

# Bile acid changes after metabolic surgery are linked to improvement in insulin sensitivity

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[Correction added on 6 February 2020, after first online publication: Cefalù has been amended to Cefalo]

**Background:** Metabolic surgery is associated with a prompt improvement in insulin resistance, although the mechanism of action remains unknown. The literature on bile acid changes after metabolic surgery is conflicting, and insulin sensitivity is generally assessed by indirect methods. The aim of this study was to investigate the relationship between improvement in insulin sensitivity and concentration of circulating bile acids after biliopancreatic diversion (BPD) and Roux-en-Y gastric bypass (RYGB).

**Methods:** This was a prospective observational study of nine patients who underwent BPD and six who had RYGB. Inclusion criteria for participation were a BMI in excess of 40 kg/m<sup>2</sup>, no previous diagnosis of type 2 diabetes and willingness to participate. Exclusion criteria were major endocrine diseases, malignancies and liver cirrhosis. Follow-up visits were carried out after a mean(s.d.) of 185.3(72.9) days. Fasting plasma bile acids were assessed by ultra-high-performance liquid chromatography coupled with a triple quadrupole mass spectrometer, and insulin sensitivity was measured by means of a hyperinsulinaemic–euglycaemic clamp.

**Results:** A significant increase in all bile acids, as well as an amelioration of insulin sensitivity, was observed after metabolic surgery. An increase in conjugated secondary bile acids was significantly associated with an increase in insulin sensitivity. Only the increase in glycodeoxycholic acid was significantly associated with an increase in insulin sensitivity in analysis of individual conjugated secondary bile acids.

**Conclusion:** Glycodeoxycholic acid might drive the improved insulin sensitivity after metabolic surgery.

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## Introduction

Bariatric surgery is the only currently available obesity treatment resulting in long-term weight loss<sup>1</sup>. Several studies<sup>2–4</sup> have shown remission of type 2 diabetes and improvements in insulin resistance long before weight reduction, and this has led to introduction of the term metabolic surgery. The mechanism behind the rapid improvement in insulin resistance remains unclear.

Bile acids bind to the farnesoid X receptor (FXR) and to the G-protein-coupled bile acid receptor (TGR5), two receptors whose activation mediates effects on energy and glucose homeostasis<sup>5</sup>. The primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are produced by hepatocytes and conjugated with glycine or taurine to form conjugated bile acids before secretion into the small

intestine. A large portion of the conjugated bile acids is reabsorbed in the terminal ileum, and a smaller portion is converted to secondary bile acids in the large intestine by the gut microbiota and then reabsorbed or lost with stool<sup>6</sup>. Some types of metabolic surgery lead to a reduced gastric volume combined with bypassing parts of the small intestine, thus leading to a more rapid delivery of nutrients into the jejunum or ileum, and bile coming in contact with the nutrients more distally in the intestine. Higher levels of bile acids have been reported in patients after Roux-en-Y gastric bypass (RYGB)<sup>7</sup>, and patients who have undergone bariatric surgery display changes in the gut microbial composition<sup>8</sup> known to metabolize bile acids<sup>9</sup>. Interestingly, in two knock-out mice models, one lacking the nuclear FXR, which binds bile acids, and the other missing the cell membrane TGR5, activated by bile acids,

improvement in insulin sensitivity after metabolic surgery was attenuated or absent<sup>10,11</sup>. Hence, bile acids may play a primary role in the amelioration of insulin sensitivity that follows metabolic surgery.

A major drawback in previous studies is that insulin sensitivity was measured by indirect methods. The hyperinsulinaemic–euglycaemic clamp is considered the standard method. The aim of the present study was to investigate, by use of the clamp technique, whether changes in insulin sensitivity are related to changes in plasma levels of bile acids, in patients who have undergone biliopancreatic diversion (BPD) or RYGB.

## Methods

Study protocols were approved by the regional ethics committee at the Catholic University Hospital of the Sacred Heart, Rome, Italy. All participants gave written informed consent to take part in the study.

### Study participants

Patients were recruited at the obesity centre at the Catholic University Hospital of the Sacred Heart, Rome, Italy. The patients underwent either RYGB or BPD for treatment of obesity. No randomization to the different surgical treatments was undertaken. Inclusion criteria for participating in the study were a BMI greater than 40 kg/m<sup>2</sup>, no previous diagnosis of type 2 diabetes, with glycated haemoglobin (HbA1c) 7 per cent or less, and willingness to be included in the study. Exclusion criteria were major endocrine diseases, malignancies and liver cirrhosis.

