

ONLINE APPENDIX

Supplementary Table 1. Clinical and biological characteristics of the lean controls, obese diabetic and non diabetic subjects before gastric surgery.

	Control n = 13	OB/nD n = 23	OB/D n = 7
Age (years)	36 ± 3 ^(A)	42 ± 2 ^(A, B)	49 ± 5 ^(B)
Adiposity markers			
Body Weight (kg)	59.0 ± 1.9 ^(A)	127.4 ± 4.3 ^(B)	121.5 ± 11.6 ^(B)
BMI (kg/m ²)	21.7 ± 0.4 ^(A)	48.3 ± 1.6 ^(B)	45.4 ± 3.5 ^(B)
Adipocyte diameter (µm)	nd	115.6 ± 1.7 ^(A)	120.1 ± 3.4 ^(B)
REE (kcal)	nd	1830.3 ± 65.2 ^(A)	1762.1 ± 85.9 ^(A)
Fat mass%	26.5 ± 1.4 ^(A)	48.2 ± 1.1 ^(B)	46.6 ± 2.1 ^(B)
Fat-free mass%	69.5 ± 1.3 ^(A)	49.7 ± 1.1 ^(B)	50.9 ± 1.9 ^(B)
Leptin (ng/ml)	9.24 ± 1.46 ^(A)	50.5 ± 4.1 ^(B)	52.2 ± 8.3 ^(B)
Plasma glucose homeostasis and insulin sensitivity			
Glycaemia (mmol/l)	4.3 ± 0.2 ^(A)	5.4 ± 0.2 ^(A)	9.4 ± 1.4 ^(B)
Insulinemia (µU/ml)	3.8 ± 0.3 ^(A)	16.6 ± 1.8 ^(B)	19.4 ± 10.9 ^(B)
Adiponectin (µg/ml)	13 ± 1.43 ^(A)	6.8 ± 0.6 ^(B)	5.4 ± 0.8 ^(B)
Plasma lipid homeostasis			
Total cholesterol (mmol/l)	4.8 ± 0.3 ^(A)	4.5 ± 0.2 ^(A)	4.6 ± 0.3 ^(A)
Total triglycerides (mmol/l)	0.8 ± 0.1 ^(A)	1.5 ± 0.2 ^(B)	1.8 ± 0.5 ^(B)
HDL cholesterol (mmol/l)	1.8 ± 0.1 ^(A)	1.2 ± 0.1 ^(B)	1.3 ± 0.1 ^(B)
Inflammatory markers			
Plasma hsCRP (mg/dl)	nd	2.7 ± 0.8 ^(A)	4.4 ± 2.4 ^(A)
Plasma IL-6 (pg/ml)	nd	4 ± 0.4 ^(A)	5.8 ± 1.3 ^(A)
Plasma orosomucoid (g/l)	nd	1.05 ± 0.05 ^(A)	0.95 ± 0.08 ^(A)

All values are expressed as mean ± SEM; nd: not determined. Fat mass%, Fat-free mass%: values expressed as a percentage of body weight. REE: resting energy expenditure. Wilcoxon Rank Sum tests for changes between the groups. Data not sharing the same letter within a horizontal line are significantly different (P<0.05).

Supplementary Table 2. Evolution of the clinical and biological characteristics of diabetic (OB/D) and non-diabetic (OB/nD) subjects before surgery, 3 months and 6 months after surgery

