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Original article

Inulin-type fructans modulate intestinal *Bifidobacterium* species populations and decrease fecal short-chain fatty acids in obese women

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SUMMARY

Background & aims: : Inulin-type fructans (ITF) prebiotics promote changes in the composition and activity of the gut microbiota. The aim of this study was to determine variations on fecal short chain fatty acids (SCFA) concentration in obese women treated with ITF and to explore associations between *Bifidobacterium* species, SCFA and host biological markers of metabolism.
Methods: Samples were obtained in a randomized, double blind, parallel, placebo-controlled trial, with 30 obese women randomly assigned to groups that received either 16 g/day ITF ($n = 15$) or maltodextrin ($n = 15$) for 3 months. The qualitative and quantitative analysis of *Bifidobacterium* spp. was performed in feces by PCR-DGGE and q-PCR, and SCFA profile was analyzed by gas chromatography. Spearman correlation analysis was performed between the different variables analyzed.
Results: The species *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum* and *Bifidobacterium adolescentis* were significantly increased at the end of the treatment in the prebiotic group ($p < 0.01$) with being *B. longum* negatively correlated with serum lipopolysaccharide (LPS) endotoxin ($p < 0.01$). Total SCFA, acetate and propionate, that positively correlated with BMI, fasting insulinemia and homeostasis model assessment (HOMA) ($p < 0.05$), were significantly lower in prebiotic than in placebo group after the treatment period.
Conclusions: ITF consumption selectively modulates *Bifidobacterium* spp. and decreases fecal SCFA concentration in obese women. ITF could lessen metabolic risk factors associated with higher fecal SCFA concentration in obese individuals.

1. Introduction

Obesity is a complex and multifactorial disorder that has become one of the prevalent health issues of the 21st century. Indeed, obesity is considered as a triggering factor for many metabolic diseases including diabetes, hypertension, ischemic heart disease and stroke [1].

In the last years, the gut microbiota has been proposed as an environmental factor that could be implicated in adiposity and metabolic diseases. The "energy harvest theory" evidences a

Abbreviations: DGGE, denaturing gradient gel electrophoresis; GC, gas chromatography; ITF, inulin-type fructans; HOMA, homeostasis model assessment; LPS, lipopolysaccharide; short-chain fatty acids, SCFA.

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dysbiosis in microbial gut metabolic function and composition between lean and obese individuals in animal models and humans, suggesting that these differences explain the ability of the host to extract energy from the diet and store this energy in the adipose tissue [2,3]. Microbial metabolites such as short-chain fatty acids (SCFA) can be used as energy sources by the host but can also act as regulators of energy intake and energy metabolism. Their potential role in the modulation of adiposity has been proposed [4–6]. Human studies have reported higher fecal concentrations of SCFA in overweight and obese humans compared with their normal-weight counterparts [7–9]. However, the changes in gut microbial composition at phylum and genus/species level that could explain differences between overweight and obese people remain matter of debate [2,7,10].

There is also a growing interest in alternative nutritional interventions for weight control and prevention of obesity. For instance, the administration of prebiotics represents, next to probiotic supplementation, a current strategy to modulate the composition/activity of the microbial gut ecology. A dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health [11]. Among prebiotic nutrients, inulin-type fructans (ITF) are well characterized and their administration promotes growth of beneficial microorganisms like *Bifidobacterium* [11]. These microorganisms are involved in the reduction of intestinal endotoxin concentration, improve glucose tolerance and low grade inflammation in prebiotic treated mice, according to previous studies performed in our group [12,13]. In healthy individuals, ITF intake promotes satiety and modulates gut peptides regulating food intake [14,15].

The aim of the present study was to analyze changes promoted by ITF administration in obese women focusing on intestinal *Bifidobacterium* species, fecal SCFA and biological markers of metabolism.

