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Running title: Serum lipidomics in gastric bypass versus banding

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Abstract

Background/Objectives: Circulating phospholipids and sphingolipids are implicated in obesity related-comorbidities such as insulin resistance and cardiovascular disease. How bariatric surgery affects these important lipid markers is poorly understood. We sought to determine whether Roux-en-Y gastric bypass (RYGB), which is associated with greater metabolic improvement, differentially affects the phosphosphingolipidome compared to adjustable gastric banding (AGB).

Subjects/Methods: Fasting sera were available from 59 obese women (BMI range 37-51 kg/m^2; n=37 RYGB and 22 AGB) before surgery, then at 1 (21 RYGB, 12 AGB) and 3 months follow-up (19 RYGB, 12 AGB). HPLC-MS/MS was used to quantify 131 lipids from 9 structural classes. DXA measurements and laboratory parameters were also obtained. The associations between lipids and clinical measurements were studied with P-values adjusted for the false discovery rate (fdr).

Results: Both surgical procedures rapidly induced weight loss and improved clinical profiles, with RYGB producing better improvements in fat mass, and serum TC, LDL-C, and orosomucoid (fdr<10%). Ninety-three (of 131) lipids were altered by surgery—the majority decreasing—with 29 lipids differentially affected by RYGB during the study period. The differential effect of the surgeries remained statistically significant for 20 of these lipids after adjusting for differences in weight loss between surgery types. The RYGB signature consisted of phosphatidylcholine species not exceeding 36 carbons, and ceramides and sphingomyelins containing C22 to C25 fatty acids. RYGB also led to a sustained increase in unsaturated ceramide and sphingomyelin species. The RYGB-specific lipid changes were associated with decreases in body weight, total and LDL-C, orosomucoid and increased HOMA-S (fdr<10%).

Conclusions: Concomitant with greater metabolic improvement, RYGB induced early and sustained changes in phosphatidylcholines, sphingomyelins, and ceramides that were independent of greater weight loss. These data suggest that RYGB may specifically alter sphingolipid metabolism, which, in part, could explain the better metabolic outcomes of this surgical procedure.

Introduction

Morbid obesity is associated with numerous comorbidities including diabetes, nonalcoholic fatty liver disease (NAFLD), and atherosclerosis. Bariatric surgery is an effective treatment for obesity that results in sustained weight loss and improvements in several cardiovascular risk factors (ref. 1). As bariatric surgery is able to resolve T2D in a large number of patients, and even alter the hormonal response to meal ingestion prior to weight loss, many studies have focused on the beneficial effects of gastric bypass on glucose homeostasis (ref. 2). However, the benefits of bariatric surgery also extend to improvements in NAFLD and cardiovascular disease (ref. 3, ref. 4). Systematically evaluating the evolution of biomarkers between different surgeries thus provides a useful model for identifying mechanisms, and eventually novel therapies, for the treatment of a number of obesity comorbidities.

The success of bariatric surgery depends on the procedure used. Roux-en-Y gastric bypass (RYGB) involves the creation of a small gastric pouch and diversion of most of the stomach, the duodenum, and part of the proximal jejunum, which are further anastomosed to the distal jejunum. Adjustable gastric banding (AGB) involves restriction of the proximal stomach. Compared to AGB, RYGB results in greater weight loss and better improvements in numerous risk factors, including clinical lipid measurements (ref. 1, ref. 5, ref. 6). While both AGB and RYGB restrict the stomach, the latter is also malabsorptive and alters the physiology of the retained and bypassed parts of the small intestine, and it is these alterations that are hypothesized to explain the greater weight loss following RYGB (ref. 7). There remains continued debate regarding how much RYGB contributes to long-term improvements in glucose control over and above weight loss *per se*, thus there is need to further define surgery-specific effects on metabolism (ref. 8–10). Lipidomic analysis may provide deeper insight into these effects.

With the advent of modern lipidomic technologies, over 500 molecular lipid species have been quantified in human plasma (ref. 11). Phospholipids and sphingolipids, collectively called the phosphosphingolipidome, and which contain the bioactive ceramides, are among the most diverse lipid categories and may act as important biomarkers (ref. 12). For example, plasma levels of sphingolipids and phospholipids are increased in obesity-associated nonalcoholic steatohepatitis (NASH) (ref. 13) and outperform neutral lipids and eicosanoids for predicting liver injury (ref. 14). Serum phospholipids and sphingolipids may reflect synthesis and efflux from metabolically relevant tissues, but can also directly participate in pathophysiology as a source of triglycerides in hepatic steatosis (ref. 15), or by altering the cholesterol efflux from macrophages, which is suspected to be an important mechanism in the development of atherosclerosis (ref. 16). Ceramides (Cer) are especially implicated in the pathogenesis of insulin resistance (ref. 17), and it was recently shown that infusion of Cer(d18:1/24:0) into mice can induce peripheral insulin resistance (ref. 18). Little is known about how bariatric surgery affects these important lipids.

Separating the effects of RYGB and AGB on serum lipids, particularly after adjusting for differences in weight loss, provides an informative model for deciphering the specific effects of RYGB on metabolism. As the weight loss differences between procedures are less drastic early after surgery, and given the metabolic effects occur almost immediately, the evolution of serum lipids was determined after 1 and 3 months of follow-up. Compared to AGB, we hypothesized that RYGB would have a surgery-specific effect on circulating phospholipids and sphingolipids concomitant with the greater metabolic response following this procedure.

Methods

Clinical cohort

Starting from July 2011 until July 2014, female bariatric surgery candidates with a BMI greater than 40kg/m^2 or greater than 35kg/m^2 with at least one severe obesity-related comorbidity were recruited into this prospective observational study. Patients were treated in the Obesity Unit of Pitié-Salpetrière Hospital, Institute of Cardiometabolism and Nutrition (ICAN), Paris, France. Patients underwent either adjustable gastric banding (AGB) or Roux-en-Y Gastric Bypass (RYGB) based upon their choice and the agreement of a multidisciplinary clinical panel. After excluding patients who were converted from AGB to RYGB (5 subjects), 59 subjects had sufficient clinical data and serum available for lipidomic analysis to be included in the current study. Ethical approval was obtained from the Research Ethics Committee of Pitié-Salpêtrière Hospital (CPP Ile-de-France). Informed written consent was obtained from all subjects. The Microbaria protocol is registered as clinical trial NCT01454232.

Clinical and anthropometric measurements were taken before (M0), one (M1), and three months (M3) after surgery. Anthropometric parameters were estimated by a whole-body fan-beam DXA scanner (Hologic Discovery W, software v12.6, 2; Hologic, Bedford, MA), as previously described (ref. 19). Variables included in this study were total fat-free mass (FFM, in kg) and total fat mass (FM, in kg and percent).

Biological analysis

Blood samples were collected after an overnight fast to measure routine biochemical parameters, as described previously (ref. 13). Serum glucose, total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (γGT) were measured enzymatically. Low-density lipoprotein-cholesterol (LDL-C) was estimated by the Friedewald formula. ApoA1 and ApoB were measured by immunonephelometry. Serum insulin was assayed by Bi-INSULIN IRMA (CisBio International, Gif-sur-Yvette, France); leptin and adiponectin by radioimmunoassay (Linco Research, Saint Louis, MI, USA); interleukin-6 (IL-6) by ELISA (QuantikineUS, R&D System Europe Ltd, Abingdon, UK); and high-sensitivity C-reactive protein (CRP) and orosomucoid by an IMMAGE automatic immunoassay system (Beckman-Coulter, Fullerton, CA, USA). Insulin sensitivity was measured by the McAuley index (ref. 20) and HOMA-%S (HOMA2-S) (ref.

21). The latter was calculated using HOMA-CIGMA software, and the McAuley index was calculated as = exp[2.63 – 0.28ln(insulin) – 0.31ln(triglycerides)].

Lipidomics

Targeted lipidomics analysis of phospholipids and sphingolipids was conducted by HPLC-MS/MS, as described previously (ref. 13, ref. 16). Serum was prepared from whole blood after an overnight fast. Whole blood was rested for 30 minutes at 4°C and then centrifuged for 10 minutes at 3000 rpm at 4°C. Serum was aliquoted into dry tubes and immediately stored at -80°C. Lipids were extracted by acidified methanol:chloroform with internal standards for each lipid class and fatty acid saturation level (Avanti Polar Lipids, Alabaster, AL, USA). Serum had not undergone any freeze-thaw cycles prior to extraction. Samples were extracted in 4 batches, with repeated measures of any one subject included in the same batch. One hundred and fifty-four lipids were quantified, using 19 external standards (Avanti Polar Lipids, Alabaster, AL, USA) as previously described (ref. 13, ref. 16), and include Phosphatidylcholines (PC), Phosphatidylethanolamines (PE), lyso-phosphatidylcholines (LPC) and –ethanolamines (LPE), phosphatidylinositols (PI), phosphatidylserines (PS), phosphatidylglycerols (PG), phosphatidic acids (PA), sphingomyelins (SM). Ceramides (Cer) could be further classified as dihydroceramides (DHCer), labeled as Cer(d18:0) in standard nomenclature, and sphingosine- or sphingadienine-containing ceramides, that is Cer(d18:1) and Cer(d18:2), respectively. Unfortunately, this methodology could not identify the sphingosine and fatty acid component of each SM. A previous publication from the Lipid MAPS consortium, however, provides the proportion of different sphingosine-fatty acid combinations for each measured SM in their large representative sample (ref. 11). Using these estimates, presumed fatty acid content was assigned for each SM species if greater than 60% of that SM species could be attributed to one sphingosine-fatty acid pair and if it did not contain a mixture of saturated and unsaturated fatty acids. For example, SM(42:1) was hypothesized to be SM(d18:1/24:0) as this specific sphingosine-fatty acid combination comprised 100% of the reported SM(42:1). Finally, measurements below the level of quantitation cannot be treated as missing, and as simple 0-imputation underestimates the true value, multiplicative log-normal-randomized imputation was computed with the zCompositions package in R(ref. 22). Only lipids with at least 80% quantitated values and detected at all 3 time points were included, leaving 131 lipids in the analysis.

Analyses of serum free fatty acids (FFA) were performed on a UPLC Waters Acquity (Waters Corp, Saint-Quentin-en-Yvelines, France) coupled to an Orbitrap-based instrument: a Q-Exactive (Thermo Fisher Scientific, Illkirch, France). Briefly, 50 µl of serum was extracted with 400µl of frozen acetonitrile containing 0.1% of formic acid and a mix of labelled internal standards (16 amino acids). Mass spec data were processed using XCMS and CAMERA packages in R software. The resulting dataset was filtered, normalized and annotated based on standard guidelines (ref. 23, ref. 24). FFA were annotated using an in-house database built using commercially available standards.

Statistical analysis

All analyses were conducted in R version 3.2.3 with the indicated (packages). Lipidomics data were log-transformed. Distributions for clinical variables and model residuals were examined and, when necessary, variables were log or square-root transformed. Tables report untransformed means and standard errors for easier interpretation. Means at baseline were compared using Welch's t-test. Permutational MANOVA of the Euclidean distance matrix was used to test for multivariate differences between groups (vegan). The longitudinal effects of surgery were analyzed using 2 factor mixed effects ANOVA including a random intercept for subject (lme4, car). Due to their estimation by maximum likelihood, mixed effects models are robust to non-informative dropout of patients, which was present in this study, and thus efficiently use all available data (ref. 25). With the exception of the post-hoc tests described below, all p-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate. Padj, or the false discovery rate, has a different interpretation than the p-value—the proportion of expected false positive results at a given threshold—and is often set to different thresholds than the strict convention of 0.05 used for the type 1 error rate. With this in mind, Padj <0.1 was used for interaction effects, which are often tested at less conservative thresholds than main effects, and for correlation matrices, which tend to have a high number of redundant comparisons and thus may suffer from adjustment-induced loss of power. Following a significant interaction, data were stratified by surgery type and all pair-wise comparisons between means at M0, M1, and M3 were tested with the family-wise error rate maintained at alpha=0.05 using one-step generalized linear hypothesis tests (multcomp). Linear regressions controlling for baseline lipid concentration were used to test for the difference between RYGB and AGB on the change in each lipid to M3 while controlling for the change in body weight. That is, a regression model of the form:

$$Lipid_{M3} = \beta_0 + \beta_1(Lipid_{M0}) + \beta_2(Weight_{M0}) + \beta_3(\Delta Weight_{M3-M0}) + \beta_4(Surgery_{RYGB=1,AGB=0})$$

For non-significant interactions, a main effect of time was considered significant at Padj <0.05, and post-hoc tests were applied across time. All fold-changes were based on differences in means. Between-lipid correlations and lipid-clinical correlations were calculated using biweight mid-correlation, an outlier-robust analog of the Pearson correlation (WGCNA).

