



HAL
open science

Serum lipidomics reveals early differential effects of gastric bypass compared to banding on phospholipids and sphingolipids independent of differences in weight loss

Brandon D. Kayser, Marie Lhomme, Maria Carlota Dao, Farid Ichou, Jean-Luc Bouillot, Edi E. Prifti, Anatol Kontush, Jean-Marc Chevallier, Judith Aron-Wisnewsy, Isabelle Dugail, et al.

► To cite this version:

Brandon D. Kayser, Marie Lhomme, Maria Carlota Dao, Farid Ichou, Jean-Luc Bouillot, et al.. Serum lipidomics reveals early differential effects of gastric bypass compared to banding on phospholipids and sphingolipids independent of differences in weight loss. *International Journal of Obesity*, 2017, 10.1038/ijo.2017.63 . hal-01497688

HAL Id: hal-01497688

<https://hal.sorbonne-universite.fr/hal-01497688v1>

Submitted on 29 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Serum lipidomics reveals early differential effects of gastric bypass compared to banding on
2 phospholipids and sphingolipids independent of differences in weight loss

3
4 Running title: Serum lipidomics in gastric bypass versus banding

5
6 Brandon D Kayser^{1,2,3}, Marie Lhomme¹, Maria Carlota Dao^{1,2,3}, Farid Ichou¹, Jean-Luc Bouillot⁴, Edi
7 Prifti¹, Anatol Kontush^{1,5,6}, Jean-Marc Chevallier⁷, Judith Aron-Wisnewsky^{1,2,3}, Isabelle Dugail^{1,2,3}, Karine
8 Clément^{1,2,3}

9
10 ¹Institute of Cardiometabolism and Nutrition, ICAN, Assistance Publique Hôpitaux de Paris, Pitié-
11 Salpêtrière hospital, Paris, France. ²INSERM, UMR S U1166, Nutriomics Team, Paris, France.

12 ³Sorbonne Universités, UPMC University Paris 06, UMR_S 1166, Nutriomics Team, Paris, France.

13 ⁴Assistance Publique-Hôpitaux de Paris, Visceral Surgery Department, Ambroise Paré, Paris, France.

14 ⁵INSERM, UMR_S U1166, Dyslipidemia, Inflammation, and Atherosclerosis Team, Paris, France.

15 ⁶Sorbonne Universités, UPMC Université Paris 06, UMR_S 1166, ICAN, Dyslipidemia, Inflammation, and
16 Atherosclerosis Team, Paris, France. ⁷Assistance Publique-Hôpitaux de Paris, Visceral surgery
17 Department, Hôpital Européen Georges-Pompidou, Paris, France.

18
19 Key words: Bariatric surgery, ceramides, sphingomyelins, weight loss, obesity

20 Abstract word count: 300

21 Manuscript word count: 4,726

22 Figure: 3

23 Tables: 1

24 Correspondence: Prof. Karine Clément.
25 Institute of Cardiometabolism and Nutrition (ICAN)
26 43-83 boulevard de l'Hôpital, E3M bureau 637
27 75013 Paris, France.
28 Phone: +33 01 40 77 97 28. Email: karine.clement@inserm.fr

29 This paper describes original research that has not been submitted to other publications, nor is it
30 currently under review elsewhere. We declare no conflicts of interest.

33 Abstract

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

39 **Subjects/Methods:** Fasting sera were available from 59 obese women (BMI range 37-51 kg/m²; n=37
40 RYGB and 22 AGB) before surgery, then at 1 (21 RYGB, 12 AGB) and 3 months follow-up (19 RYGB,
41 12 AGB). HPLC-MS/MS was used to quantify 131 lipids from 9 structural classes. DXA measurements
42 and laboratory parameters were also obtained. The associations between lipids and clinical
43 measurements were studied with P-values adjusted for the false discovery rate (fdr).

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

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

57

58

59

60

61

62 Introduction

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

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

83 With the advent of modern lipidomic technologies, over 500 molecular lipid species have been
84 quantified in human plasma (ref. 11). Phospholipids and sphingolipids, collectively called the
85 phosphosphingolipidome, and which contain the bioactive ceramides, are among the most diverse lipid
86 categories and may act as important biomarkers (ref. 12). For example, plasma levels of sphingolipids
87 and phospholipids are increased in obesity-associated nonalcoholic steatohepatitis (NASH) (ref. 13) and
88 outperform neutral lipids and eicosanoids for predicting liver injury (ref. 14). Serum phospholipids and
89 sphingolipids may reflect synthesis and efflux from metabolically relevant tissues, but can also directly
90 participate in pathophysiology as a source of triglycerides in hepatic steatosis (ref. 15), or by altering the

91 cholesterol efflux from macrophages, which is suspected to be an important mechanism in the
92 development of atherosclerosis (ref. 16). Ceramides (Cer) are especially implicated in the pathogenesis
93 of insulin resistance (ref. 17), and it was recently shown that infusion of Cer(d18:1/24:0) into mice can
94 induce peripheral insulin resistance (ref. 18). Little is known about how bariatric surgery affects these
95 important lipids.

