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Gut microbiome composition is linked to whole grain-induced immunological improvements

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Associated Data

Supplementary Materials

Abstract

The involvement of the gut microbiota in metabolic disorders, and the ability of whole grains to affect both host metabolism and gut microbial ecology, suggest that some benefits of whole grains are mediated through their effects on the gut microbiome. Nutritional studies that assess

the effect of whole grains on both the gut microbiome and human physiology are needed. We conducted a randomized cross-over trial with four-week treatments in which 28 healthy humans consumed a daily dose of 60 g of whole-grain barley (WGB), brown rice (BR), or an equal mixture of the two (BR+WGB), and characterized their impact on fecal microbial ecology and blood markers of inflammation, glucose and lipid metabolism. All treatments increased microbial diversity, the Firmicutes/Bacteroidetes ratio, and the abundance of the genus *Blautia* in fecal samples. The inclusion of WGB enriched the genera *Roseburia*, *Bifidobacterium* and *Dialister*, and the species *Eubacterium rectale*, *Roseburia faecis* and *Roseburia intestinalis*. Whole grains, and especially the BR+WGB treatment, reduced plasma interleukin-6 (IL-6) and peak postprandial glucose. Shifts in the abundance of *Eubacterium rectale* were associated with changes in the glucose and insulin postprandial response. Interestingly, subjects with greater improvements in IL-6 levels harbored significantly higher proportions of *Dialister* and lower abundance of Coriobacteriaceae. In conclusion, this study revealed that a short-term intake of whole grains induced compositional alterations of the gut microbiota that coincided with improvements in host physiological measures related to metabolic dysfunctions in humans.

Keywords: inflammation, gut microbiota, metabolic disorders, whole grains

Introduction

Obesity is associated with an increased risk in cardiovascular disease, type-2 diabetes, non-alcoholic fatty-liver disease and some cancers, and constitutes a major health concern worldwide (Cornier *et al.*, 2008; Hu, 2011). A diet high in whole grains and dietary fibers has been shown to improve metabolic parameters related to these metabolic disorders (Liu *et al.*, 1999; Fung *et al.*, 2002; Liu *et al.*, 2003; Murtaugh *et al.*, 2003; Jensen *et al.*, 2004; Nettleton *et al.*, 2008). The mechanisms responsible for the benefits of whole grains are not completely understood. It has been proposed that the dietary fiber present in whole grains increases the viscosity of the digesta and binds to bile acids in the small intestine, thus contributing to decreased sugar and lipid (cholesterol) absorption (Behall *et al.*, 2004; Alminger and Eklund-Jonsson, 2008). In addition, phytochemicals and other bioactive compounds in whole grains might provide metabolic benefits (Adom and Liu, 2002; Nilsson *et al.*, 2006; Harris and Kris-Etherton, 2010). Furthermore, the metabolic inflammation associated with obesity and related diseases is now considered to trigger metabolic dysfunctions (Gregor and Hotamisligil, 2011), and the benefits of whole grains might be due to an anti-inflammatory action (Nilsson *et al.*, 2008b; Rosén *et al.*, 2011). In this respect, bacterial fermentation of undigestible constituents of whole grains in the gastrointestinal tract has been suggested to be partly responsible for the benefits of whole grains (Nilsson *et al.*, 2008a; North *et al.*, 2009; Harris and Kris-Etherton, 2010).

A consideration of the gut microbiome in the context of the health effects of whole grains has become especially relevant in light of recent research that indicated an etiological role of gut bacteria in metabolic disorders. Obesity and type 2 diabetes have been linked to alterations in the intestinal microbiota in both the humans and animal models (Ley *et al.*, 2006; Turnbaugh *et al.*, 2006; Cani *et al.*, 2007; Larsen *et al.*, 2010; Vijay-Kumar *et al.*, 2010). If these aberrations contribute to human disease is still unclear, but pathophysiological indicators are reduced in

animal models when animals are kept germ-free or when treated with antibiotics, and manifestations of disease can be transmitted through the gut microbiota (Ley *et al.*, 2005; Cani *et al.*, 2008; Vijay-Kumar *et al.*, 2010; Henao-Mejia *et al.*, 2012). Proposed mechanisms by which microbiota contribute to metabolic aberrations are the induction of lipolysis leading to increased fat storage (Bäckhed *et al.*, 2007), hepatic *de-novo* synthesis of triglycerides (Bäckhed *et al.*, 2004) and the alteration of bile acid metabolites with consequences to lipid metabolism in the host (Claus *et al.*, 2011). Furthermore, the gut microbiome might exacerbate the systemic inflammation associated with obesity and related metabolic disorders (Hotamisligil, 2006; Ding *et al.*, 2010), possibly through the induction of endotoxemia driven by lipopolysaccharide translocation through the intestinal epithelium (Cani *et al.*, 2007; Amar *et al.*, 2008; Cani *et al.*, 2008; Li and Hotamisligil, 2010).

The interplay between the gut microbiota and host metabolism and the ability of whole grains to affect both of these aspects suggest that one mechanism by which whole grains confer their benefits might be through a modulation of the gut microbiome. Recent research has revealed that the composition and metabolism of the gut microbiota can be modulated through prebiotics and fiber (Flint *et al.*, 2007; Louis *et al.*, 2007), and these carbohydrates have been shown to improve metabolic markers in experimental models (Cani *et al.*, 2007; Neyrinck *et al.*, 2011). Despite these encouraging findings, human studies that investigate the effects of whole grains and cereal fibers on host metabolism have neglected, until now, to characterize the gut microbiome and explore its potential contribution to health improvements (Tilg and Kaser, 2011). In addition, although the effect of fiber on the gut microbiota has been recently studied in experimental animals (Neyrinck *et al.*, 2011; Van den Abbeele *et al.*, 2011), information on how whole grains impact human gut microbiome composition is lacking.

The aims of this study were to characterize the impact of the incorporation of whole grains to an otherwise unrestricted diet on gut microbial ecology in healthy human subjects, and to investigate whether a connection with metabolic and immunological improvements exists. For this purpose, we performed a human crossover study with three four-week whole grain treatments, and collected fecal and blood samples at baseline and at the end of each treatment. The effect of whole grains on fecal microbiota composition was characterized by pyrosequencing of 16S rRNA gene tags, and inflammatory and metabolic markers related to metabolic dysfunctions in humans were measured in blood samples. The molecular characterization of fecal microbiota in parallel to host phenotyping allowed an investigation of associations between diet-induced metabolic changes and shifts in the gut microbiome.

Materials and methods

Subjects

The human trial was approved by the Institutional Review Board of the Kansas State University (IRB Approval Number: 5298), and written informed consent was obtained from all subjects. Healthy participants (see Supplementary materials for inclusion/exclusion criteria) were recruited through leaflets distributed on-campus by the College of Human Nutrition at the

Kansas State University, Manhattan, KS. Twenty-eight participants, 17 females and 11 males (age 25.9±5.5 years), took part in the study.

Test meals

Whole grain Prowashonupana Barley (Sustagrain Barley Quick Flakes, ConAgra Mills, Omaha, NE, USA) and whole-grain brown rice (Insta Grains Brown Rice Flakes, Briess, Chilton, WI, USA) flakes were used in this study. Three test meals with different amounts of total dietary fiber were included: a barley treatment (WGB), consisting of 60 g of barley (18.7 g total dietary fiber); a brown rice and barley treatment (BR+WGB), consisting of 30 g each barley and BR (11.5 g total dietary fiber); and a BR treatment, consisting of 60 g of BR (4.4 g total dietary fiber). Subjects were provided with individual bags containing a daily dose of the corresponding treatment (60 g of flakes). Nutritional information of the whole-grain flakes used in the study is available in the Supplementary materials and Supplementary Table S1.

Study design

The study was conducted as a randomized crossover trial over 17 weeks (Figure 1). The first week served as a baseline period, after which each subject underwent three four-week dietary treatments (BR, BR+WGB, WGB) in random order, and interspaced by two-week washout periods. The study was conducted under free-living conditions, and no dietary restrictions were imposed except that subjects were expected to be non-vegetarian. Subjects were instructed to consume the 60 g of flakes daily either plain, with yogurt or with milk, without time restrictions. Weekly symptom diaries were completed by the subjects in which they self-reported bowel movement, discomfort, flatulence, bloating, stool consistency and general well-being on a scale from 1 to 5 (1 being optimal/normal and 5 worst/abnormal).

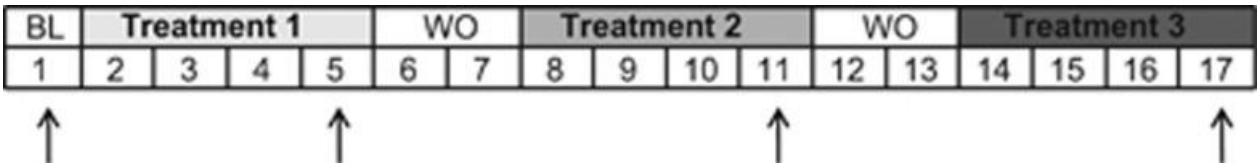

Figure 1 Experimental design. Time line of the randomized crossover trial. Three four-week dietary treatments were assessed in succession. The treatments were interspaced by two-week washout (WO) periods. Blood and stool samples (indicated by arrows) were collected during the baseline (BL) and at the end of each treatment period.

Subject parameters and determination of metabolic and immunological markers

Subject parameters were measured at the Human Metabolism Laboratory at Kansas State University. Total body composition was assessed at baseline with dual-energy X-ray absorptiometry (Prodigy GE-Lunar, GE, Waukesha, WI, USA). Blood samples were drawn at baseline and at the end of each dietary treatment after a 12 h overnight fast. An initial blood sample was drawn (time 0). A standard drink containing 75 g of glucose (Fisher Scientific,

Pittsburg, PA, USA) was consumed within 10 min, and blood samples were collected at 15, 30, 45, 60, 90 and 120 min for the determination of postprandial glucose and insulin responses. Blood was immediately placed in tubes containing K_2-EDTA (Vacutainer, BD, Franklin Lakes, NJ, USA) and centrifuged at 1000–1500*x g* for 13 min at 5–10 °C. Aliquots of plasma were transferred into tubes for storage at −80 °C until further testing.

Glucose and insulin were measured in plasma samples in duplicate using an automated analyzer (YSI 2300, YSI Life Sciences, Yellow Springs, OH, USA) and the Human Gut Hormone Immunoassay kit (Milliplex, EMD Millipore, Billerica, MA, USA) with a dual laser flow cytometer (Luminex, EMD Millipore, Billerica, MA, USA), respectively. A lipid profile, consisting of total cholesterol, high-density lipoprotein (HDL) and non-HDL cholesterol was performed on the preprandial samples (time 0) using the Cholestech LDX System (Alere, Waltham, MA, USA). Three markers of inflammation were measured in plasma samples by enzyme-linked immunosorbent assays (in duplicate): lipopolysaccharide-binding protein (LBP) (USCN Life Science and Technology, Huston, TX, USA), high-sensitive C-reactive protein (hs-CRP) (Symansis, Timaru, New Zealand), and interleukin 6 (IL-6) (R&D Systems, Minneapolis, MN, USA).

Short-chain fatty acids were quantified in fecal samples by gas chromatography as described in the Supplementary materials.

