Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY APPENDIX

Effect of Diet versus Gastric Bypass on Metabolic Function in Diabetes

Mihoko Yoshino, M.D., Ph.D, Brandon D. Kayser, Ph.D, Jun Yoshino, M.D., Ph.D, Richard I. Stein, Ph.D, Dominic Reeds, M.D., J. Christopher Eagon, M.D., Shaina R. Eckhouse, M.D., Jeramie D. Watrous, Ph.D, Mohit Jain, M.D, Ph.D, Rob Knight, Ph.D., Kenneth Schechtman, Ph.D., Bruce W. Patterson, Ph.D., and Samuel Klein, M.D.

From the Center for Human Nutrition (M.Y., B.D.K., J.Y., R.I.S., D.R., K.S., B.W.P., S.K.) and the Department of Surgery (J.C.E., S.R.E.), Washington University School of Medicine, Saint Louis, Missouri; the Departments of Medicine and Pharmacology (J.D.W., M.J.), and Departments of Pediatrics and Computer Science and Engineering (R.K.), University of California San Diego, San Diego, California.

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Methods

Study Visits and Metabolic Testing

Body composition analyses, assessment of resting energy expenditure, a three-stage, hyperinsulinemic-euglycemic pancreatic clamp procedure, a mixed-meal metabolic test, and a 24-hour assessment of plasma glucose, insulin and c-peptide concentration profiles were conducted in the Clinical and Translational Research Unit and the Center for Clinical Imaging Research at Washington University School of Medicine before and after participants achieved a targeted (~18%) weight loss. All inpatient testing occurred during two study visits, approximately 1-2 weeks apart. Assessment of resting energy expenditure and the clamp procedure were conducted during the first inpatient admission to the research unit, and the mixed-meal metabolic test plus 24-hour assessment of plasma substrates and hormones were conducted during the second inpatient admission to the research unit. In addition, fecal samples were collected from each subject before and after weight loss.

Body Composition

Body fat mass and fat-free mass were determined by using dual-energy X-ray absorptiometry, and intra-abdominal adipose tissue volume and intrahepatic triglyceride content were determined by using magnetic resonance spectroscopy.[1]

Dual Glucose Tracer Mixed-meal Metabolic Test and 24-hour Blood Sampling

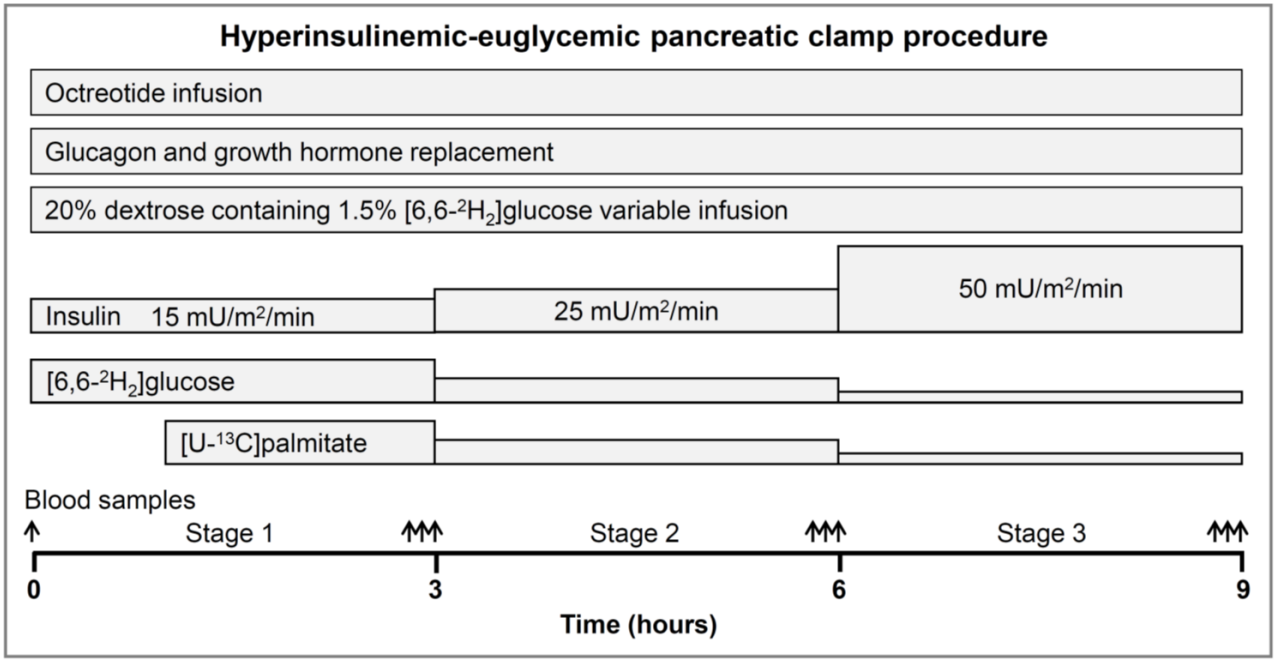
Participants were admitted to the Washington University Clinical and Translational Research Unit in the afternoon and consumed a standard evening meal containing 50% of calories as carbohydrate, 30% as fat, and 20% as protein. In the morning, after participants had fasted overnight, a catheter was inserted into a forearm vein for infusion, and a second catheter was inserted into a radial artery to obtain arterial blood samples. At 0600 h, a primed, continuous infusion of [6,6-$^{2}H_{2}$]glucose (priming dose, 22.5 μmol/kg; infusion rate, 0.25 μmol/kg/min) was started and continued until 1300 h. At 0900 h, after 3 hours of tracer infusion, participants ingested a 450 kcal liquid meal, containing 49 g of glucose mixed with 0.9 g [U-^{13}C]glucose, 18 g of fat, and 22 g of protein, which was provided in 7

