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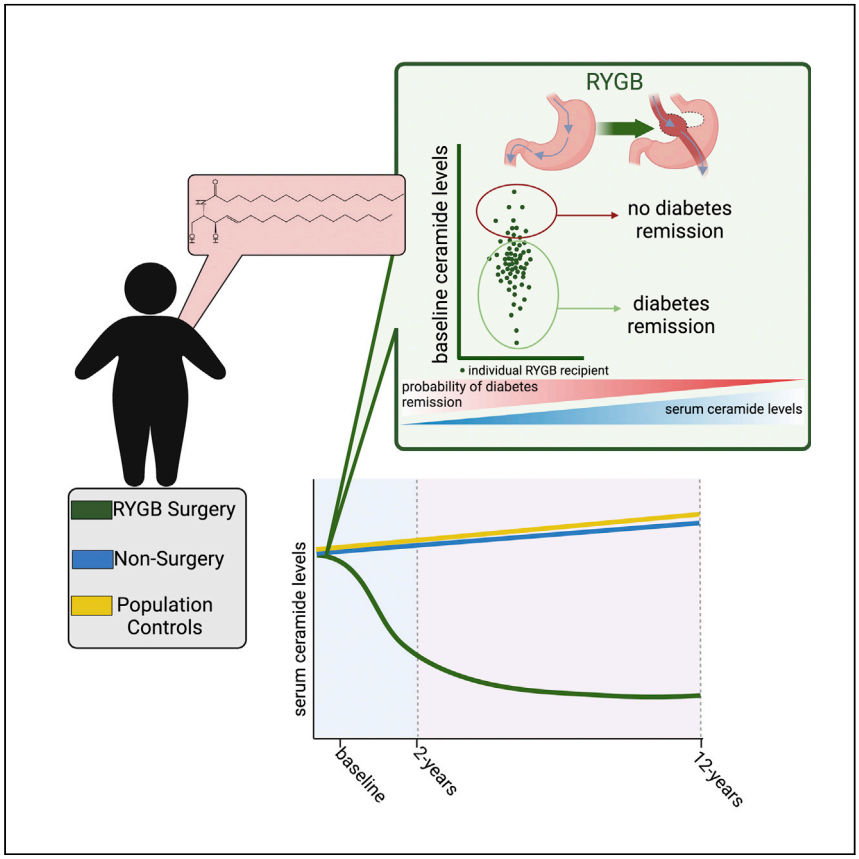
Following Roux-en-Y gastric bypass surgery, serum ceramides demarcate patients that will fail to achieve normoglycemia and diabetes remission



Poss et al. find that Roux-en-Y gastric bypass surgery decreases circulating ceramides for up to 12 years after surgery. Moreover, the authors identify ceramides as markers of poor glycemic control and inverse predictors of diabetes remission.

Annelise M. Poss, Benjamin Krick, J. Alan Maschek, ..., William L. Holland, Mary C. Playdon, Scott A. Summers

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Highlights

Serum ceramides decrease 2 and 12 years after RYGB surgery

Ceramides positively associate with HbA1c and HOMA-IR

Baseline ceramides inversely predict diabetes remission 2 years after surgery

Translation to Patients

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Following Roux-en-Y gastric bypass surgery, serum ceramides demarcate patients that will fail to achieve normoglycemia and diabetes remission

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SUMMARY

Background: Obesity is a prevalent health threat and risk factor for type 2 diabetes. In this study, we evaluate the relationship between ceramides, which inhibit insulin secretion and sensitivity, and markers of glucose homeostasis and diabetes remission or recursion in patients who have undergone a Roux-en-Y gastric bypass (RYGB).

Methods: The Utah Obesity Study is a prospective cohort study, with targeted ceramide and dihydroceramide measurements performed on banked serum samples. The Utah Obesity Study consists of 1,156 participants in three groups: a RYGB surgery group, a non-surgery group denied insurance coverage, and severely obese population controls. Clinical examinations and ceramide assessments were performed at baseline and 2 and 12 years after RYGB surgery.

Findings: Surgery patients (84% female, 42.2 ± 10.6 years of age at baseline) displayed lower levels of several serum dihydroceramides and ceramides at 2 and 12 years after RYGB. By contrast, neither the control group (77% female, 48.7 ± 6.4 years of age at baseline) nor the non-surgery group (95% female, 43.0 ± 11.4 years of age at baseline) experienced significant decreases in any species. Using a linear mixed effect model, we found that multiple dihydroceramides and ceramides positively associated with the glycemic control measures HOMA-IR and HbA1c. In surgery group participants with prevalent diabetes, ceramides inversely predict diabetes remission, independent of changes in weight.

Conclusions: Ceramide decreases may explain the insulin sensitization and diabetes resolution observed in most RYGB surgery patients.

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Context and Significance

Roux-en-Y gastric bypass surgery induces rapid and sustained weight loss and improved metabolic health, including the unique phenomenon of type 2 diabetes remission. However, the precise mechanisms linking Roux-en-Y gastric bypass to improved metabolic parameters is poorly understood. Ceramides are a deleterious lipid subtype that accumulate in states of metabolic distress. We found that they decrease dramatically after Roux-en-Y gastric bypass. This decrease in ceramides is sustained for up to 12 years after the procedure. Moreover, ceramides measured at the time of surgery inversely predict whether patients will achieve diabetes remission. These data suggest that ceramides could be an important clinical tool for gauging metabolic responses to surgery in this patient population.

INTRODUCTION

The global prevalence of obesity has increased dramatically over the last 50 years,[1–3] and the condition contributes to noncommunicable diseases including type 2 diabetes (T2D) and cardiovascular disease.[4] As a result of these debilitating comorbidities, individuals with severe obesity face a dramatic decrease in lifespan (approximately 20 years).[4] The harmful relationship between obesity and health are unlikely to be explained solely by adipose tissue mass. Instead, obesity likely reflects the presence of a lipotoxic state, where the ectopic deposition of deleterious lipid metabolites in non-adipose tissues drives the cellular dysfunction that underlies disease.[5,6] Studies in preclinical models suggest that ceramides are among the more

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harmful and bioactive lipid species that accumulate in lipotoxicity, because they alter metabolism and survival[7,8] and impair insulin secretion and sensitivity.[7,8] We, thus, sought to evaluate the relationship between ceramides and measures of glycemic control in patients undergoing Roux-en-Y gastric bypass (RYGB) surgery—a procedure that is associated with a multitude of metabolic benefits.[9–11]

RYGB is a restrictive-malabsorptive procedure that accounts for 44% of bariatric procedures performed in the United States.[12] The procedure entails surgical division of the stomach into two sections to form a pouch out of the proximal stomach, which restricts the volume of enteral intake. Surgeons divide the proximal jejunum and create a long Roux limb, which they connect to the gastric pouch, with the bile inflow from the duodenum and proximal jejunum reconnected to the Roux limb approximately 100–150 cm distal to the site of the attachment. The procedure results in the malabsorption of ingested fat and carbohydrates[13] and has demonstrated durable weight loss, greatly decreasing obesity-related morbidity and mortality.[9–11] A meta-analysis of randomized controlled trials indicates that patients who undergo bariatric surgery—as compared with those undergoing non-surgical weight loss interventions—lose on average 26 kg more body weight and have higher T2D remission rates than non-surgical patients.[9] For most patients, RYGB results in sustained weight loss and diabetes remission for more than a decade.[10,11] However, RYGB is an invasive procedure with variation in the extent and permanence of weight loss and comorbidity resolution, with a fraction of patients regaining weight and/or re-developing diabetes or other comorbidities in the years after the surgery.[10,14] The studies described herein sought to determine the long-term trajectory of ceramides in patients after RYGB and to discern their relationships with sustained, glycemic improvements, and diabetes remission.

Ceramide synthesis occurs in the endoplasmic reticulum. The first reaction of the multistep pathway (Figure 1A), catalyzed by serine palmitoyltransferase, condenses fatty (typically palmitoyl-CoA) and amino (typically serine) acids to produce the sphingoid backbone that is characteristic of sphingolipids.[15] In a subsequent reaction, one of six (dihydro)ceramide synthases (CERS1–6) adds a variable acyl chain to the sphingoid scaffold,[16] producing much of the variety in the sphingolipid pool. These CERS enzymes demonstrate distinct substrate selectivity and tissue expression patterns. The dihydroceramide products of the CERS reactions can be desaturated in the d4 position of the sphingoid backbone by dihydroceramide desaturase-1 to produce the more abundant and deleterious ceramides. Ceramides and dihydroceramides can be trafficked to the Golgi apparatus, where they may be converted into more abundant complex sphingolipids such as sphingomyelins (with the addition of phosphocholine), or gangliosides (with the addition of sugar moieties). Ceramides can be re-formed through sphingomyelin hydrolysis or re-acylating sphingosine generated by ceramidase-mediated ceramide breakdown. The ceramide synthesis pathway is, thus, a complex and dynamic metabolic pathway yielding a multitude of distinct species with unique bioactive signaling roles.

