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Effect of the Synbiotic (*B. animalis* spp. *lactis* Bb12 + Oligofructose) in Obese Subjects. A Randomized, Double-Blind, Controlled Clinical Trial

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Abstract Rats fed high fat diets have alterations of their intestinal microbiota, gut barrier function, circulating lipopolysaccharide (LPS) levels and biomarkers of oxidative stress, inflammation, and glucose/insulin metabolism, resulting in a higher risk of type-2 diabetes. These deleterious effects are prevented by antibiotics or prebiotics. The aim of this study was to determine whether the administration of a synbiotic improves metabolic alterations and low grade inflammation in obese subjects. A randomized, double-blind, controlled clinical trial (www.ClinicalTrials.gov, Access Number NCT01235026) was carried out in 40 obese patients. Subjects were distributed in two groups to receive 8g oligofructose + 1 g of lyophilized *Bifidobacterium lactis* Bb12 (1010 CFU/g) or 9g maltodextrin as placebo, twice a day for six weeks. Body composition, blood lipids, antioxidant capacity of plasma, biomarkers of inflammation (usCRP, IL-6) and LPS exposure (LPS-Binding Protein, LBP, and sCD14), areas under curves of glycemia and insulinemia and fecal microbiota (qPCR) were quantified at baseline and after treatment. 38 subjects (34.8 ± 9.2y; BMI: 36.7 ± 5.3 kg.m-2) completed the study. A positive correlation was observed at baseline between usCRP, IL-6, LBP, sCD14 and the percentage of body fat; correlations also existed between usCRP, IL-6 and LBP values while sCD14 only correlated with IL-6. Compared with placebo, the administration of synbiotic increased the fecal levels of *Bifidobacterium* spp. but did not affect body composition, lipid profile, antioxidant status and areas under curves of glycemia and insulinemia, nor the plasma concentrations of usCRP, IL-6 and LBP. Plasma concentrations of sCD14 were significantly lower after treatment in the symbiotic group compared with the placebo 3 group (5.98 µg/ml [5.01-6.96] vs. 7.26 [6.34-8.09] µg/ml (Means [CI95%), respectively; p=0.043). The synbiotic increased fecal bifidobacteria in obese subjects without improvement the biochemical, inflammatory and metabolic markers; more studies are required to elucidate the role of the symbiotic on plasma sCD14.

Keywords: *obesity, Bifidobacterium lactis Bb12, oligofructose, intestinal microbiota, lipopolysaccharide, sCD14*

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1. Introduction

Obesity is associated with metabolic disorders that predispose individuals to the development of type-2 diabetes and cardiovascular diseases [1,2]. The intestinal microbiota (IM) is currently accepted as an actor in the development of obesity and its complications [3], being particularly involved in the extraction of energy from non-digestible foodstuffs and in the storage of fat by adipocytes [4,5]. Compared with normal weight individuals, the Bacteroidetes/Firmicutes ratio of the IM is decreased in obese mice and humans and is restored after weight loss [6,7]. The association between IM alterations (dysbiosis) and obesity and its metabolic complications has been confirmed by other authors, even if the bacterial populations involved vary depending on the studies and the laboratory methods [8,9,10]. Interestingly, Vrieze et al. recently showed that the transplant of IM from lean donors to recipients with metabolic syndrome improved their peripheral and hepatic insulin sensitivity and increased the butyrate-producing bacteria in their IM suggesting for the first time a causal relation between the IM and insulin resistance in humans [11].

It has also been proposed that the fat content of the diet rather than obesity *per se* affects the IM [12]. The administration of high fat diets to mice increases the gram-negative at the expense of the gram-positive bacteria

in their IM, resulting in higher luminal concentrations of lipopolysaccharide (LPS); this is associated with impairments of the gastrointestinal barrier function and the development of metabolic endotoxemia [13,14]. It is probable that the LPS from the Enterobactericeae or Desulfovibrionaceae families are implicated in these events, as their endotoxin activities are significantly higher than that from *Bacteroides* species [15,16]. Circulating endotoxin activates the LPS receptors CD14 and TLR4 in the plasma membrane of immune cells, increasing the release of proinflammatory mediators (IL-6, TNFα) and oxidative stress and leading finally to insulin resistance and metabolic complications such as hepatic steatosis [13,14]. Intraluminal LPS could also gain access to the circulation associated with chylomicrons, as supported by the observation that human volunteers fed a fat-rich meal exhibit transient increases of plasma LPS, LPS-binding protein (LBP), soluble CD14 (sCD14) and IL-6 [17].