2. Methods

2.1. Subjects

The fecal samples analyzed here were obtained during a previous double blind randomized trial that determined the impact of ITF on gut microbiota and host metabolism of obese women [16]. Briefly, obese women between 18 and 65 years old and with a BMI > 30 kg/m^2 were selected and divided in two groups ($n = 15$/group) that received a daily supplement of 16 g of ITF (Synergy 1 namely, inulin/oligofructose 50/50 mix)-(prebiotic group) or the placebo maltodextrin (placebo group) during three months. Both products were kindly provided by Orafti, Oreye, Belgium. A detailed protocol of the trial together with the exclusion criteria for the recruitment was registered in clinicaltrials.gov as NCT00616057. Fecal samples were collected before and after the intervention period and stored at −20 °C until the DNA extraction was performed. The Commission dEthique Biomédicale Hospitalo-facultaire from the Université Catholique de Louvain (Brussels, Belgium) provided ethical approval for this study and written informed consent was obtained from each participant.

2.2. DNA extraction for Bifidobacterium spp. analysis

The DNA was isolated as previously described [16], using the repeated bead beating procedure with a modified protocol for the QuiAmp Stool Mini Kit (Quiagen, Hilden, Germany). The PCR-DGGE fingerprinting technique and quantitative PCR (q-PCR) were performed to characterize *Bifidobacterium* spp. in fecal samples.

2.3. PCR–DGGE analysis

To amplify the 16S rRNA gene of the *Bifidobacterium* genus, a nested PCR approach was used. Specifically, a 520–bp fragment was first amplified with *Bifidobacterium* genus specific primers BIF164F-BIF662R as described previously [17]. The first PCR round was followed by a second amplification with primers 338F-GC with a GC clamp of 40 bp and 518R [18]. All amplification products were checked by electrophoresis on a 1.5% agarose gel.

Denaturing gradient gel electrophoresis (DGGE) was performed with the use of a PhorU system (Ingeny, Goes, The Netherlands) in 0.5× TAE buffer at 60 °C. PCR products were loaded onto 8% v/v polyacrylamide gels in 0.5× TAE. The electrophoretic conditions were the following: 16 h at 120 V in a 45–65% urea–formamide denaturant agent gradient. The gels were stained in 1× TAE buffer with SYBR Gold (Invitrogen) for one hour and visualized with UV radiation.

Specific bands were excised and DNA was re-amplified using 338F/518R primers and amplification products checked by agarose gel electrophoresis. PCR products were subjected to sequencing using the services of Macrogen (Amsterdam, The Netherlands). The sequences were compared with those available in the GenBank database using NCBI BLAST (http://www.ncbi.nlm.nih.gov).

2.4. q-PCR analysis

The q-PCR was used to characterize fecal *Bifidobacteria* using group and species-specific primers [19,20]. PCR amplification and detection of the 16S rRNA gene was performed on MicroAmp optical plates sealed with MicroAmp optical caps (Applied Biosystems, Foster City, CA, USA) in a 7500 Fast Real-Time PCR System (Applied Biosystems) using Fast Start SYBR Green Master (Roche Diagnostics, Barcelona, Spain). Thermal cycling consisted of an initial cycle of 95 °C 10 min, followed by 40 cycles of 95 °C 15 s, and 1 min at the appropriate primer-pair temperature. The bacterial concentration of each sample was calculated by comparing the Ct obtained from standard curves in which the Ct values were plotted as a linear function of the base-10 logarithm of the number of cells calculated by plate counting. The standard curves were made with pure cultures of *Bifidobacterium adolescentis* CECT 5781, *Bifidobacterium animalis* IPLA R1, *Bifidobacterium bifidum* IPLA IF 10/10, *Bifidobacterium breve* NCIMB 8807, *Bifidobacterium longum* IPLA 8809 and *Bifidobacterium pseudocatenulatum* LMG 11041 which were grown in MRSC broth (MRS broth [BioKar Diagnostics, Beauvais, France] supplemented with 0.25% [wt:vol] L-cysteine [Sigma Chemical Co., St. Louis, MO]) under anaerobic conditions. Samples were analyzed in duplicate. In the negative samples, the value of the detection limits obtained for the corresponding primer pair was assigned (ranging between 10^3 and 10^4 cells/g depending on the bacterial group).

