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.

References

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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

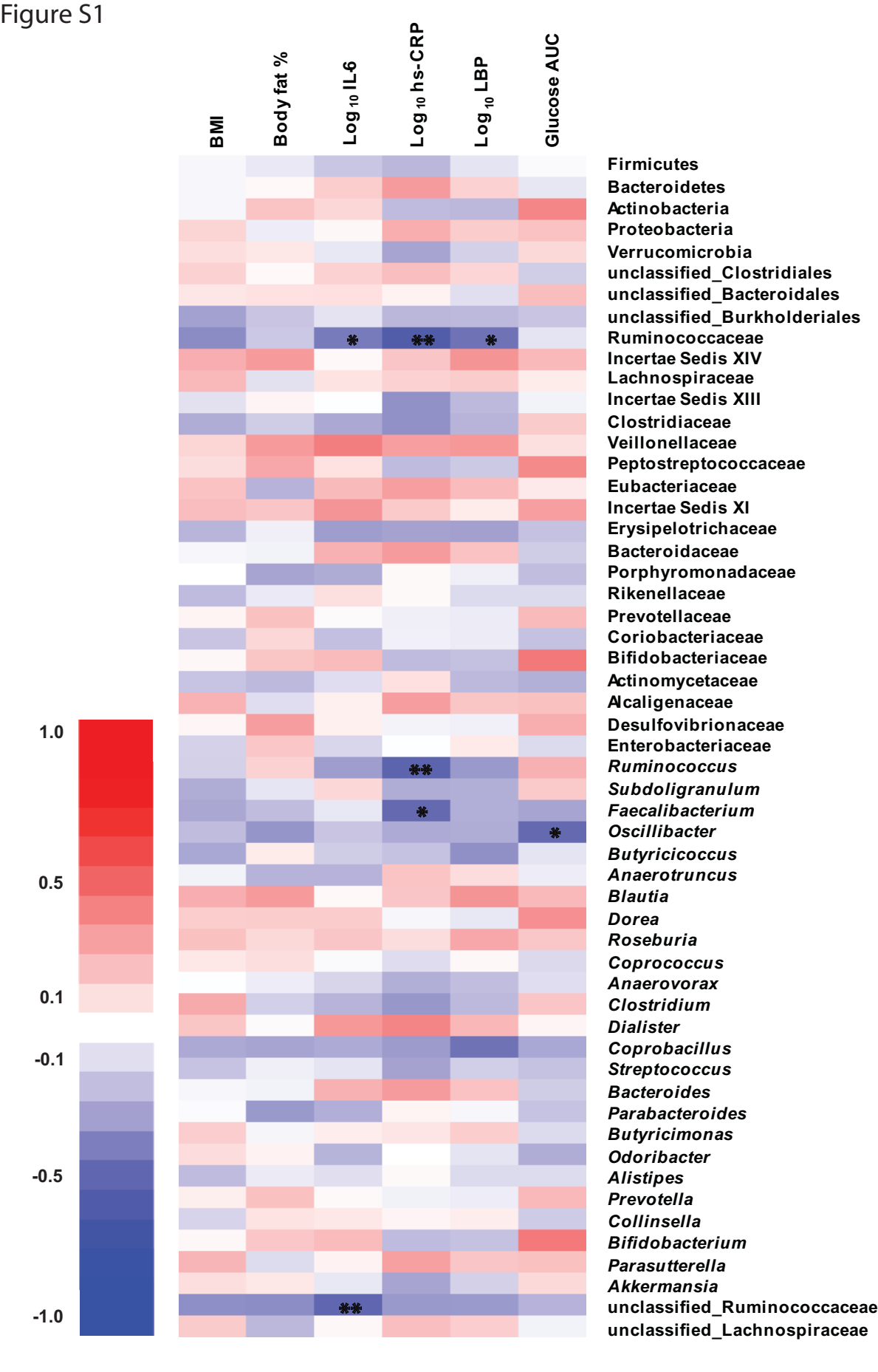
BMI
Body fat %
Log 10 IL6
Log 10 hs-CRP
Log 10 LBP
Glucose AUC
Firmicutes
Bacteroidetes
Actinobacteria
Proteobacteria
Verrucomicrobia
unclassified_Clostridiales
unclassified_Bacteroidales
unclassified_Burkholderiales
Ruminococcaceae
Incertae Sedis XIV
Lachnospiraceae
Incertae Sedis XIII
Clostridiaceae
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
unclassified_Ruminococcaceae
unclassified_Lachnospiraceae
1.0
0.5
0.1
-0.1
-0.5
-1.0