Probiotics and prebiotics have been proposed as tools for the nutritional management of gut dysbiosis and the modulation of metabolic parameters [18]. In mice fed a high fat diet, oligofructose (OFS) administration increases gut bifidobacteria and improves insulin sensitivity while decreasing metabolic endotoxemia and low-grade inflammation [19]. Synbiotics (i.e. the combination of pre- and probiotics) are expected to exert their health promoting effect in a synergistic form, compared with their individual administration; their use appears as promising in obese subjects with metabolic disturbances. For example, the administration of *B. longum* + inulin/OFS has been shown to improve the IM and plasma concentrations of pro-inflammatory cytokines in elder subjects [20].

Based on these antecedents, we proposed that the six weeks administration of a synbiotic product (*B. lactis* Bb12+ OFS) would contribute to improve the low grade inflammation, LPS exposure and insulin resistance in obese subjects.

2. Subjects and Methods

2.1. Ethics

The study protocol was approved by the Ethics Committee for Research in Humans of the Institute of Nutrition and Food Technology (INTA), Univ. of Chile, in compliance with the Helsinki Declaration. All subjects were informed about the aims and procedures of the study and those who agreed to participate and met the inclusion and exclusion criteria signed a written informed consent form. Subjects were selected from the database of the Laboratory of Energy Metabolism (INTA) and from the Felix de Amesti Health Center of the Ministry of Health in the South Eastern Health District in Santiago

2.2. Sample Size

The sample size was calculated using the decrease of plasma IL-6 concentrations as the primary outcome. To detect a 25% decrease of plasma IL-6 levels with the intervention, with a power ß of 80% and α of 0.05, a sample size of 40 subjects was necessary.

2.3. Inclusion and Exclusion Criteria

The following inclusion criteria were considered: obese individuals of either sex, with BMI > 29 and <50 kg.m-2, between 18 and 55 years of age and non-smokers. The exclusion criteria were the presence of current intestinal diseases or antecedents of chronic intestinal diseases and/or malabsorption (celiac or inflammatory bowel disease, cancer, etc), a history of alcoholism, use of drugs that could interfere with the IM (antibiotics, anti-inflammatory drugs, laxatives, prokinetics, etc.) during the three weeks preceding the beginning of the study, treatment with hypoglycemiant drugs or immunodeficiencies (HIV, chemotherapy, radiotherapy, organ transplant). The recruited subjects were considered to have the metabolic syndrome when at least three of the following criteria were present, according to the Adult Treatment Panel III [21]: abdominal obesity (waist circumference>102 cm in males or >88 cm in females), hypertriglyceridemia (serum triglycerides ≥150 mg/dL), low levels of high-density lipoprotein cholesterol (HDL<40 mg/dL in males or <50 mg/dL in females), high blood pressure (≥130/≥85 mmHg) and hyperglycemia (blood glucose ≥110 mg/dl).

2.4. Experimental Design

A clinical, randomized, double-blind, placebo-controlled trial was carried out, that was registered (www.ClinicalTrials.gov, Access Number NCT01235026) previous to the volunteer recruitment. On admission, subjects were randomized into one of two groups (synbiotic or placebo), stratified by sex and age. Each subject of the synbiotic group had to ingest one gram of *Bifidobacterium lactis*Bb12 lyophilisate containing 10^{10} CFU (gift of Nestlé Chile, Santiago, Chile) and 8 g of OFS (Raftilin, Orafti) twice a day for 6 weeks while those of the placebo group received maltodextrin in the same amounts. Digestive symptoms as well as stool frequency and consistency were registered daily during the study using *ad hoc* forms and the Bristol Chart. Anthropometric data and measurements of systolic and diastolic pressures were obtained from all the subjects, and whole body composition was determined by air displacement plethysmography (Bod-Pod, Body Composition System; Life Measurement Instruments, Concord, CA, USA) before and after treatment. A food survey was carried out by a trained dietitian to quantify fat consumption.