2.5. Fecal SCFA analysis by GC

Supernatants from 1 ml of the homogenized fecal samples were obtained by centrifugation (10,000 *g*, 30 min, 4 °C) and filtration (0.2 μm). A chromatographic system composed of a 6890 N GC (Agilent Technologies Inc., Palo Alto, CA, USA) connected with an ion flame detector and a mass spectrometry 5973N detector (Agilent) was used for quantification and identification of fecal SCFA as described previously [20].

2.6. Measure of anthropometric/biological parameters

Blood was collected in EDTA tubes before and after the intervention period in both groups and classical biological parameters:

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glycaemia, insulinaemia, HbA1c, total cholesterol, high-density and low-density lipoproteins (HDL and LDL, respectively) cholesterol and triglycerides were measured as described elsewhere [16]. The BMI, body composition, waist and hip circumferences, homeostasis model assessment (HOMA) index and the serum LPS levels were measured as previously described [16].

2.7. Statistical analysis

Raw data are expressed as mean ± SEM. Treatment effect was assessed based on differential values obtained by subtracting the value at T3 months from the value at T0 for each patient. Differential values are expressed as mean ± SEM. As most of the parameters had not a normal distribution (assessed using a Shapiro–Wilk test with R software), the Mann–Whitney test was used to compare differential values between groups and Wilcoxon signed-rank test to compare differential values of the same group before and after treatment. Correlations were analyzed by using Spearman's correlation in GraphPad Prism (V.5.00 for Windows, GraphPad Software, San Diego, California, USA). The level of significance was set at $p < 0.05$.

3. Results

3.1. Subjects

The baseline characteristics for the 30 patients involved in the clinical trial have been previously described [16] and no differences between placebo and prebiotic group were reported at the beginning of the study.

3.2. Changes in fecal Bifidobacteria populations

The PCR-DGGE fingerprinting technique was performed to check possible different profiles between obese women included in the prebiotic group at the beginning and at the end of the intervention period. Different patterns were observed between the analyzed individuals although in general the band profile of each patient was homogeneous before and after the intervention period. Nevertheless with some donors, the enrichment of certain bands or the appearance/disappearance of bands was evidenced at the end of the ITF treatment (Fig. 1). The main species found in the DGGE-profiles were *B. adolescentis*, *B. longum* and *B. pseudocatenulatum*, as summarized in Fig. 1. Species identification is presented in Table S1 (see supplementary data). The q-PCR technique at genus and species taxonomic level was performed to confirm the results obtained by PCR-DGGE, as well as to quantify other species, like *B. animalis*, *B. breve* and *B. bifidum*, not appearing in PCR-DGGE gels. The counts of total *Bifidobacteria* and the *Bifidobacterium* species determined by q-PCR were in the same order of magnitude in fecal samples of the placebo and the prebiotic groups at the beginning of the study (Table 1) but the q-PCR analysis evidenced a selective increase of the genus *Bifidobacterium* in the group of obese women receiving ITF, after the intervention period. Moreover, a significant increase in certain species such as *B. adolescentis* (~0.6 log units), *B. longum* (~0.7 log units) and *B. pseudocatenulatum* group (~1.2 log units) was observed after the intervention period in the prebiotic group but not in the placebo group (Table 1).

