

Intake of Whole-Grain and Fiber-Rich Rye Bread Versus Refined Wheat Bread Does Not Differentiate Intestinal Microbiota Composition in Finnish Adults with Metabolic Syndrome^{1–4}

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Abstract

Whole-grain (WG) foods rich in indigestible carbohydrates are thought to modulate the composition of the intestinal microbiota. We investigated in a randomized, parallel, 2-arm 12-wk intervention whether consumption of WG and fiber-rich rye breads compared with refined wheat breads affected the microbiota composition in Finnish individuals aged 60 ± 6 y with metabolic syndrome. Fecal samples from 51 participants (25 males, 26 females) before and after the intervention were processed for the microbiota analysis using a phylogenetic microarray and quantitative polymerase chain reactions targeting the 16S rRNA gene. The intake of whole grains calculated from food records was higher in the group consuming rye breads (75 g) than in that consuming refined wheat breads (4 g; $P < 0.001$), confirmed by fasting plasma alkylresorcinol concentrations, a biomarker of whole grain intake. The intestinal microbiota composition did not significantly differ between the groups after the intervention. However, we detected a 37% decrease of Bacteroidetes ($P < 0.05$) in parallel to a 53% decrease in the alkylresorcinol concentration ($P < 0.001$) in the group consuming refined wheat breads. In this group, the abundance of bacteria related to *Bacteroides vulgatus*, *B. plebeius*, and *Prevotella tannerae* decreased, whereas that of bacteria related to *Collinsella* and members of the *Clostridium* clusters IV and XI increased. In a multivariate regression analysis, the abundance of *Bacteroides* spp. was best explained by different fat compounds among dietary variables, whereas the main sugar-converting butyrate-producers were mostly associated with the intake of whole- and refined-grain bread and fiber. Our results indicate that the quality of grains has a minor effect on the intestinal microbiota composition in participants with metabolic syndrome and suggest that the dietary influence on the microbiota involves other dietary components such as fat. *J. Nutr.* 143: 648–655, 2013.

Introduction

The amount and nature of ingested carbohydrates are assumed to affect the composition and activity of the intestinal microbiota that dominate the large intestine. A low-carbohydrate diet

has been observed to significantly decrease the main butyrate producers, *Roseburia* spp. and *Eubacterium rectale*, compared with an isoenergetic high-protein or normal diet (1). However, the amount of indigestible carbohydrates that reaches the large intestine is more likely to affect the microbiota than the total carbohydrate content of diet per se. Whole grains are rich in various indigestible carbohydrates, including cellulose, arabinoxylan, β -glucan, and fructan. Arabinoxylan is one of the main dietary fibers in wheat and rye (2,3). Refined grains lack these compounds mainly due to the removal of the bran layer of the grain. Some intestinal bacteria can ferment arabinoxylan in model systems, but in vivo data in humans are scarce (4). Hence, the amount of whole-grain (WG)¹⁰ foods in the diet is expected to largely control the amount of fermentable substrates available for the large intestinal microbiota. Furthermore, other nutrients

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³ This trial was registered at www.ClinicalTrials.gov as NCT00573781.

⁴ Supplemental Tables 1–3 and Figures 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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¹⁰ Abbreviations used: AR, alkylresorcinol; HITChip, Human Intestinal Tract Chip; PLS, partial least square; RB, rye bread group; RDA, redundancy analysis; WG, whole-grain; WWB, white wheat bread group.

and staple foods might also play a role in modifying the microbiota composition, as suggested by cross-sectional studies that indicated an adaptation of specific bacterial groups to the content of complex carbohydrates, fat, and protein in the diet (5–7).

An increasing number of studies indicate that diet affects the development of metabolic disorders, such as type 2 diabetes, possibly via an effect on the intestinal microbiota (8–10). However, there are only a limited number of human intervention studies relating WG food consumption with the intestinal microbiota composition. A 2-wk, randomized, cross-over study reported slightly increased numbers of *Clostridium leptum* but showed no difference in Bifidobacteria or *Bacteroides* in 17 healthy participants when a diet based on whole grains was compared with one based on refined grains (11). Similarly, in another strictly controlled intervention study, a diet supplemented with wheat bran did not notably change the intestinal microbiota composition (12). In contrast, Costabile et al. (13) observed that the numbers of Bifidobacteria increased after consumption of a WG wheat breakfast cereal compared with one based on wheat bran for 3 wk. While this result suggests that whole grains are more bifidogenic than wheat bran alone, no other differences were reported for the analyzed bacterial groups between the treatments. Recently, a randomized cross-over study showed that adding WG barley flakes to the diet for 4 wk increased the abundance of *Bifidobacterium*, *Blautia*, and *Roseburia* spp (14). Finally, a study entailing 69 participants with metabolic syndrome showed that a diet supplemented with a purified cereal fiber extract had no effect on the intestinal microbiota composition or fermentation profile (15). Most of the inconclusive present results are based on targeted intestinal microbiota analysis with a limited set of dominant or otherwise relevant bacterial groups. Thus, they do not provide a global view of the microbiota during dietary change.

