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ORIGINAL ARTICLE

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Metabolic remission precedes possible weight regain after gastric bypass surgery

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Abstract

Objective: Some patients regain weight to a variable extent from 1 year after Roux-en-Y gastric bypass surgery (RYGB), though rarely reaching preoperative values. The aim of the present study was to investigate whether, when, and to what extent metabolic remission occurs.

Methods: Fasting metabolite and lipid profiles were determined in blood plasma collected from a nonrandomized intervention study involving 148 patients before RYGB and at 2, 12, and 60 months post RYGB. Both short-term and long-term alterations in metabolism were assessed. Anthropometric and clinical variables were assessed at all study visits.

Results: This study found that the vast majority of changes in metabolite levels occurred during the first 2 months post RYGB. Notably, thereafter the metabolome started to return toward the presurgical state. Consequently, a close-to-presurgical metabolome was observed at the time when patients reached their lowest weight and glucose level. Lipids with longer acyl chains and a higher degree of unsaturation were altered more dramatically compared with shorter and more saturated lipids, suggesting a systematic and reversible lipid remodeling.

Conclusions: Remission of the metabolic state was observed prior to notable weight regain. Further and more long-term studies are required to assess whether the extent of metabolic remission predicts future weight regain and glycemic deterioration.

Oksana Rogova, Katharina Herzog, Nils Wierup, and Peter Spégel contributed equally to this study.

INTRODUCTION

The prevalence of obesity has continuously increased for several decades and has nearly tripled since 1975, resulting in the present global occurrence of 13% [1]. Obesity is associated with an increased risk of multiple diseases, including cardiovascular disease, cancer, and type 2 diabetes (T2D), and is therefore strongly linked to an increase in all-cause mortality [2].

Bariatric surgery (BS) produces rapid and sustained weight loss. Several different procedures have been developed, out of which sleeve gastrectomy and Roux-en-Y gastric bypass surgery (RYGB) are the most common [3]. In RYGB, a small pouch (15–20 mL) is constructed of the upper stomach and connected directly to the jejunum, thus bypassing the distal stomach, duodenum, and upper parts of jejunum. An interesting benefit of RYGB is the remission of T2D that is observed in about 80% of cases, whereas glycemic control is improved in an additional 10% of patients [4]. Identification of the mechanisms underlying T2D remission has been challenging because of the massive alterations in physiology and metabolism elicited by BS. On the one hand, remission has been attributed to alterations in gut hormone secretion, gut microbiota, and nutrient absorption [5, 6]. On the other hand, reduced caloric intake has also been implicated, which is supported by studies showing low-calorie diets (LCD) to elicit remission of T2D [7].

Among individuals undergoing RYGB, 87% regain weight within 5 years post surgery [8], though not reaching preoperative levels, whereas untreated people with obesity gain weight throughout their adult lives. However, weight regain is a blunt instrument with respect to its ability to monitor the remission of an unhealthy metabolism as it is a slow process, reflecting the cumulative effect of small changes in energy balance over long periods of time.

A more sensitive marker for the reestablishment of an unhealthy metabolism may be found in the metabolome, reflecting the myriad of metabolic processes linked to energy balance. Multiple studies have identified altered metabolite levels long before the development of diabetes [9] or obesity [10]. We have previously studied the acute effect of RYGB on alterations in the metabolome. Our data have shown that a substantial number of RYGB-induced metabolic effects had already leveled off 6 weeks post surgery [11]. However, whether a more pronounced, or even complete, metabolic remission is observed in the longer term is still to be established.

In the present study we examined both the short- and long-term effect of RYGB on metabolism using a large and well-characterized cohort of 148 individuals in a repeated measures design. Our aim was to investigate metabolic remission in relation to commonly assessed clinical variables following RYGB.

Study Importance

What is already known?

- Metabolic alterations underlying weight regain and remission of diabetes post bariatric surgery are poorly understood.

What does this study add?

- We found that initial changes in metabolism elicited by bariatric surgery to a large extent are already reversed within a year post surgery.
- Bariatric surgery-induced changes in lipid levels follow an acyl carbon number- and degree of unsaturation-dependent pattern.

How might these results change the direction of research or the focus of clinical practice?

- Metabolic remission may provide a more sensitive tool for monitoring of changes in metabolic health than blood glucose and BMI.

METHODS

Participants and study design

The study involved an initial cohort of 167 individuals, of whom 148 qualified for RYGB during the years 2012 to 2019 in Sweden. All measurements were done after an overnight fast. Baseline parameters and blood samples were obtained at the first visit to the clinic, before introduction of the presurgical LCD, which was, on average, 2 months before surgery. On the day of operation, only body weight was measured to ascertain the expected weight loss. At surgery, patients were given 1.5 g of cefuroxime and, on discharge from hospital, prescriptions for vitamin B12, vitamin D, and calcium. No further antibiotics were administered. Blood was sampled at baseline ($n = 148$ out of which $n = 26$ diagnosed with T2D) and then at 2 ($n = 95$), 12 ($n = 115$), and 60 months ($n = 11$) post surgery. Clinical variables, measured as previously described [12], were obtained at baseline ($n = 148$) and at 2 ($n = 147$), 12 ($n = 151$), and 24 months ($n = 85$) post RYGB. The rate of weight loss was more pronounced directly following surgery than after the LCD, so at the 2-month study point, metabolism was catabolic (eating less than half daily expenditure). Weight loss then slowed down. Clinical variables are summarized in Supporting Information Table S1. The study was approved by the Human Ethical Committee in Lund, Sweden (Dnr. 2009/3), and it adhered to the standards of the Declaration of Helsinki. All patients gave signed consent.

Lipidomics

Analysis of lipids was performed as previously described [11] (Supporting Information Table S2). Samples were analyzed in 10 batches, matched for glycemic status (T2D or normoglycemic), using constrained randomization [13] with separate randomization between and within study participants. With this approach, samples from an

individual are analyzed in random order but close to each other within the analysis sequence. Therefore, the impact of instrumental drift and batch effects is minimized. A quality control (QC) sample, created by mixing aliquots of all samples, was analyzed twice prior to the first sample injection and then every 10th injection to allow for a continuous monitoring of method performance. Methods details are provided in the online Supporting Information.

Metabolomics

Metabolite profiling was performed by gas chromatography/mass spectrometry (GC/MS) [14] and ultrahigh-performance liquid chromatography/quadrupole time of flight-MS (UHPLC/QTOF-MS) as previously described in detail [11]. The analysis order was the same as for the lipidomics analysis. Because of limited sample volumes, an extract of fetal calf serum (FCS) was used as the QC sample in the GC/MS analysis. Methods details are provided in the online Supporting Information.