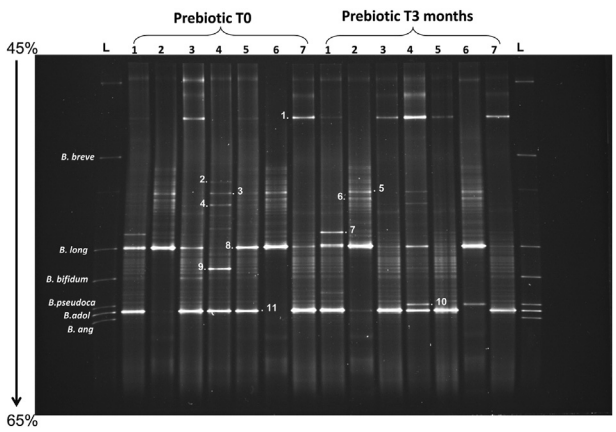

Fig. 1. PCR-DGGE profiles of 7 representative fecal samples obtained before (prebiotic T0) and after (prebiotic T3 months) treatment (1, 2, 3, 4, 5, 6, 7). L: ladder. *B. breve*: *Bifidobacterium breve*, *B. long*: *Bifidobacterium longum*, *B. bifidum*: *Bifidobacterium bifidum*, *B. pseudoca*: *Bifidobacterium pseudocatenulatum*, *B adol*: *Bifidobacterium adolescentis*, *B. ang*: *Bifidobacterium angulatum*. Numbers inside the gels refer to sequenced DNA bands, whose tentative identification are indicated in Table S1.

3.3. Correlations between Bifidobacteria species and anthropometric/biological parameters

Regarding the existence of potential links between the *Bifidobacterium* species and some biological markers of metabolic syndrome, a Spearman correlation analysis was performed. All the statistically significant correlations are shown in Fig. 2. Changes in *B. bifidum* and *B. adolescentis* were inversely associated with fat mass percentage whereas *B. breve* negatively correlated with total cholesterol and LDL cholesterol. Moreover *B. longum* negatively correlated with serum LPS.

3.4. Fecal SCFA profiles and correlations with anthropometric/biological parameters

Fecal SCFA profiles were studied through GC analysis and no significant differences were observed between placebo and prebiotic group at the beginning of the study. However, important differences were found among individuals, in each group. As expected, the most abundant SCFA in feces were acetate followed by propionate and butyrate (data not shown). From all organic compounds analyzed, acetate, propionate and total SCFA decreased significantly after the intervention period in the prebiotic group (Fig. 3, Fig. S1, see supplementary data). Total branched SCFA expressed as the sum of isobutyrate and isovalerate tended to decrease in the prebiotic group although no significant difference was observed. Caproate concentration increased, specifically in five patients of the placebo group but this effect did not occur in the prebiotic group (Fig. S1). The Spearman correlation analysis performed between the fecal SCFA concentration and biological parameters revealed that acetate and propionate positively correlated with BMI, fasting insulinemia and HOMA (Table 2). Butyrate and total SCFA significantly correlated with fasting insulinemia and HOMA but not with BMI, whereas propionate also positively correlated with fasting glycemia and HbA1c. In contrast, there were no significant correlations between serum lipids and SCFA concentration (data not shown).

4. Discussion

Several hypotheses have been reported to explain the association between specific gut microbiota and metabolic disease, including increased energy harvest, changes in host gene expression, alterations in gut permeability and interactions with the endocannabinoid system [21]. In addition, an increasing body of evidence has suggested that prebiotic administration could be effective in the treatment of obesity and metabolic syndrome in animal models but the relevance of this issue remains to be proven in humans [12]. Moreover, prebiotic supplementation has been

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Table 1
Bifidobacterium genus and *Bifidobacterium* species before (T0) and after (T3 months) treatment.