In summary, it has not yet been established whether or how the type of cereal foods affects intestinal microbiota. Hence, we investigated the effects of refined low-fiber wheat bread and WG and high-fiber rye bread intake on the intestinal microbiota composition in 51 participants with metabolic syndrome. The dietary difference was mainly achieved by changing the bread type in diet, i.e., by affecting the quality of one of the staple foods. We performed a comprehensive, deep, and high throughput analysis of the intestinal microbiota composition using a phylogenetic microarray and additionally addressed associations of the intestinal microbiota with nutrient and food intake.

Methods

Participants and study design. Participants were recruited into a 12-wk, parallel, controlled dietary intervention study from the Kuopio area of Finland as previously described (16,17). Fifty-two participants in 2 intervention groups provided fecal samples. Of these, one participant was excluded because of diagnosed inflammatory bowel disease. Thus, 51 participants (25 males, 26 females) were studied for effects of diet on the intestinal microbiota composition and clinical variables and for associations between diet and microbiota.

Inclusion criteria for the participants were age 40–65 y, a BMI of 26–39 kg/m², and at least 3 other features of metabolic syndrome: impaired glucose tolerance (2-h glucose 7.8–11.0 mmol/L) or impaired fasting glucose (glucose 5.6–6.9 mmol/L), waist circumference >102 cm (men) or >88 cm (women), fasting serum TG concentration >1.7 mmol/L, fasting serum HDL cholesterol concentration <1.0 mmol/L (men) or <1.3 mmol/L (women), and blood pressure >130/85 mm Hg or medication for hypertension. Exclusion criteria were: BMI >40 kg/m²; fasting serum TG concentration >3.5 mmol/L; fasting serum total cholesterol concentration

>8 mmol/L; type 1 or 2 diabetes; abnormal liver, thyroid, or renal function; alcohol abuse [>16 portions/wk (women) or >24 portions/wk (men)], and inflammatory bowel disease. The participants were randomized to a rye bread (RB) diet ($n = 27$) or a refined white wheat bread (WWB) diet ($n = 24$). The dietary groups were matched for gender, BMI, age, and fasting plasma glucose concentration. The protocol for the study was approved by the Ethics Committee of the Hospital District of Northern Savo. Written informed consent was obtained from all participants.

Intervention diets. Participants in the RB group consumed rye breads with a high-fiber content (7–15%) and those in the WWB group consumed refined wheat breads with a low fiber content (4%). Most of the total grain intake consisted of bread (aiming to cover 20–25% of total energy intake). The test breads were chosen on the basis of our previous postprandial studies with rye and whole-meal wheat breads showing a beneficial low-insulin response (18,19). The breads in the RB group were a selection of commercial WG rye breads (50% share of all the breads), endosperm rye bread (40% share), and a whole-meal wheat bread (10% share). In addition, the participants in the RB group were asked to consume whole-meal pasta [3.5 dL/wk (measured as uncooked)] and were given high-fiber oat biscuits for voluntary intake. In the WWB group, the test breads were a selection of commercial refined wheat breads and the intake of rye products was restricted to 1–2 portions/d. The participants were provided with the test products and advised by a registered dietician on the practical management of the diet. Assessment of dietary compliance was based on questionnaires where the participants recorded their consumption of the test products daily. Apart from the grain products, the participants' habitual diet and lifestyle habits were not controlled but were advised to keep unchanged during the trial.

Dietary analyses. Participants filled in 4-d food records at baseline and at the end of the intervention (i.e., wk 11). Dietary data for the intake of nutrients and food groups was analyzed by Micro-Nutrica software version 2.0 (Finnish Social Insurance Institute). Nutrient intake-based dietary evaluation was complemented by calculating intake of food items. The food items were grouped into several categories such as WG breads, refined white breads, other grain products, vegetables, fruits, spreads, dairy products, meat, fish, and drinks (a more detailed description of the food groups is in Supplemental Table 1). In addition, intakes of grain fiber and WG ingredients (i.e., whole grains) were calculated from food records using data from food labels and common recipes.

Clinical measurements and biochemical analyses. Clinical variables were measured at baseline and at the end of the intervention. Measurements and analyses of fasting glucose, insulin, serum cholesterol fractions and TGs, and markers of glucose metabolism and inflammation were described earlier (16,17).

The plasma total alkylresorcinol (AR) concentration, a biomarker of WG intake (20,21), was analyzed at baseline and at the end of the intervention to evaluate the compliance to the intervention diets. Fasting plasma samples were analyzed for AR homologs C17:0–C25:0 according to a GC-MS–single ion monitoring method, using molecular ions for quantification (22). Total concentration of AR was calculated from the sum of homologs.

