Gut microbiota after gastric bypass in human obesity: increased richness and associations of bacterial genera with adipose tissue genes

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ABSTRACT

Background: Roux-en-Y gastric bypass (RYGB) surgery is one of the most efficient procedures for treating morbid obesity and results in weight-loss and improvements in metabolism and inflammation.

Objective: We examined the impact of RYGB on modifications of gut microbiota and its potential associations with changes in gene expression in white adipose tissue (WAT).

Design: Gut microbiota were profiled from fecal samples by using pyrosequencing in morbidly obese individuals, explored before (0 mo), 3 mo after, and 6 mo after RYGB. WAT gene expression was studied at 0 and 3 mo. We explored associations between microbial genera and differentially expressed genes in WAT and clinical markers.

Results: The richness of gut microbiota increased after RYGB; 37% of increased bacteria belonged to Proteobacteria. The associations between gut microbiota composition and WAT gene expression increased after RYGB. Fourteen discriminant bacterial genera (7 were dominant and 7 were subdominant) and 202 WAT genes changed after RYGB. Variations in bacterial genera correlated with changes in both clinical phenotype and adipose tissue gene expression. Some genes encode metabolic and inflammatory genes. Almost half of the correlations were independent of the change in calorie intake.

Conclusion: These results show an increase in gut microbiota richness and in the number of associations between gut microbiota and WAT genes after RYGB in obesity. Variations of gut microbiota were associated with changes in WAT gene expression. These findings stimulate deeper explorations of the mechanisms linking gut microbiome and WAT pathological alterations in human obesity and its changes after weight loss. Am J Clin Nutr doi: 10.3945/ajcn.113.058743.

INTRODUCTION

Obesity is a low-grade inflammatory disease, partly a result of white adipose tissue (WAT) secretions, which may contribute to changes in metabolic homeostasis (1, 2). In this context, gut microbiota components have been suggested to be important players linking obesity to metabolic and inflammatory alterations (3). Several, but not all, studies observed that gut microbiota in obese subjects had different profiles compared with lean ones (4, 5). Weight loss associated with partial recovery in bacterial components similar to those of lean profiles (6).

Roux-en-Y gastric bypass (RYGB) surgery, the most effective treatment of severe obesity, induces both dramatic and sustained weight loss and improvement or remission of related complications (7, 8). RYGB represents a valuable model to better understand pathophysiological mechanisms associated with these improvements. Along with the amelioration of systemic inflammation, a profound WAT remodeling is observed. It includes changes in histologic characteristics and gene expression (9, 10) and modifications in transcriptional control of inflammation (11). RYGB also induces anatomic modifications of the gastrointestinal tract, which results in functional changes such as gut motility, pH, and alterations of bile acid flow and gut hormones secretions (12).

A few teams have evaluated the gut microbiota composition after RYGB using different approaches. These studies converge to the existence of major postsurgical changes in humans (13–15) and rodent models (16). Using a pyrosequencing approach on a small subset of patients, Zhang et al (13) showed an increase in Gammaproteobacteria after RYGB. Our team showed, in a larger cohort, an increase in Escherichia coli after RYGB. Some associations were found between changes in reverse transcriptase–polymerase chain reaction (RT-PCR)–detected bacterial genera and bioclinical

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5 Abbreviations used: KEGG, Kyoto Encyclopedia of Genes and Genomes; RT-PCR, reverse transcriptase–polymerase chain reaction; RYGB, roux-en-Y gastric bypass; TGF-β, transforming growth factor-β; WAT, white adipose tissue.

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variables (15). Bacterial taxa, such as Bacteroides/Prevotella group and E. coli (member of Gammaproteobacteria) species, increased at 3 mo, whereas Lactobacillus/Leuconostoc/Pediococcus group and Bifidobacterium genus decreased. Whereas some bacterial groups associated predominantly with nutritional status and changes in metabolic variables, others did with inflammatory markers. Importantly, some of these associations were highly dependent on changes in calorie intake (Bacteroides/Prevotella or Lactobacillus/Leuconostoc/Pediococcus). In contrast, the strong association between the increase in E. coli species and metabolic variables (eg, BMI, fat mass, and leptin) remained significant after adjustment for total calorie intake. Faecalibacterium prausnitzii species were lower in subjects with type 2 diabetes and associated negatively with inflammatory markers, both before and after RYGB, independently of changes in calorie intake. Furthermore, it was recently shown in mice model subjected to diet-induced weight loss that the abundance of several cecal bacterial groups associated with levels of selected inflammatory and metabolic gene expression (eg, Slc25a25, Saa3, Pai1, and Fiaf) in inguinal WAT (17).