At inclusion and follow-up, blood samples were obtained from patients after overnight fasting and anthropometric data were collected. Blood chemistry analysis was undertaken at the central laboratory of the Catholic University Hospital of the Sacred Heart, Rome.

### Hyperinsulinaemic–euglycaemic clamp

Study participants were fasted overnight and underwent a hyperinsulinaemic–euglycaemic clamp to assess peripheral insulin sensitivity<sup>12</sup>. On the morning of the test, venous access was established into the antecubital vein for infusions. A second access was inserted into a hand vein and the hand was placed in a heated air box (60°C) to obtain arterialized blood samples. The patients received a primed constant insulin infusion (6 pmol per min per kg) and a variable glucose infusion, which was adjusted so that blood glucose concentration was clamped at the fasting value for 2 h. Adjustment of the

variable glucose infusion was undertaken every 5 min when needed on the basis of blood glucose measurements. Whole-body glucose uptake (*M*) was calculated during the last 40 min of clamping during steady-state euglycaemic hyperinsulinaemia.

### Measurement of fasting bile acids

A targeted platform based on ultra-high-performance liquid chromatography coupled with a triple quadrupole mass spectrometer was applied to quantify fasting bile acids in both unconjugated and conjugated forms, as described previously<sup>13</sup>. Briefly, 4 µl of bile acid internal standard (IS) mixture was added to 20 µl of plasma. The IS mixture contained the following compounds (each at a concentration of 50 ng/ml): CA, CDCA, deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), glycolithocholic acid (GLCA), glyoursodeoxycholic acid (GUDCA), taurocholic acid, taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid and the deuterated forms (CA-d4, CDCA-d4, DCA-d4, LCA-d4, UDCA-d4, GCA-d4, GUDCA-d4, GCDCA-d4, GLCA-d4). The bile acids were obtained from Sigma-Aldrich (St Louis, Missouri, USA) and the deuterated forms of bile acids from QMx Laboratories (Thaxted, UK). Precipitation of proteins was carried out by adding 24 µl of acetonitrile. The samples were then vortexed for 5 s and underwent 3 min of ultrasound treatment before being centrifuged for 5 min at 12 100 g. Evaporation of the samples was performed with nitrogen, and they were then reconstituted with 4 µl of methanol and further diluted with 6 µl of water. Analysis of the samples was undertaken on this solution but also on a further dilution with methanol (1 to 20). An Acquity ultra-high-performance liquid chromatography system (Milford, Massachusetts, USA) and Xevo triple quadrupole mass spectrometer (Waters, Manchester, UK) were used for bile acid analysis. An Acquity HSS T3 (2.1 Å~100 mm, 1.7 µm) column (Waters) kept at 35°C was used for chromatography. A volume of 3 µl was injected. A gradient elution with 0.1 per cent formic acid in water (v/v) (A) and 0.1 per cent formic acid in acetonitrile:methanol (3:1, v/v) (B) at a flow rate of 0.5 ml/min was used for separation. The gradient programme was set to: 0 min 15 per cent B, 1 min 30 per cent B, 16 min 70 per cent B, 18–20 min 100 per cent B. The equilibrium time was 5 min between runs. A negative polarity with 2.0 kV capillary voltage was used when mass spectrometry was carried out. A desolvation temperature of 650°C and a source temperature of 150°C were applied together