Data processing

Annotation of metabolites and lipids followed the Metabolomics Standards Initiative. Integration of lipidomics data was performed using MZmine2 (version 5.1). Lipids were annotated based on in-house libraries and structure-retention time correlation plots [15] (level 2a; Supporting Information Table S3). Peak annotation in the GC/MS data was performed in AMDIS based on information on retention index and mass spectra from analysis of standards (level 1) or data in the NIST MS library (level 2b; Supporting Information Table S5). Raw data files were exported in NetCDF format to a MATLAB-based script for targeted integration [16].

Data from UHPLC/QTOF-MS analyses were analyzed by targeted feature extraction using the Profinder software (B.08.00, Agilent Technologies Inc., Santa Clara, California) with in-house libraries and the Metlin library (level 1, 2a or 2b; Supporting Information Table S6). Metabolites and lipids with an average signal to noise ratio of <10 in the QC samples or with >5% missing data in a single batch were excluded from the analysis. Missing data were then imputed as the lowest detected level divided by two. When the same metabolite was detected by several platforms, data from the platform showing the lowest variation in the QC samples were kept.

Statistical analysis

Data were analyzed in R (version 4.0.3) using several tidyverse packages. Metabolite and lipid peak areas were log2-transformed prior to analysis. Principal component analysis (PCA; prcomp, stats) was conducted using centered and unit-variance scaled data. Effects of post-surgical time on clinical variables and metabolite levels were estimated using linear mixed-effect models (lmer, lme4) and illustrated in heat maps (pheatmap). The models were constructed using individuals as blocking factor and they included T2D diagnosis and sex as covariates and the interactions between these variables. Data are presented as mean value ± standard error if not stated otherwise. *P* values were corrected for multiple comparisons using the false discovery rate method. The level of significance was set at a false discovery rate of 5% ($q < 0.05$). Orthogonal partial least squares-effect projections (OPLS-EP) [13] models were calculated using the opls function (ropls). Correlations between loadings (getLoadingsMN, ropls) and lipid acyl chain carbon number and degree of unsaturation, including quadratic terms and their interaction, were estimated by linear models (lm, stats) and visualized using plot_model (sjPlot). All other graphs were produced using ggplot (ggplot2).

Data and resource availability

The metabolomics data were deposited in the Lund University Diabetes Centre repository (https://www.ludc.lu.se/resources/repository) under the accession number LUDC2023.05.1.

RESULTS

Clinical variables

Clinical and anthropometric data for the patients receiving RYGB are presented in Figure 1 and Supporting Information Table S1. Data collected at 60 months post RYGB were excluded from analysis because of low sample size; data for the entire study are shown in Supporting Information Figure S1. The average decrease in body mass index (BMI) in the preoperative phase was -2.50 ± 0.11 kg/m^2 ($n = 148$, $p < 2 \times 10^{-16}$), which was a result of the presurgical LCD. The reduction in BMI at 2 and 12 months post surgery was -7.06 ± 0.17 ($n = 147$, $p < 2 \times 10^{-16}$) and -14.66 ± 0.34 ($n = 147$, $p < 2 \times 10^{-16}$), respectively, relative to baseline. A similar pattern was observed for waist circumference. BMI did not change further between 12 and 24 months post RYGB. For normoglycemic participants, the reduction in BMI was more substantial among women than men, whereas this was not observed in those with T2D.

As expected, levels of glycated hemoglobin decreased (-18.81 ± 3.29 mmol/mol, $p < 2 \times 10^{-16}$, $n = 25$) in patients diagnosed with T2D during the first 12 months post surgery but then increased slightly in the following 12 months ($+3.29 \pm 2.10$ mmol/mol, $p = 0.044$, $n = 22$). Glycated hemoglobin levels were slightly decreased in normoglycemic individuals (-3.10 ± 0.42 mmol/mol, $p < 2 \times 10^{-16}$, $n = 117$) from baseline to 12 months post RYGB. In line with this, levels of glucose were also marginally decreased (-0.28 ± 0.13, $p = 0.0021$, $n = 81$) in normoglycemic individuals from baseline to 2 months post RYGB. In patients with T2D, blood glucose levels decreased during the first 2 months following RYGB (-2.93 ± 1.36 mmol/L, $p = 4.89 \times 10^{-5}$, $n = 14$) and then remained at this level throughout the study period. Only 3 out of 26 patients who were diagnosed with T2D pre surgery