Because the RT-PCR method used in our previous study (15) does not provide a full picture of gut microbiota composition, we extended our previous results by using a pyrosequencing method to examine additional relations between modifications of fecal bacterial composition and changes in bioclinical variables and looked for potential associations with large-scale WAT gene expression in obese patients.

SUBJECTS AND METHODS

Subjects and blood samples

Thirty obese women (7 diabetic, 23 nondiabetic) enrolled in a bariatric surgery program, were recruited at Pitie’-Salpeˆtrie`re Hospital, Paris, France. Clinical characteristics of these patients were described in details previously (15). Samples were obtained before surgery (0 mo) and 3 and 6 mo after surgery. The Ethics Committee of Hôtel-Dieu Hospital approved the clinical protocol. All subjects provided written informed consent.

Dietary assessment

At each visit, caloric intake and macronutrient portions were evaluated by a registered dietitian during a 1-h questioning period, as described in detail in our previous publication (15).

Adipose tissue samples and microarray analysis

Subcutaneous adipose tissue samples in the periabdominal area were obtained 0 and 3 mo after RYGB surgery. Detailed information on WAT sampling is described elsewhere (see Supplementary Table 1 under “Supplemental data” in the online issue). Technical procedures were described previously (18). Total RNA in these 30 biopsy samples were extracted with the use of the RNeasy total RNA Mini kit (Qiagen). Total RNA concentrations and quality were confirmed with the use of the Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA (200 ng) samples from each sample were amplified and transcribed into fluorescent cRNA with the use of Agilent’s Low RNA Input Linear Amplification kit (Agilent Technologies). Cyanine-5 dye was incorporated into all samples, and an in-house obese reference pool (19) was labeled with cyanine-3 dye. All samples were hybridized to Agilent whole human genome microarrays. Arrays were scanned with the use of a GenePix 4000A Scanner (Axon Instruments-Molecular Devices).

Isolation of fecal DNA and multiplex pyrosequencing

Fecal samples were obtained in the morning. stool samples were self-collected in sterile boxes and stored at $-20^\circ$C until further analysis. During the follow-up, fecal samples were obtained for 26 subjects at 3 mo and for 14 subjects at 6 mo. A complete set of stool samples (collected at 0, 3, and 6 mo) was finally obtained from 11 individuals. DNA was extracted from (200-mg aliquots) feces as previously described (20).

The following universal 16S rRNA gene primers were used for the PCR reaction: V3F (TGCGGRAGGCAGCAG) (21) and V4R (GGACTACCAGGTTATCTAAT) (22) to target the V3-V4 region, which gives the lowest taxonomic assignment error rates (23).

Barcode sequences (GsFLX key) TCAG and MIDGsFLX (12 nucleotides) were attached between the 454 GsFLX adaptor sequence and the forward primer V3F. The GsFLX key and the 454 GsFLX adaptors were attached to the reverse primer.

The concentration and quality of the PCR products were assessed with Picogreen to obtain equal amounts of each sample, and then 16S rRNA gene amplicons were sequenced on a Roche GS FLX 454 sequencer and processed with standard protocol from the manufacturer. To check the variability of the different technical processes, we also sequenced some DNA samples in replicate at different time points (0 and 3 mo) by different companies (Genoscreen and MWG-Eurofins) (see Supplementary Figure 1 under “Supplemental data” in the online issue).

Bioinformatics and statistical analysis

Analysis of gene expression in host WAT

All microarray data from subcutaneous WAT samples were filtered and normalized with use of the Limma package (version 3.6.9). Unique annotated genes with their gene identifier (GeneID) were kept for the next steps, which were used for variance analysis between 0 and 3 mo with the Monte Carlo test, with 999 replicates using the ADE4 package (version 1.4–14). Essential gene functional information was obtained by using an opensource database (http://smd.stanford.edu). Details of the bioinformatic procedure were previously described (9), and detailed methodologic results are described elsewhere (see Supporting text Section S1 under “Supplemental data” in the online issue).