Table 1 Patient characteristics	Before intervention			After intervention		
	All (n = 15)	BPD (n = 9)	RYGB (n = 6)	All (n = 15)	BPD (n = 9)	RYGB (n = 6)
Age (years)	44.3(8.3)	44.7(8.1)	43.7(9.4)	44.8(8.4)	45.1(8.3)	44.3(9.4)
Sex ratio (M : F)	10 : 5	4 : 5	6 : 0¶	10 : 5	4 : 5	6 : 0¶
Height (cm)	171.5(9.2)	167.3(9.1)	177.8(5.1)§	171.3(9.2)	167.0(8.9)	177.8(5.1)§
BMI (kg/m <sup>2</sup> )	51.6(9.6)	55.8(9.5)	45.3(5.7)§	37.7(7.4)*	39.1(8.5)†	35.5(5.5)‡
Weight (kg)	151.0(24.8)	156.3(28.7)	143.0(16.7)	110.4(22.2)*	109.4(26.8)†	112.0(15.1)‡
Interval to follow-up (days)				185.3(72.9)	183.7(61.8)	187.8(93.6)
Weight loss (kg)				-40.6(19.4)	-46.9(21.8)	-31.0(10.78)
Weight loss (%)				-27(11)	-30(12)	-22(7)
Plasma glucose (mg/dl)	97.5(17.0)	101.7(20.7)	91.3(6.3)	76.8(11.1)*	76.7(13.8)‡	77.0(6.3)‡
HbA1c (%)	5.9(0.36)	6.0(0.38)	5.8(0.33)	5.5(0.21)†	5.6(0.22)‡	5.4(0.19)‡
HbA1c (mmol/mol)	41.4(3.7)	42.1(4.0)	40.3(3.3)	36.5(2.6)†	37.3(2.5)‡	35.3(2.3)‡
M (mg per kg per min)	2.71(1.45)	2.34(1.21)	3.27(1.70)	5.95(2.21)*	6.09(2.29)‡	5.74(2.28)‡
Type 2 diabetes	2	1	1	0	0	0
Total cholesterol (mg/dl)	189.6(28.2)	197.0(31.3)	178.5(20.2)	138.1(28.6)†	124.7(17.3)†§	158.2(31.7)§
HDL cholesterol (mg/dl)	47.1(9.5)	47.9(7.9)	46.0(12.4)	41.7(12.3)	38.9(13.2)	45.5(11.0)
LDL cholesterol (mg/dl)	119.5(22.1)	127.9(16.3)	107.8(25.6)	74.9(27.5)†	61.6(14.2)‡§	92.7(32.0)§
Triglycerides (mg/dl)	134.9(58.5)	141.4(52.7)	126.3(69.7)	109.6(49.3)	116.2(61.1)	99.7(25.2)
AST (units/l)	35.3(21.0)	34.5(25.6)	36.6(13.2)	29.8(18.1)	28.1(11.4)	31.8(25.0)
ALT (units/l)	45.7(37.4)	48.6(48.1)	41.8(19.3)	37.5(27.7)	39.3(24.7)	34.6(35.0)

Values are mean(s.d.) for continuous variables. BPD, biliopancreatic diversion; RYGB, Roux-en-Y gastric bypass; HbA1c, glycated haemoglobin; M, whole-body glucose uptake; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase. \* $P < 0.001$ , † $P < 0.010$ , ‡ $P < 0.050$  versus same patient group before operation (Wilcoxon signed-rank test); § $P < 0.050$  versus BPD at the same time point (Mann-Whitney  $U$  test); ¶ $P < 0.050$  versus BPD at the same time point (Fisher's exact test).

with the following gas flow settings: cone gas flow 150 l/h (nitrogen), desolvation gas 1100 l/h (nitrogen), collision gas 0.15 ml/min (argon). A selected reaction monitor with auto dwell time function (dwell time 20–95 ms, 20 points/peak) was used to detect the analytes. A calibration curve from 1.4 pg/ml to 642 ng/ml with a series of 1 : 3 dilutions was used. The IS method was applied to quantify the analytes.

## Statistical analysis

Continuous data are presented as mean(s.d.). Wilcoxon signed-rank test was used to compare differences between baseline and follow-up after each procedure. The Mann-Whitney  $U$  test was used for comparisons of continuous variables between the different surgery groups at each time point. Fisher's exact test was used for comparisons of categorical variables between patients who underwent BPD and RYGB at the same time point. A principal component analysis and a heat map clustering analysis were carried out on bile acid data before and after intervention, and  $\log_2$  values were calculated. Spearman correlation analysis was undertaken between fold changes of bile acids (after versus before intervention)

and fold changes in M (after versus before intervention) to select variables for further linear regression analysis. Before linear regression analysis,  $\log_2$  values of the fold changes were calculated to achieve linear relationships.  $P < 0.050$  was considered statistically significant. Principal component analysis and clustering analysis with heat map construction were carried out with MetaboAnalyst (<https://www.metaboanalyst.ca/>)<sup>14</sup>. Other statistical analyses were undertaken with SPSS® version 21.0 (IBM, Armonk, New York, USA).