	Placebo group ($n = 15$)			Prebiotic group ($n = 15$)			Mann–Whitney test
	T0	T3 months	Δ	T0	T3 months	Δ	*p* value
Bifidobacterium spp.	9.36 ± 0.16	9.05 ± 0.20	−0.31 ± 0.15	9.16 ± 0.10	9.79 ± 0.12***	0.63 ± 0.12	0.0001
B. adolescentis	6.39 ± 0.37	6.57 ± 0.39	0.18 ± 0.22	6.52 ± 0.23	7.14 ± 0.35**	0.62 ± 0.17	0.0381
B. animalis	5.54 ± 0.24	5.48 ± 0.38	0.14 ± 0.33	5.99 ± 0.32	6.23 ± 0.40	0.24 ± 0.31	0.6943
B. bifidum	5.36 ± 0.35	5.79 ± 0.45	0.42 ± 0.41	4.85 ± 0.14	5.29 ± 0.23	0.44 ± 0.26	0.7107
B. breve	5.60 ± 0.06	5.53 ± 0.04	−0.08 ± 0.05	5.62 ± 0.05	5.55 ± 0.03	−0.07 ± 0.06	0.9085
B. longum	8.14 ± 0.27	7.73 ± 0.33	−0.45 ± 0.16	7.94 ± 0.17	8.67 ± 0.19***	0.73 ± 0.12	<0.0001
B. pseudocatenulatum	7.33 ± 0.43	6.79 ± 0.45	−0.54 ± 0.23	7.20 ± 0.41	8.49 ± 0.41**	1.29 ± 0.35	0.0005

Raw data are given as the mean ± SEM in log(CFU/g feces). $^{**}p < 0.01$, $^{***}p < 0.001$ according to Wilcoxon signed-rank test.
Differential values (T3 months − T0) (Δ) are given as the mean ± SEM in log(CFU/g feces). *p* values according to the Mann–Whitney test performed on differential values (placebo *vs* prebiotic) to assess treatment effect.

traditionally associated with the stimulation of *Bifidobacteria* and *Lactobacilli* but recent research work suggests that prebiotic action may also involve other beneficial microbial groups [11,13,22].

Our research group has recently shown that a three-months ITF treatment promoted a selective modulation of the gut microbiota composition in obese women at phylum and species taxonomic levels with *Bifidobacterium* spp. being an outstanding group which experienced a significant increase [16]. In the current study, we confirm this augmentation and we report that ITF treatment in obese women promotes the selective increase of certain *Bifidobacterium* species. Indeed, the prebiotic treatment significantly raises the counts of *B. adolescentis*, *B. longum* and *B. pseudocatenulatum* which are predominant species in adult gut microbiota. The stimulation of certain species of *Bifidobacterium* after ITF intake in humans has only been described previously in few reports [23,24], that also demonstrated the enrichment of *B. adolescentis* and *B. longum*. However in those cases, healthy and not obese individuals were investigated.