Results

Baseline and longitudinal clinical variables between surgery types

Thirty-seven and 22 patients underwent RYGB and AGB, respectively, with approximately 45% lost to follow-up at M1 and M3 for each group (see Table 1 for clinical characteristics). Baseline characteristics did not differ between patients with complete or incomplete data during follow-up (data not shown), indicating that the imbalance across time would not bias the results. The mean age was 34.5 (±1.6) and 37.3 (±1.9) years for AGB and RYGB, respectively, and was not significantly different between groups. At baseline, and compared to AGB, RYGB patients had 2.9 kg/m^2 higher BMI, 11.1 IU/L higher γGT, 0.6mM higher fasting glucose (with 24% T2D in RYGB and 5% in AGB), 0.22mM higher fasting triglycerides, and 0.4% higher HbA1c, all $P<0.05$. However, no variables were significantly different after adjusting for the false discovery rate (all $P_{adj}>0.1$). 22% of RYGB patients were treated with metformin or statins at baseline, while no AGB patients were on these medications ($P=0.051$). There were no statistically significant differences in the prevalence of T2D. Thus, RYGB tended towards higher obesity and worse diabetes risk factors.

Regarding the effect of bariatric surgery, RYGB generally resulted in better weight and body composition improvement than AGB, as expected (Table 1). RYGB patients decreased from a mean BMI of 46.5 at baseline to 37.9 at M3. Following AGB, mean BMI decreased from 43.6 to 38.3. Total FM and FFM decreased to a greater extent in RYGB than AGB, and percent FM decreased by 3.4% and 2.4% at M3, respectively. Leptin also fell more rapidly in RYGB. Clinical biochemistries improved to a greater extent after RYGB than AGB. Specifically, Apo-A1, LDL-C, and TC decreased by both M1 and M3 in RYGB, but returned to baseline in AGB at M3. Fasting insulin decreased by 40% then 51% in RYGB, and 43% in AGB at M3 only. HOMA2-S decreased more rapidly and to a greater extent in RYGB than AGB, but the McAuley index improved equivalently in both groups.

γGT was the only liver enzyme to decrease significantly in AGB by M3. With RYGB, γGT was significantly decreased, while both ALT and AST were significantly increased from baseline at both M1 and M3. Orosomucoid, or alpha-1-acid glycoprotein, an acute phase protein, decreased by 20% in RYGB, but was unchanged by AGB. Other parameters, such as CRP, HbA1c, triglycerides, and adiponectin, were altered to the same extent in each surgical procedure. In summary, both surgeries resulted in improvements in the majority of measured parameters, but RYGB resulted in greater weight loss, including both lean and fat masses, and persistent improvements in TC, LDL-C, and orosomucoid.

<u>Limited associations between baseline clinical variables and the phosphosphingolipidome</u>

Given the slight clinical differences between patients undergoing the two surgeries, the relationships between clinical parameters and the phosphosphingolipidome were examined at baseline to identify potential confounding factors. There were no significant differences in the serum concentrations of any of the lipids between surgical groups ($P_{\text{multivariate}}$=0.33; Fig 1A; supplementary table 1). Baseline metformin or statin treatment could potentially confound the longitudinal effect on circulating lipids, but neither metformin ($P_{\text{multivariate}}$=0.45; data not shown; supplementary table 1) nor statins ($P_{\text{multivariate}}$=0.24; Fig 1B; supplementary table 1) were associated with lipidomic measurements. Exploratory analysis revealed that lipids were primarily organized by their structural classes and thus were analyzed in this manner. Shown in Figure 1C, total Cer, SM, and PC were strongly positively associated with Apo-B, LDL-C, and TC (all r>0.49), whereas total PE (r=0.52), PG (r=0.59), LPE (r=0.38), and PI (r=0.49) were positively associated with fasting triglycerides. Total PC was associated with Apo-A1 and triglycerides (r=0.46 and 0.51), and total PG was inversely correlated with the McAuley insulin sensitivity index (r=-0.45). Given the absence of an association with surgery status and baseline lipidomics, and the somewhat sparse associations with other clinical variables, the longitudinal effect of each surgery is unlikely confounded by baseline differences between the two groups.

<u>Procedure-independent and -dependent changes in phospholipids and sphingolipids</u>

For the longitudinal analysis, the main effects of surgery and the interaction between the two surgeries and time were evaluated. A main effect of time (and no interaction) was detected for 64 lipids (Padj<0.05), whereby 54 of these lipids decreased from baseline and included all classes except PA (Fig 1D-1F, Supplementary Table 2). The vast majority of these lipids were decreased at both M1 and M3, which created a markedly similar pattern of change at each time point (Fig 1E). Eight of the ten most statistically significantly decreased lipids were PE species. Only Cer and SM species, and one PC species, were significantly increased following surgery, and included Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1) (Fig 2E and 2F, Supplementary Table 2).

A significant interaction (Padj<0.1) was detected for 29 lipids (Fig 1D), indicating that they changed differentially between the two surgery types. The majority of lipids were decreased following each surgery and consisted of a number of PC, SM, and Cer, and also PE(38:3) (Fig 2A, Supplementary Table 2). PC species, which did not exceed 36 carbons, decreased by M1 and remained suppressed at M3 by 20 to 64% in RYGB, whereas they either returned or tended to return to baseline values in AGB. Ceramides, which included DHCer, Cer(d18:1), and Cer(d18:2) containing 22 to 24 carbon fatty acids, were also decreased by M1 and remained decreased by 35-60% at M3 in RYGB, but returned to baseline in AGB. All SM with 1 double-bond decreased and remained decreased with RYGB, but they returned to baseline values in AGB (with the exception of SM-32:1). Thus, RYGB selectively induces a sustained decrease in these lipids. Interestingly, 4 surgery-dependent lipids were increased during follow-up. SM(42:3), SM(42:4), and SM(36:2), all polyunsaturated, increased at M1 and remained elevated by 24-33% in RYGB, but were only elevated by 17-23% in AGB at M1. Cer(d18:1/26:1) was increased by over 75% in RYGB, but did not differ at any time in AGB.

Body weight decreased to a greater extent in RYGB than AGB, therefore the kinetic differences between RYGB and AGB at M3 were also tested after adjusting for weight loss. We observed two-thirds of the RYGB-specific lipids were differentially altered by RYGB independent of differences in weight loss (Fig 2B, Supplementary Table 3). These data reveal a PC and sphingolipid “signature” of RYGB that is independent from the greater weight loss induced by this procedure.

<u>The RYGB lipid signature is related to differences in metabolic outcomes</u>

Having identified a group of surgery-dependent lipid species, characterizing the lipid-lipid and clinical-lipid associations could elucidate potential mechanisms for the RYGB-specific lipid improvements. After assigning putative fatty acid content to each SM, marked agreement between the changes from baseline to M3 in SM, Cer(d18:1) and Cer(d18:2) were observed based on the carbon length and saturation in RYGB patients (Fig 3A). Particularly strong agreement is observed for sphingolipid species with C22 to C24 fatty acids attached to the sphingoid backbone, which all decreased following RYGB throughout the 3 months follow up, suggesting a coordinated decrease in Cer and the corresponding SM.

Finally, we sought to determine whether the RYGB dependent lipid modifications were related to the clinical parameters that changed to a greater extent in RYGB by M3 (Fig 3B). With the exception of an inverse association with Cer(d18:1/26:1), there were no statistically significant associations with changes in FM or %FM, which corroborates a body fat-independent effect of RYGB on the identified lipids. The RYGB-specific decreases in SM, some PC, and Cer species were most strongly associated with the decrease in TC, LDL-C, orosomucoid, leptin, body weight, FFM, and to a lesser extent, HOMA2-S. A number of these lipids were also associated with the decline in γGT. On the other hand, the increase in the three unsaturated SM and Cer(d18:1/26:1) were associated with amelioration of the aforementioned clinical parameters, demonstrating heterogeneity in the clinical relevance of individual sphingolipid species. The potential effect of differences in lipolysis were tested by measuring fasting serum saturated free fatty acids (FFA). Both surgeries increased C16, C18, and C20 FFA after one month, but there was no statistically significant interactions between surgery type and time (Fig 3C). FFA(16:0) (palmitate) was higher in RYGB than AGB throughout the study period (surgery main-effect $P<0.01$).

Discussion

The objective of this study was to identify the differential effects of bariatric surgeries on the serum phosphosphingolipidome. The most significant finding is that RYGB patients had decreases in a number of PC, SM, and longer chain Cer species by both 1 and 3 months post-op, whereas nearly all of these same lipids returned to baseline within 3 months following AGB. Importantly, the majority of RYGB-specific changes remained independent of the greater weight loss following RYGB. A number of unsaturated SM and Cer were actually increased following bariatric surgery. The RYGB lipidomic signature was associated with improvements in cholesterol, body weight, orosomucoid, γGT, and to some extent insulin sensitivity. These findings may reveal a specific effect of RYGB on a number of biologically relevant lipids.

At baseline, PC, SM, and Cer were positively associated with total cholesterol, LDL-C, and ApoB, which are biomarkers of atherosclerosis, while PG, PE, and PI were associated with triglycerides. These findings may be attributed to the distribution of lipids in lipoprotein fractions: 50% and 60% of SM and Cer, respectively, are found in LDL (ref. 26). The lack of associations with other clinical phenotypes is striking given previous reports (ref. 13, ref. 14, ref. 27). It is possible that at the extreme end of obesity, the phosphosphingolipidome poorly differentiates clinical phenotypes based on simple clinical chemistries.

The majority of lipids, representing nearly all classes measured, decreased equivalently between both surgical groups. The broad effect of surgically-induced weight loss has been described by others. A previous, though much smaller study of only 5 subjects, also reported decreases in LPC, PC, PE, PI, SM and Cer at 3 months following RYGB (ref. 28). RYGB has also been shown to induce a sustained decrease in a number of Cer species for up to 6 months (ref. 29). In addition, a number of SM, PC, and LPC species were among the most altered lipids following RYGB as soon as 4 days after surgery, and this occurred to a greater extent in patients with diabetes remission compared to non-remitters 2 years after surgery (ref. 30). However, the current study extends these previous reports in an important way: RYGB could be shown to have substantial weight loss-independent effects on specific lipid classes.

Given the greater metabolic improvement induced by RYGB compared to AGB, we reasoned that RYGB-specific lipid alterations would identify clinically relevant biomarkers. To this end, the current study reveals a distinct effect of RYGB to decrease a subclass of PCs shorter than 36 carbons, and induce both decreases and increases in a number of SM and longer chain Cer and DH-Cer. The majority of these changes remained significant after adjust for the greater weight loss in RYGB. It is noteworthy that these 3 classes were identified given their shared biochemical synthesis: DH-Cer are desaturated to Cer, and SM are formed by Cer and PC as source of phosphocholine (Figure 3C). While the sphingosine-fatty acid content of our detected SM could only be presumed, we observed a remarkable consistency across the fatty acid lengths decreased in RYGB between SM and Cer. The similarity was most striking for C22 - C24 fatty acids. Interestingly, a previous metabolomics study by our group identified Cer(d18:1/24:0) as one of the metabolites decreased at 3 and 6 months following RYGB, further supporting the particular effect on longer Cer species (ref. 31). Phospholipids and sphingolipids are related to a number of cardiometabolic diseases. For example, PC synthesis is a regulator of VLDL secretion and hepatic steatosis, and further, circulating PC is an important source of triglycerides in steatosis (ref. 15, ref. 32). The SM and PC species differentially affected by RYGB are increased in coronary artery disease and associated with increased mortality (ref. 33, ref. 34). Bariatric surgery has been shown to improve NAFLD (ref. 3) and reduce cardiovascular mortality (ref. 4), therefore the changes in PC and SM could be involved. The role of Cer may be more difficult to interpret.

