Clinical Nutrition 34 (2015) 501–507

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

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

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ARTICLE INFO

Article history:
Received 10 April 2014
Accepted 3 June 2014

Keywords:
Bifidobacterium
ITF
SCFA
Gut microbiota
Obesity

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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http://dx.doi.org/10.1016/j.clnu.2014.06.001

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: