Minor Changes in the Composition and Function of the Gut Microbiota During a 12-Week Whole Grain Wheat or Refined Wheat Intervention Correlate with Liver Fat in Overweight and Obese Adults

Mara PH van Trijp,[1] Sophie Schutte,[1] Diederik Esser,[1] Suzan Wopereis,[2] Femke PM Hoevenaars,[2] Guido JEJ Hooiveld,[1] and Lydia A Afman[1]

[1]Nutrition, Metabolism & Genomics Group, Division of Human Nutrition and Health, Wageningen University, Wageningen, The Netherlands; and [2]TNO, Netherlands Organization for Applied Scientific Research, Zeist, The Netherlands

ABSTRACT

Background: Whole grain wheat (WGW) products are advocated as a healthy choice when compared with refined wheat (RW). One proposed mechanism for these health benefits is via the microbiota, because WGW contains multiple fibers. WGW consumption has been proposed to ameliorate nonalcoholic fatty liver disease, in which microbiota might play a role.

Objectives: We investigated the effect of WGW compared with RW intervention on the fecal microbiota composition and functionality, and correlated intervention-induced changes in bacteria with changes in liver health parameters in adults with overweight or obesity.

Methods: We used data of a 12-wk double-blind, randomized, controlled, parallel trial to examine the effects of a WGW (98 g/d) or RW (98 g/d) intervention on the secondary outcomes fecal microbiota composition, predicted microbiota functionality, and stool consistency in 37 women and men (aged 45–70 y, BMI 25–35 kg/m^2). The changes in microbiota composition, measured using 16S ribosomal RNA gene sequencing, after a 12-wk intervention were analyzed with nonparametric tests, and correlated with changes in liver fat and circulating concentrations of liver enzymes including alanine transaminase, aspartate transaminase, γ-glutamyltransferase, and serum amyloid A.

Results: The WGW intervention increased the mean ($\pm$ SD) relative abundances of *Ruminococcaceae_UCG-014* (baseline: 2.2 $\pm$ 4.6%, differential change over time (Δ) 0.51 $\pm$ 4.2%), *Ruminiclostridium_9* (baseline: 0.065 $\pm$ 0.11%, Δ 0.054 $\pm$ 0.14%), and *Ruminococcaceae_NK4A214_group* (baseline: 0.37 $\pm$ 0.56%, Δ 0.17 $\pm$ 0.83%), and also the predicted pathway acetyl-CoA fermentation to butyrate II (baseline: 0.23 $\pm$ 0.062%, Δ 0.035 $\pm$ 0.059%), compared with the RW intervention (*P* values <0.05). A change in *Ruminococcaceae_NK4A214_group* was positively correlated with the change in liver fat, in both the WGW ($\rho = 0.54$; *P* = 0.026) and RW ($\rho = 0.67$; *P* = 0.024) groups.

Conclusions: In middle-aged overweight and obese adults, a 12-wk WGW intervention increased the relative abundance of a number of bacterial taxa from the family *Ruminococcaceae* and increased predicted fermentation pathways when compared with an RW intervention. Potential protective health effects of replacement of RW by WGW on metabolic organs, such as the liver, via modulation of the microbiota, deserve further investigation. This trial was registered at clinicaltrials.gov as NCT02385149. *J Nutr* 2021;151:491–502.

Keywords: fatty liver, human, gut microbiota, overweight/obesity, refined wheat, whole grain wheat, fermentation

Introduction

The consumption of whole grains (WGs) is associated with lower risk of diseases such as type 2 diabetes, cardiovascular diseases, obesity, and certain types of cancer in observational studies (1–3). In addition, consumption of WGs rather than refined grain has been proposed to prevent nonalcoholic fatty liver disease (NAFLD) (4). The presence of numerous bioactive compounds makes WGs, including whole grain wheat (WGW), nutritionally superior to refined wheat (RW) products (5). In refined products, both the bran and germ in the wheat kernel,

which are rich sources of dietary fiber, polyphenols, B-complex vitamins, betaine, choline, and minerals, have been removed (5, 6). However, multiple previous WG interventions in human subjects have reported conflicting results in improvements in health parameters such as insulin sensitivity and cholesterol concentrations (7–10).

