A Mixture of *trans*-Galactooligosaccharides Reduces Markers of Metabolic Syndrome and Modulates the Fecal Microbiota and Immune Function of Overweight Adults[1–3]

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Abstract

Metabolic syndrome is a set of disorders that increases the risk of developing cardiovascular disease. The gut microbiota is altered toward a less beneficial composition in overweight adults and this change can be accompanied by inflammation. Prebiotics such as galactooligosaccharides can positively modify the gut microbiota and immune system; some may also reduce blood lipids. We assessed the effect of a galactooligosaccharide mixture [Bi²muno (B-GOS)] on markers of metabolic syndrome, gut microbiota, and immune function in 45 overweight adults with ≥3 risk factors associated with metabolic syndrome in a double-blind, randomized, placebo (maltodextrin)-controlled, crossover study (with a 4-wk wash-out period between interventions). Whole blood, saliva, feces, and anthropometric measurements were taken at the beginning, wk 6, and end of each 12-wk intervention period. Predominant groups of fecal bacteria were quantified and full blood count, markers of inflammation and lipid metabolism, insulin, and glucose were measured. B-GOS increased the number of fecal bifidobacteria at the expense of less desirable groups of bacteria. Increases in fecal secretory IgA and decreases in fecal calprotectin, plasma C-reactive protein, insulin, total cholesterol (TC), TG, and the TC:HDL cholesterol ratio were also observed. Administration of B-GOS to overweight adults resulted in positive effects on the composition of the gut microbiota, the immune response, and insulin, TC, and TG concentrations. B-GOS may be a useful candidate for the enhancement of gastrointestinal health, immune function, and the reduction of metabolic syndrome risk factors in overweight adults.   J. Nutr. 143: 324–331, 2013.

Introduction

Metabolic syndrome is a combination of disorders, such as glucose intolerance, central obesity, dyslipidemia, and hypertension, which increases the risk of developing cardiovascular disease, type 2 diabetes, and cancer (1). The development of metabolic syndrome is a complex process that involves genetic, environmental, and dietary factors [e.g., long-term stress, positive energy balance (excessive energy intake and low physical activity), diets comprising high fat and sugar and low micronutrients, disruption of chronobiology] and is associated with pathways that connect metabolism with the immune system and vice versa. Individuals affected by metabolic syndrome do not all present the same combination of disorders. Although excessive weight can sometimes be regarded as benign (2), it is generally accepted as being an important factor in metabolic

syndrome. Regardless of an individual's weight, the chronic low-grade inflammatory condition that accompanies metabolic syndrome has been implicated as a major factor in both the onset of the syndrome and its associated pathophysiological consequences (3).

Recent studies have shown that the gut microbiota varies in both its composition (e.g., reduced levels of bifidobacteria and increased levels of less desirable bacteria) and its metabolic activity in obese individuals compared with lean individuals (4–6). It seems that the "obese microbiota" can modulate host energy homeostasis and adiposity through a number of different mechanisms, including harvesting energy from food (7), LPS-induced chronic inflammation (8), modulation of tissue fatty acid composition (9), and gut-derived peptide secretion (10).

Modulation of the gut microbiota by dietary means is the basis for the probiotic (11) and prebiotic (12) concepts. The majority of scientific data on prebiotic effects comes from studies with either inulin-type fructooligosaccharides (FOS)[7] or

[7] Abbreviations used: BP, blood pressure; B-GOS, Bi²muno; FBC, full blood count; FISH, fluorescence in situ hybridization; FOS, fructooligosaccharide; G-CSF, granulocytes colony-stimulating factor; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NDO, nondigestible oligosaccharide; sIgA, secretory IgA; TC, total cholesterol.

galactooligosaccharides. The capacity of these prebiotics to selectively stimulate the growth of bifidobacteria, and in some cases lactobacilli, and elicit a significant change in the overall composition of the gut microbiota has been repeatedly demonstrated (13). With regard to obesity and related metabolic disorders, the majority of available data related to prebiotics comes from animal models and dietary supplementation with FOS. These studies suggest that prebiotics are able to regulate food intake and weight gain, glucose homeostasis, dyslipidemia, steatosis, and hypertension (14). However, definitive evidence of an effect of prebiotics in obese humans is scarce and no study to our knowledge has looked at the effects of prebiotics on the gut microbiota, immune response, and markers of metabolic syndrome together. Furthermore, the effects of galactooligosaccharide supplementation in overweight individuals with respect to weight management, modulation of the gut microbiota, or metabolic syndrome have not been reported thus far, to our knowledge. In the present study, we investigated the effect of administering $Bi^{2}muno$ (B-GOS) on the fecal microbiota and on markers of metabolic syndrome and immune function in overweight adults in a double-blind, randomized, placebo-controlled, crossover study.

