p*	*p*	*p*
	0.989	0.592	*0.028*	0.142	*0.001*	*0.005*	0.775	0.940
tCDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	−0.105	−0.038	*−0.246*	0.090	*0.275*	0.218	−0.037	0.032
	p	*P*	*p*	*p*	*p*	*p*	*p*	*p*
	0.362	0.743	*0.031*	0.432	*0.016*	0.059	0.772	0.778
tDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.086	0.078	−0.209	*0.235*	*0.304*	*0.292*	0.130	−0.179
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.455	0.499	0.068	*0.039*	*0.008*	*0.010*	0.304	0.117
tLCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.039	0.012	0.071	0.111	*0.243*	0.208	−0.104	−0.008
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.733	0.919	0.537	0.332	*0.035*	0.071	0.411	0.947
gCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.150	0.223	−0.039	0.220	*0.474*	*0.466*	0.123	−0.129
	p	*p*	*p*	*P*	*p*	*p*	*p*	*p*
	0.189	0.051	0.737	0.053	*< 0.001*	*< 0.001*	0.329	0.261
gCDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.038	0.108	0.017	0.161	*0.349*	*0.325*	0.079	−0049
	p	*P*	*p*	*p*	*p*	*p*	*p*	*p*
	0.740	0.352	0.882	0.158	*0.002*	*0.004*	0.533	0.673
gLCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.254	0.101	0.047	*0.355*	*0.307*	*0.355*	*0.266*	−0.152
	p	*p*	*p*	*p*	*p*	*p*	*p*	*p*
	0.025	0.383	0.686	*0.001*	*0.007*	*0.002*	*0.032*	0.183

BMI, body mass index; *VFA*, visceral fat area; *DBP*, diastolic blood pressure; *GLYC*, glycaemia; *INUSL*, insulinemia; *HOMA*, homeostasis model assessment of insulin resistance; *HbA1c*, glycated hemoglobin; *FMD*, flow-mediated dilation; *CA*, cholic acid; *CDCA*, chenodeoxycholic acid; *DCA*, deoxycholic acid; *LCA*, lithocholic acid; *tCA*, taurocholic acid; *tCDCA*, taurochenodeoxycholic acid; *tDCA*, taurodeoxycholic acid; *tLCA*, taurolithocholic acid; *gCA*, glycocholic acid; *gCDCA*, glycochenodeoxycholic acid; *gCA*, glycolithocholic acid

subjects than in lean subjects [19, 20]. Our data suggest that circulating BA levels are not influenced by body weight.

After SG, we observed BA composition changes with a significant reduction of primary BA and increase of secondary BA. SG-induced BA changes were independent from weight loss (i.e., BMI reduction), and only primary BA reduction was positively associated with insulin-resistance improvement. At first glance, our results seem to be discordant to current

Table 3 Correlations among total bile acids, BMI, metabolic parameters and FMD

	GLYC	INSUL	HOMA	Hb1AC	FMD
Total CA	*r*	*r*	*r*	*r*	*r*
	0.256	*0.408*	*0.438*	0.182	− 0.046
	p	*p*	*p*	*p*	*p*
	0.024	*0.024*	*< 0.001*	0.148	0.687
Total CDCA	*r*	*r*	*r*	*r*	*r*
	0.208	*0.397*	*0.389*	0.122	− 0.067
	p	*p*	*p*	*p*	*p*
	0.068	*< 0.001*	*0.001*	0.333	0.560
Total LCA	*r*	*r*	*r*	*r*	*r*
	0.232	*0.277*	*0.278*	0.118	0.019
	p	*p*	*p*	*p*	*p*
	0.041	*0.015*	*0.015*	0.347	0.871