equally divided aliquots given every 5 minutes for 30 minutes (0900-0930 h). Blood samples were collected immediately before starting the glucose tracer infusion, before initiating meal ingestion (times -20, -10 and 0 minutes), every 10 minutes for the first 60 minutes, and then every 20 minutes for the remaining 3 hours to determine plasma substrate, hormone concentrations, and glucose tracer-to-tracee ratios. After completing the mixed meal metabolic test, participants remained in the Clinical and Translational Research Unit until 0900 h the next day to complete the remainder of the 24-hour metabolic study. Liquid meals containing 50% carbohydrate, 35% fat, and 15% protein were provided at 1300 h, 1700 h, and 2100 h, which were consumed in seven equally divided aliquots every 5 minutes for 30 minutes. The energy content of the meal was individualized based on each subject's estimated total daily energy requirement, calculated as 1.25 times measured resting energy expenditure assessed by using a metabolic cart (TrueOne 2400, ParvoMedics, Sandy, UT). Blood samples were obtained every hour from 1300 h to 0100 h and then every 2 hours from 0100 h until 0900 h the next morning to determine plasma glucose, free fatty acid, insulin and C-peptide concentrations.

Hyperinsulinemic-euglycemic Pancreatic Clamp Procedure

Participants were admitted to the Clinical and Translational Research Unit in the afternoon and consumed a standard evening meal, containing 50% of calories as carbohydrate, 30% as fat, and 20% as protein. The following morning, after participants had fasted for about 10 h overnight, a catheter was inserted into a forearm vein for infusion, and a second catheter was inserted into a radial artery to obtain arterial blood samples. At 0600 h, a 9-h three-stage hyperinsulinemic-euglycemic pancreatic clamp procedure was started by infusing octreotide (45 ng/kg fat free mass/min) and replacement doses of glucagon (1.5 ng/kg fat free mass/min) and growth hormone (6 ng/kg fat free mass/min), which were maintained until the end of the study (a diagram below describes the stages of the clamp procedure). An intravenous infusion of insulin was started at 0600 h at a rate of 15 mU/m^2 body surface area/min (stage 1) for 3 hours (initiated with an insulin infusion of 60 mU/m^2 body surface area/min for 5 minutes and then 30 mU/m^2 body surface area/min for 5 minutes), and was increased to 25 mU/m^2

body surface area/min (stage 2) for the next 3 hours (initiated with an insulin infusion of 100 mU/m^2 body surface area/min for 5 minutes and then 50 mU/m^2 body surface area/min for 5 minutes), and then increased to 50 mU/m^2 body surface area/min (stage 3) for the final 3 hours (initiated with an insulin infusion of 200 mU/m^2 body surface area/min for 5 minutes and then 100 mU/m^2 body surface area/min for 5 minutes). A primed, constant infusion of [6,6-$^2H_2$]glucose (priming dose 22.5 µmol/kg body weight and 0.25 µmol/kg body weight/min infusion) was started at 0600 h, followed by an infusion of [U-^{13}C]palmitate (6 nmol/kg fat free mass/min) at 0700 h. The infusion rate of [6,6-$^2H_2$]glucose and [U-^{13}C]palmitate was reduced by 50% and 75% during stages 2 and 3 of the hyperinsulinemic-euglycemic pancreatic clamp procedure, respectively, to account for the expected decrease in endogenous glucose production and adipose tissue lipolytic rates. Plasma glucose concentration was kept constant during stages 1 and 2 and maintained at ~100 mg/dL during stage 3 of the clamp procedure by infusing 20% dextrose enriched to 2% with [6,6-$^2H_2$]glucose.



