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Randomized Control Trials

Link between gut microbiota and health outcomes in inulin -treated obese patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial

Sophie Hiel [a, 1], Marco A. Gianfrancesco [b, 1], Julie Rodriguez [a, 1], Daphnée Portheault [c], Quentin Leyrolle [a], Laure B. Bindels [a], Carolina Gomes da Silveira Cauduro [c], Maria D.G.H. Mulders [d], Giorgia Zamariola [e], Anne-Sophie Azzi [f], Gaetan Kalala [g], Barbara D. Pachikian [h], Camille Amadieu [a], Audrey M. Neyrinck [a], Audrey Loumaye [i], Patrice D. Cani [a, j], Nicolas Lanthier [k], Pierre Trefois [l], Olivier Klein [d], Olivier Luminet [e], Jérôme Bindelle [g], Nicolas Paquot [b], Miriam Cnop [c, f], Jean-Paul Thissen [i], Nathalie M. Delzenne [a, *]

[a] Metabolism and Nutrition Research Group, Louvain Drug Research Institute, UCLouvain, Université catholique de Louvain, Brussels, Belgium
[b] Laboratory of Diabetology, Nutrition and Metabolic disease, Liège, Université de Liège, Belgium
[c] ULB Center for Diabetes Research, Université Libre de Bruxelles, Belgium
[d] Center for Social and Cultural Psychology, Université libre de Bruxelles, Belgium
[e] Research Institute for Psychological Sciences, UCLouvain, Université catholique de Louvain, Louvain-La-Neuve, Belgium
[f] Division of Endocrinology, Erasmus Hospital, Université Libre de Bruxelles, Brussels, Belgium
[g] Gembloux Agro-Bio Tech, Université de Liège, Gembloux, Belgium
[h] Centre d'Investigation Clinique en Nutrition, UCLouvain, Université catholique de Louvain, Louvain-La-Neuve, Belgium
[i] Pole of Endocrinology, Diabetes and Nutrition, Institut de Recherche Expérimentale et clinique, UCLouvain, Université catholique de Louvain, Brussels, Belgium
[j] WELBIO- Walloon Excellence in Life Sciences and BIOtechnology, UCLouvain, Université catholique de Louvain, Brussels, Belgium
[k] Laboratory of Hepatogastroenterology, Institut de recherche expérimentale et clinique, UCLouvain, Université catholique de Louvain, Brussels, Belgium
[l] Medical Imaging Department, Cliniques Universitaires St-Luc, Brussels, Belgium

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SUMMARY

Background: The gut microbiota is altered in obesity and is strongly influenced by nutrients and xenobiotics. We have tested the impact of native inulin as prebiotic present in vegetables and added as a supplement on gut microbiota-related outcomes in obese patients. Metformin treatment was analyzed as a potential modulator of the response.

Methods: A randomized, single-blinded, multicentric, placebo-controlled trial was conducted in 150 obese patients who received 16 g/d native inulin versus maltodextrin, coupled to dietary advice to consume inulin-rich versus -poor vegetables for 3 months, respectively, in addition to dietary caloric restriction. Anthropometry, diagnostic imaging (abdominal CT-scan, fibroscan), food-behavior questionnaires, serum biology and fecal microbiome (primary outcome; 16S rDNA sequencing) were analyzed before and after the intervention.

Results: Both placebo and prebiotic interventions lowered energy intake, BMI, systolic blood pressure, and serum γ-GT. The prebiotic induced greater weight loss and additionally decreased diastolic blood pressure, AST and insulinemia. Metformin treatment compromised most of the gut microbiota changes and metabolic improvements linked to prebiotic intervention. The prebiotic modulated specific bacteria, associated with the improvement of anthropometry (i.e. a decrease in *Desulfovibrio* and *Clostridium sensu stricto*). A large increase in *Bifidobacterium* appears as a signature of inulin intake rather than a driver of prebiotic-linked biological outcomes.

* Corresponding author. Metabolism and Nutrition Research Group, Louvain Drug Research Institute, UCLouvain, Université catholique de Louvain, avenue E. Mounier box B1.73.11, B-1200, Brussels, Belgium.
E-mail address: nathalie.delzenne@uclouvain.be (N.M. Delzenne).
[1] These authors contributed equally to this work.

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Conclusions: Inulin-enriched diet is able to promote weight loss in obese patients, the treatment efficiency being related to gut microbiota characteristics. This treatment is more efficacious in patients who did not receive metformin as anti-diabetic drugs prior the intervention, supporting that both drug treatment and microbiota might be taken into account in personalized nutrition interventions.
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Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
CRP	C-reactive protein
DEBQ	Dutch Eating Behavior Questionnaire
DPP-IV	dipeptidyl-peptidase IV
BMI	body mass index
GLP-1	glucagon-like peptide 1
γGT	γ-glutamyl transferase
HOMA-IR	homeostasis model assessment of insulin resistance
ITF	inulin-type fructan
NAFLD	nonalcoholic fatty liver disease
PCA	principal component analysis
PLS-DA	partial least square discriminant analysis
Q-EDD	Questionnaire for Eating Disorder Diagnoses
SCFA	short-chain fatty acids

1. Introduction

According to the World Health Organization (WHO) more than 1.9 billion adults (18 years and older) were overweight in 2016. Among these, over 650 million were obese. High body mass index (BMI) is as a risk factor for an extending set of chronic diseases, including cardiovascular diseases, type 2 diabetes, nonalcoholic fatty liver disease (NAFLD), chronic kidney disease, many cancers and musculoskeletal disorders [1,2]. Given the multifactorial origin of obesity, pharmacological treatments alone fail to curb the global epidemic and new strategies are needed to prevent and treat obesity worldwide. Over the past fifteen years, the gut microbiota has been identified as an interesting target in the management of obesity and related metabolic disorders. Diet has a crucial impact on human health, and also is a key environmental component that shapes gut microbiota [2,3]. Some vegetables are particularly rich in dietary fibers prone to act as prebiotics, meaning that they are selectively metabolized by host microorganisms and confer health benefits [4]. Among prebiotics, inulin-type fructans (ITF) have been widely studied as a dietary supplement for their beneficial health properties in the context of obesity [5]. Very little is known, however, about the health impacts related to the consumption of vegetables naturally rich in fructans. In plants, fructans are found in leaves and roots, acting as carbohydrate storage. Interestingly, native inulin is present in a variety of vegetables commonly cultivated in Belgium, including Jerusalem artichoke, salsify, artichoke, asparagus, garlic, leek, onion, chicory root, etc [6]. In a recent study performed in healthy adults, we showed that nutritional intervention based on a selection of locally cultivated inulin-rich vegetables led to an increased intake of well-tolerated dietary fibers, associated with beneficial gut microbiota changes and food-related behavior [7]. Here, we hypothesized that combining native inulin supplementation with dietary advice to increase ITF-rich vegetable intakes and restrict caloric intake may be an efficient way to control weight gain and associated metabolic disorders. Therefore, we conducted a parallel intervention study in three Belgian university hospitals on the impact of a dietary prebiotic intervention based on inulin-rich vegetables coupled with inulin supplements in obese adults. We evaluated weight loss, energy and nutrient intake, food-related behavior, glucose and lipid homeostasis, liver integrity, and gut microbiota composition in obese adults. We explored the potential synergistic effect of inulin supplementation with metformin, which is widely prescribed in obese patients with type 2 diabetes and exerts beneficial effects through the gut microbiota modulation [8]. We postulated that metformin in addition to inulin-rich food could modulate study outcomes.

