Supplemental material

Human gut microbiota after bariatric surgery alters intestinal morphology and glucose absorption in mice independent of obesity

Supplemental Methods

Bariatric surgical procedure. Laparoscopic Sleeve Gastrectomy **(**LSG) was performed following removal of the greater curvature and fundus of the stomach. Briefly, the greater curvature of the stomach was mobilized from the antrum to the Hiss angle. A gastric tube was therefore created using repeated 60mm firing of linear staplers/cutters over a 34-French bougie, starting 5cm from the pylorus to the Hiss angle. Bilio-Pancreatic Diversion with a Duodenal Switch (BPD-DS) was carried out following a LSG created along a 34-French bougie, starting 7 cm from the pylorus. In addition, the duodenum was transected 3 cm distal to the pylorus and anastomosed to a 250 cm alimentary limb with a 100 cm common channel. Duodeno-ileal anastomosis was hand-sewn, whereas ileo-ileal anastomosis was semi-mechanical.

Ileal surgery and ileal glucose infusion. Male rats were anaesthetized (ketamine, 60 mg/kg; xylazine, 8 mg/kg) prior to surgical procedures. Gut catheters were placed into the luminal compartment 2 cm distal to the ileocecal valve. Vascular surgeries were performed as described in the 'Intestinal and vascular surgery' part of 'Material & Methods' section. After four days of recovery, rats were fasted overnight (16h, from 16:00 to 8:00) and a bolus of glucose at 2 different doses (0.25 or 4 g/kg) was infused via the ileal catheter. Blood glucose was monitored at different time points (0, 20, 30, 40 min) after ileal glucose infusion.

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*Mass spectrometry***.** To assess circulating 3-OMG in circulation, deproteinised plasma samples were derivatised by acetylation and injected into an Agilent 1290 Infinity II HPLC with an Agilent 6495C iFunnel QQQ mass spectrometer for detection. Analytes were separated on an Agilent RRHD Eclipse Plus C18 (100 mm x 2.1 mm, i.d., 1.8 µm) column using mobile phases consisting of (A) 0.1% (v/v) formic acid in water and (B) 0.1% (v/v) formic acid in acetonitrile and a constant flow of 0.4 mL/min. For Paracetamol quantification, deproteinised samples were injected into the same equipment using the same column and mobile phases and a constant flow of 0.3 mL/min. Autosampler and column were maintained at 10˚C and 30 ˚C, respectively, throughout the analysis.

Short-chain Fatty Acids (SCFA). SCFA were assessed in the fæces of donor patients and in the cecal content of germ-free mice colonized with the the fecal microbiota of patients pre- or post-LSG or BPD-DS. Immediately after collection, fæces were weighed and 1 mL of distilled water/100 mg of material was added. Fecal suspensions were homogenized for 2 min with a Bead Ruptor 12 (Omni International, Kennesaw, GA, USA) and centrifuged at 18,000 × *g* for 10 min at 4 °C. The supernatant was collected, and an equal volume of ethyl acetate spiked with internal standard 4-methylvaleric acid was added and samples were acidified with phosphoric acid 10%. To extract SCFA, samples were mixed 2 min at 2400 rpm using a VWR VX-2500 Multitube Vortexer (VWR, Radnor, PA, USA), then centrifuged at 18,000 × *g* for 10 min at 4 °C. The organic phase was transferred to an autosampler vial for gas chromatography analysis. A 5-point calibration curve were prepared with a mix of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and internal standard 4-methylvaleric acid. SCFA quantification was performed on a Shimadzu GC 2010 Plus equipped with a Nukol Supelco capillary GC column (30 m × 0.25 mm id, 0.25 µm) and a FID detector.

Histology. Proximal (ie, ~15 cm distal to the pylorus) and distal (ie, ~10 cm proximal to ileocecal sphincter) small intestine were arranged in Swiss-rolls and fixed in Carnoy's solution containing ethanol, chloroform and glacial acetic acid (6:3:1) for 3 h. After thorough washing in 100% ethanol, samples were immersed in 70% ethanol for 72 hours. Five µm cross-sections were mounted onto slides and stained with hematoxylin and eosin (H&E). Images of H&E-stained slides were acquired with a Nikon Eclipse Ni & DS-QI2 microscope and analyzed using NIS-Elements. Morphometry was conducted with Image J.

Messenger RNA extraction and RT qPCR. Total RNA was obtained from ~50 mg of upper or distal small intestine via mechanical homogenization at 4.5 m/s for 30 s using a FastPrep-24 tissue homogenizer (MP Biomedicals) and glass beads in tryzol. RNA was extracted using the Directzol RNA Miniprep kit (Zymo Research) and cDNA synthesis was made from 1 µg of tissue RNA using High Capacity cDNA Reverse Transcriptase kit (Applied Bioscience). Transcript expression was measured using TaqMan Assays with AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific). The ΔΔCt for target genes was calculated using 18S as a reference gene. The following primers were used in this study: Glut1 (*Slc2a1*, Mm00441480_m1), Glut2 (*Slc2a2*, Mm00446229_m1), Sglt1(*Slc5a1*, Mm00451203_m1) and RN18S (Mm03928990_g1).

Immunoblotting analysis. Approximately 40 mg of upper or lower upper intestine were processed to yield total protein lysates, which were quantified using a BCA protein assay kit (Thermo Fisher Scientific). Twenty-five mg of protein were mixed with Laemmli buffer and loaded into 12% polyacrylamide gels. Samples were resolved by electrophoresis using a mini-Protean system (Bio-Rad) and transferred to polyvinylidene fluoride (PVDF) membranes, which were immunoblotted with antibodies for non-phospho (active) β-catenin Ser33/37/Thr41 (D13A1) (Cell Signaling, 1:1000, cat# 8814), phosphorylated (inactive) β-catenin (D10A8) (Cell Signaling, 1:1000, cat#