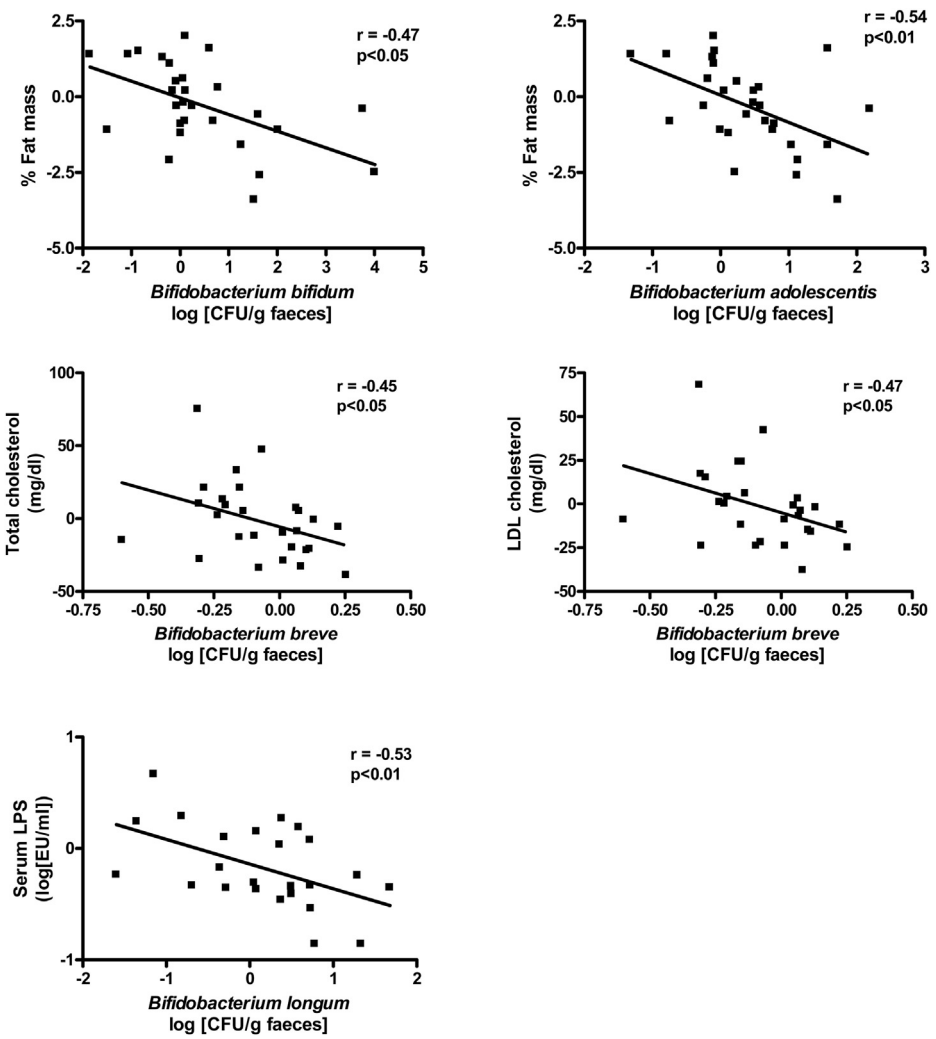

Fig. 2. Correlations between *Bifidobacterium* species and anthropometric/biological parameters in patients of the placebo ($n = 15$) and prebiotic ($n = 15$) groups at both times (T0 and T3 months). *r* and *p* values are given following the Spearman correlation test. LDL, low density lipoprotein; LPS lipopolysaccharide.

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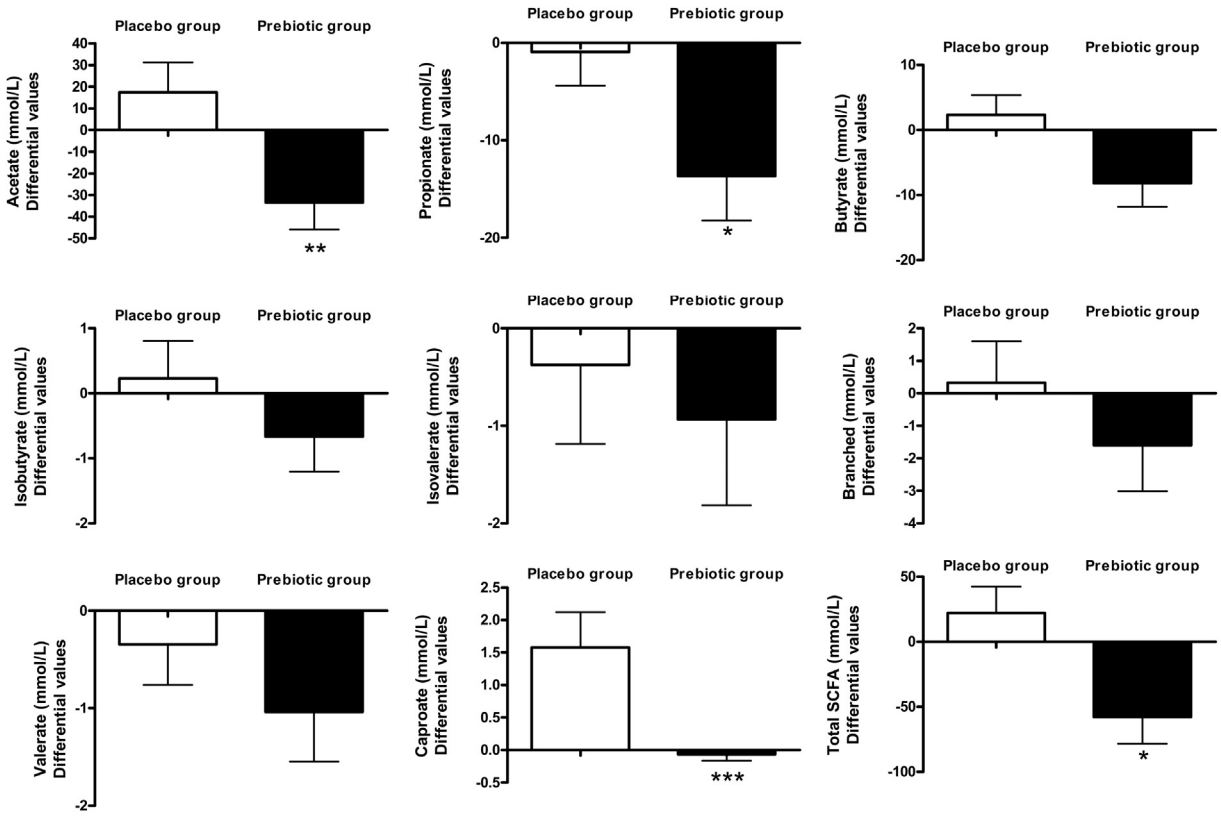