Plasma glucose and insulin concentrations achieved during each stage of the clamp procedure conducted after weight loss were purposely controlled to match the values obtained before weight loss in both the Diet and Surgery groups. Blood samples were collected immediately before starting the

glucose tracer infusion, and every 10 min during the final 20 minutes (3 samples) of each stage of the clamp procedure to determine plasma glucose and insulin concentrations, and both glucose and palmitate tracer-to-tracee ratios. The assessment of hepatic and adipose tissue insulin sensitivity was performed during low-dose (15 mU/m^2 body surface area/min) and medium-dose (25 mU/m^2 body surface area/min) insulin infusion to submaximally suppress glucose production and lipolysis. The assessment of skeletal muscle insulin sensitivity was performed during high-dose (50 mU/m^2 body surface area/min) insulin infusion to adequately stimulate glucose disposal.[2] Resting energy expenditure was determined by a metabolic cart with online expiratory gas exchange analysis (TruOne 2400; ParvoMedics, Sandy, UT) from 0630 h to 0700h during the clamp procedure.[3]

Weight Loss Interventions

Diet group. Participants in the Diet group received weekly individual dietary and behavioral education sessions during the entire study period. All meals were provided to participants as liquid shakes (Optifast HP® Shake Mix, Fremont, MI) and prepackaged entrees (30% carbohydrate; 40% protein; 30% fat; 200 kcal/meal) prepared by the Clinical and Translational Research Unit Metabolic Kitchen to enhance compliance with low-calorie dietary therapy. Participants were instructed to keep daily food records and enter their data into the MyFitnessPal application (MyFitnessPal, Inc). Food records were evaluated by the study dietitian and behavioral psychologist, and the daily energy content of the meals was adjusted weekly as needed to achieve about a 1% decrease in body weight per week. After participants achieved about an 18% weight loss, energy intake was adjusted to prevent continued weight loss and maintain a stable body weight (<2% change) for 3 weeks before repeat testing was performed at 23±7 weeks after starting diet therapy.

Surgery group. The gastric bypass procedure was performed by using standard laparoscopic techniques.[4] A linear stapler was used to completely divide the stomach into a small (15-20 ml) proximal gastric pouch and a large distal gastric remnant. A Roux limb was constructed by transecting the jejunum 30-50 cm distal to the ligament of Treitz. The proximal cut end of the jejunum

(biliopancreatic limb) was anastomosed 75 -150 cm distally on the Roux limb by using a linear stapler. The proximal end of the Roux limb was anastomosed to the proximal gastric pouch by using a stapled technique. The first week after surgery participants consumed a liquid diet only. Progression to regular foods occurred 2-4 weeks after surgery. A study dietitian consulted with participants weekly by phone or in person to monitor body weight, review dietary intake and adjust dietary intake in order to meet the targeted weight loss goal. After participants achieved about an 18% weight loss, energy intake was adjusted to prevent continued weight loss and maintain a stable body weight (<2% change) for 3 weeks before repeat testing was performed at 16±4 weeks after gastric bypass surgery.

Management of Blood Glucose and Diabetes Medications

Participants were provided with a glucometer and glucose test strips at the time of study enrollment and checked their blood glucose concentrations twice daily (before breakfast and before bedtime) by fingerstick throughout the study. Participants were instructed to stop taking oral and injectable diabetes medications at different time points before each admission to the Clinical and Translational Research Unit. Oral diabetes medications were discontinued 3 days before and insulin was discontinued 1 day before each admission, whereas glucagon-like peptide 1 receptor agonists were discontinued 2 weeks before the first admission and not restarted unless needed for glycemic control after second research unit admission. During the period of medication withdrawal, blood glucose values obtained at home were reviewed regularly by the study physician. Participants were instructed to contact study medical staff when blood glucose was >200 mg/dl before breakfast or >300 mg/dl before bedtime to obtain instructions for possible additional medical therapy. Blood glucose values were reviewed weekly throughout the study by the study nurse and physicians, and diabetes medications were adjusted every 1-2 weeks, as needed.

Sample Analyses

Blood samples. Plasma glucose concentrations were determined by using an automated glucose analyzer (YSI 2300 STAT plus; Yellow Spring Instrument Co, Yellow Springs, OH) and both

plasma insulin and c-peptide concentrations were determined by using electrochemiluminescence technology (Elecsys 2010, Roche Diagnostics). Plasma concentrations of leptin and high-molecular weight adiponectin were measured by using a commercially available radioimmunoassay kit (EMD Millipore, Burlington, MA) and enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN), respectively. Plasma free fatty acid concentrations were measured by using a commercially available diagnostic kit (FUJIFILM Wako Diagnostics, Mountain View, CA). Plasma branched-chain amino acids (valine, leucine, and isoleucine) and short-chain acylcarnitines (propionyl carnitine [C3] and 2-methylbutyryl/isovaleryl carnitine [C5]) were analyzed by using liquid chromatography tandem mass spectrometry (LC-MS/MS). These metabolites were chromatographically separated by using a Thermo Vanquish UPLC system with a Zic-pHILIC (2.1 x 150 mm) 5µm column, and mass spectra acquired on a Thermo QExactive orbitrap mass spectrometer, as previously described.[5] Metabolites were identified by matching accurate mass, retention time, and MS/MS fragmentation patterns to an in-house library of commercially available standards. Plasma bile acids concentrations were analyzed by adding isotopically labeled internal standards and using LC-MS/MS as previously described.[6] Plasma glucose and palmitate tracer-to-tracee ratios were determined by using gas chromatography-mass spectrometry.[7]

