DIFFERENTIAL ADAPTATION OF HUMAN GUT MICROBIOTA TO BARIATRIC SURGERY-INDUCED WEIGHT LOSS: LINKS WITH METABOLIC AND LOW-GRADE INFLAMMATION MARKERS

Running Title: Gut microbiota profile in RYGB induced weight loss

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**Objective**  Obesity alters gut microbiota ecology and associates with low-grade inflammation in humans. Roux-en-Y gastric bypass (RYGB) surgery is one of the most efficient procedures for the treatment of morbid obesity resulting in drastic weight loss and improvement of metabolic and inflammatory status. We analyzed the impact of RYGB on the modifications of gut microbiota and examined links with adaptations associated with this procedure.

**Research design and methods**  Gut microbiota was profiled from faecal samples by qPCR in 13 lean controls and in 30 obese individuals (with 7 type 2-diabetics) explored before (M0), 3 (M3) and 6 months (M6) after RYGB.

**Results**  Four major findings are highlighted: (i) *Bacteroides/Prevotella* group was lower in obese subjects than in controls at M0 and increased at M3. It was negatively correlated with corpulence, but the correlation depended highly on caloric intake, (ii) *Escherichia coli* species increased at M3 and inversely correlated with fat mass and leptin levels, independently of changes in food intake, (iii) Lactic acid bacteria including *Lactobacillus/Leuconostoc/Pediococcus* group and *Bifidobacterium* genus decreased at M3, (iv) *Faecalibacterium prausnitzii* species was lower in subjects with diabetes and associated negatively with inflammatory markers at M0 and throughout the follow-up after surgery, independently of changes in food intake.

**Conclusions**  These results suggest that components of the dominant gut microbiota rapidly adapt in a starvation-like situation induced by RYGB while the *F. prausnitzii* species is directly linked to the reduction in low-grade inflammation state in obesity and diabetes, independently of calorie intake.

Obesity is characterized by increased fat mass accumulation and the development of co-morbidities including other metabolic and cardiovascular diseases. Even though some but not all environmental factors have been elucidated, the increasing epidemic of obesity appears virtually impossible to control and the mechanisms associated with fat mass expansion need to be identified. Obesity is considered as a low-grade inflammatory disease with adipose tissue contributing to this state via the secretion of molecules capable of altering metabolic homeostasis (1;2). A novel factor identified to play a role in human obesity, and associated metabolic risks, is the commensal microbiota of the intestine (3).

A role for the intestinal microbiota in harvesting energy from food (4) and regulating body fat storage (5) was proposed in rodents. Germ-free mice colonized by microbiota increase their body fat and develop insulin-resistance in spite of a 30% decrease in food intake. These changes were associated with a dysbiosis in obese mice: an increased representation of the Firmicutes phylum and a reduced representation of the Bacteroidetes phylum (6). Other studies suggested a contribution of the gut microbiota-produced lipopolysaccharides (LPS) to inflammation and development of metabolic syndrome (7-9). In humans, increased endotoxemia (circulating LPS) was found to be associated with increased fat consumption (10). In obese patients losing weight throughout low calorie diets, diminished Bacteroidetes and increased
Firmicutes were found to be more abundant in obese subjects compared to lean controls at the end of the dietary intervention (11). However, modification of the Firmicutes/Bacteroidetes ratio observed in obese individuals was not confirmed in other studies (12). No study has clearly explored the association between changes in gut microbiota and improvement of metabolic or inflammatory phenotypes associated with weight modification over time.

Roux-en-Y gastric bypass (RYGB) surgery is an increasingly effective model to study microbiota changes in this context. RYGB leads to major improvements in metabolic and inflammatory markers (13). This procedure allows understanding the molecular adaptations underlying the observed health benefits and the potential role of calorie restriction in changes in gut microbiota pattern.