Compositional analysis of the fecal microbiota by pyrosequencing

Despite the fact that fecal samples represent microbial communities that are shed from the gut and not resident, they provide a good overview over the microbiota present in the distal colon, and are the most practical samples that can be obtained from subjects participating in nutritional trials. Subjects provided fecal samples within 24 h of blood sampling and 2 h of defecation. Fecal material and 1:10 fecal homogenates in phosphate-buffered saline (pH=7) were immediately frozen (−80 °C) and stored until further processing. Bacterial DNA was extracted from fecal homogenates as described by Martínez *et al.* (2010), using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) in combination with enzymatic and mechanical cell lysis. Pyrosequencing of amplicons obtained by PCR with universal primers targeting the V1–V3 region of the 16 S rRNA gene was performed as previously described (Martínez *et al.*, 2010), using the 454 Genome Sequencer FLX with GS FLX Titanium series reagents at the Core for Applied Genomics and Ecology (University of Nebraska). Sequences obtained during this study are deposited in the MG-RAST server under the accession numbers 4498555.3, 4498556.3 and 4498557.3.

Sequence processing was performed combining features of QIIME (Caporaso *et al.*, 2010) and the Ribosomal Database Project pipeline (Cole *et al.*, 2009). Three-thousand quality-controlled sequences per sample were randomly selected and used for taxonomic classification. Sequences were assigned to a bacterial phylum, family and genus using the Classifier tool of the Ribosomal Database Project (Wang *et al.*, 2007). In addition, sequences were assigned to operational taxonomic units with 97% sequence homology as described in the Supplementary materials. Chao1 species richness estimator, and Shannon's and Simpson's (defined as 1-Dominance) diversity indices were computed with QIIME.

Query for genes encoding β-glucanases in genomes of human gut microbes

Bacterial genomes available in the Joint Genome Institute database were used to identify large-bowel associated bacteria with β-glucanase encoding activity. The integrated microbial genomes platform of Joint Genome Institute was used to conduct this survey. A list of the strains included in the survey is presented in the Supplementary materials. For the species identified to contain β-glucanase genes, their abundance in the fecal microbiota of our subjects was quantified by BLASTn.

Statistics

Results are presented as means±s.d. Differences in bacterial taxa and host phenotypes among treatments were determined by one-way analysis of variance with repeated measures in combination with Tukey's *post-hoc* tests, and $P<0.05$ was considered statistically significant. If the data were not normally distributed, values were subjected to transformations such as square root or logarithm with base 10 to achieve normality. If normality could not be achieved through transformations, the non-parametric Kruskal-Wallis test was performed. When only two groups of data were compared, Student's *t*-tests were performed. Correlations between host parameters and bacterial populations were assessed by Pearson's correlation tests using GraphPad Prism version 5.00 (GraphPad Software, La Joya, CA, USA). Associations between inflammatory markers and gut microbiome composition were also analyzed through linear models using SAS (SAS Institute Inc., Cary, NC, USA). Additional information on the statistical methods can be found in the Supplementary materials.

Results

Physiologic, metabolic and microbiome characteristics of the study population

Twenty-eight volunteers, 11 males and 17 females, participated in the nutritional trial, and subjects' parameters are presented in Table 1. Based on percent body fat, 13 subjects were classified as overweight, using as cutoff values >31% body fat for women and >25% for men. This Metabolic and immunological markers included in the study were plasma fasting glucose and insulin levels, glycemic and insulin postprandial response, a lipid panel (total cholesterol, HDL, non-HDL), and inflammatory markers (hs-CRP, IL-6 and LBP). The rationale for the inclusion of these markers is their suitability in determining the progression of metabolic aberrancies and the risk of cardiovascular disease (Schumann *et al.*, 1990; Spranger *et al.*, 2003; Cardellini *et al.*, 2005; Ridker, 2009), and their association with obesity (Sun *et al.*, 2010). Accordingly, positive correlations between body fat and all three inflammatory markers were observed (Figures 2a–c). LBP and hs-CRP were highly correlated ($r=0.90$, $P<0.0001$) (Figure 2d). The linear model identified body fat as a significant factor affecting IL-6 ($P<0.01$), hs-CRP ($P<0.0001$) and LBP ($P<0.0001$). Furthermore, significant positive correlations existed between IL-6 and postprandial glucose response (Figure 2a). Together, these associations substantiate the

link between adiposity, a low-grade systemic inflammation, and glucose metabolism (Hotamisligil, 2006).

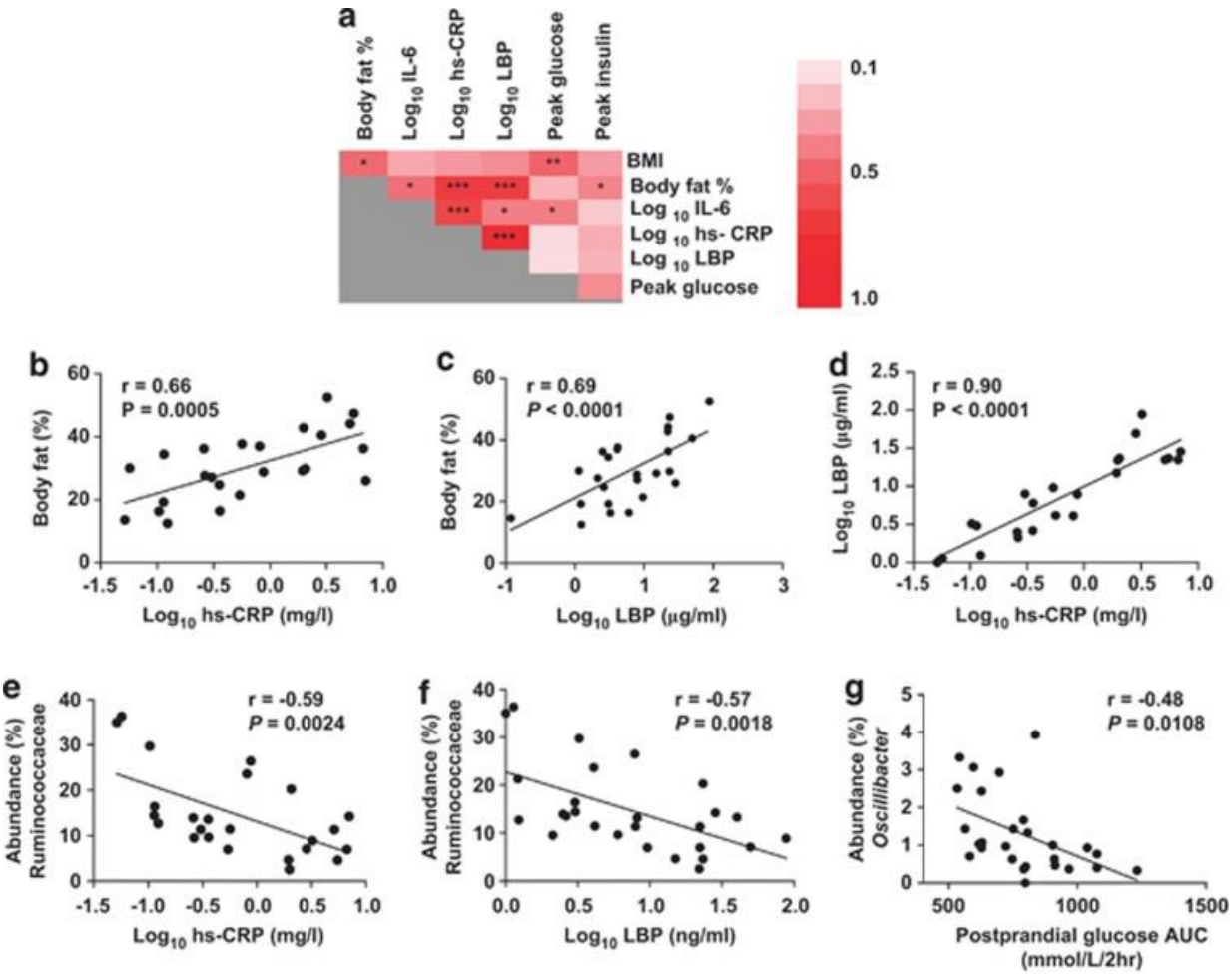

Figure 2

Associations among host physiological characteristics and their correlation with bacterial populations in fecal samples at baseline. Heatmap displaying correlation coefficients between metabolic and physiological parameters of the study population at baseline (**a**). Correlations between hs-CRP with body fat (**b**), LBP with body fat (**c**), hs-CRP and LBP (**d**), hs-CRP and Ruminococcaceae (**e**), LBP with Ruminococcaceae (**f**) and *Oscillibacter* with postprandial AUC glucose (**g**). Pearson's correlation (*r*) and the corresponding *P*-values are presented.

Table 1. Baseline characteristics of the 28 subjects, and differentiated by gender and percent body fat (values are presented as mean±s.d.)

	Overall	*Gender*			*Body fat*[a]		
	***All subjects* (n=*28*)**	***Male* (n=*11*)**	***Female* (n=*17*)**	***P-value***	***Overweight* (n=*13*)**	***Normoweight* (n=*15*)**	***P-value***
Age	25.9±5.4	26.7±5.4	25.4±5.8	NS	28.6±6.6	23.6±3.0	< 0.05
Weight (kg)	72.3±18.3	87.7±17.1	62.3±10.5	< 0.001	79.7±19.5	65.9±14.9	< 0.05
BMI ($kg\ m^{-2}$)	25.1±4.5	27.4±4.8	23.6±3.7	< 0.05	27.9±4.4	22.7±3.0	< 0.001
Body fat mass (kg)	20.8±10.3	20.2±11.6	21.2±9.8	NS	29.7±8.1	13.1±3.6	< 0.001
Body fat (%)	29.6±11.0	22.8±8.5	34.0±10.8	< 0.01	39.2±7.4	21.3±6.3	< 0.001
Cholesterol ($mmol\ l^{-1}$)							
Total cholesterol	4.86±1.12	4.07±0.69	5.22±1.67	< 0.01	4.76±1.26	4.75±1.07	NS
Non-high-density lipoprotein	3.13±1.03	2.78±0.74	3.29±1.38	NS	3.23±1.21	2.94±0.85	NS
High-density lipoprotein	1.65±0.42	1.30±0.28	1.84±0.57	< 0.001	1.53±0.45	1.65±0.42	NS
Fasting plasma glucose ($mmol\ l^{-1}$)	5.17±0.74	5.14±0.72	5.15±1.42	NS	5.44±0.93	4.94±0.40	NS
Fasting plasma insulin ($\mu lU\ ml^{-1}$)	43.44±18.86	42.76±20.55	44.93±21.14	NS	49.77±19.92	40.34±18.38	NS
Inflammatory markers							
IL-6 ($pg\ ml^{-1}$) (min–max)	1.75±1.43 (0.06–5.17)	1.18±0.81 (0.33–2.59)	2.01±1.60 (0.06–5.17)	NS	2.29±1.34 (0.65–4.89)	1.28±1.33 (0.06–5.17)	NS
Hs-CRP ($mg\ l^{-1}$) (min–max)	1.69±2.24 (0.002–7.039)	0.32±0.22 (0.052–0.805)	2.38±2.46 (0.002–7.039)	< 0.01	2.47±2.36 (0.115–6.696)	0.97±1.87 (0.002–7.039)	NS
LBP ($\mu g\ ml^{-1}$) (min–max)	15.09±19.85 (0.12–88.05)	4.42±3.03 (1.00–9.61)	20.91±22.41 (0.12–88.05)	< 0.01	23.98±24.90 (2.12–88.05)	7.48±8.60 (0.12–28.51)	NS

Abbreviations: hs-CRP, high-sensitive C-reactive protein; IL, interleukin; LBP, lipopolysaccharide-binding protein; NS, not significant.

[a]Women with over 31% body fat, and men with over 25% body fat were considered as overweight individuals. All others were considered lean.