2. Methods

2.1. Subjects

Male and female subjects were recruited in three university hospitals in Belgium (Hôpital Erasme in Brussels, Centre Hospitalier Universitaire in Liège and Cliniques universitaires Saint-Luc in Brussels). This study was a 3-month long, multicentric, single-blind, placebo-controlled trial. All participants were blinded during the entire trial. The inclusion criteria were: BMI >30 kg/m^2, aged from 18 to 65 years, Caucasian ethnicity, presence of at least one obesity-related metabolic disorder (prediabetes/diabetes, dyslipidemia, hypertension, elevated alanine aminotransferase (ALT)/aspartate aminotransferase (AST)/γ-glutamyl transferase (γGT) suggestive of NAFLD). Following the screening, subjects were randomly assigned to the prebiotic or placebo arm (random sequence generated using MS Excel®, simple randomization). Randomization sequences were not revealed to the study staff. Subjects and research staff (excepted dieticians who provided dietary advices and recipes books) were blinded to the treatments. For each university hospital center, one person was responsible for enrolling and assigning participants to interventions. The exclusion criteria included the use of antibiotics, pro/prebiotics, dietary fiber supplements, or any molecules that modifies intestinal transit time within 6 weeks enrolment, pregnancy in progress or planned within 6 months, presence of psychiatric problems and/or use of antipsychotics, following a special diet (e.g., vegetarian, vegan), recent (<6 weeks) or ongoing diets (e.g., high-protein, high-fiber diet), excessive alcohol consumption (more than 3 glasses/day), type 1 diabetes, general dislike for vegetables.

This study was approved by the “Comité d’éthique Hospitalo-facultaire de Saint-Luc”. Written informed consent was obtained from all participants before inclusion. The trial protocol was published on protocols.io (dx.doi.org/10.17504/protocols.io.baidica6) and the trial was registered at ClinicalTrials.gov under identification number NCT03852069.

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2.2. Outcomes

The primary outcome of this trial was the effect of the prebiotic intervention on the gut microbiota composition. The secondary outcome of this trial was the effect of the prebiotic intervention on the BMI. Other secondary endpoints were anthropometric and clinical parameters.

2.3. Dietary intervention

The subjects were included for a period of three months and randomized to consume either 16 g/d of native inulin (extracted from chicory root, Cosucra, Belgium) or 16 g/d of maltodextrin (Cargill, Belgium), provided in identical packaging. Empty and unused packets were returned to measure compliance. During the first week, patients were asked to ingest half the dose to promote adaptation to the fiber. In addition, participants received a cookbook with recipes based on vegetables either rich or poor in fructans and were advised to consume at least one meal proposed in the recipe per day. According to a previous study [6], we selected a list of vegetables enriched in fructans, including artichoke, asparagus, black radish, brussels sprouts, butternut, cauliflower, celeriac, celery, endive, garlic, Jerusalem artichoke, leek, onion, parsnip, pumpkin, salsify, shallot, spaghetti squash, tuberous parsley, turnip and zucchini; artichokes, Jerusalem artichokes, onions and salsify being the most enriched vegetables. Patients in the placebo arm were asked to consume daily recipes based on vegetables poorly enriched in fructans including beans, cress, cucumber, eggplant, lettuce, lamb's lettuce, mushroom, peas, pepper, spinach, swiss chard and tomato. Participants prepared their own meals. A telephone follow-up was performed three times during the study (after 2 weeks, 1 month and 2 months) to enquire about possible side effects.

2.4. Energy and nutrient intake

The participants met a dietician before and monthly during the intervention. At baseline, the dietician calculated energy expenditure of the participants in order to prescribe a hypocaloric diet corresponding to −30% of the calculated energy expenditure. At all visits, one-week recall questionnaires were completed to evaluate dietary intake. Energy and nutrient intake were calculated from the one-week recall using the Nubel Pro program (Nubel asbl, Brussels, Belgium).

2.5. Gut microbiota composition

Stool samples were available for 95 patients: 47 for the placebo group and 48 for the group treated with the prebiotic. 53 metformin-naive (prebiotic = 26, placebo n = 27) and 42 metformin-treated (prebiotic = 22, placebo n = 20). They were collected at baseline and at the end of the 3-month of intervention and stored at room temperature with a DNA stabilizer (Stratec biomolecular, Berlin, Germany) for maximum three days, then transferred to −80 °C for the analysis of the gut microbiota composition. Genomic DNA was extracted from faeces using a PSP® spin stool DNA kit (Stratec biomolecular, Berlin, Germany). Sequencing, subsequent bioinformatics and biostatistics analyses were performed as previously described [9]. Raw sequences was provided on SRA database (accession number: PRJNA595949).

2.6. Anthropometric characteristics

Weight, height, waist and hip circumference, blood pressure and body composition were measured at baseline and after three months of intervention. Body composition was assessed by bioimpedance devices (BIA 101, Akern, Italy; Biocorpus, Medi Cal, Germany; Tanita BC-418 MA, Tanita, UK). The measure of resistance obtained was used to calculate total body fat according to the method described by Sun et al. [10]. Subcutaneous and visceral fat area were obtained by abdominal CT-scan exam and liver stiffness (elasticity) was measured by Fibroscan.

2.7. Behavioral assessment

Two behavioral questionnaires were used: the Questionnaire for Eating Disorder Diagnoses (Q-EDD [11]) and the French version of the Dutch Eating Behavior Questionnaire (DEBQ [12]). The Q-EDD was used to assess binge-eating behavior coded as “0” (no binge eating), “1” (sub-clinical binge-eating) or “2” (clinical binge-eating). In the DEBQ, 16 questions measure emotional eating, restrained eating and external eating on a 1–6 Likert scale.

2.8. Blood parameters

Glycaemia, HbA1c, AST, ALT, γGT, total cholesterol, high-density (HDL) and low-density lipoproteins (LDL) cholesterol and triglycerides were measured in fasting plasma samples. The remainder of the blood was centrifuged at 2000×*g* for 10 min at 4 °C and the plasma was frozen at −80 °C. Insulin and C-peptide levels were measured by ELISA kit (Mercodia, Uppsala, Sweden). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the following formula: fasting plasma insulin (in mU/mL) * fasting plasma glucose (in mg/dL)/405. Dipeptidyl-peptidase IV (DPP-IV) activity was assessed as previously described [13]. Plasma concentrations of the cytokines (IL-17A, IL-1β, MCP-1, IFN-γ, IL-12p70, IL-8 and TNF-α) were determined using multiplex immunoassay (Milliplex Human Cytokine, Merck Millipore) with Luminex® technology (BioplexH, Bio-rad, Belgium). C-reactive protein (CRP) levels were measured using the R&D Systems quantikine ELISA kit (R&D Systems, Minneapolis, USA).

2.9. Statistical analysis

The sample size (number of volunteers) was determined with JMP software before trial initiation based on the primary endpoint of the trial. The calculation of sample size are made to observe an effect size of 2.0 for the relative abundance of *Bifidobacterium* genus taking into account an alpha of 0.05 and a power of 80%. Data are expressed as mean ± SD. For baseline characteristics, Mann–Whitney test was used for continuous variable and chi-squared test was used for categorical variables. Between group differences were analyzed by mixed model repeated measures ANOVA with time and treatment as fixed effects and patient and hospital as random effects using JMP Pro 14 software. Within group analyses were evaluated using a Wilcoxon paired test (from baseline to 3 months of intervention). A significance level of $p < 0.05$ was used for all the analyses. For gut microbiota analysis, p-values of within group comparisons were corrected to control for the false discovery rate (FDR) for multiple tests according to the Benjamini and Hochberg procedure and a significance level of $q < 0.1$ was used. Beta-diversity clustering was analyzed using ANOSIM for categorical variables (hospital, patient, time and treatment).