Subjects and Methods

Materials. Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich or BDH Chemicals. Fluorescent, Cy3-labeled probes for fluorescence in situ hybridization (FISH), and cell culture media and supplements were obtained from Sigma-Aldrich.

Subjects and study design. More than 200 overweight volunteers predisposed to the development of metabolic syndrome and its associated increased risk of cardiovascular disease were invited for screening. From this pool of volunteers, written informed consent was obtained from 48 subjects who presented ≥3 risk factors for metabolic syndrome (15) [i.e., fasting glucose (>5.6 mmol/L), high blood pressure (BP), dyslipidemia [low HDL cholesterol (HDL-C) (<1 mmol/L), high TG (>1.3 mmol/L), and large waist circumference (>94 cm in men, >80 cm in women)]. Further criteria for inclusion in the study were that subjects: were between 18 and 65 y of age; had a BMI >25 kg/m²; had not had a myocardial infarction/stroke or cancer in the past 12 mo; were not diabetic (diagnosed or fasting glucose >7 mmol/L) or suffering from other endocrine disorders; did not suffer from chronic coronary, renal, bowel disease/gastrointestinal disorders or have a history of choleostatic liver or pancreatitis; were not receiving drug treatment for hyperlipidemia, hypertension, inflammation, or hypercoagulation or using drugs that affected intestinal motility or absorption; had no history of alcohol/drug abuse; were not planning or currently on a weight-reducing regime; were not taking any dietary antioxidant or other phytochemical, prebiotic, or probiotic supplements; were not pregnant or lactating; had not taken antibiotics for 1 mo prior to the start of the trial; were not anemic (hemoglobin: men >140 g/L; women >115 g/L); and did not smoke. Of the 48 subjects, 3 volunteers were withdrawn from the study after completing the second visit, because they were reluctant to fully comply with the study protocol. The trial protocol was reviewed and approved by The University of Reading Research Ethics Committee.

Volunteers were randomly assigned to 1 of 2 groups: one started the trial with the placebo (maltodextrin) and the other with B-GOS, with both compounds provided in powder form (5.5 g/d) and supplied by Clasado. Subjects were asked to reconstitute the contents of the sachets immediately before consumption by mixing the powder with water and to consume the product every day at approximately the same time. They consumed the products for 12 wk, followed by a 4-wk washout period, before switching to the other intervention for the final 12 wk. Volunteers were required to visit The University of Reading on 7 separate occasions during this period. Their BP, BMI, and waist circumference were recorded and blood sampled at each visit (2–3 wk before the start, on d 0,

and after 6, 12, 16, 22, and 28 wk). During all visits (except 2–3 wk before the start), fecal samples were obtained and the use of any medication (including vitamin/mineral supplements) and adverse events recorded. In addition, habitual diet was assessed by prevalidated, 4-d, food diaries (2 weekend and 2 weekdays) before the start of the study and then twice during each intervention period. Information collected in the food diaries was analyzed using Dietplan 6 (Forestfield Software).

Preparation and collection of fecal samples. Fecal samples were collected, diluted, homogenized, and used to enumerate gut bacteria by FISH as previously described (16). A portion of each fecal sample was removed prior to dilution and used for secretory IgA (sIgA) and calprotectin analyses and to freeze-dry and determine the dry weight.

Preparation and collection of whole blood. Whole blood was collected in several different tubes depending on the analyses. EDTA-coated tubes were used for cytokine, CRP, insulin, and full blood count (FBC) analyses. FBC analyses were performed by the Pathology Department at the Royal Berkshire NHS Foundation Trust, Reading, UK, using a Sysmex XE-2100 automated hematology analyzer (Sysmex UK). Serum separation tubes-coated tubes were used for lipid analyses and fluoride oxalate tubes were used for glucose analysis.

FISH. The differences in bacterial populations were assessed using FISH analysis with oligonucleotide probes designed to target specific diagnostic regions of 16S rRNA as previously described (16). The probes used were Bif164 (17), Bac303 (18), Lab158 (19), Erec482 and His150 (20), Srb687 (21), Ato291 (22), Ecyl387 (23), Ehal469 (24), Bet42a (25), Prop853 (26), and Fprau645 (27), specific for *Bifidobacterium* spp., *Bacteroides* spp., *Lactobacillus/Enterococcus* spp., the *Clostridium coccoides/Eubacterium rectale* group, the *Clostridium histolyticum* group, *Desulfovibrio* spp., the *Atopobium* cluster, *Eubacterium cylindroides*, *Eubacterium hallii*, β-Proteobacteria, *Clostridium* cluster IX, and the *Faecalibacterium prausnitzii* cluster, respectively.