Figure S2

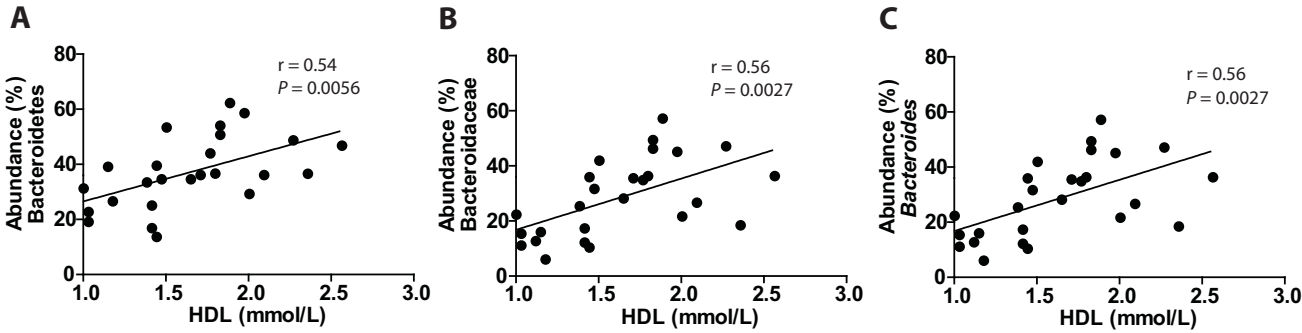

Figure S3

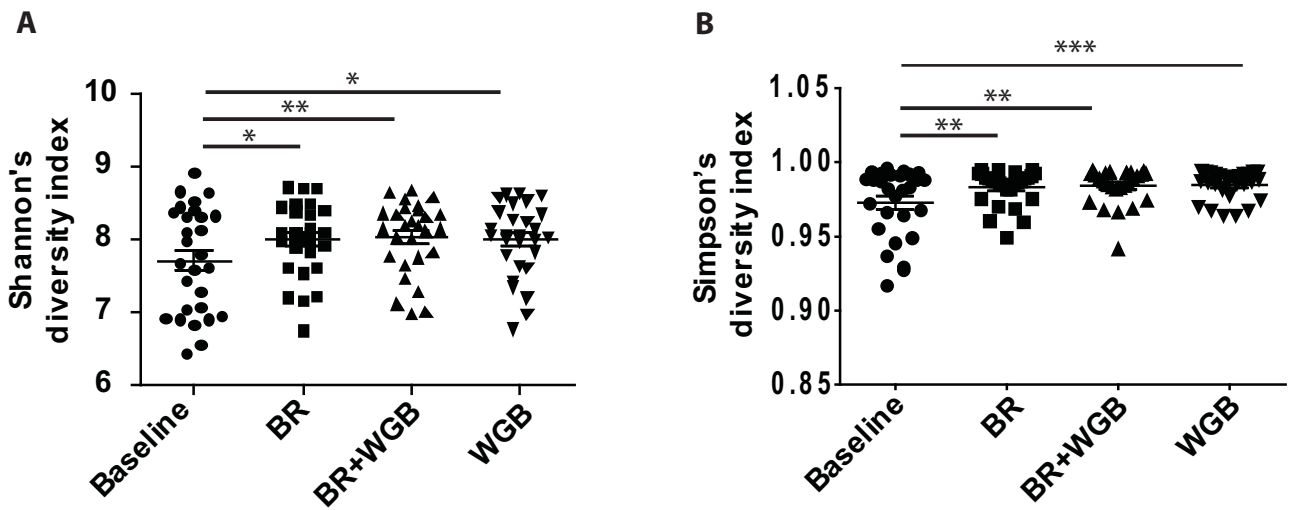

Figure S4