2.5. Samples

Blood samples were obtained in the fasted state from all subjects at baseline and at the end of the six week treatment period. The plasma lipid profile (total cholesterol, HDL, LDL and triglycerides), blood sugar, insulinemia and ultrasensitive C-reactive protein (usCRP) were assessed and the concentrations of IL-6, LBP and sCD14 were determined by Elisa, using commercial kits according to the instructions provided by the manufacturer (Cell Sciences, USA). The antioxidant capacity of plasma was determined by FRAP as described by Benzie and Strain [22]. A two hour glucose/insulin tolerance test for was performed in all subjects after ingestion of 300 ml of a solution containing 75 g glucose and the areas under the curves of glycemia and insulinemia were calculated. A

fresh stool sample was also obtained at the same time to characterize the fecal microbiota.

2.6. Fecal Microbiota Analysis by qPCR

Bacterial fecal DNA was extracted and purified with a commercial kit (QIAmp DNA Stool Mini Kit, Quiagen, Hilden, Germany) according to the instructions provided by the manufacturer. DNAs from all fecal samples were processed by qPCR for detection of total bacteria and of the *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacteroides* populations. For this purpose, 50 ng of DNA were amplified using the corresponding primers, as shown in Table 1 [23-27], using the Light Cycler Fast Start DNA Master SYBR Green I kit in a Light Cycler (Roche Diagnostics, Mannheim, Germany). The log 16sDNA copy/g stool for each bacterial group was calculated using the corresponding standard curves.

Table 1. list of the primers used in the study for the characterization of the intestinal microbiota

Bacterial population	Primers (5' -> 3')	bp	Reference
Total bacteria	F: TCCTACGGGAGGCAGCAGT R: GGACTACCAGGGTATCTAATCCTGTT	467	23
Lactobacillus	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	341	24, 25
Bifidobacterium	F: GGGTGGTAATGCCGGATG R: CCACCGTTACACCGGGAA	523	26
Bacteroides	F: GCATCATGAGTCCGCATGTTC R: TCCATACCCGACTTTATTCCTT	287	27
Enterococcus	F: CCCTTATTGTTAGTTGCCATCATT R: ACTCGTTGTACTTCCCATTGT	144	24

2.7. Statistical Analysis

The statistical analysis was carried out by using the Statistica Software Package, version 11.0 (StatSoft, Tulsa, OK, USA). Data with normal distribution, as determined by the Shapiro-Wilk test, were expressed as means and 95% confidence interval (CI) while those with a skewed distribution were logarithmically transformed and expressed as geometric means and CI. The presence of outliers was detected by the Grubbs test. Changes with time between the synbiotic and placebo groups were analyzed in the normally distributed variables by two-way ANOVA for repeated measurements and subsequent contrast analysis when necessary. Skewed data whose distribution could not be normalized by log transformation were expressed as median and interquartile range and analyzed by non parametric ANOVA and subsequent Mann-Whitney U test when necessary. Correlations were determined by the Spearman Rank Test.

3. Results

Table 2. Anthropometric and nutritional parameters at baseline and at the end of the study in the Placebo and Synbiotic groups. Means [CI95%]; *Geometric means [CI95%]; **Median [IQR]. p significance for the corresponding parametric or no-parametric analysis of variances