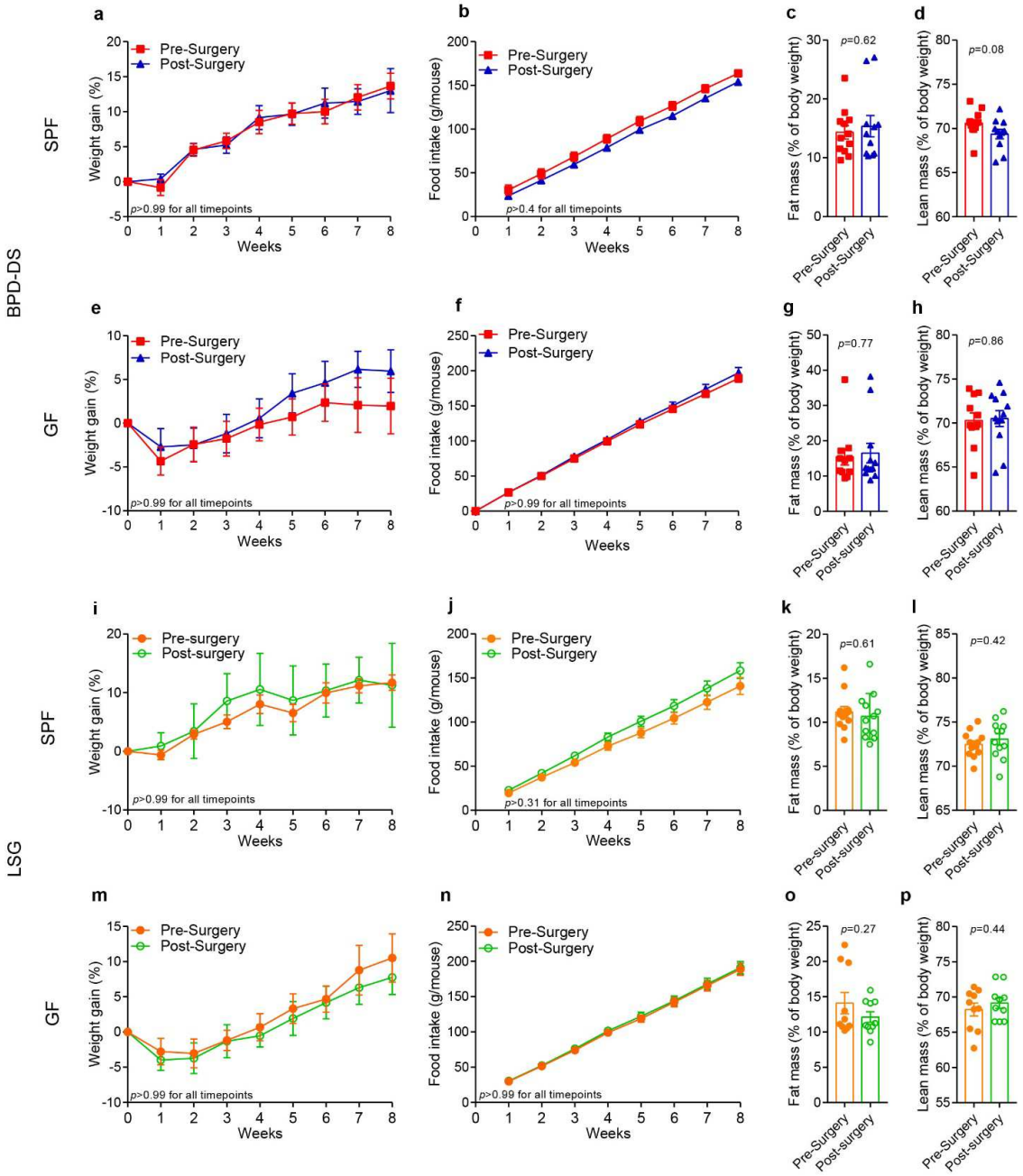
8480) and β-actin (13E5) (Cell Signaling, 1:1000, cat# 4970) as a loading control. Membranes were thereafter incubated with HRP-linked anti-rabbit secondary antibody (Cell Signaling, 1:10000, cat# 7074). Image documentation was carried out after incubation with Clarity Western ECL Substrate (Bio-Rad) in a ChemiDoc Imager (Bio-Rad).

Metabolic phenotyping. All tests in mice were conducted after at least 7 weeks of continuous microbial colonization via oral gavage. A glucose tolerance test (GTT, 4 g glucose/kg oral or 2 g glucose/kg *i.p.*) or insulin tolerance test (ITT, 0.8 UI/kg, *i.p.*) was done after 6 h of fasting (from 8:00 to 14:00). For pharmacological inhibition of Sglt1 in mice, GTT was performed 1h after *i.p.* injection with phloridzin (P3449, Sigma-Aldrich, 0.04 g/kg) or (vehicle 10% DMSO and 10% ethanol in 0.9% saline). A glucose-stimulated insulin secretion test (GSIS, 4 g glucose/kg oral) was done after 12 h of fasting (from 21:00 to 9:00). Blood glucose measurements were taken by tail blood. Insulin and c-peptide were assessed using a mouse insulin/c-peptide multiplex ELISA kit (Millipore, Cat. #MMHMAG-44K). Body fat composition was measured using whole body MRI (Bruker Minispec LF90-II).

*Bacterial profiling***.** Isolation of DNA from fæcal pellets was done using mechanical and enzymatic lysis. The V3-4 region of the 16S rRNA gene was PCR amplified with barcode tags compatible with Illumina technologies and the Illumina MiSeq platform was used to sequence amplified DNA products. Briefly, 50 ng of DNA was used as template with 1U of Taq, 1x buffer, 1.5 mM MgCl2, 0.4 mg/mL BSA, 0.2 mM dNTPs, and 5 pmoles each of 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACNVGGGTWTCTAAT) Illumina adapted primers. The reaction was carried out at 94 ºC for 5 minutes, 5 cycles of 94 C for 30 seconds, 47C for 30 seconds and 72C for 40 seconds, followed by 25 cycles of 94 ºC for 30 seconds, 50 ºC for 30 seconds and 72 ºC for 40 seconds, with a final extension of 72 ºC for 10 minutes. Resulting PCR products were visualized on a 1.5%

agarose gel. Details are available at www.surettelab.ca/protocols. A custom pipeline was used to process the FASTQ files. DADA2 [1] was used to assign reads to Amplicon Sequence Variants (ASVs) and assign taxonomy with the Ribosomal Database Project (RDP) Bayesian classifier using the Silva 132 database [2]. ASVs were clustered at 99% identity. Custom R scripts were used to calculate taxonomic relative abundance values, α- and β-diversity, and to perform statistical tests. In heat maps, relative abundance of each taxon was expressed as $\log_{10}$ fold change from its median across the entire cohort. All relative abundance values of 0 were assigned 1×10^{-6} in heat maps, one order of magnitude lower than the lowest detectable taxon in the dataset, to allow the logarithmic transformation of the fold change.