Compositional analysis of the intestinal microbiota. Fecal spot samples were collected by the participants at home at baseline and the end of the intervention. The samples were stored in -70°C after delivering to the Department of Clinical Nutrition either as cooled or being frozen in a home freezer (-18°C) for less than a day. Extraction of bacterial DNA from the fecal samples was performed using mechanical lysis (23). Compositional analysis of the intestinal microbiota was performed using the Human Intestinal Tract Chip (HITChip), a phylogenetic microarray produced by Agilent Technologies as previously described (24). In brief, the HITChip consists of >4800 oligonucleotide probes targeting 1033 distinct phylotypes based on the V1 and V6 hypervariable regions of the 16S rRNA (24). Phylogenetic assignment of the probes and quality control of the HITChip array data have been

described in detail elsewhere (23,24). The array data were first min-max normalized and then a background subtraction was carried out as in Zilliox et al. (25), using the mean background level plus 1 SD as noise threshold. All analyses were done on duplicate arrays and the data only passed the quality control when the Pearson's coefficient was >98% (24). The amount of total bacteria and methanogenic archaea in the samples was determined with qPCR as previously described (26).

Statistical analyses. Statistical analyses were performed with scripts in R, version 2.15.1 (R Development CT. Vienna, Austria: R Foundation for Statistical Computing; 2012. R: A Language and Environment for Statistical Computing). All statistical analyses were carried out with a 10-base logarithm-transformed data.

For the HITChip, dietary, and clinical data, a comparison between the groups was carried out with the nlme package in R (27) using a linear mixed model, with subject modeled as a random effect, and time, intervention group, and their interaction as fixed effects. The contrasts were then estimated with the multcomp package (28). Pairwise *t* tests were used to estimate the significance of changes within group as well as to analyze the qPCR data. After their estimation, all *P* values of fixed effects and different comparisons were subjected to Benjamini-Hochberg false discovery rate correction. The adjusted *P* values < 0.05 were regarded as significant. Statistical over-representation (enrichment) of genus-like groups among the significant phylotypes was tested using Fisher's exact test (29). The dietary, clinical, and qPCR data are expressed as means ± SDs for variables with normal distribution and as median (minimum-maximum) for initially skewed distributions.

Hierarchical clustering of the HITChip microbiota profiles was carried out by using nonbackground-subtracted, oligo-level data using correlation as the distance measure and complete linkage clustering algorithm. Multivariate analysis was carried out with bootstrap aggregated (bagged) redundancy analysis (RDA) and partial least squares (PLSs) that allow identifying sets of covariates whose joint effect explains the dependent variable(s). RDA was applied to find sets of bacteria that separate dietary groups. PLS was used to find a set of nutrients or food groups explaining the variation of these sets of bacteria. In this setting, the WG variable was dropped out from analysis, because it was a control variable between the dietary groups. Overall associations between the microbiota and dietary variables were analyzed with PLS, where the dependent variable was the RDA component separating the 2 dietary groups in food intake data. Bootstrap aggregated (bagged) RDA and PLS were implemented as R scripts with 10,000 bootstrap data sets, using vegan package (30) to estimate the RDA or PLS solutions for each set and using Procrustes rotations to solve the rotational ambiguity. In PLS, *R*²

(proportion of variation) was estimated with a bootstrap 0.632+ method (31). The latent dimensionality of PLS was set to 2. Missing values in the data matrices were imputed with a probabilistic principal component analysis (32). Bi-weight mid-correlations were used to correlate bacterial genus-like groups with nutrients and food groups. Here, a looser threshold for significance was employed for exploratory purposes by including correlations that had an >80% chance of being true positives.

Results

Characteristics of participants and diet. The participants were 60 ± 6 y of age at baseline and fulfilled the set criteria for metabolic syndrome as having a BMI of 31 ± 4 kg/m², fasting plasma glucose concentration of 6.1 ± 0.5 mmol/L, waist circumference of 111 ± 9 cm (men) or 101 ± 8 cm (women), and systolic or diastolic blood pressure of 140 ± 13 or 88 ± 8 mm Hg, respectively (53% of the participants had medication for hypertension). The markers of metabolic syndrome did not differ between the study groups, which were in line with the results from the original larger study group (16,17). The weight of the participants remained the same throughout the intervention (89 ± 14 kg). The intervention did not induce differences between the groups in the markers of glucose metabolism and inflammation, as also previously reported (16,17), although there was improvement in the high sensitivity C-reactive protein concentration within the RB group (16).

The intake of energy and nutrients was the same in both groups at baseline (Table 1).