In preclinical models, genetic or pharmacological inhibition of enzymes required for ceramide biosynthesis ameliorates insulin resistance and T2D.[17,18] In humans, circulating ceramides reflect tissue levels and are potent, cholesterol-independent, clinical markers of diabetes and cardiovascular disease.[19–23] Indeed, because of their predictive value, a small number of clinics have started using blood-based ceramide scores as markers of adverse health outcomes.[24] A handful of small ($n \leq 20$) studies have measured circulating ceramides within the first 6 months after bariatric surgery.[25–27] Indeed, these studies have generally suggested that a decrease in

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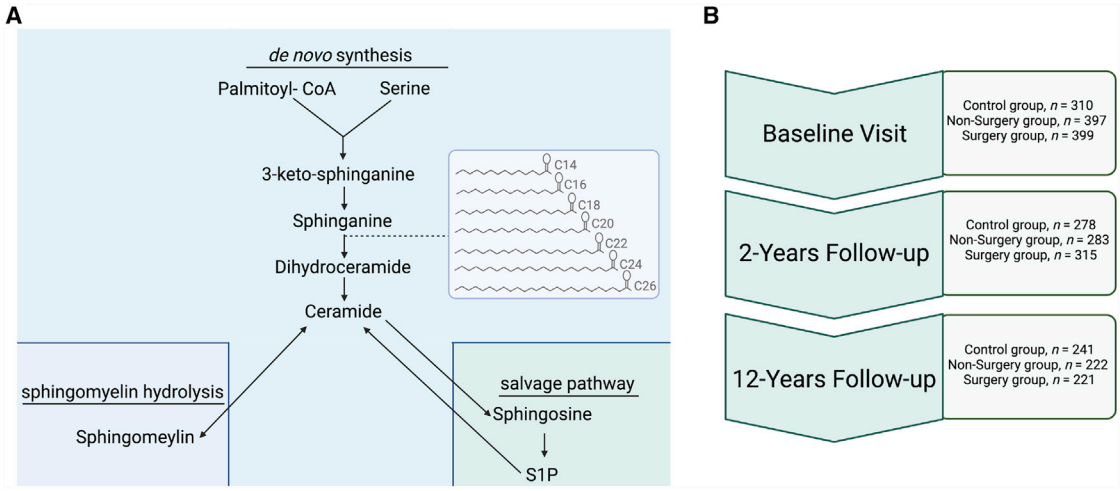

Figure 1. Ceramide synthesis pathway and UOS study design schematics

(A) Ceramides are produced through a ubiquitous *de novo* synthesis pathway. They are the major precursor of complex sphingolipids, and they can be re-formed through the degradation of sphingomyelins or the re-acylation of sphingosine. Much of the diversity of the sphingolipid species comes through the addition of acyl-chains to sphinganine (insert), through a reaction catalyzed by the CERS enzymes. Although the sphingolipidome is diverse, evidence is mounting that distinct acyl chain and lipid species may have discrete biological roles and tissues of origin.

(B) UOS sample collection occurred at baseline (pre-surgery), as well as at 2 years and 12 years after the procedure. Our workflow included available biospecimen procurement, targeted liquid chromatography tandem mass spectrometry sphingolipid measurement, and data analysis to characterize the response of ceramides and dihydroceramides to RYGB and the relationship between ceramides and diabetes remission. Figure generated with BioRender.com.

ceramides follows the surgical procedure.[25–27] However, these studies are small, lack obese control groups, and occur during the extreme post-operative weight loss period. We sought to characterize the effect of RYGB on ceramides following the nadir of weight loss using the Utah Obesity Study (UOS), a prospective study that follows RYGB surgery patients (n = 399), non-surgery controls (n = 397), and population controls with severe obesity (n = 310) for 12 years after the surgery (study schematic in Figure 1B). Herein we report the findings of these studies, which revealed dramatic relationships between post-surgical ceramides, measures of insulin sensitivity and glucose control, and diabetes.

RESULTS

The UOS leverages a powerful study design that allows for an extensive assessment of clinical outcomes and blood biomarkers after the nadir of maximum weight loss, thus providing an opportunity to interrogate durable post-surgical metabolic alterations. By measuring sphingolipids at baseline, as well as at 2 and 12 years after surgery, we assessed sustained alterations in sphingolipids that were neither a result of the immediate stress response to surgery nor a transient response to the dramatic weight loss that typically occurs in the first year after gastric bypass.

Cohort characteristics

General cohort characteristics are presented in Table 1. UOS participants were predominantly female (77% control, 95% non-surgery, 84% surgery), which is typical in the bariatric surgery population.[28] The surgery group lost an average of 35.0% of body weight (in kilograms) at 2 years, with patients showing subsequent weight gain (8.1%) during the interval between 2 and 12 years after surgery. A total of 289 surgery group participants re-gained weight in the 2- to 12-year follow-up period.

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Table 1. Clinical characteristics of the UOS participants at baseline, 2 years, and 12 years of follow-up

	Baseline			2 Years			12 Years		
	Control	Non-surgery	Surgery	Control	Non-surgery	Surgery	Control	Non-surgery	Surgery
Age (years)	49.55 ± 10.83	42.82 ± 11.27	42.70 ± 10.87	51.76 ± 10.74	47.07 ± 11.16	46.41 ± 10.72	60.31 ± 10.69	54.07 ± 10.94	54.97 ± 10.41
n	310	397	399	278	283	315	221	222	241
Sex									
Female, *n* (%)	*205* (78)	*280* (86)	*277* (74)	*185* (78)	*200* (86)	*213* (83)	*145* (76)	*161* (88)	*166* (83)
BMI (kg/m^2)	43.65 ± 6.40	46.21 ± 7.70	47.31 ± 7.71	43.68 ± 7.36	43.96 ± 8.61	30.52 ± 6.49	42.81 ± 9.28	41.77 ± 9.38	34.92 ± 8.33
Smoking status									
Yes, *n* (%)	*36* (12)	*102* (26)	*109* (28)	*25* (9)	*49* (18)	*65* (21)	*35* (17)	*60* (29)	*65* (28)
Anti-hypertensive medication									
Yes, *n* (%)	*120* (33)	*123* (31)	*138* (35)	*123* (45)	*103* (39)	*45* (15)	*96* (50)	*73* (44)	*44* (23)
Systolic blood pressure	128.75 ± 18.80	125.19 ± 17.68	126.30 ± 19.17	127.60 ± 19.30	125.13 ± 18.76	115.91 ± 18.24	125.80 ± 18.74	121.22 ± 19.61	119.95 ± 18.08
Diastolic blood pressure	72.21 ± 10.48	71.92 ± 10.82	71.76 ± 11.25	71.76 ± 11.21	72.71 ± 11.02	70.03 ± 9.84	69.68 ± 9.30	70.75 ± 10.86	70.55 ± 10.33
Lipid-lowering medication									
Yes, *n* (%)	40 (15)	46 (14)	53 (16)	55 (23)	39 (18)	18 (7)	65 (38)	44 (35)	19 (13)
Total cholesterol (mg/dL)	188.62 ± 35.64	185.15 ± 37.61	188.18 ± 33.57	189.75 ± 38.10	182.03 ± 39.79	166.24 ± 32.62	176.93 ± 37.14	181.47 ± 37.99	177.26 ± 31.55
LDL-C (mg/dL)	106.20 ± 30.45	102.21 ± 29.97	104.32 ± 28.97	113.36 ± 33.95	103.95 ± 31.50	88.44 ± 28.77	102.42 ± 34.57	104.02 ± 33.24	97.20 ± 28.70
HDL-C (mg/dL)	47.23 ± 10.92	44.60 ± 10.73	46.63 ± 11.52	44.67 ± 11.47	43.32 ± 11.55	56.91 ± 13.72	47.82 ± 12.56	52.44 ± 16.92	61.29 ± 18.39
VLDL-C (mg/dL)	33.12 ± 17.71	35.37 ± 22.99	34.17 ± 19.90	29.56 ± 15.01	31.69 ± 26.95	19.04 ± 9.76	16.34 ± 22.75	14.51 ± 15.02	8.46 ± 6.50
Triglycerides (mg/dL)	176.12 ± 86.48	193.83 ± 123.43	186.19 ± 97.29	158.98 ± 77.35	179.24 ± 196.75	104.37 ± 53.06	143.58 ± 81.21	138.81 ± 77.31	102.92 ± 43.48
Diabetes characteristics									
Diabetes status									
Yes, *n* (%)	90 (29)	108 (27)	86 (22)	89 (32)	71 (25)	18 (6)	79 (41)	55 (30)	24 (12)
HbA1c	5.93 ± 1.09	5.99 ± 1.27	5.79 ± 1.04	6.11 ± 1.02	5.95 ± 0.92	5.59 ± 0.75	6.39 ± 1.59	5.98 ± 1.34	5.68 ± 0.85
HOMA-IR	3.65 ± 3.78	4.82 ± 4.27	5.01 ± 4.80	5.04 ± 4.05	4.89 ± 5.31	1.26 ± 1.33	6.15 ± 11.43	3.98 ± 7.30	2.07 ± 2.27
HOMA-B	152.81 ± 190.72	203.35 ± 209.54	232.99 ± 249.98	199.40 ± 143.21	249.64 ± 241.82	162 ± 329.96	214.66 ± 502.00	169.11 ± 126.18	153.09 ± 206.40
Insulin	13.79 ± 13.06	17.88 ± 14.45	19.52 ± 16.63	18.53 ± 11.41	19.02 ± 14.59	6.03 ± 6.15	20.74 ± 32.38	13.86 ± 18.67	9.05 ± 8.77
Glucose	106.65 ± 31.60	107.83 ± 41.19	101.91 ± 31.28	106.09 ± 35.54	98.77 ± 31.08	83.31 ± 22.10	107.69 ± 45.05	99.28 ± 38.03	89.68 ± 23.08
Glucose-lowering medication	61 (20)	78 (20)	65 (16)	73 (28)	60 (23)	14 (5)	68 (35)	49 (27)	24 (22)
Diabetes age of onset	47.19 ± 10.79	39.87 ± 11.15	40.24 ± 12.09	49.52 ± 13.87	48.86 ± 6.34	35.75 ± 9.32		45.96 ± 10.89	39.55 ± 18.21
Weight at diabetes diagnosis	251.40 ± 47.19	266.00 ± 60.40	265.59 ± 66.76	281.47 ± 73.35	288.50 ± 40.20	318.00 ± 145.69	266 ± 57.03	287.50 ± 67.17	291.14 ± 86.48
Body habitus									
Weight (kg)	123.35 ± 22.94	129.73 ± 24.98	133.96 ± 26.89	123.63 ± 25.30	122.84 ± 27.20	86.81 ± 21.66	118.75 ± 32.45	113.41 ± 27.29	96.93 ± 26.04
Waist circumference (cm)	130.55 ± 15.88	134.36 ± 17.15	135.89 ± 17.96	131.77 ± 16.98	129.63 ± 19.42	101.77 ± 18.84	131.55 ± 20.99	127.11 ± 22.10	113.88 ± 22.96