Fecal samples. DNA extraction, amplification of the V4 region of the 16S rRNA gene, and Illumina amplicon sequencing were performed at the University of California San Diego Center for Microbiome Innovation, according to the Earth Microbiome Project protocols.[8] Sequence data were processed by using Quantitative Insights Into Microbial Ecology2[9] and sequences were deposited in the European Nucleotide Archive maintained at the European Bioinformatics Institute EBI-ENA (accession number EBI: ERP119960). Microbial sub-Operational Taxonomic Units were identified from sequences trimmed to 150 nucleotides by using the Deblur algorithm.[10] Taxonomic assignment was performed with the GreenGenes v13.8 99% taxonomic reference database using the naïve Bayes classifier implemented in the q2-feature-classifier plugin,[11] and bespoke taxonomic weights were provided to the

classifier by using the precompiled GreenGenes v13.8 V4 human-stool weights produced by the q2-clawback plugin.[12]

Beta diversity was calculated based on the sub-Operational Taxonomic Units relative abundance table by using the Bray-Curtis dissimilarity. Differential abundance testing was performed separately for each taxonomic level on features detected in at least 30% of samples with a mean relative abundance of 0.01%[13] across all samples. Raw counts were modelled by using a negative binomial generalized linear model with sequencing depth as an offset. To analyze the within-subject change between groups by using fixed effects, a nested interaction formula was used with participant and time nested within treatment groups, and a custom contrast was specified to test the difference in the within-group change i.e. the group by time interaction. Means and 95% confidence intervals were estimated for the within-group differences at the phylum level. P-values were calculated for the group by time interaction at the genus level, and were adjusted for multiple comparisons by using the Benjamini-Hochberg false discovery rate and a 5% false discovery rate was considered statistically significant. Statistical analyses were performed by using R (The R Foundation for Statistical Computing, Vienna, Austria).

Calculations

Diabetes medication score. A diabetes medication score for each subject was calculated based on the number and dose of diabetes medications being taken, as described previously.[14] For each medication, with the exception of insulin, a numerical score was calculated as the daily dose relative to the maximum recommended dose. For insulin, a numerical score was calculated as the daily insulin dose relative to a standard dose of 1U of insulin per kg of body weight per day. A composite diabetes medication score was calculated as the sum of each medication score.

Insulin and glucose kinetics after mixed meal ingestion and over 24 hours. The insulin secretion rate was determined by using a stochastic deconvolution of the plasma C-peptide concentration.[15-17] The clearance rate of insulin was calculated as the ratio of the insulin secretion rate to the prevailing

plasma insulin concentration. The 4-hour postprandial and 24-hour area under the curves for plasma glucose, free fatty acid, C-peptide, insulin, and insulin secretion rate were calculated by using the trapezoid method. Total (endogenous and meal-derived) glucose rate of appearance, ingested glucose rate of appearance, and endogenous glucose rate of appearance were calculated as previously described.[4]

Multiorgan insulin sensitivity. Hepatic insulin sensitivity (suppression of glucose rate of appearance into the systemic circulations) and adipose tissue insulin sensitivity (suppression of palmitate rate of appearance into the systemic circulations) was calculated during stages 1 and 2 of the hyperinsulinemic-euglycemic pancreatic clamp procedure, and skeletal muscle insulin sensitivity (stimulation of glucose rate of disappearance from the systemic circulations) was calculated during stage 3 of the clamp procedure as previously described.[2] Whole-body insulin sensitivity was calculated as the glucose infusion rate during stage 3 of the clamp procedure relative to prevailing plasma insulin concentrations.

β-cell function. β-cell function was assessed as the product of β-cell glucose sensitivity (the ratio of postprandial total areas-under-the-curve of insulin secretion rate to total areas-under-the-curve of postprandial plasma glucose) and whole-body insulin sensitivity (glucose infusion rate divided by the plasma insulin concentration during stage 3 of the clamp procedure).[4,7]

Statistical Analyses

The difference in the treatment response between the Diet and Surgery groups was evaluated by using analysis of covariance with the post-intervention value as the dependent variable, the study group as the independent variable, and the baseline value as the covariate. Because the two groups were well-matched on potential confounding variables, no additional covariates were included. The adequacy of the fit of all models was evaluated by assessing regression residuals (Table S1-3). A p-value ≤ 0.05 was considered statistically significant for the single predefined primary outcome (hepatic insulin sensitivity). All other (secondary) outcomes should be regarded as exploratory, therefore only the 95%

confidence intervals are provided for statistical inference. The within-group change in each outcome was evaluated by determining the mean and 95% confidence interval of the difference between the pre- and post-intervention values, which were estimated by using paired t-tests. Statistical analysis was performed by using SPSS version 23 (IBM SPSS, Chicago, IL).