Supplemental figures:


BPD-DS
LSG
SPF
GF
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
Pre-Surgery
Post-Surgery
Pre-surgery
Post-surgery
Weight gain (%)
Food intake (g/mouse)
Fat mass (% of body weight)
Lean mass (% of body weight)
Weeks
p>0.99 for all timepoints
p>0.4 for all timepoints
p>0.31 for all timepoints
p=0.62
p=0.08
p=0.77
p=0.86
p=0.61
p=0.42
p=0.27
p=0.44

Supplemental figure 1: Body features in mice colonised with the fæcal microbiota of patients before and after BPD-DS or LSG. (a, e) Weight gain, **(b, f)** food intake, and **(c, d, g, h)** body composition in specific pathogen-free (SPF) and germ-free (GF) female mice colonised with fæcal slurries from women before and after Biliopancreatic Diversion with Duodenal Switch. **(i, m)** Weight gain**, (j, n)** food intake, and **(k, l, o, p)** body composition in SPF and GF female mice colonised with fæcal slurries from women before and after Laparoscopic Sleeve Gastrectomy (LSG). Data are presented as the mean ±SEM. Unpaired student's t-test was used to assign statistical significance to pairwise comparisons. For repeated measurements throughout time, repeated measures two-way ANOVA followed by pairwise comparisons with a Tukey post-test were performed. Statistical significance was accepted at $p<0.05$. Each square, triangle and circle represents a biological replicate (n=11-12).

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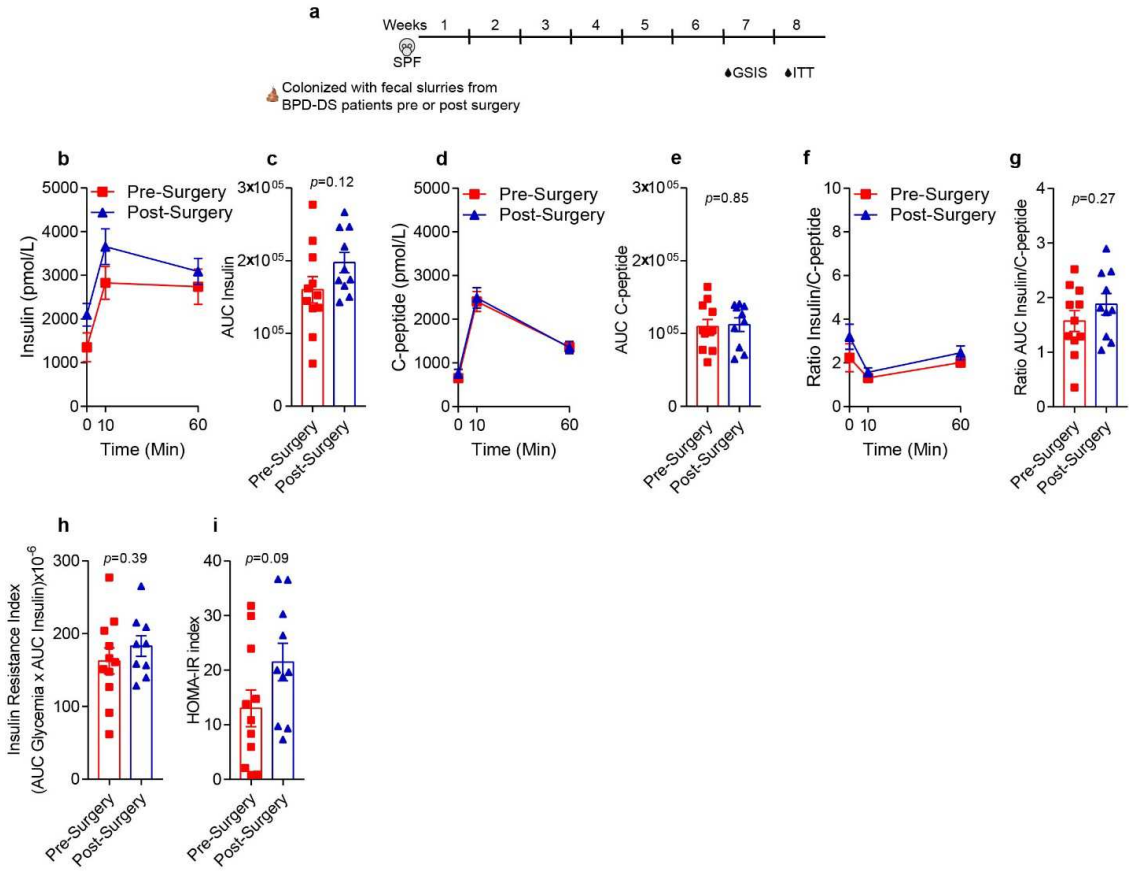
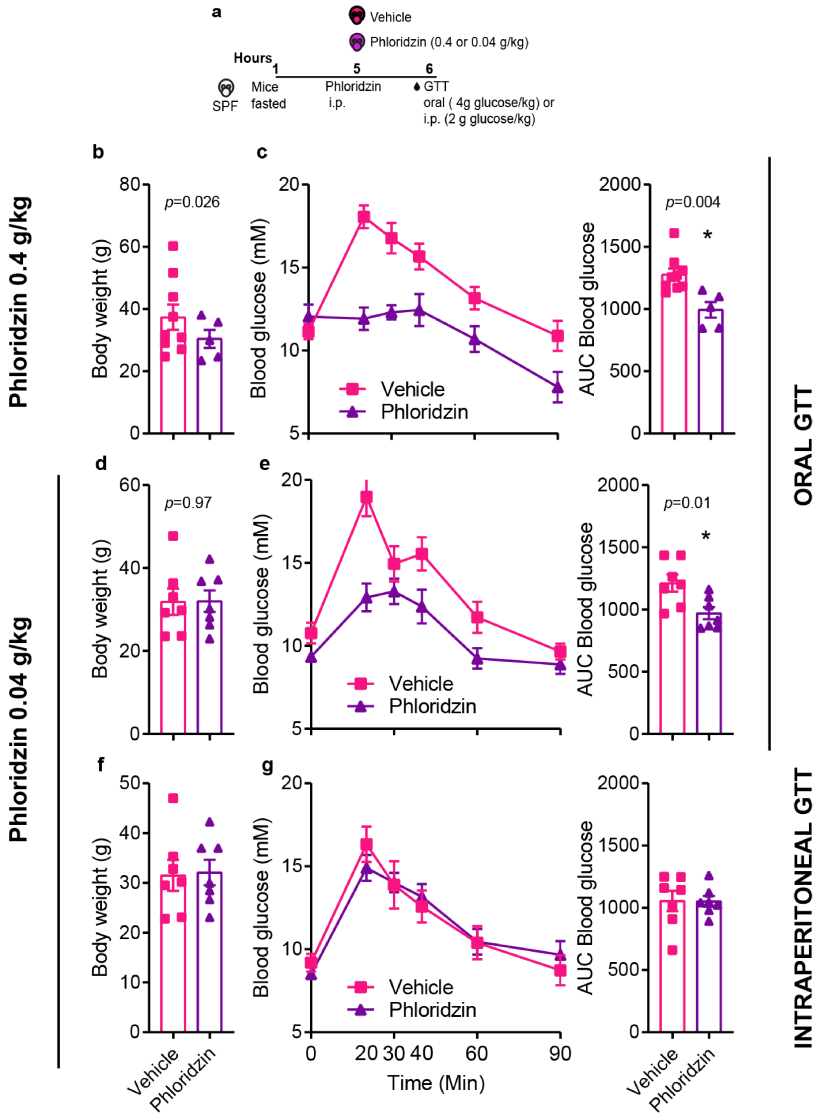
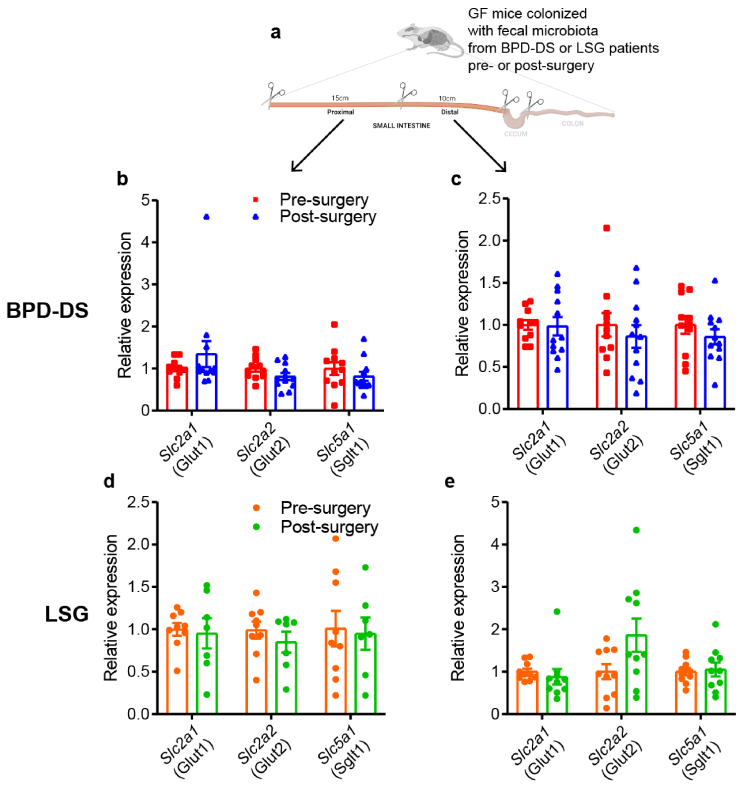