As expected based on the diet modification, the daily intakes of total and grain fiber and whole grains differed between the groups during the intervention (*P* < 0.05), such that the intakes were lower at the end of the intervention in the WWB group than in the RB group (*P* < 0.001) (Table 1). Within the WWB group, the intake of total fiber, grain fiber, and whole grains decreased by 2, 3, and 51 g, respectively (*P* < 0.05). Within the RB group, the intake of grain fiber increased (*P* < 0.001) by 5 g and that of whole grains increased (*P* < 0.01) by 8 g, but there was no change in the total fiber intake. The fasting plasma concentration of AR differed between the groups during the intervention (*P* < 0.05) by decreasing 53% in the WWB group (*P* < 0.001) and

TABLE 1 Intake of nutrients and plasma AR concentration at wk 0 and 11 in RB and WWB groups¹

| | RB group (<i>n</i> = 27) | | WWB group (<i>n</i> = 24) | | <i>P</i> -interaction ² |
|---------------------|---------------------------|----------------|----------------------------|------------------------------|------------------------------------|
| | wk 0 | wk 11 | wk 0 | wk 11 | |
| Energy intake, kJ/d | 6830 ± 2220 | 7870 ± 2880* | 7200 ± 1840 | 8590 ± 1930** | 0.61 |
| Carbohydrate, E% | 46 ± 7 | 46 ± 9 | 48 ± 5 | 46 ± 6 | 0.61 |
| Protein, E% | 19 ± 3 | 19 ± 3 | 19 ± 4 | 18 ± 2 | 0.90 |
| Fat, E% | 33 ± 5 | 33 ± 7 | 31 ± 6 | 34 ± 6 | 0.31 |
| SAFA, E% | 12.1 ± 2.2 | 12.1 ± 3.0 | 12.0 ± 2.8 | 12.8 ± 2.8 | 0.59 |
| MUFA, E% | 11.3 ± 2.1 | 10.5 ± 3.6 | 9.9 ± 2.3 | 10.3 ± 2.6 | 0.31 |
| PUFA, E% | 5.8 (3.6–10.1) | 4.6 (1.3–9.6)* | 5.2 (3.3–9.9) | 4.9 (3.0–8.8) | 0.61 |
| 18:2n6, E% | 3.9 (2.5–7.3) | 3.2 (1.0–8.5)* | 3.6 (1.9–6.5) | 3.2 (1.1–6.5) | 0.61 |
| 18:3n3, E% | 1.0 (0.4–1.4) | 0.8 (0.2–1.3)* | 0.7 (0.3–1.9) | 0.7 (0.2–1.1) | 0.61 |
| Total fiber, g/d | 24 (12–38) | 24 (17–42) | 21 (14–39) | 19 (8–28) [†] ** | 0.02 |
| Grain fiber, g/d | 14 (3–32) | 19 (15–38)*** | 13 (7–20) | 10 (6–15) [†] *** | <0.001 |
| Whole grains, g/d | 67 (4–211) | 75 (41–164)** | 55 (17–101) | 4 (0–24) [†] * | <0.001 |
| Plasma AR, nmol/L | 94 (22–263) | 83 (32–476) | 51 (23–226) | 24 (11–124) [†] *** | 0.02 |

¹ Values are means ± SDs or median (minimum-maximum). Different from RB at wk 11: [†]*P* < 0.001 (linear mixed model with false discovery rate correction). Different from wk 0: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (pairwise *t* test with false discovery rate correction). AR, alkylresorcinol; E%, percentage of total energy intake; RB, rye bread; WWB, white wheat bread.

² *P* value for group × time interaction (linear mixed model with false discovery rate correction).

TABLE 2 Intake of grain foods at wk 0 and 11 in RB and WWB groups¹

| | RB group (n = 27) | | WWB group (n = 24) | | P-interaction ² |
|----------------------|-------------------|--------------|--------------------|--------------------|----------------------------|
| | wk 0 | wk 11 | wk 0 | wk 11 | |
| | g/d | | | | |
| WG breads | 94 (0–267) | 92 (50–157) | 78 (15–111) | 0 (0–15)**.†† | 0.002 |
| High-fiber breads | 0 (0–75) | 0 (0–17) | 0 (0–180) | 0 (0–0) | 0.63 |
| Refined white breads | 15 (0–215) | 0 (0–12)†† | 46 (0–236) | 188 (101–280)**.†† | < 0.001 |
| Endosperm rye bread | 0 (0–0) | 60 (0–175)†† | 0 (0–0) | 0 (0–0)** | < 0.001 |
| Whole-meal pasta | 0 (0–24) | 12 (0–56)†† | 0 (0–0) | 0 (0–40)* | 0.008 |
| Other pasta | 0 (0–68) | 0 (0–50) | 0 (0–50) | 0 (0–113) | 0.56 |
| Oat biscuit | 0 (0–0) | 8 (0–68)†† | 0 (0–0) | 0 (0–8)** | < 0.001 |
| Other WG products | 17 (0–56) | 2 (0–34)†† | 7 (0–79) | 3 (0–32)† | 0.56 |
| Other grain products | 41 (4–121) | 13 (0–118)†† | 52 (14–143) | 35 (12–89)† | 0.91 |

¹ Values are median (minimum–maximum). Different from RB at wk 11: * $P < 0.05$, ** $P < 0.001$ (linear mixed model with false discovery rate correction). Different from wk 0: † $P < 0.05$, †† $P < 0.001$ (pairwise t test with false discovery rate correction). RB, rye bread; WG, wholegrain; WWB, white wheat bread.

² P value for group \times time interaction (linear mixed model with false discovery rate correction).

being lower than in the RB group at the end of the intervention ($P < 0.001$), confirming the compliance of the participants regarding consumption of grain products.