Participants and Study Design

Thirty-three men and women with obesity and type 2 diabetes were enrolled in this study. Eighteen participants were enrolled in the low-calorie diet (Diet) group and 15 participants scheduled to have Roux-en-Y gastric bypass surgery at Barnes-Jewish Hospital (St. Louis, Missouri, USA) were enrolled in the Surgery group (Supplemental Figure 1 CONSORT Diagram). Four participants in the Diet group dropped out of the study (2 before starting the weight loss intervention, and 2 during the weight loss intervention) and 3 were withdrawn because they were unable to achieve the minimum targeted 16% weight loss. Four participants in the Surgery group dropped out of the study before surgery. Accordingly, 11 participants in the Diet and Surgery groups completed the study. All participants completed a comprehensive screening evaluation consisting of medical history and physical examination, a resting electrocardiogram, and standard blood tests. Eligibility criteria was used to determine eligibility for participation. All participants provided written informed consent before participating in this study, which was approved by the Institutional Review Board of Washington University School of Medicine in St. Louis, MO.

Study Eligibility Criteria

All participants met the following eligibility criteria:

Inclusion criteria: 1) men and women aged 25 to 65 years, inclusive at the time of informed consent; 2) body mass index between 34.0 and 55.0 kg/m^2 ; 3) diagnosis of type 2 diabetes based on HbA1C >6.5%, results of oral glucose tolerance test or medical history plus current use of anti-diabetes medications; 4) scheduled for Roux-en-Y gastric bypass surgery at Barnes-Jewish Hospital (Saint Louis, Missouri, USA) for Surgery group participants; and 5) provided written informed consent.

Exclusion criteria: 1) evidence of significant organ system dysfunction (e.g., severe pulmonary or kidney disease) or a disease that could affect the study outcome measures other than obesity and type 2 diabetes, or significantly increase the risk of the study procedures; 2) cancer or cancer that has been in remission for <5 years; 3) previous intestinal resection; 4) regular use of tobacco products; 5) use of medication that can affect the study outcome measures; 5) regular exercise for more than 90 minutes per week; 6) women who are pregnant or lactating; 6) conditions that render subject unable to complete all testing procedures (e.g. aversion to needles, metal implants that prevent magnetic resonance imaging); 7) persons unable or unwilling to follow the study protocol; and 8) persons not able to grant voluntary informed consent