The potential health benefits of WGW have also been ascribed to fiber. WGW is a source of both fermentable and nonfermentable dietary fibers, such as (hemi)cellulose including arabinoxylan and β-glucan, lignin, and the oligosaccharides raffinose, stachyose, and fructan (11). Dietary fibers are not hydrolyzed or absorbed in the small intestine, and therefore can directly interact with the colonic gut microbiota, resulting in the production of metabolites that are relevant to health (12, 13). Diet affects the microbiota, and consequently dietary changes (for instance, via interventions) can possibly modulate the intestinal microbiota composition and functionality (13). Cereal fibers and other components in WGW such as iron (14) have shown an ability to modulate the gut microbiota composition in vitro and in vivo (15, 16). Moreover, multiple human trials demonstrated an increase in specific bacteria associated with host health benefits after WGW interventions, including enhanced abundance of *Bifidobacterium* (17, 18) and *Lactobacillus* (18, 19). Two trials reported an increase in butyrate-producing bacteria upon WGW or WG consumption (19, 20). However, other human trials did not find effects of WGs on the microbiota composition (21–23). The effects of WGW and WGs on both the composition and functions of the gut microbiota in humans are therefore not yet well understood. Some previous trials investigated only a selected subset of bacteria (17, 18, 24) and could have failed to capture the full effects of WGW and RW on the gut microbiota. It remains to be elucidated if the gut microbiota mediates the health benefits of WGW in humans.

The fermentable dietary fibers can be broken down by the gut microbiota (11, 25), producing SCFAs, mainly acetate, butyrate, and propionate. SCFAs can reach the liver and the peripheral circulation, where they can affect organ function and metabolism (26, 27). In vitro studies and animal experiments have shown that SCFAs increase fat oxidation in liver tissue (28, 29), suggesting a link between microbial-produced SCFAs and liver fat. Modulation of the gut microbiota is thought to play a role in the development of NAFLD and nonalcoholic

steatohepatitis (30–33). In the review of Ross et al. (4) the effect of WGs on the microbiota was proposed as a potential mechanism in the prevention of NAFLD. The presence of a perturbed metabolic health status, such as being middle-aged and being overweight or obese, is often a prerequisite for the development of NAFLD (4).

Recently, we performed the Graandioos study (7, 34), and observed that a 12-wk RW intervention increased intrahepatic triglycerides (IHTGs) and decreased microbiota diversity in middle-aged adults with overweight and obesity, whereas a WGW intervention prevented an increase in liver fat (7). We have previously reported the effect of WGW and RW on the abundances of a preselected subset of bacteria (7). In the current article we describe the effects of this randomized, controlled, double-blind, parallel trial with a 12-wk WGW intervention compared with an RW intervention on the complete fecal microbiota composition with additional analyses, as well as the effects of the interventions on predicted microbial functionality. Moreover, we investigated the relation between intervention-induced changes in bacterial composition and changes in various liver health parameters, such as IHTGs and liver enzymes, to examine the potential role of microbiota in the preventive effect of WGW in hepatic fat accumulation.

Methods

Subjects

Fifty middle-aged Dutch men and postmenopausal women (aged 45–70 y, BMI 25–35 kg/m^2) with mildly elevated plasma total cholesterol (>5 mmol/L) participated. Subjects using cholesterol-lowering medication or subjects that used antibiotics <1 mo prior to day 1 of the study were excluded (for details see reference 7). Subjects had no history of medical/surgical events that could affect the study outcomes. During the screening visit, subjects filled in an FFQ to determine habitual dietary intake. WG consumption was quantified by research dietitians using The Dutch Food Composition Database (NEVO) table 2010. This trial was approved by the Medical Ethical Committee of Wageningen University and registered at clinicaltrials.gov under NCT02385149. All participants gave written consent before participation.

Study design and procedures

This study was a randomized, controlled, double-blind, parallel trial (**Supplemental Figure 1**). Recruitment and study logistics are described in reference 7. Before the start of the intervention, a 4-wk run-in period with uncolored RW products was included to reduce WGW intake variation at baseline. Afterwards, subjects were randomly assigned to a 12-wk intervention with either WGW products or colored RW products. Age, gender, BMI, and cholesterol concentrations were stratified among the intervention groups. The randomization was conducted using block randomization (Microsoft Excel) by a researcher who was not involved in the study. Participants were asked to continue their dietary pattern and dietary habits during the intervention period. They were not allowed to lose or gain weight during the intervention. Before and after the 12-wk intervention, feces were collected. At test days, liver fat accumulation and metabolic health parameters were measured. Participants recorded stool consistency weekly using the Bristol Stool Chart, which describes seven types of stools ranging from 1: hard/lumpy to 7: watery without solid pieces. The day prior to the test day, participants consumed a standardized low-fat evening meal, refrained from alcohol or strenuous exercise, and were not allowed to eat or drink anything except water after 20:00 to ensure a fasting state.