The specificity for very long chain Cer in the RYGB signature highlights the complicated role of Cer acylation in metabolism (ref. 35). A family of ceramide synthase genes—CerS1 to CerS6—that have different fatty acid affinities and different tissue expression levels determine *de novo* Cer fatty acid content (ref. 36). Recent experiments in mice that have genetically manipulated CerS2, CerS5, and CerS6—where the first produces longer chain Cer and the latter two produce C16 ceramides—showed that elevation in C16:0, but not C24:0 or C24:1, induce insulin resistance (ref. 37–39). Furthermore, Cer(d18:1/18:0) appears to be the most detrimental in skeletal muscle (ref. 40). A large epidemiology study recently showed that Cer(18:1/16:0) was associated with increased risk of cardiovascular mortality, whereas elevated Cer(d18:1/24:0) showed a protective relationship (ref. 41). Thus our results present a paradox: serum C16 and C18 Cer were transiently increased following surgery-induced weight loss despite rapid improvements in HOMA-S. The observed post-surgery rise in fasting serum FFA could potentially explain this transient rise in long-chain Cer. On the other hand, CerS2 is the major liver isoform and produces C20 to C26 Cer (ref. 36). The liver is likely a major contributor to serum Cer (and SM) levels due to secretion into lipoproteins, which is increased by *de novo* sphingolipid synthesis (ref.

42, ref. 43). The distinct decrease in circulating C22 to C24 ceramides may therefore reflect a specific effect of RYGB on hepatic Cer synthesis, secretion, or both. Indeed, a number of, though not all, studies in humans have shown similar relationships between ceramides and impaired glucose homeostasis ranging from C16 to C24 Cer (ref. 27, ref. 44). Importantly, enrichment of LDL with either Cer(d18:1/16:0) or Cer(d18:1/24:0) in mice produced equivalent degrees of insulin resistance and inflammation, both *in vitro* and *in vivo* (ref. 18). Thus, while there is little doubt that Cer(d18:1/16:0) and Cer(d18:1/18:0) are likely the most deleterious species, the findings in the current study emphasize the importance of better understanding the role of serum or lipoprotein ceramide acyl chain length, which could help better understand the effects of RYGB, diabetes and cardiovascular risk in general.

While the effect of RYGB remained independent of changes in weight, nevertheless, weight loss was associated with decreases in PC, SM, and Cer, consistent with an important role of obesity and increased sphingolipid levels. It is unclear why decreases in FFM would be better correlated to the changes in measured lipids compared to FM. This relationship may simply reflect a proportionally greater loss of FFM in RYGB and thus simply a coincident association rather than an effect of changes in FFM *per se* (ref. 45). TC, LDL-C, and orosomucoid remained decreased by month 3 in RYGB, but were unchanged by AGB. This same temporal pattern was observed in the RYGB-specific Cer and SM species. Inflammation is a potent inducer of sphingolipid accumulation (ref. 46), and given the reduced levels of orosomucoid in the RYGB group, a greater reduction in hepatic inflammation could contribute to these specific lipid improvements. LDL-ceramides are increased in T2D and were selectively decreased following diet-induced weight loss (ref. 18), thus the temporal association between LDL-C and ceramides could also reflect this partitioning. The direct associations between reductions in Cer(d18:1/23:0) and Cer(d18:1/24:0) and improvements in HOMA2S are difficult to interpret, as described above, but again, warrant further investigation. Greater reductions in saturated FFA exposure could alter ceramide synthesis (ref. 47), however changes in fasting FFA were not different between the surgeries, suggesting that differential effects on lipolysis do not explain the altered sphingolipid responses. Finally, the changes in Cer-26:1, SM-36:2, SM-42:3, and SM-42:4 were entirely dependent upon changes in body weight, unlike the saturated ones, indicating that circulating levels of saturated and unsaturated sphingolipids may be influenced by different mechanisms.

Several limitations must be discussed. While the short-term follow-up of this study was specific to our research hypothesis, our results cannot immediately be generalized to longer follow-up. Analyses beyond 1, 2, or even 5 years will be necessary to determine if these lipid changes are sustained and how they are related to other clinical improvements. As the patients in the short 3 month follow-up are still losing weight, an important question is the role of ongoing weight loss vs. a sustained lower body weight. This again emphasizes the need for longer term studies. Our sample only included women; similar studies in men are warranted to exclude sex-specific differences. Furthermore, as this study was not randomized, we cannot exclude the possibility that the apparent effects of surgery are confounded by baseline differences in the surgery groups, whether measured or unmeasured. Finally, changes in calorie or nutrient consumption and absorption or communication between the intestine and liver, e.g. bile acids and FXR signaling (ref. 48), could also be important contributors to sphingolipid metabolism and secretion. Indeed, a recent report on a smaller subset of the current cohort indicates greater decreases in total energy and meat and fish intake in the RYGB group compared to AGB (ref. 49). The very limited number of subjects with both dietary intake and lipidomics data unfortunately prevented more in-depth analysis. Follow-up studies controlling for energy and nutrient intake, as well as the rate of weight loss, will be necessary to attribute a unique effect of RYGB on sphingolipid metabolism.

In summary, RYGB patients demonstrated greater and sustained decreases in a number of PC, SM, and longer chain Cer compared to AGB, the majority of which occurred independent of differences in weight loss. A previously unidentified increase in unsaturated SM and Cer following weight loss was also observed. While surgically induced weight loss, regardless of surgery type, has an important effect on circulating phospholipids and sphingolipids, the RYGB-specific lipid signature is associated with concomitant decreases in body weight, circulating cholesterol, insulin sensitivity and orosomucoid. Longer follow-up is warranted to determine the long-term effects of RYGB on these lipids, but the current findings suggest an improved sphingolipid profile in the reduction of cardiometabolic risk following RYGB.

Supplementary information

Supplementary information is available at the *International Journal of Obesity's* website.

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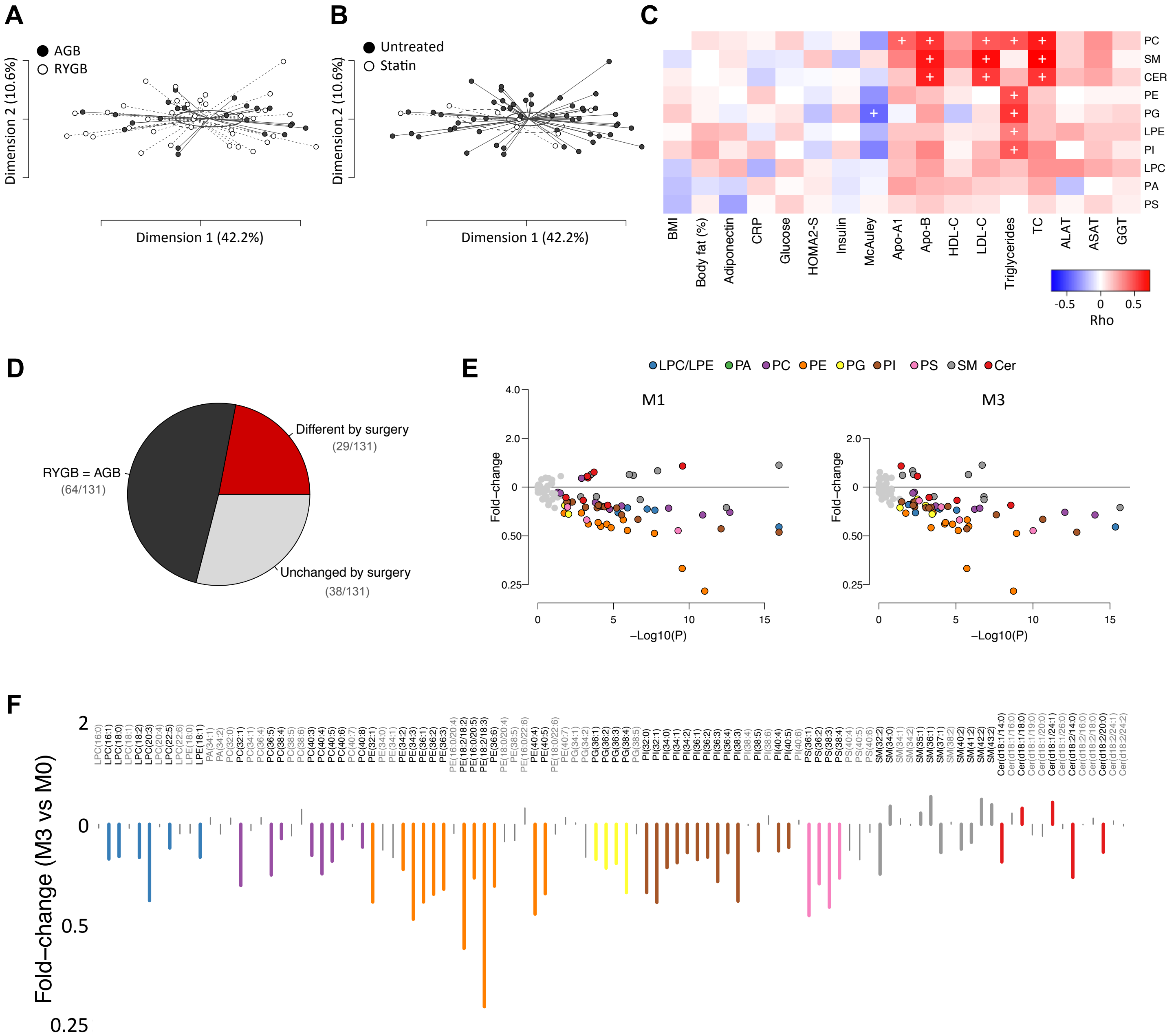
Table 1. Clinical parameters at baseline and during follow-up. ANOVA P are adjusted for the false discovery rate. *P<0.05 compared to baseline, adjusted for the family-wise error rate. #Chi-squared test. Values are mean (SE) for continuous variables and % prevalence for categorical. TC = Total cholesterol, T2D = Type 2 Diabetes. P = Welch's t-test for surgery differences at baseline, Padj = False discovery rate adjustment of t-test. P_I= Interaction (time x surgery), P_S= Main-effect of surgery, P_T= Main-effect of time. McAuley index = exp[2.63 – 0.28ln(insulin) – 0.31ln(triglycerides)].