## Results

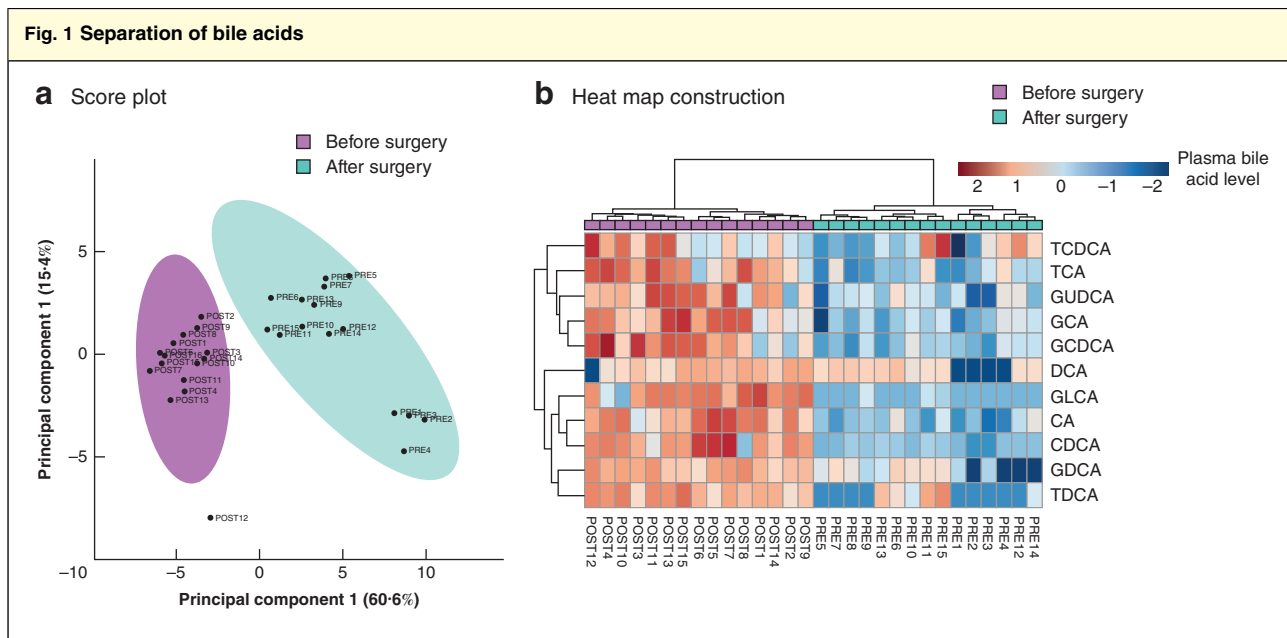
Fifteen patients were included in the study who underwent either RYGB (6) or BPD (9) for treatment of obesity. Follow-up visits were carried out after a mean(s.d.) of 185.3(72.9) days.

Patient characteristics before and after metabolic surgery are presented in Table 1. A significantly higher proportion of men were present in the RYGB group (6 of 6) compared with the BPD group (4 of 9;  $P = 0.044$ ), and the RYGB group was also significantly taller (177.8(5.1) versus 167.3(9.1) cm;  $P = 0.018$ ). The BPD group had a