	OB/nD (n = 23)			OB/D (n=7)		
	Basal	3 months	6 months	Basal	3 months	6 months
Food intake						
Food intake (kcal)	2118 ± 111 ^(A)	1171 ± 99 ^(A)	1406 ± 55 ^(B)	1469 ± 69 ^(A)	784 ± 94 ^(B)	1192 ± 131 ^(C)
Adiposity markers						
Body Weight (kg)	127.4 ± 4.3 ^(A)	108.0 ± 4.0 ^(B)	98.0 ± 3.8 ^(C)	121.5 ± 11.6 ^(A)	105.3 ± 11.0 ^(B)	99.3 ± 10.7 ^(C)
BMI (kg/m ²)	48.3 ± 1.6 ^(A)	40.9 ± 1.4 ^(B)	37.2 ± 1.4 ^(C)	45.4 ± 3.5 ^(A)	39.3 ± 3.4 ^(B)	36.9 ± 3.2 ^(C)
Adipocyte diameter (µm)	115.6 ± 1.7 ^(A)	111.2 ± 1.2 ^(B)	101.6 ± 3.9 ^(C)	120.1 ± 3.4 ^(A)	107.4 ± 1.3 ^(A)	109.3 ± 3.7 ^(A)
REE (kcal)	1830.3 ± 65.2 ^(A)	1830.3 ± 55.7 ^(A)	1566.4 ± 49.3 ^(B)	1762.1 ± 85.9 ^(A)	1887.3 ± 152.1 ^(B)	1492.2 ± 80.1 ^(C)
Fat mass%	48.2 ± 1.1 ^(A)	44.9 ± 1.1 ^(B)	41.3 ± 1.4 ^(C)	46.6 ± 2.1 ^(A)	43.3 ± 2.5 ^(B)	41.4 ± 2.3 ^(C)
Fat-free mass%	49.7 ± 1.1 ^(A)	52.7 ± 1.0 ^(B)	56.0 ± 1.3 ^(C)	50.9 ± 1.9 ^(A)	53.9 ± 2.3 ^(B)	55.6 ± 2.0 ^(C)
Leptin (ng/ml)	50.5 ± 4.1 ^(A)	24.1 ± 2.5 ^(B)	23.0 ± 2.6 ^(B)	52.2 ± 8.3 ^(A)	30.0 ± 6.4 ^(B)	30.4 ± 8.6 ^(B)
Plasma glucose homeostasis and insulin sensitivity						
Glycaemia (mmol/l)	5.4 ± 0.2 ^(A)	4.8 ± 0.1 ^(B)	4.6 ± 0.1 ^(B)	9.6 ± 1.7 ^(A)	5.6 ± 0.6 ^(B)	5.5 ± 0.6 ^(B)
HbA1C (%)	5.8 ± 0.1 ^(A)	5.6 ± 0.1 ^(B)	5.6 ± 0.1 ^(B)	8.5 ± 1.3 ^(A)	6.4 ± 0.3 ^(B)	6.4 ± 0.2 ^(B)
Insulinemia (mmol/l)	16.6 ± 1.8 ^(A)	9.5 ± 1.0 ^(B)	6.3 ± 0.8 ^(C)	8.4 ± 1.7 ^(A)	8.0 ± 1.4 ^(A)	5.6 ± 0.7 ^(A)
HOMA-B%	67.0 ± 2.8 ^(A)	77.3 ± 2.6 ^(B)	89.8 ± 6.0 ^(C)	40.3 ± 8.4 ^(A)	65.8 ± 7.7 ^(A)	74.2 ± 15.0 ^(A)
HOMA-S%	145.4 ± 6.3 ^(A)	168.6 ± 5.7 ^(B)	180.3 ± 21.3 ^(B)	81.5 ± 20.7 ^(A)	142.7 ± 17.3 ^(A)	148.7 ± 25.7 ^(A)
HOMA-IR	0.7 ± 0.04 ^(A)	0.6 ± 0.02 ^(B)	0.8 ± 0.11 ^(A)	1.6 ± 0.40 ^(A)	0.8 ± 0.10 ^(A)	0.7 ± 0.10 ^(A)
Adiponectin (µg/ml)	6.8 ± 0.6 ^(A)	7.5 ± 0.7 ^(A)	8.4 ± 0.7 ^(B)	5.4 ± 0.8 ^(A)	8.7 ± 1.9 ^(A)	8.4 ± 2.3 ^(A)
Plasma lipid homeostasis						
Total cholesterol (mmol/l)	4.53 ± 0.18 ^(A)	4.23 ± 0.19 ^(A)	4.31 ± 0.17 ^(A)	4.59 ± 0.31 ^(A,B)	4.23 ± 0.23 ^(A)	4.47 ± 0.26 ^(B)
Triglycerides (mmol/l)	1.50 ± 0.19 ^(A)	1.47 ± 0.18 ^(A)	1.42 ± 0.16 ^(A)	1.81 ± 0.52 ^(A)	1.77 ± 0.47 ^(A)	1.70 ± 0.55 ^(A)
HDL cholesterol (mmol/l)	1.21 ± 0.06 ^(A,B)	1.15 ± 0.06 ^(A)	1.28 ± 0.06 ^(B)	1.27 ± 0.1 ^(A)	1.23 ± 0.18 ^(A)	1.36 ± 0.2 ^(A)
Inflammatory markers						
hsCRP (mg/dl)	2.7 ± 0.8 ^(A)	2.9 ± 1.2 ^(A)	2.9 ± 1.0 ^(A)	4.4 ± 2.4 ^(A)	1.2 ± 0.6 ^(A)	1.9 ± 0.7 ^(A)
IL-6 (pg/ml)	4 ± 0.4 ^(A)	4.3 ± 0.4 ^(A)	3.7 ± 0.4 ^(A)	5.8 ± 1.3 ^(A)	3.8 ± 0.6 ^(A)	2.7 ± 0.5 ^(A)
Orosomucoide (g/l)	1.05 ± 0.05 ^(A)	0.94 ± 0.04 ^(A,B)	0.88 ± 0.04 ^(B)	0.95 ± 0.08 ^(A)	0.92 ± 0.09 ^(A)	0.81 ± 0.05 ^(A)