Clinical characteristics of participants in the UOS (control, baseline n = 310, 2-year n = 278, 12-year n = 221; non-surgery, baseline n = 397, 2-year n = 283, 12-year n = 221; surgery, baseline n = 399, 2-year n = 315, 12-year n = 241). Continuous variables presented as mean ± SD and categorical variables presented as number (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-B, homeostatic model of β-cell function; HOMA-IR, homeostatic model of insulin resistance; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

Effect of RYGB on dihydroceramides and ceramides

We measured six ceramide species, including the Cer(d18:1/16:0) and Cer(d18:1/18:0) species that have been identified in rodents as likely causal drivers of

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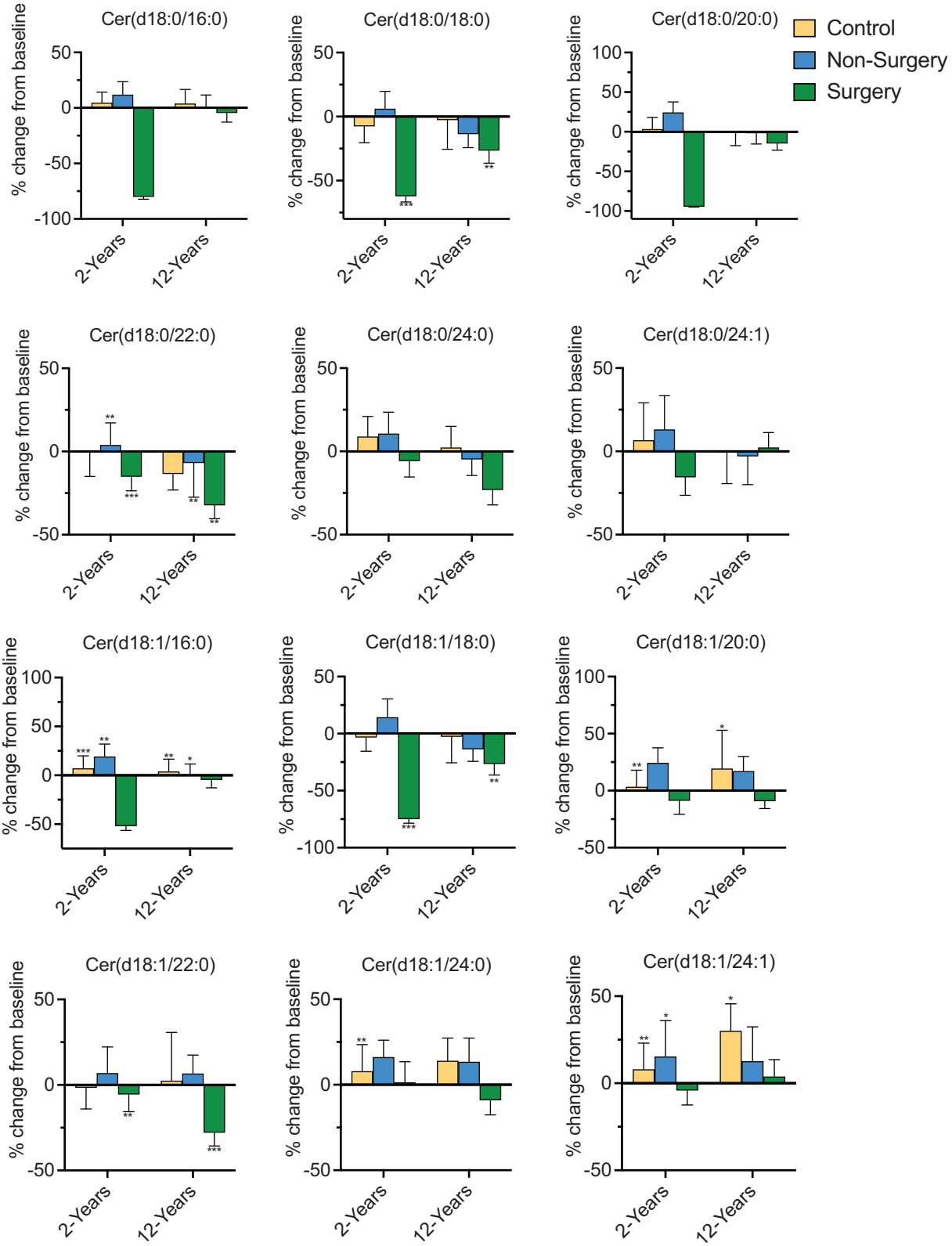

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metabolic dysfunction and insulin resistance.[29,30] We also quantified six dihydroceramides, which are immediate precursors of ceramides in the *de novo* synthesis pathway and are often good markers of flux through the enzymatic cascade, as well as being implicated as predictive biomarkers of diabetes.[23] Means and interquartile ranges for the 12 lipids are provided in Table S1. Using multilevel modeling, we demonstrate that surgery decreased levels of several sphingolipid species, either during the (i) baseline to 2-year or (ii) 2-year to 12-year post RYGB intervals. The sphingolipids that were most responsive to surgery included Cer(d18:0/18:0), Cer(d18:0/22:0), Cer(d18:1/18:0), and Cer(d18:1/22:0). For other ceramides, surgery blunted an increase in lipid levels over time; for example, surgery blocked the increase in the potentially pathogenic Cer(d18:1/16:0) species observed in the control and non-surgery groups at the 2- and 12-year time points, as compared with baseline measurements. Surgery similarly blunted increases in Cer(d18:1/20:0), Cer(d18:1/24:0), and Cer(d18:1/24:1). The changes in individual ceramide and dihydroceramides are depicted as a percent change from baseline to 2 years and baseline to 12 years for the control (yellow), non-surgery (blue), and surgery (green) groups (Figure 2). Significance was derived from the multilevel model using $\log_{10}$-transformed lipid concentrations with the raw concentrations visualized in Figure S1. Although not all differences attained statistical significance, many other species followed a similar pattern, with surgery tending to decrease their levels in comparison with the control and non-surgery groups. Interestingly, some species, such as Cer(d18:1/22:0), exhibited a greater magnitude of change from baseline to 12 years than from baseline to 2 years, whereas others showed the larger changes at the earlier time interval. The reason for these differential patterns is unclear. Table S2 contains estimates and 95% confidence intervals, raw p values, and Benjamini-Hochberg-adjusted p values for change in ceramide concentration for each study group at each time interval from the multilevel models. To further characterize the relationship between ceramides and weight loss, we performed cross-sectional logistic regression—sequentially adjusting for covariates as noted above—to monitor the association of each lipid species with significant weight loss (defined as a 20% weight decrease from baseline to 2 years, which has been previously reported as the average durable weight loss after RYGB[31]) (Table S3).