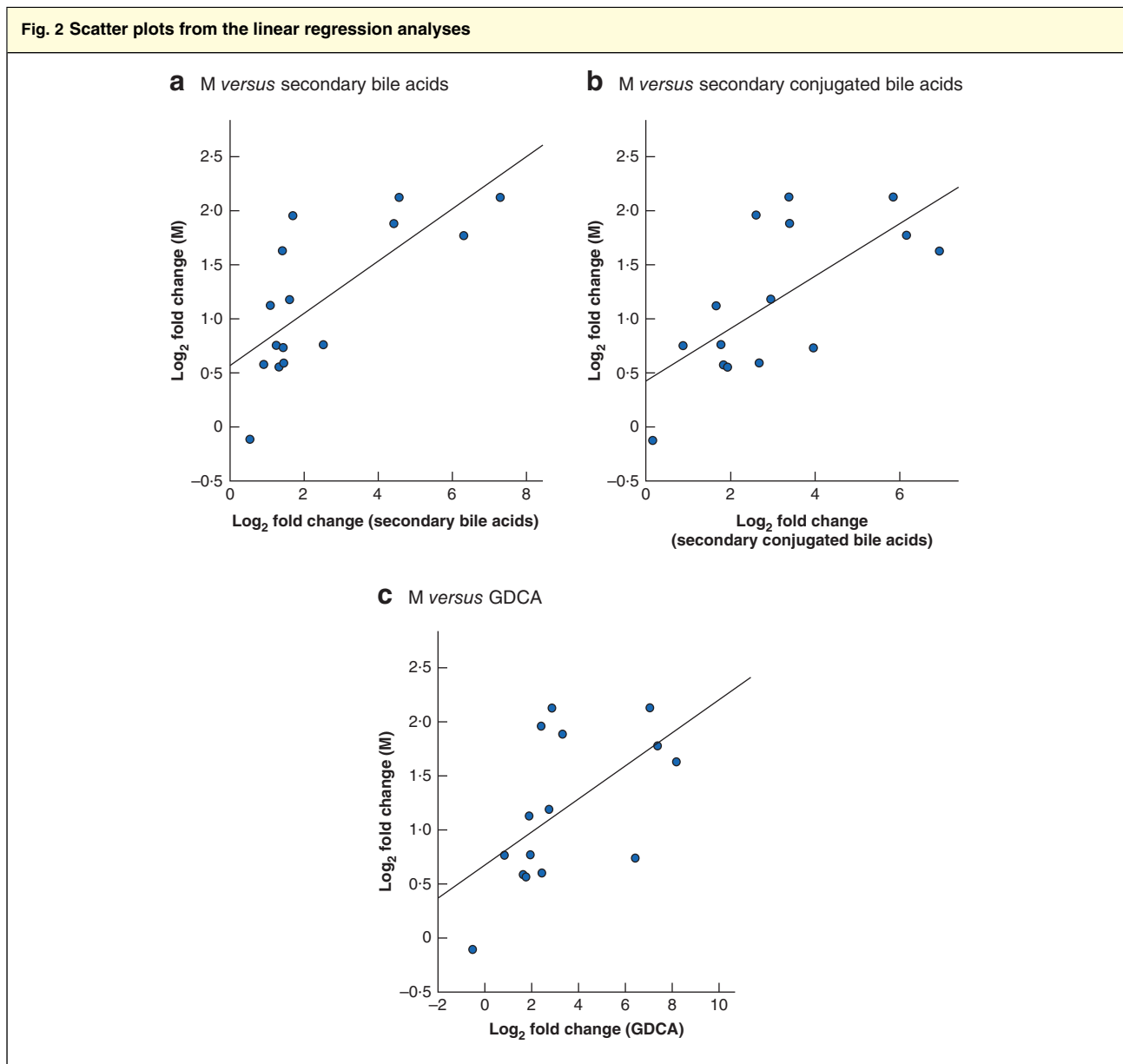
**Table 2** Bile acid levels before and after surgery