During the intervention, the daily intake of energy similarly increased by 1040 kJ ($P < 0.05$) and 1390 kJ ($P < 0.01$) in the RB and WWB groups, respectively. However, the percentage of energy from the energy-yielding nutrients did not change, except for PUFA, 18:2n6, and 18:3n3, which decreased in the RB group ($P < 0.05$).

An analysis of the food groups showed differences mainly in the intake of grain products, as expected. The intake of WG breads decreased and that of refined white breads increased in the WWB group compared with the RB group ($P < 0.001$) (Table 2). In the RB group, the intake of endosperm rye bread, whole-meal pasta, and oat biscuits increased ($P < 0.001$). There were no other differences in the mean intake of food items between the groups during the intervention, but individual-specific differences were noted, reflecting the individuality in the habitual dietary patterns (Supplemental Table 2).

Intestinal microbiota composition. The composition of the intestinal microbiota was analyzed by hybridizing the 16S rRNA amplicons on the HITchip phylogenetic microarray, which targets more than 1000 intestinal phylotypes covering most of the so-far-known diversity (24). Hierarchical clustering of the microbiota profiles was performed to gain an overview of the similarity of the total intestinal microbiota within and between the participants. Despite the change in diet, the microbiota had high individual specificity and temporal stability (within-subject Pearson correlation = 0.92 ± 0.03 , with no difference between the groups). The microbiota composition of none of the participants changed to the extent that it would have hampered the clustering according to participant (Supplemental Fig. 1). The probabilistic principal component analysis that addressed the maximal variation in the HITchip data also did not show segregation of the samples by the intervention group or pre- and postintervention samples (data not shown). Based on qPCR analysis of 16S rRNA amplicons, the amount of total bacteria and methanogenic archaea was 11.7 ± 0.2 and $8.2 \pm 1.2 \log_{10}$ genome copies/g feces, respectively. These values did not differ between the groups or before and after the intervention.

Linear models were applied to identify the bacteria whose relative abundance significantly differed between the groups or

whose abundance significantly changed within a group during the intervention. Comparative analyses were performed on different phylogenetic levels by summing up the probe signal intensities to phylotype (species-like), genus-like, or phylum levels. The microbiota composition did not differ between the groups either at baseline or after the intervention, except for the phylotype *Bryantella formatexigens*, which was 16% higher in the WWB group at the end of the intervention ($P = 0.04$).

When analyzing the within-group effects, the microbiota composition changed in the WWB group. The participants' microbiota showed a decrease ($P < 0.05$) of Bacteroidetes spp. paralleled by an increase in the members of *Clostridium* cluster IV (Firmicutes) as well as *Collinsella* and *Atopobium* spp. that belong to the Actinobacteria (Fig. 1). The relative proportion of Bacteroidetes phylum decreased 37% during the intervention. On the genus-like level, 9 taxa were enriched based on 55 phylotypes that showed a change in the WWB group

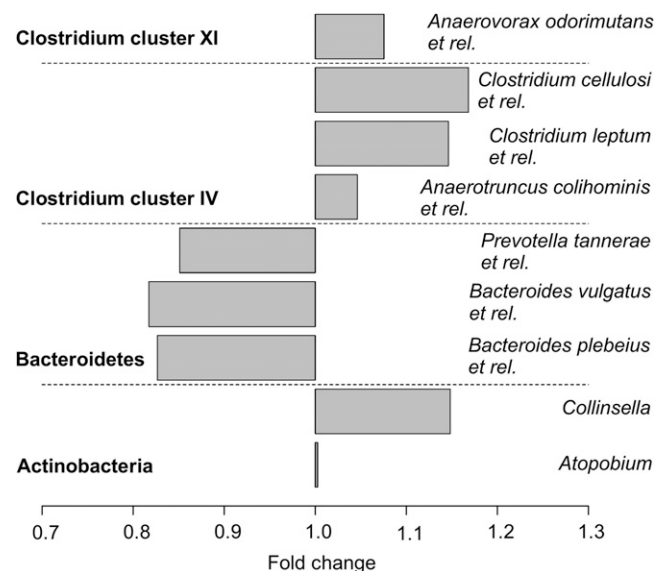


FIGURE 1 Magnitude and direction of change in the 9 significantly enriched, genus-like, phylogenetic bacterial groups in the WWB group during the intervention. A higher phylogenetic level of each group is indicated on the left with order for Firmicutes and phylum for others. WWB, white wheat bread.

(Supplemental Table 3). Among the enriched genera, *Bacteroides plebeius* and relatives (*et rel.*) *B. vulgatus et rel.* and *Prevotella tannerae et rel.*, decreased, whereas mainly uncultured taxa belonging to Clostridium clusters IV (*Clostridium leptum et rel.*, *Clostridium cellulosi et rel.*, and *Anaerotruncus colihominis et rel.*) and XI (*Anaerovorax odorimutans et rel.*) as well as to Actinobacteria (*Atopobium* and *Collinsella*) increased (Fig. 1). The largest mean decrease was detected in *B. vulgatus et rel.* (19% decrease) and the largest increase in Clostridium cluster IV (17% increase for *Clostridium cellulosi et rel.*). In the RB group, the intervention diet did not change the relative abundance of any bacterial taxa (this was observed at all phylogenetic levels) (data not shown).