For counts of total bacteria, the nucleic acid stain 4′,6-diamidino-2-phenylindole was used. The number of cells obtained is expressed as per gram of dry weight feces.

Measurement of cytokine production by whole blood cultures. Blood (collected at the beginning and the end of each intervention) was diluted (1:10, v:v) in RPMI/Gln/Ab medium and plated onto 24-well plates in the presence of LPS (1 mg/L) and incubated at 37°C in an air: carbon dioxide (19:1) atmosphere for 24 h. Following incubation, the plates were centrifuged at $400 \times g$ for 5–6 min and the supernatants collected and frozen in aliquots (200 µL). Concentrations of cytokines [IL-6, IL-10, IL-8, TNFα, and granulocytes colony-stimulating factor (G-CSF)] were measured by ELISA using commercially available kits (Universal Biologicals Cambridge, except G-CSF, which was from R&D Systems Europe). The names of kits used and the catalog numbers are as follows: AssayMax Human IL-6 ELISA kit (EI 1006–1), AssayMax Human IL-10 ELISA kit (EI 3010–1), AssayMax Human IL-8 ELISA kit (EI 1008–1), AssayMax Human TNF-α ELISA kit (ET 2010–1), and Quantikine Human G-CSF (DCS50). Kits were used according to the manufacturers' instructions. The limits of detection for these assays were as follows: IL-6, <10 ng/L; IL-10, <100 ng/L; IL-8, <1 ng/L; TNFα, <10 ng/L; and G-CSF, <20 ng/L (data were supplied by the manufacturers of the kits).

Analysis of calprotectin, CRP, insulin, and sIgA. Fecal calprotectin, a marker of intestinal inflammation (PhiCal Calprotectin ELISA kit, K 6930, BioSupply UK), plasma CRP (AssayMax Human CRP ELISA kit, EC 1001–1, Universal Biologicals Cambridge), and plasma insulin (Insulin ELISA kit, K 6219, Alere) as well as fecal and salivary sIgA (sIgA ELISA Kit, K 8870, Oxford Biosystems) were all measured by ELISA using commercially available kits and instructions provided by the manufacturers. The limits of detection for these assays were as follows: calprotectin, 2.915 µg/L; CRP, <100 ng/L; insulin, 3 pmol/L; and sIgA, 13.4 µg/L (data were supplied by manufacturers of the kits).

Analysis of plasma total cholesterol, HDL-C, LDL cholesterol, TG, and glucose. Whole blood was collected into appropriate tubes as

described above and centrifuged for 10 min at $1600 \times g$ and 4°C. Plasma was removed and stored at −20°C until analysis. Samples were analyzed by using an ILab 600 biochemical analyzer and enzymatic colorimetric kits [IL Test TM Cholesterol, IL Test TM Triglycerides, and IL Test TM Glucose (0018250740), IL Test TM LDL-Cholesterol (0018255740), IL Test TM HDL-Cholesterol (0018256040), Instrumentation Laboratories].

Statistical analyses. All statistical tests were performed using SAS version 9.2. A mixed model was used allowing for the crossover nature of the study. The change from baseline for each measurement (variable) was used as a response and differences were sought between placebo and intervention having taken period effect and gender into consideration. Interaction terms were also considered in the modeling; the intervention period interaction was included as a test for carryover effects as well as interactions between intervention and gender. Where interaction terms and gender were not significant, they were removed from the model. Period and intervention terms remained in the model irrespective of the significance associated with them, because they define the crossover design of the study. Variables were split into primary (lipids, glucose, insulin, BP) and secondary (everything else). Due to the number of variables considered, P values were corrected for multiple testing using the Bonferroni correction. Following the Bonferroni adjustment, significance levels were set at $P < 0.005$ for the primary variables and $P < 0.0012$ for the secondary. Homogeneity of variance was tested using Levene's test. In the case of significantly different variances, Welch's test was used.

Significant differences were not observed for any of the measured variables between the 2 groups at the start of each intervention period (baseline samples) and carry-over effect was not significant regardless of time, intervention, or variable. Values in the text are given as mean ± SD.