Because metformin modulates gut microbiota [8] and a large proportion of the study participants were treated with metformin, we performed an *a posteriori* exploratory analysis of the impact of ITF-rich diet according to metformin treatment. In order to examine differences in gut microbiota between metformin-treated versus non-treated (metformin-naive) patients at baseline, multidimensional analyses were used. We performed principal

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component analysis (PCA) and partial least square discriminant analysis (PLS-DA) and selected genera with a cos^2 >0.2 in PCA and loadings >0.3 in PLS-DA. We compared selected genera, anthropometric and clinical parameters at baseline but also changes between (3 months vs baseline) by using statistical tests described above. In order to analyze the relationships between the changes (3 months vs baseline) in genera relative abundance and anthropometric and clinical outcomes, spearman correlations were performed (GraphPad Prism version 8.1.2 for windows).

3. Results

3.1. Subject characteristics

The recruitment was conducted from January 2016 to May 2018. One hundred and fifty subjects were randomized in the study. One hundred and forty-eight received allocated intervention, and a total of 110 subjects completed the study. Among them, 4 subjects were excluded from the analysis because they were not available for their 3-months appointment, and one patient was excluded because he did not meet the inclusion criteria. One hundred and six subjects (placebo n = 55, prebiotic n = 51) were included in the analysis (Supplemental Fig. 1). There was 96% and 93% compliance in the prebiotic and placebo group, respectively. Ninety-seven patients provided fecal samples for gut microbiota analysis. Of these, 2 were removed from the analysis due to the low number of reads detected during amplicon sequencing. At baseline, the groups were similar in terms of age, sex, weight and BMI (Supplemental Table 1). Metformin has been shown to be a confounding factor when assessing gut microbiota composition in type 2 diabetic patients [8]. For this reason, we analyzed the data separately in metformin-treated and metformin-naive patients. Of 106 patients, 59 patients were metformin-naive (prebiotic = 27, placebo n = 32) and 47 were metformin-treated (all diabetic, prebiotic = 24, placebo n = 23). In the metformin-naive group, 12 participants were diabetic (placebo = 8, prebiotic = 4), 20 prediabetic (placebo = 9, prebiotic = 11), and 27 non-diabetic (placebo = 15, prebiotic = 12).

3.2. Prebiotic treatment induces changes in gut microbiota composition at phylum, family, genus, and OTU levels

In the whole cohort, the interventions did not modify the overall composition of gut microbiota assessed by the measure of both α- and β-diversity indexes. As shown by the principal coordinate analysis of the Bray Curtis index and the Weighted Unifrac index (Fig. 1A, Supplemental Fig. 2). The distribution of the β-diversity was mainly due to intrapersonal variability, explaining 95% and 73% of the variability respectively for the Bray Curtis and Weighted Unifrac indexes (ANOSIM analysis). Phylum and family levels of bacteria revealed changes occurring in the prebiotic group only (Fig. 1B). Indeed, in this group, we observed a significant increase in Actinobacteria phylum and Bifidobacteriaceae family ($q < 0.001$). At the gender level (Table 1), prebiotic treatment largely increased *Bifidobacterium,* increased *Catenibacterium* and decreased *Desulfovibrio and Roseburia* ($q < 0.1$). In addition, *Dorea*, Erysipelotrichaceae *incertae sedis*, *Escherichia/Shigella* and *Lactobacillus* were increased, whereas *Butyricimonas*, *Clostridium sensu stricto* and *Clostridium* cluster XIVa decreased after the prebiotic intervention ($p < 0.05$). At the OTU (Operational Taxonomic Unit) level, the prebiotic treatment increased *B. bifidum*, *Bifidobacterium longum* subsp. *longum* and *B. adolescentis* - in accordance with the increase in *Bifidobacterium* genus - as well as *Anaerostipes hadrus*, while it decreased the abundance of *Blautia* sp (Supplemental Fig. 3). In the placebo group, only minor changes occurred (none of them at the q value), with lessened *Flavonifractor* and increased *Dorea*.

3.3. Nutritional and behavioral outcomes in placebo and prebiotic groups

Both prebiotic and placebo interventions led to a reduced energy and overall nutrient intake (protein, lipid and carbohydrates) (Table 2). Fiber intake assessed by questionnaires (that does not take into account the native inulin supplement), was increased after 3 months only in the prebiotic group.

Regarding possible undesirable side effects, we evaluated the apparition of gastrointestinal symptoms during the study (Supplemental Fig. 4). In both groups, nausea, cramp, reflux and rumbling remained unchanged throughout the intervention. Treatment with prebiotic significantly increased flatulence and bloating, especially at the start of the intervention, both symptoms being decreasing with the duration of the treatment.

We also assessed psychological variables related to eating behaviour before and after the 3-months intervention (Table 2). External eating decreased significantly within placebo group while restrained eating increased in both groups. None of the treatments modified emotional eating. Concerning binge eating, the proportion of patients with score 1 (subclinical binge eating) was decreased in the placebo group after 3 months of intervention (17.9% at baseline, 7.7% at 3-months, $p < 0.05$), while in the prebiotic group, the difference was marginally significant (14.1% at baseline, 6.4% at 3 months, p-value = 0.08). As there were very few, patients with clinical binge eating (score 2, n = 3), they were excluded from the analysis.

3.4. Contribution of prebiotic and placebo interventions to the improvement of clinical outcomes

The 3-month intervention was effective, resulting in weight loss in both placebo and prebiotic groups (Table 3). Compared to placebo, the prebiotic intervention reduced body weight and BMI to a greater extent (mixed model ANOVA: interaction $p < 0.05$, Table 3). Fat mass and waist circumference decreased in both groups. The intervention decreased subcutaneous abdominal fat in the placebo group, while visceral fat was not modified in either group. Systolic blood pressure improved in both groups, whereas diastolic blood pressure was significantly decreased in the prebiotic group only. Fibroscan measurements did not reveal changes in the fibrosis marker (elasticity) due to prebiotic treatment. γ-GT decreased in both groups whereas AST was reduced in the prebiotic group only. DPP-IV activity decreased in the prebiotic group. Among markers of glucose homeostasis, fasting insulin was differentially modulated by the treatments (mixed model ANOVA: interaction $p < 0.05$). Indeed, insulinemia, even if not significantly modified, increased in the placebo group while decreasing in the prebiotic group. This suggests the prebiotics improved insulin sensitivity, although HOMA-IR was not significantly changed. Some parameters were not modified by the interventions, such as blood lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) or pro-inflammatory markers measured in the plasma (Table 3, Supplementary Table 2).

3.4. Metformin, more than diabetes or gender, altered the gut microbiota composition at baseline

We performed a series of analysis in order to evaluate whether some variables can affect the gut microbiota prior to intervention. For instance, analysis of bacteria at a phylum level and PCA based on bacterial genera did not reveal any changes in the gut microbiota composition, according to the gender, in both placebo and prebiotic groups prior to intervention (Supplemental Fig. 5A). Then, PCA showed that metformin-naive