96 Separating the effects of RYGB and AGB on serum lipids, particularly after adjusting for
97 differences in weight loss, provides an informative model for deciphering the specific effects of RYGB on
98 metabolism. As the weight loss differences between procedures are less drastic early after surgery, and
99 given the metabolic effects occur almost immediately, the evolution of serum lipids was determined after
100 1 and 3 months of follow-up. Compared to AGB, we hypothesized that RYGB would have a surgery-
101 specific effect on circulating phospholipids and sphingolipids concomitant with the greater metabolic
102 response following this procedure.

103 Methods

104 Clinical cohort

105 Starting from July 2011 until July 2014, female bariatric surgery candidates with a BMI greater
106 than 40kg/m² or greater than 35kg/m² with at least one severe obesity-related comorbidity were recruited
107 into this prospective observational study. Patients were treated in the Obesity Unit of Pitié-Salpêtrière
108 Hospital, Institute of Cardiometabolism and Nutrition (ICAN), Paris, France. Patients underwent either
109 adjustable gastric banding (AGB) or Roux-en-Y Gastric Bypass (RYGB) based upon their choice and the
110 agreement of a multidisciplinary clinical panel. After excluding patients who were converted from AGB to
111 RYGB (5 subjects), 59 subjects had sufficient clinical data and serum available for lipidomic analysis to
112 be included in the current study. Ethical approval was obtained from the Research Ethics Committee of
113 Pitié-Salpêtrière Hospital (CPP Ile-de-France). Informed written consent was obtained from all subjects.
114 The Microbaria protocol is registered as clinical trial NCT01454232.

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

120 Biological analysis

121 Blood samples were collected after an overnight fast to measure routine biochemical parameters, as
122 described previously (ref. 13). Serum glucose, total cholesterol (TC), high-density lipoprotein-cholesterol
123 (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl
124 transpeptidase (γGT) were measured enzymatically. Low-density lipoprotein-cholesterol (LDL-C) was
125 estimated by the Friedewald formula. ApoA1 and ApoB were measured by immunonephelometry. Serum
126 insulin was assayed by Bi-INSULIN IRMA (CisBio International, Gif-sur-Yvette, France); leptin and
127 adiponectin by radioimmunoassay (Linco Research, Saint Louis, MI, USA); interleukin-6 (IL-6) by ELISA
128 (QuantikineUS, R&D System Europe Ltd, Abingdon, UK); and high-sensitivity C-reactive protein (CRP)
129 and orosomucoid by an IMMAGE automatic immunoassay system (Beckman-Coulter, Fullerton, CA,
130 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 $= \exp[2.63 - 0.28\ln(\text{insulin}) - 0.31\ln(\text{triglycerides})]$.

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.

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

166

167 Statistical analysis

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

187 difference between RYGB and AGB on the change in each lipid to M3 while controlling for the change in
188 body weight. That is, a regression model of the form:

189

$$190 \quad Lipid_{M3} = \beta_0 + \beta_1(Lipid_{M0}) + \beta_2(Weight_{M0}) + \beta_3(\Delta Weight_{M3-M0}) + \beta_4(Surgery_{RYGB=1,AGB=0})$$

191

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

196

197

198 Results

199 Baseline and longitudinal clinical variables between surgery types

200 Thirty-seven and 22 patients underwent RYGB and AGB, respectively, with approximately 45%
201 lost to follow-up at M1 and M3 for each group (see Table 1 for clinical characteristics). Baseline
202 characteristics did not differ between patients with complete or incomplete data during follow-up (data
203 not shown), indicating that the imbalance across time would not bias the results. The mean age was
204 34.5 (± 1.6) and 37.3 (± 1.9) years for AGB and RYGB, respectively, and was not significantly different
205 between groups. At baseline, and compared to AGB, RYGB patients had 2.9 kg/m² higher BMI, 11.1
206 IU/L higher γ GT, 0.6mM higher fasting glucose (with 24% T2D in RYGB and 5% in AGB), 0.22mM higher
207 fasting triglycerides, and 0.4% higher HbA1c, all $P < 0.05$. However, no variables were significantly
208 different after adjusting for the false discovery rate (all $P_{adj} > 0.1$). 22% of RYGB patients were treated
209 with metformin or statins at baseline, while no AGB patients were on these medications ($P = 0.051$).
210 There were no statistically significant differences in the prevalence of T2D. Thus, RYGB tended towards
211 higher obesity and worse diabetes risk factors.

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

221 γ GT was the only liver enzyme to decrease significantly in AGB by M3. With RYGB, γ GT was
222 significantly decreased, while both ALT and AST were significantly increased from baseline at both M1
223 and M3. Orosomucoid, or alpha-1-acid glycoprotein, an acute phase protein, decreased by 20% in
224 RYGB, but was unchanged by AGB. Other parameters, such as CRP, HbA1c, triglycerides, and
225 adiponectin, were altered to the same extent in each surgical procedure. In summary, both surgeries

226 resulted in improvements in the majority of measured parameters, but RYGB resulted in greater weight
227 loss, including both lean and fat masses, and persistent improvements in TC, LDL-C, and orosomucoid.