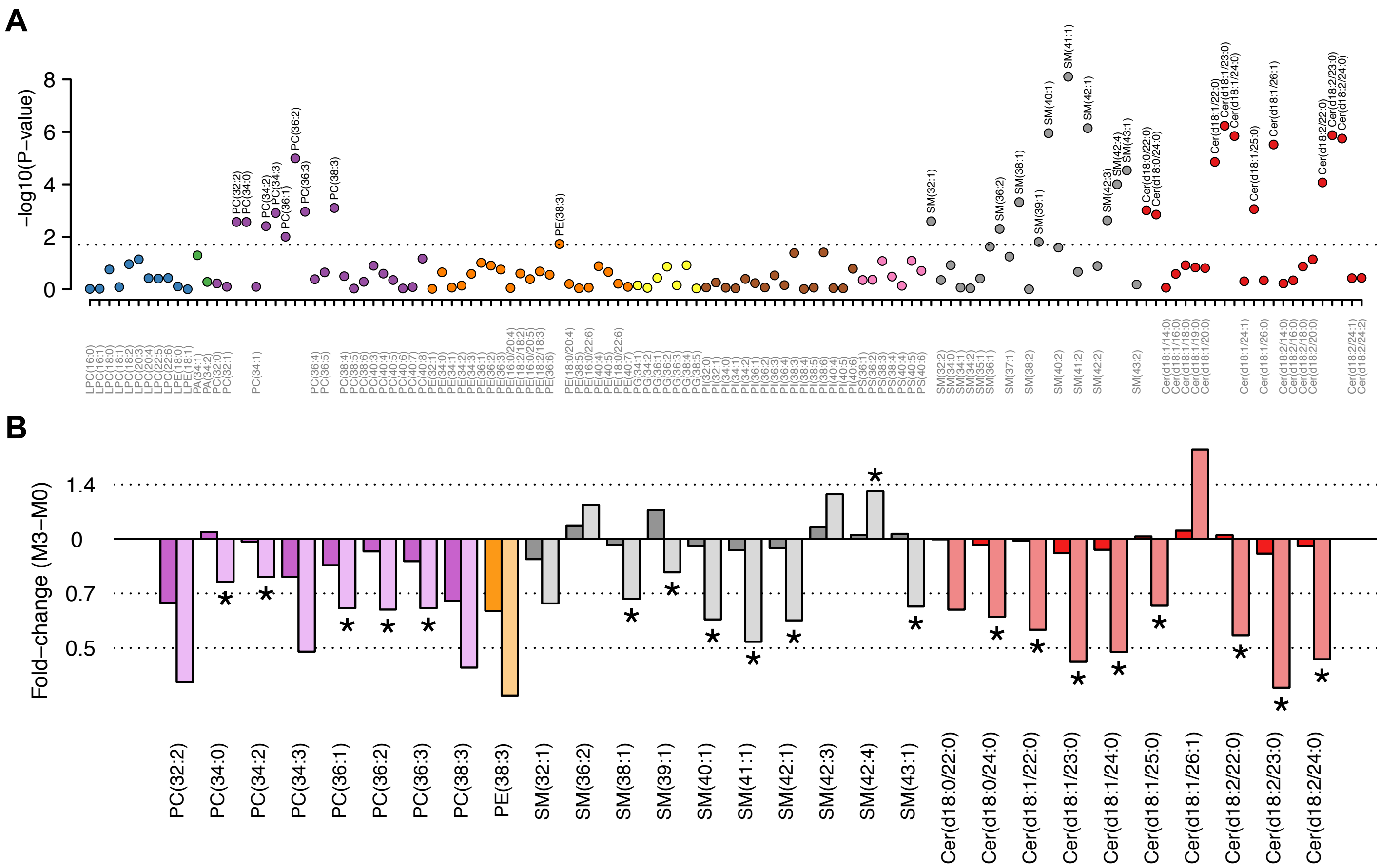
Figure 1. PCoA of baseline lipidomics with 95% confidence ellipses in A: AGB and RYGB, and B: Untreated and statin-treated subjects. C: Correlation heatmap between baseline clinical parameters and lipid classes, + is Padj<0.1. D: Summary of ANOVA results. E: Volcano plots of change in lipids without an interaction averaged across the two surgeries at month 1 (*left*) and month 3 (*right*). F: Fold-change from M0 to M3 for lipids without a significant interaction. Bold text and colored lines are lipids significantly different from baseline (Padj<0.05).

Figure 2. A: Manhatten plot for the Time by Surgery interaction in the mixed effect ANOVA. Dotted line is the 10% Benjamini-Hochberg false discovery rate. B: Change from baseline to M3 in AGB (dark) and RYGB (light) for lipids with a significant interaction. * indicates Padj <0.05 after adjusting for weight loss.

Figure 3. A: Change in sphingolipids from baseline to M3 organized by fatty acid content for Cer and presumed fatty acid content for SM (see Methods). B: Heatmap of correlations between deltas of lipids and clinical parameters (M3-M0) that had a significant interaction in ANOVA, + indicates a Padj <0.1. Lipids and clinical variables are clustered with average-linkage hierarchical clustering. C: Change in saturated free fatty acids following surgery. Time points with different letters are statistically significantly different (P<0.05) for both surgeries as there was no significant interaction. D: RYGB "signature" overlaid on a simplified diagram of phospholipid and sphingolipid synthesis. Bold or colored names are analytes measured in the current study. 1,2-DAG, 1-2-Diacylglycerol; CDP-DAG, Cytidine Diphosphate Diacylglycerol; PA, Phosphatidic Acid; PI, Phosphatidylinositol; PG, Phosphatidylglycerol; PS, Phosphatidylserine; PE, Phosphatidylethanolamine.



A
AGB
RYGB
Dimension 2 (10.6%)
Dimension 1 (42.2%)
B
Untreated
Statin
Dimension 2 (10.6%)
Dimension 1 (42.2%)
C
PC
SM
CER
PE
PG
LPE
PI
LPC
PA
PS
BMI
Body fat (%)
Adiponectin
CRP
Glucose
HOMA2-S
Insulin
McAuley
Apo-A1
Apo-B
HDL-C
LDL-C
Triglycerides
TC
ALAT
ASAT
GGT
-0.5
0
0.5
Rho
D
Different by surgery
(29/131)
RYGB = AGB
(64/131)
Unchanged by surgery
(38/131)
E
LPC/LPE
PA
PC
PE
PG
PI
PS
SM
Cer
M1
M3
Fold−change
−Log10(P)
F
Fold−change (M3 vs M0)
2
0
0.5
0.25


A
−log10(P−value)
8
6
4
2
0
PC(32:2)
PC(34:0)
PC(34:2)
PC(34:3)
PC(36:1)
PC(36:2)
PC(36:3)
PC(38:3)
PE(38:3)
SM(32:1)
SM(36:2)
SM(38:1)
SM(39:1)
SM(40:1)
SM(41:1)
SM(42:1)
SM(42:3)
SM(42:4)
SM(43:1)
Cer(d18:0/22:0)
Cer(d18:0/24:0)
Cer(d18:1/22:0)
Cer(d18:1/23:0)
Cer(d18:1/24:0)
Cer(d18:1/25:0)
Cer(d18:1/26:1)
Cer(d18:2/22:0)
Cer(d18:2/23:0)
Cer(d18:2/24:0)
B
Fold−change (M3−M0)
1.4
0
0.7
0.5
PC(32:2)
PC(34:0)
PC(34:2)
PC(34:3)
PC(36:1)
PC(36:2)
PC(36:3)
PC(38:3)
PE(38:3)
SM(32:1)
SM(36:2)
SM(38:1)
SM(39:1)
SM(40:1)
SM(41:1)
SM(42:1)
SM(42:3)
SM(42:4)
SM(43:1)
Cer(d18:0/22:0)
Cer(d18:0/24:0)
Cer(d18:1/22:0)
Cer(d18:1/23:0)
Cer(d18:1/24:0)
Cer(d18:1/25:0)
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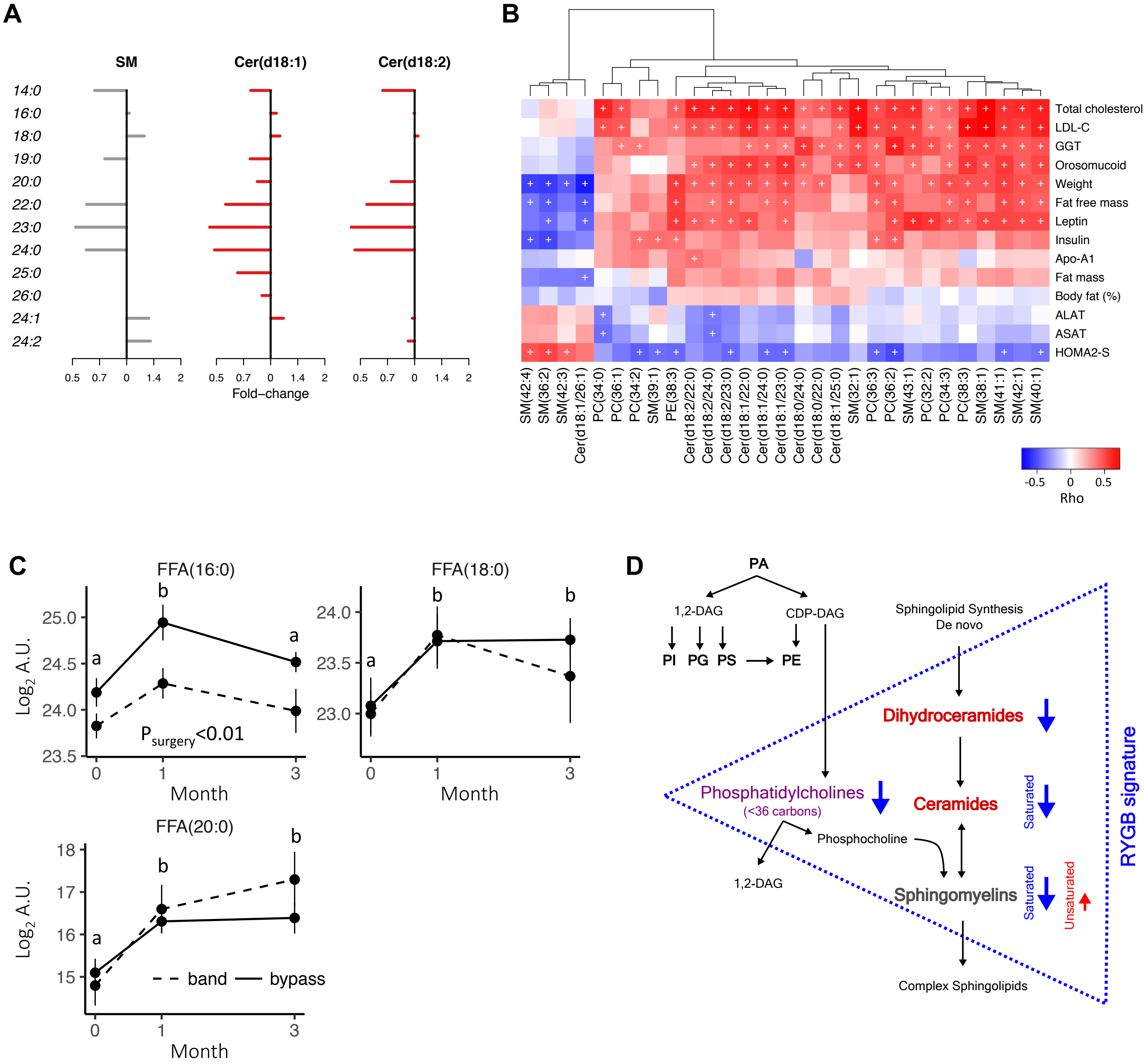
Cer(d18:2/24:0)


A
SM
Cer(d18:1)
Cer(d18:2)
14:0
16:0
18:0
19:0
20:0
22:0
23:0
24:0
25:0
26:0
24:1
24:2
Fold−change
B
Total cholesterol
LDL-C
GGT
Orosomucoid
Weight
Fat free mass
Leptin
Insulin
Apo-A1
Fat mass
Body fat (%)
ALAT
ASAT
HOMA2-S
Rho
C
FFA(16:0)
FFA(18:0)
FFA(20:0)
Month
band
bypass
D
PA
1,2-DAG
CDP-DAG
PI PG PS PE
Sphingolipid Synthesis De novo
Dihydroceramides
Phosphatidylcholines
(<36 carbons)
Ceramides
Phosphocholine
1,2-DAG
Sphingomyelins
Saturated
Unsaturated
Complex Sphingolipids
RYGB signature