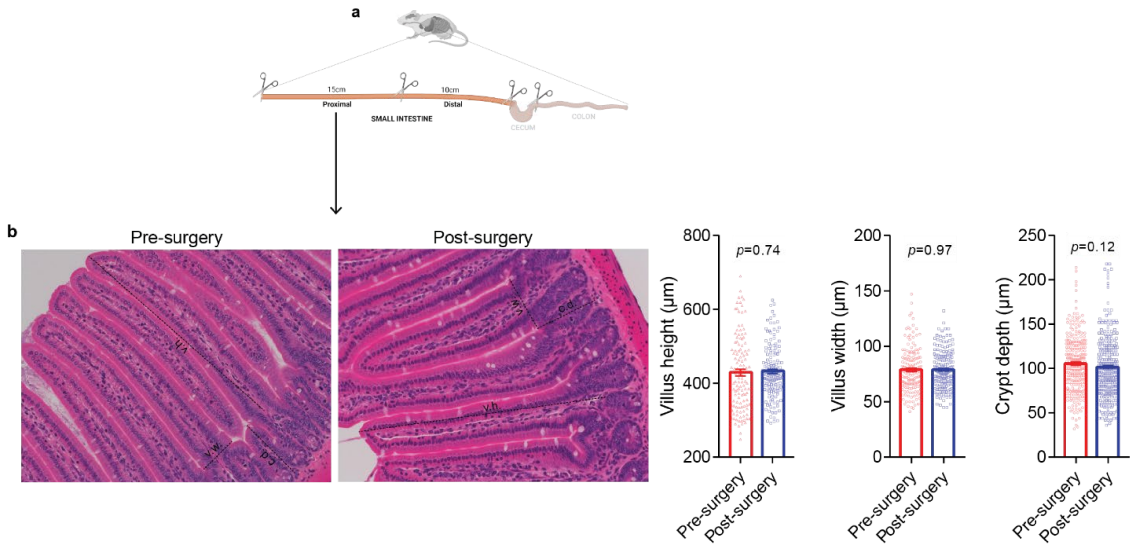
Supplemental Figure 2: Glucose-stimulated insulin and c-peptide levels in mice colonised with the fæcal microbiota of patients before and after BPD-DS. (a) Timeline of metabolic profiling in specific pathogen-free (SPF) female mice colonised with fæcal slurries from women before and after Biliopancreatic Diversion with Duodenal Switch (BPD-DS). **(b, c)** Plasma insulin, **(d, e)** c-peptide, and **(f, g)** insulin/c-peptide ratio during glucose-stimulated insulin secretion (GSIS) tests and area under the curves (AUC). **(h)** Insulin resistance index. **(i)** HOMA-IR. Unpaired student's t-test was used to calculate *p* values, which were considered significant at $p<0.05$. Each square and triangle represents a biological replicate (n=10-12).