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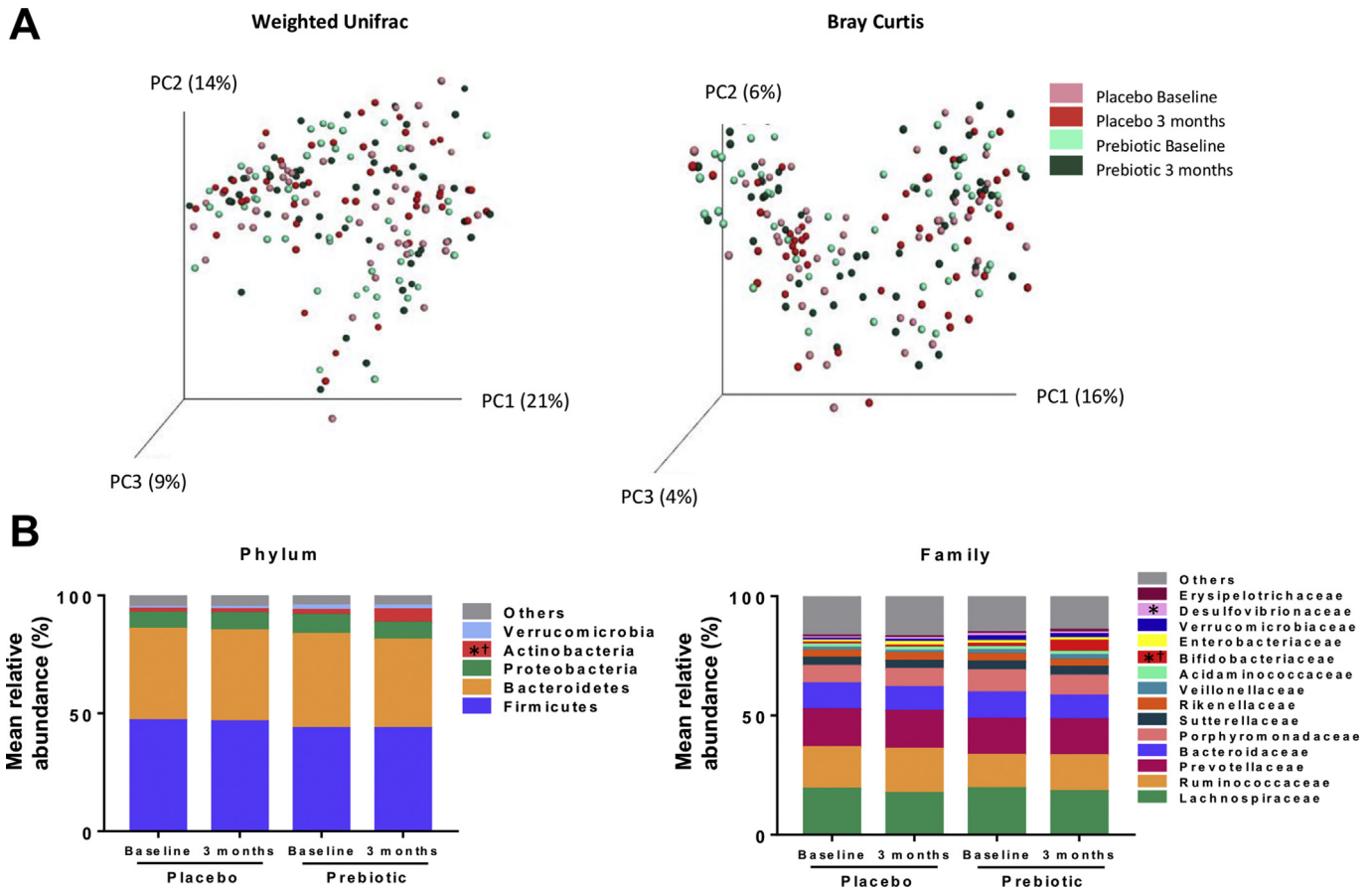

Fig. 1. (A) Principal coordinates analysis (PCoA) of the β-diversity indexes: Weighted UniFrac and Bray Curtis (B) Relative abundances of bacterial taxa accounting for more than 1%, at the phylum and family levels, assessed using Illumina 16S rRNA gene sequencing in obese patient receiving prebiotic (n = 48) or placebo (n = 47) for 3 months. Matched-pairs Wilcoxon signed-rank tests were performed to compare changes from baseline (Within-group variations; *p < 0.05 in the prebiotic group; †corrected q-value < 0.1 in the prebiotic group).

diabetic patients had a gut microbiota profile similar to metformin-naive non-diabetic patients, while it was different from metformin-treated diabetic patients. This suggests that metformin-treatment, rather than diabetes itself, was responsible for the observed differences in gut microbiota composition (Supplemental Fig. 5B). Indeed, by repeating a new PCA analysis based on metformin-naïve and metformin-treated patients, we observed a clear separation in clusters of patients at baseline, according to metformin treatment ($p < 0.001$, Supplemental Fig. 5C). PCA and PLS-DA analyses highlighted 32 genera as drivers of the difference in the gut microbiota composition between metformin-treated and metformin-naive patients (Fig. 2, Supplemental Fig. 4).

Furthermore, the metformin-treated group had higher levels of *Akkermansia*, *Clostridium cluster XIVa*, *C. cluster XIVb*, *Escherichia/Shigella*, *Klebsiella* and unclassified Enterobacteriaceae, and lower level of *C. cluster XI*, *C. cluster XVIII*, *Roseburia* and unclassified Lachnospiraceae than the metformin-naive group (Fig. 2A).

Table 1
Significant changes in relative abundance of gut bacteria at the genus level in obese patients receiving prebiotic or placebo for 3 months.[1]

	Placebo			Prebiotic		
	Baseline	3 months	Change	Baseline	3 months	Change
Bifidobacterium	0.79 ± 1.29	0.83 ± 1.47	**0.04 ± 1.24**	1.27 ± 2.16	4.68 ± 6.20*†	**3.41 ± 6.43**
Butyricimonas	0.59 ± 0.55	0.65 ± 0.66	**0.06 ± 0.42**	0.84 ± 1.04	0.67 ± 0.91*	**−0.16 ± 0.66**
Catenibacterium	0.25 ± 0.56	0.19 ± 0.43	**−0.06 ± 0.30**	0.08 ± 0.21	0.23 ± 0.67*†	**0.15 ± 0.49**
Clostridium sensu stricto	0.41 ± 2.17	0.10 ± 0.19	**−0.20 ± 2.25**	0.10 ± 0.25	0.05 ± 0.13*	**−0.06 ± 0.15**
Clostridium cluster XlVa	0.23 ± 0.56	0.35 ± 1.17	**0.11 ± 0.66**	0.42 ± 1.17	0.29 ± 0.80*	**−0.14 ± 0.44**
Desulfovibrio	0.27 ± 0.5	0.33 ± 0.70	**0.07 ± 0.63**	0.45 ± 1.20	0.14 ± 0.50*†	**−0.31 ± 1.05**
Dorea	0.42 ± 0.38	0.64 ± 0.72*	**0.22 ± 0.66**	0.45 ± 0.85	0.55 ± 0.62*	**0.11 ± 0.97**
Erysipelotrichaceae *incertae sedis*	0.21 ± 0.29	0.28 ± 0.44	**0.07 ± 0.42**	0.45 ± 0.88	0.68 ± 1.27*	**0.23 ± 0.63**
Escherichia/Shigella	0.40 ± 0.73	0.57 ± 1.52	**0.17 ± 1.66**	0.52 ± 1.01	0.69 ± 1.99*	**0.17 ± 2.12**
Flavonifractor	0.16 ± 0.19	0.11 ± 0.10*	**−0.06 ± 0.16**	0.21 ± 0.24	0.19 ± 0.24	**−0.02 ± 0.27**
Lactobacillus	0.03 ± 0.11	0.02 ± 0.04	**−0.01 ± 0.11**	0.11 ± 0.38	0.25 ± 0.64*	**0.15 ± 0.63**
Roseburia	2.69 ± 2.63	2.31 ± 2.44	**−0.41 ± 2.89**	2.97 ± 2.38	1.96 ± 2.13*†	**−1.01 ± 2.72**

[1]Values are means ± SD (placebo: n = 47; prebiotic: n = 48). Matched-pairs Wilcoxon signed-rank tests were performed to compare changes from baseline (Within-group variations; *p < 0.05; †corrected q-value < 0.1).
"Change" column, in bold, correspond to the difference between the end of the protocol (M3) and baseline.