Ceramides associate with measures of poor glycemic control

We probed the temporal relationships between individual dihydroceramides or ceramides versus measures of glycemic control (i.e., hemoglobin A1c [HbA1c] and homeostatic model of insulin resistance [HOMA-IR]) using linear mixed effect models. We used a linear mixed effect model, leveraging the unique longitudinal study design of the UOS and pooling all study visits. This approach allowed us to evaluate the trajectory of HOMA-IR or HbA1c over time in association with the temporal trajectory of individual lipid species. We note that we adjust for study group and BMI at each visit in order to account for post-surgical changes in body habitus. All six dihydroceramides (Cer[d18:0/16:0], Cer[d18:0/18:0], Cer[d18:0/20:0], Cer[d18:0/22:0], Cer[d18:0/24:0], and Cer[d18:1/24:1]) and ceramides Cer(d18:1/22:0) and Cer(d18:1/24:0) were positively associated with HbA1c

Figure 2. Percent change in dihydroceramide and ceramide concentrations from baseline to 2-year and baseline to 12-year in the UOS

Figure depicts the percent change in six ceramide and six dihydroceramide species over two time intervals (baseline to 2-year; baseline to 12-year) for each of the study groups: control (yellow), non-surgery (blue) and surgery (green). Covariates adjusted for include age, sex, baseline BMI, race, ethnicity, marital status, income, education, smoking status, blood pressure, triglycerides, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, anti-hypertensive medication, and lipid-lowering medication. Asterisks denote false discovery rate-adjusted significance from the multivariable adjusted multilevel model: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. The median is graphed, and the error bar represents the 95% confidence interval.

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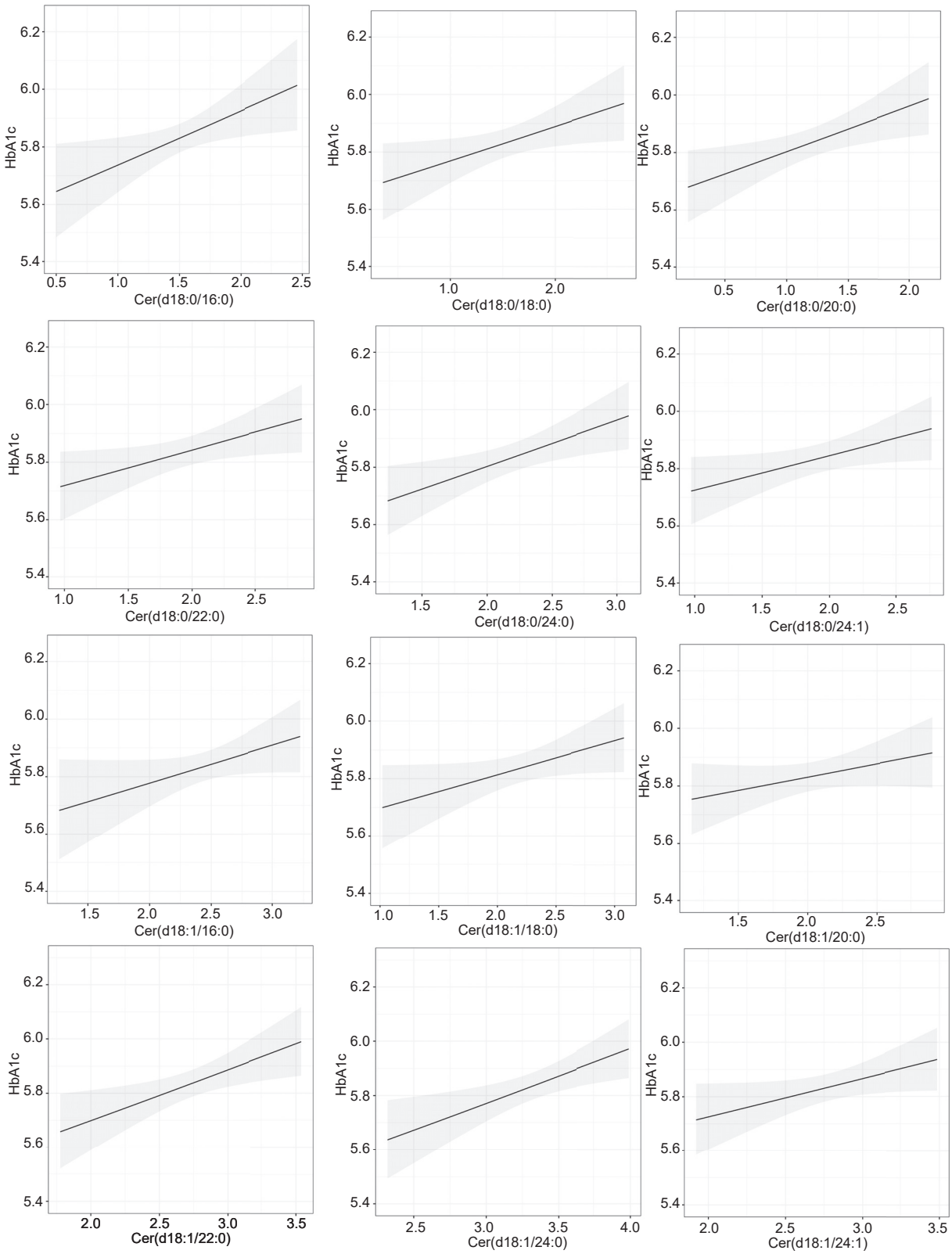

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(Figure 3). Similarly, many dihydroceramides (Cer[d18:0/16:0], Cer[d18:0/20:0], Cer[d18:0/22:0], and Cer[d18:0/24:0]) and ceramides (Cer[d18:1/22:0] and Cer [d18:1/24:0]) showed positive relationships with HOMA-IR (Figure 4). These data support the hypothesis that ceramides induce insulin resistance, as suggested by preclinical studies in mice and cells.[17,18,32] Table S4 contains estimates, 95% confidence intervals and Benjamini-Hochberg-corrected p values for these analyses. In contrast, serum dihydroceramides and ceramides did not associate with the homeostasis model of beta cell function (HOMA-B) (Table S4). The positive significant association with HOMA-IR coupled with the null association with HOMA-B suggests that circulating ceramides in this study associate with insulin resistance, but not insulin secretion.

Low baseline ceramides predict 2-year diabetes remission and demarcate patients who experience durable diabetes remission

Since bariatric surgery is one of the few clinical interventions that can lead to diabetes remission, the UOS provides a unique opportunity to evaluate relationships between ceramides and disease resolution. Indeed, 73% of the patients with diabetes (16.5% of the total surgery group; n = 66), experienced either a transient or sustained resolution of diabetes (Figure 5A). Of the 67 participants with serum ceramide data available at baseline and 2 years, 18 had persistent diabetes and 49 achieved diabetes remission (i.e., a 73% remission rate). Transient remission was defined as the disappearance of diabetes at 2 years, but subsequent re-diagnosis at 12 years (n = 9), while sustained remission refers to the resolution of diabetes at the 2- and 12-year time points (n = 40). By comparison, 27% of patients (18 patients) failed to achieve any resolution of diabetes.

Baseline dihydroceramides and ceramides predict 2-year diabetes remission (Figure 5B). Table S5 presents the odds ratios, 95% confidence intervals, and p values for the association of baseline and 2-year ceramides with diabetes remission. Associations were significant when adjusting for diabetes remission related factors (duration of diabetes, baseline HbA1c, diabetes medication). We also tested whether inclusion of ceramides in a diabetes remission clinical predictive model improved prediction as assessed by the receiver operator characteristic-area under the curve (ROC-AUC) C-statistic. We incorporated ceramides and dihydroceramides in a stepwise fashion into a baseline clinical model that includes HbA1c, diabetes duration, and diabetes medication. We retained sphingolipids that improved model fit according to the C-statistic. While no single ceramide species improved the clinical model, the inclusion of all ceramide species had a substantial effect (Figure S4). We repeated the analysis cross-sectionally by including 2-year ceramides, which also improved the clinical model (Figure S3).