Variables	Placebo (n=20)	Symbiotic (n=18)	p
Daily caloric intake (Kcal)	3140 [2730-3549]	3109 [2636-3581]	*NS*
Daily fat intake (g)	122.7 [109.9-148.4]*	120.3 [98.5-145.4]*	*NS*
Daily fat intake (%)	38.6 [35.3-41.8]	37.4 [32.6-42.1]	*NS*
% Male	15.0	11.1	*NS*
Age (years)	33.9 [29.7-38.1]	36.1 [31.2-41.0]	*NS*
Height (m)	1.62 [1.58-1.65]	1.57 [1.53-1.61]	*NS*
Weight (kg) Baseline	93.6 [86.8-100.4]	91.8 [83.6-100.0]	*NS*
Final	94.1 [87.2-101.1]	92.1 [84.1-100.1]	*NS*
BMI (kg/m^2) Baseline	35.7 [33.9-37.5]	37.0 [34.1-39.9]	*NS*
Final	35.8 [34.1-37.6]	37.2 [34.4-39.9]	*NS*
Body fat mass (kg) Baseline	43.0 [39.2-46.8]	43.6 [37.4-49.8]	*NS*
Final	43.5 [39.6-47.3]	43.7 [37.6-49.8]	*NS*
Body fat mass (%)Baseline	45.5 [42.8-49.2]**	46.8 [43.4-50.3]**	*NS*
Final	46.1 [42.2-48.0]**	46.8 [43.4-50.2]**	*NS*
Waist circumf.(cm) Baseline	108.5 [103-111]**	110 [104-115]**	*NS*
Final	108.2 [98.8-117.8]**	108.0 [95.5-120.5]**	*NS*
Systolic BP (mm Hg) Baseline	129 [120-131]**	121 [115-125]**	*NS*
Final	121 [104-138]**	120 [110-130]**	*NS*
Diastolic BP (mm Hg) Baseline	85 [78.5-89.0]**	80.5 [80.0-85.0]**	*NS*
Final	80 [70.5-89.5]**	80 [80.0-90.0]**	*NS*
Subjects with Metabolic Syndrome	15 (75%)	14 (77.8%)	*NS*

Of the 40 subjects recruited in the study protocol, two were excluded *a posteriori*: one due to the fact that his BMI was greater than 50 on the recruitment and was erroneously included in the study protocol; the other subject became pregnant. The anthropometric, nutritional and biochemical characteristics of the 38 remaining subjects at baseline are shown in Table 2 and Table 3; at this time, no differences between the groups were observed for these parameters. According to the Adult Treatment Panel III [21], 75% of the subjects in the placebo group and 77.8% in the synbiotic group had metabolic syndrome; they had a high daily intake of fat and energy. Biomarkers of inflammation (usCRP and IL-6), LPS exposure (LBP) and oxidative stress (FRAP) at baseline are described in Table 4; no differences between groups were observed at this stage. A positive correlation was observed between the percentage of body fat and the usCRP (ρ=0.59), IL-6 (ρ=0.53), LBP (ρ=0.42) and sCD14 (ρ=0.36); in addition, Il-6 was shown to correlate also with usCRP (ρ=0.61), LBP (ρ=0.53) and sCD14 (ρ=0.33) and usCRP correlated with LBP (ρ=0.65). FRAP values did not correlate with any of the biomarkers of

inflammation nor with LPS exposure. On the other hand, a positive correlation was observed between HOMA values and BMI (ρ=0.45). These results suggest that both groups were homogenous when evaluated at the beginning of the study.

Table 3. Biochemical parameters at baseline and at the end of the study in the Placebo and Synbiotic groups. Means [CI95%]; *Geometric means [CI95%]; **Median [IQR]. p significance for the corresponding parametric or no-parametric analysis of variances