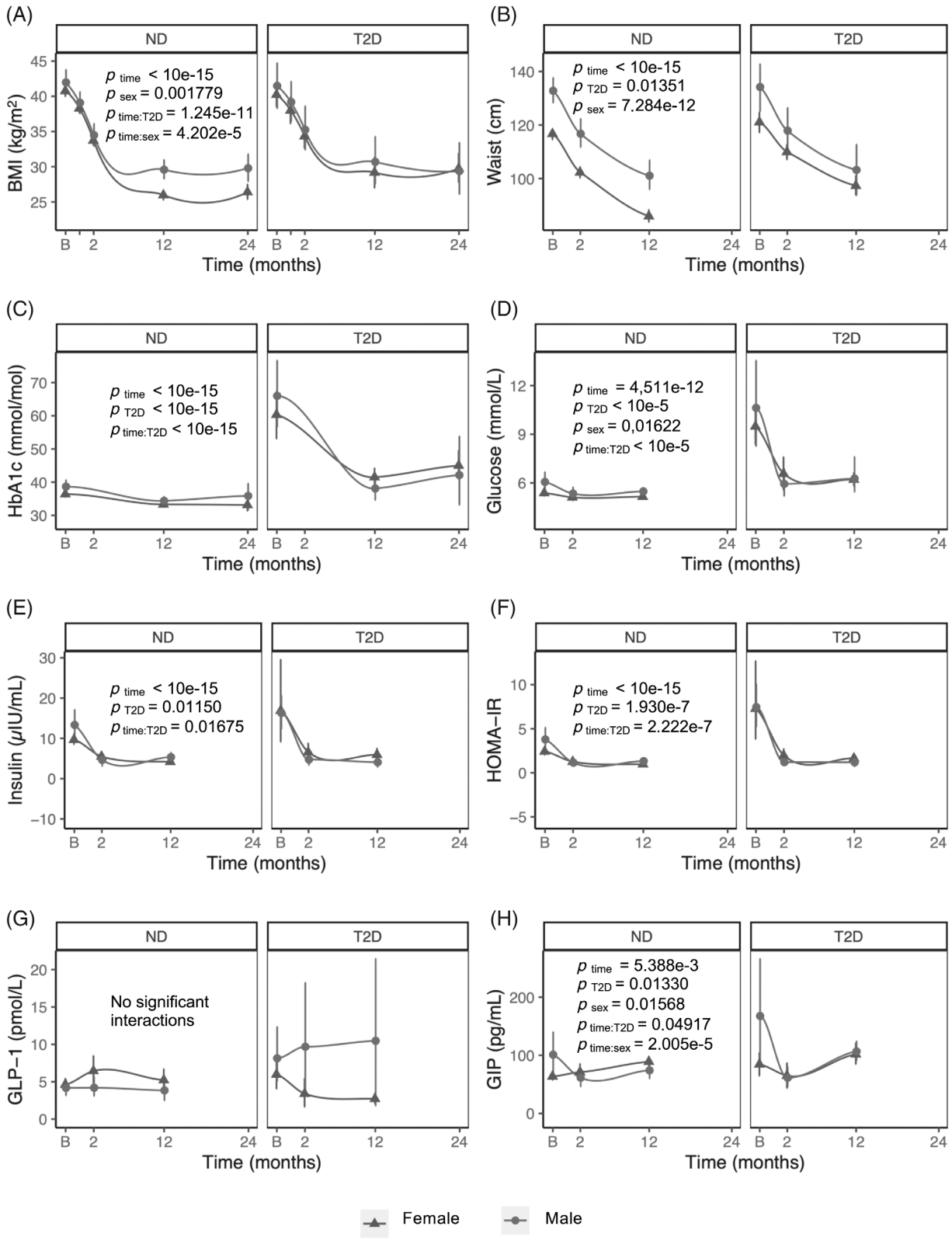

FIGURE 1 Alterations in clinical variables, (A) BMI, (B) waist circumference, (C) HbA_{1c}, (D) glucose, (E) insulin, (F) HOMA-IR, (G) active GLP-1, and (H) active GIP over the 2-year study period, presented separately for patients with T2D ($n = 26$) and without T2D (nondiabetic, ND; $n = 122$) prior to the surgery. The p values are derived from linear mixed-effect models (variable ~ Time + T2D + Sex + Time:T2D + Time:Sex) nested by patient ID. Trajectories were created using loess smoothing and confidence intervals (95%) determined by nonparametric bootstrapping. GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; T2D, type 2 diabetes

still received glucose-lowering medication a year post RYGB. Insulin levels were decreased 2 months post RYGB in both normoglycemic individuals (-3.95 ± 0.67 μU/mL, $p = 5.36 \times 10^{-16}$, $n = 78$) and in individuals diagnosed with T2D (-11.09 ± 7.35 μU/mL, $p = 0.029$, $n = 14$), and this did not change at the following study visits. Homeostatic model assessment of insulin resistance declined during the first 2 months post RYGB, after which it stabilized at the improved level. We could not observe any changes in fasting active glucagon-like peptide-1 levels. However, fasting active glucose-dependent insulinotropic polypeptide (GIP) levels were higher in men than in women at baseline in both patients diagnosed with T2D (83.18 ± 33.90 pg/mL, $p = 0.043$, $n = 14$) and normoglycemic individuals (37.54 ± 11.88 pg/mL, $p = 0.022$, $n = 81$). At 2 months post surgery, active GIP levels decreased in male patients and reached the same level as observed in women. GIP levels then increased between 2 and 12 months post RYGB in patients both with ($+40.50 \pm 14.17$ pg/mL, $p = 2.0 \times 10^{-5}$, $n = 13$) and without ($+17.88 \pm 7.21$ pg/mL, $p = 0.0099$, $n = 72$) a T2D diagnosis at baseline.

Lipids

First, we performed a PCA independently on the 126 lipids (Supporting Information Table S3) measured in the two lipidomics experiments performed in positive and negative electrospray ionisation mode (ESI + and ESI-, respectively), to provide an overview of regulation of lipid metabolism (Figure 2A,B and Supporting Information Figure S2). The largest changes in the lipidome were observed between baseline and 2 months post RYGB, with changes occurring in the following 10 months being smaller and in the opposite direction. Next, to benefit from the paired structure of the study design, we performed a PCA on changes in lipid levels between study visits (Figure 2C,D). This analysis made it clear that the initial changes occurring within 2 months post RYGB were reversed in the following 10 months. Consequently, the metabolic effects of the surgery observed 1 year post RYGB were small (Figure 2E,F).

Next, to provide a description of alterations in levels of individual lipid species, we analyzed data using linear mixed-effect models (Figure 3 and Supporting Information Table S4); results including the 60-month visit are found in Supporting Information Figure S3. Again, the most significant changes in lipid levels were observed during the first 2 months post surgery. During this period, levels of the majority of lipid species decreased and only a few sphingomyelin (SM), lysophosphatidylcholine, and lysophosphatidylethanolamine species increased. Notably, the levels of most lipids changed in the opposite direction between 2 and 12 months post RYGB. From these analyses it could also be observed that lipids with longer acyl chains and a higher degree of unsaturation were more likely to demonstrate increased levels and those with shorter and more saturated acyls to show decreased levels during the first 2 months post RYGB, with the opposite being observed during the following 10 months.

To enable a deeper understanding of the impact of RYGB on systematic lipid remodeling, we next analyzed data using OPLS-EP [13] (Figure 4). Metabolomics data produced by untargeted mass spectrometry are, especially when analyses are conducted in multiple batches over several days, affected by systematic variation, so-called drift, in the intensity of detected metabolites [13]. OPLS-EP, similar to traditional OPLS, is capable of isolating the drift-associated covariation pattern and separating it from the variation of interest. Hence, an attractive feature of the OPLS approach is that one single analysis provides information on all lipids, as compared with assessing all lipids individually by means of, for example, linear mixed-effect models. Predictive loadings [17] extracted from the OPLS-EP model revealed RYGB-induced alterations in lipid levels to vary within lipid classes. This variation, at least in the case of SMs and triglycerides (TGs), could be described from the carbon number and degree of unsaturation of the acyl groups (Figure 4). Notably, the pattern observed in the first 2 months post RYGB was largely reversed in the following 10 months. However, altered lipid levels were still observed 12 months post surgery.

Metabolites

Data on levels of 60 metabolites (Supporting Information Tables S5 and S6) were initially analyzed using PCA (Figure 5A,B, Supporting Information Figures S4 and S5). Again, we found that samples acquired 2 months post RYGB differed the most from the baseline samples, with samples acquired 12 months post RYGB reflecting a metabolome that was more similar to the baseline state. However, the pattern was less distinct as compared with the clear clustering observed in the lipidomics data, although becoming clearer in a PCA performed on changes in metabolite levels (Figure 5C,D). In line with results from the lipidomics data, changes during the short and long term were in the opposite direction, with the cumulative effect being relatively smaller (Figure 5E,F).