Pyrosequencing revealed that the baseline fecal microbiota of the participants was dominated by the phyla Firmicutes and Bacteroidetes, with lower proportions of Verrucomicrobia and Actinobacteria, in agreement with previous molecular characterizations of the human fecal microbiota (Ley *et al.*, 2006; Martínez *et al.*, 2010). We investigated whether associations between host phenotypes and microbial populations existed (Supplementary Figure S1). No significant correlation was observed between any bacterial group and body fat or BMI, although overweight subjects harbored significantly lower abundances of Ruminococcaceae (10.8±5.4% versus 17.9±9.9%, $P<0.05$) and *Faecalibacterium* (1.8±1.8% versus 3.7±2.5%, $P<0.05$). The analysis revealed negative correlations between the family Ruminococcaceae and all the three inflammatory markers at baseline (Figures 2e and f, and Supplementary Figure S1). Within this family, the genera *Faecalibacterium* and *Ruminococcus* displayed negative correlations with hs-CRP ($r=-0.48$, $P<0.05$, and $r=-0.60$, $P<0.01$, respectively). The analysis also revealed a negative association between *Oscillibacter* and postprandial glucose area under the curve (Figure 2f). Regarding the markers of lipid metabolism, proportions of Bacteroidetes, Bacteroidaceae and *Bacteroides* were positively correlated to plasma HDL values ($r=0.54$, $P<0.05$; $r=0.56$, $P<0.05$; $r=0.56$, $P<0.05$; respectively) (Supplementary Figure S2).

Effects of whole grains on fecal microbial communities

Sequence data obtained by pyrosequencing were used to establish the effects of whole grains on the gut microbiota composition. This analysis revealed that whole grains had a measurable effect on gut microbiota composition. All three treatments significantly increased the bacterial diversity measured by Shannon's and Simpson's indices but not by Chao1 (Supplementary Figure S3). These results indicated an increase in community evenness (Shannon's and Simpson's), but not in total species richness (Chao1).

In accordance to previous studies that assessed the effect of diet on the gut microbiome (Martínez *et al.*, 2010; Davis *et al.*, 2011), substantial inter-individual variation was observed in response to whole grains (Supplementary Table 2). Despite this variability, several diet-induced shifts reached statistical significance in the entire study population. The proportion of the phylum Firmicutes increased, while Bacteroidetes were reduced (Table 2). The decrease in Bacteroidetes was largely caused by a reduction of the genus *Bacteroides* (Table 2).

Table 2

Abundance of dominant bacterial taxa (% of total microbiota) in fecal samples as determined by 454 pyrosequencing (values are presented as mean±s.d.)

	Baseline	***BR***	***BR+WGB***	***WGB***	**P-*value***	***Confirmation by linear model***
Phylum						

	Baseline	***BR***	***BR+WGB***	***WGB***	**P-*value***	***Confirmation by linear model***
Firmicutes	57.30±14.13	65.06±11.40[a]	65.53±10.64[a]	65.42±12.05[a]	0.003	Yes
Bacteroidetes	37.99±14.35	30.74±11.62[a]	29.85±11.93[a]	30.32±12.22[a]	0.01	Yes
Verrucomicrobia	1.82±1.98	1.34±1.53	0.68±0.80	0.59±0.80	NS	Yes
Actinobacteria	1.24±0.97	1.42±1.78	2.23±3.32	2.05±2.73	NS	Yes
Family						
Bacteroidaceae	28.55±15.73	22.89±10.37	21.19±11.87[a]	23.48±12.62	0.013	Yes
Lachnospiraceae	22.21±7.90	22.62±7.91	23.11±6.56	22.65±7.63	NS	Yes
Ruminococcaceae	14.64±8.76	17.32±8.90	16.53±8.06	15.82±8.32	NS	Yes
Incertae Sedis XIV	5.79±3.15	7.63±4.47	8.16±3.97[b]	8.62±4.32[b]	0.001	Yes
Porphyromonadaceae	3.40±3.07	2.69±3.42	2.76±3.10	1.95±1.55[a]	0.022	No
Prevotellaceae	2.97±9.24	2.34±6.56	3.59±10.10	2.39±6.50	NS	Yes
Verrucromicrobiaceae	1.85±4.58	0.77±1.53	0.68±1.28	0.59±0.80	NS	Yes
Rikenellaceae	1.77±2.09	1.68±1.85	1.12±1.06	1.35±1.68	NS	Yes
Veillonellaceae	1.59±1.13	1.52±1.19	1.86±1.19	1.97±1.60	NS	Yes
Genus						
Bacteroides	28.55±15.73	22.89±10.37	21.19±11.87[a]	23.48±12.62	0.022	Yes
Blautia	5.68±3.15	7.61±4.47	8.14±3.97[b]	8.61±4.32[b]	0.001	Yes
Ruminococcus	4.20±4.91	5.35±5.05	4.171±5.75	3.46±4.32	NS	Yes
Faecealibacterium	2.82±2.38	3.06±2.29	3.86±3.22	3.86±3.19	NS	Yes
Prevotella	2.79±8.89	1.99±6.24	3.34±9.84	2.02±6.30	NS	Yes
Dorea	2.59±2.01	3.12±2.22	3.08±1.80	2.75±1.86	NS	Yes
Parabacteroides	2.58±3.05	2.06±3.23	2.10±3.14	1.59±1.44	NS	Yes
Roseburia	1.98±1.35	1.70±1.25	2.42±1.58	3.06±2.91[e]	0.01	Yes

	Baseline	***BR***	***BR+WGB***	***WGB***	**P-*value***	***Confirmation by linear model***
Akkermansia	1.85±4.58	0.77±1.53	0.68±1.28	0.59±0.80	NS	Yes
Coprococcus	1.82±2.09	1.91±2.08	1.47±2.22	1.35±1.78	NS	Yes
Alistipes	1.76±2.08	1.67±1.85	1.11±1.05	1.34±1.67	NS	Yes
Oscillibacter	1.27±1.04	1.24±1.00	1.08±0.83	0.96±0.61	NS	Yes
Bifidobacterium	0.99±1.88	1.02±1.64	1.95±3.16	1.84±2.54[d]	0.011	No
Subdoligranulum	0.94±1.03	1.17±1.43	1.42±1.73	1.09±1.02	NS	Yes
Dialister	0.75±1.17	0.60±0.89	0.94±1.21	1.14±1.69[d]	0.027	No
Odoribacter	0.26±0.24	0.28±0.35	0.28±0.41	0.15±0.18[b]	0.002	No
Operational Taxonomic Units (OTU number, closest hit in database, % identity with 16S rRNA gene)						
1737, *Odoribacter splanchnicus*, 99%	0.15±0.14	0.13±0.18	0.15±0.24	0.07±0.10[b]	0.001	No
679, Eubacterium rectale, 94%	0.25±0.32	0.31±0.42	0.43±0.57	0.57±0.63[b,e]	< 0.0001	Yes
956, *Roseburia faecis*, 99%	0.12±0.17	0.06±0.07	0.26±0.31	0.53±0.92[b,f]	< 0.0001	Yes
770, *Roseburia intestinalis*, 100%	0.09±0.12	0.04±0.05	0.17±0.18[d]	0.30±0.42[a,f]	< 0.0001	Yes
3, *Blautia wexlerae*, 100%	1.07±0.78	1.58±1.11	1.49±0.98	1.82±1.14[c,g]	< 0.0001	Yes
179-188, *Blautia spp*.	1.81±1.13	2.38±1.69	2.75±1.75[a]	2.80±2.04[b]	0.006	Yes
44-19-1999-93, *Eubacterium rectale*, 98%	2.48±2.67	2.75±3.27	3.65±3.45	4.83±3.98[a,e,g]	0.001	Yes

Open in a separate window

Abbreviations: BR, brown rice; NS, not significant; OUT, operational taxonomic unit; WGB, whole grain barley.

Significantly different to baseline: [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$.

Significantly different to BR: [d]$P<0.05$, [e]$P<0.01$, [f]$P<0.001$.

Significantly different to BR+WGB: [g]$P<0.05$.

The increase in Firmicutes was more comprehensive and shifts in the abundance of several taxa were detected. All three dietary treatments increased the abundance of the genus *Blautia* and two operational taxonomic units within this genus (Table 2), although significance was only achieved when WGB was included in the treatment. Several compositional shifts were strictly associated with the consumption of WGB, namely the genera *Roseburia*, *Bifidobacterium* and *Dialister* and the species *E. rectale*, *R. faecis* and *R. intestinalis* (Table 2), and many of these taxa increased gradually with WGB intake. The linear regression model confirmed all of these significant changes except for the species *Bifidobacterium*, and *Dialister*. Other taxa clearly responded to WGB, but because of inter-individual variation, these shifts did not reach statistical significance. For example, *Bacteroides coprocola*, which was only detected in three subjects, showed a 10-fold increase with WGB consumption in only two of the subjects (Supplementary Table S2). Although both whole grains led to an equivalent increase in the Firmicutes/Bacteroides ratio, no family or genus showed a significant increase for BR, suggesting that this test meal induced diverse alterations in the gut microbiome that are not consistent among subjects.

No significant differences were detected in the amounts of short-chain fatty acids for any of the treatments. It is possible that an increase in short-chain fatty acids could not be detected in fecal samples they are for the most part absorbed in the gastrointestinal tract (Millet *et al.*, 2010).

Distribution of *β*-glucanase genes in human gut microbes

WGB contains a high amount of β-glucans (14.1%), while none were detected in BR (Supplementary Table S1). In order to test if the ability to hydrolyze β-glucans could explain the specific shifts in the fecal microbiota induced through WGB, we investigated distribution of β-glucanase genes in 112 strains originating from the human gut. This analysis revealed that β-glucanase genes are present in a variety of gut bacterial species from a broad taxonomic range, including ten *Bacteroides*, four *Bifidobacterium*, three *Collinsella*, two *Clostridium*, two *Coprococcus*, two *Eubacterium*, one *Ruminococcus*, two *Roseburia*, and one *Akkermansia* species (Supplementary Table S2). Of these species, only *E. rectale*, *R. faecis* and *R. intestinalis* were significantly increased through WGB, indicating that the mere presence of β-glucanase encoding genes does not predict the changes in community composition in response to the diet.

Whole grain-induced metabolic and immunological changes

The daily consumption of 60 g of whole grains for 4 weeks improved immunological and metabolic markers in the human subjects. The findings for the entire study population are shown in Supplementary Table S3, and differentiated by gender and body fat in Supplementary Tables S4 and S5. A significant decrease in plasma IL-6 levels for the BR+WGB treatment versus baseline values was detected (Figure 3a). Quantitatively, this reduction was highest in overweight subjects (Figure 3b). In women, all three treatments significantly reduced IL-6 (Figure 3c). The linear model analysis confirmed the anti-inflammatory effect of whole grains and revealed a significant reduction of IL-6 for BR+WGB and WGB treatments ($P<0.01$,

P<0.05). Despite not achieving statistical significance due to high inter-individual variation, hs-CRP plasma levels were halved during the BR+WGB period compared with the baseline (Supplementary Tables S3-S5).