A substantial individuality characterized the microbiota responses. Although the changes in the majority of the participants in the WWB group were unidirectional, in some individuals, the implicated taxa did not respond at all or even changed to the opposite direction (Supplemental Fig. 2). On the other hand, the abundance of *B. vulgatus* that mostly contributed to the decrease of Bacteroidetes in the WWB group also decreased in about one-half of the participants in the RB group (Supplemental Fig. 3). Among the genus-like bacterial groups, *Bifidobacterium* spp. varied the most during the intervention (fold change per individual ranged from 0.17 to 9.12), but the degree of variation was independent of the dietary group.

Associations of the intestinal microbiota with nutrient and food intake. In the RDA analysis of the HITchip data, the bacteria contributing to the separation between the diet groups were dominated by the members of Bacteroidetes (Supplemental Fig. 4), in line with the changes we identified within the WWB group. The abundance of 12 *Bacteroides-Prevotella* taxa differed or tended to differ ($P = 0.01-0.10$) between the diet groups [referred to as the *Bacteroides* cluster below, consisting of *B. vulgatus* ($P < 0.05$), *P. tannerae* ($P < 0.05$), *B. intestinalis* ($P < 0.05$), *B. plebeius* ($P < 0.05$), *P. oralis* ($P < 0.05$), *P. ruminicola* ($P = 0.06$), *B. ovatus* ($P = 0.08$), *B. uniformis* ($P < 0.05$), *B. stercoris* ($P < 0.05$), *B. fragilis* ($P = 0.10$), *B. splachnicus* ($P = 0.10$), and *P. melaninogenica* ($P = 0.06$)]. To find an explanation for the variation in the *Bacteroides* cluster, their joint abundance in relation to dietary variables was analyzed with PLS. The diet explained 82% of the variation. Within the dietary variables, the percentage of the energy intake from PUFA and 18:2n6 was most associated with variation of the *Bacteroides* cluster (proportion of variation ~30%), followed by energy from 18:3n3 (Fig. 2). WG and refined breads also had >10% of their variation associated with *Bacteroides* cluster variation.

When studying the overall associations between the microbiota composition and dietary intake for the entire data set, a total of 37% of the variation in diet was associated with the microbiota composition, with the butyrate-producing *Faecalibacterium prausnitzii* having clearly the highest impact (PLS explained 72% of the variation of *F. prausnitzii*), followed by another butyrate producer, *Eubacterium rectale* (Supplemental Fig. 5). Among the 15 bacterial genera that were the most associated with diet, 7 are capable of producing butyrate from sugar but not from lactate (*F. prausnitzii*, *E. rectale*, *Lachnospira pectinoschiza*, *Roseburia intestinalis*, *E. cylindroides*, *Lachnospira bovis*, and *E. ventriosum*). A total of 97% of the variation in these butyrate producers was explained by diet, so the intake of WG breads, refined white breads, total fiber, and grain fiber explained their variation the most (40–52%) (Fig. 3).

To explore the diet-microbiota associations identified in the PLS analysis, we analyzed bivariate mid-correlations between

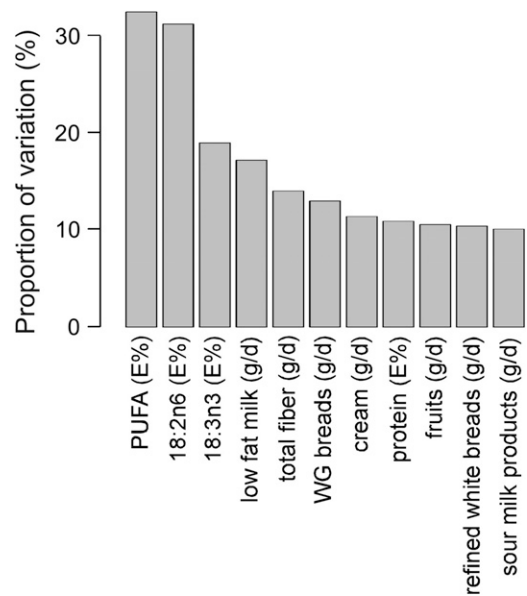


FIGURE 2 Variation in nutrient and food group intakes associated with the relative abundance of the *Bacteroides* cluster in bootstrap aggregated PLS analysis. Only nutrients and food groups with $\geq 10\%$ of associated variation are shown. E%, percentage of total energy intake; PLS, partial least square; WG, whole grain.