Our present work analyzed the microbiota profiles in the faeces of morbidly obese subjects before and after RYGB. We examined the association between gut microbiota changes and a range of body composition, metabolic and inflammatory markers. These results provide new insight regarding gut microbiota changes in obese subjects after RYGB and highlight some bacterial groups as possible factors associated with changes in nutritional status, others with metabolic and inflammatory parameters.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Thirty obese subjects (27 women/3 men) enrolled in a bariatric surgery program were recruited at the Center of Reference for Medical and Surgical Care of Obesity (Pitié-Salpêtrière hospital, Paris, France). The patients had the criteria for obesity surgery: Body Mass Index BMI $\geq 40$ kg/m$^2$ with at least two co-morbidities (hypertension, type-2 diabetes, dyslipidemia or obstructive sleep apnea syndrome). The subjects’ weight was stable ($\pm 2$kg) for at least 3 months prior to surgery. Subjects were exempted from acute or chronic inflammatory diseases, infectious diseases, viral infection, cancer and/or known alcohol consumption. No antibiotics were taken before surgery or during the post-surgery follow-ups. Clinical and biological parameters were assessed prior to Roux-en-Y surgery (i.e. basal or M0) and at 3 and 6 months post-surgery (M3 and M6, respectively). Oral glucose tolerance test (OGTT) was performed in the 23 non-diabetic subjects (OB/nD subgroup). All had a glycemia $< 11$ mM two hours after 75 g oral glucose. Seven subjects had type-2 diabetes (OB/D subgroup), with a fasting glycemia over 7 mM and/or the use of an anti-diabetic drug. Two individuals necessitated insulin therapy while the other 5 subjects were treated with metformin and hypolipidemic drugs (either fibrate or statins). Thirteen normal weight healthy women volunteers living in the same area as the obese subjects were recruited as a lean control group. The Ethics Committee of the Hôtel-Dieu Hospital approved the clinical protocol. All subjects gave a written informed consent.

**Dietary Assessment.** At each visit, caloric intake and macronutrient portions were evaluated by a registered dietician during a one hour questioning. Multivitamins and iron supplements were provided to avoid deficiencies, a well-known secondary effect of bariatric surgery (14). Serum iron, ferritin, the coefficient of saturation of iron in transferrin, vitamins (A, D, E, B1, B12 and B9), micronutrients and calcium were measured using routine bio-clinical tests. Serum analyses showed that these parameters were in the normal range at all time points (data not shown).

**Body composition, metabolic and inflammatory parameters.** Body composition was determined before and after the surgery by DXA (GE Lunar Prodigy Corporation, Madison, WI, USA) and resting energy expenditure (REE) was measured by indirect calorimetry (Deltatrac, Datex, France). Periumbilical surgical biopsies of
subcutaneous adipose tissues were obtained and adipocyte diameter was measured as described (15). Blood samples were obtained at each time point after 12 hours fasting to measure plasma lipids (total cholesterol, HDL-cholesterol and triglycerides), insulin, glucose, leptin, adiponectin and inflammatory markers (hsCRP, IL-6, orosomucoid).

**Faecal samples.** Faecal samples were obtained in the morning before breakfast. Whole stools were self-collected in sterile boxes and stored at -20°C within 4 hours. Samples were treated in the laboratory as 200 mg aliquots and stored at -80°C until further analysis. The 30 obese subjects and 13 healthy controls delivered samples at M0. During follow-ups, faecal samples were obtained for 26 subjects (including 6 diabetics) at M3 and for 15 subjects (including 5 diabetics) at M6. A complete course of stool samples (M0, M3 and M6) was finally obtained for 10 individuals.

**DNA extraction from faecal samples.** DNA was extracted from (200 mg aliquots) faeces as previously described (16). After the final precipitation, DNA was resuspended in 150 µL of TE buffer and stored at -20°C prior to further analysis.

**Oligonucleotide primers and probes.** Primers and probes used in this study are presented in Table-S6. TaqMan® qPCR was adapted to quantify total bacteria population in addition to the dominant (> 1% of faecal bacteria) bacterial species *Clostridium leptum* (*C. leptum*), *Clostridium coccoides* (*C. coccoides*), *Bacteroides/Prevotella* and *Bifidobacterium*. Quantitative PCR using SYBR-Green® was performed for the sub-dominant bacterial species: *Escherichia-Coli* (*E. coli*) (20) as well as for the *Faecalibacterium prausnitzii* (*F. prausnitzii*). The TaqMan® probes were synthesized by Applied-Biosystems Applera-France. Primers were purchased from MWG (MWG-Biotech AG, Ebersberg, Germany).

**Real-time qPCR.** Real-time qPCR was performed using an ABI 7000 Sequence Detection System with software version 1.2.3 (Applied-Biosystems, Foster City, Ca, USA). Amplification and detection were carried out in 96-well plates with TaqMan® Universal PCR 2× MasterMix (Applied-Biosystems) or with SYBR-Green® PCR 2× Master Mix (Applied-Biosystems). Each reaction was run in duplicate in a final volume of 25 µL with 0.2 mM final concentration of each primer, 0.25 mM final concentration of each probe and 10 µL of appropriately diluted DNA samples. Amplifications were carried out using the following ramping profile: 1 cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 1 min. For SYBR-Green® amplifications, a melting step was added to improve amplification specificity. Total numbers of bacteria were inferred from averaged standard curves as described (17).