We observed a decrease in all ceramide species from baseline to 2 years in participants who experienced diabetes remission (Figure S4). The relationship was less robust for dihydroceramides (data not shown). We conducted a sensitivity analysis to further evaluate the relationships between ceramides and the durability of diabetes remission, finding that the sphingolipids were associated inversely with

Figure 3. Marginal effects plots depicting relationships between dihydroceramides/ceramides and glycated hemoglobin

Log_{10} transformed serum dihydroceramide and ceramide concentrations positively associate with HbA1c levels. Each plot shows the magnitude of increase in HbA1c (y axis) associated with a given increase in a specific ceramide species (x axis), with the shaded area representing the 95% confidence interval. For this analysis all three study groups were pooled together, but the model adjusted for study group and BMI at each time point in addition to age, sex, baseline BMI, race, ethnicity, marital status, income, education, smoking status, blood pressure, triglycerides, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, anti-hypertensive medication, lipid-lowering medication.

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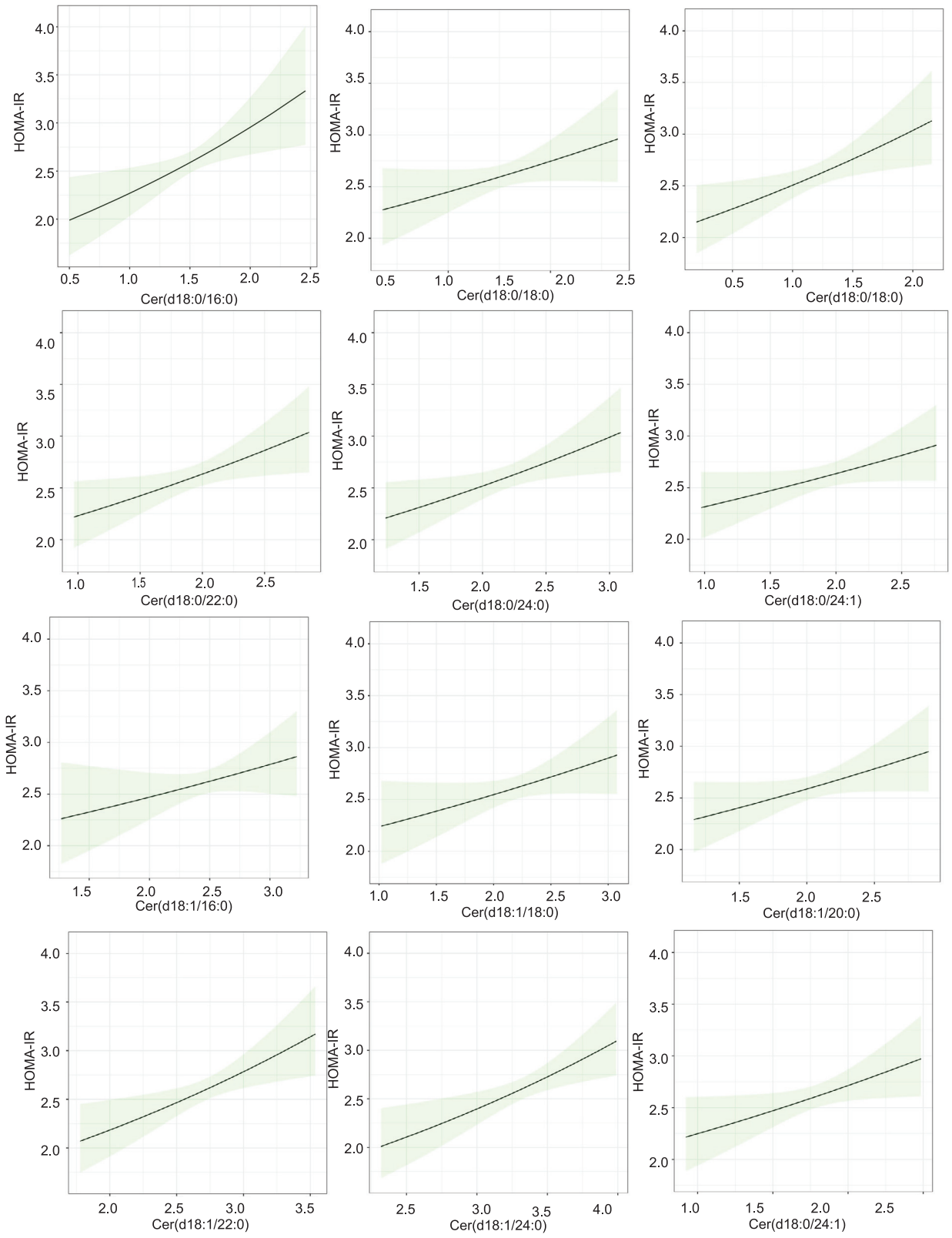

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sustained diabetes remission (Figure 5C). These data suggest that ceramide levels may decrease in individuals who have undergone RYGB surgery who are to achieve prolonged (i.e., 12-year) resolution of diabetes, but remain elevated in those that were going to re-develop the disease (Figure 5C). However, these sensitivity analysis results did not reach significance, perhaps owing to the small number of individuals in each of these unique subgroups. Nonetheless, these provocative data suggest that, even before re-diagnosis, patients achieving only transient remission may already exhibit a ceramide pattern similar to the persistent diabetes group, suggesting that ceramides contribute to diabetes etiology. This pattern in post-surgical ceramide levels was independent of weight, and BMI was unrelated to diabetes status in the surgical patients ($p = 0.949$) (Figure 5D). Thus, monitoring ceramides after surgery has predictive potential to reveal ultimate outcomes on diabetes remission and in remission durability.

Ceramides do not associate with weight re-gain

To explore the relationship between ceramides and post-RYGB weight re-gain in the 2- to 12-year period, we performed a linear regression with individual lipid species as the exposure and percent weight re-gain as the outcome. We observed no significant associations at the false discovery rate of less than 0.05 between ceramides at 2 years or 12 years with post-RYGB weight re-gain (Table S6). For the control and non-surgery groups, the average percent body weight change was 0.6% and 3.5% from baseline to 2 years, respectively. The subsequent weight gain for these groups during the 2- to 12-year interval was 2.9% and 6.5%, respectively.

Ceramides do not associate with serum cholesterol or triglycerides

In this cohort, RYGB decreases levels of circulating lipids—including low-density lipoproteins, very low-density lipoprotein cholesterol, and triglycerides—while increasing levels of high-density lipoprotein cholesterol.[10] We evaluated correlations between these lipids with ceramides and dihydroceramides at baseline and 2 years (Table S7; Figures S5A and S5B). We also correlated percent change in lipid concentration from baseline to 2 years with ceramides and dihydroceramides (Table S7, Figure S5C). These data align with previous publications indicating that sphingolipid species and lipoprotein levels are conditionally independent of one another.[22]

DISCUSSION

We applied a highly quantitative targeted lipidomics method to measure serum sphingolipids in patients undergoing RYGB to understand the relationship between serum ceramides and durable glycemic control. The work demonstrated that bariatric surgery decreased the levels of several dihydroceramides and ceramides, which positively associated with HOMA-IR and HbA1c. Remarkably, ceramides measured at the baseline visit predicted which patients would fail to achieve diabetes remission. Moreover, ceramides measured 2 years after surgery seemed to differentiate those who would achieve sustained diabetes remission at the

Figure 4. Marginal effects plots for dihydroceramide and ceramide concentrations and the homeostatic model of insulin resistance in the UOS

Log_{10}-transformed serum dihydroceramide and ceramide concentrations positively associate with HOMA-IR values. Each plot shows the magnitude of increase in HOMA-IR (y axis) associated with a given increase in a specific ceramide species (x axis), with the shaded area representing the 95% confidence interval. For this analysis all three study groups were pooled together, but the model adjusted for study group and BMI at each time point in addition to age, sex, baseline BMI, race, ethnicity, marital status, income, education, smoking status, blood pressure, triglycerides, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, anti-hypertensive medication, and lipid-lowering medication.