All values are expressed as mean ± SEM. Fat mass%, Fat-free mass%: values expressed as a percentage of body weight. REE: resting energy expenditure). HOMA: homeostasis model assessment; HOMA-B%: estimate the pancreatic β-cell function; HOMA-S%: evaluate the insulin sensitivity and HOMA-IR: represent the insulin resistance. Paired wilcoxon tests for changes in these parameters between various time points.

† Comparison in 5 diabetic subjects without insulin injection, exogenous insulin may introduce a wrong interpretation of the HOMA test. Data not sharing the same letter within a horizontal line are significantly different (P<0.05).

Supplementary Table 3. Composition of microbiota compared in diabetic (OB/D) and non-diabetic (OB/nD) subjects before surgery, and 3 months and 6 months after surgery.

	<i>All-Bacteria</i> [*]	<i>Firmicutes</i>				<i>Bacteroidetes</i>		
		<i>Clostridium coccoides</i> group [†]	<i>Lactobacillus/Leuconostoc/Pediococcus</i> group [†]	<i>Clostridium leptum</i> group [†]	<i>Fecalibacterium prausnitzii</i> species ^{†,‡}	<i>Bifido-bacterium</i> genus [†]	<i>Bacteroides/Prevotella</i> group [†]	<i>E. coli</i> species [†]
OB/nD								
M0 (23)	11.29 ± 0.1	- 1.58 ± 0.2 ^A	- 2.75 ± 0.3 ^A	- 0.86 ± 0.3 ^A	- 1.45 ± 0.2 ^A	- 2.37 ± 0.2 ^A	- 1.61 ± 0.1 ^A	- 3.42 ± 0.3 ^A
M3 (20)	11.00 ± 0.2	- 1.90 ± 0.1 ^A	- 3.26 ± 0.2 ^B	- 1.04 ± 0.3 ^A	- 1.85 ± 0.2 ^B	- 3.10 ± 0.2 ^B	- 0.91 ± 0.1 ^B	- 1.76 ± 0.2 ^B
M6 (11)	11.09 ± 0.2	- 1.50 ± 0.3 ^A	- 3.57 ± 0.3 ^B	- 0.37 ± 0.2 ^A	- 1.40 ± 0.1 ^A	- 2.85 ± 0.3 ^B	- 1.02 ± 0.2 ^B	- 2.13 ± 0.3 ^A
OB/D								
M0 (7)	11.17 ± 0.1	- 1.46 ± 0.4 ^A	- 2.62 ± 0.5 ^A	- 1.63 ± 0.8 ^A	- 2.79 ± 0.5 ^B	- 2.22 ± 0.4 ^A	- 1.61 ± 0.2 ^A	- 2.49 ± 0.3 ^A
M3 (6)	10.73 ± 0.4	- 1.92 ± 0.7 ^A	- 2.81 ± 0.3 ^A	- 0.69 ± 0.5 ^A	- 1.78 ± 0.7 ^B	- 2.45 ± 0.2 ^A	- 0.86 ± 0.4 ^A	- 1.18 ± 0.3 ^A
M6 (4)	10.99 ± 0.2	- 1.70 ± 0.2 ^A	- 3.63 ± 0.4 ^A	- 0.57 ± 0.3 ^A	- 1.82 ± 0.4 ^B	- 3.06 ± 0.7 ^A	- 0.75 ± 0.1 ^A	- 1.27 ± 0.2 ^A