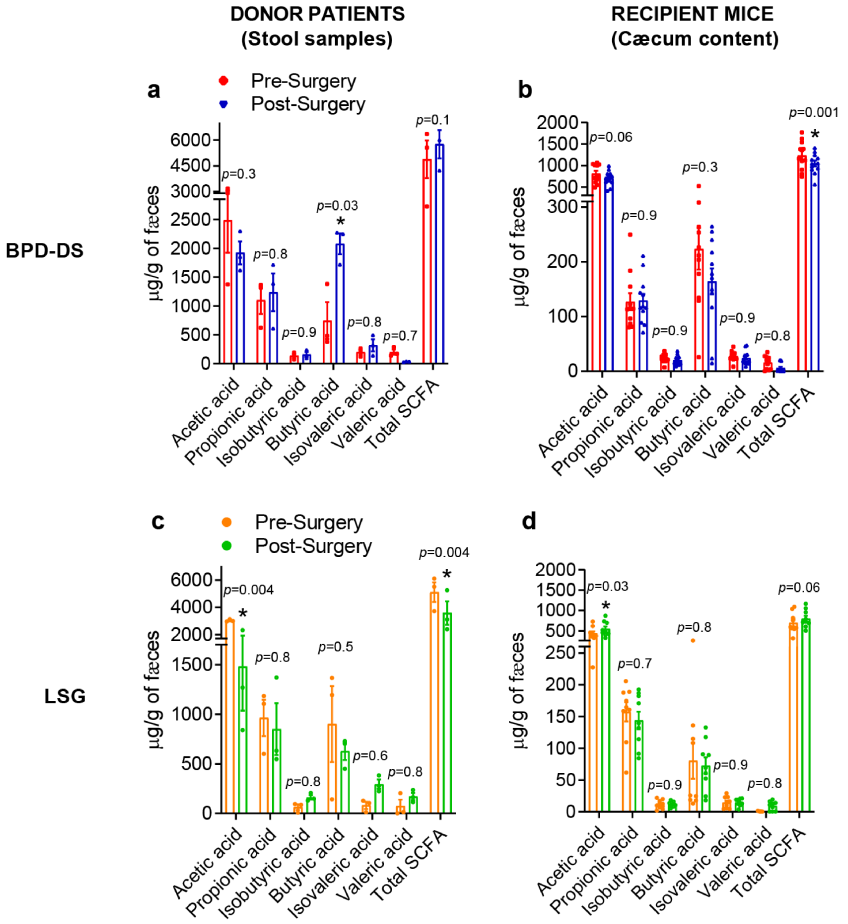
Supplemental figure 3: A low dose of phloridzin attenuates blood glucose upon oral, but not intraperitoneal, GTT in mice. (a) Female specific pathogen-free (SPF) mice were intraperitoneally injected with 0.4 or 0.04 g/kg phloridzin or vehicle and next subjected to oral or intraperitoneal glucose tolerance tests (GTT). Body weight, glucose excursion during oral GTT and area under GTT curves (AUC) after injection with **(b, c)** 0.4 g/kg and **(d, e)** 0.04 g/kg phloridzin. **(f, g)** Body weight, glucose excursion during intraperitoneal GTT and AUC after injection with 0.04 g/kg. Data are presented as the mean ±SEM. Unpaired student's t-test was used to calculate *p* values, which were considered significant at $p<0.05$. Each square or triangle represents a biological replicate (n=5-9)



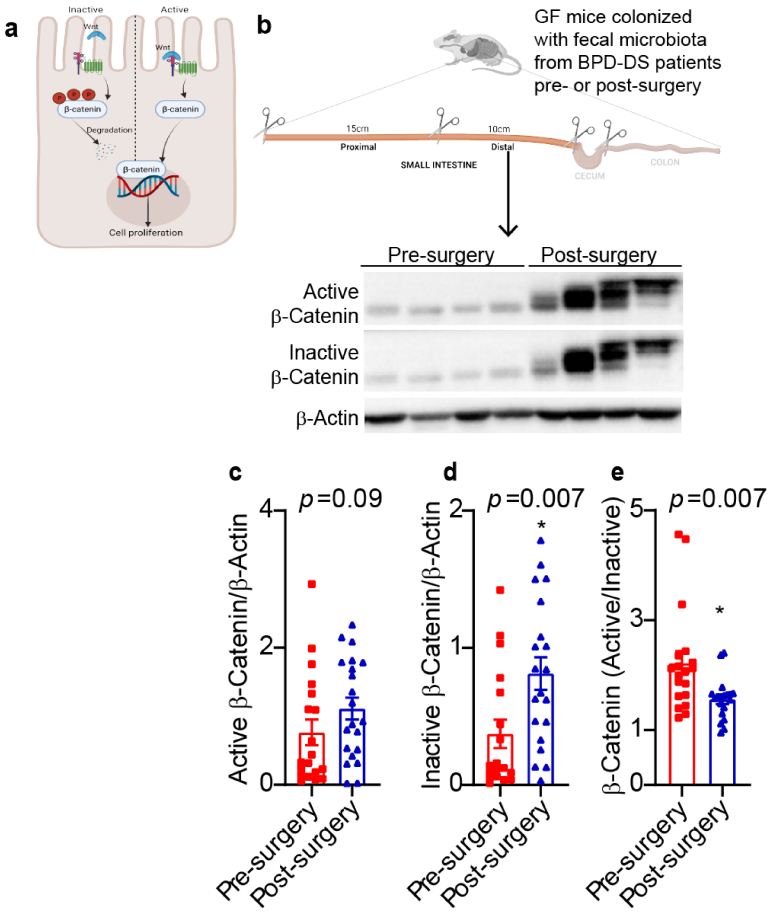
Supplemental figure 4: Transcript levels of glucose transporters in the gut of mice colonised with the fæcal microbiota of patients before and after BPD-DS or LSG. (a) mRNA was purified from proximal and distal small intestine sections from female germ-free (GF) mice colonised with fæcal microbiota of women before and after Bioliopancreatic Diversion with Duodenal Switch (BPD-DS) or Laparoscopic Sleeve Gastrectomy, reverse transcribed and used for RT-qPCR analysis. The mRNA expression of genes encoding Glut1 (*Slc2a1*), Glut2 (*Slc2a2*) and Sglt1 (*Slc5a1*) were analyzed using the ΔΔCt method and 18S as reference gene in the proximal and distal intestine of mice colonised with the fæcal microbiota before and after **(b, c)** BPD-DS or **(d, e)**. Data are presented as the mean ±SEM. Unpaired student's t-test was used to calculate *p* values, which were considered significant at $p<0.05$. Each square, triangle or circle represents a biological replicate (n=7-11)