Results

Participants. A total of 45 volunteers (16 males, 29 females) completed the study. Anthropometric and physiological data for the volunteers at the start of the trial are shown in **Table 1**. All participants had a BMI >25 kg/m^2; 98% of the participants had central obesity, 78% had plasma insulin concentrations >40 pmol/L, 27% had plasma glucose concentrations >5.6 mmol/L, 42% had BP >130/85 mm Hg, 93% had plasma total cholesterol

(TC) concentrations >5.0 mmol/L, 76% had low plasma HDL-C (<1.03 mmol/L in men and <1.29 mmol/L in women), and 40% had plasma TG concentrations >1.7 mmol/L. Body weight and BP remained stable throughout the study for all participants regardless of intervention (data not shown). The habitual diets of participants at the start of the trial, as assessed by food diaries, provided 17.2 ± 3.4%, 35.9 ± 5.1%, and 42.3 ± 6.6% of energy from protein, total fat, and carbohydrate, respectively. Energy and nutrient intake did not differ between the 2 groups (i.e., placebo intervention first compared with B-GOS intervention first) at the start of the trial. Diet diaries indicated that the participants' habitual diets remained unchanged throughout the study (data not shown).

In addition, FBC analyses, which included counts of white blood cells, platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils, remained unchanged throughout the study for all participants regardless of intervention (data not shown).

Populations of fecal bacteria. The composition of the fecal microbiota of the overweight volunteers who participated in this study was determined by using FISH to enumerate 12 different bacterial groups of interest and total bacteria. There were no differences in populations of fecal bacteria in the 2 groups at the start of each intervention period (**Table 2**). The 2 dietary interventions had no significant effects on counts of total bacteria, *Lactobacillus*/*Enterococcus* spp., *Clostridium coccoides*/*Eubacterium rectale* group, *Atopobium* cluster, *E. cylindroides*, *E. hallii*, *β-Proteobacteria*, *Clostridium* cluster IX, and *F. prausnitzii* cluster during the study. However, after 6 wk and at the end of the study period, differences between interventions were observed with respect to the number of bifidobacteria, bacteroides, and *C. histolyticum* group bacteria detected ($P < 0.0001$). That is, B-GOS increased the number of bifidobacteria in feces, whereas representation of the *Bacteroides* spp. and *C. histolyticum* group was lower (Table 2). The number of *Desulfovibrio* spp. tended to be lower during the B-GOS period than during the placebo period at 6 wk ($P = 0.0018$) and was lower at the end of 12 wk ($P < 0.0001$). In addition, the number

TABLE 1 Anthropometric and physiological data for the participants at the start of the trial[1]

Characteristic	Male (*n* = 16)	Female (*n* = 29)
Age, *y*	42.8 ± 12.1	46.4 ± 11.8
BMI, kg/m^2	30.7 ± 5.3	32.1 ± 6.3
>25 kg/m^2, *n* (%)	16 (100)	29 (100)
Waist circumference, *cm*	104 ± 11.0	99.2 ± 14.5
>94 cm (male) or >84 cm (female), *n* (%)	15 (94)	29 (100)
Fasting insulin, *pmol/L*	77.7 ± 33.6	65.0 ± 29.2
>40 pmol/L, *n* (%)	13 (81)	22 (76)
Fasting glucose, *mmol/L*	5.5 ± 0.8	5.2 ± 0.6
>5.6 mmol/L, *n* (%)	5 (31)	7 (24)
Systolic BP, *mm Hg*	128 ± 10.1	126 ± 15.8
Diastolic BP, *mm Hg*	80.9 ± 8.6	80.5 ± 10.0
BP >130/85 mm Hg, *n* (%)	6 (38)	13 (45)
TC, *mmol/L*	6.6 ± 1.2	6.1 ± 1.3
>5 mmol/L, *n* (%)	14 (88)	28 (97)
HDL-C, *mmol/L*	1.2 ± 0.2	1.5 ± 0.3
<1.03 mmol/L (male) or <1.29 mmol/L (female), *n* (%)	10 (63)	24 (83)
TG, *mmol/L*	2.1 ± 0.9	1.3 ± 0.4
>1.7 mmol/L, *n* (%)	11 (69)	7 (24)

[1] Values are mean ± SD unless noted otherwise. BP, blood pressure; HDL-C, HDL cholesterol; TC, total cholesterol.