**Normalization of quantitative PCR data.** All-Bacteria results were presented as the mean of the log10 value ± SEM. To recount for water content in faecal samples, data for each faecal sample was normalized as previously described (16). The level for each bacterial species or group was subtracted from all bacteria content. The data are presented as log number of bacteria per gram of stool.

**Day-to-day variations.** To evaluate the stability of results of microbiota, we added a supplementary experiment to estimate day-to-day variations of faecal samples. Five healthy lean women (BMI: 23 ± 1; age 28 ys ± 0.3) were included in this study. Faecal samples were collected during 2 consecutive days in the same conditions and with the same method of collection and treatment as cited above. (See Supplementary Table S5 in the online appendix available at http://diabetes.diabetesjournals.org.)

**Biochemical assays.** Plasma glucose was measured by the glucose oxidase method (Beckman Fullerton, Palo Alto, CA). Plasma
insulin was determined by using reactif kit from Abbott (Rungis, France). Plasma triacylglycerol and free fatty acids (FFAs) were measured with Biomérieux kits (Marcy l'Etoile, France), total cholesterol, HDL and LDL-cholesterol with Labtest kits (Aix-en-Provence, France). Leptin, adiponectin, hsCRP, IL-6, TNFα were determined by using ELISA kits from R&D Systems Inc (Minneapolis, MN).

**Statistical Analysis.** All values are expressed as mean ± SEM. Insulin resistance (HOMA-IR), insulin sensitivity (HOMA-S%) and beta-cell function (HOMA-B%) provided in Table-S2 were estimated. The composition of microbiota is expressed as mean of the log10 of the normalized PCR values. Wilcoxon Rank Sum tests were used to assess statistical significance of differences between lean controls, OB/nD and OB/D subjects at baseline. Paired Wilcoxon tests were performed to analyze changes in these parameters between various time points after surgery.

Principal component analysis (PCA) combined with co-inertia analysis was used to explore complex and potentially redundant relationships involving a relatively large number of clinical, biological and microbiological variables at baseline, and following RYGB. Co-inertia analysis is a coupling method for comparing different types of parameters presenting different variances. The significance of the associated variations of biological and clinical parameters and of bacterial counts, during the follow-up after surgery, was evaluated by Monte Carlo tests. Significant associations were visualized by a circle of correlations, while their intensity was expressed by computing Spearman correlation coefficients. The significance of the strongest dynamic associations of clinical-biological parameters and of bacterial counts after surgery, among those identified by PCA and co-inertia analysis, was further evaluated by building linear mixed-effects models (LME) to test for inter-variables redundancies and adjust for potential confounding factors. All LME models were fit by maximizing the restricted log-likelihood (REML) of their estimated coefficients. All statistical analyses were performed using the R software (http://www.r-project.org). PCA and co-inertia analyses were performed with ADE-4 package (18). LME modeling was performed by relying on statistical functions available in the nlme package (19). All statistical computations were considered significant when resulting p-values were: < 0.05 threshold.

**RESULTS**

**Clinical and biological characteristics before RYGB.** The clinical characteristics of lean and obese diabetic (OB/D) or non-diabetic (OB/nD) subjects are presented in supplemental data (Table-S1 in the online appendix). While mean age between controls and OB/nD subjects were not statistically different, OB/D subjects were older. RYGB improves markedly clinical, metabolic and inflammatory phenotypes. Along with the drastic reduction in food consumption, RYGB resulted in significant changes in body weight and fat mass from M0 to M3 and M6 (Table 1). For the majority of parameters, improvements occurred rapidly in the first three months. At M6, the subjects had lost 22% of their initial weight (p<0.01). Fat mass decreased and the percentage of Fat-free mass increased. These changes were associated with a decrease in adipocyte cell diameter (p<0.05) and leptin serum concentrations (p<0.01). These improvements were observed in both groups (OB/nD and OB/D) when considered separately (Table-S2). However, the improvements found in inflammatory parameters (hsCRP, orosomucoid) in the whole group of obese subjects (Table 1), disappeared when considered separately (Table-S2). A slight
decrease in orosomucoid remained in the OB/nD group. Plasma glucose, insulin levels, glycosylated hemoglobin (HbA1C) and HOMA-IR decreased significantly post-RYGB, however adiponectin concentration did not change significantly at M3 (Table 1). An improvement in insulin sensitivity (HOMA-S%) of the OB/nD group as well as an amelioration of blood glucose homeostasis was found in the 7 diabetic subjects (Table-S2). Anti-diabetic drugs were stopped in diabetic subjects as well as hypolipidemic treatment in all obese individuals.