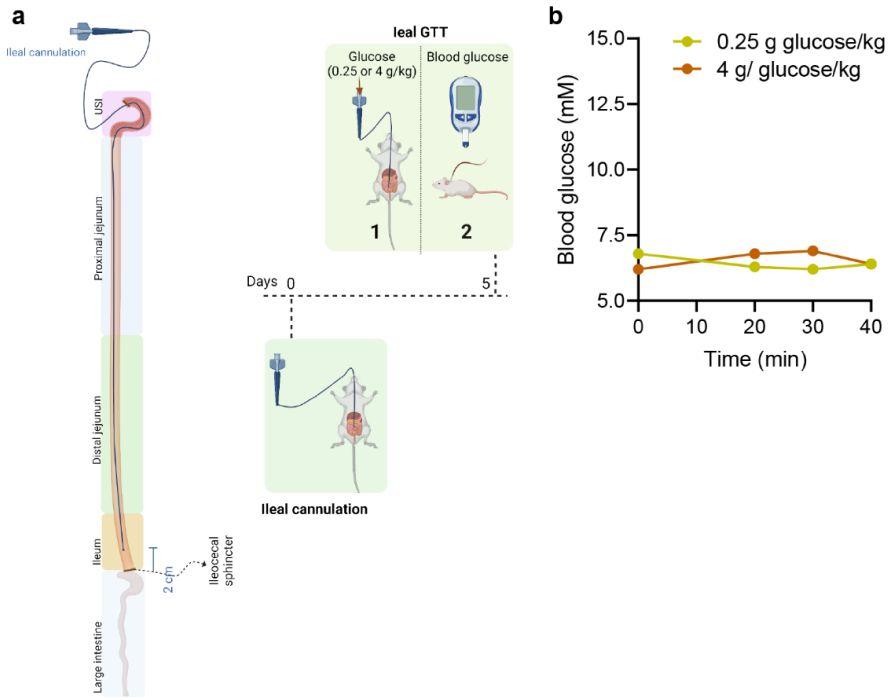
Supplemental figure 5: Intestinal morphology in the proximal small intestine of mice colonised with the fæcal microbiota of patients before and after BPD-DS. (a) Schematic representation of method used to separate and harvest different intestinal sections from mice. **(b)** Representative images and morphometric analysis of hematoxylin/eosin-stained proximal small intestine sections harvested from female germ-free (GF) mice colonised with the fæcal microbiota of female patients before and after Bioliopancreatic Diversion with Duodenal Switch (BPD-DS). Each dot represents a villus or crypt (ie, technical replicates, n=122-296).



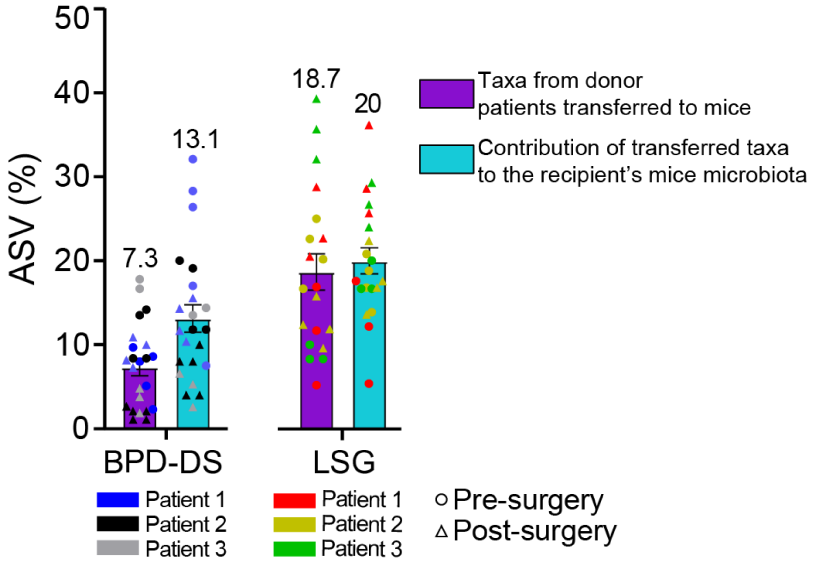
Supplemental figure 6: Short-chain fatty acids in patients and recipient mice before and after BPD-DS or LSG. (a, c) Short-chain fatty acids (SCFAs) found in stool samples from women before and after Biliopancreatic Diversion with Duodenal Switch (BPD-DS) or Laparoscopic Sleeve Gastrectomy (LSG) (n=3). **(b, d)** SCFAs in the cæcum of female mice colonised with fæcal slurries from women before and after BPD-DS or LSG (n= 11-12). Data are presented as the mean ±SEM. Unpaired student's t-test was used to calculate *p* values, which were considered significant at $p<0.05$. Each square or triangle or circle represents a biological replicate.