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Table 2
Changes in nutritional and behavioral outcomes in obese patients receiving prebiotic or placebo for 3 months.[1]

	Placebo			Prebiotic			Mixed model ANOVA		
	Baseline	3 months	**Change**	Baseline	3 months	**Change**	**Time**	**Treatment**	**Interaction**
Nutritional outcomes									
Energy, kcal/d	2040.7 ± 452.3	1707.7 ± 440.6*	**−333.0 ± 343.6**	2062.4 ± 648.4	1637.2 ± 444.5*	**−425.2 ± 450.9**	<0.0001	NS	NS
Protein, g/d	89.2 ± 20	82.9 ± 19.4*	**−6.2 ± 17.6**	86.4 ± 20.5	77.8 ± 18.5*	**−8.6 ± 16.0**	<0.0001	NS	NS
Lipid, g/d	80.7 ± 28.2	64.9 ± 28.3*	**−15.9 ± 20.2**	87.3 ± 43.6	60.6 ± 21*	**−26.8 ± 36.3**	<0.0001	NS	NS
Carbohydrates, g/d	216.2 ± 55.6	181.9 ± 50.8*	**−34.2 ± 55.2**	216.9 ± 65.7	181.4 ± 61.9*	**−36.0 ± 47.9**	<0.0001	NS	NS
Dietary fiber, g/d	22.2 ± 6.2	22 ± 6.2	**−0.2 ± 6.1**	22 ± 10.5	25 ± 9.2*	**2.9 ± 7.4**	<0.0001	NS	NS
Behavioral outcomes									
Emotional eating	3.2 ± 1	3.1 ± 1.2	**−0.1 ± 0.7**	3.4 ± 1.1	3.2 ± 1	**−0.2 ± 0.9**	NS	NS	NS
Restrained eating	2.8 ± 0.7	3.4 ± 0.5*	**0.5 ± 0.7**	2.9 ± 0.8	3.4 ± 0.8*	**0.5 ± 0.9**	<0.0001	NS	NS
External eating	3.2 ± 0.8	2.9 ± 0.8*	**−0.3 ± 0.7**	3.1 ± 0.9	2.9 ± 1	**−0.2 ± 0.6**	0.002	NS	NS

[1]Values are means ± SD (placebo: n = 55; prebiotic: n = 51). Matched-pairs Wilcoxon signed-rank tests were performed to compare changes from baseline (Within-group variations; *p < 0.05). Between-groups variations were analyzed by mixed model ANOVA (with time and treatment as fixed effects and patient and hospital as random effects). "Change" column, in bold, correspond to the difference between the end of the protocol (M3) and baseline.

Table 3
Changes in anthropometric and clinical outcomes in obese patients receiving prebiotic or placebo for 3 months.[1]

	Placebo			Prebiotic			Mixed model ANOVA		
	Baseline	3 months	Change	Baseline	3 months	Change	Time	Treatment	Interaction
Body weight, kg	103.5 ± 15.6	102.3 ± 15.6*	**−1.2 ± 2.9**	103.2 ± 22.1	100.5 ± 21.4*	**−2.7 ± 4.0**	<0.0001	NS	0.035
BMI, kg/m^2	35.8 ± 5.2	35.4 ± 5.1*	**−0.4 ± 1.0**	36.9 ± 5.2	36.0 ± 5.1*	**−1.0 ± 1.4**	<0.0001	NS	0.031
Fat mass, kg	40.2 ± 11.7	39.6 ± 10.8*	**−0.6 ± 2.4**	42.3 ± 10.2	40.8 ± 10.3*	**−1.5 ± 3.5**	0.0008	NS	NS
Waist, cm	113.6 ± 12.9	111.4 ± 12.3*	**−2.6 ± 4.1**	114.1 ± 14.9	111.9 ± 15.5*	**−2.2 ± 4.2**	<0.0001	NS	NS
Waist/hip ratio	0.97 ± 0.09	0.96 ± 0.09	**0.01 ± 0.03**	0.94 ± 0.08	0.93 ± 0.08	**−0.01 ± 0.04**	0.016	NS	NS
Visceral fat, cm^2	249.2 ± 104.9	237.6 ± 105.8	**−11.6 ± 37.0**	217.1 ± 101.8	211.1 ± 94.9	**−6.0 ± 38.8**	0.037	NS	NS
Subc. fat, cm^2	350.9 ± 131.2	331.6 ± 119.8*	**−19.3 ± 33.3**	370.7 ± 128.6	370.2 ± 135.8	**−0.6 ± 41.0**	0.018	NS	0.025
SBP, mm Hg	137.4 ± 16.4	130.9 ± 14.6*	**−6.5 ± 12.5**	135.8 ± 14.4	131.6 ± 17*	**−4.2 ± 13.1**	<0.0001	NS	NS
DBP, mm Hg	83.9 ± 12	82 ± 8.7	**−1.9 ± 10.2**	85.6 ± 11.4	80.4 ± 13.6*	**−5.1 ± 12.5**	0.002	NS	NS
Elasticity, kPa	7.2 ± 4.3	6.2 ± 2.6	**−1.0 ± 3.1**	6.3 ± 3.5	5.8 ± 3.2	**−0.6 ± 1.6**	0.019	NS	NS
Total chol, mg/dl	187.6 ± 46.7	183.8 ± 45.1	**−3.3 ± 19.8**	197.3 ± 45.1	192.4 ± 47.1	**−4.9 ± 26.2**	NS	NS	NS
LDL-chol, mg/dl	109.9 ± 44.3	106.6 ± 42.7	**−3.3 ± 19.4**	122 ± 38.2	121.5 ± 41.5	**−0.5 ± 22.5**	NS	NS	NS
HDL-chol, mg/dl	46.5 ± 11.7	47 ± 11.4	**0.5 ± 6.8**	48.2 ± 11	47.3 ± 10.4	**−1.0 ± 5.4**	NS	NS	NS
TG, mg/dl	170.3 ± 98.3	161.3 ± 83.2	**−9.0 ± 70.3**	160.6 ± 133.4	147.3 ± 70.5	**−13.3 ± 118.3**	NS	NS	NS
AST, U/l	29.4 ± 16.5	28.9 ± 13.4	**−0.5 ± 10.0**	23.5 ± 7.5	21.8 ± 8*	**−1.7 ± 5.4**	NS	0.0075	NS
ALT, U/l	41.5 ± 29.5	41.4 ± 30.4	**−0.1 ± 14.0**	31.6 ± 15.2	30.3 ± 14.4	**−1.3 ± 10.2**	NS	0.023	NS
γGT, U/l	50.2 ± 47	46.6 ± 47*	**−3.6 ± 16.6**	37.7 ± 26	35.6 ± 29.8*	**−2.1 ± 19.6**	NS	NS	NS
DPP-IV, mUI/ml	18.6 ± 7.5	18.4 ± 8.2	**−0.1 ± 3.2**	17.7 ± 5.3	16.7 ± 5.4*	**−1.0 ± 2.4**	0.042	NS	NS
CRP, mg/l	4.2 ± 5	3.9 ± 4.2	**−0.3 ± 4.9**	4.1 ± 4.1	5.1 ± 7.1	**1.0 ± 5.5**	NS	NS	NS
HbA1c, %	6.3 ± 1.4	6.10 ± 1	**−0.2 ± 1.0**	5.9 ± 0.9	5.9 ± 1	**0.0 ± 0.2**	NS	NS	NS
Glycemia, mg/dl	115.3 ± 35.3	112 ± 37.5	**−3.2 ± 17.4**	111 ± 40.8	111.9 ± 65.4	**4.1 ± 35.2**	NS	NS	NS
Insulin, mU/L	14.8 ± 9.4	16.3 ± 9.4	**1.5 ± 6.7**	17.8 ± 11.9	15.9 ± 9.4	**−1.9 ± 6.6**	NS	NS	0.0094
C-peptide, pM	1126.8 ± 488.9	1079.8 ± 426.6	**−46.9 ± 268.9**	1002.2 ± 427.2	984.3 ± 490.5	**−22.7 ± 289.4**	NS	NS	NS
HOMA-IR	4.5 ± 4	4.9 ± 4.4	**0.4 ± 2.4**	5.1 ± 4.8	5 ± 5.3	**−0.1 ± 3.4**	NS	NS	NS

[1]Values are means ± SD (placebo: n = 55; prebiotic: n = 51). Matched-pairs Wilcoxon signed-rank tests were performed to compare changes from baseline (Within-group variations; *p < 0.05). Between-groups variations were analyzed by mixed model ANOVA (with time and treatment as fixed effects and patient and hospital as random effects). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Chol, cholesterol; CRP, C-reactive protein; DBP, diastolic blood pressure; DPP-IV, dipeptidyl-peptidase IV; γGT, γ-glutamyl transferase; HDL, High-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; Inter, Interaction; LDL, Low-density lipoprotein; SBP, systolic blood pressure; Subc, subcutaneous; TG, Triglyceride.
"Change" column, in bold, correspond to the difference between the end of the protocol (M3) and baseline.