TABLE 2 Populations of bacteria in feces of overweight adults during 12-wk B-GOS and placebo interventions[1]

Group	Baseline[2]	B-GOS		Placebo	
		wk 6	wk 12	wk 6	wk 12
		log₁₀ cells/g dry weight feces			
Total bacteria	11.4 ± 0.2	11.4 ± 0.2	11.5 ± 0.2	11.4 ± 0.2	11.5 ± 0.2
Bifidobacterium spp.	9.8 ± 0.4	10.1 ± 0.4*	10.2 ± 0.3*	9.7 ± 0.3	9.8 ± 0.4
Bacteroides spp.	10.2 ± 0.3	10.2 ± 0.3*	10.1 ± 0.3*	10.3 ± 0.3	10.4 ± 0.3
Lactobacillus/Enterococcus spp.	9.9 ± 0.3	10.0 ± 0.4	10.1 ± 0.3	10.0 ± 0.3	10.0 ± 0.3
E. rectale/C. coccoides group	10.4 ± 0.2	10.4 ± 0.3	10.5 ± 0.3	10.2 ± 0.2	10.4 ± 0.3
C. histolyticum group	10.1 ± 0.3	10.1 ± 0.3*	10.0 ± 0.3*	10.3 ± 0.3	10.3 ± 0.3
Desulfovibrio spp.	8.8 ± 0.3	8.7 ± 0.3	8.7 ± 0.3*	8.8 ± 0.2	9.0 ± 0.3
Atopobium cluster	10.1 ± 0.3	10.0 ± 0.3	10.0 ± 0.3	10.0 ± 0.3	9.9 ± 0.3
E. cylindroides	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.4	9.8 ± 0.3	9.8 ± 0.3
E. hallii	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.3	9.8 ± 0.2	9.8 ± 0.3
β-Proteobacteria	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3	9.3 ± 0.3
Clostridium cluster IX	10.2 ± 0.2	10.2 ± 0.2	10.2 ± 0.3	10.2 ± 0.2	10.2 ± 0.2
F. prausnitzii cluster	10.3 ± 0.3	10.3 ± 0.3	10.3 ± 0.3	10.4 ± 0.2	10.3 ± 0.3

[1] Values are mean ± SD, $n = 45$. Following Bonferroni adjustment for multiple testing, significance was set at $P < 0.0012$. No differences were observed between the 2 groups of participants at the beginning of each intervention period. The period-intervention interaction, which tested for a carryover effect, the gender-intervention interaction, and gender main effect were nonsignificant and therefore removed from the model. *Different from placebo at the same time point, $P < 0.0001$. B-GOS, Bi²muno.
[2] Mean of all volunteers at the beginning of each intervention period.

of *β-Proteobacteria* found in fecal samples tended to be lower ($P = 0.0019$) with B-GOS intervention than with placebo at 12 wk (Table 2).

Cytokine production by whole blood cultures. There were no significant effects (data not shown) of time or intervention on the production of the measured cytokines (G-CSF, IL-6, IL-10, IL-8, and TNFα).

Fecal calprotectin, plasma CRP, and fecal and salivary sIgA. Changes in the concentrations of fecal calprotectin and plasma CRP at 6 and 12 wk for the placebo and B-GOS interventions from baseline are shown in **Figure 1**. The concentration of calprotectin was lower during the B-GOS intervention than during the placebo intervention both after 6 wk and at the end of the 12-wk study ($P < 0.0001$). The concentration of plasma CRP did not differ between the interventions at 6 wk; however, it was lower at the end of the 12 wk following B-GOS administration ($P < 0.0012$).

Neither intervention had any effect on sIgA concentrations in saliva (data not shown). However, the change in the concentration of fecal sIgA at the end of the 12 wk was significantly greater during the B-GOS period (808 ± 176 μg/g dry weight

feces) than during the placebo period ($-384 ± 69$ μg/g dry weight feces) ($P < 0.0001$).

Plasma glucose and insulin. Glucose concentrations did not significantly differ at the beginning of each intervention and they remained unaffected regardless of intervention type or time during the trial period (**Table 3**). The plasma insulin concentration tended to be lower during the B-GOS period than during the placebo period after 6 wk ($P = 0.008$) and was lower at the end of the 12-wk study period ($P < 0.005$) (Table 3).