Intervention products

Participants received either WGW or RW products to replace their habitual intake of grain products. Four slices of bread (in total 100 g/d),

and one serving of ready-to-eat-cereals (33.4 g/d) were consumed daily. In total, this added up to 98 g of RW or WGW flour per day. RW products were colored with roasted wheat malt and caramelized sugar to match the appearance of WGW products. On the macronutrient and energy level, the RW and WGW products were similar, except for fiber content. The WGW products contained 17.6 g fiber/100 g, and the RW products 7.2 g fiber/100 g. The nutritional composition (macronutrients, vitamins, and minerals) of the intervention products is provided in **Supplemental Table 1**. During the run-in and intervention periods, consumption of additional WG food products was not allowed, including products from other grain sources (i.e., brown rice), in both the WGW and RW groups. All participants received a list of WG products to avoid during the intervention. Subjects were allowed to complement their daily diet with additional refined grain products.

Clinical chemistry and IHTG accumulation

Lipid content in the liver was quantified with proton magnetic resonance spectroscopy using a 3-T whole-body MRI scanner (7). Plasma alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyltransferase (GGT), C-reactive protein (CRP), and serum amyloid A (SAA) were included as liver health markers, which were analyzed as described previously (7). β-Hydroxybutyrate was also included as marker because it can be synthesized in the liver via the metabolism of butyrate. As a biomarker for WGW intake, plasma alkylresorcinol was analyzed as described previously (7).

Fecal samples and microbiota profiling

Feces were collected at home ≤ 3 d before the test day using collection pots and equipment and were stored at $-20°C$ for ≤ 3 d. Samples were transferred to $-80°C$. Fecal material was mechanically homogenized, and genomic DNA was isolated with the use of an AGOWA mag Mini kit (AGOWA, Berlin, Germany) according to the manufacturer's instructions. The V4 hypervariable region of the 16S ribosomal RNA (rRNA) gene was PCR amplified using F515/R806 primers. PCR products were purified, followed by paired-end sequencing on an Illumina MiSeq platform, as described previously (7). Twenty-two technical replicate samples were included in the dataset, representing 2 aliquots taken from the same fecal sample at the same time from which all consecutive steps were performed separately to isolate DNA. Raw sequencing data were first demultiplexed by trimming barcodes and primer sequences. Afterwards, the data were processed and amplicon sequence variants (ASVs) were picked with NG-Tax using default settings (35, 36). Chimeras were detected and filtered when the forward and reverse read of that ASV was identical to two different ASVs, and the abundance of the matched ASVs were ≥ 2 times the abundance of that specific ASV. The SILVA reference database version 128 was used to assign taxonomy, with a confidence $>80\%$ for genus-level classification.

Microbiota composition analyses

R version 3.5.1 (R Foundation) was used for all analyses (37). Raw counts were transformed to relative abundance. Technical replicates were compared by calculating pairwise Pearson correlation using the genus-level relative abundance. Afterwards, bacterial abundance in technical replicates was averaged. α-Diversity was calculated using Faith phylogenetic diversity based on branch length connecting taxa in those samples and the root node of the phylogenetic tree. Pairwise weighted UniFrac (38) distance-based principal coordinate analysis was used to visualize overall microbial community variation (39). Permutational multivariate analysis of variance (PERMANOVA) (40) was used to test for significant differences in overall community composition between groups.