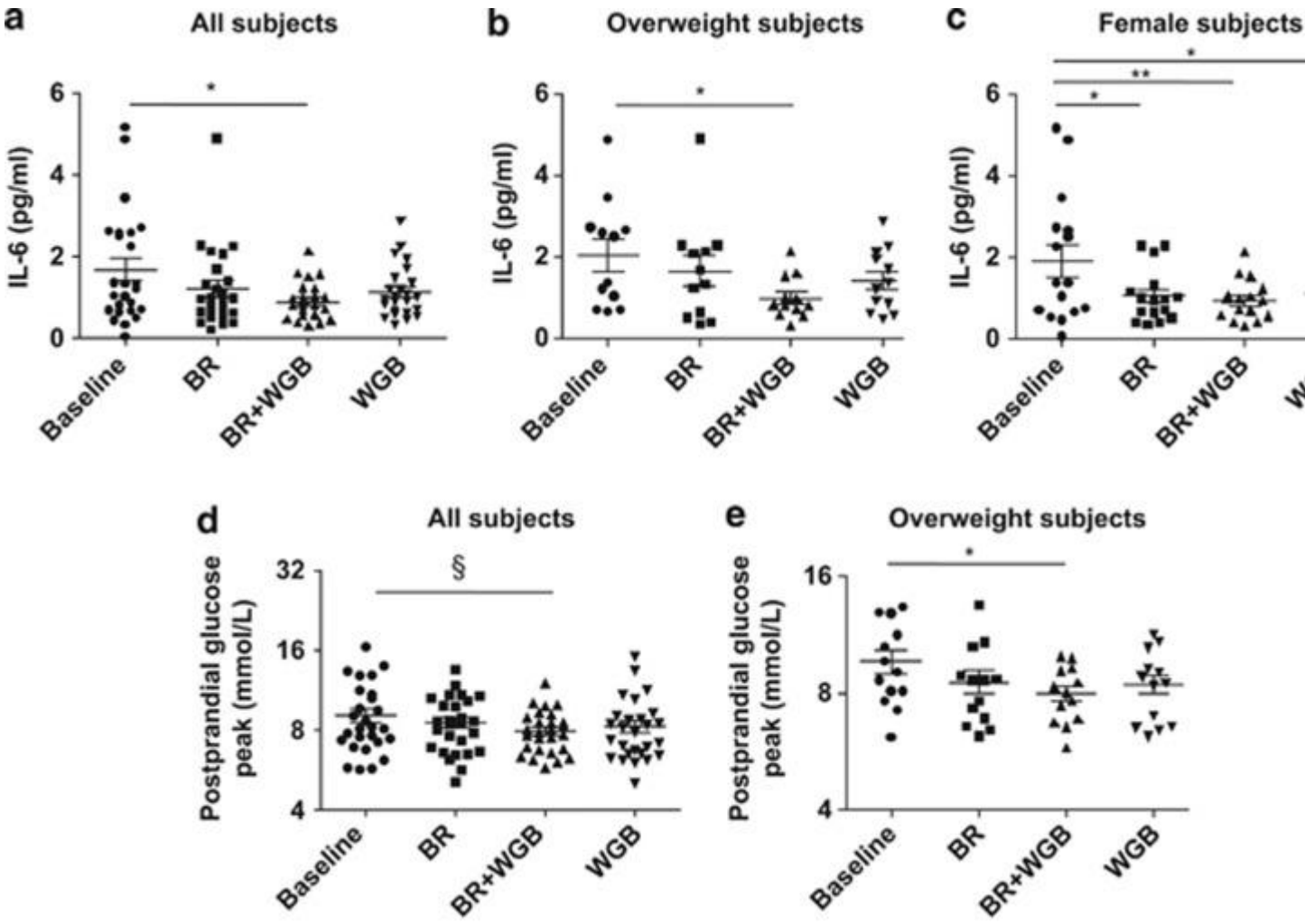

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Figure 3

Immunological and metabolic improvements induced through whole-grain consumption. Plasma IL-6 levels in the entire subject population (**a**), in overweight participants (**b**), and in females (**c**). Maximum postprandial glucose levels in the entire subject population (**d**) and overweight subjects (**e**) during the three treatments (BR, BR+WGB, WGB) and at baseline. **P*< 0.05, ***P*< 0.01, §*P*< 0.1.

Whole-grain consumption significantly improved glucose and lipid metabolism. Postprandial peak glucose levels were significantly lowered in overweight subjects during the BR+WGB period (*P*<0.05), and the reduction approached significance in the entire study population (*P*<0.1) (Figures 3d and e). Fasting glucose levels were significantly decreased in women and

overweight subjects, and in females, total cholesterol was significantly reduced (Supplementary Tables S4 and S5).

Links between whole grain-induced metabolic improvements and fecal microbial community structure

To determine whether effects of whole grains were related to the gut microbiome, a correlation analysis was performed between bacterial shifts and changes in the metabolic markers that occurred during the BR+WGB period. We focused the analysis on the BR+WGB treatment as it induced the most significant metabolic improvements (Figure 3). This analysis revealed that increases in the abundance of *E. rectale* were associated with improvements in the postprandial glucose and insulin response (Supplementary Figures S4A and SB). The association between *E. rectale* and maximum postprandial glucose levels approached significance (Supplementary Figure S4C).

In addition, we categorized subjects into the three groups (terciles) according to the magnitude of the improvements in IL-6, hs-CRP, fasting glucose and glucose peak through BR+WGB. The baseline proportions of the bacterial groups between the three groups were compared. This analysis revealed that the gut microbiota of subjects with the highest improvement in IL-6 (3rd tercile) contained significantly higher percentages of Veillonellaceae (Figure 4a), and within this family, the genus *Dialister* (Figure 4b). Conversely, Coriobacteriaceae were significantly decreased in subjects with the highest improvement in IL-6 (Figure 4c). No significant differences in microbiome composition were detected between the terciles generated for hs-CRP, fasting glucose and postprandial glucose peak.

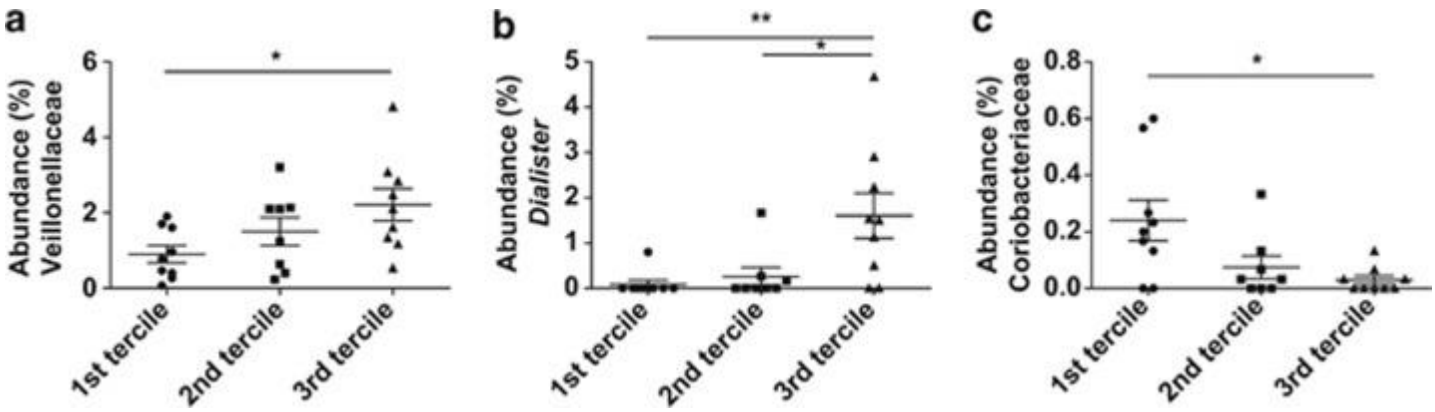

Figure 4

Abundance of specific taxa in subjects that showed differences in their IL-6 response to whole grains. Subjects were classified into terciles according to the magnitude of the change in plasma IL-6 levels induced by whole-grain consumption (BR+B treatment versus baseline). The proportions of bacterial taxa in fecal samples during the baseline were compared in the three terciles and significant differences existed in the proportions of Veillonellaceae (**a**), Dialister (**b**) and Coriobacteriaceae (**c**) in fecal samples during baseline. *P< 0.05, **P< 0.01.

Gastrointestinal symptoms

Self-reported symptoms diaries revealed that 60 g of WGB significantly increased all the gastrointestinal symptoms surveyed, especially flatulence, while 30 g caused only a slight increase in flatulence (Supplementary Table S6). The addition of BR to the diet did not result in any reported changes in symptoms.

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Discussion

The metabolic and immunological benefits of whole grains have been shown in various studies (Fung *et al.*, 2002; Behall *et al.*, 2004; Jensen *et al.*, 2004; Nilsson *et al.*, 2006, 2008b), and a contribution of the gut microbiome to these effects has been suggested (North *et al.*, 2009). However, the assessment of bacterial participation in these processes has been limited to hydrogen breath measurements, and the effects of whole grains on the gut microbiome structure have not been investigated. In this study, we showed that whole grains have a significant effect on the composition of the fecal microbiota that coincided with metabolic and immunological improvements in healthy human individuals.

All whole-grain test meals caused an increase in community diversity within the subjects, driven by an increase in evenness of bacterial species. Therefore, WGB and BR seem to differ in their effects on the gut microbiota when compared with prebiotics and dietary fibers, which have not been shown to increase community diversity (Martínez *et al.*, 2010; Davis *et al.*, 2011; Van den Abbeele *et al.*, 2011). These differences might be due to compositional complexity of whole grains, which contain a variety of carbohydrates, potentially affecting a wider scope of bacterial taxa. Interestingly, a higher microbial diversity in fecal samples was also observed in children from Burkina Faso, who consumed a diet high in whole grains, legumes and vegetables, when compared with Europeans (De Filippo *et al.*, 2010). In addition, weaning in human infants leads to a drastic increase in diversity likely caused by the incorporation of more diverse arrays of dietary carbohydrates (reviewed in Koropatkin *et al.*, 2012). Therefore, it appears that bacterial diversity in the gut can be increased by providing a broader range of undigestible substrates, and our findings showed that this can be achieved by intake of whole grains.

This study revealed shifts in the fecal microbiota that were induced by both BR and WGB, while others were specific to WGB intake. Both whole grains increased the Firmicutes/Bacteroidetes ratio and the abundance of the genus *Blautia*. The overall shift in microbiota structure in favor of an expansion of Firmicutes could be the result of an increased carbohydrate intake (Duncan *et al.*, 2008). However, in a previous study, we did not observe an increase in the Firmicutes/Bacteroidetes ratio with the consumption of crackers containing resistant starches (Martínez *et al.*, 2010), although the dose of carbohydrates and fiber in these crackers exceeded that of the whole-grain test meals. Interestingly, a decrease of *Bacteroides* was also shown to be associated with a long-term consumption of diets rich in whole grains, dietary fibers and vegetables in African children and US individuals (De Filippo *et al.*, 2010; Wu *et al.*, 2011). These and our findings suggest that other components included in whole grains and other plant-derived food products might influence community structure at the phylum level, specifically

decreasing Bacteroidetes. The reason for the increase in the genus *Blautia* through whole grains might be due to a syntrophic effect. *Blautia* species are acetogenic and might benefit from the production of hydrogen, which is a product of glycan fermentation, and, therefore, likely induced by whole grain consumption (Nakamura *et al.*, 2010; Koropatkin *et al.*, 2012).

We detected several bacterial taxa that displayed a specific increase with WGB, several with a clear dose response. This is likely due to its high content of β-glucans. Accordingly, the bacteria that specifically responded to WGB harbor genes encoding for β-glucanases and utilize the substrate *in vitro* (Hughes *et al.*, 2008; Tasse *et al.*, 2010). However, the *in vivo* findings cannot solely be explained based on functional and genomic attributes of community members, as *Bacteroides* species decreased during WGB consumption, but possess β-glucanase genes and can utilize β-glucans *in vitro* (Crittenden *et al.*, 2002; Tasse *et al.*, 2010; Zhao and Cheung, 2011). A possible explanation for the *in vivo* findings could entail preferences towards distinct β-glucan structures and molecular weights. The β(1–4) to β(1–3) linkage ratio in barley is 2.3-3, while *Bacteroides* species have been shown to especially possess β(1–3)-glucanase activity (Salyers *et al.*, 1977). Moreover, barley-derived β-glucan fractions of high molecular weight have also been shown to be poorly fermented by *Bacteroides* (Hughes *et al.*, 2008). However, previous human trials with prebiotics and resistant starches have also revealed that the ability of a species to utilize substrates *in vitro* does not predict population shifts *in vivo* (Martínez *et al.*, 2010; Davis *et al.*, 2011; Koropatkin *et al.*, 2012). Therefore, although the findings obtained suggest that β-glucans are the main cause for the shifts in composition induced by WGB, the exact mechanisms by which these changes are restricted to only a small number of taxa are likely to be due to competitive interactions.