	RYGB			AGB			T-test (M0)		ANOVA		
	M0	M1	M3	M0	M1	M3	P	Padj	P_I	P_S	P_T
Sample size	37	21	19	22	21	19					
Age (years)	37.3 (1.9)			34.5 (1.6)			0,3	0,77			
BMI (kg/m^2)	46.5 (1.0)	41.1 (1.1)*	38.0 (1.2)*	43.6 (0.7)[P]	40.4 (1.0)*	38.3 (1.0)*	0,01	0,2	p<0.001		
Weight (kg)	124.9 (3.0)	109.8 (3.5)*	99.7 (3.4)*	117.8 (2.8)	106.6 (2.5)*	100.7 (2.3)*	0,09	0,36	p<0.001		
Fat mass (kg)	62.2 (1.8)	54.9 (2.2)*	47.1 (2.1)*	58.2 (1.8)	52.2 (1.8)*	48.0 (2.0)*	0,13	0,46	0,04		
Fat free mass (kg)	58.9 (1.2)	52.2 (1.5)*	50.3 (1.3)*	55.5 (1.4)	52.4 (1.1)	50.5 (8.4)*	0,07	0,36	p<0.001		
Fat mass (%)	50.1 (0.5)	50.0 (0.8)*	46.7 (0.8)*	49.6 (0.9)	48.5 (1.0)*	47.2 (1.0)*	0,65	0,93	0,04		
Glucose (mM)	5.7 (0.3)	4.9 (0.1)*	4.8 (0.2)*	5.1 (0.1)[P]	4.8 (0.1)*	4.8 (0.2)*	0,04	0,27	0,22	0,54	p<0.001
HbA1c (%)	6.1 (0.2)	5.6 (0.1)*	5.5 (0.1)*	5.7 (0.1)[P]	5.5 (0.1)*	5.4 (0.1)*	0,049	0,27	0,13	0,54	p<0.001
Insulin (µIU/ml)	22.5 (2.3)	13.4 (1.2)*	11.0 (1.2)*	22.1 (4.1)	24.5 (7.6)	12.5 (1.7)*	0,75	0,93	0,04		
HOMA2-β	185 (15)	165 (15)	155 (15)*	196 (15)	230 (36)	155 (19)*	0,44	0,84	0,36	0,54	0,04
HOMA2-S	42.9 (5.3)	59.0 (5.5)*	82.8 (11.1)*	44.4 (5.3)	41.6 (4.7)	70.6 (14.3)*	0,62	0,93	0,04		
McAuley Index	5.9 (0.3)	6.2 (0.2)*	7.2 (0.3)*	6.4 (0.3)	6.4 (0.4)*	7.7 (0.6)*	0,24	0,66	0,41	0,57	p<0.001
TC (mM)	4.7 (0.2)	4.1 (0.2)*	4.2 (0.2)*	4.6 (0.2)	4.7 (0.2)	4.6 (0.3)	0,53	0,92	0,06		
HDL-C (mM)	1.1 (0.1)	0.9 (0.0)	1.0 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	0,82	0,93	0,22	0,57	0,06
LDL-C (mM)	3.0 (0.2)	2.5 (0.2)*	2.6 (0.2)*	3.0 (0.1)	3.1 (0.2)	3.0 (0.2)	0,87	0,93	0,09		
Apo-A1 (mM)	1.4 (0.0)	1.1 (0.0)*	1.2 (0.1)*	1.4 (0.1)	1.4 (0.1)	1.4 (0.1)	0,93	0,95	0,06		
ApoB (mM)	0.91 (0.0)	0.84 (0.1)*	0.85 (0.0)	0.85 (0.0)	0.89 (0.1)*	0.87 (0.1)	0,41	0,84	0,4	0,96	0,07
Triglycerides (mM)	1.3 (0.1)	1.5 (0.1)	1.2 (0.1)*	1.1 (0.1)[P]	1.0 (0.1)	0.9 (0.1)*	0,049	0,27	0,68	0,12	0,01
CRP (mg/dl)	8.2 (0.9)	3.1 (0.5)*	3.5 (0.6)*	10.0 (1.8)	4.6 (1.0)*	3.8 (0.6)*	0,49	0,9	0,11	0,54	p<0.001
IL-6 (pg/ml)	4.9 (0.4)	4.3 (0.4)	3.9 (0.3)	4.1 (0.5)	5.5 (1.1)	3.0 (0.3)	0,23	0,66	0,18	0,81	0,11
Orosomucoid (mg/ml)	1.00 (0.1)	0.92 (0.1)	0.80 (0.1)*	0.99 (0.0)	0.91 (0.0)	1.00 (0.1)	0,81	0,93	0,04		
Adiponectin (µg/ml)	4.3 (0.3)	5.2 (0.4)	5.5 (0.6)*	4.5 (0.4)	4.1 (0.4)	5.3 (1.1)*	0,81	0,93	0,11	0,57	p<0.001
Leptin (ng/ml)	82.6 (4.8)	44.5 (5.5)*	30.6 (4.4)*	82.9 (5.6)	49.5 (5.8)*	35.3 (3.5)*	0,84	0,93	0,09		
ALAT (IU/l)	26.9 (2.6)	49.2 (6.0)*	40.6 (6.8)*	23.6 (3.4)	28.6 (8.8)	18.1 (1.7)	0,19	0,63	0,03		
ASAT (IU/l)	24.5 (1.1)	32.7 (2.4)*	36.1 (5.4)*	24.3 (1.3)	24.9 (2.7)	22.6 (1.7)	0,95	0,95	0,04		
γGT (IU/l)	36.9 (3.8)	44.8 (7.2)	26.1 (4.6)*	25.8 (2.4)[P]	23.9 (2.6)	19.4 (2.4)*	0,02	0,2	0,07		
T2D	9			1			[#]0.147				
Statin therapy	8			0			[#]0.051				
Metformin	8			0			[#]0.051				

Table 1

Supplementary Table 1: Baseline lipidomics between surgery type, metformin, and statin therapy.

Data are means and SE with t-test P values adjusted for the Benjamini-Hochberg (BH) false discovery rate

	Surgery group					Metformin treatment					Statin treatment				
	RYGB		AGB		T-test	Untreated		Metformin		T-test	Untreated		Statin		T-test
Lipid	Mean	SE	Mean	SE	Padj	Mean	SE	Mean	SE	Padj	Mean	SE	Mean	SE	Padj
Cer.d18.0.22.0	0.012	0.001	0.009	0.001	0.62	0.011	0.001	0.012	0.002	0.98	0.011	0.001	0.011	0.002	0.78
Cer.d18.0.24.0	0.013	0.001	0.010	0.001	0.62	0.012	0.001	0.013	0.001	0.94	0.012	0.001	0.013	0.002	0.66
Cer.d18.1.14.0	0.012	0.001	0.012	0.001	0.86	0.012	0.001	0.012	0.002	0.98	0.012	0.001	0.011	0.001	0.66
Cer.d18.1.16.0	0.180	0.007	0.161	0.008	0.71	0.170	0.005	0.192	0.022	0.98	0.173	0.006	0.175	0.014	0.92
Cer.d18.1.18.0	0.152	0.008	0.128	0.008	0.62	0.139	0.006	0.166	0.017	0.82	0.142	0.007	0.147	0.006	0.64
Cer.d18.1.19.0	0.006	0.000	0.005	0.000	0.86	0.005	0.000	0.005	0.001	0.98	0.005	0.000	0.005	0.001	0.95
Cer.d18.1.20.0	0.102	0.006	0.089	0.006	0.76	0.096	0.004	0.106	0.013	0.98	0.098	0.005	0.092	0.005	0.90
Cer.d18.1.22.0	0.552	0.028	0.476	0.029	0.71	0.519	0.021	0.547	0.084	0.98	0.528	0.022	0.495	0.063	0.84
Cer.d18.1.23.0	0.476	0.022	0.422	0.023	0.76	0.455	0.017	0.463	0.059	0.98	0.462	0.018	0.420	0.051	0.71
Cer.d18.1.24.0	1.518	0.091	1.284	0.076	0.71	1.417	0.065	1.519	0.256	0.98	1.455	0.069	1.273	0.183	0.66
Cer.d18.1.24.1	0.678	0.031	0.631	0.027	0.86	0.651	0.021	0.717	0.098	0.98	0.666	0.025	0.620	0.044	0.79
Cer.d18.1.25.0	0.074	0.004	0.069	0.003	0.86	0.072	0.003	0.077	0.013	0.98	0.074	0.003	0.063	0.005	0.64
Cer.d18.1.26.0	0.019	0.001	0.017	0.001	0.86	0.018	0.001	0.018	0.003	0.98	0.019	0.001	0.016	0.002	0.65
Cer.d18.1.26.1	0.012	0.001	0.012	0.001	0.86	0.012	0.001	0.013	0.002	0.98	0.012	0.001	0.011	0.001	0.69
Cer.d18.2.14.0	0.005	0.000	0.005	0.000	0.92	0.005	0.000	0.006	0.002	0.98	0.006	0.000	0.004	0.001	0.64
Cer.d18.2.16.0	0.032	0.002	0.031	0.002	0.86	0.031	0.001	0.035	0.008	0.98	0.032	0.002	0.028	0.003	0.66
Cer.d18.2.18.0	0.028	0.001	0.027	0.002	0.86	0.027	0.001	0.031	0.004	0.98	0.028	0.001	0.028	0.002	0.84
Cer.d18.2.20.0	0.031	0.002	0.029	0.002	0.86	0.030	0.001	0.032	0.004	0.98	0.030	0.001	0.030	0.002	0.87
Cer.d18.2.22.0	0.201	0.013	0.183	0.014	0.86	0.194	0.010	0.198	0.030	0.98	0.197	0.010	0.176	0.021	0.75
Cer.d18.2.23.0	0.100	0.006	0.094	0.007	0.86	0.099	0.005	0.096	0.015	0.98	0.100	0.005	0.086	0.013	0.66
Cer.d18.2.24.0	0.261	0.017	0.240	0.015	0.86	0.253	0.012	0.253	0.039	0.98	0.259	0.013	0.217	0.030	0.64
Cer.d18.2.24.1	0.168	0.009	0.170	0.010	0.89	0.168	0.007	0.172	0.023	0.99	0.171	0.008	0.150	0.012	0.66
Cer.d18.2.24.2	0.014	0.001	0.013	0.001	0.86	0.014	0.001	0.013	0.001	0.98	0.014	0.001	0.012	0.001	0.71
LPC.16.0	33.669	1.283	32.335	1.451	0.86	32.420	1.003	37.964	2.710	0.82	32.684	1.073	36.282	1.720	0.62
LPC.16.1	1.184	0.058	1.041	0.062	0.77	1.108	0.048	1.275	0.090	0.82	1.111	0.048	1.253	0.095	0.64
LPC.18.0	10.999	0.551	10.490	0.650	0.86	10.647	0.457	11.845	1.041	0.97	10.734	0.475	11.288	0.684	0.66
LPC.18.1	8.487	0.435	7.439	0.386	0.76	7.901	0.338	9.334	0.732	0.82	7.925	0.333	9.182	0.864	0.64
LPC.18.2	11.226	0.618	9.967	0.608	0.84	10.695	0.512	11.147	0.764	0.98	10.704	0.494	11.089	1.185	0.86
LPC.20.3	1.103	0.054	0.928	0.056	0.62	1.006	0.044	1.239	0.084	0.67	1.019	0.045	1.160	0.090	0.64
LPC.20.4	2.438	0.148	2.143	0.072	0.86	2.206	0.087	3.107	0.374	0.70	2.209	0.084	3.086	0.409	0.62
LPC.22.5	0.177	0.012	0.159	0.010	0.86	0.162	0.009	0.224	0.021	0.31	0.165	0.009	0.206	0.020	0.62
LPC.22.6	0.560	0.040	0.532	0.036	0.94	0.513	0.024	0.781	0.114	0.70	0.520	0.025	0.735	0.121	0.62
LPE.18.0	0.638	0.050	0.516	0.025	0.62	0.580	0.037	0.668	0.057	0.82	0.585	0.038	0.639	0.033	0.62
LPE.18.1	0.530	0.048	0.380	0.028	0.42	0.464	0.037	0.535	0.053	0.82	0.467	0.037	0.522	0.059	0.64
PA.34.1	0.129	0.012	0.123	0.006	0.87	0.126	0.008	0.130	0.030	0.98	0.123	0.008	0.152	0.030	0.71
PA.34.2	0.206	0.024	0.195	0.012	0.90	0.203	0.017	0.195	0.034	0.98	0.203	0.017	0.193	0.034	0.90
PC.32.0	9.909	0.401	9.710	0.444	0.93	9.793	0.303	10.103	1.131	0.98	9.847	0.301	9.761	1.164	0.87
PC.32.1	18.197	1.630	15.859	1.521	0.86	17.329	1.305	17.303	2.457	0.98	16.937	1.189	19.804	4.273	0.84
PC.32.2	3.677	0.354	3.531	0.261	0.92	3.640	0.234	3.512	1.029	0.98	3.583	0.235	3.876	1.012	0.98
PC.34.0	1.715	0.074	1.606	0.073	0.86	1.661	0.053	1.757	0.214	0.98	1.679	0.057	1.644	0.164	0.87
PC.34.1	178.210	8.299	168.718	9.807	0.86	172.227	6.884	190.253	15.983	0.98	172.674	6.791	187.402	18.078	0.72
PC.34.2	477.127	18.847	470.804	18.164	0.99	477.802	14.149	455.435	44.753	0.98	478.692	14.442	449.760	39.750	0.78
PC.34.3	20.438	1.294	18.830	1.173	0.86	19.808	1.008	20.032	2.362	0.98	19.684	0.989	20.821	2.671	0.85
PC.36.1	40.728	2.203	37.900	2.611	0.86	39.406	1.823	41.382	4.667	0.98	39.309	1.785	41.999	5.263	0.85
PC.36.2	260.574	9.340	253.915	12.103	0.87	260.211	7.514	244.579	26.338	0.98	260.477	7.664	242.881	24.118	0.72
PC.36.3	155.650	6.403	150.704	8.546	0.86	154.702	5.511	148.095	13.970	0.98	154.619	5.541	148.624	13.454	0.86
PC.36.4	196.256	8.872	198.102	9.467	0.88	193.580	6.981	218.390	17.822	0.97	193.253	6.949	220.477	17.946	0.64