Figure S1. CONSORT Flow Diagram

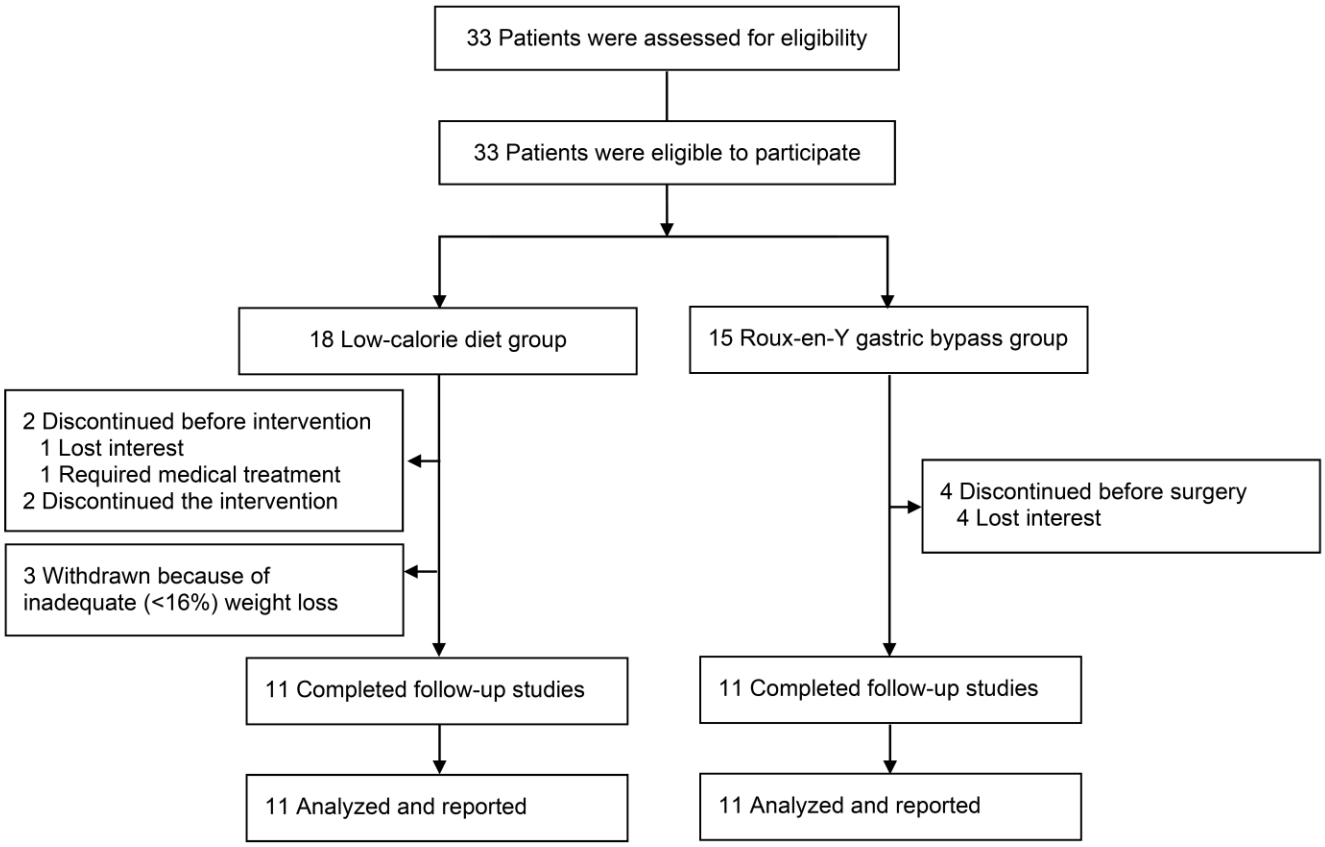
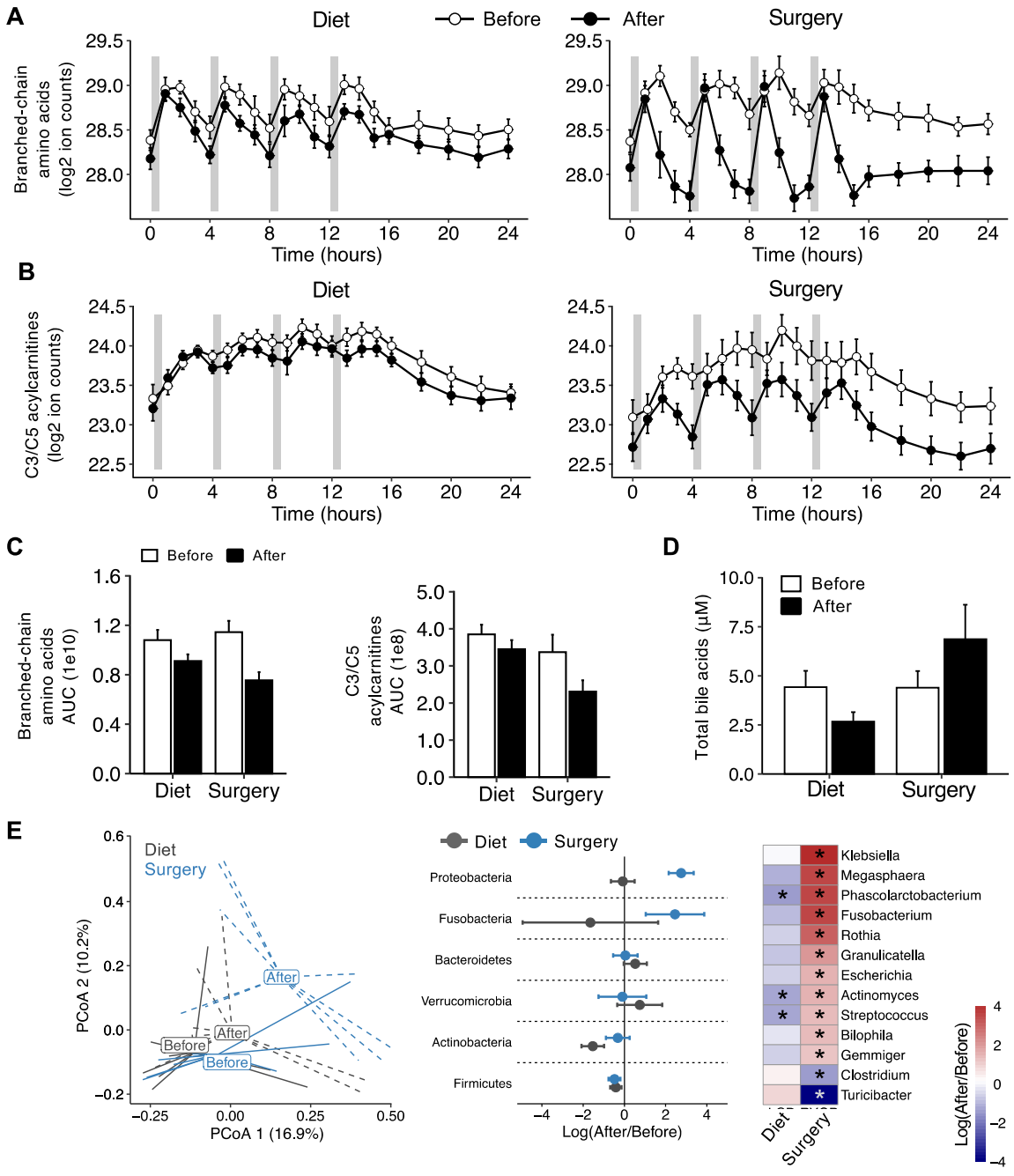


Figure S2.