Plasma lipid profile. Plasma concentrations of HDL-C and LDL cholesterol (LDL-C) remained unchanged regardless of intervention type or time during the trial period (Table 3). In addition, both interventions had no effect on plasma concentration of TC after 6 wk, but by the end of the 12-wk study, B-GOS resulted in a lower concentration compared with placebo ($P < 0.001$) (Table 3). Plasma TG concentrations were lower ($P < 0.0001$ at 6 wk and $P < 0.0005$ at 12 wk) following administration of B-GOS compared with the placebo throughout the study period (Table 3). However, in the case of TG, the effect after 6 wk was significant ($P < 0.005$) only in males (**Fig. 2**). The effect of gender was not significant by the end of the trial period (data not shown).
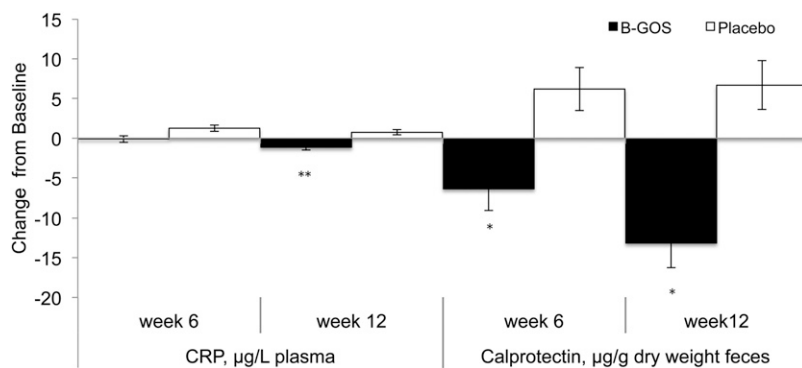

FIGURE 1 Changes in fecal calprotectin and plasma CRP concentrations in overweight adults who received B-GOS and placebo interventions for 1 wk each. Values are mean ± SD, $n = 45$. Data were analyzed in a mixed model taking the period and gender effect and the interaction between intervention and gender into consideration. Following Bonferroni adjustment for multiple testing, significance was set at $P < 0.0012$. Asterisks indicate different from placebo at that time: *$P < 0.0001$, **$P < 0.0012$. B-GOS, Bi²muno.

TABLE 3 Plasma lipids, glucose, and insulin in overweight adults during 12-wk B-GOS and placebo interventions[1]

Intervention/ time point	TC	TG	HDL-C	LDL-C	Glucose	Insulin	TC:HDL-C ratio
			mmol/L			*pmol/L*	
Placebo							
Baseline	6.2 ± 1.3	1.6 ± 0.7	1.4 ± 0.4	4.2 ± 1.1	5.2 ± 0.9	64.8 ± 30.6	4.5 ± 1.1
wk 6	6.3 ± 1.1	1.8 ± 0.9	1.4 ± 0.3	4.2 ± 1.1	5.4 ± 0.8	69.1 ± 33.9	4.6 ± 1.1
wk 12	6.2 ± 1.2	1.6 ± 0.7	1.4 ± 0.4	4.3 ± 1.0	5.6 ± 0.8	70.1 ± 36.8	4.6 ± 1.3
B-GOS							
Baseline	6.3 ± 1.3	1.6 ± 0.8	1.4 ± 0.3	4.2 ± 1.1	5.4 ± 0.6	67.3 ± 30.9	4.8 ± 1.3
wk 6	6.1 ± 1.2	1.6 ± 0.7*	1.4 ± 0.3	4.3 ± 1.0	5.6 ± 0.7	63.0 ± 29.0	4.6 ± 1.0
wk 12	5.9 ± 1.1*	1.5 ± 0.6**	1.4 ± 0.4	4.1 ± 1.0	5.6 ± 0.7	58.1 ± 29.7**	4.3 ± 1.1*

[1] Values are mean ± SD, n = 45. Following Bonferroni adjustment for multiple testing, significance was set at $P < 0.005$. No differences were observed between the 2 groups of participants at the beginning of each intervention period. The period-intervention interaction, which tested for a carryover effect, was nonsignificant and therefore removed from the model. The gender-intervention interaction ($P < 0.0001$) and gender main effect ($P < 0.005$) were significant only in the case of TG at 12 wk. Asterisks indicate different from placebo at the same time point: *$P < 0.0001$, **$P < 0.005$. B-GOS, Bi²muno; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol.

The changes from baseline in the TC:HDL-C ratio at 6 and 12 wk for placebo and B-GOS interventions are shown in Table 3. After 6 wk, the interventions had no significant effect. However, at the end of the 12 wk, B-GOS resulted in a lower TC:HDL-C ratio than the placebo ($P < 0.0001$) (Table 3); this effect was more pronounced in males, as the intervention-gender interaction was significant at the end of each 12-wk intervention period ($P < 0.005$) (Fig. 2).

Discussion

Excessive weight and diabetes are associated with a poor inflammatory status, leading to impaired insulin action and adipose-tissue plasticity (3). Although the origin of this inflammation is unclear, it seems that poor diet (high in fat, low in fiber) has direct pro-/antiinflammatory effects, depending on its fatty acid composition and fiber content, and indirect effects through the gut microbiota and its ability to modulate metabolic endotoxemia, intestinal permeability, and inflammation.