GLYC, glycemia; *INUSL*, insulinemia; *HOMA*, homeostasis model assessment of insulin resistance; *FMD*, flow-mediated dilation; *CA*, cholic acid; *CDCA*, chenodeoxycholic acid; *LCA*, lithocholic acid

literature. Several papers have shown a significant increase of serum BA concentrations after bariatric surgery [12]. However, data on surgery-induced BA concentrations' changes and on the associations between BA concentration variations and glucose metabolism improvement following SG are inconsistent and derive from studies carried on simple size population smaller than our population. In line with our results, Belgaumkar et al. [21] found significant decrease of primary BA and increase of secondary BA 6 months after SG in 18 obese subjects. Steinert [19] et al. investigating only sevens subjects showed a decrease of serum BA early after SG followed by an increase 1 year after surgery. Haluzíková et al. [22] found a significant increase of total BA in 17 females 2 years after surgery, and also Nakatani et al. [23] described an increase of total, primary, and secondary BA in 15 subjects about 3 months after SG. Regarding other bariatric surgery procedures, the majority of studies indicate that circulating BA increase after Roux-en-Y gastric bypass (RYGB), the most performed procedure; however, there are some reports with differing results [12]. BA increase after RYGB is usually associated with glucose metabolism improvement [12]. However, it is difficult to compare the effects of SG and RYGB because of different anatomical alteration to the gut resulting from them.

The most similar to our study has been recently published by Nemati R et al. [24], who enrolled 61 diabetic obese subjects who were evaluated before and 1 year after SG or RYGB. They showed that both primary and secondary BA increase after both types of surgery and that the increase in serum BA was associated with decreases in HbA1c but neither with weight loss nor with HOMA index. However, the population included in this study is different form our population: they included in the study only diabetic subjects and only 29 of them underwent SG, while only 10% of the subjects included in our study were affected by diabetes, and all of the participants included in the study underwent SG.

On the basis of our data, we cannot conclude that BA concentrations' changes are involved in SG-induced insulin-resistance and/or diabetes improvement or remission. We found a direct association between primary BA reduction and HOMA-index reduction, and no association between secondary BA increase and insulin-resistance parameters changes. In our opinion, the direct association between primary BA reduction and insulin-resistance improvement could mean that it is not BA which influence glucose metabolism but it is insulin-resistance which regulates primary BAs synthesis. BA are synthesized from cholesterol in the hepatic

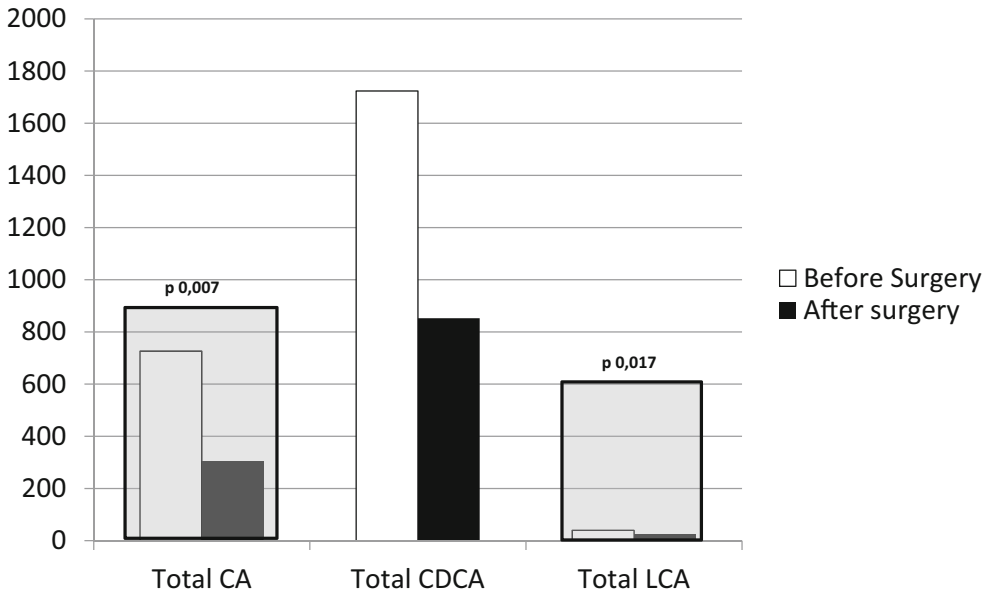

Fig. 3 Serum total bile acid concentrations' changes before and after surgery

Table 4 Correlations among surgery induced variations (Δ) of serum bile acids concentrations and metabolic parameters and FMD