	Before intervention			After intervention			Reference values from Schmid <i>et al.</i> <sup>15</sup>
	All	BPD	RYGB	All	BPD	RYGB	
<b>Primary bile acids</b>	0.89(0.39)	0.98(0.42)	0.75(0.31)	5.14(2.09)†	4.70(1.71)†	5.81(2.59)‡	–
CA (μmol/l)	0.09(0.08)	0.09(0.09)	0.09(0.08)	1.43(1.01)*	1.10(0.73)†	1.93(1.23)‡	0.87(0.20)
GCA (μmol/l)	0.21(0.09)	0.23(0.09)	0.18(0.10)	0.90(0.48)*	0.76(0.47)†	1.11(0.44)‡	0.32(0.53)
TCA (μmol/l)	0.04(0.02)	0.04(0.02)	0.04(0.02)	0.11(0.04)*	0.11(0.05)†	0.12(0.04)‡	0.07(0.01)
CDCA (μmol/l)	0.12(0.03)	0.12(0.03)	0.13(0.02)	1.31(0.86)*	1.19(0.58)†	1.50(1.20)‡	0.57(0.13)
GCDCA (μmol/l)	0.18(0.08)	0.19(0.07)	0.17(0.10)	1.03(0.71)*	1.19(0.83)†	0.78(0.41)‡	0.74(0.09)
TCDC A (μmol/l)	0.24(0.23)	0.30(0.28)	0.14(0.08)	0.35(0.24)†	0.34(0.27)	0.37(0.22)‡	0.19(0.02)
<b>Secondary bile acids</b>	0.79(0.60)	0.70(0.68)	0.93(0.47)	2.56(0.70)†	2.58(0.58)†	2.52(0.90)‡	–
DCA (μmol/l)	0.55(0.46)	0.47(0.49)	0.67(0.43)	1.37(0.73)†	1.43(0.83)‡	1.28(0.60)‡	0.40(0.37)
GDCA (μmol/l)	0.19(0.16)	0.16(0.18)	0.22(0.14)	0.89(0.35)*	0.84(0.35)‡	0.96(0.38)‡	0.35(0.05)
TDCA (μmol/l)	0.05(0.07)	0.05(0.09)	0.03(0.04)	0.17(0.08)*	0.18(0.10)†	0.16(0.07)‡	0.08(0.01)
GLCA (μmol/l)	0.01(0.00)	0.01(0.00)	0.01(0.00)	0.13(0.07)*	0.13(0.07)†	0.11(0.07)‡	0.16(0.03)
<b>Tertiary bile acids</b>							
GUDCA (μmol/l)	0.03(0.02)	0.03(0.02)	0.02(0.01)	0.14(0.09)*	0.13(0.09)†	0.14(0.09)‡	0.13(0.03)
<b>Secondary : primary ratio</b>	0.99(0.89)	0.66(0.67)	1.48(1.01)	0.59(0.30)	0.64(0.32)	0.50(0.26)	–
<b>Conjugated : unconjugated ratio</b>	2.40(2.35)	3.13(2.73)	1.31(1.10)	1.20(0.85)	1.34(1.01)	0.99(0.56)	–

Values are mean(s.d.). BPD, biliopancreatic diversion; RYGB, Roux-en-Y gastric bypass; CA, cholic acid; GCA, glycocholic acid; TCA, taurocholic acid; CDCA, chenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; TCDC A, taurochenodeoxycholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; TDCA, taurodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glyoursodeoxycholic acid. \**P* < 0.001, †*P* < 0.010, ‡*P* < 0.050 *versus* same patient group before operation (Wilcoxon signed-rank test).



**a** Score plot of the two principal components from the principal component analysis. **b** Heat map with clustering analysis on autoscaled features. POST, after surgery; PRE, before surgery; TCDC A, taurochenodeoxycholic acid; TCA, taurocholic acid; GUDCA, glyoursodeoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; DCA, deoxycholic acid; GLCA, glycolithocholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; GDCA, glycodeoxycholic acid; TDCA, taurodeoxycholic acid.



Scatter plot of  $\log_2$  fold change of whole-body glucose uptake (M) versus **a**  $\log_2$  fold change of secondary bile acids ( $y = 0.564 + 0.242x$ ;  $r^2 = 0.535$ ), **b**  $\log_2$  fold change of secondary conjugated bile acids ( $y = 0.427 + 0.243x$ ;  $r^2 = 0.465$ ) and **c** glycodeoxycholic acid (GDCA) ( $y = 0.660 + 0.154x$ ;  $r^2 = 0.336$ ).

significantly higher BMI than the RYBG group ( $55.8(9.5)$  versus  $45.3(5.7)$  kg/m<sup>2</sup>;  $P = 0.034$ ).

### Improvement in insulin sensitivity and metabolic parameters after metabolic surgery

Several metabolic parameters, including BMI, fasting plasma glucose concentration, HbA1c and M, were significantly improved after RYGB and BPD (Table 1).

A significant reduction in serum concentrations of total cholesterol and low-density lipoprotein cholesterol was observed after BPD, but not after RYGB, when the two groups were analysed separately (Table 1). However, no significant difference in percentage weight loss was observed between patients who underwent BPD and those who had RYGB (30(12) and 22(7) per cent respectively;  $P = 0.224$ ).