LPS, a component of the cell wall of Gram-negative bacteria that has been implicated in triggering low-grade inflammation (i.e., metabolic endotoxemia), is known to be increased in obese mice when they are fed a high-fat diet (27). Other studies in mice have shown that shifts of the gut microbiota toward a more beneficial composition (i.e., to a higher proportion of Gram-positive relative to Gram-negative bacteria) can decrease body weight, inhibit inflammation, and improve gut permeability (28,29). Beneficial colonic bacteria, namely lactobacilli and bifidobacteria, are Gram-positive bacteria that do not contain LPS within their cell membranes (30). Therefore, selective increases in numbers of these bacteria in overweight adults with metabolic syndrome can only be regarded as beneficial. This has been supported by several studies that have shown excessive weight gain induced following a high-fat diet (27,28,31,32) or genetic deletions (i.e., leptin-deficient models) (33) results in gut microbiota changes that reduce levels of bifidobacteria. Similar trends were observed in a study involving both genetic (apoA-I–deleted) and diet-induced murine models of metabolic syndrome, where bifidobacteria were reduced and *Desulfovibrio* spp. increased (34). In the latter study, diet was found to be a stronger contributor to microbial changes than the genetic alterations. In the only available human study comparing the microbiotas of lean and overweight adults, significantly fewer bifidobacteria and more bacteroides were observed in the overweight subjects (35).

Nondigestible oligosaccharides (NDOs) that elicit a prebiotic effect are attracting attention as vehicles for dietary management and the control of obesity and/or metabolic syndrome. Although evidence comes mostly from animal models, NDOs have been shown to regulate food intake and weight gain, dyslipidemia, insulin resistance, hypertension, and liver steatosis (14). Animal models and in vitro experiments have shown that specific immunomodulatory effects are induced by prebiotics (36,37). This immunomodulation is usually thought to be related to the microbiota. However, recent evidence suggests that, depending on the chemical structure of the tested prebiotic, a direct interaction can take place between the prebiotic and the host through binding of the prebiotic to specific receptors on cells of the immune system (38,39).

FIGURE 2 Changes in the plasma TG concentration and the TC:HDL-C ratio in overweight men and women who received B-GOS and placebo interventions for 12 wk each. Values are mean ± SD, n = 45. Data were analyzed in a mixed model taking the period and gender effect and the interaction between intervention and gender into consideration. Following Bonferroni adjustment for multiple testing, significance was set at $P < 0.005$. *Different from corresponding female, $P < 0.005$; **different from corresponding placebo, $P < 0.0001$. B-GOS, Bi²muno; HDL-C, HDL cholesterol; TC, total cholesterol.
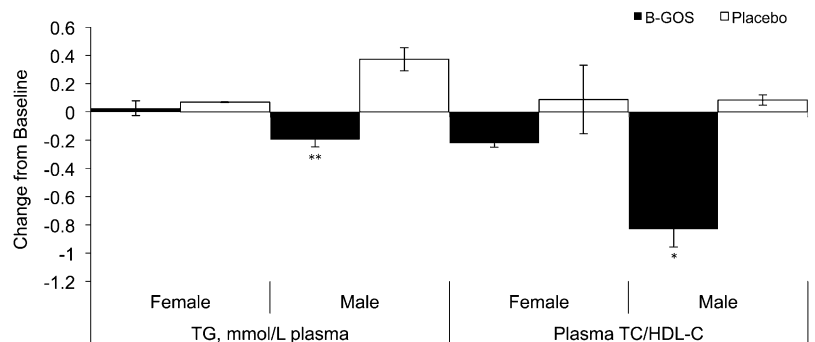
The present study assessed the effects of a novel *trans*-galactooligosaccharides mixture (B-GOS), compared with a placebo (maltodextrin), on major groups of bacteria found in the feces, immune function, and markers of metabolic syndrome in overweight adults. To the best of our knowledge, this is the first study that has looked at the effect of an NDO on such components in overweight humans. B-GOS, at the daily intake used in this study, has previously been shown to exert a significant and specific prebiotic effect in various human populations such as healthy adults, sufferers of irritable bowel syndrome, and older adults (16,40,41). Upon the ingestion of B-GOS, the selective fermentation of this prebiotic within the human gut results in a significant increase in the number of bifidobacteria in the feces at the expense of less beneficial groups of Gram-negative bacteria such as bacteroides and *Desulfovibrio* spp.; these phenomena were observed in the present study.