**Basal bacterial groups counts: decreased amount of Bacteroides/Prevotella in obesity and of F. prausnitzii in diabetes.** Average counts for each bacterial group of control, OB/nD and OB/D subjects are presented in Table 2 and Figure 1. The Bacteroides/Prevotella group was lower in obese subjects (OB/nD: p=0.039 and OB/D: p=0.038) compared to lean ones. However, while the population of C. leptum tended to be lower in obese subjects, the differences did not reach statistical significance probably due to the high inter-individual variability in this bacterial population subgroup. F. prausnitzii species qPCR system could reliably distinguish between the control and OB/D microbiota. Their counts in the OB/D microbiota were significantly lower when compared with those of control group (p<0.01) and OB/nD subjects (p<0.05). These results suggest that while obesity leads to modification in Bacteroides/Prevotella group, diabetes seems to influence the abundance of F. prausnitzii (Fig. S1).

**Bacterial changes after RYGB: increased amount of Bacteroides/Prevotella and E. coli, decreased Bifidobacterium and Lactobacillus/Leuconostoc/Pediococcus group.** Changes of bacteria amounts were observed in the obese group (OB/nD and OB/D together) after surgery but with a different pattern depending on the bacterial group (Fig. 1). Table-S3 illustrates the progression of all bacterial populations within the microbiota before (M0) and after RYGB (M3 and M6) in each obese group, separated by the diabetic status. In the OB/D subjects, a similar pattern of changes as the one characterizing the OB/nD was observed, but changes for certain bacterial groups did not reach statistical significance. The Bacteroides/Prevotella population, whose level was lower in obese subjects before RYGB, increased at M3 and remained stable until M6 (Fig. 1) at a level close to that of the controls. Importantly, the obese subjects remained obese at M6 (BMI 37.1±1.3 vs. 21.1±0.4, obese and lean subjects, respectively). At M3, E. coli showed a rapid and significant increase reaching a level higher than that of controls. An opposite pattern was observed for both the Bifidobacterium and Lactobacillus/Leuconostoc/Pediococcus. Levels of both populations decreased at M3 and M6 and, in the case of Bifidobacterium, a level lower than that measured in controls.

Interestingly, the level of F. prausnitzii, which was lower in OB/D subjects before RYGB, increased at M3 and remained stable at M6 (Table-S3). The populations of Clostridia (C. leptum and C. coccoides) were stable post-RYGB.

**Association between microbiota composition and clinical phenotypes before RYGB (Table-S4-A).** In OB/nD and OB/D subjects, we observed significant relationships between the amount of F. prausnitzii, E. coli and Bacteroides/Prevotella and metabolic and inflammatory parameters. The strongest associations were found for the amount of F. prausnitzii, which was negatively correlated with serum concentrations of inflammatory circulating markers (hsCRP Rs-0.54, p<0.01 and IL-6 Rs-0.65, p<0.001). This negative correlation was consistently significant when analyzed alone in the OB/nD subjects. No
significant association was correlated with age for any analysis.

**Time dependant associations between metabolic phenotypes and bacterial populations (Table-S4-B).** Statistical linear mixed-effects (LME) models were used to distinguish within-subject from between-subject sources of variation and to describe how trajectories in clinical and bacterial population mean responses, showed related changes over time. Analyses firstly included the entire population of obese subjects, regardless of their diabetic status, and secondly in the OB/nD group or OB/D alone. The corpulence parameters including body weight, BMI, body fat mass, serum leptin concentrations were correlated negatively with the counts of Bacteroides/Prevotella and E. coli, while positively with the amounts of Bifidobacterium population, independently of the diabetic states.

In the OB/nD group, Bacteroides/Prevotella counts correlated negatively with calorie intake (p<0.01), which drastically changed after the bypass. Analysis, performed in the OB/nD group, associating calorie intake and each of the adiposity-related parameters as fixed-effects in a combined LME model, confirmed the negative relationship between Bacteroides/Prevotella counts and the decrease in food consumption post-RYGB (p<0.05). This result was independent of corpulence. The combined model could not demonstrate significant independent relationships with any of the adiposity-related parameters, thus indicating that variations in Bacteroides/Prevotella population after surgery are related mostly to calorie intake in this cohort.

Unlike the Bacteroides/Prevotella population, the relationship between calorie intake and *E. coli* counts lost statistical significance in the combined model. This suggests that *E. coli* could be considered as a marker of corpulence variation after surgery, independent of energy intake. The relationships between the microbiota and these clinical parameters, explored through PCA, is illustrated in Figure 2A, which displays the strong negative correlation between *E. coli* counts and leptin serum concentration (Rs-0.53, p<0.001). This correlation is reinforced in Figure 2B, which concomitantly illustrates the kinetic evolution between *E. coli* population and leptin as a mirror image.