Analysis of gut microbiota 16S rRNA gene molecular inventories

The quality of raw sequencing data were trimmed according to published recommendations (24). The trimmed sequences were assigned to genera, by using the default setting (confidence parameter: 80%) of the Classifier software (25), to obtain a rapid classification. Rarefaction curves and a richness estimator were computed for each sample by using the Vegan package (Community Ecology Package, R package version 1.17–3). We indeed focused on richness [ie, the number of taxa present in a specific ecosystem among dominant microbiota (26)]. Taxonomic microbiota profiles were submitted to Principal Component Analysis; the different sampling points (0, 3, and 6 mo) were used as
an instrumental variable. The link between different time points after RYGB and genera abundance was determined by using Monte Carlo tests with 999 replicates, using the ADE4 package, as described previously (27). On the basis of Principal Component Analysis Instrumental Variable, discriminant genera were first selected with 95% CIs. Wilcoxon tests were used to confirm significant changes in the relative abundance of discriminant genera after RYGB. Detailed methodologic results are described elsewhere (see Supporting text Section S2 under “Supplemental data” in the online issue).

Correlations between gene expression in WAT, gut microbiota, and clinical variables modulated by RYGB

Spearman’s correlation tests were used to explore associations between all bacterial genera and gene expression in WAT at 0 and 3 mo. A heatmap was built to show the correlations. The same approach was applied to explore associations between delta changes in discriminant bacterial genera and discriminant gene expression in WAT (ie, genes significantly changed after RYGB). Similarly, a heatmap was built to illustrate the correlations. Six gene clusters were established by using the hierarchical clustering method included in the heatmap function; cluster names were a summary of containing genes’ functions in literature. The annotation procedure for genes correlated at least to one bacterial genus was applied for Kyoto Encyclopedia of Genes and Genomes (KEGG) biological categories by using FunNet software (http://www.funnet.info).

Correlations between variations in bacterial genera and clinical variables were also evaluated by using Spearman’s tests. Partial Spearman’s tests were used to examine whether the correlations were influenced by variations in calorie intake. Statistical computations were considered significant when the resulting \( P \) values were \( <0.05 \). \( P \) values of correlations tests were adjusted by using Benjamini and Hochberg tests with a false discovery rate \(<5\%\) for multiple comparisons considering the correlations between bacterial genera and gene expression in WAT at 0 and 3 mo; and with a false discovery rate \(<20\%\) for multiple comparisons regarding the correlations between changes of discriminant bacterial genera and discriminant gene expression in WAT.

RESULTS

Gastric bypass associated with an increase in gut microbiota richness

We found that a total of 58 genera, initially nondetectable before RYGB, were detected after surgery in all patients. Of these, 37% belonged to the phylum Proteobacteria and several species of these genera were initially isolated from the periodontal environment (eg, *Aggregatibacter*, *Filifactor*, and *Pyramidobacter*), from the oral cavity (such as *Leptotrichia*) and finally from oropharyngeal environment (such as *Cardiobacterium*, *Cryptobacterium*, and *Kingella*). As a consequence, the gut microbiota richness (Figure 1), estimated by Chao1 and abundance-based

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Observed and estimated richness of gut microbiota before and after RYGB. A and B: Chao1 and ACE indexes estimated an increase in bacterial richness after RYGB (at M3 and M6). C: Rarefaction curves showed an increase in bacterial richness after RYGB (especially at M3). ACE, abundance-based coverage estimators; M0, 0 mo (baseline); M3, 3 mo; M6, 6 mo; RYGB, roux-en-Y gastric bypass.
coverage estimators estimators and observed by rarefaction curves, increased after RYGB.

Number of associations between gut microbiota composition and WAT gene expression significantly increased after RYGB

Before RYGB (0 mo), only 8 WAT genes were correlated with 28 bacterial genera, whereas 562 WAT genes were correlated with 102 bacterial genera after RYGB at M3 (Figure 2). Correlations between WAT genes expression and bacterial genera abundance were significantly stronger after RYGB ($P < 0.01$). The increased periodontal bacterial genera were not correlated with WAT genes.