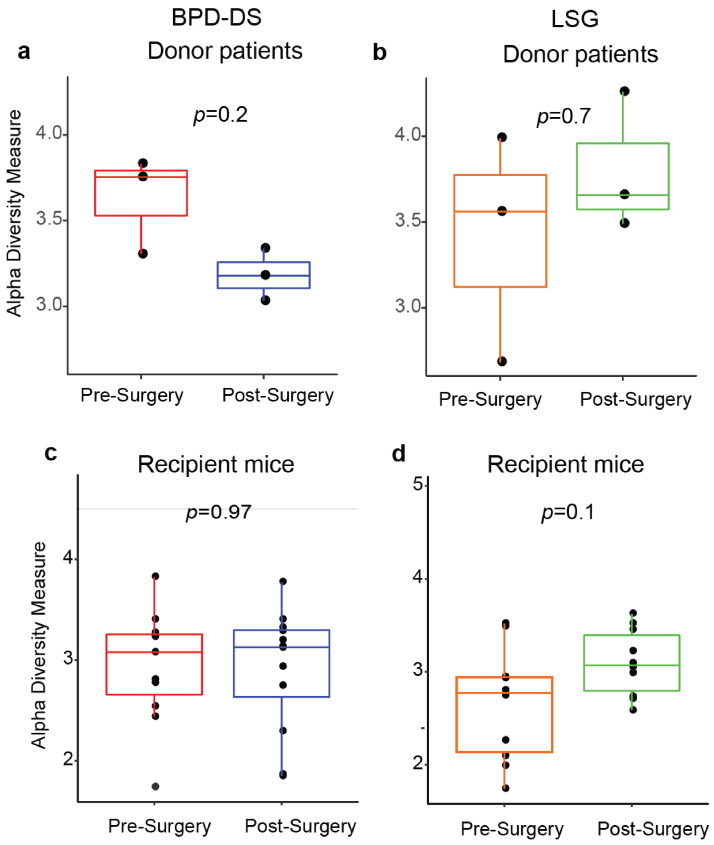
Supplemental Figure 7: Protein expression of active and inactive β-catenin in the gut of mice colonised with the fæcal microbiota of patients before and after BPD-DS (a) Upon phosphorylation, β-catenin is primed to proteasomal degradation and remains inactive. Receptor activation by a wnt ligand instigates downstream signals to dephosphorylate and activate β-catenin, which in turn regulates cell proliferation. **(b)** Protein lysates were obtained from distal small intestine sections from female germ-free (GF) mice colonised with fæcal microbiota of women before and after Bioliopancreatic Diversion with Duodenal Switch (BPD-DS) and immunoblotted against active and inactive β-catenin as well as β-actin as a loading control. Membrane picture is representative of the entire cohort and one band was cropped out of the image on each side. Bands were analyzed by densitometry, and protein expression of **(c)** active and **(d)** inactive β-catenin was normalized by the loading control. **(d)** The ratio Active:Inactive β-catenin was calculated as a readout of Wnt/ β-catenin pathway activation status. Data are presented as the mean ±SEM. Unpaired student's t-test was used to calculate *p* values, which were considered significant at $p<0.05$. Each square or triangle represents a biological replicate (n=17-21)



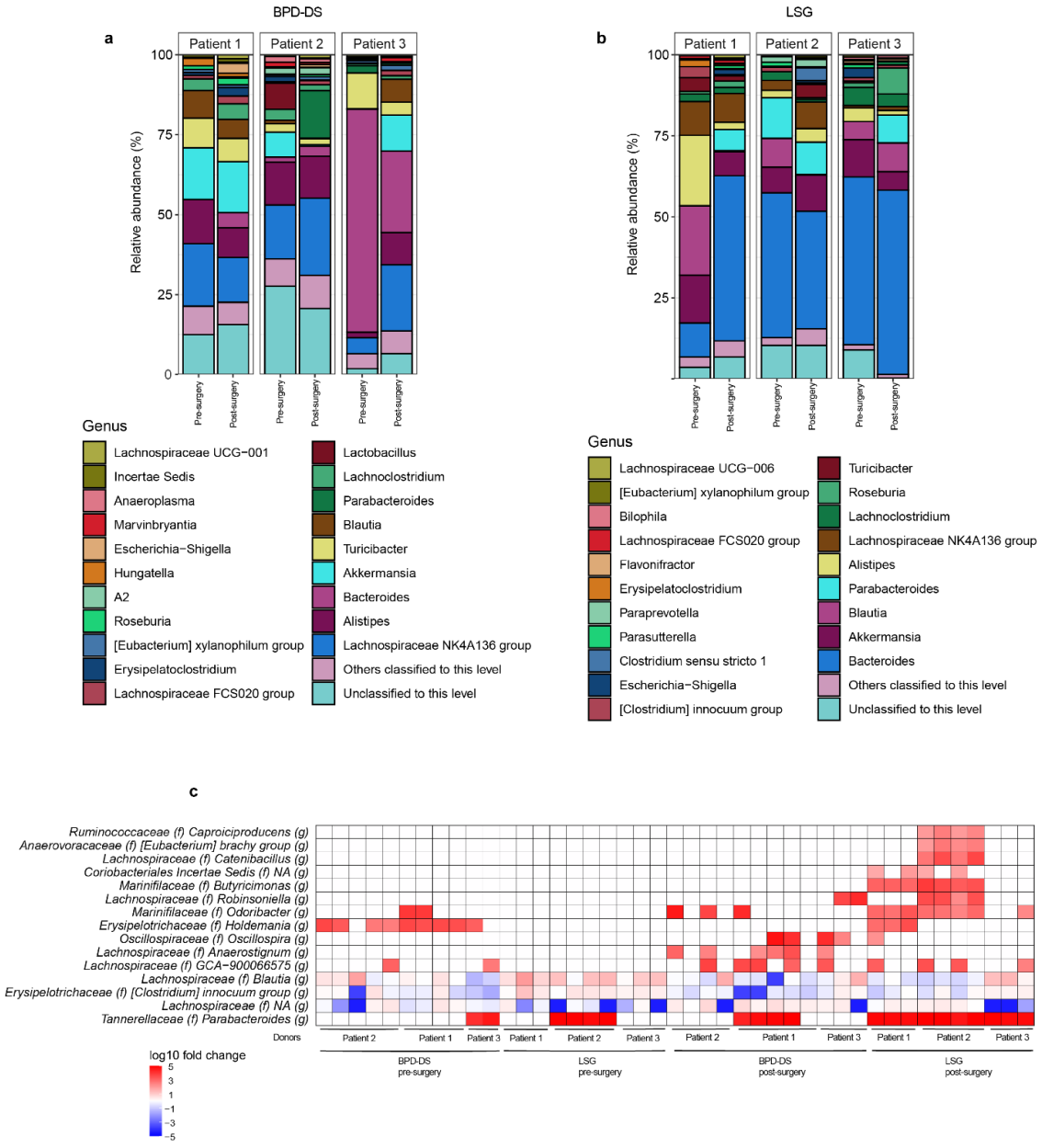
Supplemental Figure 8: Blood glucose after intraluminal glucose infusion in the ileum of rats. **(a)** A catheter was placed into the ileum (2 cm distal to the ileocecal valve) of specific pathogen-free (SPF) male rats. One day after ileal cannulation, rats were infused with 0.25 or 4 g/kg glucose targeting the distal ileum and large intestine. Blood glucose was monitored at different time points (0, 5, 10, 20, 30, 40 min) after glucose infusion. **(b)** Glucose excursion curves following glucose infusion into the ileum and large intestine (n=1).