With respect to individual metabolites, we observed that the levels of carnitine and short-chain acylcarnitines (3:0, 4:0, 5:0, 8:1) decreased, whereas those of medium- and long-chain acylcarnitines (10:0, 12:1, 14:1, 16:0, 18:1) and acetylcarnitine increased 2 months post RYGB (Figure 6 and Supporting Information Table S7). As for the majority of lipids and metabolites investigated, these changes were transient, and between 2 and 12 months post RYGB, levels of acylcarnitines changed in the opposite direction. As a result, only minor changes in levels of these metabolites remained 12 months post RYGB (Figure 6); a similar profile was observed 60 months post RYGB (Supporting Information Figure S6). Furthermore, we observed a reduction in levels of sugars and sugar-related metabolites, such as glucose, fructose, and inositol, the majority of detected essential amino acids, including the branched-chain (leucine, isoleucine, valine) and aromatic (phenylalanine, tryptophane and tyrosine) amino acids, lactate, 2-hydroxybutyrate, urea, and uric acid during the first 2 months post surgery. On the contrary, levels of the ketone body 3-hydroxybutyrate, serine, and the Krebs cycle intermediates citrate and malate were increased 2 months post surgery. Again, these metabolites changed in the opposite direction between 2 and

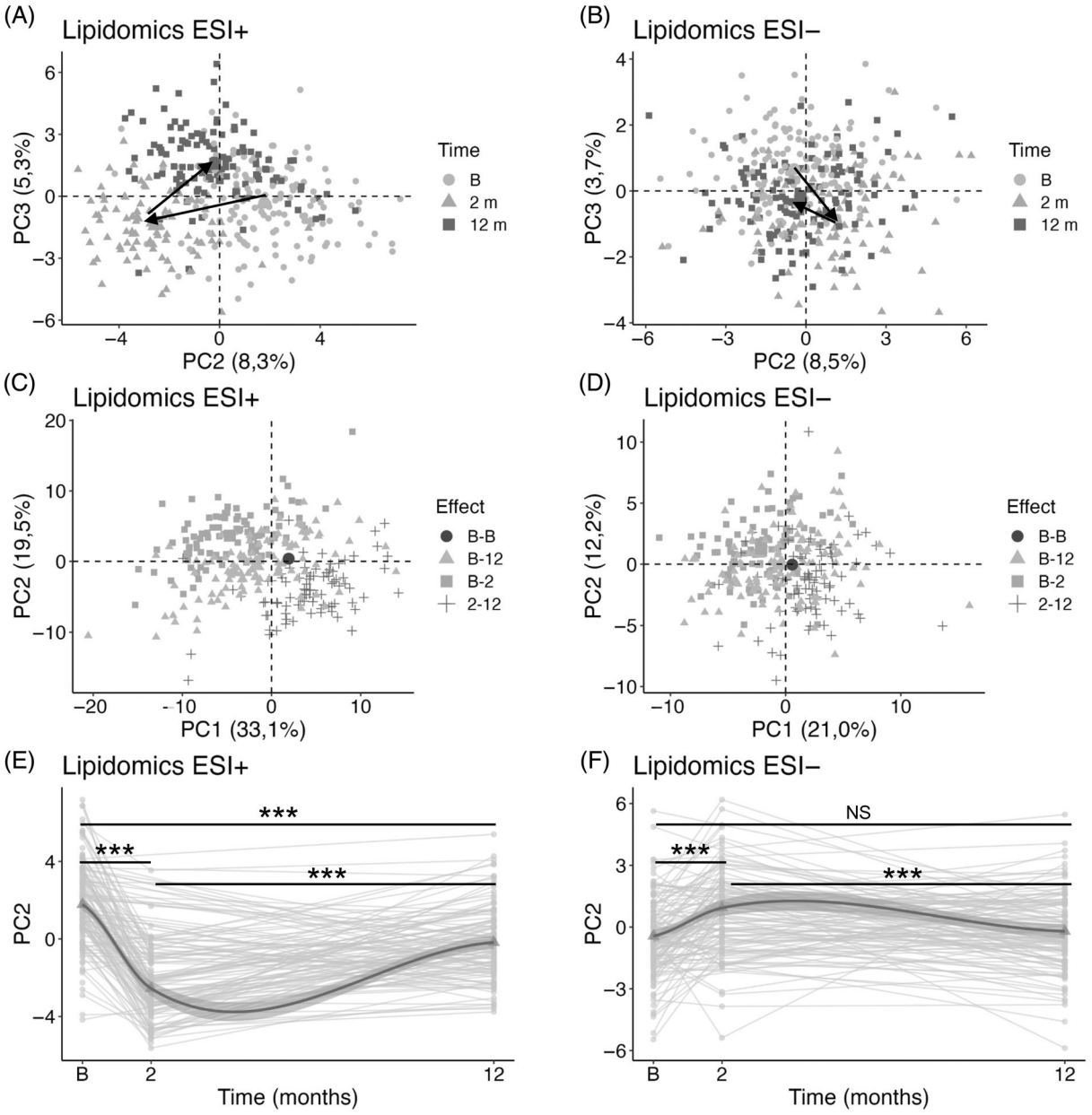

FIGURE 2 PCA score plots for lipidomics data acquired in positive (ESI+; 80 annotated lipids) and negative (ESI-; 46 annotated lipids) ESI mode. PCA was conducted on (A,B) metabolite levels and (C,D) changes in metabolite levels (effects) within defined time ranges: short term (2 months vs. baseline), long term (12 vs. 2 months), cumulative (12 months vs. baseline), and reference (baseline vs. baseline). (E,F) Changes in the lipidome for individual study participants are illustrated in the score trajectory plots, with trajectories estimated as described in Figure 1. *P* values are shown for the factor time, using the model: score ~ Time + T2D + Sex + Time:T2D + Time:Sex, nested by patient ID. ***$p < 0.001$; NS, $p > 0.05$. ESI, electrospray ionization; NS, not significant; PCA, principal component analysis; T2D, type 2 diabetes

12 months post RYGB. Whereas some metabolites, such as malate, reached baseline levels at 12 months post RYGB, several metabolites still showed altered levels 12 months post RYGB, although at a smaller magnitude than those observed during the first 2 months.