Fig. 3. Fecal short-chain fatty acid (SCFA) differential values (T3 months − T0) expressed in mmol/L. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ according the Mann–Whitney test (placebo *vs* prebiotic) to assess treatment effect. $n = 15$ of each group.

Regarding the significant correlations obtained between *Bifidobacterium* species and some anthropometric/biological parameters, *B. longum* correlated negatively with serum LPS, opening the possibility that this microorganism could be involved in the improvement of metabolic endotoxemia in humans. This result is in accordance with previous experimental animal studies suggesting that prebiotic treatment promote *Bifidobacteria* which play a role in the alleviation of endotoxemia, namely by reducing gut permeability [25]. The Spearman correlation analysis also revealed that changes in *B. adolescentis,* as well as *B. bifidum*, although only the first species significantly increased after the prebiotic treatment, negatively correlated with fat mass. Additionally, *B. breve* negatively correlated with total cholesterol and LDL cholesterol. These correlations, although proving no causal relationship, suggest the possibility to use certain species of *Bifidobacterium* as antiobesity candidates, and are in agreement with previous research works suggesting that *Bifidobacterium* spp. may decrease the tendency to obesity and diabetes in healthy humans [7,26].

A wide variation in fecal SCFA concentrations was observed among the 30 volunteers participating in the study. In spite of this, total SCFA and the main end products of bacterial fermentation (acetate and propionate) decreased significantly after the prebiotic treatment. At a glance, these results were unexpected because it is well known, from *in vitro* and *in vivo* studies, that ITF fermentation by saccharolytic bacteria produces SCFA [11]. However, it should be taken into account that this study analyzes changes promoted by ITF administration in a cohort of obese women and not in healthy volunteers. The fecal concentration of total SCFA can be dependent on increased microbial gut populations, linked to shifts in cross-feeding mechanisms between microorganisms, or can be dependent on their mucosal absorption and transit time, albeit the two later explanations remain theories. In view of data comparing lower body weight evolution and fat mass development in germ-free mice than in conventionalized animals, the energy harvest hypothesis has been developed, leading to the idea that SCFA may promote adiposity by different mechanisms [27]. Changes in the

Table 2
Associations between SCFA and anthropometric/biological parameters in patients of the placebo ($n = 15$) and prebiotic ($n = 15$) groups at both times (T0 and T3 months).