## Fasting plasma levels of bile acids are increased after metabolic surgery

Data on levels of bile acids before and after metabolic surgery are presented together with reference data<sup>15</sup> from a mixed population in *Table 2*. All bile acid concentrations were significantly increased after metabolic surgery when the two surgery groups were analysed together (*Table 2*). This was also seen in separate analyses for the two surgery groups, except for TCDCa in the BPD group where the increase after surgery was not statistically significant. In addition, a good separation of bile acids for before *versus* after surgery was observed in the principal component analysis, with two factors explaining 15.4 and 60.6 per cent of the variance respectively (*Fig. 1a*). A heat map construction with a hierarchical cluster analysis of plasma bile acid levels was also able to separate between before and after surgery (*Fig. 1b*).

No differences in individual fasting bile acid levels were observed between the RYGB and BPD groups, before or after the intervention. In addition, no differences in the ratios of conjugated: unconjugated bile acids and secondary: primary bile acids were observed, when the surgery groups were analysed together or separately (*Table 2*).

## Changes in bile acid levels are associated with, and can predict, changes in insulin sensitivity

The change in all secondary bile acid levels correlated positively with the change in M ( $\rho = 0.801$ ,  $P < 0.001$ ). When the secondary bile acids were divided into groups of conjugated and unconjugated bile acids, both the increase in DCA ( $\rho = 0.678$ ,  $P = 0.008$ ) and the increase in conjugated secondary bile acids ( $\rho = 0.592$ ,  $P = 0.020$ ) correlated positively with the increase in M. However, when the changes in individual bile acid concentrations were analysed, only the change in GDCA showed a positive correlation with the change in M ( $\rho = 0.663$ ,  $P = 0.007$ ).

Linear regression analysis revealed that the changes in concentrations of secondary bile acids (*Fig. 2a*), secondary conjugated bile acids (*Fig. 2b*) and GDCA (*Fig. 2c*), but not DCA, could predict the change in M. The increase in the secondary bile acids accounted for 53.5 per cent of the variation in the change of M, and could statistically predict the change in M with the formula:  $\log_2$  fold change in M =  $0.564 + (0.242 \times \log_2$  fold change in secondary bile acids) ( $P = 0.002$ ). Analysis of only the change in conjugated secondary bile acids showed that they accounted for 46.5 per cent of the variability in the change of M and could statistically predict the change in M with the formula:  $\log_2$  fold change in M =  $0.427 + (0.243 \times \log_2$  fold change in secondary conjugated bile acids) ( $P = 0.005$ ). In

addition, the individual increase in GDCA accounted for 33.6 per cent of the variation in change of M and the change in GDCA could statistically predict the change in M with the formula:  $\log_2$  fold change in M =  $0.660 + (0.154 \times \log_2$  fold change in GDCA) ( $P = 0.023$ ).

## Discussion

The present results showed that plasma levels of total bile acids were increased after both BPD and RYGB surgery, with a marked improvement in insulin sensitivity. The improvement in insulin sensitivity after metabolic surgery was closely associated with, and predicted by, the increases in secondary conjugated bile acids, particularly GDCA.

The results, moreover, showed an increase in fasting circulating levels of both primary and secondary bile acids and almost all individual bile acids after BPD and RYGB. These results are in line with some previous studies<sup>16–18</sup> showing increases in total fasting bile acid concentrations after RYGB and BPD. A previous study<sup>19</sup>, however, reported an early decrease in total bile acid levels after RYGB, whereas some metabolic procedures, such as banding and sleeve gastrectomy, did not appear to affect total bile acid levels<sup>20–22</sup>. The studies<sup>22,23</sup> of subgroups or individual bile acids after metabolic surgery displayed more diverse results. Concentrations of primary conjugated bile acids and CA decreased, and only the secondary bile acids and mainly GUDCA increased after sleeve gastrectomy<sup>22</sup>, whereas Werling and colleagues<sup>23</sup> showed that the levels of unconjugated bile acids and glycine-conjugated bile acids increased, and mainly GLCA decreased, after RYGB. The results of the present study, however, indicated that the levels of almost all fasting circulating bile acids increased after two types of metabolic surgery, RYGB and BPD.