Variables	Placebo (n=20)	Symbiotic (n=18)	p
Total cholesterol (mg/dL) Baseline	174.0 [163.3-195.0]	164.6 [159.1-170.0]	NS
Final	166.3 [150.5-182.1]	158.6 [142.2-174.9]	NS
HDL (mg/dL) Baseline	37.4 [32.3-42.6]	33.3 [30.4-36.3]	NS
Final	37.3 [32.9-41.7]	33.9 [29.8-38.1]	NS
LDL (mg/dL) Baseline	113.5 [103.2-126.3]**	111.6 [104.4-114.1]**	NS
Final	101.8 [87.1-129.4]**	95.4 [88.2-107.0]**	NS
Triglycerides (mg/dL) Baseline	123.1 [102.0-144.3]	110.7 [92.4-128.9]	NS
Final	114.5 [94.5-134.4]	120.1 [94.8-145.3]	NS
Glucose (mg/dL) Baseline	111.1 [103.1-119.2]	109.1 [99.9-118.3]	NS
Final	104.7 [103-109.1]	107.7 [99.7-115.7]	NS
Insulin (µU/ml) Baseline	9.11 [6.68-12.6]*	8.58 [6.17-11.9]*	NS
Final	13.1 [10.24-26.2]*	9.66 [7.8-14.9]*	NS
HOMA index Baseline	2.61 [1.88-3.60]*	2.29 [1.68-3.13]*	NS
Final	4.7 [2.8-6.7]*	3.13 [1.94-4.33]*	NS
AUC glucose Baseline	19040 [16899-21182]	17470 [15234-19707]	NS
Final	18148 [16273-20022]	19229 [16513-219144]	NS
AUC insulin Baseline	10725 [7983-13468]	10023 [7371-12674]	NS
Final	12368 [8951-15785]	11743 [8505-14981]	NS

Table 4. Biomarkers of inflammation, LPS exposure and antioxidant capacity at baseline and at the end of the study in the Placebo and Symbiotic groups. Means [CI95%]; *Geometric means [CI95%]; **Median [IQR]. p: significance for the corresponding parametric or no-parametric analysis of variances

Variables	Placebo	Symbiotic	p
usCRP (mg/L) Baseline	3.01 [1.31-5.72]**	6.04 [2.99-9.41]**	NS
Final	2.28 [2.01-4.58]**	4.01 [2.85-6.33]**	NS
IL-6 (pg/ml) Baseline	1.68 [1.22-2.34]*	1.57 [1.30-1.88]*	NS
Final	1.41 [0.79-2.02]*	2.28 [1.28-3.28]*	NS
LBP (µg/mL) Baseline	26.3 [23.4-29.2]	24.5 [19.1-29.9]	NS
Final	28.1 [25.0-31.0]	26.1 [20.8-31.3]	NS
FRAP (µmoles Fe2+/L) Baseline	818 [705-932]	789 [665-912]	NS
Final	1003 [887-1120]	1057 [922-1191]	NS

The placebo and synbiotic treatments were administered to the volunteers during six weeks. No adverse effects were reported during the time course of the study and no changes in gastrointestinal wellbeing (abdominal pain, bloating, borborygms, rectal gas, defecation strain or pain) or in stool frequency and consistency were reported by the subjects from the synbiotic group, compared with those receiving the placebo (data not shown). The anthropometric and biochemical parameters as well as the biomarkers of inflammation, LPS exposure and oxidative stress at the end of the 6-week period are described in Tables 2, 3 and 4, respectively. The statistical analysis does not indicate any significant differences between groups at six weeks nor any significant "Treatment X Time" interactions for the studied parameters. However, as shown in Figure 1, a significant increase of plasma sCD14 was observed after the six-week period of treatment in the placebo group while no change was observed in the subjects ingesting the synbiotic (p=0.044); as a result, sCD14 plasma levels were significantly lower in the synbiotic group than in the placebo group at six weeks (p=0.043).