Number in parentheses represents the numbers of studied samples.

Control: healthy female volunteers, OB/nD: obese subjects without diabetes, OB/D: obese subjects with diabetes,

Data not sharing the same letter within a column are significantly different (P<0.05) to the human population.

* All-Bacteria results obtained by qPCR were expressed as mean of the log₁₀ value ± SEM.

† Results were expressed as mean of the log₁₀ value ± SEM of normalized data, calculated as the log no. of targeted bacteria minus the log of all-bacteria number.

‡ *Faecalibacterium prausnitzii* is the major component of the *Clostridium leptum* group.

Supplementary Table 4. Correlation between clinical parameters and microbiota before and after gastric surgery.

A Correlation before gastric surgery.

B Dynamic correlation during the follow-up after gastric surgery.

A

	Body weight	BMI	Body fat mass	Adipocyte diameter	Leptin	Fasting glucose	HbA1C	Insulin	HOMA-IR	hs CRP	IL-6	orosomucoid
<i>Bacteroides /Prevotella</i>												
OB/nD + OB/D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
OB/nD	NS	NS	NS	R _s 0.52*	NS	NS	NS	NS	NS	R _s -0.43*	NS	NS
<i>C. leptum</i>												
OB/nD + OB/D	NS	NS	NS	NS	NS	R _s -0.39*	NS	NS	R _s -0.39*	R _s -0.44*	NS	NS
OB/nD	NS	NS	NS	NS	NS	NS	NS	NS	NS	R _s -0.43*	NS	NS
<i>C.coccoides</i>												
OB/nD + OB/D	NS	NS	NS	R _s 0.39*	NS	NS	NS	NS	NS	NS	NS	NS
OB/nD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<i>E. coli</i>												
OB/nD + OB/D	NS	NS	NS	NS	NS	NS	R _s 0.36*	NS	NS	NS	NS	NS
OB/nD	NS	NS	NS	NS	NS	NS	NS	R _s 0.52*	NS	NS	NS	NS
<i>Faecalibacterium prausnitzii</i>												
OB/nD + OB/D	NS	NS	NS	NS	NS	R _s -0.47*	R _s -0.39*	NS	R _s -0.47*	R _s -0.54†	R _s -0.65‡	NS
OB/nD	NS	NS	NS	NS	NS	NS	NS	NS	NS	R _s -0.58†	R _s -0.60†	NS