Effect of weight loss on plasma branched-chain amino acids, C3 and C5 acylcarnitines and bile acids, and the composition of the gut microbiome in the Diet and Surgery groups.



A, plasma branched-chain amino acids and *B,* C3/C5 acylcarnitines measured serially for 24 hours (gray bars represent time of meal consumption) and *C*, their respective 24-hour areas-under-the-curve

before and after weight loss induced by low-calorie diet (Diet, n=11) or Roux-en-Y gastric bypass (Surgery, n=7). The effect of weight loss on the 24-hour pattern of plasma branched-chain amino acids and C3/C5 acylcarnitines were different between groups, manifested by higher peaks in plasma branched-chain amino acids and C3/C5 acylcarnitine concentrations early after meal ingestion, followed by rapid reductions in circulating levels and lower plasma areas-under-the-curves after weight loss in the Surgery group than the Diet group. The ANCOVA-derived mean post-weight loss group difference is -0.34 (-0.16 to -0.05, 95% confidence interval) for branched-chain amino acids and -0.46 (-0.66 to -0.27, 95% confidence interval) for C3/C5 acylcarnitines. Plotted values are the mean ± SEM. *D,* total fasting plasma bile acids concentrations before and after weight loss in the Diet (n=11) and Surgery (n=9) groups. Post-weight loss values were higher in the Surgery group than the Diet group; ANCOVA-derived mean post-weight loss group difference is 0.76 (0.11 to 1.42, 95% confidence interval). *E left,* principal coordinates analysis of Bray-Curtis dissimilarity of sub-operational taxonomic units in the Diet (gray) and Surgery (blue) groups before and after weight loss; labels are group means and segments are the distance to individual samples. *E middle*, relative change in gut microbial phyla after weight loss in the Diet (black) and Surgery (blue) groups; values are the mean change within each group ± 95% confidence interval estimated by using negative binomial regression. *E right*, gut microbial genera that changed differently after weight loss induced by Surgery than Diet (Group by Time interaction false discovery rate <0.05). *Value after weight loss significantly different (false discovery rate <5%) from value before weight loss. Sample sizes for stool microbiome analyses are n=10 in the Diet and n=9 in the surgery groups. Abbreviations: AUC, areas-under-the-curve; PCoA, principal coordinates analysis.

Table S1. Remaining Regression Output from Analysis of Covariance Related to Table 1

Variables	Intercept	Coefficient of baseline value for outcome variable	Treatment group coefficient*
Body weight (kg)	4.77	0.782	-1.09
Body mass index (kg/m^2)	1.44	0.788	-0.392
Fat mass (kg)	-3.95	0.793	0.579
Body fat (%)	-11.3	1.11	0.998
Fat free mass (kg)	-0.508	0.932	-1.32
Intra-abdominal adipose tissue volume (cm^3)	-85.0	0.681	157
Intrahepatic triglyceride content (%)	2.09	0.132	1.43
Free fatty acids (mg/dl)	7.93	0.371	-0.851
Glucose (mg/dl)	76.5	0.186	0.768
Insulin (µU/ml)	9.56	0.119	-3.48
HbA1c (%)	4.81	0.097	0.517
High molecular weight adiponectin (µg/ml)	0.937	0.754	0.072
Leptin (ng/l)	5.34	0.384	-8.53

*Diet group was coded as the reference in the treatment group coefficient.

Intercept, beta coefficient relating the Before value (the covariate) to the After value, and the treatment group beta coefficient (the estimated difference between the mean After value in the Surgery group relative to the After value in the Diet group). To convert the values for glucose to millimole per liter, multiply by 0.05551. To convert the values for insulin to picomole per liter, multiply by 6.0. To convert the values for free fatty acid to grams per liter, multiply by 0.01.

Table S2. Remaining Regression Output from Analysis of Covariance Related to Table 2

Variables	Intercept	Coefficient of baseline value for outcome variable	Treatment group coefficient*
4-h meal glucose AUC (mg/dl × min × 10^3)	15.1	0.332	8.56
4-h meal insulin AUC (μU/ml × min × 10^3)	6.86	0.246	1.34
Total glucose Ra 4-h AUC (μmol/kg FFM/min × min)	2540	0.472	138
Ingested glucose Ra 4-h AUC (μmol/kg FFM/min × min)	2030	0.498	-19.1
Endogenous glucose Ra 4-h AUC (μmol/kg FFM/min × min)	1390	0.077	159
24-h glucose AUC (mg/dl × min × 10^3)	96.9	0.233	32.3
24-h free fatty acid AUC (mg/dl × min× 10^3)	4.59	0.448	2.67
24-h insulin AUC (μU/ml × min × 10^3)	27.1	0.218	1.47
24-h insulin secretion rate AUC (pmol/min × 10^3)	245	0.568	-31.4
24-h insulin clearance rate (L/min)	1.80	0.516	0.114

*Diet group was coded as the reference in the treatment group coefficient.