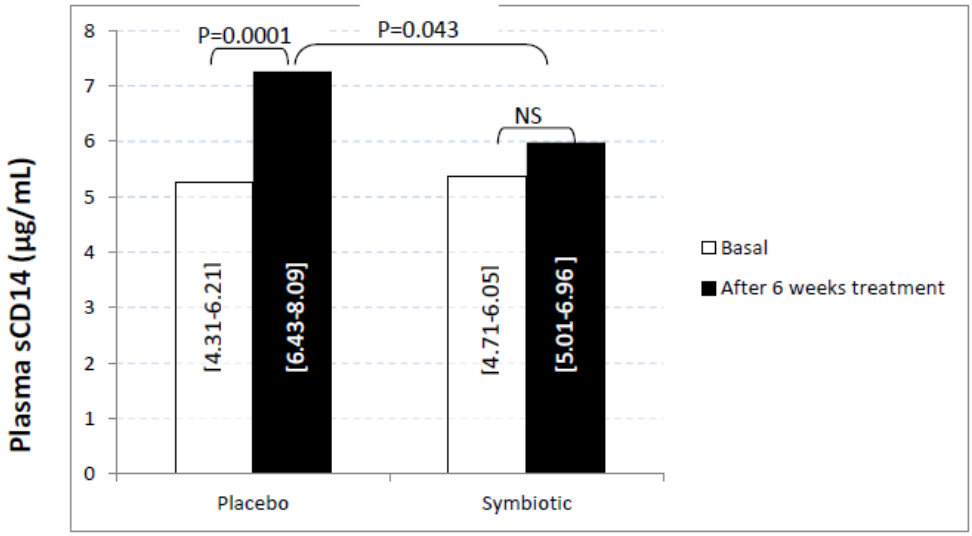

Figure 1. Changes in plasma sCD14 after 6 week of supplementation with the placebo or symbiotic product in obese subjects. (Anova for repeated measurements: F=4.38; p=0.044) (Means [CI95%])

Results of the analysis of the fecal bacterial populations are shown in Table 5. The number of 16S rDNA copies per gram of stool of *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Enterococcus* was similar when comparing both groups at baseline. Administration of the synbiotic significantly increased the concentration of *Enterococcus* (p=0.006) and the proportion of *Bifidobacterium* (p=0.049), without affecting the other bacterial populations studied.

Table 5. Bacterial populations of the fecal microbiota from the subjects from the placebo or symbiotic treated groups. (log 16S rDNA copies/g). * indicates that the difference between the final measurement and baseline value in the symbiotic group differs significantly (p<0.05) from the placebo group (ANOVA)

	Placebo		Symbiotic		p
	Baseline	Final	Baseline	Final	
Total bacteria	9.83 ± 0.46	9.87 ± 0.20	9.63 ± 0.54	9.65 ± 0.33	NS
Lactobacillus log 16S rDNA copia/g% 0.053 [0.014-0.38]	6.71 ± 0.63	6.54 ± 0.70 0.054 [0.013-0.25]	6.76 ± 0.64 0.15 [0.03-0.59]	7.09 ± 0.68 0.17 [0.09-1.15]	NS NS
Bifidobacterium log 16S rDNA copia/g% 0.45 [0.18-2.41]	7.46 ± 1.00	7.33 ± 0.90 0.49 [0.07-2.33]	7.36 ± 0.79 0.72 [0.14-1.53]	7.94 ± 0.87 1.56 [0.72-4.42]	0.087 0.049
Bacteroides log 16S rDNA copia/g% 8.61 [2.16-16.5]	8.51 ± 1.02	8.48 ± 0.96 6.02 [0.93-16.3]	8.29 ± 1.05 7.23 [2.28-13.4]	8.22 ± 0.85 7.24 [0.66-15.2]	NS NS
Enterococcus log 16S rDNA copia/g% <0.00	4.94 ± 0.64	5.23 ± 0.72 <0.00	4.80 ± 0.37 <0.00	5.54 ± 0.89* <0.00	0.006 NS

4. Discussion

It is currently accepted that the IM of patients suffering obesity or type-2 diabetes is altered, affecting particularly the Firmicutes/Bacteroides ratio. However, whether obesity *per se* or the fat content of the diet are responsible for this dysbiosis is under discussion [12]. Cani et al. also reported IM alteration, impaired gut barrier function and metabolic endotoxemia in mice fed a high-fat diet, resulting in the development of complications such as insulin resistance and diabetes [13,14]. The administration of OFS to these animals increased the intestinal counts of bifidobacteria, normalized the functional alterations and prevented the development of the metabolic complications [19]. However, it is remarkable that in these studies the animals received a diet containing 70% of the caloric intake as fat, i.e. a supra-physiological amount unlikely in the human diet, even in that consumed by obese subjects. For this reason, it is unclear whether the alterations observed in this model can be directly extrapolated to humans. For example, Leber et al. did not detect endotoxemia in obese subjects [28] and in a recent study, we did not detect any changes of the gut barrier function in obese subjects despite the alterations observed in their IM [8], a finding recently confirmed by Verdam et al. [29].