228 Limited associations between baseline clinical variables and the phosphosphingolipidome

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

245 Procedure-independent and -dependent changes in phospholipids and sphingolipids

247 For the longitudinal analysis, the main effects of surgery and the interaction between the two
248 surgeries and time were evaluated. A main effect of time (and no interaction) was detected for 64 lipids
249 ($P_{\text{adj}}<0.05$), whereby 54 of these lipids decreased from baseline and included all classes except PA (Fig
250 1D-1F, Supplementary Table 2). The vast majority of these lipids were decreased at both M1 and M3,
251 which created a markedly similar pattern of change at each time point (Fig 1E). Eight of the ten most
252 statistically significantly decreased lipids were PE species. Only Cer and SM species, and one PC
253 species, were significantly increased following surgery, and included Cer(d18:1/16:0), Cer(d18:1/18:0),
254 and Cer(d18:1/24:1) (Fig 2E and 2F, Supplementary Table 2).

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

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

273

274 The RYGB lipid signature is related to differences in metabolic outcomes

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

283 Finally, we sought to determine whether the RYGB dependent lipid modifications were related to
284 the clinical parameters that changed to a greater extent in RYGB by M3 (Fig 3B). With the exception of
285 an inverse association with Cer(d18:1/26:1), there were no statistically significant associations with
286 changes in FM or %FM, which corroborates a body fat-independent effect of RYGB on the identified
287 lipids. The RYGB-specific decreases in SM, some PC, and Cer species were most strongly associated
288 with the decrease in TC, LDL-C, orosomucoid, leptin, body weight, FFM, and to a lesser extent, HOMA2-
289 S. A number of these lipids were also associated with the decline in γ GT. On the other hand, the
290 increase in the three unsaturated SM and Cer(d18:1/26:1) were associated with amelioration of the
291 aforementioned clinical parameters, demonstrating heterogeneity in the clinical relevance of individual
292 sphingolipid species. The potential effect of differences in lipolysis were tested by measuring fasting
293 serum saturated free fatty acids (FFA). Both surgeries increased C16, C18, and C20 FFA after one
294 month, but there was no statistically significant interactions between surgery type and time (Fig 3C).
295 FFA(16:0) (palmitate) was higher in RYGB than AGB throughout the study period (surgery main-effect
296 $P < 0.01$).

299 Discussion

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

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

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

325 Given the greater metabolic improvement induced by RYGB compared to AGB, we reasoned that
326 RYGB-specific lipid alterations would identify clinically relevant biomarkers. To this end, the current study
327 reveals a distinct effect of RYGB to decrease a subclass of PCs shorter than 36 carbons, and induce

328 both decreases and increases in a number of SM and longer chain Cer and DH-Cer. The majority of
329 these changes remained significant after adjust for the greater weight loss in RYGB. It is noteworthy that
330 these 3 classes were identified given their shared biochemical synthesis: DH-Cer are desaturated to
331 Cer, and SM are formed by Cer and PC as source of phosphocholine (Figure 3C). While the
332 sphingosine-fatty acid content of our detected SM could only be presumed, we observed a remarkable
333 consistency across the fatty acid lengths decreased in RYGB between SM and Cer. The similarity was
334 most striking for C22 - C24 fatty acids. Interestingly, a previous metabolomics study by our group
335 identified Cer(d18:1/24:0) as one of the metabolites decreased at 3 and 6 months following RYGB,
336 further supporting the particular effect on longer Cer species (ref. 31). Phospholipids and sphingolipids
337 are related to a number of cardiometabolic diseases. For example, PC synthesis is a regulator of VLDL
338 secretion and hepatic steatosis, and further, circulating PC is an important source of triglycerides in
339 steatosis (ref. 15, ref. 32). The SM and PC species differentially affected by RYGB are increased in
340 coronary artery disease and associated with increased mortality (ref. 33, ref. 34). Bariatric surgery has
341 been shown to improve NAFLD (ref. 3) and reduce cardiovascular mortality (ref. 4), therefore the
342 changes in PC and SM could be involved. The role of Cer may be more difficult to interpret.

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

357 42, ref. 43). The distinct decrease in circulating C22 to C24 ceramides may therefore reflect a specific
358 effect of RYGB on hepatic Cer synthesis, secretion, or both. Indeed, a number of, though not all, studies
359 in humans have shown similar relationships between ceramides and impaired glucose homeostasis
360 ranging from C16 to C24 Cer (ref. 27, ref. 44). Importantly, enrichment of LDL with either Cer(d18:1/16:0)
361 or Cer(d18:1/24:0) in mice produced equivalent degrees of insulin resistance and inflammation, both *in*
362 *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
363 likely the most deleterious species, the findings in the current study emphasize the importance of better
364 understanding the role of serum or lipoprotein ceramide acyl chain length, which could help better
365 understand the effects of RYGB, diabetes and cardiovascular risk in general.

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

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

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

410 Supplementary information

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

412

413 Acknowledgements

414 Thank you to Valentine Lemoine for patient recruitment, Florence Marchelli for data collection, and
415 Sophie Festis for DEXA analyses as well as to Agathe Arlotti for research program coordination. We
416 would also like to thank the nurses and technicians, and of course, the patients themselves for their
417 invaluable contribution. This work was supported by Programme Hospitalier de Reserche Clinique
418 (PHRC Microbaria AOM10285/P100111), Horizon 2020 Framework Program (EPoS, grant #634413),
419 and the European Foundation for the Study of Diabetes Albert Renold travel fellowship (BDK).