the abundance of each genus-like bacterial group and nutrient and food intakes. None of the detected correlations reached $r = 0.5$ or the default threshold for significance ($P < 0.05$). When using the looser threshold ($P < 0.2$) for exploratory purposes, the following weak correlations were observed: *B. vulgatus et rel.*, *B. ovatus et rel.*, *P. tannerae et rel.*, and *P. oralis et rel.* correlated ($r \geq 0.30$) with the intake of 18:2n6, other fat-derived compounds, and margarine. Of the butyrate producers derived from the PLS analysis, *F. prausnitzii et rel.* correlated inversely with the intake of refined white breads ($r = -0.35$), and *R. intestinalis et rel.* correlated positively with that of total fiber ($r = 0.33$), grain fiber ($r = 0.34$), and WG breads ($r = 0.35$) and negatively with that of refined white breads ($r = -0.30$). The other butyrate producers, when analyzed individually, did not correlate with any nutrients or foods, except for *E. ventriosum*, which correlated with energy intake from alcohol ($r = 0.33$).

Discussion

In this 12-wk, randomized intervention study in participants with metabolic syndrome, we investigated if replacing the habitual intake of WG rye bread with refined WWB would affect intestinal microbiota composition. To our knowledge, this study is the first to couple community-level analysis of the intestinal microbiota to accurate dietary records and controlled change in the intake of grain products. Furthermore, this study addressed the impact of staple foods on the intestinal microbiota composition as opposed to added grain fiber fractions or fiber supplements previously analyzed (4,33).

The major difference between the intervention diets was the type of bread as well as the amount of whole grains consumed. The participants in both groups consumed nearly the same daily amount of grain products but of notably different quality. In the RB group, a total of 187 g/d of grain products was consumed, of which 92 g was WG breads and 60 g was high-fiber endosperm rye bread. The RB intervention diet included 75 g/d of whole grains, which is 1.5 times the amount recommended by U.S.

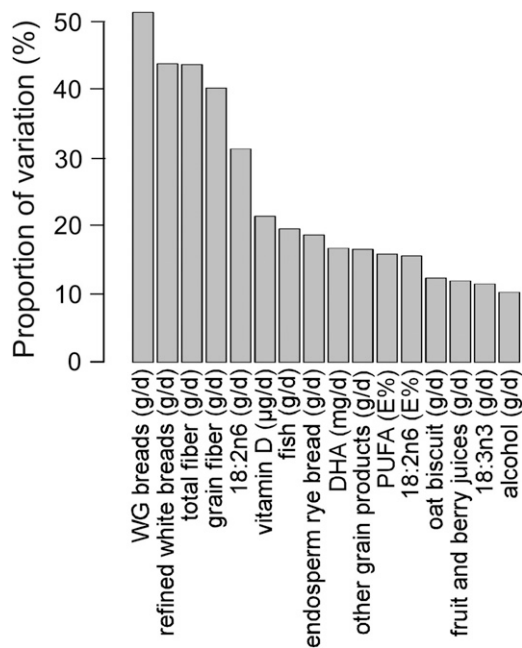


FIGURE 3 Variation in nutrient and food group intakes associated with the relative abundance of butyrate producers in bootstrap aggregated PLS analysis. Only nutrients and food groups with $\geq 10\%$ of associated variation are shown. E%, percentage of total energy intake; PLS, partial least square; WG, whole grain.

dietary guidelines (34). In contrast, in the WWB group, 226 g/d of grain products was consumed, of which 188 g was refined white breads. Despite the substantial difference in the whole grain intake, which was confirmed by the difference in plasma AR concentration, the microbiota composition did not significantly differ between the groups. Similarly to our comprehensive microbiota analysis, no effect on the dominant intestinal bacteria was observed in a strictly controlled, 2-wk intervention study with 151 g/d of whole grains, consisting mainly of wheat, compared with refined grains (11). Instead, the addition of 60 g/d of WG barley or brown rice in a diet for 4 wk increased and decreased the abundance of Firmicutes and Bacteroides, respectively, in healthy American individuals (14). Unfortunately, the participants' habitual diet was not described. Our intervention cannot be directly compared with other grain intervention studies in which participants' baseline diet is composed of refined grain products. Based on our baseline observations and a national survey (35), Finns consume a relatively high amount of WG rye bread (on average 86 g/d). Long-term diets seem to be associated with compositional differences of the microbiota (5–7). Hence, the high, long-term consumption of whole grains might have affected our study population's microbiota and its responsiveness to changes in the WG content of the diet. Furthermore, preliminary data show that individuals with metabolic syndrome differ in their microbiota composition from healthy individuals (36). Thus, the generalization of our results to populations with low WG intake or individuals without metabolic syndrome has to be made with caution.

Only the abundance of *Bryantella formatexigans* of the 1033 phylotypes detected by the HITChip was found to be significantly different with the WWB diet compared with the RB diet. *B. formatexigans*, belonging to the *Clostridium* cluster XIVa, requires carbohydrates and formate for growth and is able to ferment cellulose into acetate (37). Within-group microbiota changes were observed in only the WWB group, in which the

participants substituted refined white wheat bread for rye bread, whereas in the RB group only minor changes in the diet occurred. Thus, the habitual high-WG food consumption might explain the lack of change in the microbiota composition within the RB group.