**Time dependant associations between inflammatory parameters and changes in bacterial populations: importance of *F. prausnitzii*.** *F. prausnitzii* showed a consistent correlation with low-grade inflammation. After the surgery, the circulating inflammatory parameters (hsCRP, IL-6 and orosomucoid) reduced and association was found with an increase in *F. prausnitzii*. *F. prausnitzii* variation was strongly and negatively correlated with changes of hsCRP, IL-6 and orosomucoid serum levels when non-diabetic and diabetic obese subjects were grouped together. The correlations with hsCRP and IL-6 were maintained in the OB/nD group (Table-S4-B). These relationships were independent of calorie intake.

**DISCUSSION**

Analysis of the dynamic changes post-RYGB provided important information regarding potential associations between gut microbiota composition, food intake, metabolic adaptations and inflammation. In spite of the relatively small sample size of the diabetic group and some incompleteness in the collection of faecal samples, this study compared not only the different profile of gut microbiota between lean and obese diabetic or non-diabetic subjects, but revealed for the first time that changes of gut microbiota in the same individual before and after RYGB associate with a series of phenotypes. While gut bacteria groups correlated with energy intake, body corpulence and metabolic changes, others as *F. prausnitzii* associated
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with changes in the inflammatory state and diabetes.

Our observation was made in severe obesity and might not be extended to moderately obese subjects. However, the lower proportion of Bacteroides/Prevotella in obese subjects before RYGB and their increase after weight loss are in agreement with landmark studies in less obese populations (11;20). Correlation studies in LME kinetic models provide important information showing that these populations of bacteria were strongly associated with body composition and metabolic parameters. After RYGB, the higher the increase in the proportions of Bacteroides/Prevotella, the better the reduction in body fat mass and plasma leptin. These associations were dependent on energy intake. The estimated Firmicutes/Bacteroidetes ratio diminished substantially during weight loss, an observation also made in our study mostly due to the increase in Bacteroides/Prevotella. A degree of controversy was raised with regards to this result. No changes in the proportion of Bacteroidetes or Firmicutes/Bacteroidetes were found, while the total numbers of bacteria decreased in other studies (12;21). These discrepancies could be attributed to substantial differences in clinical protocols with varying levels and duration of calorie restriction and fat mass loss. RYGB could be considered as a unique model that can be clinically followed over time in the same individual. RYGB was found to be associated with a decrease in Firmicutes together with an increase in Gamma-Proteobacteria in three adults (22). In this first study, the individual faecal samples before and after weight loss were not paired and no information was provided about the amount weight lost and associated phenotypic changes.

Here, our patients were followed for 6 months with a marked reduction in food intake at M3 and M6 post RYGB. It is important to note that there is a well known uncertainty and possible underestimation of food intake in obese subjects (23). Nevertheless, after a short fasting period (1 to 3 days), the subjects started to increase their food intake that was composed of liquid or semi liquid foods for one week. During the 3 months after RYGB, starch-based food is often the principal food item with solid foods being progressively reintroduced. With reduced calorie intake and changes in body composition, leptin dropped and its variations was negatively associated with the amount of Bacteroides/Prevotella and E. coli, while positively with Bifidobacteria and Lactobacilli/Pediococcus. Among its diverse physiological functions, the major adipocyte secreted hormone, leptin, has a critical role in the initiation of adaptation responses to starvation (24). Leptin levels fall rapidly with the onset of energy deprivation at M3 (-50% of basal values) and relatively stabilized at M6 while BMI, fat mass and adipocyte sizes continued to shrink, in line with results found in other studies (25). This phenomenon is recognized as signaling the shift between sufficient and insufficient body energy. The ability of sustained fasting to induce dissociation between circulating leptin levels and adipose tissue mass could also reflect a permissive effect of insulin on leptin secretion. This may have conferred a survival advantage during evolution, as leptin stimulates energy expenditure and inhibits appetite (26). In agreement with Bajzer et al. (27), changes in gut microbiota post-RYGB could be linked to maximizing energy harvest as a host adaptation to the starvation-like condition. The fact that for most gut bacteria, changes were observed at M3 and remained stable at M6 (Table-S3) while corpulence and metabolic factors continued to improve favors this particular interpretation. This is also strengthened by the results of another study from our laboratory that demonstrates early changes in faecal bacteria population, which started at one week after caloric restriction
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((unpublished study). Interestingly, compared with germ-free animals, hepatic ketogenesis is enhanced during 24-h fasting in mice (CONV-D) after a microbiota transplant from the distal gut of conventionally raised lean counterparts fed with carbohydrate (28). The CONV-D mice showed an increase of short chain fatty acids and the proportion of Bacteroidetes switched from 20.6% at the fed state to 42.3% at the fasted state, while the proportion of Firmicutes reduced from 77.1% to 52.6%. We also found that the relationship between some gut microbiota changes and corpulence and metabolic parameters might be not statistically dependent on dietary changes as in the case of E. coli and Bifidobacterium. This is an indirect indication that microbiota components could participate in metabolic changes associated with this surgical procedure.