	ΔVFA	ΔDBP	ΔGLYC	ΔINSUL	ΔHOMA	ΔLDL	ΔFMD
ΔCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.282	− 0.066	*0.256*	0.084	0.190	− 0.208	0.012
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.014	0.594	*0.024*	0.484	0.113	0.072	0.917
ΔCDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.176	0.052	*0.228*	0.177	0.225	− 0.210	0.004
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.131	0.679	*0.045*	0.139	0.060	0.069	0.969
ΔDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.245	− 0.056	0.178	0.037	0.115	− 0.120	0.090
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.034	0.668	0.120	0.761	0.340	0.303	0.434
ΔLCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.132	− 0.024	0.081	− 0.010	0.015	− 0.038	0.094
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.261	0.844	0.482	0.934	0.899	0.742	0.413
ΔtCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.087	− 0.001	0.176	0.117	0.161	− 0.120	− 0.062
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.455	0.993	0.123	0.382	0.179	0.300	0.992
ΔtCDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.205	− 0.054	0.206	*0.252*	*0.282*	*− 0.261*	− 0.059
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.077	0.665	0.070	*0.034*	*0.017*	*0.023*	0.609
ΔtDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.133	− 0.167	0.167	0.148	0.198	− 0.001	0.016
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.254	0.176	0.144	0.216	0.098	0.938	0.889
ΔtLCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.047	− 0.080	− 0.022	0.060	0.051	− 0.026	− 0.098
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.688	0.671	0.851	0.618	0.672	0.821	0.396
ΔgCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.173	0.061	0.160	0.160	0.217	*− 0.239*	− 0.012
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.139	0.625	0.161	0.183	0.069	*0.038*	0.916
ΔgCDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.145	− 0.067	0.182	0.142	0.219	*− 0.226*	0.052
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.215	0.564	0.111	0.237	0.066	*0.050*	0.648
ΔgLCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.018	*− 0.251*	− 0.007	0.076	0.094	− 0.162	− 0.020
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.881	*0.040*	0.954	0.529	0.436	0.163	0.712

VFA, visceral fat area; *DBP*, diastolic blood pressure; *GLYC*, glycemia; *INUSL*, insulinemia; *HOMA*, homeostasis model assessment of insulin resistance; *LDL*, low-density lipoproteins; *FMD*, flow-mediated dilation; *CA*, cholic acid; *CDCA*, chenodeoxycholic acid; *DCA*, deoxycholic acid; *LCA*, lithocholic acid; *tCA*, taurocholic acid; *tCDCA*, taurochenodeoxycholic acid; *tDCA*, taurodeoxycholic acid; *tLCA*, taurolithocholic acid; *gCA*, glycocholic acid; *gCDCA*, glycochenodeoxycholic acid; *gCA*, glycolithocholic acid

parenchymal cells, and cholesterol 7 a-hydroxylase (CYP7A1) is the rate limiting enzyme of primary BA biosynthesis [25]. Actually, studies in rodents have shown that BA pool size and composition are regulated by insulin: CYP7A1 expression and activity are negatively controlled by BA, via BA binding to FXR; FXR, which inhibits CYP7A1 expression, is itself regulated negatively by insulin; thus, BA synthesis could be enhanced during increased circulating insulin

Table 5 Correlations among surgery induced variations (Δ) of total serum bile acid concentrations and metabolic parameters and FMD