420 References

- 421 1. Sjöström L, Lindroos A-K, Peltonen M, Torgerson J, Bouchard C, Carlsson B *et al.* Lifestyle,
422 diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *New England Journal of*
423 *Medicine* 2004;**351**:2683-2693.
- 424 2. Bradley D, Magkos F, Klein S. Effects of bariatric surgery on glucose homeostasis and type 2
425 diabetes. *Gastroenterology* 2012;**143**:897-912.
- 426 3. Caiazzo R, Lassailly G, Leteurtre E, Baud G, Verkindt H, Raverdy V *et al.* Roux-en-Y gastric
427 bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year
428 controlled longitudinal study. *Ann Surg* 2014;**260**:893-8; discussion 898.
- 429 4. Sjöström L, Peltonen M, Jacobson P, Sjöström CD, Karason K, Wedel H *et al.* Bariatric surgery
430 and long-term cardiovascular events. *JAMA* 2012;**307**:56-65.
- 431 5. Buchwald H, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ *et al.* Weight and type 2
432 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009;**122**:248-
433 256.e5.
- 434 6. Heffron SP, Parikh A, Volodarskiy A, Ren-Fielding C, Schwartzbard A, Nicholson J *et al.* Changes
435 in Lipid Profile of Obese Patients following Contemporary Bariatric Surgery: A Meta-Analysis. *Am J*
436 *Med* 2016
- 437 7. Seeley RJ, Chambers AP, Sandoval DA. The Role of Gut Adaptation in the Potent Effects of
438 Multiple Bariatric Surgeries on Obesity and Diabetes. *Cell Metabolism* 2015;**21**:369-378.
- 439 8. Bradley D, Conte C, Mittendorfer B, Eagon JC, Varela JE, Fabbrini E *et al.* Gastric bypass and
440 banding equally improve insulin sensitivity and β cell function. *J Clin Invest* 2012;**122**:4667-4674.
- 441 9. Jackness C, Karmally W, Febres G, Conwell IM, Ahmed L, Bessler M *et al.* Very low-calorie diet
442 mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and β -cell
443 Function in type 2 diabetic patients. *Diabetes* 2013;**62**:3027-3032.
- 444 10. Sjöholm K, Sjöström E, Carlsson LMS, Peltonen M. Weight Change–Adjusted Effects of Gastric
445 Bypass Surgery on Glucose Metabolism: Two- and 10-Year Results From the Swedish Obese
446 Subjects (SOS) Study. *Dia Care* 2015dc151407.
- 447 11. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH *et al.* Lipidomics

- 448 reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 2010;**51**:3299-3305.
- 449 12. Quehenberger O, Dennis EA. The human plasma lipidome. *New England Journal of Medicine*
450 2011;**365**:1812-1823.
- 451 13. Anjani K, Lhomme M, Sokolovska N, Poitu C, Aron-Winewsky J, Bouillot B *et al.* Circulating
452 phospholipid profiling identifies portal contribution to NASH signature in obesity. *J Hepatology*
453 2015;**62**:905-912.
- 454 14. Gordon DL, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S *et al.* Biomarkers of NAFLD
455 progression: a lipidomics approach to an epidemic. *J Lipid Res* 2015;**56**:722-736.
- 456 15. van der Veen JN, Lingrell S, Vance DE. The membrane lipid phosphatidylcholine is an unexpected
457 source of triacylglycerol in the liver. *J Biol Chem* 2012;**287**:23418-23426.
- 458 16. Camont L, Lhomme M, Rached F, Le Goff W, Nègre-Salvayre A, Salvayre R *et al.* Small, Dense
459 High-Density Lipoprotein-3 Particles Are Enriched in Negatively Charged Phospholipids Relevance
460 to Cellular Cholesterol Efflux, Antioxidative, Antithrombotic, Anti-Inflammatory, and Antiapoptotic
461 Functionalities. *Arteriosclerosis, thrombosis, and vascular biology* 2013;**33**:2715-2723.
- 462 17. Bikman BT, Summers SA. Ceramides as modulators of cellular and whole-body metabolism. *J Clin*
463 *Invest* 2011;**121**:4222-4230.
- 464 18. Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC *et al.* Ceramides contained in LDL
465 are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance.
466 *Diabetes* 2013;**62**:401-410.
- 467 19. Ciangura C, Bouillot JL, Lloret-Linares C, Poitou C, Veyrie N, Basdevant A *et al.* Dynamics of
468 change in total and regional body composition after gastric bypass in obese patients. *Obesity*
469 *(Silver Spring)* 2010;**18**:760-765.
- 470 20. McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA *et al.* Diagnosing
471 insulin resistance in the general population. *Diabetes Care* 2001;**24**:460-464.
- 472 21. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care*
473 2004;**27**:1487-1495.
- 474 22. Palarea-Albaladejo J, Martin-Fernandez JA. Values below detection limit in compositional chemical
475 data. *Anal Chim Acta* 2013;**764**:32-43.
- 476 23. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N *et al.* Procedures

477 for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid
478 chromatography coupled to mass spectrometry. *Nat Protoc* 2011;**6**:1060-1083.

479 24. Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA *et al*. Proposed minimum
480 reporting standards for chemical analysis Chemical Analysis Working Group (CAWG)
481 Metabolomics Standards Initiative (MSI). *Metabolomics* 2007;**3**:211-221.

482 25. Little RA, Rubin DB. *Statistical analysis with missing data*. John Wiley & Sons: Hoboken, New
483 Jersey, 2002,

484 26. Wiesner P, Leidl K, Boettcher A, Schmitz G, Liebisch G. Lipid profiling of FPLC-separated
485 lipoprotein fractions by electrospray ionization tandem mass spectrometry. *J Lipid Res*
486 2009;**50**:574-585.