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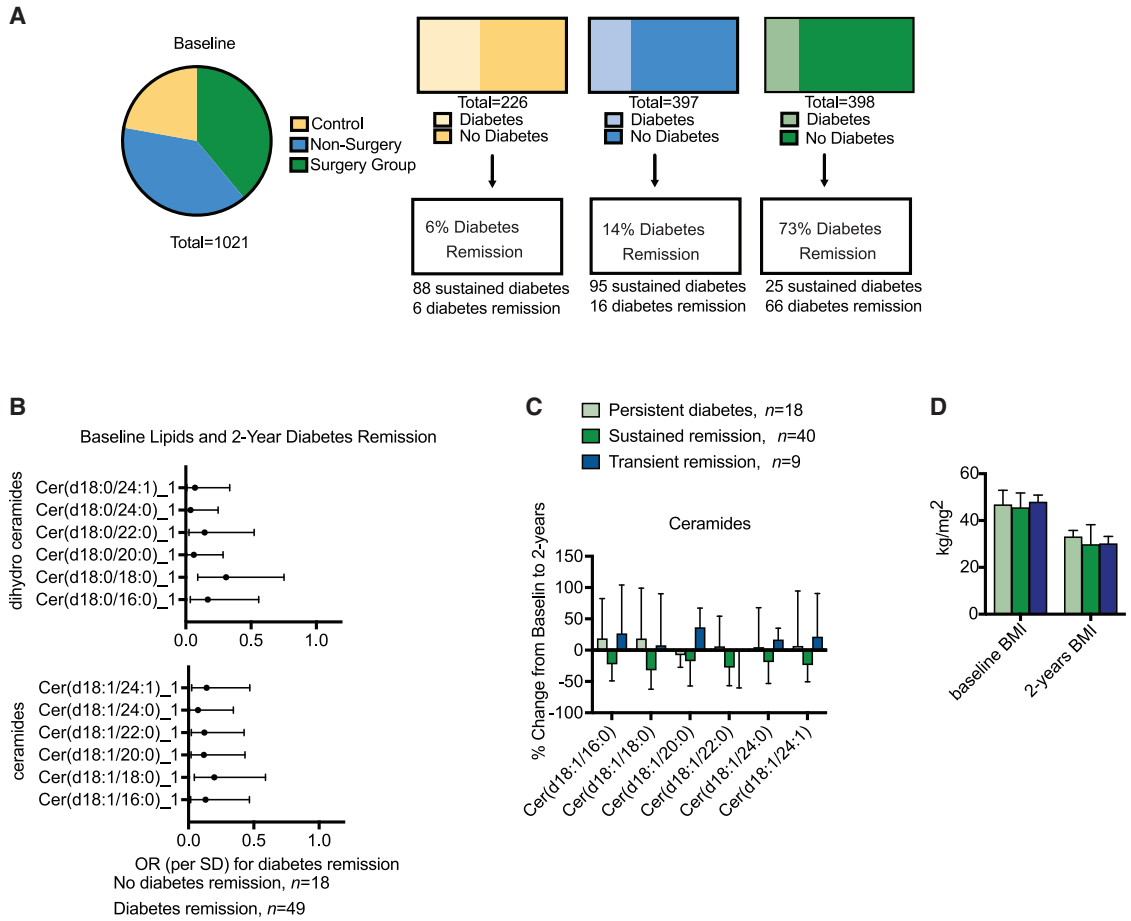

Figure 5. Assessment of dihydroceramides and ceramides in relation to diabetes remission in surgery group participants

Ceramides and dihydroceramides are assessed in relation to RYGB-induced diabetes remission.

(A) Characteristics of each study group, including the proportion of subjects that had diabetes at baseline and the proportion that that achieved diabetes remission at the 2-year follow-up visit.

(B) Forest plot of odds ratios (ORs) for 2-year diabetes remission indicating a significant inverse relationship between 2-year diabetes remission and baseline ceramides and dihydroceramides.

(C) Baseline and 2-year ceramides in subjects with diabetes at baseline. We stratified ceramides, reporting the change from baseline at 2-year in people that experienced persistent diabetes throughout the study (i.e., remained diabetic at the 2- and 12-year visits; light green), those who experienced sustained diabetes remission (i.e. had resolution of diabetes at the 2- and 12-year visits; dark green), and those who experienced transient diabetes remission (i.e., displayed resolution of diabetes and 2 years, but were re-diagnosed with diabetes by the 12-year visit; navy). The median is graphed, and the error bar is the 95% confidence interval.

(D) BMI at baseline and 2 years for patients who underwent surgery with prevalent diabetes at baseline. The median is graphed, and the error bar is the 95% confidence interval. Please note the *n* listed for each analysis next to the graphs. Some study participants did not have biospecimens available for ceramide profiling, hence the difference between figure A and the subsequent figures.

12-year visit from those who would ultimately fail to sustain normoglycemia. These data indicate that lipotoxic ceramides associate with diabetes development. Moreover, they support the hypotheses generated from studies in preclinical models that ceramides may play causal roles in insulin resistance and diabetes progression.

These results are consistent with several studies in much smaller cohorts ($n \leq 20$) that demonstrated acute reductions in serum ceramides in the months that follow bariatric surgery (i.e., up to 6 months).[25–27] One study reported a significant decrease in very long chain (VLC) (C22–24) ceramides at 1 and 30 days after laparoscopic sleeve

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gastrectromy.[25] That study similarly reported a positive association between VLC ceramide species and HOMA-IR.[25] In two studies of RYGB recipients with 6-month follow-up, one reported a post-operative decrease in Cer(d18:1/14:0), Cer(d18:1/16:0), Cer(d18:1/20:0), and Cer(d18:1/24:0), while the other reported decreases in Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/24:0), and Cer(d18:1/24:1).[26,27] Neither of these studies reported Cer(d18:1/22:0) concentrations, which we identify as one of the most dynamic and most dramatically lowered species in the UOS surgery group. These prior studies captured ceramides during the extreme post-operative weight loss period, while the UOS measurements occur after the nadir of weight loss and includes both severely obese non-surgery (i.e., met the criteria for surgery but did not receive it) and severely obese population (i.e., did not pursue bariatric surgery) control groups. Since the UOS is both larger and longer, it provides opportunities to monitor the long-term trajectory of serum ceramides after RYGB, while also permitting an analysis of relationships with disease outcomes. For example, the study population permits us to evaluate ceramides in subjects experiencing wide swings in glucose homeostasis, including some that experience sustained diabetes remission. The data obtained from this robust study population indicate that ceramides associate with glucose homeostasis and support measurements of serum ceramides as markers of long-term metabolic outcomes after surgical weight loss. Importantly, the analysis demonstrates the magnitude of the ceramide-diabetes relationship in a severely obese population, which is the most critical population to target for risk stratification. Furthermore, the findings are consistent with cross-sectional studies showing that ceramides associate with markers of insulin resistance and hyperglycemia and that ceramides are biomarkers of incident T2D.[20,23]

Several studies have shown that diabetes remission is not explained by weight loss alone or degree of weight loss.[11,33] Indeed, several patients in the UOS achieved sustained decreases in body weight, but failed to achieve prolonged resolution of diabetes. The data presented herein implicate ceramides as a potential contributor to this elusive lipotoxic mechanism. For example, Figure 5 demonstrates that ceramides, but not weight, demarcated patients who failed to achieve prolonged diabetes remission.

RYGB most dramatically decreased levels of ceramides and dihydroceramides containing the C18 or C22 acyl side chains, suggesting a diminution of biosynthetic flux in the tissues that produce these species (Figure 1A). The CERS1 and four enzymes—which are primarily expressed in brain and skeletal muscle—add the 18 carbon chains to the sphingoid scaffold. Indeed, Cer(d18:1/18:0) is the most abundant ceramide in skeletal muscle, and the lipid has been implicated in insulin resistance and mitochondrial dysfunction.[30] Moreover, the dihydroceramide Cer(d18:0/18:0) has been shown to predict diabetes incidence up to 9 years before disease manifestation.[23] These data are consistent with our findings; these species were diminished by RYGB and correlated with HOMA-IR. CERS2 and CERS3, which are primarily expressed in the kidney, liver, and skin, produce the dihydroceramides and ceramides containing 22 carbon acyl chains. Ceramides containing this side chain are also associated with insulin resistance and diabetes incidence in human prospective studies.[23]

One of the most important clinical outcomes of RYGB is the remarkably high rate of diabetes remission.[11] Thus, the UOS presents a unique opportunity to gauge the relationship between ceramides and the disappearance or reappearance of diabetes. In our analyses, all ceramide species at baseline significantly associated

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with 2-year diabetes remission and also associated with a failure to achieve sustained normoglycemia 12 years after surgery. Moreover, the addition of all ceramide species to an ROC-AUC clinical predictive model containing baseline HbA1c, duration of diabetes, and diabetes medication use resulted in an improvement of the C-statistic. Ceramides containing the 16:0 acyl-chain are produced by the CERS6 enzyme that is highly expressed in adipose tissue.[29] In rodents, deletion of CERS6 decreased Cer(d18:1/16:0) and dramatically improves insulin sensitivity in glucose tolerance.[29] Mechanistically, this seems to occur in part because of the ability of this lipid to induce mitochondrial fission, which decreases their ability to effectively and efficiently oxidize glucose and other substrates.[34] Although our sample size for the diabetes remission analyses was relatively small, our data support the involvement of this lipid in glucose control in humans, suggesting that its accumulation can contribute to diabetes in post-surgical patients, regardless of whether they achieved weight loss.