Within the WWB group, a significant microbiota change occurred even at the phylum level. The change mostly manifested within a specific *Bacteroides* cluster in which a subset of phylotypes was affected (Supplemental Table 3), possibly reflecting variable metabolic or competitive properties within a group. Although significant, the magnitude of the changes in the bacterial abundance was modest (from a 19% decrease to a 17% increase) and specific to the individual, which is in line with previous observations (6,12). Of the significantly altered genera in the WWB group, *B. vulgatus* decreased the most. *B. vulgatus* can utilize rye arabinoxylan in vitro (38). Thus, removal of rye bread from the diet could explain the observed decrease in *B. vulgatus*, as many *Bacteroides* and *Prevotella* spp. have conserved and well-described molecular machineries to degrade and ferment a great variety of indigested polysaccharides such as xylan (39,40), a component of arabinoxylan. Comparisons of the microbiota in westernized (Italy and US) and nonwesternized (rural Africa and Venezuela) countries have revealed the latter to be enriched with *Prevotella* spp., probably due to long exposure to diets rich in plant-derived, complex carbohydrates (5,7). However, in our study, there was a trend for decreased abundance of *B. vulgatus* also among the participants consuming rye bread. Accordingly, in multivariate regression analysis, the main diet-dependent explanatory factor for the abundance of the *Bacteroides* cluster was not the intake of WG breads or grain fiber, but that of fat-derived compounds. Their intake alongside the corresponding food items, such as spreads, margarine, and fish, varied largely among the individuals independent of the group. Although most of the ingested fat is absorbed in the small intestine, dietary fat might affect the colonic microbiota via modulation of bile acids (41). Previously, intake of fat was observed to be positively associated with *Bacteroides*-dominated microbiota and Enterotype 1 when analyzing long-term habitual diets (6) and negatively with the butyryl-CoA synthetase gene involved in butyrate production as well as butyrate producers (42). However, an intervention study showed no effect of SFA or MUFA on numbers of *Bacteroides* and *Prevotella* after 6 mo (43). In mice, a series of studies has shown that high-fat feeding affects the intestinal microbiota (44). Recently, the quality of ingested fat was also shown to influence the cecal microbiota via altered bile acid composition (45). Hence, several recent studies suggest that fat is a potential factor affecting the composition of the intestinal microbiota.

Certain members of *Clostridium* clusters IV and XI were slightly increased in the WWB group during the intervention. The Clostridial clusters IV and XIVa contain the main carbohydrate-utilizing butyrate producers in the human gut, with *F. prausnitzii*, *R. intestinalis*, and *E. rectale* being the most abundant (46). The abundance of these groups did not differ between the diets. Both rye and white wheat bread contain resistant starch (47,48), which may have affected the microbiota parallel to the quantified nondigestible carbohydrates. Recently, a positive association between butyrate-producing bacteria and insulin sensitivity was observed in participants with metabolic syndrome (49). Furthermore, an increase in the abundance of *E. rectale* was associated with improvement in postprandial glucose and insulin responses (14). Although the abundance of the main butyrate producers and state of glucose metabolism (17) remained the same during our intervention, increased insulin sensitivity has been observed after daily intake of

insoluble cereal fiber for 6 wk (50) without changes in dominant groups of intestinal microbiota (15). This may suggest that altered microbiota composition can contribute but is not necessary to improve insulin resistance.

Although diet altogether explained the majority of the variation in the *Bacteroides* cluster and certain butyrate producers in the PLS, any single nutrient or food group did not strongly correlate with the individual implicated bacterial groups. However, the observed correlations, although weak, were mainly in line with those derived from the PLS analysis. Our results suggest that multivariate analyses constitute a biologically more informative approach than analyses of each taxa separately, because they also capture subtle differences. Moreover, different members of the microbiota do not operate in isolation but as part of the community that is known to possess a high rate of functional redundancy and cross-feeding among the species. For example, the utilization of complex grain polysaccharides consisting of nonsoluble and soluble particles is carried out by a concerted action of different primary and secondary degraders (46). In this study, the detected high variation of Bifidobacteria was not associated with intervention group or any dietary variable, even in PLS analysis. The intake of oligosaccharide- or other prebiotic-containing foods (33) was, unfortunately, not controlled and may have contributed to the variation.

In conclusion, a high compared with low intake of whole grains for 12 wk did not differentiate the intestinal microbiota composition in participants with metabolic syndrome. However, across the entire cohort, we identified changes in the microbiota composition that were associated mostly with the intake of fat-derived compounds and to a lesser extent with that of WG foods. Our results highlight the fact that intentional modulation of the microbiota by withdrawal or supplementation of carbohydrate-containing staple foods is not straightforward, because the baseline microbiota as well as intake of minor dietary components, such as fatty acids, contributes to the outcome. To clarify the effect of diet on the intestinal microbiota composition, different types and sources of dietary fiber as well as the amount and quality of fat should be carefully controlled in further intervention studies.

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