Dominant and subdominant microbial genera changed after RYGB

We then examined significant changes in gut bacteria components before and after RYGB. Principal Component Analysis showed that the bacterial profiles at 0 mo significantly differed from those at 3 and 6 mo (Figure 3A). No significant difference was found between 3 and 6 mo. From the total variance, 48% were affected by subject effect. The between-class analysis showed that 8.1% of the variance was significantly explained by the surgery effects ($P < 0.01$). This analysis led to extraction of discriminating components, which could separate the bacterial profiles in subjects before and after RYGB. Twenty-one genera were found to discriminate 0-, 3-, and 6-mo fecal samples with 95% CIs (Figure 3A). The Wilcoxon’s test confirmed that 14 of these 21 genera significantly changed after RYGB ($P < 0.05$) (see Supplementary Figure 3 under “Supplemental data” in the online issue). Seven dominant genera that changed before and after surgery are shown in Figure 3 (B–H). Dominant genera represented $\geq 1\%$ of obtained gut microbiota sequences. Gut bacterial genera belonging to Firmicutes, such as Lactobacillus, Dorea, and Blautia, decreased after RYGB, as well as Bifidobacterium belonging to the phylum Actinobacteria (Figure 3, B–E). Bacteroides and Alistipes, of the phylum Bacteroidetes, significantly increased after RYGB (Figure 3, F and H). In agreement with previous observations ($15$), the genus Escherichia, belonging to Proteobacteria, increased after bypass (Figure 3G). The remaining 7 were subdominant genera, namely Turicibacter, Filifactor, Gemella, Neisseria, Phenyllobacterium, Peptostreptococcus, and Campylobacter.

Association between the discriminating genera and bioclinical variables

We then examined putative associations between the variations in 14 bacterial genera (ie, 7 dominant and 7 subdominant genera) and RYGB-induced changes in numerous bioclinical variables during the first 3 mo after RYGB (Figure 4). Several positive and negative associations observed herein markedly extended the data we previously published obtained on a limited number of bacterial groups assessed by RT-PCR ($15$).

Importantly, variations in most of the bacterial genera (13/14) were correlated with changes in total calorie intake and at least

![Figure 2](image-url)
one marker related to corpulence (most frequently BMI). After statistical adjustment for calorie intake, ~52.4% of these correlations (22/42) remained significant (see Supplementary Figure 4 under “Supplemental data” in the online issue).

Associations between the discriminating bacterial genera and WAT gene

Hundreds of associations were found between changes in the 14 genera mentioned earlier and the changes in WAT gene expression during the first 3 mo after RYGB (Figure 5). Each bacterial genus correlated with >28 WAT genes. The most numerous correlations were attributed to *Lactobacillus*, which correlated with 71 WAT genes, followed by *Escherichia* (correlated with 66 genes), *Blautia* (64 genes), *Alistipes* (61 genes), *Turicibacter* (52 genes), *Phenyllobacterium* (50 genes), *Dorea* (38 genes), *Bacteroides* (36 genes), *Filifactor* (33 genes), *Peptostreptococcus* (33 genes), *Bifidobacterium* (30 genes), *Campylobacter* (30 genes), *Gemella* (28 genes), and *Neisseria* (28 genes). After adjustment for calorie intake, 51.8% (320/620) of these correlations remained significant.

A total of 153 WAT genes were associated with at least one bacterial genera, the annotation of these genes is presented by KEGG (see Supplementary Figure 6 under “Supplemental data” in the online issue). With the use of this annotation, upregulated genes belonged to transforming growth factor-β (TGF-β) signaling pathway [such as bone morphogenetic protein 6 (BMP6), in cluster 6, *thrombospondin*], whereas the most downregulated genes belonged to metabolic pathways such as 24-dehydrocholesterol reductase. WAT genes most frequently associated with changes in gut bacteria were grouped in functionalities mostly related to transport and binding, signaling, enzymatic activity (including remodeling enzymes), and cell-structural components (including cell/matrix adhesion components). Transcription factors were also found (based on available Gene Ontology knowledge). As presented in Figure 5, these genes were clustered in 6 main cluster groups (see Supplementary Table 2 under “Supplemental data” in the online issue). WAT genes in clusters 1 and 2 were mostly downregulated after RYGB and correlated positively with downregulated bacterial genera. WAT genes in cluster 3 encoded diverse functions. Although, few correlations were found between these genes and bacterial genera changes, some correlations were evidenced after adjustment for calorie intake (see Supplementary Figure 5 under “Supplemental data” in the online issue). WAT genes grouped in cluster 4 were mostly annotated as binding proteins related to energy homeostasis and to many enzymatic activities, such as...
ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1 (ATP6V0E1), ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D (ATP6V1D), ATP binding cassette, sub family A, member 9 (ABCA9). These genes were downregulated after surgery and mostly correlated negatively with upregulated bacterial genera.