RYGB procedure per se may contribute to changes in gut microbiota composition. RYGB creates a small gastric pouch and the distal stomach and proximal small intestine are bypassed by attaching the distal end of the mid-jejunum to the proximal gastric pouch. The bile and pancreatic limb is attached along the Roux limb. Gastric acidity is bypassed leading in a reduction of chloride acid flux in the gut. Patients were also under the proton pump inhibitor therapy during the first 3 months, which can also influence the gastric pH. The resulting increased pH, together with the downstream delivery of bile acids, could contribute to modify faecal bacteria population. In in-vitro culture studies, the growth of Bacteroidetes was found to be inhibited when reducing pH, while growth of E. coli was facilitated by increased pH (29). We did not measure pH in our subjects’ faecal samples, but the decreased acidity in the gut after RYGB could favor an increase in Bacteroides/Prevotella and E. coli counts. The changes in E. coli strongly correlated negatively with leptin variation (Fig. 2A). However, leptin can also be secreted by cells in the lower half of the stomach glands (30). The signaling molecules mechanistically involved in driving these links need to be elucidated. Another consequence of pH change post RYGB could be the decrease in Lactobacillus/Pediococcus and Bifidobacterium (31). This is not consistent with studies in mice suggesting that a beneficial effect of Bifidobacterium species in the improvement of obesity-related metabolic and inflammatory condition (8). The Bifidobacterium genus, however, is complex. In adolescents losing moderate amounts of weight the counts of B. bifidum and of B. breve diminished while B. catenulatum increased (32). Information of the intake of prebiotic or probiotic are not available in the present study; hence, we cannot exclude a possible influence of functional ingredients included in foods (as yoghurts) on these bacteria.

Shorter and longer terms studies are needed to explore the dynamics of subjects undergoing bypass surgery with attention given to food intake behavior, measures of metabolic mediators (such as SCFA and free fatty acids), measures of faecal pH to explore the dependency between changes in food intake, the influence of the surgery per se and gut bacterial groups. A comparison with patients only subjected to a restrictive surgical procedure (i.e. gastroplasty) would be useful in this respect.

The other important information provided here was that F. prausnitzii was associated with inflammatory markers. F. prausnitzii has been identified as a conserved and dominant species of the human faecal microbiota of healthy individuals (33). F. prausnitzii might play a role in preventing local bowel inflammation and infection in acute inflammatory disease. A reduction of F. prausnitzii has been described in inflammatory bowel disease and in infectious colitis (34). Our study suggests that F. prausnitzii could also play a role in low-grade
inflammation pathologies like obesity and diabetes (35-37). The relationship between *F. prausnitzii* and inflammatory markers was observed both in OB/nD and OB/D patients and remained after adjustment for BMI. The proportions of *F. prausnitzii* were lower in type 2 diabetic subjects displaying a worsening of their low-grade inflammation (38) and higher insulin resistance. A negative association was also seen between *F. prausnitzii* and HOMA-IR, which could be explained by the amelioration of glucose metabolism in the diabetic group. However in the present study, this was not true for adiponectin, another marker of insulin sensitivity. The discrepancy between HOMA-IR and adiponectin during weight loss has been well documented (39).

*F. prausnitzii* population variation was associated with modulation of urinary metabolites of diverse structure indicating that this species is a highly active member of the microbiome, influencing host pathways (40). *F. prausnitzii* exhibits anti-inflammatory effects, partly due to secreted metabolites able to block Nuclear factor kappa B (NF-κB) activation and the secretion of proinflammatory mediators (41). Oral administration of *F. prausnitzii* or of supernatant from *F. prausnitzii* cultures increased the production of IL-10 by blood mononuclear cells and reduced the production of the proinflammatory mediator like IL-12 in the colon. The modulation of NF-κB by pharmacological agents such as statins or salicylates has been proposed as a tool to improve insulin sensitivity in type 2 diabetic patients (42;43). Our study raises the question regarding the role of *F. prausnitzii* as a mediator of low-grade inflammation in obesity and diabetes and open avenues for future investigation exploring its contribution in insulin resistance.