PC.36.5	26.113	2.631	25.568	2.957	0.93	25.258	2.158	30.068	4.689	0.83	25.053	2.133	31.373	4.969	0.64
PC.38.3	53.502	2.097	50.848	3.174	0.86	52.563	1.864	52.191	5.585	0.98	52.914	1.828	49.951	6.019	0.82
PC.38.4	131.164	5.033	131.332	4.745	0.92	129.278	3.657	143.648	12.404	0.98	129.103	3.629	144.762	12.586	0.66
PC.38.5	57.699	2.742	58.072	3.394	0.92	56.642	2.270	65.463	5.447	0.82	56.665	2.283	65.314	5.220	0.64
PC.38.6	87.945	4.972	92.526	6.133	0.86	87.034	4.022	106.349	10.999	0.82	87.771	4.140	101.655	9.959	0.64
PC.40.3	0.237	0.011	0.277	0.019	0.62	0.254	0.011	0.240	0.027	0.98	0.257	0.011	0.217	0.020	0.64
PC.40.4	3.077	0.151	3.054	0.201	0.98	3.044	0.120	3.227	0.463	0.98	3.083	0.119	2.976	0.474	0.84
PC.40.5	9.667	0.456	9.685	0.670	0.97	9.528	0.401	10.605	1.091	0.98	9.636	0.405	9.917	1.070	0.87
PC.40.6	30.369	1.522	31.735	2.072	0.86	30.145	1.295	35.552	3.324	0.83	30.342	1.298	34.297	3.521	0.66
PC.40.7	4.664	0.245	4.757	0.328	0.92	4.646	0.213	5.030	0.483	0.98	4.599	0.209	5.335	0.505	0.64
PC.40.8	0.894	0.053	0.864	0.056	0.92	0.872	0.041	0.951	0.115	0.98	0.871	0.042	0.958	0.111	0.71
PE.32.1	0.168	0.027	0.129	0.024	0.86	0.155	0.021	0.141	0.047	0.98	0.150	0.021	0.173	0.054	0.82
PE.34.0	0.034	0.004	0.025	0.003	0.77	0.030	0.003	0.033	0.006	0.98	0.030	0.003	0.033	0.005	0.64
PE.34.1	1.925	0.243	1.570	0.238	0.86	1.769	0.190	1.946	0.497	0.98	1.748	0.191	2.076	0.470	0.64
PE.34.2	1.960	0.237	1.604	0.215	0.86	1.839	0.177	1.755	0.555	0.98	1.817	0.178	1.893	0.540	0.87
PE.34.3	0.115	0.013	0.088	0.012	0.77	0.105	0.010	0.106	0.026	0.98	0.103	0.010	0.116	0.026	0.71
PE.36.1	1.823	0.267	1.378	0.173	0.86	1.653	0.197	1.684	0.479	1.00	1.642	0.198	1.754	0.453	0.72
PE.36.2	8.760	0.968	7.121	0.904	0.86	8.218	0.742	7.711	2.147	0.98	8.092	0.747	8.512	2.067	0.84
PE.36.3	1.897	0.220	1.521	0.214	0.86	1.768	0.175	1.684	0.404	0.98	1.746	0.177	1.830	0.360	0.66
PE.36.4.16.0.20.4	1.112	0.123	0.981	0.133	0.86	1.042	0.093	1.198	0.334	0.98	1.030	0.094	1.276	0.323	0.66
PE.36.4.18.2.18.2.18.2.18.3	0.107	0.013	0.087	0.012	0.86	0.100	0.010	0.094	0.019	0.98	0.101	0.010	0.091	0.020	0.87
PE.36.5.16.0.20.5	0.125	0.019	0.105	0.021	0.86	0.119	0.016	0.109	0.022	0.98	0.115	0.016	0.129	0.025	0.64
PE.36.5.18.2.18.3	0.015	0.002	0.013	0.002	0.86	0.014	0.001	0.019	0.003	0.82	0.014	0.001	0.019	0.003	0.62
PE.36.6	0.028	0.003	0.027	0.004	0.87	0.028	0.003	0.028	0.006	0.98	0.027	0.003	0.031	0.006	0.66
PE.38.3	0.859	0.095	0.719	0.106	0.86	0.814	0.078	0.762	0.187	0.98	0.797	0.077	0.870	0.201	0.82
PE.38.4.18.0.20.4	7.444	0.636	6.833	0.786	0.86	7.120	0.512	7.826	1.670	0.98	7.004	0.517	8.569	1.522	0.64
PE.38.5	1.749	0.172	1.496	0.193	0.86	1.624	0.139	1.848	0.382	0.98	1.597	0.140	2.026	0.340	0.64
PE.38.6.16.0.22.6	3.326	0.316	3.132	0.498	0.86	3.194	0.294	3.634	0.688	0.98	3.136	0.291	4.008	0.695	0.64
PE.40.4	0.131	0.017	0.102	0.014	0.86	0.116	0.012	0.143	0.048	0.98	0.116	0.012	0.144	0.047	0.82
PE.40.5	0.541	0.064	0.455	0.067	0.86	0.494	0.049	0.604	0.158	0.98	0.488	0.049	0.642	0.158	0.64
PE.40.6.18.0.22.6	3.519	0.328	3.341	0.539	0.86	3.371	0.310	3.977	0.738	0.98	3.300	0.305	4.428	0.760	0.64
PE.40.7	0.570	0.054	0.515	0.091	0.86	0.530	0.051	0.674	0.125	0.98	0.519	0.051	0.748	0.106	0.62
PG.34.1	0.143	0.012	0.125	0.014	0.86	0.135	0.010	0.142	0.022	0.98	0.131	0.009	0.170	0.033	0.66
PG.34.2	0.051	0.005	0.042	0.005	0.86	0.048	0.004	0.045	0.011	0.98	0.045	0.003	0.061	0.020	0.85
PG.36.1	0.119	0.009	0.099	0.010	0.76	0.108	0.008	0.133	0.016	0.82	0.106	0.007	0.148	0.022	0.62
PG.36.2	0.160	0.013	0.143	0.015	0.86	0.151	0.010	0.172	0.035	0.98	0.148	0.009	0.192	0.041	0.71
PG.36.3	0.024	0.002	0.022	0.002	0.88	0.023	0.002	0.021	0.006	0.98	0.023	0.002	0.026	0.006	0.87
PG.38.4	0.013	0.001	0.012	0.001	0.86	0.012	0.001	0.014	0.005	0.98	0.012	0.001	0.016	0.005	0.85
PG.38.5	0.013	0.001	0.014	0.002	0.99	0.014	0.001	0.010	0.003	0.98	0.013	0.001	0.013	0.003	0.86
PI.32.0	0.396	0.061	0.407	0.054	0.86	0.416	0.048	0.302	0.071	0.98	0.416	0.047	0.297	0.085	0.64
PI.32.1	0.693	0.078	0.677	0.076	0.98	0.702	0.062	0.590	0.117	0.98	0.686	0.057	0.694	0.216	0.85
PI.34.0	0.189	0.027	0.211	0.024	0.81	0.207	0.021	0.135	0.027	0.83	0.209	0.021	0.127	0.031	0.64
PI.34.1	4.367	0.382	4.667	0.476	0.86	4.609	0.331	3.651	0.519	0.98	4.595	0.327	3.735	0.627	0.67
PI.34.2	2.590	0.153	2.708	0.151	0.86	2.693	0.118	2.260	0.293	0.98	2.708	0.115	2.163	0.325	0.64
PI.36.1	3.580	0.308	4.312	0.445	0.76	4.005	0.285	2.886	0.408	0.82	4.019	0.287	2.796	0.283	0.62
PI.36.2	8.276	0.496	8.834	0.487	0.86	8.697	0.387	7.125	0.878	0.84	8.755	0.392	6.756	0.627	0.62
PI.36.3	2.287	0.157	2.328	0.196	0.97	2.366	0.134	1.897	0.229	0.94	2.362	0.134	1.926	0.245	0.64
PI.36.4	3.586	0.186	3.555	0.293	0.88	3.553	0.173	3.710	0.419	0.98	3.542	0.164	3.779	0.558	0.87
PI.38.3	6.603	0.415	6.928	0.703	0.99	6.899	0.407	5.609	0.680	0.98	6.942	0.405	5.336	0.638	0.64
PI.38.4	25.778	0.838	25.164	1.378	0.86	25.000	0.751	29.052	2.186	0.82	25.416	0.791	26.398	1.961	0.84
PI.38.5	1.972	0.093	2.072	0.200	0.99	2.023	0.105	1.926	0.178	0.98	2.010	0.105	2.004	0.202	0.92
PI.38.6	0.426	0.027	0.483	0.033	0.76	0.451	0.023	0.418	0.057	0.98	0.450	0.022	0.425	0.071	0.84
PI.40.4	0.467	0.021	0.523	0.046	0.86	0.491	0.024	0.471	0.051	0.98	0.497	0.024	0.431	0.044	0.66

PI.40.5	1.273	0.073	1.365	0.166	0.98	1.304	0.085	1.330	0.170	0.98	1.320	0.085	1.223	0.154	0.87
PI.40.6	1.300	0.086	1.614	0.180	0.76	1.439	0.098	1.280	0.144	0.98	1.448	0.098	1.221	0.135	0.71
PS.36.1	0.225	0.021	0.300	0.027	0.42	0.253	0.018	0.252	0.053	0.98	0.254	0.018	0.245	0.049	0.87
PS.36.2	0.065	0.005	0.081	0.005	0.42	0.071	0.004	0.072	0.016	0.98	0.071	0.004	0.072	0.015	0.95
PS.38.3	0.034	0.004	0.038	0.003	0.76	0.034	0.002	0.044	0.015	0.98	0.034	0.002	0.045	0.014	0.78
PS.38.4	0.345	0.055	0.372	0.029	0.71	0.328	0.021	0.523	0.235	0.98	0.327	0.021	0.531	0.234	0.84
PS.40.4	0.019	0.003	0.018	0.001	0.86	0.017	0.001	0.030	0.013	0.98	0.017	0.001	0.029	0.013	0.71
PS.40.5	0.036	0.006	0.030	0.002	0.88	0.029	0.002	0.061	0.027	0.98	0.030	0.002	0.060	0.027	0.64
PS.40.6	0.089	0.014	0.087	0.007	0.86	0.078	0.005	0.155	0.055	0.84	0.079	0.005	0.153	0.056	0.64
SM.32.1	10.975	0.584	10.551	0.496	0.93	10.807	0.453	10.879	0.915	0.98	10.772	0.451	11.103	0.952	0.84
SM.32.2	0.611	0.037	0.679	0.036	0.71	0.649	0.029	0.555	0.063	0.98	0.651	0.030	0.541	0.040	0.64
SM.34.0	5.973	0.313	5.613	0.280	0.86	5.824	0.247	5.933	0.488	0.98	5.802	0.237	6.075	0.670	0.85
SM.34.1	122.247	4.888	121.903	4.046	0.93	121.816	3.524	124.048	11.722	0.98	122.557	3.583	119.324	10.838	0.86
SM.34.2	19.723	0.897	20.485	0.636	0.86	20.106	0.648	19.378	1.852	0.98	20.260	0.669	18.396	1.341	0.66
SM.35.1	4.323	0.223	4.122	0.183	0.88	4.230	0.173	4.364	0.333	0.98	4.244	0.176	4.273	0.245	0.85
SM.36.1	28.534	1.202	26.597	0.958	0.86	27.433	0.862	30.224	2.830	0.98	27.750	0.948	28.200	1.361	0.82
SM.36.2	15.086	0.687	15.145	0.570	0.88	15.035	0.488	15.570	1.747	0.98	15.204	0.539	14.491	0.798	0.85
SM.37.1	5.704	0.288	5.898	0.250	0.86	5.931	0.218	4.788	0.415	0.82	5.878	0.213	5.127	0.604	0.64
SM.38.1	15.426	0.681	14.772	0.662	0.86	15.036	0.527	16.113	1.407	0.98	15.164	0.550	15.298	0.981	0.87
SM.38.2	8.203	0.396	8.475	0.352	0.86	8.336	0.291	8.103	0.949	0.98	8.448	0.315	7.392	0.315	0.62
SM.39.1	1.223	0.077	1.137	0.055	0.92	1.223	0.058	0.989	0.095	0.83	1.211	0.057	1.063	0.136	0.71
SM.40.1	31.461	1.454	29.603	1.238	0.86	30.351	0.968	33.427	4.470	0.98	30.628	0.987	31.662	4.387	0.99
SM.40.2	24.009	0.951	25.616	0.910	0.76	24.697	0.737	24.043	2.044	0.98	24.930	0.758	22.556	1.450	0.64
SM.41.1	12.293	0.484	11.969	0.547	0.89	12.100	0.371	12.631	1.325	0.98	12.118	0.372	12.519	1.322	0.90
SM.41.2	12.793	0.470	13.763	0.483	0.76	13.266	0.377	12.447	0.931	0.98	13.327	0.383	12.055	0.736	0.64
SM.42.1	15.849	0.761	15.418	0.722	0.93	15.523	0.514	16.740	2.433	0.98	15.586	0.511	16.336	2.487	0.98
SM.42.2	60.147	2.146	63.101	1.905	0.84	60.954	1.521	63.129	5.975	0.98	61.666	1.689	58.587	3.249	0.79
SM.42.3	25.976	1.051	29.292	0.893	0.42	27.417	0.761	25.913	2.978	0.98	27.841	0.835	23.206	1.089	0.62
SM.42.4	3.332	0.160	3.800	0.141	0.42	3.554	0.120	3.204	0.386	0.98	3.624	0.124	2.759	0.184	0.51
SM.43.1	1.240	0.065	1.174	0.068	0.86	1.204	0.054	1.288	0.088	0.98	1.200	0.053	1.317	0.092	0.64
SM.43.2	3.593	0.201	3.455	0.184	0.95	3.521	0.161	3.672	0.252	0.98	3.507	0.157	3.759	0.327	0.66