Genes in clusters 5 and 6 were upregulated after RYGB and correlated mostly negatively with bacterial genera. Most of them encoded for structural components, such as extracellular matrix genes (SPOK1, Sparc osteonectin, spodin 1, and SDK1), signaling molecules [such as tumor necrosis factor receptor superfamily member 8 (TNFRSF8), C1q and tumor necrosis factor-related protein 2 (C1QTNF2), a positive regulator of lipid oxidation with MAPK activity, and bone morphogenetic protein 6 (BMP6) in cluster 6], and transcription factors (such as the nuclear receptor NR1D1).

DISCUSSION

Our results extend what was previously observed in obesity (15), because with the use of pyrosequencing methods, we showed that RYGB-induced weight loss associated with increased richness of gut microbiota. Causal mechanisms remain unknown, but our result suggests that dietary habit modifications could be accounted for. RYGB induces a drastic reduction in food intake with modifications in the nutrient supply, such as a reduction in fat (28) and an increase in polysaccharide consumption. De Filippo et al (29) observed that diet containing a higher proportion in polysaccharides promotes a higher microbial richness and diversity in children. In countries where people decrease their fat intake in favor of polysaccharides, the overall diversity of gut microbiota is greater compared with that of consumers of Western diets (30).

In agreement with observations in humans (13–15), and in rodents (31), we observed a significant augmentation in Proteobacteria after RYGB that could be related to metabolic improvements and dietary changes after surgery. A high-fat diet is responsible for increased blood concentrations of bacterial DNA (32). In contrast, interventions aimed at improving insulin resistance and systemic inflammation (eg, the consumption of probiotics) reverse bacterial translocation from intestine toward tissue and decrease blood bacterial DNA (32). Proteobacteria phylum accounts for >80% of...
3 mo after RYGB. RYGB, roux-en-Y gastric bypass. Spearman’s correlation tests were used to explore associations between 14 discriminating gut microbiota genera and changes in adipose tissue gene expression correlations evaluated by coefficient of correlations (more intensive color indicates higher coefficient). The blue asterisks indicate the 7 dominant genera. indicates significant negative correlations, and the black color indicates the nonsignificant correlations. The intensity of one color indicates the degree of correlations were adjusted by multiple tests with false discovery rate at 20%. The red color indicates significant positive correlations, the green color indicates the association between changes in the most discriminating bacterial genera and clinical variables after RYGB. The significant findings. Gut microbiota phylogenetic richness is lower in obese than in lean subjects (5). In other chronic diseases, such as ulcerative colitis, there is a loss in gut bacterial richness and in the interactions between gut microbiota and the host (ie, mucosal epithelium) (36). A lower number of correlations between colon mucosal microbiota and host gene transcripts were observed in twins from discordant pairs with ulcerative colitis than in healthy twins. This work suggested that, in some pathological conditions, the crosstalk between gut microbiota and host biology might be altered.

We found associations between discriminant bacterial genera and WAT genes modified after RYGB. The KEGG annotation shows that some upregulated genes are involved in the TGF-β signaling pathway, whereas downregulated genes are more related to metabolic pathways. The TGF-β signaling pathway is related to tissue remodeling (37, 38). The genes in this pathway were correlated negatively with *Lactobacillus*, which decreased after RYGB. *Lactobacillus* species have been associated with weight changes in humans and animals. Yet, according to the species and the animal or human model, it induced either loss or gain of weight (39). Herein *Lactobacillus* was explored at the genus but not at the species level. *Lactobacillus* genera positively correlated with a decrease in BMI and leptin, and the association between *Lactobacillus* genera and leptin was independent of calorie intake (see Supplementary Figure 4 under “Supplemental data” in the online issue). The reasons why *Lactobacillus* associates with genes belonging to remodeling pathways need to be determined.