Supplemental Figure 9: Colonisation efficiency in mice colonised with the fæcal microbiota of patients before and after BPD-DS or LSG. The purple columns show the percentage of ASVs found in the inocula (fæces from donor patients) and that were also identified in the fæces of recipient mice (ie, transmitted taxa). The ocean blue columns show the percentage contribution of transmitted taxa to the recipient mice's microbiota. Each circle or triangle represents a mouse (independent biological replicates), where the shape indicates pre- or post-surgery timepoint and the colour indicates the origin of the inoculum. Data are presented as the mean ±SEM



Supplemental Figure 10: Alpha-diversity in stool samples of mice colonised with the fæcal microbiota of patients before and after BPD-DS or LSG. Taxonomically annotated 16S rRNA gene sequences were quantified and used to calculate the Shannon Index to infer within-sample diversity. Shannon index was calculated with taxa obtained from stool samples of women before and after **(a)** BPD-DS and **(b)** LSG (n=3). Shannon index was also calculated for fæcal bacterial communities found in the in female germ-free (GF) mice colonised with fæcal microbiota from women before and after **(a)** BPD-DS or **(b)** LSG (n=10-12). Unpaired student's t-test was used to calculate *p* values, which were considered significant at $p<0.05$. Each dot represents a biological replicate (n=10-12).



Supplemental figure 11: Bacterial relative abundance profile in the fæces of mice colonised with the fæcal microbiota of patients before and after BPD-DS or LSG. Relative abundance of the top 20 most abundant bacterial genera in the fæces of female germ-free (GF) recipient mice colonised with the fæcal microbiota of women before and after **(a)** Bioliopancreatic Diversion with Duodenal Switch (BPD-DS) or **(b)** Laparoscopic Sleeve Gastrectomy (LSG). **(c)** Heat maps depicting the taxa significantly different between the pre- and post- surgery groups (p<0.05,

Wilcoxon rank sum test). ASVs were clustered at 99% similarity and grouped by genus. The relative abundance of each taxon is expressed as $\log_{10}$ fold change from its median level across the entire cohort (both surgeries combined). All relative abundance values of 0 were assigned 1×10^{-6}, one order of magnitude lower than the lowest detectable taxon in the dataset, to allow the logarithmic transformation of the fold change. The heat map is clustered along both axes. Each square, triangle and circle represent a biological replicate n=11-12.

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Supplemental tables:

Supplemental Table 1: Medicaments used by donor patients

Donor	Surgery type	Medicaments pre-surgery	Medicaments post-surgery
1	BPD-DS	Metformin 500 mg, Levothyroxine 0.2 mg, Formoterol, Budesonide, Fluconazole 150 mg	
2	BPD-DS	Metformin 850 mg, Insulin isophene 100 U, Insulin lispro 5-10 U of TDI, Pantoprazole 40 mg	Pancrelipase 10000 IU
3	BPD-DS	Metformin 500 mg, Rosuvastatin 5 mg, Acetaminophen 650 mg	
1	LSG	Ranitidine 150 mg, Hydrocodone-Phenylephrine syrup, Levonogestrel IUD (20 µg/day), Acetaminophen 500 mg, Acyclovir topical cream	Acetaminophen 500 mg, Velacyclovir 500 mg
2	LSG	Metformin 850 mg, Canagliflozin 300 mg, Rosuvastatin 5 mg, Morphine 5 mg, Acetaminophen 325 mg	Acetaminophen 650 mg Buprenorphine skin patch 1µg/h.
3	LSG	Rabeprazole 20 mg	Lansoprazole 30 mg Estradiol 0.06% topical cream

Note: Data post-surgery was obtained 12 months after bariatric procedure. TDI stands for Total Daily Insulin. IUD stands for Intrauterine Device. BPD-DS and LSG are acronyms for Biliopancreatic Diversion with Duodenal Switch and Vertical Sleeve Gastrectomy, respectively.

Supplemental Table 2: Caloric intake by donor patients

			Patient 1	Patient 2	Patient 3
BPD-DS	Pre-surgery	Energy (kcal)	3072.5	1992.4	2818.6
		% calories from fat	39.7	28.9	44.4
		% calories from protein	17.4	19.6	15.9
		% calories from carbohydrate	41.9	52.9	42.9
	Post-surgery	Energy (kcal)	2793.2	1836.2	2295.5
		% calories from fat	39.6	29.2	43.7
		% calories from protein	17.0	18.8	14.4
		% calories from carbohydrate	41.7	52.8	44.8
		Caloric restriction (kcal)	279.2	156.2	523.0
		Caloric restriction (%)	9.1	7.8	18.6
LSG	Pre-surgery	Energy (kcal)	1873.6	1800.0	3432.7521
		% calories from fat	35.4	38.3	32.2342
		% calories from protein	18.9	24.3	21.3723
		% calories from carbohydrate	47.3	38.9	48.72
	Post-surgery	Energy (kcal)	2402.8	1115.0	not available
		% calories from fat	41.7	42.7	
		% calories from protein	20.3	18.8	
		% calories from carbohydrate	41.0	40.0	
		Caloric restriction (kcal)	-529.2	685.0	
		Caloric restriction (%)	128.2	61.9	

Note: Data post-surgery was obtained 12 months after bariatric procedure. BPD-DS and LSG are acronyms for Biliopancreatic Diversion with Duodenal Switch and Vertical Sleeve Gastrectomy, respectively.

References:

1 Callahan BJ, McMurdie PJ, Rosen MJ, *et al.* DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

2 Quast C, Pruesse E, Yilmaz P, *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2012;**41**:D590–6.