487 27. Meikle PJ, Wong G, Barlow CK, Weir JM, Greeve MA, MacIntosh GL *et al*. Plasma lipid profiling
488 shows similar associations with prediabetes and type 2 diabetes. *PLoS One* 2013;**8**:e74341.

489 28. Graessler J, Bornstein TD, Goel D, Bhalla VP, Lohmann T, Wolf T *et al*. Lipidomic profiling before
490 and after Roux-en-Y gastric bypass in obese patients with diabetes. *Pharmacogenomics J*
491 2014;**14**:201-207.

492 29. Huang H, Kasumov T, Gatmaitan P, Heneghan HM, Kashyap SR, Schauer PR *et al*. Gastric
493 Bypass Surgery Reduces Plasma Ceramide Subspecies and Improves Insulin Sensitivity in
494 Severely Obese Patients. *Obesity* 2011;**19**:2235-2240.

495 30. Arora T, Velagapudi V, Pournaras DJ, Welbourn R, le Roux CW, Ore?i M *et al*. Roux-en-Y Gastric
496 Bypass Surgery Induces Early Plasma Metabolomic and Lipidomic Alterations in Humans
497 Associated with Diabetes Remission. 2015

498 31. Mutch DM, Fuhrmann JC, Rein D, Wiemer JC, Bouillot JL, Poitou C *et al*. Metabolite profiling
499 identifies candidate markers reflecting the clinical adaptations associated with Roux-en-Y gastric
500 bypass surgery. *PLoS One* 2009;**4**:e7905.

501 32. Noga AA, Zhao Y, Vance DE. An unexpected requirement for phosphatidylethanolamine N-
502 methyltransferase in the secretion of very low density lipoproteins. *J Biol Chem* 2002;**277**:42358-
503 42365.

504 33. Schlitt A, Blankenberg S, Yan D, von Gizycki H, Buerke M, Werdan K *et al*. Further evaluation of
505 plasma sphingomyelin levels as a risk factor for coronary artery disease. *Nutr Metab (Lond)*

- 506 2006;**3**:5.
- 507 34. Sigruener A, Kleber ME, Heimerl S, Liebisch G, Schmitz G, Maerz W. Glycerophospholipid and
508 sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC)
509 study. *PLoS One* 2014;**9**:e85724.
- 510 35. Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related
511 metabolic disorders. *Nat Rev Endocrinol* 2016
- 512 36. Levy M, Futerman AH. Mammalian ceramide synthases. *IUBMB Life* 2010NA.
- 513 37. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B *et al*. CerS2 haploinsufficiency inhibits
514 beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell*
515 *Metab* 2014;**20**:687-695.
- 516 38. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM *et al*. Obesity-
517 induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose
518 intolerance. *Cell Metab* 2014;**20**:678-686.
- 519 39. Gosejacob D, Jäger PS, Vom Dorp K, Frejno M, Carstensen AC, Köhnke M *et al*. Ceramide
520 synthase 5 is essential to maintain C16:0 ceramide pools and contributes to the development of
521 diet induced obesity. *J Biol Chem* 2016
- 522 40. Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH *et al*. Muscle sphingolipids
523 during rest and exercise: a C18:0 signature for insulin resistance in humans. *Diabetologia*
524 2016;**59**:785-798.
- 525 41. Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhanen D *et al*. Plasma ceramides
526 predict cardiovascular death in patients with stable coronary artery disease and acute coronary
527 syndromes beyond LDL-cholesterol. *Eur Heart J* 2016;**37**:1967-1976.
- 528 42. Merrill AH, Lingrell S, Wang E, Nikolova-Karakashian M, Vales TR, Vance DE. Sphingolipid
529 biosynthesis de novo by rat hepatocytes in culture. Ceramide and sphingomyelin are associated
530 with, but not required for, very low density lipoprotein secretion. *Journal of Biological Chemistry*
531 1995;**270**:13834-13841.
- 532 43. Watt MJ, Barnett AC, Bruce CR, Schenk S, Horowitz JF, Hoy AJ. Regulation of plasma ceramide
533 levels with fatty acid oversupply: evidence that the liver detects and secretes de novo synthesised
534 ceramide. *Diabetologia* 2012;**55**:2741-2746.