* p< 0.05, † p< 0.01, ‡ p< 0.001

B

	Body weight	BMI	Body fat mass	Leptin	Calorie intake	Fasting glucose	Insulin	Adiponectin	hsCRP	IL-6	Orosomucoid
<i>Bacteroides /Prevotella</i>											
OB/nD + OB/D	Rs -0.33†	Rs -0.35†	Rs -0.32†	Rs -0.43†	Rs -0.33*	Rs -0.28*	NS	NS	Rs -0.27*	NS	Rs -0.31*
OB/nD	Rs -0.3*	Rs -0.3*	NS	Rs -0.39†	Rs -0.39*	Rs -0.36*	NS	NS	Rs -0.32*	NS	
<i>C.leptum</i>											
OB/nD + OB/D	NS	NS	NS	NS	NS	NS	NS	NS	Rs -0.31*	NS	NS
OB/nD	NS	NS	NS	NS	NS	NS	NS	NS	Rs -0.29*	NS	NS
<i>Faecalibacterium prausnitzii</i>											
OB/nD + OB/D	NS	NS	NS	NS	NS	NS	NS	NS	Rs -0.39†	Rs -0.35†	Rs -0.32*
OB/nD	NS	NS	NS	NS	NS	NS	NS	NS	Rs -0.37†	Rs -0.34†	Rs -0.30*
<i>E. coli</i>											
OB/nD + OB/D	Rs -0.42‡	Rs -0.47§	Rs -0.41‡	Rs -0.53¶	Rs -0.47‡	NS	NS	NS	NS	NS	Rs -0.38†
OB/nD	Rs -0.41†	Rs -0.47‡	Rs -0.45‡	Rs -0.55§	Rs -0.44†	Rs -0.35*	Rs -0.22*	Rs -0.33*	NS	NS	Rs -0.37*
<i>Bifidobacterium</i>											
OB/nD + OB/D	Rs 0.19†	Rs 0.17†	NS	Rs 0.34†	Rs 0.28*	NS	Rs 0.30*	NS	Rs 0.29*	NS	Rs 0.36†
OB/nD	NS	NS	NS	NS	NS	NS	Rs 0.29*	NS	NS	NS	Rs 0.36*
<i>Lactobacillus/Leuconostoc/Pediococcus</i>											
OB/nD + OB/D	NS	NS	NS	NS	Rs 0.29*	Rs 0.24*	Rs 0.28*	NS	NS	NS	NS
OB/nD	NS	NS	NS	NS	Rs 0.30†	NS	Rs 0.30*	NS	NS	NS	NS

* p< 0.05, † p< 0.01, ‡ p< 0.001, §p< 0.0001, ¶p< 0.00001

Supplementary Table 5. Day to day variations for 7 bacterial groups from 5 lean subjects

	<i>E.coli</i>	<i>Bacteroides/Pr evotella</i>	<i>Bifidobacterium</i>	<i>Lactobacillus/ Pediococcus</i>	<i>F. prausnitzii</i>	<i>C leptum</i>	<i>C coccoides</i>
D1	-4.01±0.86	-1.1±0.25	-2.35±0.17	-4.04±0.37	-0.8±0.32	0.27±0.53	-1.49±0.33
D2	-3.8±0.54	-0.94±0.42	-2.55±0.37	-4±0.5	-0.87±0.25	-0.11±0.33	-1.81±0.2
p value	1	0.8125	0.8125	0.625	1	0.3125	0.0625

Values are expressed as mean ± SEM; (n=5). D1: first day, D2: second day. Paired Wilcoxon stands for analyzing parameters changes between various time points.