In conclusion, this study presents exciting findings on the role of ceramides in glucose homeostasis by leveraging a unique and powerful study design in combination with state-of-the-art lipidomics technology. RYGB decreases several ceramide species, particularly in patients who achieved diabetes remission or improved glycemic control. Remarkably, such changes remained significant 12 years after surgery, long after the nadir of weight loss. Moreover, they reinforced the bidirectionality of the ceramide-glucose intolerance relationship, with ceramides positively associated with increased HOMA-IR and HbA1c and inversely associated with sustained diabetes remission. Indeed, the determination that 2-year ceramide measurements predicted outcomes at 12-year supports the use of post-surgical ceramide measurements as a means for gauging patient health. Ultimately, the data reveal a mechanism for RYGB in diabetes remission and suggest that other ceramide-lowering therapeutic strategies may prove efficacious for preventing or treating diabetes.

Limitations of the study

The long-term follow-up (12-year) after RYGB surgery in the UOS presents an exciting and unique opportunity to look at markers of long-term clinical outcomes. In particular, the UOS has a well-constructed cohort structure that includes two non-surgical control groups, which allow us to account for lifestyle changes pursued by non-surgery patients. The data are robust and suggest potent roles for ceramides as causal factors in insulin resistance and disease. Nonetheless, the analysis has a number of noteworthy weaknesses. First, we lack a replication cohort, as other prolonged follow-up analyses of bariatric surgery patients are lacking. Second, the UOS is racially and ethnically homogeneous, limiting the generalizability of our findings. Nonetheless, we highlight findings in the Strong Heart Study, comprising large numbers of American Indians; the authors of that study reported similar relationships between ceramides, HOMA-IR, and HbA1c.[20] Third, the UOS lacks a lean control group for comparison. The unavailability of lean counterparts limits some analyses, as virtually all participants meet the criteria for metabolic syndrome at baseline (data not shown). Additionally, we conducted all analyses in an intent-to-treat paradigm, and we did not exclude non-surgery and control participants who pursued weight loss surgeries or interventions outside of the study. Indeed, we know that a small number of patients in the non-surgical group did seek surgery at some later point, despite being denied initially. We did not exclude those patients, and our findings are thus a conservative estimate of the effects of RYGB surgery. Last, the UOS lacks an acute post-operative time point

(e.g., weeks to months) to track early changes in the lipidome, although previous smaller studies have explored this and reported corrobative findings.[25–27]

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2022.05.011.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.M.P., S.A.S., M.C.P., and W.L.H.; Methodology, B.H., B.K., and A.M.P.; Software, B.H., B.K., A.M.P., and P.K.; Validation, B.K.; Resources, J.A.M., B.H., S.C.H., and T.D.A.; Data Curation, J.A.M., S.C.H., and T.D.A.; Writing-Original Draft, S.A.S. and A.M.P.; Writing-Review and Editing, S.A.S. and A.M.P.; Visualization, A.M.P. and B.K.; Funding Acquisition, W.L.H. and S.A.S. We also wish to acknowledge A.M.P., B.K., B.H., and M.C.P. for their roles in performing, overseeing, and replicating statistical analyses. A.M.P., B.K., P.K., M.C.P., S.C.H.,

and S.A.S. all had unrestricted access to the data. All authors read and approved the final article and take responsibility for its content.

DECLARATION OF INTERESTS

S.A.S. is a cofounder of, consultant to, and shareholder in Centaurus Therapeutics. A.M.P., B.K., J.A.M., B.H., J.E.C., Y.L., T.S.T., P.K., A.I., S.C.H., T.D.A., M.C.P., and W.L.H. have no conflicts of interest to declare.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human serum	Study participants (Rocky Mountain Bariatric Clinic, University of Utah)	N/A
Chemicals, peptides, and recombinant proteins		
Sphingomyelin (d18:1/17:0)	Avanti	Cat #860585
dihydro-cer (d18:0/18:1)	Avanti	Cat #860624
d7-ceramide (d18:1-d7/16:0)	Avanti	Cat #860676
d7-ceramide (d18:1-d7/18:0)	Avanti	Cat #860677
d7-ceramide (d18:1-d7/24:0)	Avanti	Cat #860678
d7-ceramide (d18:1-d7/24:1)	Avanti	Cat #860679
glucosylceramide (d18:1/17:0)	Avanti	Cat #860569
d7-PC (15:0–18:1-d7)	Avanti	Cat #791637
Deposited data		
Deidentified raw data file	This paper	https://doi.org/10.7910/DVN/6UX5UK
Software and algorithms		
R Project for Statistical Computing	https://www.r-project.org/	N/A
Original Code	This paper	https://doi.org/10.7910/DVN/6UX5UK
Graphpad Prism 7.0	www.graphpad.com	sales@graphpad.com
BioRender	www.biorender.com	N/A
Other		
Lipid extraction	Havulinna et al., 2019	https://doi.org/10.1161/ATVBAHA.116.307497
LC-MS/MS analysis	Poss et al., 2020	https://doi.org/10.1172/JCI131838

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be promptly responded to by the lead contact, Scott Summers, 15N 2030E, Salt Lake City, Utah 84112, USA. Email: scott.a.summers@health.utah.edu.

Materials availability

Raw sphingolipid data is deposited at Dataverse and is publicly available (https://doi.org/10.7910/DVN/6UX5UK). Any additional information required to that may be required to reanalyze the data reported in the paper is available from the lead contact upon request.

Data and code availability

All original code has been deposited at Dataverse and is publicly available (https://doi.org/10.7910/DVN/6UX5UK). Any additional information that may be required to reanalyze the data reported in the paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The Utah Obesity Study (UOS) is an observational, prospective study initiated in July 2000. Of 1156 patients enrolled, 835 patients with severe obesity visited a single bariatric surgical center (Rocky Mountain Associated Physicians, Salt Lake City), seeking Roux-en-Y gastric bypass (RYGB). Of these, 418 proceeded with surgery (surgery group) and 417 did not, primarily due to denials from insurance companies (non-surgery group). An

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additional population control group consisting of 321 people with severe obesity was recruited from the Utah area. We measured serum sphingolipids on available biospecimens from 399, 397, and 310 participants in the surgery, non-surgery, and control groups, respectively (Figure S2). Participants in the control group were between 18 and 72 years of age, had no history of alcohol or narcotics abuse, and had never undergone bariatric surgery. Study subjects had no history of gastric or duodenal ulcers, myocardial infarction (in the past 6 months), or active cancer (in the past 5 years). The sex distribution of all study groups is skewed toward females (control group = 78% female, non-surgery group = 86% female, surgery group = 74% female). Clinical examinations were performed at baseline, 2-year', 6 years' and 12-year' follow-up. Clinical data were reported previously.[10,14,35,36]

At each study examination, data on medical history and lifestyle behaviors were recorded in addition to measurement of clinical parameters. Information on sex, age, and race was self-reported. Gender data was not collected, but ethnicity, education, and income were self-reported. Following the baseline examination, participants in the surgery group underwent RYGB. Participants in the non-surgery and control groups underwent no weight or lifestyle interventions as part of the study, although they were not precluded from pursuing them outside of the study.

The UOS was approved by the Institutional Review Board at the University of Utah and all information was obtained with informed consent from study participants.

Study endpoints

The primary endpoints are ceramide and dihydroceramide concentrations, which are described in the supplemental methods. Additional endpoints include prevalence and remission of T2D. Patients were considered to have T2D if they met one or more of the following conditions: fasting blood glucose $\geq$126 mg per deciliter, glycated hemoglobin (hbA1c) $\geq$ 6.5%, or current use of anti-diabetic medication. Remission is defined as the post-surgical reversal of these criteria.

Blood sample collection, processing, and storage

We quantified sphingolipids in all available serum biospecimens, which were collected at baseline, 2-year, and 12-year. The Institutional Review Boards (IRB) at the University of Utah and Intermountain Healthcare approved the study protocol and each patient provided written informed consent. Serum samples were stored at −80°C until liquid chromatography-tandem mass spectrometry (LC-MS/MS) lipidomics measurement in March 2020.