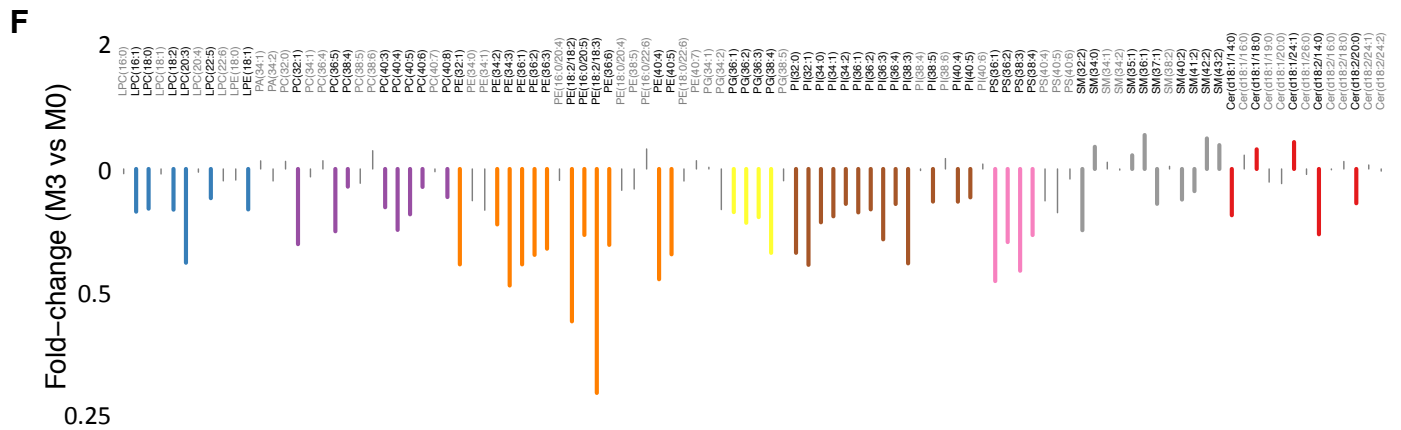
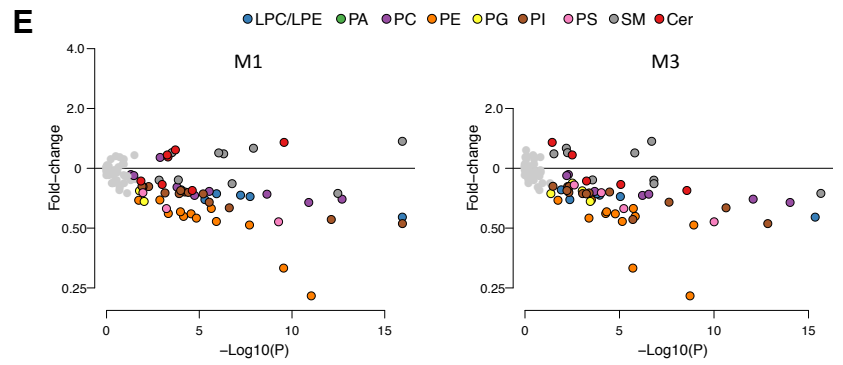
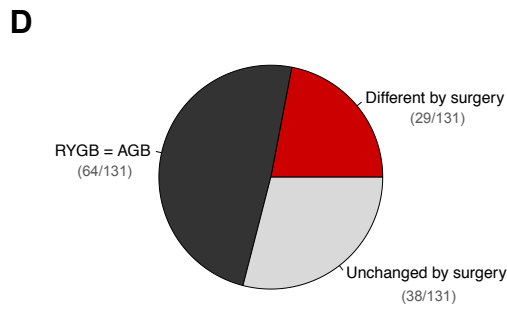
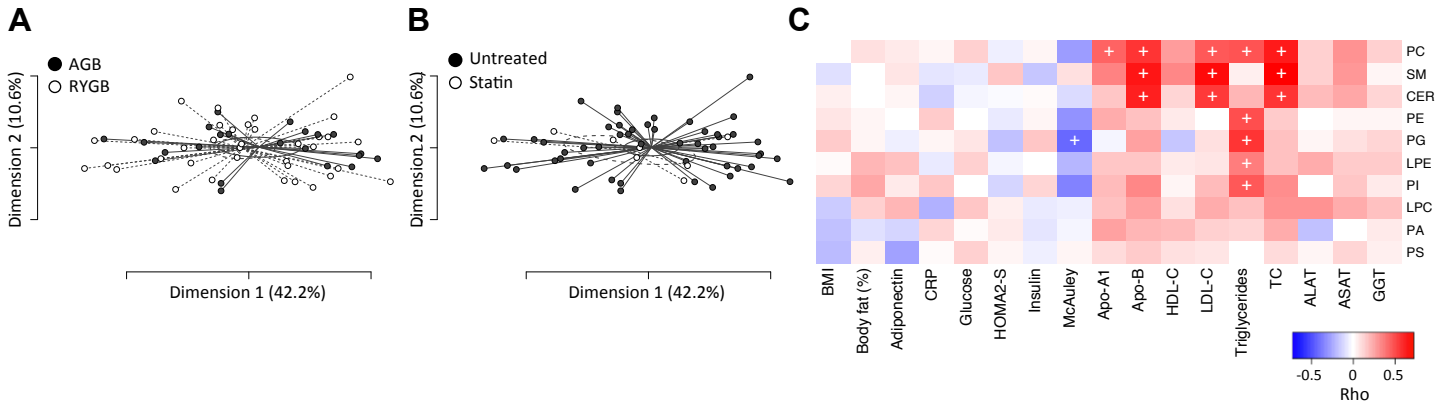
- 535 44. Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH *et al.* Serum sphingolipids:
536 relationships to insulin sensitivity and changes with exercise in humans. *Am J Physiol Endocrinol*
537 *Metab* 2015;**309**:E398-408.
- 538 45. Chaston TB, Dixon JB, O'Brien PE. Changes in fat-free mass during significant weight loss: a
539 systematic review. *Int J Obes (Lond)* 2007;**31**:743-750.
- 540 46. Memon RA, Holleran WM, Moser AH, Seki T, Uchida Y, Fuller J *et al.* Endotoxin and Cytokines
541 Increase Hepatic Sphingolipid Biosynthesis and Produce Lipoproteins Enriched in Ceramides and
542 Sphingomyelin. *Arteriosclerosis, Thrombosis, and Vascular Biology* 1998;**18**:1257-1265.
- 543 47. Chavez JA, Summers SA. Characterizing the effects of saturated fatty acids on insulin signaling
544 and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes.
545 *Archives of Biochemistry and Biophysics* 2003;**419**:101-109.
- 546 48. Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW *et al.* Intestinal farnesoid X receptor
547 signaling promotes nonalcoholic fatty liver disease. *J Clin Invest* 2015;**125**:386-402.
- 548 49. Aron-Wisnewsky J, Verger EO, Bounaix C, Dao MC, Oppert JM, Bouillot JL *et al.* Nutritional and
549 Protein Deficiencies in the Short Term following Both Gastric Bypass and Gastric Banding. *PLoS*
550 *One* 2016;**11**:e0149588.