	BMI (kg/m^2)	Fat mass (%)	HbA1c (%)	Fasting glycemia (mg/dL)	Fasting insulinemia (mg/dL)	HOMA index
Acetate (mmol/L)	0.274*	0.184	0.229	0.205	0.301*	0.276*
Propionate (mmol/L)	0.318*	0.203	0.291*	0.314*	0.329*	0.310*
Isobutyrate (mmol/L)	0.152	0.229	0.187	0.041	−0.151	−0.083
Butyrate (mmol/L)	0.120	0.099	0.216	0.219	0.278*	0.281*
Isovalerate (mmol/L)	0.126	0.197	0.136	−0.030	−0.164	−0.126
Valerate (mmol/L)	0.157	0.253	0.148	0.085	0.079	0.105
Caproate (mmol/L)	0.098	0.189	−0.028	0.075	0.051	0.093
Branched SCFA (mmol/L)	0.132	0.196	0.145	0.005	−0.148	−0.095
Total SCFA (mmol/L)	0.265	0.165	0.167	0.197	0.297*	0.288*

Data are given as correlation coefficient (r), which was calculated using Spearman correlation test with * corresponding to $p < 0.05$.

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gut microbiota of obese human individuals compared to lean individuals have also been described and in this way, previous studies have reported higher concentration of SCFA in feces of overweight and obese people [7–9]. The specific mechanisms that explain the higher fecal SCFA production in the obese population remain a matter of debate and could be due to an increase intake of dietary substrates or the consequence of an increase in the metabolic activity of certain bacterial groups. It could be possible that the decrease of SCFA levels evidenced in our cohort of obese women were related with the favorable changes obtained in fecal microbiota composition in this human group after prolonged oral intake of ITF. On the other hand, in our study, we have shown that the placebo group, after the treatment, exhibits a significant increase in caproate concentrations. This bacterial metabolite, together with valerate, is generally produced in only minor amounts and few microbial species are known to produce these products from redox-neutral substrates like sugars or lactate [28]. Regarding the possible relationship between levels of SCFA and metabolic parameters associated with obesity, we found in the present study with obese women that the main end products from gut bacterial fermentation like acetate and propionate positively correlated with BMI suggesting that SCFA might be involved in body weight increase. Acetate, propionate and butyrate were also positively associated with fasting insulinemia and HOMA index. Insulin levels and HOMA index are considered as markers of metabolic syndrome and it has been previously reported that SCFA are additional sources of energy for the body and are involved in glucose and lipid metabolism in the host. Propionate is transported to the liver where it has a role in gluconeogenesis while acetate is used as substrate for lipid synthesis [29]. Acetate and propionate are also ligands for GPR41 receptor, the activation of which increases adiposity in mice [30]. A recent article has also associated higher levels of acetate and propionate with BMI in obese women [9]. Interestingly, the prebiotic treatment with ITF in our study allows decreasing SCFA level in the feces of obese women inverting the obese profile characterized by the high production of these metabolites. One of the limitations of the interpretation of such data is that the exact fecal SCFA production is difficult to determine because it is a balance between production and absorption. A measurement of the dynamics of SCFA production and availability in obese humans could be very interesting to study with stable isotope labelled fermentation carbohydrates, for example. Moreover, larger interventional studies are necessary to evaluate if ITF supplementation could lessen metabolic risk factors associated with higher fecal SCFA concentration in obese individuals and if SCFA modulation through the diet might be a target for weight management and the pathogenesis of obesity.

In conclusion, the prebiotic ITF intervention in obese women selectively increases certain *Bifidobacterium* species that are inversely correlated with deleterious biological parameters for the host. This prebiotic treatment could also invert the higher fecal SCFA concentration observed in obese individuals and the potential mechanism of energy sparing in these individuals that could be associated with metabolic risk factors.

Conflict of interest

None.

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The authors' responsibilities were as follows-NS, EMD, and NMD designed research; NS and EMD conducted research; NS, EMD, AMN, LBB, PDC, WMdV, MG, CGdRG and NMD analyzed data or performed statistical analysis; JM, MG and CGdRG provided essential reagents or materials; JPT had responsibility for patient care and selection; NS and NMD wrote the paper. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2014.06.001.

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