Table 2: Longitudinal differences in lipid species by surgery and time point.

Data are means at each timepoint and % change from baseline. ANOVA P are adjusted for the false discovery rate. Orange indicates a significant interaction, and blue a significant main-effect of time. Bold values are statistically significant from baseline.

	RYGB - Means			AGB - Means			ANOVA		RYGB % Change		AGB % Change	
Lipid	M0	M1	M3	M0	M1	M3	P_I	P_T	ΔM1	ΔM3	ΔM1	ΔM3
sample size (n)	37	21	19	22	12	12						
Cer.d18.0.22.0	0.011	0.008	0.007	0.008	0.009	0.008	0.007		**-23**	**-36**	8	0
Cer.d18.0.24.0	0.012	0.008	0.007	0.009	0.009	0.009	0.009		**-29**	**-39**	4	-4
Cer.d18.1.14.0	0.012	0.01	0.009	0.011	0.009	0.008	0.979	0	**-16**	**-23**	**-19**	**-24**
Cer.d18.1.16.0	0.175	0.206	0.189	0.158	0.172	0.171	0.513	0.002	**17**	8	**9**	8
Cer.d18.1.18.0	0.145	0.213	0.163	0.122	0.146	0.134	0.35	0	**47**	**13**	**19**	**10**
Cer.d18.1.19.0	0.005	0.005	0.004	0.004	0.006	0.006	0.369	0.399	3	-23	27	29
Cer.d18.1.20.0	0.097	0.09	0.082	0.085	0.08	0.09	0.384	0.314	-7	-16	-5	7
Cer.d18.1.22.0	0.527	0.305	0.296	0.458	0.388	0.453	0		**-42**	**-44**	-15	-1
Cer.d18.1.23.0	0.457	0.218	0.209	0.41	0.305	0.374	0		**-52**	**-54**	**-26**	-9
Cer.d18.1.24.0	1.43	0.682	0.697	1.238	0.954	1.156	0		**-52**	**-51**	**-23**	-7
Cer.d18.1.24.1	0.652	0.796	0.768	0.618	0.674	0.705	0.745	0.001	**22**	**18**	**9**	**14**
Cer.d18.1.25.0	0.071	0.045	0.046	0.068	0.061	0.069	0.007		**-36**	**-35**	**-10**	2
Cer.d18.1.26.0	0.017	0.015	0.016	0.017	0.017	0.018	0.697	0.511	-13	-11	0	12
Cer.d18.1.26.1	0.011	0.021	0.019	0.011	0.013	0.012	0		**95**	**77**	10	5
Cer.d18.2.14.0	0.005	0.004	0.003	0.005	0.004	0.004	0.844	0	**-27**	**-34**	**-15**	**-26**
Cer.d18.2.16.0	0.031	0.036	0.031	0.03	0.032	0.03	0.697	0.159	16	-1	9	0
Cer.d18.2.18.0	0.027	0.036	0.028	0.026	0.029	0.027	0.35	0.001	**31**	5	**14**	4
Cer.d18.2.20.0	0.029	0.025	0.022	0.027	0.025	0.027	0.25	0.001	**-16**	**-26**	**-10**	**-1**
Cer.d18.2.22.0	0.188	0.102	0.102	0.173	0.139	0.177	0.001		**-46**	**-46**	-20	2
Cer.d18.2.23.0	0.094	0.038	0.037	0.09	0.063	0.082	0		**-59**	**-61**	**-30**	-9
Cer.d18.2.24.0	0.244	0.105	0.114	0.232	0.171	0.222	0		**-57**	**-53**	**-26**	-4
Cer.d18.2.24.1	0.159	0.16	0.154	0.164	0.165	0.184	0.661	0.953	1	-3	1	12
Cer.d18.2.24.2	0.013	0.014	0.012	0.013	0.013	0.014	0.661	0.306	1	-8	4	12
LPC.16.0	32.749	30.345	31.56	31.61	28.712	31.329	0.985	0.186	-7	-4	-9	-1
LPC.16.1	1.126	0.835	0.864	1	0.692	0.815	0.985	0	**-26**	**-23**	**-31**	**-19**
LPC.18.0	10.541	7.067	7.777	10.053	8.488	9.209	0.411	0	**-33**	**-26**	**-16**	**-8**
LPC.18.1	8.079	7.315	7.763	7.232	6.209	7.154	0.979	0.065	-9	-4	-14	-1
LPC.18.2	10.648	7.301	7.577	9.593	8.178	9.204	0.343	0	**-31**	**-29**	**-15**	**-4**
LPC.20.3	1.057	0.571	0.557	0.894	0.553	0.632	0.25	0	**-46**	**-47**	**-38**	**-29**
LPC.20.4	2.293	2.436	2.256	2.117	1.95	2.068	0.661	0.953	6	-2	-8	-2
LPC.22.5	0.162	0.133	0.14	0.152	0.109	0.125	0.665	0	**-18**	**-14**	**-28**	**-18**
LPC.22.6	0.513	0.535	0.476	0.505	0.443	0.477	0.661	0.185	4	-7	-12	-5
LPE.18.0	0.591	0.531	0.561	0.502	0.413	0.461	0.979	0.089	-10	-5	-18	-8
LPE.18.1	0.47	0.331	0.362	0.359	0.247	0.299	0.995	0	**-30**	**-23**	**-31**	**-17**
PA.34.1	0.115	0.116	0.134	0.119	0.12	0.103	0.195	0.915	2	17	1	-14
PA.34.2	0.18	0.158	0.164	0.186	0.199	0.182	0.768	0.499	-12	-9	7	-2
PC.32.0	9.631	9.961	10.237	9.527	9.592	9.636	0.844	0.609	3	6	1	1
PC.32.1	15.962	11.204	10.091	14.33	9.061	9.878	0.979	0	**-30**	**-37**	**-37**	**-31**
PC.32.2	3.225	1.204	1.297	3.328	1.903	2.215	0.015		**-63**	**-60**	**-43**	**-33**
PC.34.0	1.659	1.167	1.263	1.571	1.458	1.64	0.015		**-30**	**-24**	-7	4
PC.34.1	171.313	170.204	162.849	162.705	147.611	157.077	0.979	0.168	-1	-5	-9	-3

PC.34.2	464.002	372.772	364.704	463.714	434.97	455.336	0.02		**-20**	**-21**	-6	-2
PC.34.3	19.164	9.443	9.36	18.114	12.282	14.23	0.008		**-51**	**-51**	**-32**	**-21**
PC.36.1	38.843	23.63	24.991	36.256	28.056	30.692	0.048		**-39**	**-36**	**-23**	**-15**
PC.36.2	254.823	152.293	162.706	248.468	212.878	229.58	0		**-40**	**-36**	**-14**	-8
PC.36.3	150.914	94.51	97.163	145.858	111.202	126.626	0.008		**-37**	**-36**	**-24**	-13
PC.36.4	189.024	223.827	201.304	193.793	205.129	197.497	0.676	0.006	**18**	7	**6**	2
PC.36.5	22.307	15.476	14.095	22.872	18.101	19.353	0.477	0	**-31**	**-37**	**-21**	**-15**
PC.38.3	51.986	21.993	22.947	48.688	28.765	32.831	0.007		**-58**	**-56**	**-41**	**-33**
PC.38.4	127.915	116.135	111.639	129.585	125.088	124.851	0.602	0.008	**-9**	**-13**	**-3**	**-4**
PC.38.5	55.332	49.979	50.334	56.295	51.543	53.201	0.979	0.098	-10	-9	-8	-5
PC.38.6	82.806	101.739	93.922	88.777	95.082	94.847	0.768	0.064	23	13	7	7
PC.40.3	0.228	0.167	0.169	0.266	0.218	0.249	0.35	0	**-27**	**-26**	**-18**	**-7**
PC.40.4	2.949	1.921	2.002	2.931	2.294	2.232	0.513	0	**-35**	**-32**	**-22**	**-24**
PC.40.5	9.277	6.471	7.027	9.195	7.497	7.37	0.692	0	**-30**	**-24**	**-18**	**-20**
PC.40.6	29.004	26.298	25.973	30.407	28.502	27.914	0.979	0.01	**-9**	**-10**	**-6**	**-8**
PC.40.7	4.445	3.979	4.244	4.542	4.226	4.722	0.979	0.143	-10	-5	-7	4
PC.40.8	0.845	0.616	0.649	0.825	0.776	0.844	0.247	0	**-27**	**-23**	**-6**	**2**
PE.32.1	0.114	0.075	0.067	0.091	0.046	0.051	0.985	0	**-34**	**-41**	**-49**	**-44**
PE.34.0	0.027	0.02	0.019	0.021	0.019	0.022	0.477	0.159	-25	-28	-9	7
PE.34.1	1.48	1.175	1.142	1.256	0.948	1.035	0.979	0.159	-21	-23	-24	-18
PE.34.2	1.569	1.081	1.078	1.319	0.92	1.068	0.946	0.012	**-31**	**-31**	**-30**	**-19**
PE.34.3	0.093	0.046	0.043	0.074	0.042	0.046	0.513	0	**-51**	**-54**	**-43**	**-38**
PE.36.1	1.373	0.674	0.678	1.178	0.744	0.913	0.312	0	**-51**	**-51**	**-37**	**-23**
PE.36.2	7.203	3.944	3.822	6.031	4.033	4.771	0.35	0	**-45**	**-47**	**-33**	**-21**
PE.36.3	1.532	0.805	0.831	1.242	0.781	1.038	0.411	0	**-47**	**-46**	**-37**	**-16**
PE.36.4.16.0.20.4	0.911	0.922	0.862	0.819	0.662	0.754	0.979	0.827	1	-5	-19	-8
PE.36.4.18.2.18.2.18.2.18.3	0.081	0.023	0.027	0.071	0.027	0.045	0.513	0	**-72**	**-67**	**-63**	**-36**
PE.36.5.16.0.20.5	0.092	0.063	0.058	0.079	0.056	0.063	0.676	0	**-32**	**-37**	**-29**	**-20**
PE.36.5.18.2.18.3	0.012	0.002	0.003	0.009	0.003	0.004	0.47	0	**-82**	**-78**	**-66**	**-58**
PE.36.6	0.024	0.015	0.014	0.022	0.014	0.017	0.545	0	**-37**	**-41**	**-37**	**-24**
PE.38.3	0.702	0.26	0.259	0.579	0.287	0.366	0.085		**-63**	**-63**	**-50**	-37
PE.38.4.18.0.20.4	6.496	6.277	5.485	6.071	5.275	5.845	0.86	0.633	-3	-16	-13	-4
PE.38.5	1.482	1.298	1.286	1.28	1.02	1.196	0.979	0.543	-12	-13	-20	-7
PE.38.6.16.0.22.6	2.829	3.455	3.182	2.57	2.468	2.852	0.979	0.159	22	12	-4	11
PE.40.4	0.103	0.051	0.049	0.085	0.062	0.056	0.35	0	**-50**	**-52**	**-28**	**-34**
PE.40.5	0.439	0.251	0.242	0.366	0.243	0.273	0.477	0	**-43**	**-45**	**-34**	**-25**
PE.40.6.18.0.22.6	2.971	3.261	2.666	2.738	2.432	2.739	0.849	0.547	10	-10	-11	0
PE.40.7	0.479	0.506	0.482	0.409	0.347	0.457	0.979	0.929	6	1	-15	12
PG.34.1	0.125	0.135	0.129	0.109	0.095	0.106	0.939	0.964	9	3	-13	-3
PG.34.2	0.043	0.038	0.034	0.036	0.028	0.028	0.979	0.095	-10	-19	-23	-23
PG.36.1	0.107	0.091	0.079	0.088	0.073	0.075	0.661	0.008	-14	**-26**	-17	**-15**
PG.36.2	0.143	0.113	0.096	0.127	0.095	0.111	0.35	0.001	**-21**	**-33**	**-25**	**-12**
PG.36.3	0.018	0.014	0.013	0.02	0.014	0.017	0.935	0.044	-25	**-29**	-26	**-14**
PG.38.4	0.011	0.007	0.006	0.01	0.007	0.008	0.35	0.001	**-35**	**-48**	**-27**	**-15**
PG.38.5	0.011	0.01	0.01	0.011	0.01	0.012	0.979	0.373	-14	-12	-10	4
PI.32.0	0.301	0.208	0.198	0.337	0.219	0.191	0.979	0	**-31**	**-34**	**-35**	**-43**
PI.32.1	0.565	0.398	0.35	0.571	0.305	0.297	0.802	0	**-30**	**-38**	**-47**	**-48**
PI.34.0	0.15	0.109	0.112	0.185	0.145	0.135	0.979	0	**-27**	**-25**	**-21**	**-27**
PI.34.1	3.874	3.061	3.038	4.142	2.951	3.035	0.979	0	**-21**	**-22**	**-29**	**-27**
PI.34.2	2.423	2.062	2.088	2.603	1.939	1.963	0.676	0.003	**-15**	**-14**	**-25**	**-25**