3.5. Metformin treatment also alters anthropometric and biological parameters at baseline

As metformin was the variable showing the most significant impact on the gut microbiota at baseline, we investigated more its impact on other parameters. Before the dietary intervention, metformin-treated patients had a lower fat mass and decreased external eating score as compared to metformin-naive patients (Supplemental Table 3). The metformin-treated participants (all being diabetic) had higher HbA1c, fasting glycemia, and HOMA-IR.

3.6. Metformin treatment alters the benefits of prebiotic intervention

We then asked whether the changes on the gut microbiota observed with metformin, prior to intervention, could interfere with the response of prebiotic intervention. For this, we tested whether pre-existing metformin treatment was a determinant of study outcomes (Table 4). Prebiotic versus placebo effects on BMI, fat mass, AST, insulinemia and HOMA-IR were significant only in metformin-naive patients (mixed model interaction). Within-group comparisons revealed that BMI was decreased in prebiotic group regardless of the metformin use, whereas in the placebo group, BMI decreased in metformin-treated patients only. However, the effect of prebiotic versus placebo on BMI was more important in the metformin-naive group. Concerning fat mass and AST, the beneficial effects of the prebiotic were only present in the metformin-naive group. Fasting insulinemia and HOMA-IR changes were opposite in the prebiotic versus placebo group in metformin-naive patients. In metformin-naive patients, the placebo treatment increased insulinemia, whereas prebiotic did not affect this parameter. Overall, the beneficial modulation of BMI, fat mass,

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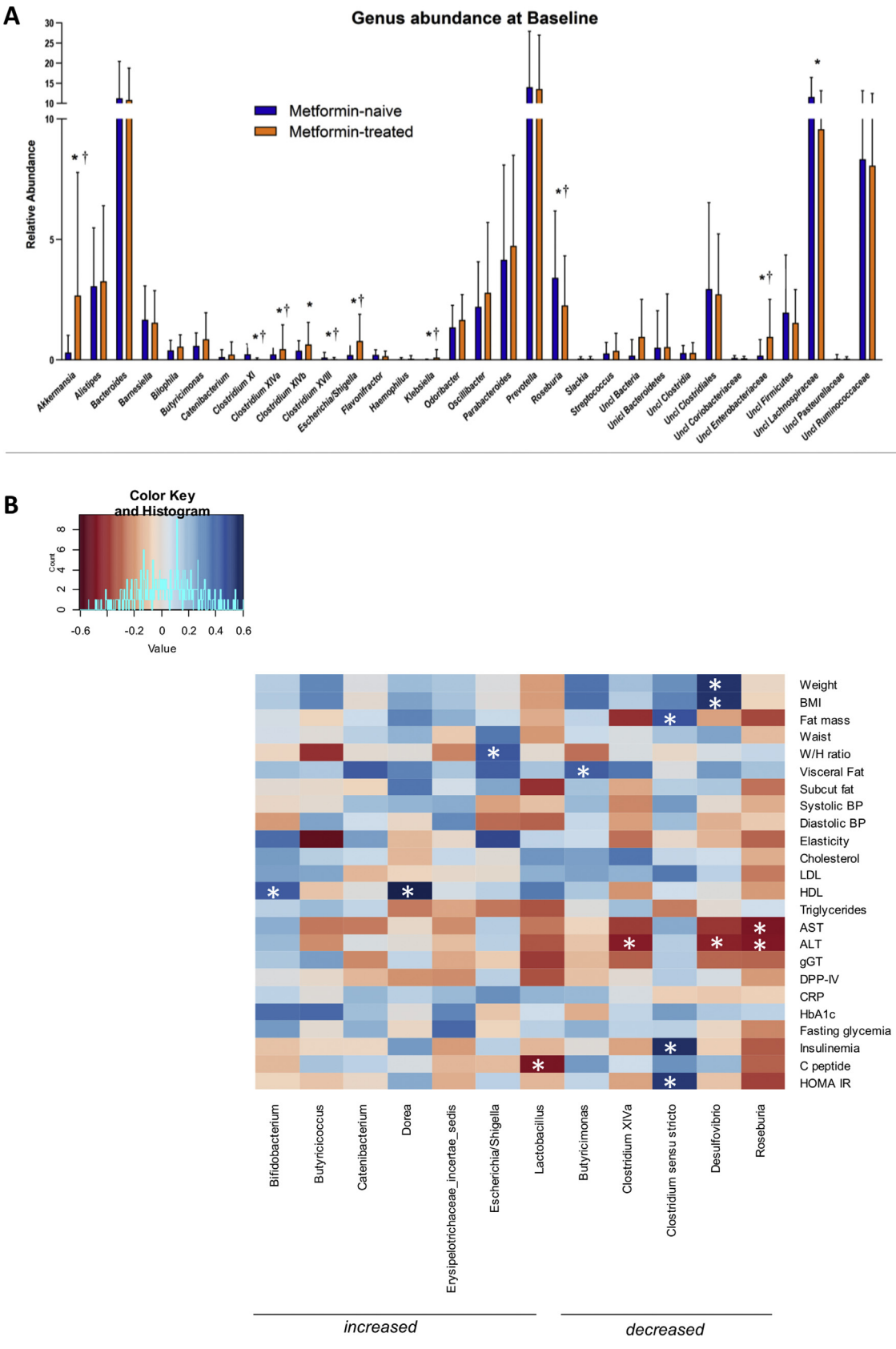

Genera regulated by prebiotic in the whole cohort and/or the metformin-naive subcohort

Fig. 2. (A) Relative abundance of bacterial genera selected from the PCA and PLS-DA analysis at baseline in metformin-treated (n = 42) and metformin-naive (n = 53) patients. Values are means ± SD. Data were analyzed by Wilcoxon test (B) Spearman's correlations between changes in main study outcomes and bacterial genera in metformin-naive patients (*p < 0.05).

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Table 4
Significant changes in anthropometric and clinical outcomes in obese patients receiving prebiotic or placebo for 3 months according to metformin treatment.[1]

	Metf	Placebo			Prebiotic			mixed model ANOVA		
		Baseline	3 months	Change	Baseline	3 months	Change	Time	Treatment	Interaction
BMI	−	36.7 ± 5.8	36.3 ± 5.9	**−0.3 ± 0.9**	37.3 ± 4.5	36.2 ± 4.1*	**−1.1 ± 1.6**	<0.0001	NS	0.035
	+	34.6 ± 3.9	34 ± 3.4*	**−0.6 ± 1.1**	36.5 ± 5.9	35.7 ± 6.1*	**−0.8 ± 1.2**	<0.001	NS	NS
Fat mass, kg	−	42.3 ± 13.2	42 ± 11.9	**−0.2 ± 2.4**	44.1 ± 10.5	41.9 ± 9.2*	**−2.2 ± 4.0**	0.009	NS	0.029
	+	37.2 ± 8.7	36.2 ± 7.9	**−1.0 ± 2.5**	40.4 ± 9.8	39.6 ± 11.5	**−0.8 ± 2.9**	0.026	NS	NS
AST, U/l	−	25.6 ± 11	26.9 ± 13.4	**1.3 ± 7.0**	25.7 ± 8.5	23.3 ± 7.7*	**−2.3 ± 5.5**	NS	NS	0.046
	+	34.2 ± 20.9	31.52 ± 13.4	**−2.6 ± 12.7**	21.1 ± 5.5	20.1 ± 8.2	**−1.0 ± 5.2**	NS	0.003	NS
Insulin, mU/L	−	14.63 ± 11	16.5 ± 10.1*	**1.9 ± 5.9**	17.6 ± 11.8	15.2 ± 8.7	**−2.5 ± 7.3**	NS	NS	0.014
	+	15.1 ± 7.1	16.1 ± 8.6	**1.0 ± 7.9**	18.1 ± 12	16.7 ± 10.3	**−1.4 ± 6.0**	NS	NS	NS
HOMA-IR	−	4.1 ± 4.4	4.7 ± 5.1	**0.6 ± 1.9**	4.2 ± 3.1	3.5 ± 2.3	**−0.7 ± 2.1**	NS	NS	0.025
	+	5.2 ± 3.3	5.2 ± 3.3	**−0.0 ± 2.8**	5.9 ± 6.3	6 ± 6.7	**0.1 ± 4.1**	NS	NS	NS