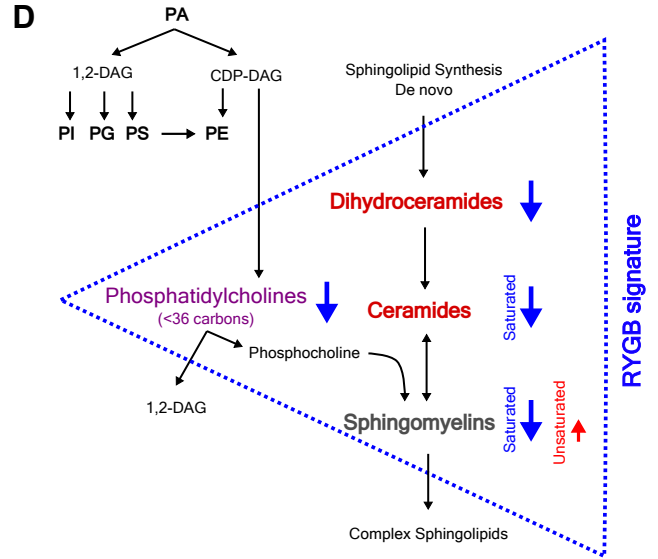
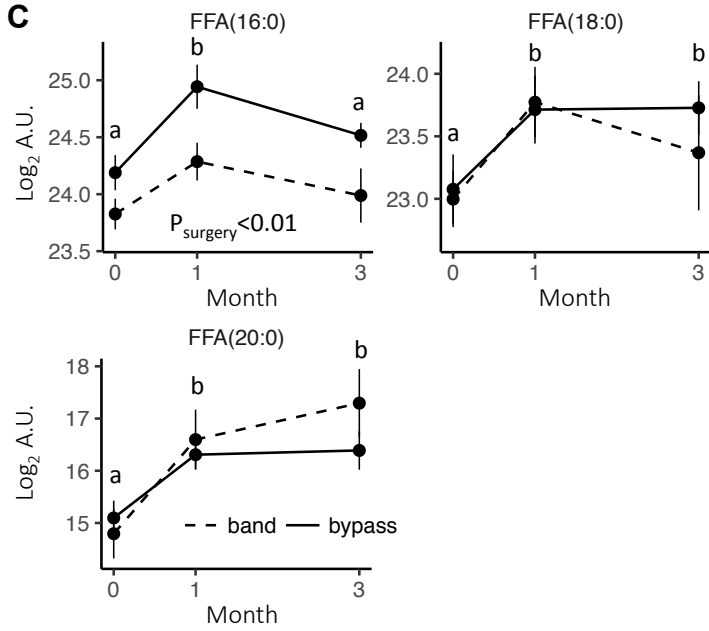
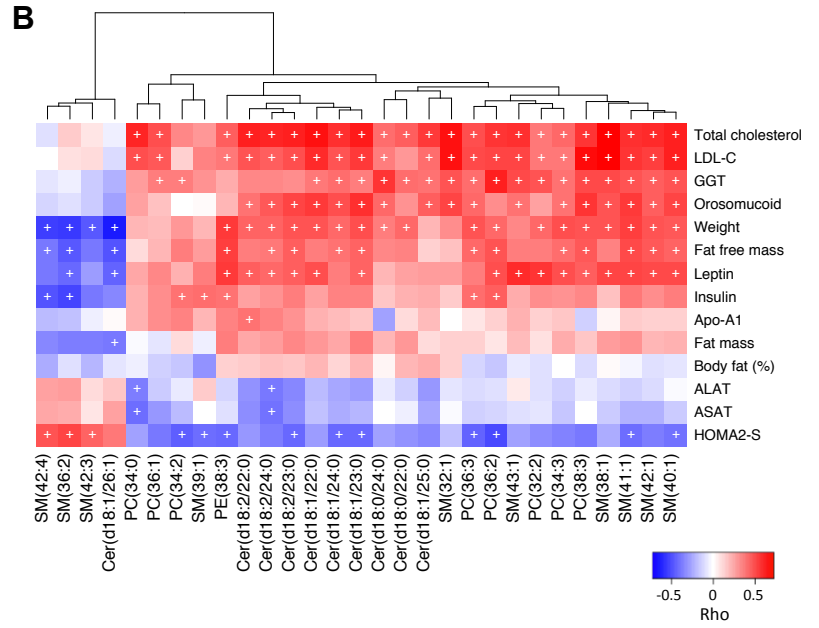
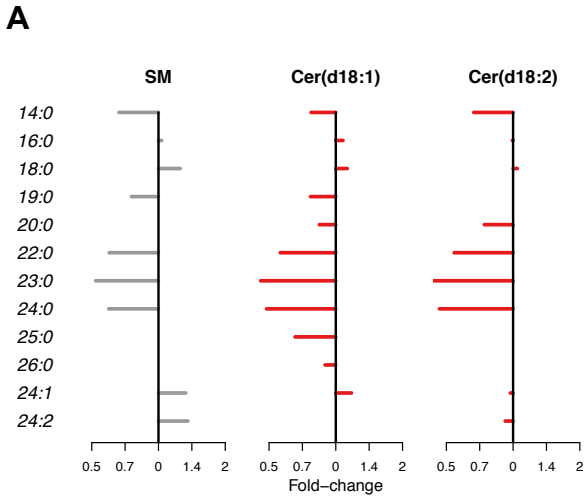
551 Table 1. Clinical parameters at baseline and during follow-up. ANOVA P are adjusted for the false
552 discovery rate. *P<0.05 compared to baseline, adjusted for the family-wise error rate. #Chi-squared test.
553 Values are mean (SE) for continuous variables and % prevalence for categorical. TC =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