Prediction of microbiota functionality and markers for SCFA production

Microbial functions were predicted based on the 16S sequences using the PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) algorithm (41). The default workflow was followed (41). Compositional count data of predicted

TABLE 1 Baseline characteristics of middle-aged overweight and obese subjects in the RW or WGW group in a subset of the total study population[1]

Variables	RW group	WGW group	P value
Gender,[2] n males (%)	9 (60)	12 (60)	0.96
Age, y	60 ± 6.0	60 ± 5.4	0.97
Body weight, kg	84 ± 7.0	86 ± 9.4	0.36
BMI, kg/m^2	27 ± 2.2	28 ± 2.0	0.14
IHTGs,[3] %	3.6 ± 2.5	5.5 ± 6.4	0.83
Total cholesterol, mmol/L	6.0 ± 0.76	6.2 ± 0.71	0.38
CRP,[3] μg/mL	2.9 ± 3.1	4.8 ± 7.3	0.95
SAA,[3] μg/mL	2.1 ± 1.9	7.2 ± 16	0.76
GGT, U/L	19 ± 13	21 ± 13	0.61
AST, U/L	19 ± 5.6	19 ± 4.7	0.60
ALT, U/L	31 ± 9.4	36 ± 12	0.18
β-Hydroxybutyrate, mmol/L	0.30 ± 0.20	0.20 ± 0.20	0.043

[1]Values are presented as group means ± SD, $n = 16$ (RW) or $n = 21$ (WGW). ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; GGT, γ-glutamyltransferase; IHTG, intrahepatic triglyceride; RW, refined wheat; SAA, serum amyloid A; WGW, whole grain wheat.
[2]Presented as the number and percentage of males.
[3]Nonparametric distribution, evaluated with the Mann–Whitney U test.

pathways and Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs were transformed to relative abundance. More details are described in **Supplemental Methods**. KEGG orthologs were selected that could be used as markers for SCFA production (see Supplemental Methods).

Statistical analysis

The nonparametric Wilcoxon test was applied for paired comparisons of bacteria and predicted pathway relative abundances within intervention group over time, due to the nonnormal distribution of the data. The unpaired Mann–Whitney U test was used to determine the differences between groups at baseline and the differential changes over time (Δ) between intervention groups. Differences in baseline characteristics and FFQ data were assessed using independent t tests, or Mann–Whitney U tests, depending on the variable distribution. Bristol Stool Chart scores were analyzed using linear mixed effects modeling with interventions, time, and the interaction as fixed effects, and subject ID as a random effect (R package lme4). Correlations between (changes of) relative abundances of bacteria at the genus level and (changes of) liver health parameters between paired samples were assessed by Spearman rank correlation tests. Analyses were performed without and with adjustment for multiple comparisons using false discovery rate (FDR). Nonadjusted P values < 0.05 were considered significant, because modest changes were expected due to the explorative character of this study.

Results

Subject characteristics and stool consistency

Participants with missing fecal samples before or after the intervention ($n = 5$) or use of antibiotics during the intervention ($n = 8$) were removed from the analysis, resulting in 16 participants in the RW group, and 21 participants in the WGW group. No significant differences in baseline characteristics were found between groups, except for β-hydroxybutyrate ($P = 0.043$), which was higher in the RW group ([Table 1]). The mean ($\pm$ SD) habitual WG intake before the start of the intervention was 60.1 ± 59.2 g/d in the RW group, and 54.4 ± 38.0 g/d in the WGW group ($P = 0.73$; **Supplemental Table 2**). No significant differences in the habitual intake of WGs, total carbohydrates, or dietary fibers were found between groups. Habitual medication use did not change during intervention,
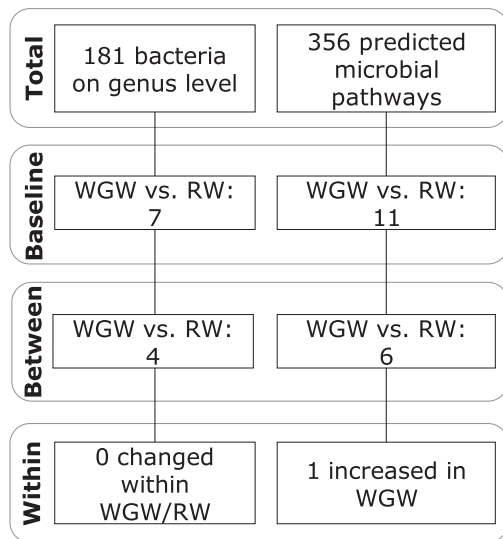
Total	181 bacteria on genus level	356 predicted microbial pathways
Baseline	WGW vs. RW: 7	WGW vs. RW: 11
Between	WGW vs. RW: 4	WGW vs. RW: 6
Within	0 changed within WGW/RW	1 increased in WGW