In addition, B-GOS (5.5 g/d) was previously shown to exert significant positive effects upon the immune system of older adults, as evidenced by an improvement in phagocytosis and NK cell activity, decreased secretion of proinflammatory cytokines (IL-6, IL-1β, and TNFα), and increased secretion of antiinflammatory IL-10 (16). In the present study, we did not observe any significant changes in the concentrations of measured cytokines, possibly due to the type of diet and amount of food ingested by the volunteers, which could affect the time it takes to modulate this type of the immune response. It seems that in the case of the majority of biomarkers measured in this study, the effect of the prebiotic was not significant until the end of the 12-wk intervention period. With respect to modulation of the gut microbiota, although the effect of B-GOS was significant after 6 wk, it was relatively small compared with what was previously reported in younger and older adults fed the prebiotic over a shorter time period (16,41). It could therefore be possible that in overweight adults, due to their dietary habits, modulation of the overall gut environment, including the immune response, takes longer than anticipated. However, significantly lower concentrations of fecal calprotectin (marker of intestinal inflammation) and plasma CRP were observed following the administration of B-GOS compared with placebo. Furthermore, an increase in levels of fecal sIgA, primarily involved in mucosal immunity and protection of barrier function and against infection, was also observed after the B-GOS intervention in the present study. The data therefore suggest that B-GOS positively influences the immune response in overweight adults at mucosal and systemic levels.

The exact mechanisms by which bifidobacteria exert their positive effects in the human gut are not fully understood. However, a number of different modes of action have been demonstrated in studies in rodent models. Administration of bifidobacteria to rodents has been shown to lead to improved barrier function, improved immune response, and a reduction in inflammatory compounds such as intestinal LPS (exposure to which can lead to metabolic endotoxemia) (42–45).

Animal studies and human feeding trials have shown that administration of FOS is effective in lowering BMI, waist circumference, total energy intake, and glucose and insulin concentrations (13,46,47). In the present study, with the exception of insulin, we did not find any significant effects on these measurements nor on BP; significantly lower concentrations of insulin were detected upon the administration of B-GOS. The observed differences of these variables after FOS and B-GOS interventions can be attributed to the differences in the chemical structures of the 2 prebiotics, and thus the way they are utilized by the gut microbiota, the metabolites, and/or interactions of which will influence systemic responses.

Prebiotics and probiotics have also been shown to effectively improve lipid profiles. Whereas animal models suggest the efficacy of these functional foods in lowering TC, TG, and LDL-C concentrations and improving HDL-C, results obtained in humans are variable. Some studies have reported improved lipid profiles through administration of either probiotics (48) or inulin (49), whereas others have shown no such effect (50,51). Most studies reporting decreased concentrations of plasma lipids and/or increased HDL-C following either probiotic or prebiotic administration have used hypercholesterolemic subjects. Our previous study with older subjects who had normal TC concentrations showed that B-GOS did not affect TC or HDL-C (16). However, in the present study, compared with placebo, B-GOS significantly reduced TC, TG, and TC:HDL-C ratios while having no effect on LDL-C and HDL-C concentrations in overweight subjects. Therefore, B-GOS administration is effective in lowering plasma lipids in a population where the majority of subjects are hypercholesterolemic. Although the exact mechanisms by which probiotics and prebiotics modify plasma lipids are unknown, a number of modes of action have been proposed, all of which point toward the involvement of a beneficial microbiota in improving plasma lipid profiles. These include enzymatic deconjugation of bile salts by bacteria, cholesterol binding in the small intestine, incorporation of lipids into bacterial cellular membranes during growth, conversion into coprostanol and fecal excretion, and inhibition of cholesterol synthesis in the liver through the production of SCFAs (52).

In conclusion, this study has shown that B-GOS administration to overweight adults with metabolic syndrome leads to a decrease in the number of Gram-negative, less beneficial bacteria in the fecal microbiota and an increase in the number of beneficial, Gram-positive bifidobacteria. This shift in the microbiota may be responsible for the significant positive effects on the immune response that we have observed, as evidenced by improvements in blood and fecal inflammatory markers (i.e., CRP and calprotectin, respectively) and increased secretion of fecal sIgA. Furthermore, there were significant effects on some metabolic syndrome markers, namely insulin, TC, TG, and the TC:HDL-C ratio, following the administration of B-GOS. Therefore, dietary intervention using B-GOS is not only an attractive option for enhancement of both the gastrointestinal and immune systems in overweight individuals, but it is also potentially beneficial in reducing some of the markers of metabolic syndrome independent of other lifestyle changes, which could be of particular importance in ameliorating the disorders associated with metabolic syndrome.

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