Because of an increasing interest in treating type 2 diabetes with gastric bypass surgery, the improvement of insulin sensitivity and the reduction of diabetes in subjects post surgery has become a primary axis of interest (44). Unraveling the immediate and long-term adaptations associated with gastric bypass surgery has been proved challenging, predominantly because the consequences of this procedure include caloric restriction, diminished nutrient absorption, reduced adipose mass, modified gut hormone signaling, and changes in whole-body glucose metabolism that can each cause numerous physiological and metabolic adaptations (45;46). Hypotheses have been postulated to explain the improved insulin sensitivity witnessed post surgery, and include the altered secretion of gut hormones (47;48), modifications in intestinal gluconeogenesis (49), and changes in intramyocellular lipid content (50). While the definitive explanation for improved insulin sensitivity post RYGB remains unclear, it is most probably a combination of the aforementioned hypotheses. Components of gut microbiota and possibly the relationship between gut hormones and *F. prausnitzii* should be also considered in this context. Taken together, the applicability of *F. prausnitzii* as a valuable therapeutic tool for the improvement of inflammation, blood glucose tolerance and insulin sensitivity, calls for more investigations.

**Author Contributions.** JP.F. researched data, contributed to discussion, wrote manuscript, reviewed/edited manuscript. LC.K. researched data, contributed to discussion, wrote manuscript, reviewed/edited manuscript. J.T. researched data, contributed to discussion, reviewed/edited manuscript. C.P. researched data, reviewed/edited manuscript. A.B. researched data, reviewed/edited manuscript. JL.B. researched data, reviewed/edited manuscript. D.M. researched data. G. C. researched data, contributed to discussion, reviewed/edited manuscript. J.D. contributed to discussion,
reviewed/edit manuscript. C.H. contributed to discussion, reviewed/edit manuscript. S.R. researched data, contributed to discussion, wrote manuscript, reviewed/edit manuscript. K.C. researched data, contributed to discussion, wrote manuscript, reviewed/edit manuscript.

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REFERENCES:


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Table 1. Clinical and biological characteristics of obese subjects before, and 3 and 6 months after gastric surgery.

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<td><strong>Food intake</strong></td>
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<td>Food intake (kcal)</td>
<td>1933 ± 101 ^A</td>
<td>1080 ± 87 ^B</td>
<td>1355 ± 54 ^C</td>
</tr>
<tr>
<td><strong>Adiposity markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>126 ± 4.2 ^A</td>
<td>107 ± 3.9 ^B</td>
<td>98 ± 3.8 ^C</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>47.6 ± 1.5 ^A</td>
<td>40.6 ± 1.3 ^B</td>
<td>37.1 ± 1.3 ^C</td>
</tr>
<tr>
<td>Adipocyte diameter (µm)</td>
<td>116.7 ± 1.5 ^A</td>
<td>110.7 ± 1.0 ^B</td>
<td>103.3 ± 3.2 ^C</td>
</tr>
<tr>
<td>REE (kcal)</td>
<td>1814.4 ± 54.8 ^A</td>
<td>1842.5 ± 53.6 ^A</td>
<td>1551.1 ± 42.9 ^B</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>47.9 ± 1.0 ^A</td>
<td>44.5 ± 1.0 ^B</td>
<td>41.3 ± 1.2 ^C</td>
</tr>
<tr>
<td>Fat-free mass %</td>
<td>50.0 ± 1.0 ^A</td>
<td>53.0 ± 0.9 ^B</td>
<td>55.9 ± 1.1 ^C</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>50.8 ± 3.7 ^A</td>
<td>25.6 ± 2.5 ^B</td>
<td>24.9 ± 2.8 ^B</td>
</tr>
<tr>
<td><strong>Plasma Glucose homeostasis and insulin sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycaemia (mmol/l)</td>
<td>6.4 ± 0.5 ^A</td>
<td>5.1 ± 0.2 ^B</td>
<td>4.8 ± 0.1 ^B</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>6.4 ± 0.3 ^A</td>
<td>5.7 ± 0.1 ^B</td>
<td>5.8 ± 0.1 ^B</td>
</tr>
<tr>
<td>Insulinemia (µU/ml)</td>
<td>17.1 ± 1.6 ^A</td>
<td>10.7 ± 0.9 ^B</td>
<td>6.9 ± 0.7 ^C</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.88 ± 0.09 ^A</td>
<td>0.63 ± 0.03 ^B</td>
<td>0.78 ± 0.09 ^A</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>6.4 ± 0.5 ^A</td>
<td>7.8 ± 0.7 ^A</td>
<td>8.3 ± 0.7 ^B</td>
</tr>
<tr>
<td><strong>Plasma lipid homeostasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.54 ± 0.16 ^A</td>
<td>4.23 ± 0.16 ^A</td>
<td>4.34 ± 0.15 ^A</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.57 ± 0.19 ^A</td>
<td>1.54 ± 0.17 ^A</td>
<td>1.48 ± 0.17 ^A</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.22 ± 0.05 ^A</td>
<td>1.17 ± 0.06 ^A</td>
<td>1.30 ± 0.06 ^B</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
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</tr>
<tr>
<td>Plasma hsCRP (mg/dl)</td>
<td>3.1 ± 0.8 ^A</td>
<td>2.5 ± 0.9 ^B</td>
<td>2.7 ± 0.8 ^B</td>
</tr>
<tr>
<td>Plasma IL-6 (pg/ml)</td>
<td>4.4 ± 0.4 ^A</td>
<td>4.2 ± 0.4 ^A</td>
<td>3.4 ± 0.4 ^A</td>
</tr>
<tr>
<td>Plasma Orosomucoid (g/l)</td>
<td>1.02 ± 0.04 ^A, ^B</td>
<td>0.94 ± 0.04 ^A</td>
<td>0.86 ± 0.03 ^B</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; (n=30). Fat mass%, Fat-free mass%: values expressed as a percentage of body weight. REE: resting energy expenditure. Paired Wilcoxon stands for analyzing parameters changes between various time points. Data not sharing the same letter within a horizontal line are significantly different (P<0.05).
Table 2. Composition of microbiota compared in lean controls, obese diabetic (OB/D) and non-diabetic (OB/nD) subjects before gastric surgery.