Our aim in the present study was to determine whether the administration of a synbiotic positively affects the markers of metabolic, inflammatory, LPS exposure and oxidative stress in obese subjects. The use of synbiotics is based on the synergy between its prebiotic and probiotic components in the lumen of the gastrointestinal tract. Oligofructose is generally recognized as safe (GRAS) and adverse effects, mainly digestive symptomatology, have only been reported in individuals consuming high amounts of this prebiotic [30]. *Bifidobacterium* is a normal member of the human resident gut microbiota; it is considered as a safe microorganism and is widely consumed in the world though some caution must be considered when administered to critically ill patients or strongly immunosupressed subjects [31]. On the other hand, the health promoting effects of these functional ingredients are widely documented [32]. Our results indicate that, as expected, a six-week intake of OFS and *B. lactis* Bb12 increased the fecal counts of *Bifidobacterium* spp. in these subjects. Interestingly, bifidobacteria are considered beneficial microorganisms which display anti-inflammatory activities of importance for the homeostasis of the gut ecosystem. The synbiotic treatment also increased the fecal concentrations of *Enterococcus* but the significance of this finding is unclear as the counts of this bacterial population remains very low since it represents less than 0.01% of the total bacteria and that, in consequence, it is unlikely that it will affect, either negatively or positively, the local colonic ecosystem. It is possible that this microorganism may use oligofructose as substrate although data about this matter are scarce as most studies evaluating the influence of oligofructose or inuline on gut microbiota did not quantitate the *Enterococcus* family alone but the *Lactobacillus-Enterococcus* group. On the other hand, some bifidobacteria have been shown to express exo-polysaccharides which may stimulate some bacterial populations of the gut microbiota including *Enterococcus* [33]. Our results also showed that the synbiotic intake did not significantly affect the BMI or the body composition of the subjects, nor their lipid profile, the glucose/insulin balance parameters and the blood pressure. Although these variables were only slightly altered in our subjects, we observed that most of their parameters of inflammation (usCRP, IL-6) and LPS exposure (sCD14, LBP) correlated between them and with body mass index or % body fat, confirming results from other studies [34]. No correlation between LPB and sCD14 was observed in our subjects, in opposition with a number of other studies. However, absence of correlation between these parameters has also been described, for example in older subjects [35]. Leber et al., who studied the effect of probiotic supplementation in patients with metabolic syndrome, did not report LPB/sCD14 correlation in these subjects while they observed a significant increase in plasma LBP without changes in sCD14 during the treatment [28]. sCD14 and LBP are both secreted by the liver but sCD14 is also released by monocytes and LBP by enterocytes. The regulation of the expression and secretion of these factors is not fully understood and it is possible that they are differentially regulated by molecules other than LPS; this could explain the absence of correlation we observed between LPS and sCD14. For example it has been proposed that supplementation with fish oil during pregnancy could affect sCD14 concentrations in breast milk [36]. On the other hand, it is noteworthy that most of