	ΔVFA	ΔDBP	ΔGLYC	ΔINSUL	ΔHOMA	ΔLDL	ΔFMD
ΔTotal CA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.174	− 0.022	*0.287*	0.177	*0.286*	− 0.194	0.005
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.135	0.857	*0.011*	0.140	*0.016*	0.089	0.968
ΔTotal CDCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.205	− 0.056	*0.241*	0.218	*0.298*	− *0.243*	0.053
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.078	0.653	*0.033*	0.067	*0.012*	*0.034*	0.646
ΔTotal LCA	*r*	*r*	*r*	*r*	*r*	*r*	*r*
	0.001	− 0.210	− 0.045	− 0.092	− 0.067	− 0.015	0.031
	p	*p*	*p*	*p*	*p*	*p*	*p*
	0.991	0.087	0.695	0.446	0.581	0.900	0.790

VFA, visceral fat area; *DBP*, diastolic blood pressure; *GLYC*, glycemia; *INUSL*, insulinemia; *HOMA*, homeostasis model assessment of insulin resistance; *LDL*, low-density lipoproteins; *FMD*, flow-mediated dilation; *CA*, cholic acid; *CDCA*, chenodeoxycholic acid; *LCA*, lithocholic acid;

levels [26]. Moreover, CYP7A1 activity could be directly regulated by insulin (negatively) and by glucose (positively); therefore, during insulin-resistance states and hyperglycemia CYP7A1 activity could be enhanced [27].

In our study, circulating secondary BA levels significantly increase after SG. This rise is probably due to a higher primary BA biotransformation to secondary BA by intestinal microbiota enzymes. Actually, SG leads to accelerated intestinal motility [28]; therefore, a greater amount of free BA arrives to the distal ileum and to the large intestine where BA biotransformation occurs. Moreover, SG, as other bariatric surgery procedure, could change gut microbiota abundance and composition [29], thus modifying the microbiota ability to metabolize BA.

Although it has been suggested that BA may have vasodilatory properties [30] and may play a role in atheroprotection [12], we did not find any significant correlation neither between BA and FMD at baseline nor between surgery-induced circulating BA changes and FDM improvement. Walsh KL et al. [31] showed that oral administration of tauroursodeoxycholic acid (TUDCA) can mitigate hyperglycemia-induced endothelial dysfunction after an oral glucose challenge in healthy humans, and Battson ML [32] et al. found that TUDCA improved endothelium-dependent dilation without affecting endothelium-independent dilation in a mouse model. Moreover, Sinisalo J et al. [33] showed that 6 weeks of ursodeoxycholic acid (UDCA) therapy improved endothelium-dependent nitric oxide-independent vasodilatation in coronary heart disease subjects under conditions of impaired nitric oxide production, while Chung et al. [34] suggested that UDCA inhibits atheromatous plaque formation in mouse models. The proposed explanation of these beneficial effects is that TUDCA inhibits neo-intimal hyperplasia in vascular smooth muscle cells [35] and inhibits endoplasmic reticulum (ER) stress in endothelial cells [33], while UDCA increases nitric oxide production and inhibits endothelin-1 production in human endothelial cells [36]. In our study, we did not measure neither TUDCA nor UDCA circulating levels, which are secondary BA representing only for 2% of total human BA composition [12]; therefore, we cannot compare our results with these previous studies.

The main limitations of our study are that we did not include a non-obese control group at baseline for comparing BA acids levels between obese and lean subjects, and that we did not measure fibroblast growth factor 19 (FGF-19) concentrations which mediate BA effects on glucose metabolism after FXR activation.

In conclusion, a relationship between glucose metabolism and circulating BA seems to exist and SG modifies circulating BA pool and composition. Certainly, further studies are needed for better understanding how glucose metabolism and BA influence each other and if BA play a role in surgery-induced diabetes improvement or remission.

Compliance with Ethical Standards

We state that the article is original and has not been previously published in any form and is not being considered for publication elsewhere in whole or in part. All authors have read and approved the manuscript and have contributed substantially to the conception and completion of the review and to paper draft.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Statement All procedures performed in this study involving human participants were in accordance with the ethical standards of the New Zealand national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Statement Informed consent was obtained from all individual participants included in the study.

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