A main objective of this study was to determine whether the effects of whole grains on the gut microbiome are associated with physiological benefits. The whole grains used in our study led to immunological and metabolic improvements, especially when BR+WGB was consumed. Plasma IL-6 was reduced, and a tendency for a decrease in plasma hs-CRP was detected. In addition to this anti-inflammatory effect, an improvement in the glycemic response during BR+WGB treatment was detected. Our findings are in agreement with previous research that established the immunological and metabolic benefits of whole grains (Casiraghi *et al.*, 2006; Kallio *et al.*, 2008; Nilsson *et al.*, 2008b; Rosén *et al.*, 2011). Most importantly, inflammation has been identified as a main cause of metabolic disorders (Hotamisligil, 2006), and the anti-inflammatory effect could provide a mechanism by which whole grains improve glucose metabolism.

The anti-inflammatory effect of whole grains might be mediated through its effect on the gut microbiota. A remarkable positive correlation between LBP and hs-CRP was identified in our study population, supporting a link between bacterial lipopolysaccharide and systemic inflammation. The associations of these markers with body-fat support the hypothesis that endotoxemia could contribute to obesity (Cani *et al.*, 2007; Delzenne and Cani, 2011). WGB led to an increase of bacterial taxa such as bifidobacteria and *Roseburia*, which have been suggested to affect immune/inflammatory and metabolic functions in animal models (Cani *et al.*, 2008; Neyrinck *et al.*, 2011). Although one could envision that these shifts might underlie the anti-inflammatory effect of whole grains, no significant correlations between these taxa and inflammatory markers were observed. However, shifts in the abundance of *E. rectale* induced through the BR+WGB diet correlated with decreased postprandial glucose and insulin responses.

This organism produces butyrate, which might contribute to the immunological benefits of whole grain consumption through its anti-inflammatory effects.

Interestingly, compositional differences at baseline were detected in the gut microbiome of subjects that differed in the magnitude of their anti-inflammatory response to whole grains. Subjects with the greatest reduction in plasma IL-6 concentration had significantly higher proportions of *Dialister* and a lower abundance of Coriobacteriaceae. These bacterial groups have been linked to chronic inflammation in previous studies. *D. invisus* and Coriobacteriaceae have been shown to be reduced and increased in patients with Crohn's disease and colitic mice, respectively (Clavel *et al.*, 2009; Würdemann *et al.*, 2009; Willing *et al.*, 2010; Joossens *et al.*, 2011). The association of *Dialister* and Coriobacteriaceae with IL-6 response suggests that these taxa may condition the capability of an individual to be immunologically responsive to whole grains.

Before the start of the treatments, associations between bacterial groups, inflammatory state and host metabolism were observed (Figure 3). Ruminococcaceae negatively correlated with markers of inflammation and were more dominant in normoweight individuals. In addition, Bacteroidetes positively correlated with HDL cholesterol. These observations could result from an impact of these taxa on host physiology, and these associations provide a rationale to develop dietary strategies that target Ruminococcaceae and Bacteroidetes to improve human metabolic and immunological functions. However, host physiology (inflammatory state, cholesterol/bile acid metabolism) might also shape the microbiome composition. If systemic inflammation and cholesterol metabolism impact levels of Ruminococcaceae and Bacteroidetes, respectively, then these interactions could explain the discrepancies related to an altered microbiome in obese versus normoweight individuals (Ley *et al.*, 2006; Duncan *et al.*, 2008; Schwiertz *et al.*, 2010). Not obesity per se, but the associated inflammatory and metabolic aberrations could shape microbiome composition and might cause variable and more complex patterns of dysbiosis.

This study has provided novel information about the relationship between whole grains, the gut microbiota and host metabolism. Whole grain-induced alterations in the characteristics and composition of the fecal microbiota coincided with immunological and metabolic benefits, and the clear associations between the reduction of IL-6 and the presence of certain bacterial taxa (*Dialister*, Coriobacteriaceae) indicate an important functional role of gut bacteria in the physiologic effects of whole grains.

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Acknowledgments

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Notes

The authors declare no conflict of interest.

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Footnotes

Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)

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Supplementary Information

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Supplementary Information

Materials and Methods

Subjects

Interviews were conducted with the volunteers to explain the protocol, determine whether they met the inclusion criteria, and record demographic data (age and gender). Exclusion criteria were treatment with antibiotics within 3 months prior to the beginning of the study or throughout its duration, being vegetarian, exercise of more than 2 h weekly, a history of a chronic gastrointestinal disorder, and the use of antihypertensive or lipid-lowering medications. Twenty-nine healthy adults were recruited to participate in this study. One female subject was excluded during the study as she required antibiotic treatment. Prior to the beginning of the study, training sessions were held to explain the protocol to the subjects.

Participants were instructed to incorporate the whole grains to their regular diet. Other instructions included withholding from strenuous physical activity and alcohol consumption on the day prior to blood drawing. Compliance with the dietary treatments was encouraged by meeting with the subjects on a weekly basis, on which occasions symptom diaries were collected and a bag with 7 daily portions of the treatment flakes were distributed.

Test meals

Prowashonupana (Sustagrain® Barley Quick Flakes, ConAgra Mills) is a waxy, hulless barley variety differing from standard barley in terms of its composition. Prowashonupana contains exceptionally high levels of total dietary fiber (30%), almost half being accounted for by β-glucan, and low levels of starch (<30%). Brown rice has high amounts of soluble starch (around 75%) and small amounts of total dietary fiber (around 7%). The processing of the barley flakes was as

follows, cleaned grain kernels were roller cut and steam treated at 100.5°C for 40 min to ensure microbiological safety and passed through flaking rolls to reduce the pieces to a thickness of 0.020 ± 0.002 inches. The flakes were then cooled down to room temperature, seized, screened and packaged. The brown rice (Insta Grains® Brown Rice Flakes, Briess) was used as provided by the manufacturer. It is currently unknown how the processing conditions of both whole grains affect their functionality when compared to the unprocessed grains.

Digestible and resistant starches in the two flakes were measured in the products (K-RSTAR, Megazyme, Ireland), as well as β-glucans (K-BGLU, Megazyme, Ireland), and total dietary fiber (Andersson et al., 2009; AACC International, 2011). The nutritional data of the flakes is presented in Table S1.

DNA extraction from fecal samples

Fecal homogenates were transferred to bead beating tubes (Biospec products, USA) containing zirconium beads (300 mg). Homogenates were centrifuged (8,000×g for 5 min at room temperature) and the bacterial cell pellets were washed twice by re-suspension in ice-cold PBS. 100 µl of lysis buffer (200 mM NaCl, 100 mM Tris, 20 mM EDTA, 20 mg/ml Lysozyme, pH 8.0) containing 20 mg/ml of Lysozyme (Sigma-Aldrich) were added, and enzymatic lysis was conducted at 37°C for 30 min. 1.6 ml of buffer ASL from QIAamp DNA Stool Mini Kit (Qiagen, Germany) was added to each sample, after which the samples were mechanically homogenized in a MiniBeadbeater-8 (BioSpec Products, USA) for 2 min at maximum speed. DNA was purified from 1.2 ml of the resulting supernatant with the QIAamp DNA Stool Mini Kit following the manufacturer's instructions.

Compositional analysis of the fecal microbiota by pyrosequencing

Sequences were binned by primer barcodes using QIIME (Caporaso *et al*., 2010). Sequences that were shorter than 300 bp or longer than 550 bp, contained one or more ambiguous nucleotides, had one or more mismatches to

the primer or barcode, had an average quality scores below 25, or contained homopolymer runs over 6 bp, were removed. Chimeras were removed using the Blast Fragments Algorithm included in QIIME.

OTU picking was performed by aligning sequences using the RDP Infernal Alignment tool and clustered with the Complete Linkage Clustering algorithm (RDP). As current OTU picking algorithms tend to generate too many clusters (Ghodsi *et al.*, 2011), abundance of OTUs identified to be associated with host phenotypes or dietary treatments were confirmed using BLASTn. For this purpose, 5 representative sequences per OTU were taxonomically assigned and aligned by ClustalW within their respective phylum. A distance matrix was generated and phylogenetic trees (one per phylum) were constructed using the Neighbor-joining algorithm (MEGA 4.0) (Tamura *et al.*, 2007). OTUs were assigned visually as clusters within the phylogenetic trees, and membership was confirmed by sequence comparisons and restricted to sequences with >97% similarity. Consensus sequences were generated for each OTU. To quantify each OTU, a local database was created in BioEdit (Hall, 1999) with all the sequences. BLASTn with >97% similarity and >95% length overlap was used to determine the number of sequences belonging to individual OTUs. OTUs that shared a majority of their sequences were merged.

Genome queries for β-glucanase activity

The web-based Integrated Microbial Genomes (IMG) database of the Joint Genome Institute (JGI) was used to identify gut organisms with beta-glucanase function. The following bacteria were included: *Bacteroides caccae* ATCC 43185, *Bacteroides coprocola* M16, *Bacteroides dorei* 5_1_36/D4, *Bacteroides dorei* DSM 17855, *Bacteroides eggerthii* 1_2_48FAA, *Bacteroides eggerthii* DSM 20697, *Bacteroides finegoldii* DSM 17565, *Bacteroides fragilis* 3_1_12, *Bacteroides fragilis* 638R, *Bacteroides fragilis* NCTC 9343, *Bacteroides fragilis* YCH46, *Bacteroides intestinalis* 341, *Bacteroides ovatus* 3_8_47FAA, *Bacteroides ovatus*

ATCC 8483, *Bacteroides ovatus* SD CC 2a, *Bacteroides ovatus* SD CMC 3f, *Bacteroides stercoris* ATCC 43183, *Bacteroides thetaiotaomicron* VPI-5482, *Bacteroides uniformis* ATCC 8492, *Bacteroides vulgatus* ATCC 8482; *Bacteroides vulgatus* PC510, *Bacteroides xylanisolvens* SD CC 1b, *Bacteroides xylanisolvens* XB1A, *Bifidobacterium adolescentis* ATCC 15703, *Bifidobacterium adolescentis* L2-32, *Bifidobacterium catenulatum* DSM 16992, *Bifidobacterium longum* DJO10A, *Bifidobacterium longum* NCC2705, *Bifidobacterium longum* subps. *infantis* 157F-NC, *Bifidobacterium longum* subps. *infantis* ATCC 16697, *Bifidobacterium longum* subps. *infantis* JCM 1217, *Bifidobacterium longum* subsp. *longum* ATCC 55813, *Bifidobacterium longum* subsp. *longum* BBMN68, *Bifidobacterium longum* subsp. *longum* CCUG 52486, *Bifidobacterium longum* subsp. *longum* F8, *Bifidobacterium longum* subsp. *longum* JDM301, *Bifidobacterium longum* subsp. *longum* KACC 91563, *Bifidobacterium pseudocatenulatum* DSM 20438, *Blautia hansenii* VPI C7-24, *Blautia hydrogenotrophica* DSM 10507, *Bryantella formatexigens* I-52, *Butyrivibrio crossotus* DSM 2876, Clostridiales sp. SM4/1, Clostridiales sp. 1_7_47FAA, Clostridiales sp. SS3/4, Clostridiales sp. SSC/2, *Clostridium bolteae* ATCC BAA-613, *Clostridium butyricum* 5521, *Clostridium butyricum* E4, *Clostridium leptum* DSM 753, *Clostridium ramosum* VPI 0427, *Clostridium* sp. M62/1, *Clostridium spiroforme* DSM 15579, *Collinsella aerofaciens* ATCC 25986, *Collinsella intestinalis* DSM 13280, *Collinsella stercoris* DSM 13279, *Coprococcus comes* ATCC 27758, *Coprococcus eutactus* ATCC 27759, *Dialister invisus* DSM 15470, *Dorea formicigenerans* ATCC 27755, *Dorea longicatena* DSM 13814, *Eggerthella lenta* VPI 0255, *Enterococcus fecalis* ATCC 29200, *Enterococcus fecalis* ATCC 4200, *Eubacterium biforme* DSM3989, *Eubacterium cylindroides* T2-87, *Eubacterium eligens* ATCC 27750, *Eubacterium hallii* DSM 3353, *Eubacterium limosum* KIST612, *Eubacterium rectale* ATCC 33656, *Eubacterium rectale* DSM 17629, *Eubacterium rectale* M104/1, *Eubacterium ventriosum* ATCC 27560, *Faecalibacterium prausnitzii* KLE1255, *Faecalibacterium prausnitzii* A2-165, *Faecalibacterium prausnitzii* L2-6, *Faecalibacterium prausnitzii* M21/2, *Faecalibacterium prausnitzii* SL3/3, Lachnospiraceae 1_1_57FAA,