DISCUSSION

Metabolomics studies reporting on the impact of BS on the human metabolome have produced inconclusive results and have been difficult to replicate [18]. A possible reason for these inconsistencies is the timing of sampling, which has ranged from hours up until several months [11, 18]. We have previously shown, using a repeated sampling strategy in patients undergoing RYGB, that changes in metabolism acting to reinstate the presurgical metabolic state can already be observed within 6 weeks post surgery [11]. In the present study, we expand the time frame from 6 weeks up to 1 year after surgery, with some patients being followed for 5 years.

A general problem within the field of metabolomics is the large variety of methods being used, which has consequences on the extent

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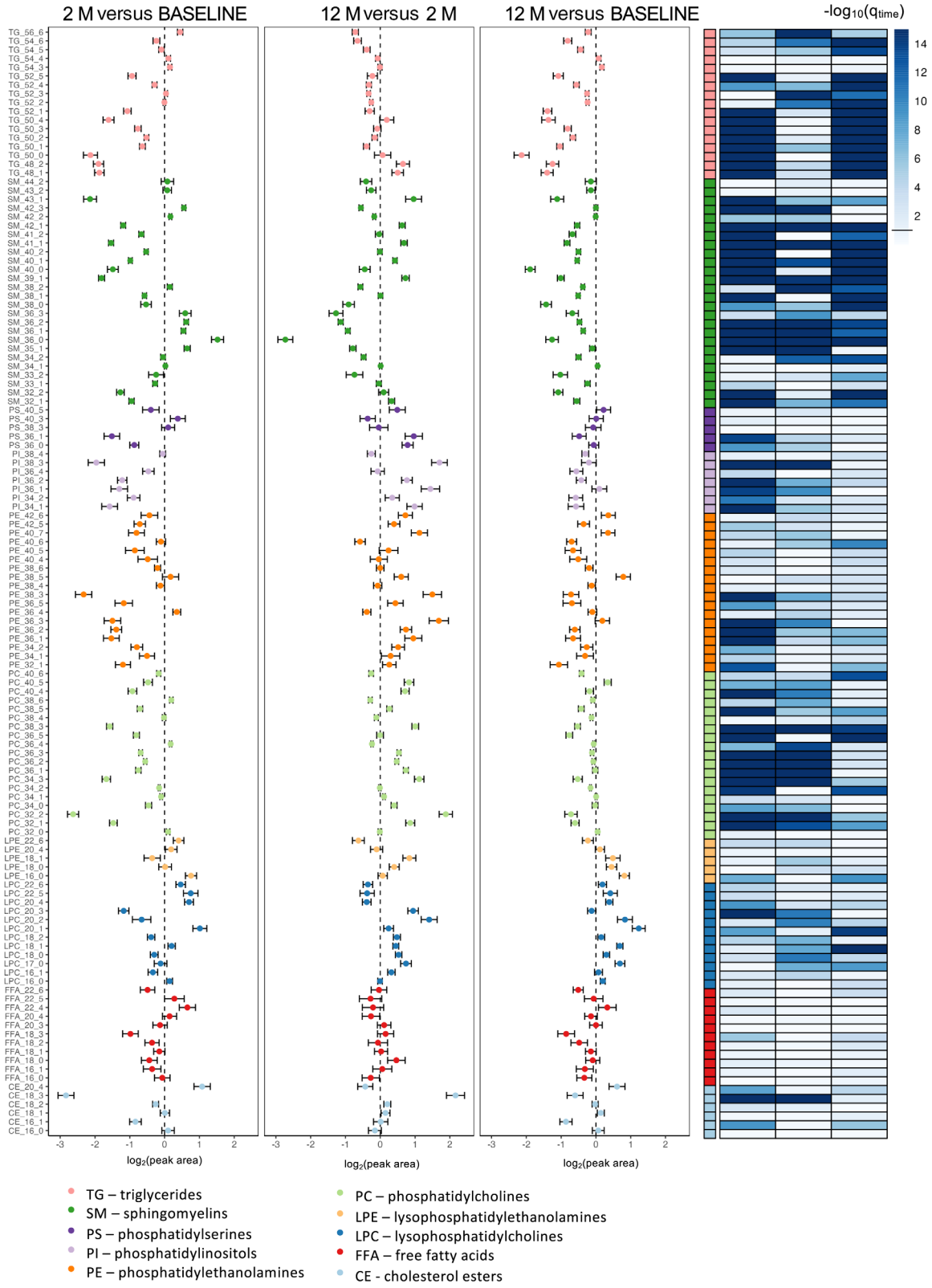

FIGURE 3 Visualization of the effect of postsurgical time on changes in lipid abundance ($\log_2$[peak area]) for time ranges as defined in Figure 2. The box-plot shows effects of time and standard errors obtained from linear mixed-effect models (Lipid ~ Time + T2D + Sex + Time:T2D + Time:Sex) nested by patient ID and the corresponding heat map shows the significance of the effect ($-\log_{10}[q_{time}]$) after correction for multiple testing using the FDR method. The line on the color scale to the right indicates the significance cutoff at $-\log(0.05)$. Effects and *q* values are reported in Supporting Information Table S4. Lipids are named as *class*_*carbon number*_*degree of unsaturation*. FDR, false discovery rate; T2D, type 2 diabetes [Color figure can be viewed at wileyonlinelibrary.com]