[1]Values are means ± SD (placebo metformin-naive: n = 32; placebo metformin-treated: n = 23; prebiotic metformin-naive: n = 27; prebiotic metformin-treated: n = 24). Matched-pairs Wilcoxon signed-rank tests were performed to compare changes from baseline (Within-group variations; *p < 0.05). Between-groups variations were analyzed by mixed model ANOVA (with time and treatment as fixed effects and patient and hospital as random effects). AST, aspartate aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; Metf, Metformin treatment.
"Change" column, in bold, correspond to the difference between the end of the protocol (M3) and baseline.

hepatic enzyme and insulin sensitivity by prebiotic intervention is influenced by metformin use.

Concerning the gut microbiota (Table 5), the bifidogenic effect of prebiotic treatment was present in both metformin-treated and naive patients, but was larger in the metformin-naive group (fold change of 7 vs 1.9). In addition to the effect on *Bifidobacterium,* the decrease in *Desulfovibrio* by prebiotic was observed independently of metformin treatment. In metformin-treated patients, only *Butyricicoccus* was increased by the prebiotic treatment. The prebiotic treatment, also increased *Catenibacterium* and *Lactobacillus* and decreased *Butyricimonas* in the metformin-naive group.

3.7. Bacterial genera associated with biological outcomes in metformin-naive inulin-treated patients

Our data suggest that the prebiotic treatment predominantly impacts the metformin-naive patients. Therefore, we explored the relationship between the inulin-driven changes in bacterial genera and study outcomes in metformin-naive patients. Overall, few associations were observed between genera variations and biological variables (Fig. 2B). Indeed, the main genus increased by prebiotic supplementation (*Bifidobacterium)* was associated with a higher HDL cholesterol, but not with other parameters. HDL increase also correlated with *Dorea*. The increase in Lactobacilli was correlated to decreased fasting C-peptide. In addition, the decrease in *Desulfovibrio* was positively associated with decreased BMI and weight, and the decrease in *Butyricimonas* correlated with the decrease in visceral fat. The decrease in *C. sensu stricto* correlated with the decrease of fat mass, HOMA-IR and insulinemia. Moreover, the increment in *Escherichia Shigella* correlated with waist/hip ratio, and *Roseburia* and *C. cluster XIVa* decreased by inulin was associated with an increase in AST and ALT.

4. Discussion

Prebiotic dietary fibers are prone to selectively modulate the gut microbiota, and to exert positive health effects, but most studies published until now were conducted with processed inulin derivatives. In the present study, the dietary advice to consume more inulin-rich vegetables increased dietary fiber intake, independently of native inulin supplementation for a period of 3-months, and enabled to reach an intake of 25 g/d of dietary fiber intake, the level recommended by the European Food Safety Authority [14].

In several studies, weight loss was comparable to the control group or was not achieved upon ITF supplementation in overweight or obese adults [15–18]. In one study, overweight and obese adults supplemented with oligofructose for 12 weeks lost around 1 kg of body weight [19]. Here, we show that inulin supplementation together with dietary advice improves the response to dieting leading to significant body weight loss in 75% patients

Table 5
Significant changes in gut microbiota composition in obese patients receiving prebiotic or placebo for 3 months according to metformin treatment.[1]

	Metf	Placebo			Prebiotic		
		Baseline	3 months	Change	Baseline	3 months	Change
Bifidobacterium	−	0.77 ± 1.01	0.88 ± 1.18	**0.11 ± 1.43**	0.82 ± 1	5.72 ± 7.72*†	**4.90 ± 7.35**
	+	0.83 ± 1.62	0.76 ± 1.82	**−0.07 ± 0.97**	1.79 ± 2.95	3.45 ± 3.48*	**1.66 ± 4.74**
Butyricimonas	−	0.47 ± 0.41	0.52 ± 0.5	**0.05 ± 0.47**	0.74 ± 0.62	0.55 ± 0.71*	**−0.19 ± 0.75**
	+	0.75 ± 0.68	0.81 ± 0.81	**0.06 ± 0.34**	0.95 ± 1.40	0.81 ± 1.11	**−0.14 ± 0.56**
Butyricicoccus	−	0.28 ± 0.2	0.37 ± 0.43	**0.09 ± 0.40**	0.34 ± 0.26	0.25 ± 0.15	**−0.09 ± 0.27**
	+	0.52 ± 0.54	0.37 ± 0.43	**−0.14 ± 0.48**	0.30 ± 0.40	0.45 ± 0.49*	**0.15 ± 0.19**
Catenibacterium	−	0.16 ± 0.4	0.16 ± 0.41	**−0.00 ± 0.30**	0.08 ± 0.18	0.21 ± 0.42*	**0.12 ± 0.27**
	+	0.37 ± 0.71	0.24 ± 0.46	**−0.13 ± 0.30**	0.08 ± 0.25	0.25 ± 0.89	**0.17 ± 0.68**
Desulfovibrio	−	0.32 ± 0.54	0.41 ± 0.81	**0.09 ± 0.73**	0.37 ± 0.72	0.11 ± 0.25*	**−0.27 ± 0.61**
	+	0.20 ± 0.46	0.22 ± 0.52	**0.02 ± 0.48**	0.54 ± 1.60	0.18 ± 0.70*	**−0.37 ± 1.43**
Lactobacillus	−	0.01 ± 0.02	0.01 ± 0.03	**0.00 ± 0.02**	0.07 ± 0.33	0.20 ± 0.37*	**0.13 ± 0.39**
	+	0.05 ± 0.17	0.02 ± 0.06	**−0.03 ± 0.17**	0.15 ± 0.44	0.31 ± 0.86	**0.17 ± 0.84**

[1]Values are means ± SD (placebo metformin-naive: n = 27; placebo metformin-treated: n = 20; prebiotic metformin-naive: n = 26; prebiotic metformin-treated: n = 22). Matched-pairs Wilcoxon signed-rank tests were performed to compare changes from baseline (Within-group variations; *p < 0.05, †corrected q-value < 0.1). Metf, Metformin treatment.
"Change" column, in bold, correspond to the difference between the end of the protocol (M3) and baseline.

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(from −0.5 to −18.5 kg with mean of −2.7 kg). In accordance with the decreased total caloric intake, restrained eating was increased in both groups, which translates the intention to restrict food intake deliberately in order to prevent weight gain or promote weight loss [20]. Interestingly, we highlighted that weight loss and improved BMI, diastolic blood pressure, AST, DPP-IV and insulinemia occurring in both groups were potentiated by the prebiotic intervention in the context of dietary caloric restriction.

Both inflammation and fibrosis are important components in NAFLD that can be controlled by lifestyle-driven weight loss [21]. AST was decreased in the prebiotic group, as previously demonstrated in studies in nonalcoholic steatohepatitis and diabetic patients [22,23]. However, plasma CRP and proinflammatory cytokines revealed that the prebiotic intervention had no effects on systemic inflammation.