the studies carried out with synbiotics, either in humans or in animal models, are oriented to the prevention or management of colorectal cancer or inflammatory bowel diseases or to the improvement of digestive wellbeing and that data about their use in individuals with metabolic disturbances and low grade inflammation, such as obesity or the metabolic syndrome, are scarce. Contrarily to our results, Safavi et al. observed that the 8-week administration of a synbiotic (a combination of *L. casei, L. rhamnosus, L. acidophilus, L. bulgaricus, S. thermophilus, B. breve* and *B. longum*, with fructo-oligosaccharides) to obese and overweight children (6-18 years) significantly decreased their BMI, serum triglycerides, total cholesterol and LDL-cholesterol [37]. It is possible that these discordant results are explained by the duration of the treatment, greater in the study of Safavi, or to the fact that they were using a combination of different strains of probiotics. A number of studies carried out in healthy animals or in animal models of type-2 diabetes or obesity have shown that the administration of probiotics (including strains of *L. rhamnosus*, *L. acidophilus*, *L. gasseri*) or prebiotics (mainly inulin, OFS or galacto-oligosaccharides) improves the lipid profile and the biomarkers of oxidative stress, immune activation and inflammation, as well as the plasma concentrations of the hormones involved in the regulation of appetite/satiety [38]. Studies with probiotic strains or with prebiotics have also been carried out in healthy volunteers (including pregnant women) or in obese individuals and in patients with type-2 diabetes or hypercholesterolemia, evaluating mainly the effects of these dietary bioactive components on lipid profile, inflammatory factors and markers of glucose/insulin homeostasis [39]. No significant effects on BMI were reported in these studies. Three of these were carried out using the *B. lactis* Bb12 strain combined with a strain of *Lactobacillus* (*L. rhamnosus* GG or *L. acidophilus* La5); two studies, using Bb12/LGG together with dietary counseling, were carried out in healthy pregnant women and showed an improvement of glucose/insulin control and a decrease in the risk of central adiposity [39,40]. The third study did not report any changes in the lipid profile of women after 6 weeks of consuming a yogurt with Bb12/La5, compared with the placebo yogurt [41]; however their lipid profile improved when compared with the subjects who did not consume any kind of yogurt (control or with probiotic) suggesting that fermented milk products exert healthy effects, independently of the presence of probiotics. Administering the same yogurt (300 g/d) for 6 weeks to patients with type 2 diabetes, Ejtahed et al. observed decreases of total cholesterol and LDL-cholesterol by 4.54% and 7.45%, respectively, compared with the control group, without changes in triglycerides and HDL-C [42]. The fasting blood glucose and hemoglobin A1c levels of the diabetic subjects were also decreased by the probiotic yogurt while their erythrocyte superoxide dismutase and glutathione peroxidase activities as well as their total antioxidant status were enhanced, suggesting that these probiotic microorganisms may contribute to improve their health status [43].

In the present study, we determine plasma sCD14 and LBP rather than plasma endotoxin considering the short half-life of plasma LPS in humans (about 3 hours), compared with those of sCD14 and LBP (24–48 h), that indicates that plasma sCD14 and LBP probably reflect long-term LPS exposure while plasma LPS could reflect the transient kinetics of LPS absorption only. In agreement with this, the release of sCD14 by cultured peripheral blood monocytes has been shown to increase significantly in the presence of low stimulatory LPS concentrations (0.01 ng/ml), suggesting that sCD14 may be considered as a biomarker of LPS exposure [44,45]. Our results show that the synbiotic treatment did not improve the plasma concentrations of usCRP, IL-6 and LBP while it contributes to maintain lower plasma concentrations of sCD14 during the study compared with the control group. Schiffrin et al. observed a decrease of serum sCD14 as well as lower expressions of TNF and IL-6 by blood leucocytes, without changes in the fecal microbiota or in the nutritional parameters, after a 12-week oral nutritional supplementation with oligosaccharides in 74 elderly subjects at risk of malnutrition [46]. Similar observations were reported by these authors in another study in elder subjects after the 4-week administration of a yogurt containing *L. johnsonii* La1, a probiotic with well-described antibacterial and immunomodulatory activities [47]. A decrease of sCD14 has also been described in cord blood of infants born to mothers receiving *Bifidobacterium lactis* HN019 for 2-5 weeks prior to delivery [48]. These results may indicate a lower exposition to plasma LPS due to modulation of the IM of these infants, with concomitant increases of gram-positive bacteria at the expense of LPS-bearing gram-negative bacteria. However, in our study we effectively observed an increase of the fecal bifidobacteria but without changes in the population of the gram-negative bacteria evaluated (*Bacteroides*). It cannot be ruled out that other gram-negative populations, not detected in our study, were modified by the treatment.

In conclusion, the administration of *B. lactis* Bb12 and oligofructose increased the fecal concentrations of bifidobacteria in obese subjects but had no effects on their anthropometric, biochemical, metabolic and inflammatory parameters. More studies are necessary to elucidate the effect of the synbiotic on plasma sCD14.

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Statement of Competing Interests

the authors have no competing interests.

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