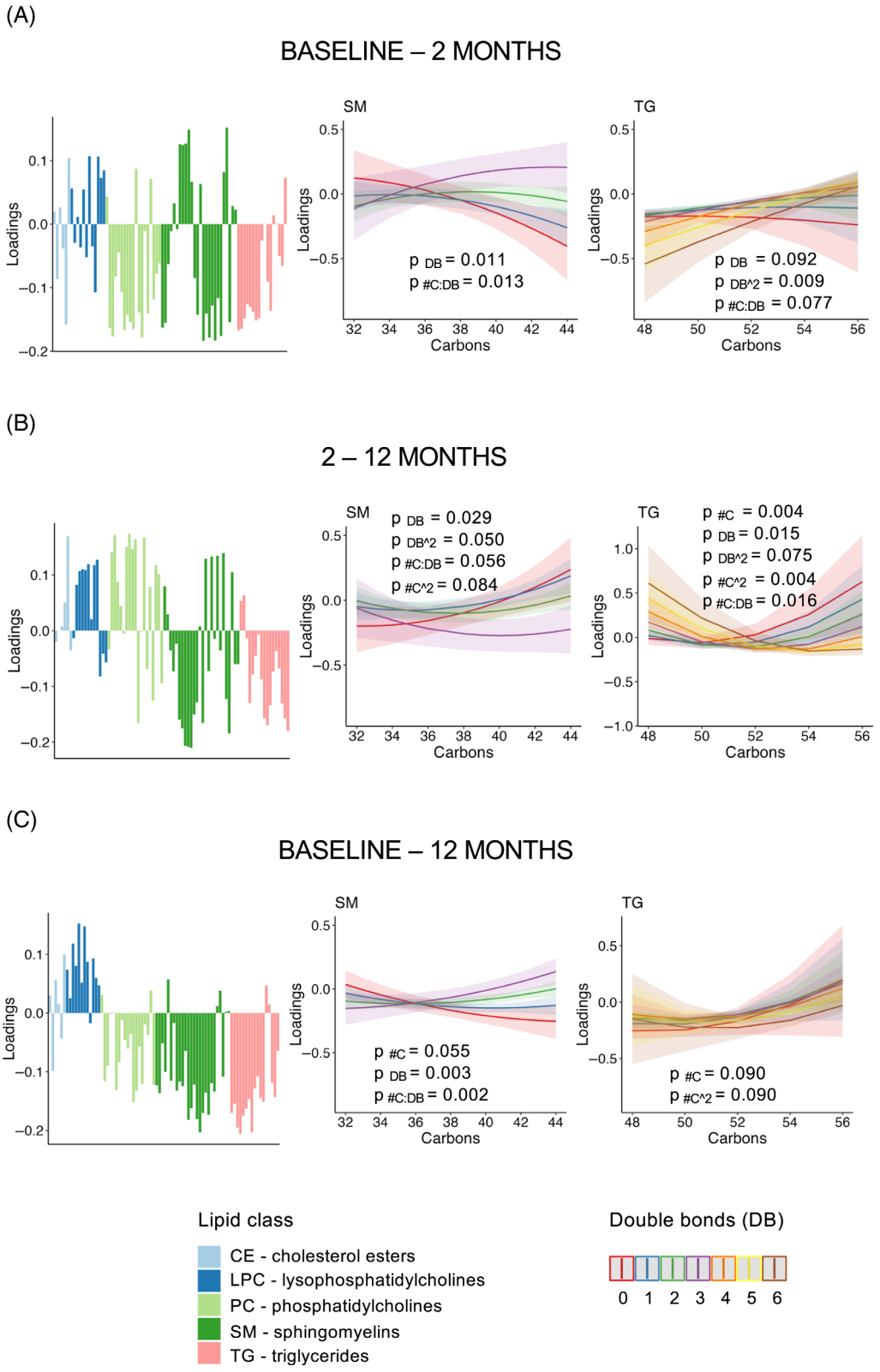

FIGURE 4 Visualization of carbon number- and degree of unsaturation-dependent regulation of lipid levels using OPLS-EP. Predictive loadings (left) and alterations in SM (center) and TG (right) levels as a function of #C and number of DB estimated using the following model: loading $\sim$ #C + #C^2 + DB + DB^2 + #C:DB. Plots show (A) 2 months post RYGB vs. baseline, (B) 12 vs. 2 months post RYGB, and (C) 12 months post RYGB vs. baseline. #C, carbon number; OPLS-EP, orthogonal projections to latent structures effect projections; RYGB, Roux-en-Y gastric bypass [Color figure can be viewed at wileyonlinelibrary.com]

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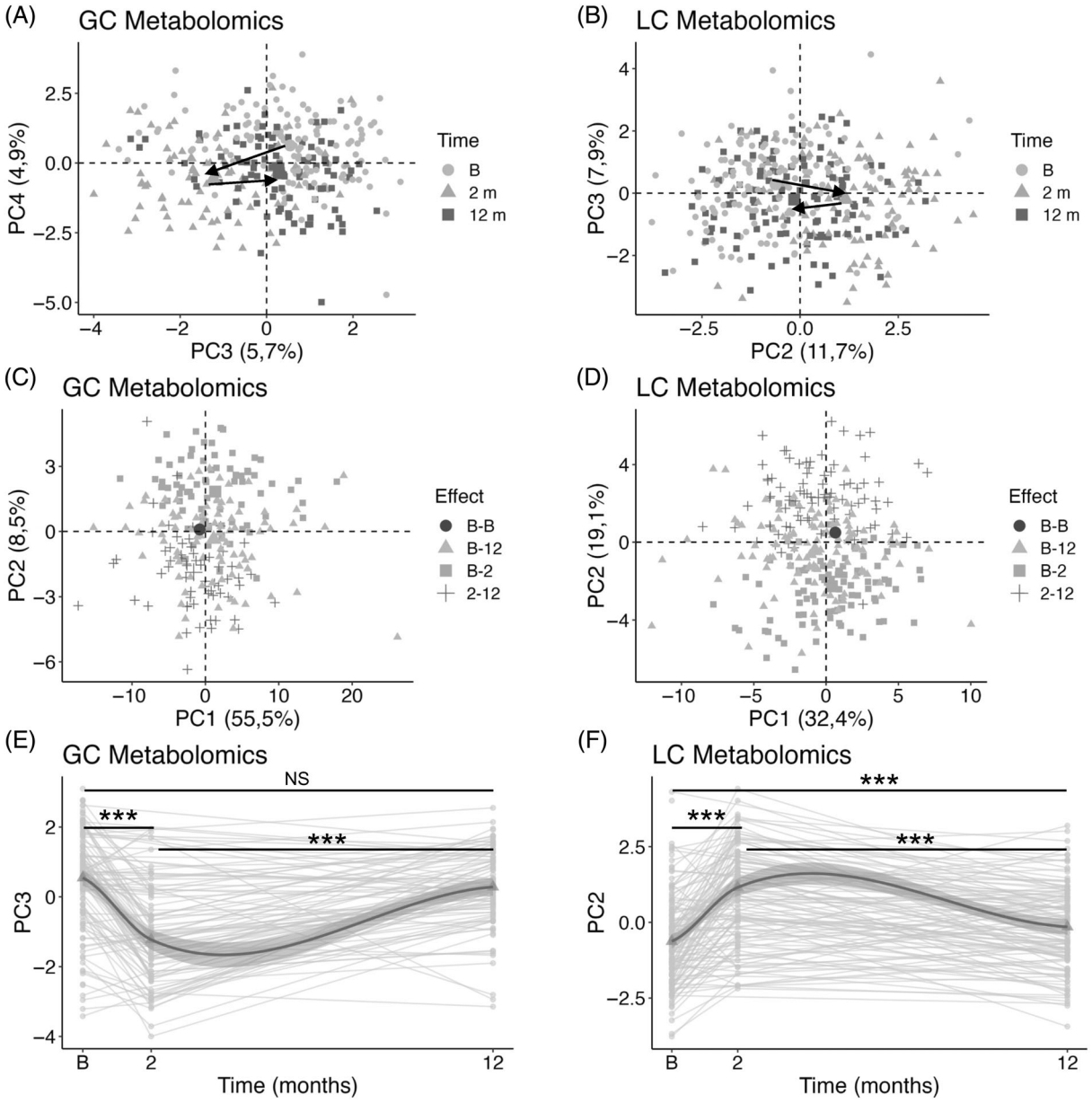