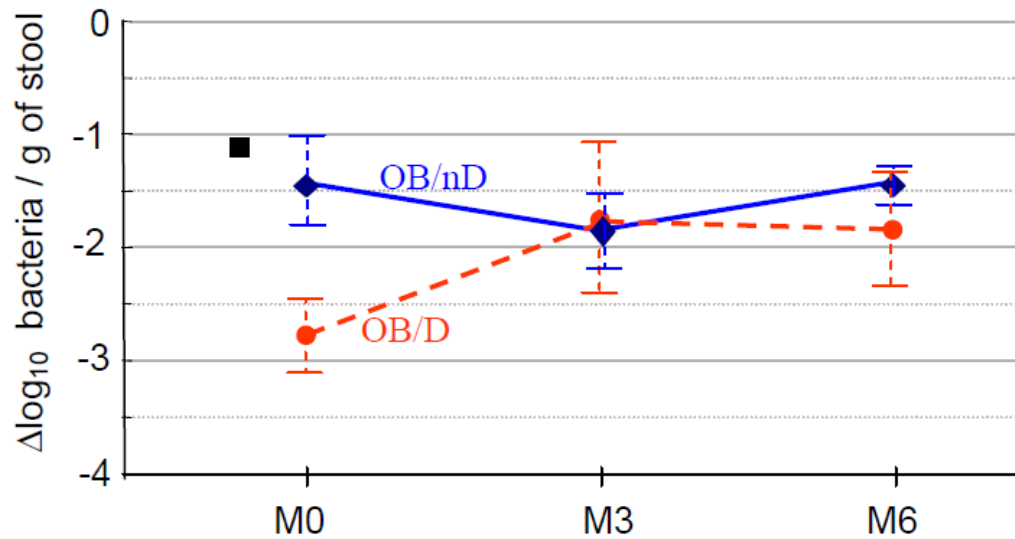
Supplementary Table 6. Group and species-specific 16S rRNA-targeted primers and probes.

<i>Target organism</i>	<i>Primer and probe</i>	<i>Sequence 5'-3'</i>
<i>All-Bacteria</i> (*)	F_Bact 1369	CGG TGA ATA CGT TCC CGG
	R_Prok1492	TAC GGC TAC CTT GTT ACG ACT T
	P_TM1389F	6FAM-CTT GTA CAC ACC GCC CGT C
<i>C. leptum</i>	F_Clept 09	CCT TCC GTG CCG SAG TTA
	R_Clept 08	GAA TTA AAC CAC ATA CTC CAC TGC TT
	P-Clep 01	6FAM-CAC AAT AAG TAA TCC ACC
<i>Bifidobacterium</i>	F_Bifid 09c	CGG GTG AGT AAT GCG TGA CC
	R_Bifid 06	TGA TAG GAC GCG ACC CCA
	P_Bifid	6FAM-CTC CTG GAA ACG GGT G
<i>C. coccoides</i>	F_Ccoc 07	GAC GCC GCG TGA AGG A
	R_Ccoc 14	AGC CCC AGC CTT TCA CAT C
	P_Erec482(*)	VIC-CGG TAC CTG ACT AAG AAG
<i>Bacteroides/Prevotella</i>	F_Bacter 11	CCT WCG ATG GAT AGG GGT T
	R_Bacter 08	CAC GCT ACT TGG CTG GTT CAG
	P_Bac303(*)	VIC-AAG GTC CCC CAC ATT G
<i>E. coli</i>	E.coli F	CAT GCC GCG TGT ATG AAG AA
	E.coli R	CGG GTA ACG TCA ATG AGC AAA
<i>Lactobacillus/Leuconostoc/Pediococcus</i>	F_Lacto 05	AGC AGT AGG GAA TCT TCC A
	R_Lacto 04	CGC CAC TGG TGT TCY TCC ATA TA
<i>F. prausnitzii</i>	Fprau 07	CCA TGA ATT GCC TTC AAA ACT GTT
	Fprau 02	GAG CCT CAG CGT CAG TTG GT

(*) modified from reference

Primers and probes used in this study are as previously described (16;17). Probe sequences are in bold.

Supplementary Figure 1: Dynamic evolution of *F. prausnitzii* population levels from M0 to M3 and M6 after gastric surgery in obese diabetic (OB/D) and non-diabetic (OB/nD) subjects.



■ Average *F. prausnitzii* population levels in control subjects.

Results were expressed as mean \pm SEM of the $\Delta\log_{10}$ value of normalized data, calculated as the log number of targeted bacteria minus the log number of All-Bacteria.