Recent studies<sup>24,25</sup> concluded that several different mechanisms, such as weight loss, calorie restriction and rerouting of nutrients, may contribute to improvement in insulin sensitivity after metabolic surgery. The present study demonstrated that the change in concentration of secondary conjugated bile acids, and especially GDCA, was associated with positive changes in M after metabolic surgery. Some associations between bile acids and insulin sensitivity have been reported previously. Ferrannini and co-workers<sup>26</sup> showed a significant correlation between the ratio of unconjugated: conjugated bile acids and M after BPD. Interestingly, an alternative hypothesis, with insulin resistance driving the production of bile acids, was suggested after a positive correlation was found between 12- $\alpha$ -hydroxylated bile acids and the homeostatic model assessment for insulin resistance<sup>27</sup>. In some studies of bile acids and metabolic surgery<sup>16,19,20</sup>, however, no links

between changes in bile acids and insulin sensitivity were found. Hence, the effect of bile acid changes on insulin sensitivity after metabolic surgery is still unclear, but the present results suggest a potential role of conjugated secondary bile acids in the amelioration of insulin sensitivity.

The two main bile acid receptors mediating the possible metabolic effects are FXR and TGR5<sup>5</sup>. Mice lacking FXR or TGR5 display attenuated or absent improvement in insulin sensitivity after metabolic surgery, indicating a possible role for these receptors in the improved insulin sensitivity seen after metabolic surgery<sup>10,11</sup>. FXR is usually activated by primary bile acids and TGR5 by secondary bile acids<sup>6</sup>. Activation of human TGR5 has been shown to be dose-dependent in Chinese hamster ovary cells and human embryonic kidney 293 cells, and different bile acids are known to have different ability to activate the receptor, with LCA the strongest, followed by DCA, CDCA, then CA<sup>28,29</sup>. The opposite pattern has been observed for FXR where CDCA is the most potent activator followed by CA, DCA and LCA, whereas UCDCA is considered a partial agonist<sup>30–33</sup>. However, during a clamp, M is, to a large extent, based on the glucose uptake in muscle tissue, as 75 per cent of glucose during the clamp is taken up by muscles and not by the liver. Interestingly, TGR5 is expressed in skeletal muscle<sup>28</sup>, whereas expression of FXR has not been reported in human muscle tissue, but it has in liver and colon<sup>6,34</sup>. In human muscle tissue, activation of TGR5 is known to lead to increased energy expenditure by conversion of thyroxine T4 into the active tri-iodothyronine T3<sup>35</sup>, possibly resulting in increased insulin sensitivity. Another possible explanation for the present findings is that more bile acids reach the colon after metabolic surgery, and that TGR5 activation in enteroendocrine cells increases glucagon-like peptide 1 release, which mediates glucose uptake in muscle<sup>36–38</sup>. Taken together, if secondary conjugated bile acids play a role in the amelioration of insulin sensitivity, this could be mediated via the TGR5 receptor, which might explain why only the secondary conjugated bile acids were associated with M in the present study.

Metabolic surgery procedures in which the bile is diverted distally in the ileum, such as BPD, should modify the bile acid pool to a greater extent than less malabsorptive procedures, such as RYGB, where the bile is diverted into the proximal jejunum. Differences in circulating bile acid levels between BPD and RYGB have been reported in humans<sup>16</sup> but BPD and RYGB groups in the present study showed remarkably similar fasting bile acid levels and profiles. Interestingly, a previous study<sup>39</sup> in the rat suggested that bile diversion in the mid-jejunum and in the mid-ileum led to the same level of plasma bile acids

and improvement in glucose tolerance, which supports the present results and suggests that the length of the alimentary limb does not have a major role in determining the increased bile acid levels after metabolic surgery. It was not possible to rule out differences in the postprandial bile acid levels into the present study. In addition, systemic circulating bile acids are not a complete reflection of composition changes in the enterohepatic circulation, which may differ between the two surgical procedures.

The present study has limitations. Follow-up visits were carried out a mean(s.d.) of 185.3(72.9) days after surgery, when the patients had achieved substantial weight loss (mean(s.d.) –40.6(19.4) kg). Hence, this study did not capture the relationship between bile acids and the immediate metabolic effect after metabolic surgery before weight loss had occurred. Other mechanisms, such as calorie restriction and weight loss, may also contribute to the amelioration of insulin sensitivity.

Patients undergoing metabolic surgery show an improvement in insulin sensitivity in parallel with a marked increase in bile acids. Changes in concentrations of secondary conjugated bile acids as a group, and particularly GDCA, were associated with improvement in insulin sensitivity, suggesting a potential role in insulin sensitivity improvement after metabolic surgery.

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