FIGURE 5 PCA score plot for metabolomics data acquired using (A) GC/MS (38 annotated metabolites) and (B) UHPLC/QTOF-MS (22 annotated metabolites). PCA calculated on changes in metabolite levels for (C) GC/MS and (D) UHPLC/QTOF-MS data. Time intervals are defined as described in Figure 2. Changes in the metabolome for individual study participants are illustrated in score trajectory plots for (E) GC/MS data and (F) UHPLC/QTOF-MS data. *P* values are determined as outlined in Figure 2. ***$p < 0.001$. GC/MS, gas chromatography/mass spectrometry; NS, not significant; PCA, principal component analysis; UHPLC/QTOF-MS, ultrahigh-performance liquid chromatography/quadrupole time of flight-mass spectrometry

to which single biomarkers can be replicated. However, as metabolism is composed of a network of a large set of interacting metabolic reactions, perturbations of metabolism are expected to be associated with synchronized alterations in groups of metabolites rather than changes in individual members of the metabolome. To identify such joint variation, we applied OPLS-EP [13] to model covariation in the effect of postsurgical time on the lipidome. These analyses revealed that the extent to which levels of TGs and SMs were altered depended on the degree of unsaturation, the carbon number, and their interaction. For instance, levels of lipids with a high carbon number increased during the first 2 months post RYGB only if they were also highly unsaturated. Notably, the opposite pattern was observed between 2 and 12 months post RYGB. Whether this remodeling of the lipidome is healthy or not cannot be resolved in the present study. However, our observations are in line with previous studies showing that short, saturated acyls are associated with an increased risk of developing prediabetes and T2D, whereas acyls with higher carbon number and degree of unsaturation are protective against the same conditions [19]. More generally, increased levels of lipids containing long-chain polyunsaturated fatty acids are associated with several

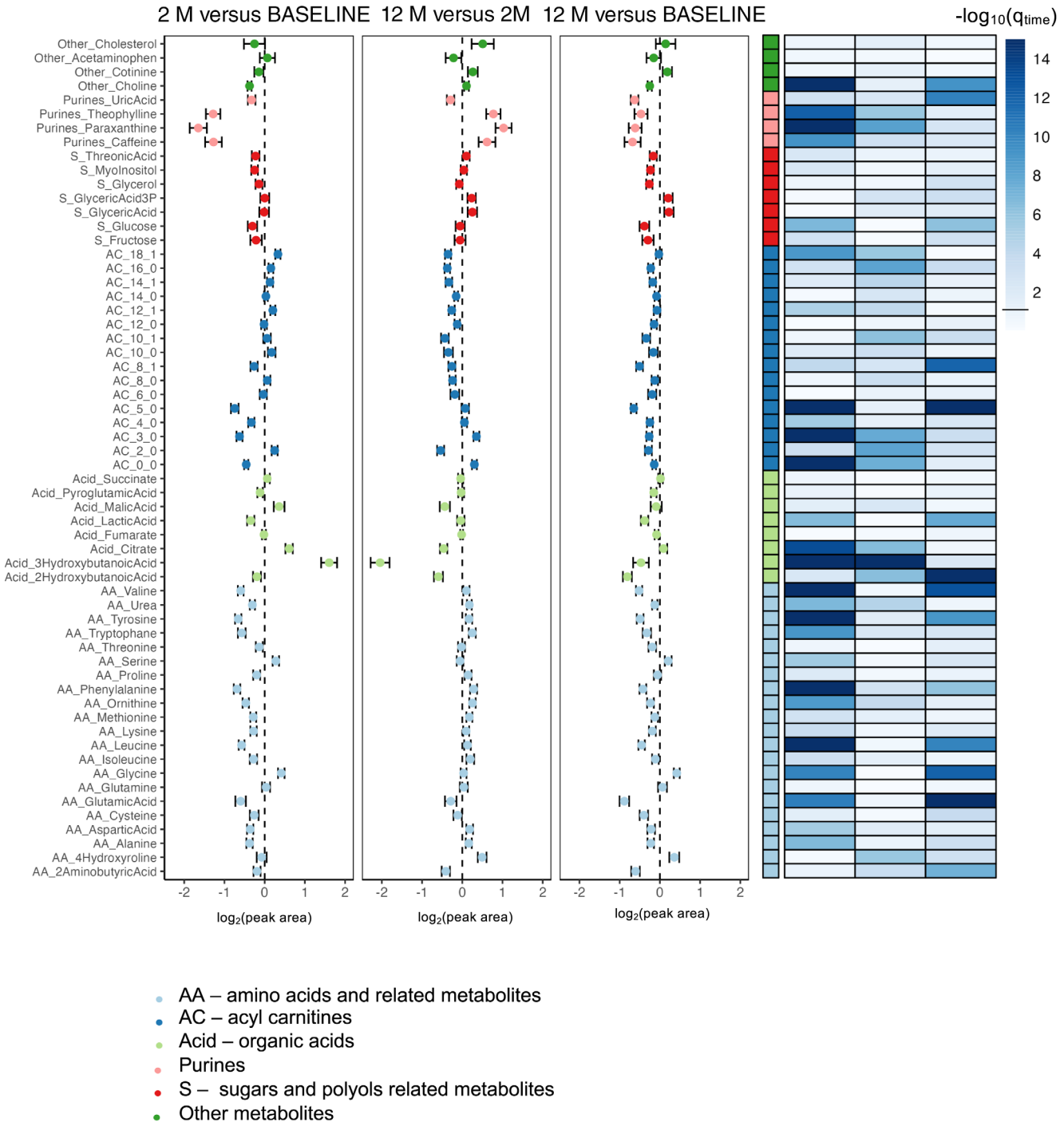

FIGURE 6 Visualization of the effect of postsurgical time on changes in metabolite abundance (log2[peak area]). Data are illustrated as outlined in Figure 3. Effects and *q* values are reported in Supporting Information Table S7. AC are named as *number of carbons in acyl chain*_*number of double bonds in acyl chain* [Color figure can be viewed at wileyonlinelibrary.com]

beneficial health effects [20]. This has been linked to increased membrane fluidity, with consequences on membrane protein function and alterations in signal transduction and membrane-linked transport [20]. Additionally, polyunsaturated fatty acids also serve important roles as signaling molecules and they are necessary for the formation of eicosanoids, which are involved in regulation of inflammation [20].