Lachnospiraceae 1_4_56FAA, Lachnospiraceae 2_1_46FAA, Lachnospiraceae 2_1_58FAA, Lachnospiraceae 3_1_46FAA, Lachnospiraceae 3_1_57FAA, Lachnospiraceae 4_1_37FAA, Lachnospiraceae 5_1_37FAA, Lachnospiraceae 6_1_63FAA, Lachnospiraceae 9_1_43BFAA, Lachnospiraceae sp 5_1_63FAA, Lachnospiraceae 8_1_57FAA, *Olsenella uli* DSM 7084, *Odoribacter splanchnicus* DSM 20712, *Parabacteroides distasonis* ATCC 8503, *Parabacteroides merdae* ATCC 43184, *Parabacteroides* sp. D13, *Phascolarctobacterium* sp YIT 12067, *Prevotella bryantii* B14, *Roseburia intestinalis* L1-82, *Roseburia intestinalis* M50/1, *Roseburia intestinalis* XB6B4, *Roseburia inulinivorans* DSM 16841, *Ruminococcaceae bacterium* D16, *Ruminococcus bromii* L2-63, *Ruminococcus gnavus* ATCC 29149, *Ruminococcus lactaris* ATCC 29176, *Ruminococcus obeum* A2-162, *Ruminococcus obeum* ATCC 29174, R*uminococcus torques* ATCC 27756, *Ruminococcus torques* L2-14, *Slackia exigua* ATCC 700122, *Slackia heliotrinireducens* DSM 20476, *Turicibacter sanguinis* PC909.

Short chain fatty acid determination

SCFAs were determined based on approaches described by Campbell and coworkers (1997), with slight modifications. Undiluted fecal samples were removed from storage at -80°C and thawed on ice, and 0.4 g were diluted in 2.8 ml water containing 5-10 mM 4-methylvaleric acid and vortexed. 0.4 ml of 25% (w/v) metaphosphoric acid was added and the sample was vortexed again, followed by centrifugation for 20 min at 15,000 x g. The supernatant was stored overnight at -20°C. Samples were thawed and centrifuged in the same conditions as before. SCFA were quantified by gas chromatography (Perkin Elmer Clarus with Perkin Elmer Elite-FFAP column) in a 4 µl injection volume, and the data was analyzed with appropriate software (TotalChrom, Perkin Elmer, USA). Moisture quantification in the fecal samples was done as follows. Approximately 0.2 g of feces was introduced into a plastic tube with a small perforation in its cap and frozen overnight at -20°C. Samples were freeze dried for at least 36 hours

until stable weight of the sample was achieved, and dry weight was calculated. SCFA were expressed on a dry basis.

Statistics

Correlations between host parameters and bacterial populations were assessed by Pearson's correlation test (GraphPad Prism v5.0). Graphs were generated for parameters that showed significant correlations and were visually inspected. If the removal of one single data-point caused the association to become non-significant, the data point was considered an outlier and removed.

Associations between inflammatory markers and members of the gut microbiome were further analyzed with the following linear models:

$$I_{ijt} = \beta_0 + \beta_1 Fat + \beta_2 Gender + \beta_3 Age + \beta_4 T2 + \beta_5 T3 + \beta_6 T4 \quad (1)$$

$$M_{hjt} = \beta_0 + \beta_1 Fat + \beta_2 Gender + \beta_3 Age + \beta_4 T2 + \beta_5 T3 + \beta_6 T4 \quad (2)$$

ijt is the inflammatory marker i for subject j in treatment t, i=1,2,3; j=1…28; hjt is the inflammatory marker h for subject j in treatment t, h=1,…,80; j=1…28; t=1,2,3,4; Fat indicates the percent body fat; Gender is a binary variable that takes values of 0 if the subject is female and 1 otherwise; Age is the age of subject in years; T2 is a binary variable that assigns 1 if the treatment is 30 grams of B and BR each and 0 otherwise; T3 is a binary variable that assigns 1 if the treatment is 60 grams of B and 0 otherwise; T4 is a binary variable that assigns 1 if the treatment is 60 grams of BR and 0 otherwise; and T1 represents no treatment and is left out of the models as the base. Fixed effects and random effects methods were used to estimate models (1) and (2). Chi-square estimates that measure the heterogeneity of the responses clustered by subject, were used as the criterion for choice between fixed and random effects estimation methods. For the models with Chi-square values associated with $P < 0.1$, random effects method was chosen.

Because hs-CRP concentrations >10 mg/l in plasma are indicative of acute inflammation unrelated to cardiovascular disease risk (Pearson et al., 2003). Therefore, 4 samples from 4 different subjects were excluded from the analysis. If the same samples also displayed abnormally high values of LBP or IL-6 levels, these data points were also considered outliers and removed. 2 and 3 samples were excluded from LBP and IL-6 analysis, respectively. One subject was excluded from the analysis of glucose parameters as incomplete data was obtained for this subject.

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Table S1. Nutritional information of the barley and brown rice flakes used in the study.

	Whole grain barley	Brown rice
Calories (kcal per 100g)	392	366
Fat (%)	6.7	3.0
Saturated fat (%)	1.7	1.0
Cholesterol (%)	0.0	0.0
Total carbohydrates (%)	64.6	80.0
Digestible starch [b] (%)	32.3	83.3
Resistant starch [b] (%)	0.2	0.5
Total dietary fiber [c] (%)	31.1	7.3
Insoluble fiber [c] (%)	22.8	6.8
Soluble fiber [c] (%)	8.3	0.5
B-glucan [d] (%)	14.1	0.0
Protein (%)	18.2	8.0

[a] Nutrient composition as provided by the manufacturers except when specifically noted.

[b] Measured with K-RSTAR Megazyme kit. (Expressed as dry basis).

[C] Measured according to AACCI Approved Method 32-25.01 with modifications from Andersson et al. (1999). (Expressed as dry basis).

[d] Measured with K-BGLU Megazyme kit. (Expressed as dry basis).

Table S2. List of bacterial species possessing β-glucanase genes and/or that responded to whole grain barley. Bacterial genomes containing β-glucanase genes were identified using the Integrated Microbial Genomes system (IMG)**.** The number and types of β-glucanases are indicated for the individual species. The number of subjects in which the species was detected and the direction of the shifts in response to WGB intake are presented. Abundances of species as a percentage of total fecal microbiota are also shown (mean ± SD).

Bacterial species	Number and type of enzymes encoded	Number of subjects in which detected	Response to WGB in individual subjects	Abundance (% of total microbiota)				P-value (ANOVA)
				Baseline (mean ± SD)	BR (mean ± SD)	BR+WGB (mean ± SD)	WGB (mean ± SD)	
Akkermansia muciniphila	2 β-glucanase precursor	10	10 no pattern	0.84 ± 1.82	0.57 ± 1.3	0.34 ± 0.86	0.41 ± 0.66	NS
Bacteroides caccae	7 β-glucanase/β-glucanase synthase	17	17 no pattern	0.2 ± 0.75	0.09 ± 0.21	0.13 ± 0.31	0.13 ± 0.43	NS
Bacteroides coprocola	4 endoglucanase 2 β-glucanase/β-glucanase synthase	3	2 ↑ 1 no pattern	0.37 ± 1.52	0.14 ± 0.5	1.06 ± 3.73	1.24 ± 4.68	NS
Bacteroides dorei	1 β-glucanase 2 β-glucanase/β-glucanase synthase	24	24 no pattern	1.88 ± 3.81	1.60 ± 2.90	1.37 ± 2.75	1.34 ± 2.84	NS
Bacteroides finegoldii	2 β-glucanase/β-glucanase synthase	4	4 no pattern	0.04 ± 0.2	0.06 ± 0.19	0.06 ± 0.33	0.02 ± 0.08	NS
Bacteroides fragilis	9 β-glucanase precursor 3 putative β-glucanase precursor	18	1 ↓ 17 no pattern	2.68 ± 8.12	1.81 ± 6.04	1.60 ± 5.05	1.21 ± 4.56	NS
Bacteroides intestinalis	2 β-glucanase/β-glucanase synthase 6 endoglucanase	25	3 ↑ 22 no pattern	0.32 ± 0.55	0.93 ± 2.66	0.38 ± 0.69	0.47 ± 0.76	NS
Bacteroides ovatus	2 β-glucanase/β-glucanase synthase 6 endoglucanase	ND						
Bacteroides thetaiotamicron	3 β-glucanase precursor 2 endoglucanase E precursor	25	25 no pattern	0.57 ± 0.79	0.72 ± 1.38	0.52 ± 0.98	0.40 ± 0.52	NS
Bacteroides uniformis	1 β-glucanase/β-glucanase synthase 8 endoglucanase	26	1 ↑ 3 ↓ 22 no pattern	4.55 ± 4.55	3.34 ± 3.3	2.77 ± 3.38	3.57 ± 4.59	NS
Bacteroides eggerthii	2 endoglucanase	7	1 ↑ 6 no pattern	0.35 ± 1.39	0.36 ± 1.03	0.32 ± 1.15	0.33 ± 0.97	NS
Blautia wexlerae	No matches found	28	6 ↑ 1 ↓ 11 no pattern	1.07 ± 0.78	1.58 ± 1.11	1.49 ± 0.98	1.82 ± 1.14¶¶¶*	< 0.0001
Blautia hydrogenotrophica	No matches found	4	4 no pattern	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.02	0.00 ± 0.01	NS
Blautia coccoides	No matches found	7	7 no pattern	0.01 ± 0.02	0.00 ± 0.01	0.00 ± 0.01	0.01 ± 0.02	NS
Blautia producta	No matches found	4	4 no pattern	0.01 ± 0.03	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	NS
Blautia hansenii	No matches found	5	5 no pattern	0.10 ± 0.26	0.05 ± 0.16	0.18 ± 0.91	0.08 ± 0.34	NS
Blautia spp. (*Ruminococccus obeum*)	1,3-beta-glucosidase	14	4 ↑ 10 no pattern	1.81 ± 1.13	2.38 ± 1.69	2.75 ± 1.75¶	2.80 ± 2.04¶¶	0.006