557
558 Figure 1. PCoA of baseline lipidomics with 95% confidence ellipses in A: AGB and RYGB, and B:
559 Untreated and statin-treated subjects. C: Correlation heatmap between baseline clinical parameters and
560 lipid classes, + is P_{adj}<0.1. D: Summary of ANOVA results. E: Volcano plots of change in lipids without
561 an interaction averaged across the two surgeries at month 1 (*left*) and month 3 (*right*). F: Fold-change
562 from M0 to M3 for lipids without a significant interaction. Bold text and colored lines are lipids
563 significantly different from baseline (P_{adj}<0.05).

564
565 Figure 2. A: Manhattan plot for the Time by Surgery interaction in the mixed effect ANOVA. Dotted line is
566 the 10% Benjamini-Hochberg false discovery rate. B: Change from baseline to M3 in AGB (dark) and
567 RYGB (light) for lipids with a significant interaction. * indicates P_{adj} <0.05 after adjusting for weight loss.

568
569 Figure 3. A: Change in sphingolipids from baseline to M3 organized by fatty acid content for Cer and
570 presumed fatty acid content for SM (see Methods). B: Heatmap of correlations between deltas of lipids
571 and clinical parameters (M3-M0) that had a significant interaction in ANOVA, + indicates a P_{adj} <0.1.
572 Lipids and clinical variables are clustered with average-linkage hierarchical clustering. C: Change in
573 saturated free fatty acids following surgery. Time points with different letters are statistically significantly
574 different (P<0.05) for both surgeries as there was no significant interaction. D: RYGB "signature" overlaid
575 on a simplified diagram of phospholipid and sphingolipid synthesis. Bold or colored names are analytes
576 measured in the current study. 1,2-DAG, 1-2-Diacylglycerol; CDP-DAG, Cytidine Diphosphate
577 Diacylglycerol; PA, Phosphatidic Acid; PI, Phosphatidylinositol; PG, Phosphatidylglycerol; PS,
578 Phosphatidylserine; PE, Phosphatidylethanolamine.





	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
Orosomuroid (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

Lipid	Surgery group					Metformin treatment					Statin treatment				
	RYGB		AGB		T-test Padj	Untreated		Metformin		T-test Padj	Untreated		Statin		T-test Padj
	Mean	SE	Mean	SE		Mean	SE	Mean	SE		Mean	SE	Mean	SE	
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	178.21	8.299	168.71	9.807	0.86	172.22	6.884	190.25	15.98	0.98	172.67	6.791	187.40	18.07	0.72
PC.34.2	477.12	18.84	470.80	18.16	0.99	477.80	14.14	455.43	44.75	0.98	478.69	14.44	449.76	39.75	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	260.57	9.340	253.91	12.10	0.87	260.21	7.514	244.57	26.33	0.98	260.47	7.664	242.88	24.11	0.72
PC.36.3	155.65	6.403	150.70	8.546	0.86	154.70	5.511	148.09	13.97	0.98	154.61	5.541	148.62	13.45	0.86
PC.36.4	196.25	8.872	198.10	9.467	0.88	193.58	6.981	218.39	17.82	0.97	193.25	6.949	220.47	17.94	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	131.16 4	5.033	131.33 2	4.745	0.92	129.27 8	3.657	143.64 8	12.40 4	0.98	129.10 3	3.629	144.76 2	12.58 6	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	106.34 9	10.99 9	0.82	87.771	4.140	101.65 5	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	122.24 7	4.888	121.90 3	4.046	0.93	121.81 6	3.524	124.04 8	11.72 2	0.98	122.55 7	3.583	119.32 4	10.83 8	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.

Lipid	RYGB - Means			AGB - Means			ANOVA		RYGB % Change		AGB % Change	
	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.36.4.18.2.18.2.18.3	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 ($P_{adj} < 0.05$).

Lipid	Baseline concentration		Baseline weight		Change in weight		Change with RYGB (vs. AGB)		
	Beta	P	Beta	P	Beta	P	Beta	P	P _{adj}
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