We observed the most distinct carbon number- and degree of unsaturation-dependent regulation for SMs. SM species are the most abundant sphingolipid in plasma, with most of the lipid being found in very low-density lipoprotein and low-density lipoprotein particles and somewhat less in high-density lipoprotein particles [21]. Increased levels of SMs, and other sphingolipids such as ceramides, are associated with cardiovascular disease, obesity, and diabetes [21]. The

mechanisms underlying these associations are not entirely resolved, although studies have implicated both altered de novo SM synthesis and activation of sphingomyelinases, which convert SMs to ceramides [21]. The complex changes observed in SM species in the present study are unlikely to reflect a more general alteration in plasma lipoprotein abundance, but they may relate to altered sphingomyelinase activity, with different sphingomyelinases selectively metabolizing lipids with different carbon numbers [22]. However, our observation of a similar trend in the regulation of TG levels questions this explanation and rather supports a more fundamental process. In line with this, a similar pattern has been observed previously, despite other lipids being detected, in response to an LCD [11], as well as in association with reduced insulin resistance and a lower risk for future T2D [23].

The lipid composition of the human body depends on a complex interplay between food intake and composition and lipid metabolism, including de novo lipogenesis. Desaturases and elongases play important roles in this interaction, serving to produce long-chain and unsaturated fatty acids to compensate for insufficient dietary supply. The activity of desaturases, which is tightly controlled by the dietary micro- and macronutrient composition [24], has been linked to insulin sensitivity [25]. In addition to this, fatty acid elongation has also been linked to obesity and insulin resistance [26, 27]. Our observation of an interaction between carbon number and the degree of unsaturation for two lipid classes supports the involvement of elongases and desaturases in RYGB-elicited lipid remodeling, as these enzymes are known to act in a repetitive and consecutive fashion.

We also observed increased levels of medium- and long-chain acylcarnitines and decreased levels of most short-chain acylcarnitines 2 months post surgery; these changes were then reversed in the following 10 months. This observation may appear counterintuitive in light of the reduced insulin resistance observed in the patients, given that increased levels of acylcarnitines previously have been found in individuals with insulin resistance and T2D [28]. In the insulin resistant state, elevated levels of intermediate- and long-chain acylcarnitines have been linked to an accelerated lipolysis combined with impaired mitochondrial β-oxidation [28]. In the present study, the increased levels of these intermediates are more likely to be linked to the excessive lipolysis associated with weight loss, in combination with an overloading of the mitochondrial oxidative capacity [29]. The increased levels of acetylcarnitine, together with a reduction in lactate, support a more efficient use of glucose in mitochondrial metabolism. In line with this, the reduction in levels of short-chain acylcarnitines C3-C5 suggests a relatively reduced catabolism of amino acids [28], in turn implying that glucose plays a more important role than amino acids in the anaplerotic reactions required for efficient mitochondrial fatty acid oxidation.

As compared with the lipids, levels of smaller and more polar metabolites showed less distinct alterations following RYGB, which may be explained by their higher interindividual and temporal variation [30]. Despite this, several notable changes in the metabolome were observed. The ketone body 3-hydroxybutyrate showed the largest increase during the first 2 months and then the largest decrease in the following 10 months post RYGB, as compared with any of the other measured metabolites. These changes support a catabolic state shortly after surgery and they are in line with our interpretation of the changes observed in acylcarnitine levels. Moreover, increased levels of the Krebs cycle intermediates citrate and malate further support a high activity in oxidative mitochondrial metabolism [31]. However, we cannot rule out that changes in these intermediates reflect RYGB-associated changes in renal function [32].

The majority of amino acids showed decreased levels during the first 2 months post RYGB, with serine and glycine being the only exceptions. Serine has been reported to be positively correlated to insulin secretion and sensitivity [33], and glycine levels have been reported to be decreased in individuals with obesity or T2D [34]. Hence, these findings are in line with the reduced weight and improved β-cell function [35] and insulin sensitivity [36] observed post RYGB.

Branched-chain and aromatic amino acids showed decreased levels in the first 2 months following surgery, after which levels of the majority of these amino acids increased in the following 10 months. These amino acids show high levels in patients with insulin deficiency and they are associated with an increased risk of developing T2D, which has been linked with an altered activity of branched-chain amino acid catabolizing enzymes in the liver and adipose tissue [37]. Hence, our results support the proposed protective role of RYGB on diabetes risk [38], but they also suggest that this protection levels off already within 1 year post surgery. Levels of alanine, which has been previously reported as a T2D biomarker [39], as well as methionine, which is associated with oxidative stress [40], showed trajectories similar to those of the branched-chain and aromatic amino acids. Notably, levels of 2-hydroxybutyrate, which increase during conditions of increased oxidative stress and which are robustly associated with an increased risk of developing T2D [41], were decreased in both the shorter and the longer term. A similar consistent decrease was observed in levels of uric acid. Hyperuricemia is commonly experienced in individuals with metabolic syndrome and is caused by increased ATP turnover and reduced renal excretion, which in turn is linked to deficient insulin signaling [42]. In line with changes in amino acids and acylcarnitines, levels of urea decreased during the first 2 months post RYGB and then increased in the following 10 months, which further supports a reduced amino acid catabolism.

Several other metabolites showed changes that are likely to be related to the observed improvement in insulin sensitivity. This includes lower levels of glucose, fructose, and inositol. Even though inositol is expected to enhance insulin sensitivity [43], decreased levels of this metabolite were likely a result of reduced production of the sugar alcohol in the kidneys, which, besides the brain, produce most of the inositol in our bodies.

Limitations of the study include lack of detailed dietary data and longer follow-up time. Even though the study was designed for a follow-up time of 5 years, we could not conduct any detailed analyses in the longer time range because of a low sample size, caused by an unforeseen low participation at the 5-year study visit. Although we

lack data to link metabolic remission to future relapse of T2D, it is described in a survey study [44] that T2D recurs in 26% to 50% of patients undergoing BS within 5 to 10 years post surgery [44, 45]. The possible impact of varying degrees of catabolism remains to be investigated.

CONCLUSION

The majority of metabolic changes that are elicited by RYGB level off within a year post surgery, at which time the patients are at their nadir with respect to measures of obesity and glycemia. Hence, metabolic remission precedes weight regain. Our study therefore suggests that both weight regain and recurrence of T2D, both of which are slow processes governed by the cumulative effect of small disturbances in energy balance, are initiated already within a year post surgery.O

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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