Bifidobacterium adolescentis	2 putative β-1,3-endoglucanase 2 endoglucanase	14	2 ↑ 12 no pattern	0.22 ± 0.42	0.36 ± 0.83	0.64 ± 1.3	0.48 ± 1.08	NS
Bifidobacterium angulatum	2 endoglucanase	ND						
Bifidobacterium longum	1 putative β-1,3-exoglucanase 2 endoglucanase	17	1 ↑ 16 no pattern	0.16 ± 0.42	0.17 ± 0.50	0.18 ± 0.33	0.23 ± 0.50	NS
Bifidobacterium pseudocatenulatum	4 endoglucanse	7	7 no pattern	0.07 ± 0.22	0.14 ± 0.65	0.08 ± 0.24	0.07 ± 0.20	NS
Clostridium butyricum	7 endoglucanase	4	4 no pattern	0.01 ± 0.03	0.00 ± 0.01	0.00 ± 0.01	0.00 ± 0.01	NS
Clostridium ramosum	2 β-glucanase/β-glucanase synthase 2 endoglucanase	ND						
Collinsella aerofaciens	2 endoglucanase	17	17 no pattern	0.08 ± 0.22	0.14 ± 0.28	0.1 ± 0.19	0.08 ± 0.18	NS
Collinsella intestinalis	2 endoglucanase	ND						
Collinsella stercoris	2 endoglucanase	ND						
Coprococcus comes	2 endoglucanase	25	1 ↑ 24 no pattern	0.29 ± 0.38	0.35 ± 0.42	0.33 ± 0.48	0.29 ± 0.45	NS
Coprococcus eutactus	1 β-glucanase/β-glucanase synthase 8 endoglucanase	13	1 ↑ 12 no pattern	0.68 ± 1.23	0.64 ± 1.23	0.62 ± 1.29	0.75 ± 1.35	NS
Dialister invisus	No matches found			0.52 ± 0.97	0.41 ± 0.72	0.56 ± 0.86	0.81 ± 1.41	
Eubacterium eligens	1 putative endoglucanase	14	2 ↑ 12 no pattern	0.22 ± 0.42	0.36 ± 0.83	0.64 ± 1.3	0.48 ± 1.08	NS
Eubacterium rectale	1 endo-1,4-β-glucanase	28	14 ↑ 14 no pattern	2.48 ± 2.67	2.75 ± 3.27	3.65 ± 3.45	4.83 ± 3.98¶ǂǂ*	0.001
Roseburia inulinivorans	1 endo-1,4-β-glucanase	28	1 ↑ 27 no pattern	0.25 ± 0.3	0.21 ± 0.32	0.14 ± 0.24	0.16 ± 0.18	NS
Roseburia faecis	Not in database	27	10 ↑ 17 no pattern	0.12 ± 0.17	0.06 ± 0.07	0.26 ± 0.31	0.53 ± 0.92¶¶ǂǂǂ	< 0.0001
Roseburia intestinalis	5 endo-1,4-β-glucanase	28	9 ↑ 19 no pattern	0.09 ± 0.12	0.04 ± 0.05	0.17 ± 0.18ǂ	0.30 ± 0.42¶ǂǂǂ	< 0.0001

ND: Not detected; NS: Not significant.

Table S3. Treatment effect on metabolic and immunological markers for all subjects. Metabolic data of the 28 participants, at baseline and at the end of the 4-week dietary treatments (BR, BR+WGB, WGB). Values are presented as mean ± SD.

	Overall				
	Baseline	BR	BR+WGB	WGB	*P-value*
Cholesterol					
Total cholesterol (mmol/l)	4.86 ± 1.15	4.76 ± 0.79	4.56 ± 0.89	4.89 ± 0.94	NS
Non-HDL (mmol/l)	3.09 ± 1.04	3.15 ± 0.84	3.00 ± 0.85	3.32 ± 0.94	NS
HDL (mmol/l)	1.63 ± 0.43	1.60 ± 0.37	1.55 ± 0.45	1.57 ± 0.36	NS
Plasma glucose					
Fasting (mmol/l)	5.15 ± 0.73	4.87 ± 0.49	4.81 ± 0.39	4.81 ± 0.50	NS
AUC ($[mmol/l]^2$)	784 ± 184	763 ± 164	746 ± 132	770 ± 179	NS
Max. peak (mmol/l)	9.08 ± 2.78	8.58 ± 2.02	7.92 ± 1.46	8.19 ± 2.35	< 0.1
Plasma insulin					
Fasting (µUI/ml)	6.77 ± 1.96	6.60 ± 2.13	6.51 ± 2.02	7.03 ± 2.07	NS
AUC ($[µUI/ml]^2$)	3463 ± 1523	3606 ± 1520	3333 ± 1035	3540 ± 1481	NS
Max. peak (µUI/ml)	44.08 ± 19.19	44.70 ± 19.56	42.86 ± 14.49	45.13 ± 21.61	NS
Inflammatory markers					
IL-6 (pg/ml)	1.68 ± 1.36	1.21 ± 0.99	0.90 ± 0.45*	1.12 ± 0.63	0.0295
Hs-CRP (mg/L)	1.60 ± 2.23	1.33 ± 1.65	0.95 ± 1.23	1.36 ± 1.88	NS
LBP (µg/ml)	14.41 ± 19.65	14.39 ± 2.09	13.23 ± 19.04	13.78 ± 18.30	NS

*$P < 0.05$ compared to Baseline.

Table S4. Treatment effect on metabolic and immunological markers in the subjects according to gender. Metabolic data of the female and male volunteers, at baseline and at the end of the 4-week dietary treatments (BR, BR+WGB, WGB). Values are presented as mean ± SD.

	Males					Females				
	Baseline	BR	BR+WGB	WGB	*P-value*	Baseline	BR	BR+WGB	WGB	*P-value*
Cholesterol										
Total cholesterol (mmol/l)	4.42 ± 1.11	4.59 ± 0.85	4.31 ± 0.91	4.46 ± 0.89	NS	5.02 ± 1.14	4.87 ± 0.76	4.73 ± 0.87	5.15 ± 0.89	0.0342
Non-HDL (mmol/l)	2.78 ± 0.74	3.01 ± 0.96*	2.90 ± 0.93	3.08 ± 0.82	0.0327	3.29 ± 1.16	3.24 ± 0.78	3.06 ± 0.83	3.47 ± 1.00	NS
HDL (mmol/l)	1.30 ± 0.28	1.41 ± 0.30	1.33 ± 0.31	1.23 ± 0.24	NS	1.84 ± 0.37	1.73 ± 0.36	1.69 ± 0.48	1.76 ± 0.25	NS
Plasma glucose										
Fasting (mmol/l)	5.14 ± 0.72	5.10 ± 0.64	4.91 ± 0.39	4.86 ± 0.34	NS	5.15 ± 0.76	4.72 ± 0.29	4.75 ± 0.40	4.77 ± 0.59	0.0344
AUC ([mmol/l]^2)	860 ± 232	851 ± 143	762 ± 166	857 ± 180	NS	739 ± 138	706 ± 155	735 ± 110	718 ± 162	NS
Max. peak (mmol/l)	10.13 ± 3.25	10.08 ± 1.67	8.21 ± 1.80	8.99 ± 2.37	< 0.1	8.40 ± 2.27	7.61 ± 1.61	7.74 ± 1.23	7.66 ± 2.26	NS
Plasma insulin										
Fasting (µUI/ml)	6.63 ± 1.75	5.93 ± 1.90	6.38 ± 1.80	6.05 ± 2.12	NS	6.85 ± 2.12	7.04 ± 2.22	6.60 ± 2.20	7.66 ± 1.83	NS
AUC ([µUI/ml]^2)	3436 ± 1787	3816 ± 1704	3399 ± 1086	3600 ± 1586	NS	3480 ± 1405	3483 ± 1442	3294 ± 1037	3505 ± 1465	NS
Max. peak (µUI/ml)	42.76 ± 20.55	48.63 ± 19.14	41.46 ± 12.37	48.54 ± 22.37	NS	44.93 ± 18.86	42.15 ± 19.98	43.76 ± 16.01	42.92 ± 21.50	NS
Inflammatory markers										
IL-6 (pg/ml)	1.18 ± 0.81	1.42 ± 1.35	1.99 ± 3.63	1.09 ± 0.58	NS	2.01 ± 1.58	1.16 ± 0.83*	1.10 ± 0.86**	1.67 ± 2.39*	0.0028
Hs-CRP (mg/L)	0.35 ± 0.22	0.92 ± 1.26	0.31 ± 0.24	0.76 ± 1.07	NS	2.35 ± 2.56	1.57 ± 1.85	1.33 ± 1.43	1.72 ± 2.19	NS
LBP (µg/ml)	4.76 ± 2.96	6.50 ± 5.48	4.42 ± 2.22	6.19 ± 4.09	NS	20.44 ± 23.19	19.32 ± 25.62	18.73 ± 22.74	18.52 ± 22.04	NS

*P < 0.05 compared to Baseline.
**P < 0.01 compared to Baseline.

Table S5. Treatment effect on metabolic and immunological markers in normoweight and overweight subjects. Metabolic data of normoweight and overweight, at baseline and at the end of the 4-week dietary treatments (BR, BR+WGB, WGB). Values are presented as mean ± SD.

	Overweight					Normoweight				
	Baseline	BR	BR+WGB	WGB	*P-value*	Baseline	BR	BR+WGB	WGB	*P-value*
Cholesterol										
Total cholesterol (mmol/l)	4.84 ± 1.26	4.84 ± 0.86	4.51 ± 0.89	5.03 ± 1.05	NS	4.75 ± 1.07	4.69 ± 0.75	4.61 ± 0.92	4.77 ± 0.84	NS
Non-HDL (mmol/l)	3.24 ± 1.21	3.35 ± 0.97	3.12 ± 0.90	3.52 ± 1.05	NS	2.94 ± 0.85	2.98 ± 0.71	2.89 ± 0.83	3.14 ± 0.83	NS
HDL (mmol/l)	1.61 ± 0.45	1.47 ± 0.32	1.37 ± 0.40	1.51 ± 0.35	NS	1.65 ± 0.42	1.72 ± 0.38	1.71 ± 0.45	1.62 ± 0.37	NS
Plasma glucose										
Fasting (mmol/l)	5.37 ± 0.93	4.87 ± 0.45*	4.88 ± 0.37	4.88 ± 0.42	0.0231	4.94 ± 0.40	4.87 ± 0.54	4.75 ± 0.42	4.74 ± 0.57	NS
AUC ($[mmol/l]^2$)	867 ± 184	800 ± 187	774 ± 128	811 ± 160	NS	707 ± 153	730 ± 140	720 ± 135	731 ± 192	NS
Max. peak (mmol/l)	9.66 ± 2.14	8.53 ± 2.11	7.99 ± 1.32*	8.43 ± 1.79	0.0428	8.58 ± 3.09	7.86 ± 1.62	7.98 ± 2.80	8.63 ± 2.01	NS
Plasma insulin										
Fasting (µUI/ml)	6.93 ± 1.70	7.10 ± 2.53	6.82 ± 1.78	7.60 ± 1.58	NS	6.62 ± 2.22	6.17 ± 1.69	6.24 ± 2.24	6.54 ± 2.36	NS
AUC ($[µUI/ml]^2$)	3730 ± 1677	3952 ± 1665	3249 ± 1128	3804 ± 1482	NS	3216 ± 1382	3284 ± 1354	3411 ± 978	3295 ± 1493	NS
Max. peak (µUI/ml)	48.39 ± 19.92	50.23 ± 22.63	43.19 ± 16.16	49.94 ± 21.17	NS	40.34 ± 18.38	39.90 ± 15.67	42.57 ± 13.45	40.96 ± 26.83	NS
Inflammatory markers										
IL-6 (pg/ml)	2.03 ± 1.32	1.64 ± 1.27	0.97 ± 0.52*	1.40 ± 0.77	0.0438	1.35 ± 1.36	0.81 ± 0.32	0.83 ± 0.38	0.86 ± 0.32	NS
Hs-CRP (mg/L)	2.26 ± 2.47	2.12 ± 1.96	1.37 ± 1.52	1.86 ± 1.87	NS	1.04 ± 1.93	0.66 ± 0.99	0.59 ± 0.83	0.94 ± 1.86	NS
LBP (µg/ml)	22.45 ± 24.90	23.56 ± 26.42	21.63 ± 23.90	22.16 ± 22.66	NS	6.36 ± 6.67	4.83 ± 5.58	5.40 ± 5.63	5.21 ± 6.83	NS

*$P < 0.05$ compared to Baseline.