Intercept, beta coefficient relating the Before value (the covariate) to the After value, and the treatment group beta coefficient (the estimated difference between the mean After value in the Surgery group relative to the After value in the Diet group). To convert the values for C-peptide to nanogram per milliliter, multiply by 0.331. Abbreviations: AUC, area-under-the-curve; FFM, fat-free mass; Ra, rate of appearance into the systemic circulation.

Table S3. Remaining Regression Output from Analysis of Covariance Related to Table 3

Variables	Intercept	Coefficient of baseline value for outcome variable	Treatment group coefficient*
Glucose Ra (Stage 1) (μmol/kg FFM/min)	3.89	0.360	0.252
Glucose Ra (Stage 2) (μmol/kg FFM/min)	2.61	0.095	0.021
Glucose Rd (Stage 3) (μmol/kg FFM/min)	47.4	0.465	-6.51
Palmitate Ra (Stage 1) (μmol/kg FFM/min)	0.659	0.084	0.327
Palmitate Ra (Stage 2) (μmol/kg FFM/min)	0.507	0.124	-0.044
Beta cell function index	2.09	0.726	-0.706

*Diet group was coded as the reference in the treatment group coefficient.

Intercept, beta coefficient relating the Before value (the covariate) to the After value, and the treatment group beta coefficient (the estimated difference between the mean After value in the Surgery group relative to the After value in the Diet group). Abbreviations: FFM, fat-free mass; Ra, rate of appearance into the systemic circulation; Rd, rate of disposal from the systemic circulation.

References

1. Smith GI, Shankaran M, Yoshino M, et al. Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. J Clin Invest 2020;130:1453-60.
2. Conte C, Fabbrini E, Kars M, Mittendorfer B, Patterson BW, Klein S. Multiorgan insulin sensitivity in lean and obese subjects. Diabetes Care 2012;35:1316-21.
3. Fabbrini E, Yoshino J, Yoshino M, et al. Metabolically normal obese people are protected from adverse effects following weight gain. J Clin Invest 2015;125:787-95.
4. Bradley D, Conte C, Mittendorfer B, et al. Gastric bypass and banding equally improve insulin sensitivity and beta cell function. J Clin Invest 2012;122:4667-74.
5. Kantz ED, Tiwari S, Watrous JD, Cheng S, Jain M. Deep Neural Networks for Classification of LC-MS Spectral Peaks. Anal Chem 2019;91:12407-13.
6. Patel A, Yusta B, Matthews D, Charron MJ, Seeley RJ, Drucker DJ. GLP-2 receptor signaling controls circulating bile acid levels but not glucose homeostasis in Gcgr(-/-) mice and is dispensable for the metabolic benefits ensuing after vertical sleeve gastrectomy. Mol Metab 2018;16:45-54.
7. Magkos F, Fraterrigo G, Yoshino J, et al. Effects of Moderate and Subsequent Progressive Weight Loss on Metabolic Function and Adipose Tissue Biology in Humans with Obesity. Cell Metab 2016;23:591-601.
8. McDonald D, Hyde E, Debelius JW, et al. American Gut: an Open Platform for Citizen Science Microbiome Research. mSystems 2018;3.
9. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852-7.
10. Amir A, McDonald D, Navas-Molina JA, et al. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems 2017;2.
11. Bokulich NA, Kaehler BD, Rideout JR, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 2018;6:90.

12. Kaehler BD, Bokulich NA, McDonald D, Knight R, Caporaso JG, Huttley GA. Species abundance information improves sequence taxonomy classification accuracy. Nat Commun 2019;10:4643.
13. Ji BW, Sheth RU, Dixit PD, et al. Quantifying spatiotemporal variability and noise in absolute microbiota abundances using replicate sampling. Nat Methods 2019;16:731-6.
14. Klein S, Fabbrini E, Patterson BW, et al. Moderate effect of duodenal-jejunal bypass surgery on glucose homeostasis in patients with type 2 diabetes. Obesity (Silver Spring) 2012;20:1266-72.
15. Sparacino G, Cobelli C. A stochastic deconvolution method to reconstruct insulin secretion rate after a glucose stimulus. IEEE Trans Biomed Eng 1996;43:512-29.
16. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368-77.
17. Sparacino G, Pillonetto G, Capello M, De Nicolao G, Cobelli C. WINSTODEC: a stochastic deconvolution interactive program for physiological and pharmacokinetic systems. Comput Methods Programs Biomed 2002;67:67-77.