METHOD DETAILS

Lipid extraction

The method for conducting high-throughput lipid extraction from serum samples was derived from a previously described method.[37] The internal standard (IS) stock solution containing sphingomyelin (d18:1/17:0) (2502 pmol/sample), dihydroceramine (d18:0/18:1) (5 pmol/sample), d7-ceramide (d18:1-d7/16:0) (6 pmol/sample), d7-ceramide (d18:1-d7/18:0) (2 pmol/sample), d7-ceramide (d18:1-d7/24:0) (152 pmol/sample), d7-ceramide (d18:1-d7/24:1) (20 pmol/sample), glucosylceramide (d18:1/17:0) (50 pmol/sample), and d7-phosphocholine (PC) (15:0–18:1-d7) (500 pmol/sample) was prepared in methanol. Serum samples were thawed at 4°C for 12 h before proceeding with lipid extraction. Samples were extracted in a 96-well format with three columns of controls: a 600-μL isopropanol double blank (DB), a process blank (PB) with 50 μL PBS, and a pooled control human serum sample (quality control [QC]) (MilliporeSigma). Serum (50 μL) was transferred into the remaining

72 wells of the 96-deep-well plate (USA Scientific). The IS mix (550 μL) and protein precipitation (PPT) solvent (ethyl acetate/isopropanol, 2:8, *v/v*) were added to each sample (with the exception of the DB) for a final volume of 600 μL per well. The plate was sealed using a silicone cap mat (Analytical Sales and Products). Samples were placed on a shaker at room temperature for 10 min followed by a 10-min centrifugation at 3000 × *g*. The supernatant was then transferred onto a 96-well plate (USA Scientific) and sealed with heat-sealing foil (Beckman Coulter), and plates were stored at 4°C preceding liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Lipid standards and other chemical reagents

Sphingomyelin (d18:1/17:0), dihydro-cer (d18:0/18:1), d7-ceramide (d18:1-d7/16:0), d7-ceramide (d18:1-d7/18:0), d7-ceramide (d18:1-d7/24:0), d7-ceramide (d18:1-d7/24:1), glucosylceramide (d18:1/17:0), and d7-PC (15:0–18:1-d7) were obtained from Avanti Polar Lipids. An Acquity CSH C18, 1.7-μm VanGuard Pre-Column and an Acquity CSH C18, 2.1 × 50 mm 1.7-μm column were obtained from Waters Corporation. 2-propanol, acetonitrile, and formic acid (all LC-MS grade) were obtained from Honeywell, Burdick & Jackson. HPLC-grade ethyl acetate was obtained from MilliporeSigma. Ammonium acetate was acquired from MPBio.

LC-MS/MS analysis

Lipid extracts were separated on an Acquity CSH C18 1.7 μm 2.1 × 50 mm column with a 1.7 μM VanGuard Pre-Column (Waters Corporation) maintained at 60°C and connected to an Agilent HiP 1290 Sampler and an Agilent 1290 Infinity Pump, equipped with an Agilent 1290 Flex Cube and an Agilent 6490 triple quadrupole (QqQ) mass spectrometer. Sphingolipids were detected using dynamic multiple reaction monitoring (dMRM) in positive ion mode. The source gas temperature was set to 210°C, with a gas (N_2) flow of 11 L/min and a nebulizer pressure of 30 psi. The sheath gas temperature was 400°C, the sheath gas (N_2) flow was 12 L/min, the capillary voltage was 4000 V, and the nozzle voltage was 500 V. The injection volume was 3 μL, and the samples were analyzed in a randomized order, with the pooled QC sample injected 8 times throughout the sample queue. With eight controls per plate, there were 80 QC injections in totality. Mobile phase A consisted of ACN/H_2O (60:40 *v/v*), and mobile phase B consisted of IPA/ACN/H_2O (90:9:1 *v/v*), both of which contained 10 mM ammonium formate and 0.1% formic acid. The chromatography gradient started at 15% mobile phase B, increased to 30% B over 1 min, increased to 70% B from 1.0 to 1.1 min, was held at 70% B until 4.5 min, and increased to 99% B from 4.5 to 4.51 min, at which point it was held until 5 min, and then returned to the starting conditions at 5.1 min. Post-time was 1.5 min, and the flow rate was 0.5 mL/min throughout. Collision energies and cell accelerator voltages were optimized using sphingolipid standards with dMRM transitions as $[M + H]^+ \rightarrow [m/z = 266.3 \text{ or } 284.4]$ for dihydroceramides; $[M\text{-}H_2O + H]^+ \rightarrow [m/z = 264.2]$ for ceramides; and $[M\text{-}H_2O + H]^+ \rightarrow [m/z = 271.3]$ for isotope-labeled ceramides. Sphingomyelins were monitored with dMRM transitions as $[M + H]^+ \rightarrow [m/z = 184.4]$. Sphingolipids without available standards were identified on the basis of high-resolution LC-MS, quasi-molecular ions, and characteristic product ions. Results from the LC-MS experiments were collected using an Agilent Mass Hunter Workstation and analyzed with Agilent Mass Hunter Quant B.07.00 software. Sphingolipids were quantitated on the basis of peak area ratios to the internal standards.

Lipid species

A total of 38 lipids were quantified including dihydroceramides (dihydro-cer(d18:0)); ceramides (cer(d18:1)); glucosylceramides (glucosyl-cer(d18:1)); dihydrosphingomyelins

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(dihydro-SM(d18:0)); sphingomyelins (SM(d18:1)); sphinganine; sphingosine; phosphatidylcholines (PC). For sphingolipid species, except for sphinganine and sphingosine, acyl chain lengths of 16, 18, 20, 22, and 24 and a carbon length of 24:1 were reported. For phosphatidylcholines, six species at the sum composition level were reported (34:2, 36:0, 36:1, 36:2, 36:3, and 36:4). The average coefficient of variation (CV) $\pm$ SD for all sphingolipid species reported is 37.3 $\pm$ 13.1. Calculated for only ceramide and dihydroceramide species, the average percent CV is 29.8 $\pm$ 7.9. Both of the CVs are in line with previous reports of sphingolipid data.[38] Lipid data was batch corrected using the Locally Weighted Scatterplot Smoother (LOESS) algorithm[39]

QUANTIFICATION AND STATISTICAL ANALYSIS

Participant characteristics were summarized as the mean $\pm$ SD for continuous variables or *n* (percentage) for categorical variables. Lipid species were summarized as mean and interquartile range using the original scale and were $\log_{10}$ transformed for analysis, owing to non-normal distributions.

For all analyses, we ran sequential models, including unadjusted, minimally adjusted (study group, age, sex, baseline BMI), and multivariable adjusted (study group, age, sex, baseline BMI, race, ethnicity, marital status, income, education level, smoking status, blood pressure, triglycerides, HDL-C, VLDL-C, LDL-C, anti-hypertensive medication, lipid-lowering medication). First, we conducted multilevel modeling to measure the changes in ceramide concentration over time (baseline to 2-year, and 2-year to 12-year) within and study groups. We modeled the relationship between sphingolipid concentrations and group over time, and provided precise beta estimates and 95% CIs of these associations.[40] A multilevel model accounts for the nested nature of the data in regards to both study group and non-linear timepoints. We specified the model with random intercepts for groups and employed fixed effects for time. Next, we also implemented a multilevel model to determine the relationship between ceramide concentrations and diabetes markers (glycated hemoglobin (HbA1c), and homeostatic model assessment of insulin resistance (HOMA-IR). This analysis included an adjustment for group, as it pooled all three study groups together, and included BMI at each time point as a covariate, thereby accounting for fluctuations in weight over time. Finally, we evaluated diabetes remission in the RYGB group. This analysis included surgery group participants with prevalent diabetes at baseline and ceramide data at baseline and 2-year (n = 67). We visualized the proportion of each study group with prevalent diabetes at baseline and the proportion of these patients that experienced remission at 2-year' follow-up. We performed a multivariable adjusted logistic regression for the association of ceramides with 2-year diabetes remission, then visualized the fold-change of lipid concentrations from baseline to 2-year for[1] diabetes remission (n = 49) versus no remission (n = 18);[2] sustained diabetes remission at study termination (12-year) (n = 40) versus transient diabetes remission (remission at 2-year but re-diagnosis before study termination, n = 9), and persistent diabetes (no diabetes remission over study duration, n = 18). For all diabetes remission analyses, the following covariates were added to the multivariable adjusted model: baseline use of diabetes medications, duration of diabetes, and baseline HbA1c.

All analyses were performed in R 3.5.1.[41] Figures were generated in R 3.5.1, GraphPad Prism, or BioRender. Associations were considered statistically significant at a false discovery rate (FDR) below 0.05 to account for multiple statistical tests.[42]