Table S6. Gastrointestinal symptoms. Weekly gastrointestinal symptoms of the 28 participating subjects, scored in a scale from 1 (best/normal) to 5 (worst/abnormal) during the baseline and at the end of each 4-week dietary treatment (BR, BR+WGB, WGB). Values are presented as mean ± SD.

	Baseline	BR	BR+WGB	WGB	*P-value*
Bowel movement	1.5 ± 0.5	1.3 ± 0.5	1.7 ± 0.6	2.0 ± 0.8$^{¶∓∓}$	< 0.01
Stool consistency	1.5 ± 0.6	1.4 ± 0.5	1.8 ± 0.6	2.0 ± 0.8$^{¶∓∓}$	< 0.01
General well-being	1.2 ± 0.3	1.2 ± 0.4	1.5 ± 0.6	2.2 ± 0.6$^{¶¶¶∓∓∓**}$	< 0.001
Flatulence	1.3 ± 0.5	1.4 ± 0.5	2.2 ± 0.9$^{¶¶¶∓∓∓}$	3.1 ± 1.0$^{¶¶¶∓∓∓***}$	< 0.001
Abdominal pain	1.1 ± 0.2	1.1 ± 0.3	1.4 ± 0.6	1.8 ± 0.8$^{¶¶¶∓∓∓*}$	< 0.001
Bloating	1.2 ± 0.4	1.2 ± 0.4	1.6 ± 0.7$^{¶∓}$	2.2 ±0.8$^{¶¶¶∓∓∓**}$	< 0.001

¶ Compared to baseline
∓ Compared to BR

Supplementary Figures

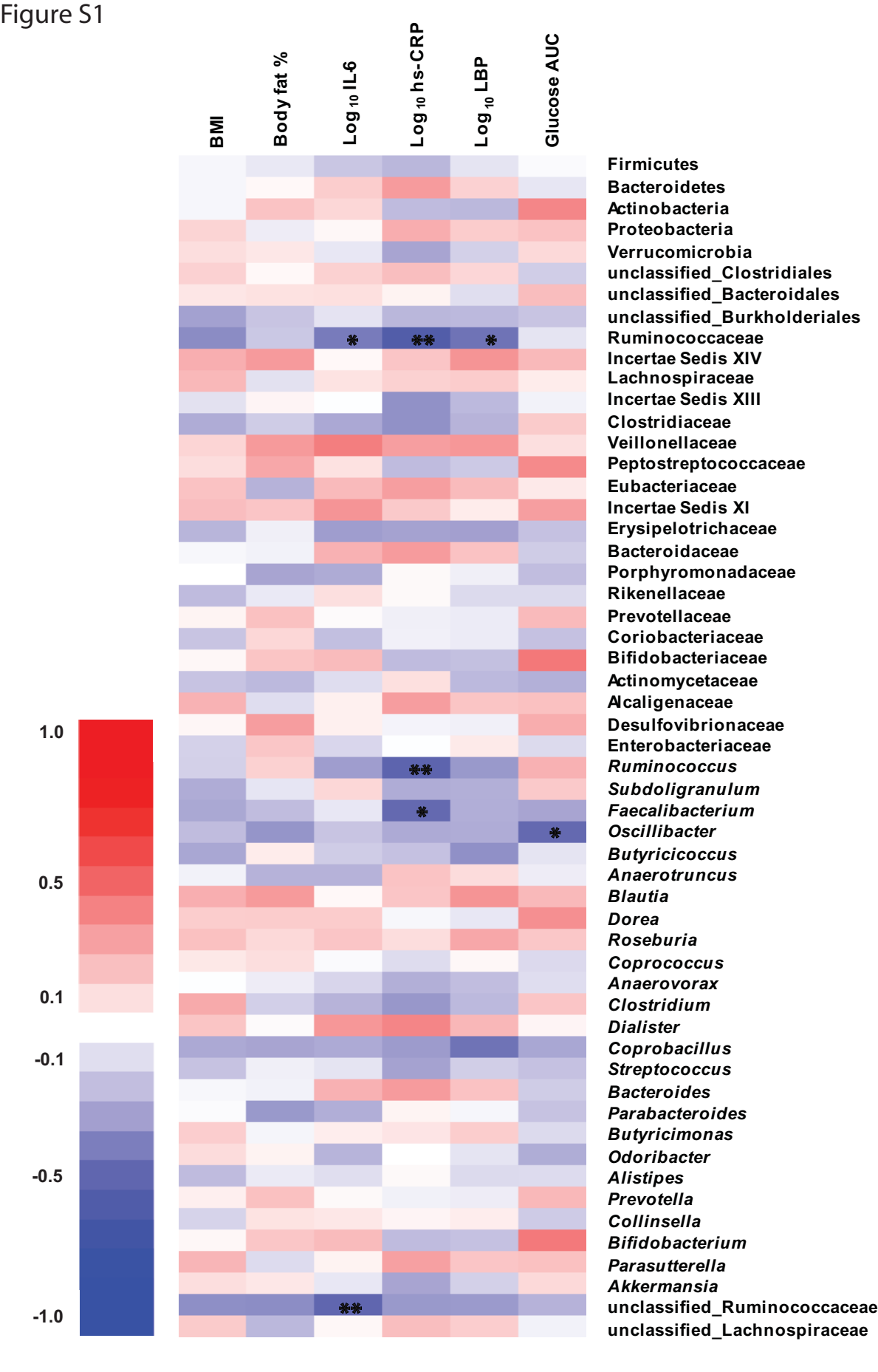
Figure S1. Association between inflammatory and metabolic markers and bacterial taxa in fecal samples. A heat map shows correlation coefficients (Pearson) between BMI, percent body fat, IL-6, hs-CRP, LBP and glucose AUC with proportions of bacterial taxa in fecal samples.

Figure S2. Associations between Bacteroidetes related taxa and HDL plasma levels at baseline. Correlations between proportions of Bacteroidetes (A), Bacteroidaceae (B) and Bacteroides (C) in fecal samples with HDL measured in plasma at baseline. Pearson's r correlation and the *P* values are presented.

Figure S3. Impact of whole grains on the fecal microbiota. Diversity of the bacterial population in fecal samples assessed by Shannon's (A) and Simpson's (B) α-diversity indices. * $P < 0.05$, ** $P < 0.01$.

Figure S4. Association between diet induced shifts in glycemic response and the proportion of *Eubacterium rectale*. Correlation of the shift of the *Eubacterium rectale* abundance with the shifts observed in postprandial AUC (A), Insulin AUC (B), and maximum glucose levels (C). Shift refers to differences between values obtained during the BR+B period and the baseline. Pearson's r correlation and the *P* values are presented.

Figure S1


BMI
Body fat %
Log 10 IL6
Log 10 hs-CRP
Log 10 LBP
Glucose AUC
Firmicutes
Bacteroidetes
Actinobacteria
Proteobacteria
Verrucomicrobia
unclassified_Clostridiales
unclassified_Bacteroidales
unclassified_Burkholderiales
Ruminococcaceae
Incertae Sedis XIV
Lachnospiraceae
Incertae Sedis XIII
Clostridiaceae
Veillonellaceae
Peptostreptococcaceae
Eubacteriaceae
Incertae Sedis XI
Erysipelotrichaceae
Bacteroidaceae
Porphyromonadaceae
Rikenellaceae
Prevotellaceae
Coriobacteriaceae
Bifidobacteriaceae
Actinomycetaceae
Alcaligenaceae
Desulfovibrionaceae
Enterobacteriaceae
Ruminococcus
Subdoligranulum
Faecalibacterium
Oscillibacter
Butyricicoccus
Anaerotruncus
Blautia
Dorea
Roseburia
Coprococcus
Anaerovorax
Clostridium
Dialister
Coprobacillus
Streptococcus
Bacteroides
Parabacteroides
Butyricimonas
Odoribacter
Alistipes
Prevotella
Collinsella
Bifidobacterium
Parasutterella
Akkermansia
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unclassified_Lachnospiraceae
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0.5
0.1
-0.1
-0.5
-1.0

Figure S2

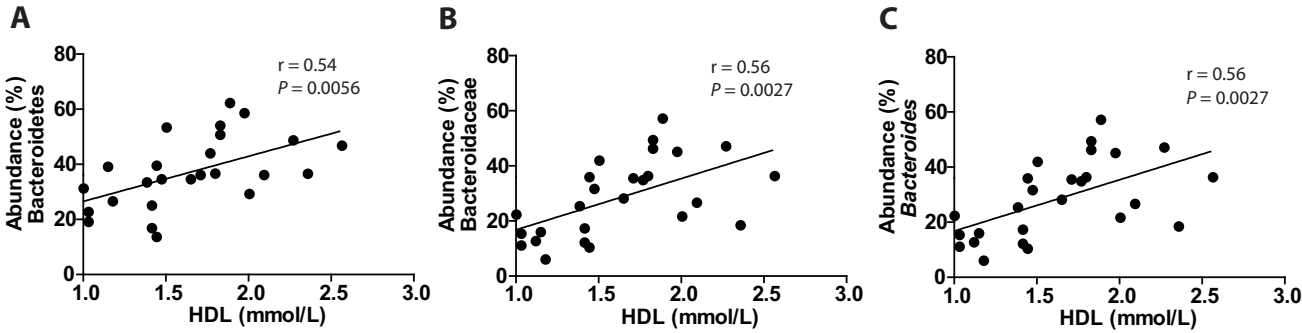

Figure S3

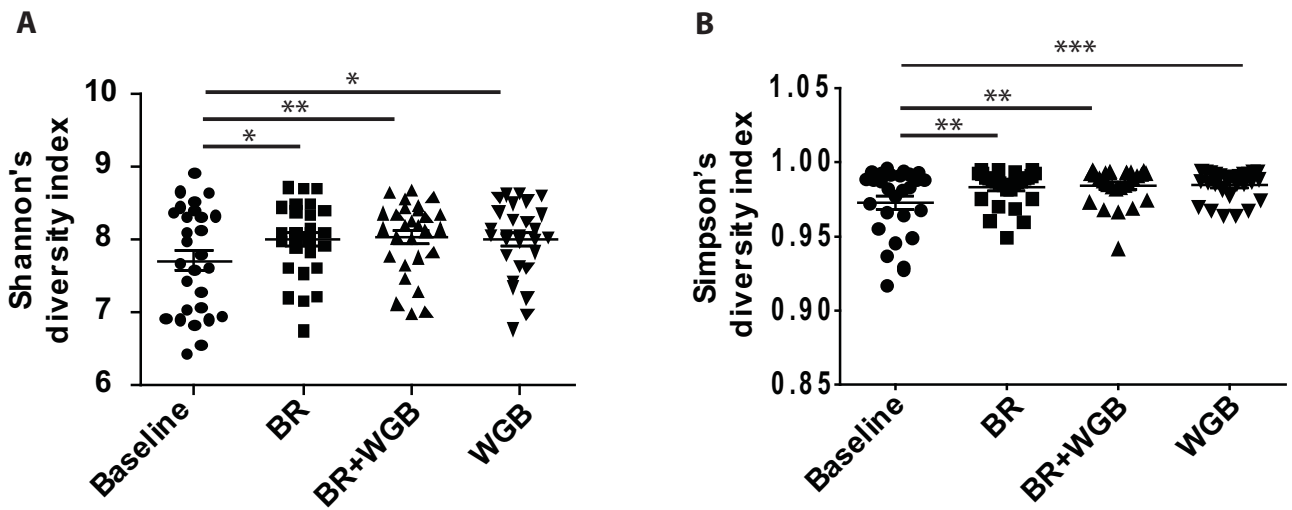

Figure S4