<table>
<thead>
<tr>
<th></th>
<th>Firmicutes</th>
<th>Bacteroidetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All-Bacteria *</td>
<td>Bacteroides/Prevotella genus†</td>
</tr>
<tr>
<td></td>
<td>Clostridium Coccoides group†</td>
<td>E. coli species †</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus/Leuconostoc/Pediococcus group†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clostridium leptum group†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Faecalibacterium prausnitzii species †,‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bifidobacterium genus†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacteroides/Prevotella group†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli species †</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>All-Bacteria *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n : represents the numbers of studied samples.</td>
<td></td>
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<tr>
<td></td>
<td>Data not sharing the same letter within a column are significantly different (P&lt;0.05).</td>
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<tr>
<td></td>
<td>*All-Bacteria results obtained by qPCR were expressed as mean of the log10 value ± SEM.</td>
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</tr>
<tr>
<td></td>
<td>† Results were expressed as mean of the log10 value ± SEM of normalized data, calculated as the log number of targeted bacteria minus the log number of All-Bacteria.</td>
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<tr>
<td></td>
<td>‡ Faecalibacterium prausnitzii is the major component of the Clostridium leptum group.</td>
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<tr>
<td>Control</td>
<td>13</td>
<td>11.74 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>11.29 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>11.17 ± 0.1</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1: Quantifications of faecal microbiota in lean controls and obese subjects before (M0) and after surgery (M3 and M6). The qPCR results were plotted as boxes and whiskers graph. The boxes (containing 50% of all values) show the median (horizontal line across the middle of the box) and interquartile range, while whiskers represent the 10th and 90th percentiles. The extreme data points are indicated as circles. Data not sharing the same letter in parenthesis within a horizontal line are significantly different (P<0.05).

Figure 2: Relationship between changes in faecal microbiota composition and clinical parameters in obese patients following RYGB surgery. Real-time qPCR quantifications were used to determine the faecal microbiota composition for the bacterial groups indicated in Table-S6. Clinical parameters included adipocyte cell size, BMI, calorie intake, HOMA-IR, leptin, and orosomucoid. 2A. Principal Component Analysis (between class analyses). Bold arrows indicate the marked inverse relationship between changes in *E. coli* population and leptin serum concentrations. 2B. Dynamics of *E. coli* population evolution and leptin concentration during the study. *E. coli* population levels are expressed as mean ± SEM of the Δlog10 value of normalized data, calculated as the log number of targeted bacteria minus the log number of All-Bacteria. Leptin results were expressed as mean ± SEM of serum concentrations.
Gut microbiota profile in RYGB induced weight loss

Figure 2

A

B

\( E. \text{coli} \)

Bacteroides / Prevotella

F. prausnitzii

Bifidobacterium

Cell size

HOMA-IR

Orosomucoid

BMI

calorie intake

leptin

\[ \Delta \log_{10} \text{bacteria/g of stool} \]

E. coli

Leptin

M0

M3

M6

A

A

B

B

B

B

0

10

20

30

40

50

60

70

80

90

100

ng/ml