Other correlations were found between bacteria and genes grouped in functions related to “binding, transport, signaling and enzymatic activity.” Genes in cluster 4 were particularly involved in “energy homeostasis and metabolism” or in “mitochondrial metabolism.” Some of these downregulated genes after RYGB were negatively associated with *Escherichia* (13, 15). This agrees with previous data. For example, contamination of germ-free mice by a single *E. coli* strain could prolong survival during starvation (40), which suggests that *E. coli* might help the host to harvest energy. In RYGB, which can be considered a transient starvation-like condition in the first weeks or months, an increase in *Escherichia* could possibly contribute to an efficient energy harvest.

Genera modified after RYGB were associated with the patients’ bioclinical phenotypes. The changes in 8 genera (3 dominant-*bifidobacterium, dorea*, and *bacteroides* and 5 subdominant species) negatively correlated with plasma orosomucoid, which diminished after RYGB. In previous data, orosomucoid concentrations were twice as high in obese than in lean subjects and correlated with C-reactive protein and insulin resistance in obesity (41). Furthermore, the variation of *Bifidobacterium* correlated negatively with adiponectin—a marker of insulin sensitivity.
known to exert antiinflammatory properties in muscle and liver (42).

Interestingly, Ravussin et al (17) observed that some selected inflammatory WAT genes (Slc25a25, Saa3, Pai1, and Angpt4) were associated with bacterial components after diet-induced weight loss in mice. These genes were not among the correlated ones in our study, but weight-loss interventions are not fully comparable in animal and human models. However, we found associations between bacterial groups and other WAT genes involved in inflammation. IL1R2 gene expression in WAT (which was downregulated after RYGB) correlated with changes in Dorea, Alstipes, and Bacteroides. We further confirmed by RT-PCR the change of IL1R2 in WAT (data not shown). IL1R2 has a membrane and a cleavable extracellular part, which is the source of sIL1R2 that buffers the proinflammatory role of IL-1α (43). However, its role is currently unknown in WAT. Another gene, STK39 (serine threonine kinase 39), in cluster 5, cell behaviors) positively correlated with Bacteroides and negatively with Dorea. These 2 bacterial genera also associated, albeit modestly, with plasma inflammation (orosomucoid). STK39 is a serine/protein kinase that plays an important role in many physiologic processes, including cell differentiation, transformation, proliferation, and inflammation. STK39 is involved in the maintenance of intestinal mucosal innate immune homeostasis in mice, because overexpressed STK39 caused an increase in intestinal permeability and exacerbated experimental colitis (44).

Whereas our study was mostly exploratory and should be confirmed both at a larger scale and longer term after surgery, our findings emphasize the need to deeply explore mechanistic aspects linking gut microbiota and WAT biology. Approximately half of the associations found between gut microbiota and WAT genes or clinical variables were dependent on the variation in calorie intake after RYGB. Thus, potential links between gut microbiota composition and surgery-induced changes in host biology might not fully depend on dietary changes. RYGB-induced modifications in environmental and systemic factors and in the digestive tract may deeply affect gut microbiota composition (12), and the effect of each of these changes probably merits extensive study using different surgery techniques (purely restrictive (12), and the effect of each of these changes probably merits extensive study using different surgery techniques (purely restrictive (12), and the effect of each of these changes probably merits extensive study using different surgery techniques (purely restrictive (12), and the effect of each of these changes probably merits extensive study using different surgery techniques (purely restrictive (12), and the effect of each of these changes probably merits extensive study using different surgery techniques (purely restrictive (12), and the effect of each of these changes probably merits extensive study using different surgery techniques (purely restrictive (12)

In conclusion, gut microbiota richness increased after RYGB, and some components were associated with WAT genes. Because different factors may be implicated in the changes in gut microbiota components and in WAT gene expression after surgery, deeper investigations need to be conducted to better understand the links we found between specific fecal bacterial genera and WAT functions.

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