In obese patients, a 6-week ITF supplementation decreased fasting insulin level and improved insulin resistance [24]. Here, fasting insulin evolved in an opposite way in the prebiotic (decrease) compared to placebo group (increase). It was previously shown that ITF supplementation in high-fat-fed rats decreased the DPP-IV activity, and this was associated with an increase in glucagon-like peptide 1(GLP-1) [25]. Therefore, the lower DPP-IV activity observed after prebiotic treatment could be linked with the improved insulin levels.

We took advantage of this cohort to analyze some variables that could alter the gut microbiota composition at baseline such as gender, diabetes or metformin treatment. Since we only found a strong effect of metformin on the gut microbiota prior to intervention, we investigated further in detail the consequence of metformin use for the prebiotic response during the intervention. Indeed, most diabetic patients were treated with metformin prior to the nutritional intervention. Importantly, we have shown that prebiotic intervention significantly improved outcomes specifically in obese patients who were not treated with metformin. BMI was decreased in prebiotic group regardless of metformin use, the effect being more pronounced in metformin-naive patients. However, prebiotic versus placebo effects was significant not only on BMI, but also on fat mass, AST, insulinemia and HOMA-IR only in metformin-naive patients. Therefore, despite the decrease in statistical power linked to the limited number of patients per subgroup, our data suggest that prebiotic intervention is more beneficial in patients not using metformin.

The key objective of our study was to unravel how changes in the gut microbiota could be implicated in the health outcomes, and our data invite us to take into account the metformin treatment as a confounding factor. Metformin has been reported to modulate gut microbiota in several studies [8,26,27]. In our study, the metformin-treated patients had more *Akkermansia, Clostridium (cluster XIVa and XIVb)*, *Escherichia/Shigella, Klebsiella,* unclassified Enterobacteriaceae and less *C. cluster XI, C. cluster XVIII, Roseburia,* unclassified Lachnospiraceae. These baseline differences correspond to previous observations for most of these genera [8].

As expected, specific modifications of gut microbiota composition occurred in the prebiotic group. We observed an important increase in the relative abundance of Actinobacteria and *Bifidobacterium (B. bifidum, B. longum subs.longum, B. adolescentis)* which is in line with the recent systematic review of human studies in adult individuals showing the effects of inulin on the gut microbiome [28] and numerous studies showing the bifidogenic effect of inulin-derived products, including data obtained in the context of obesity [15,29,30]. The bifidogenic effect of inulin was present but less pronounced in metformin-treated patients, despite the fact that this genus was not modified at baseline by metformin. Correlation analyses between microbiota and health parameters showed overall few associations in metformin-naive patients. *Bifidobacterium* genus was positively correlated to HDL level only, a parameter that was not significantly modified by the prebiotic intervention. With these results, we can hypothesize that the bifidogenic effect of the prebiotic intervention may be a signature of ITF fermentation rather than a key driver of the observed health effects.

Inulin supplementation increased abundance of *Catenibacterium,* an effect reported so far only in pigs [31]. *Catenibacterium*, a polysaccharide degrading genus has been previously associated to the adherence to a Mediterranean diet [32]. The increase of *Catenibacterium* did not persist in metformin-treated patients. An increase in *Lactobacillus*, correlated with a decrease in C-peptide, occurred only in metformin-naive patients. This increase in *Lactobacilli* confirms our previous findings in obese patients, as well as results from other ITF intervention studies in healthy subjects [33–35]. We also observed an increase in *A. hadrus,* a species that has been repeatedly found to be increased after ITF supplementation [15,29,36]. *Anaerostipes* is a butyrate-producer genus able to metabolize the end products of inulin degraders, such as *Bifidobacterium* [37].

Prebiotic treatment also decreased *C. sensu stricto, Clostridium* cluster XIVa, *Roseburia, Butyricimonas*, and *Desulfovibrio*. A decrease in *Desulfovibrio* by inulin supplementation has already been shown in healthy individuals [38]. Interestingly, in our cohort, the decrease in *Desulfovibrio,* was observed in both metformin-treated and -naive patients, and it was associated with a decrease in weight/BMI. A positive association between BMI and *Desulfovibrio* has been reported in overweight subjects compared to lean individuals [39]. *Butyricimonas* was decreased upon inulin intervention in the whole cohort and in metformin-naive subgroup only. This decrease was correlated with a lower visceral fat. In addition, the decrease in *C. sensu stricto* by prebiotic was in line with the improvement of fat mass and of markers of insulin resistance. The link between this bacterial genus and BMI has recently been shown in healthy and obese individuals [40]. Some bacterial changes upon inulin treatment correlated with the worsening of biological parameters: *Escherichia Shigella* whom increase correlated with waist/hip ratio, and of *Roseburia* and *C. cluster XIVa* of whom decrease was associated with an increase in transaminase activity signing hepatic alterations.

Overall, the changes in gut microbiota composition elicited by 3 months of inulin are subtle but wider than its well-known bifidogenic effect. The cause–effect relationship between the bacterial genera associated with improved weight/BMI by prebiotic - namely the decrease in *Desulfovibrio* and *Clostridium stricto sensu,* which is quite robust-remains to be proven.

One limitation of our study is the observation of gastrointestinal symptoms, such as flatulence and bloating in patients receiving inulin, given as supplement together with dietary advices. The bloating in prebiotic group was observed only upon the first month of treatment. For flatulence, this effect persisted during several weeks in the prebiotic group. Interestingly, these symptoms significantly decreased with time until they became not significantly different from the placebo group. This raises the question of the optimal dose of inulin to envision future intervention studies in humans, in particular in patients presenting gastro-intestinal sensitivity. In view of our previous data, we can propose that a food-based intervention with inulin rich vegetables could be an alternative to promote inulin intake, with less and even more positive outcomes on gastro-intestinal symptoms as compared to supplementation [7].

Our study highlights vegetables as an important source of beneficial fibers that may help obese patients to achieve weight loss in the context of a healthy lifestyle. Our data clearly support that inulin-supplementation should be proposed as a nutritional

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approach before introducing treatments that targets metabolic disorders among others through the modulation of the gut microbiota, like metformin. Well-powered studies are needed to evaluate the potential beneficial effects of ITF supplementation in prediabetic subjects prior to drug treatment.

In conclusion, our work clearly shows that nutritional intervention targeting the gut microbiota – even if they will never solve the problem of obesity and diabetes alone - must be considered in the medical follow-up of obese.

Author contributions

Conceptualization: NMD, JPT, NP, MC, BP, OK, OL, JB, AMN.
Data curation: SH, JR, QL, NMD, CA, MAG, MDM, GZ.
Formal analysis: JR, QL, LBB, SH, CA, AMN, MDM, GZ.
Funding acquisition: NMD, PDC, MC, NP, JPT, OK, OL, JB.
Investigation: SH, MAG, JR, BP, DP, CGSC, ASA, PT, NL, NMD, MC, JPT, NP, OK, OL, GK, MDM, GZ, AL.
Methodology: BP, JR, NL, MC, GK, JB.
Project administration: AMN, JR, BP, NMD, MC, JPT, NP, OK, OL, JB.
Resources: PDC, NMD, MC, JPT, NP, OK, OL, JB, NL.
Software: QL, LBB, JR, CA.
Supervision: NMD, JPT, MC, NP, OK, OL.
Validation: NMD, AMN, LBB, NL, MC, JPT, NP, OK, OL.
Visualization: SH, QL, JR, AMN.
Writing – original draft: SH, JR, NMD, MAG, MDM, GZ.
Writing – review & editing: JR, QL, AMN, MAG, JPT, MC, NP, OK, OL, NL, PDC, LBB, NMD.
All authors read and approved the final manuscript.

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Conflicts of interest

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.04.005.

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