PI.36.1	3.201	2.291	2.426	3.894	3.078	3.231	0.838	0	**-28**	**-24**	**-21**	**-17**
PI.36.2	7.787	5.684	6.024	8.529	6.505	7.135	0.979	0	**-27**	**-23**	**-24**	**-16**
PI.36.3	2.122	1.08	1.329	2.148	1.351	1.629	0.567	0	**-49**	**-37**	**-37**	**-24**
PI.36.4	3.415	2.925	2.915	3.265	2.415	2.493	0.935	0.004	**-14**	**-15**	**-26**	**-24**
PI.38.3	6.216	3.048	3.287	6.205	3.663	4.345	0.164	0	**-51**	**-47**	**-41**	**-30**
PI.38.4	25.294	24.671	25.153	24.372	23.423	24.004	0.985	0.911	-2	-1	-4	-2
PI.38.5	1.899	1.418	1.568	1.903	1.468	1.601	0.979	0	**-25**	**-17**	**-23**	**-16**
PI.38.6	0.395	0.453	0.472	0.457	0.399	0.395	0.16	0.932	15	19	-13	-14
PI.40.4	0.45	0.355	0.375	0.481	0.359	0.398	0.979	0	**-21**	**-17**	**-25**	**-17**
PI.40.5	1.197	0.988	1.017	1.208	0.955	1.032	0.979	0.013	**-17**	**-15**	**-21**	**-15**
PI.40.6	1.208	1.342	1.359	1.445	1.293	1.273	0.398	0.941	11	13	-10	-12
PS.36.1	0.196	0.115	0.113	0.273	0.126	0.126	0.692	0	**-41**	**-42**	**-54**	**-54**
PS.36.2	0.059	0.049	0.042	0.077	0.049	0.045	0.692	0	**-17**	**-29**	**-36**	**-42**
PS.38.3	0.028	0.018	0.019	0.035	0.022	0.014	0.274	0	**-38**	**-33**	**-37**	**-59**
PS.38.4	0.278	0.225	0.213	0.348	0.293	0.199	0.612	0.008	-19	**-23**	-16	**-43**
PS.40.4	0.015	0.014	0.013	0.017	0.018	0.014	0.948	0.373	-10	-15	8	-19
PS.40.5	0.029	0.021	0.023	0.028	0.031	0.021	0.274	0.159	-29	-20	13	-25
PS.40.6	0.073	0.079	0.076	0.082	0.102	0.067	0.453	0.183	8	3	24	-19
SM.32.1	10.466	7.535	6.943	10.315	9.268	9.07	0.015		**-28**	**-34**	-10	**-12**
SM.32.2	0.577	0.408	0.397	0.659	0.535	0.489	0.692	0	**-29**	**-31**	**-19**	**-26**
SM.34.0	5.712	7.357	6.642	5.472	6.695	5.93	0.35	0	**29**	**16**	**22**	**8**
SM.34.1	119.211	122.369	123.407	120.526	131.967	126.023	0.979	0.159	3	4	9	5
SM.34.2	19.139	19.64	18.93	20.283	22	20.35	0.979	0.159	3	-1	8	0
SM.35.1	4.14	4.963	4.427	4.041	4.682	4.445	0.665	0	**20**	**7**	**16**	**10**
SM.36.1	27.695	39.894	34.717	26.219	33.065	29.934	0.104	0	**44**	**25**	**26**	**14**
SM.36.2	14.592	20.353	18.127	14.922	18.332	16.259	0.025		**39**	**24**	**23**	9
SM.37.1	5.472	4.422	4.133	5.797	5.685	5.511	0.212	0	**-19**	**-24**	**-2**	**-5**
SM.38.1	14.937	10.571	10.198	14.465	12.814	13.936	0.004		**-29**	**-32**	**-11**	-4
SM.38.2	7.943	7.824	7.802	8.331	8.762	8.969	0.995	0.953	-2	-2	5	8
SM.39.1	1.132	0.944	0.916	1.107	1.259	1.329	0.073		-17	-19	14	20
SM.40.1	30.404	18.561	18.223	29.073	26.131	27.828	0		**-39**	**-40**	**-10**	-4
SM.40.2	23.382	17.96	18.228	25.285	24.156	24.321	0.108	0	**-23**	**-22**	**-4**	**-4**
SM.41.1	11.968	6.748	6.227	11.721	10.032	10.916	0		**-44**	**-48**	**-14**	-7
SM.41.2	12.499	10.412	10.379	13.586	12.77	13.344	0.476	0	**-17**	**-17**	**-6**	**-2**
SM.42.1	15.285	9.361	9.108	15.079	13.652	14.222	0		**-39**	**-40**	**-9**	-6
SM.42.2	58.825	71.38	72.609	62.476	72.748	69.683	0.35	0	**21**	**23**	**16**	**12**
SM.42.3	25.292	31.721	33.6	28.993	33.827	31.305	0.015		**25**	**33**	**17**	8
SM.42.4	3.204	4.111	4.347	3.747	4.385	3.84	0.001		**28**	**36**	**17**	2
SM.43.1	1.181	0.785	0.769	1.137	1.103	1.175	0		**-34**	**-35**	-3	3
SM.43.2	3.394	4.192	3.812	3.357	3.835	3.972	0.895	0.001	**24**	**12**	**14**	**18**

Supplementary table 3: Weight loss adjusted effect of surgery type on change in lipid species from M0 to M3.

Regression results for weight-loss adjusted effect of RYGB on change in RYGB-signature lipids from M0 to M3. Beta coefficient reflects change in RYGB over and above that of AGB (on the natural log scale). Orange indicates statistically significant different responses between RYGB vs. AGB after false discovery rate adjustment of P-values based on the Benjamini-Hochberg procedure (Padj<0.05).

	Baseline concentration		Baseline weight		Change in weight		Change with RYGB (vs. AGB)		
Lipid	Beta	P	Beta	P	Beta	P	Beta	P	Padj
Cer.d18.0.22.0	0.733	0.000	-0.017	0.071	0.027	0.009	-0.201	0.186	0.208
Cer.d18.0.24.0	0.712	0.000	-0.001	0.922	0.012	0.247	-0.352	0.025	0.042
Cer.d18.1.22.0	0.673	0.001	-0.015	0.100	0.023	0.021	-0.349	0.018	0.039
Cer.d18.1.23.0	0.467	0.024	-0.019	0.075	0.025	0.025	-0.439	0.009	0.030
Cer.d18.1.24.0	0.423	0.021	-0.015	0.143	0.021	0.050	-0.425	0.009	0.030
Cer.d18.1.25.0	0.527	0.003	0.003	0.724	0.004	0.686	-0.416	0.003	0.019
Cer.d18.1.26.1	0.499	0.009	0.023	0.030	-0.018	0.091	0.321	0.044	0.061
Cer.d18.2.22.0	0.306	0.123	-0.016	0.189	0.022	0.085	-0.432	0.022	0.042
Cer.d18.2.23.0	0.329	0.126	-0.022	0.105	0.027	0.064	-0.602	0.006	0.025
Cer.d18.2.24.0	0.228	0.205	-0.020	0.064	0.024	0.034	-0.488	0.005	0.022
PC.32.2	0.802	0.000	-0.010	0.389	0.020	0.123	-0.332	0.078	0.090
PC.34.0	0.269	0.111	-0.003	0.599	0.006	0.347	-0.227	0.025	0.042
PC.34.2	0.647	0.000	0.000	0.974	0.002	0.754	-0.207	0.011	0.030
PC.34.3	0.580	0.004	-0.014	0.152	0.021	0.045	-0.277	0.068	0.082
PC.36.1	0.337	0.006	0.001	0.840	0.004	0.561	-0.232	0.028	0.042
PC.36.2	0.579	0.001	-0.002	0.715	0.007	0.223	-0.326	0.001	0.016
PC.36.3	0.667	0.000	-0.004	0.498	0.007	0.280	-0.260	0.014	0.034
PC.38.3	0.531	0.018	-0.013	0.181	0.020	0.064	-0.293	0.065	0.082
PE.38.3	0.626	0.001	-0.038	0.047	0.044	0.032	-0.227	0.424	0.424
SM.32.1	0.658	0.000	-0.004	0.471	0.010	0.125	-0.177	0.061	0.080
SM.36.2	0.653	0.000	0.009	0.096	-0.006	0.298	0.097	0.231	0.248
SM.38.1	0.754	0.000	-0.003	0.584	0.004	0.495	-0.212	0.026	0.042
SM.39.1	0.183	0.175	0.010	0.276	-0.012	0.183	-0.442	0.003	0.019
SM.40.1	0.628	0.003	-0.010	0.181	0.011	0.164	-0.297	0.010	0.030
SM.41.1	0.755	0.003	-0.010	0.220	0.010	0.235	-0.412	0.002	0.019
SM.42.1	0.653	0.001	-0.007	0.320	0.008	0.316	-0.343	0.003	0.019
SM.42.3	0.422	0.025	0.006	0.341	-0.007	0.293	0.099	0.310	0.321
SM.42.4	0.813	0.000	0.008	0.213	-0.009	0.203	0.228	0.034	0.049
SM.43.1	0.723	0.001	-0.006	0.547	0.005	0.571	-0.329	0.019	0.039