FIGURE 1 Significantly different fecal bacterial taxa and bacterial pathways at baseline and after 12-wk of an RW or WGW intervention in middle-aged overweight and obese adults. The flow diagram shows the number of bacterial taxa at the genus level and the predicted pathways for which the change in relative abundance was significantly different among interventions. RW, refined wheat; WGW, whole grain wheat.

and analgesic use during the whole study period was ≤15 d in total. Stool consistency showed a nonspecific trend during the run-in and intervention periods (**Supplemental Figure 2**), without a significant effect of time ($P = 0.58$) or intervention ($P = 0.64$) on the consistency score. The main findings in this subset of the study population reflected those in the total study population, published previously ([7]), namely RW significantly increased IHTGs compared with the WGW group ($P = 0.033$), and WGW consumption decreased SAA ($P = 0.042$) and increased β-hydroxybutyrate ($P = 0.003$), compared with the RW group (**Supplemental Table 3**).

Microbiota composition and predicted pathways at baseline

The microbiota composition and predicted pathway datasets were of high quality (**Supplemental Figure 3**), and all details are provided in the **Supplemental Results**. In total, 1450 unique ASVs were identified in the microbiota dataset within 181 unique genera, and 356 pathways were predicted to be active in the dataset. Substantial interindividual variation in microbiota composition at the genus level was observed at baseline (**Supplemental Figure 4**), without clear differences between intervention groups for the average composition. Without correcting for multiple testing, 7 bacterial taxa and 11 predicted pathways were significantly different between groups at baseline (**Figure 1**, **Supplemental Table 4**). After FDR correction, none of the bacterial taxa or pathways were significantly different at baseline.

The gut microbiota diversity

No significant clustering effect was found between baseline and postintervention within and between groups based on overall microbiota profiles (PERMANOVA $P = 0.63$, **Figure 2**A). However, the overall microbiota community of most individuals showed a shift over time. The change in microbial diversity (phylogenetic diversity, PD) (**Figure 2**B) was not significantly
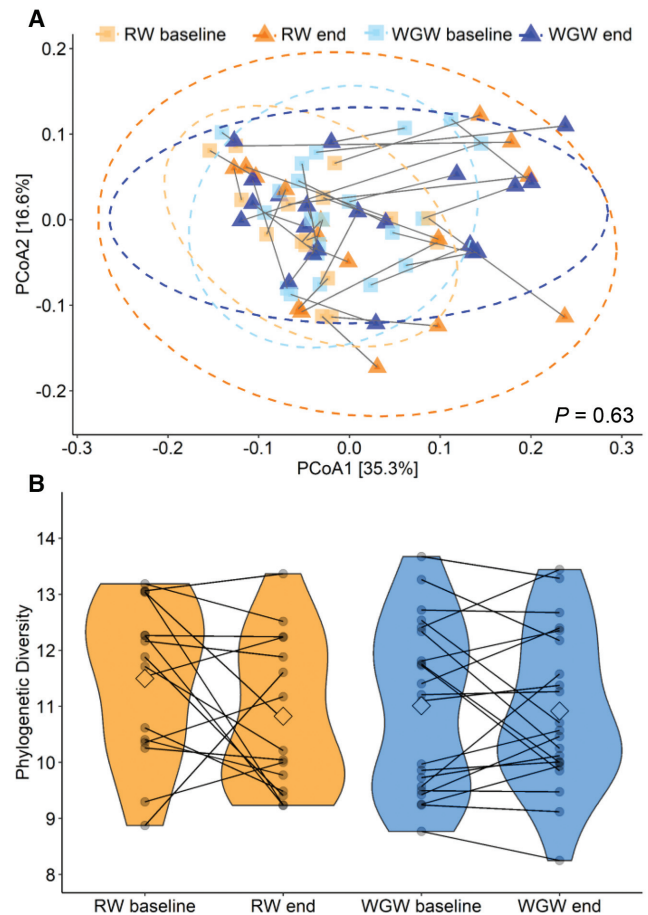
FIGURE 2 The effect of 12 wk of an RW or WGW intervention on the fecal microbiota diversity in middle-aged overweight and obese adults. (A) PCoA plot using weighted UniFrac dissimilarity to visualize the overall microbiota community variation. Color and shape highlight the intervention groups before or after the intervention. The lines connect the within-person samples over time; 95% CIs are plotted. (B) Microbial diversity as assessed using Faith phylogenetic diversity at baseline and after the 12-wk intervention (end). Individual paired samples are connected by a line. The width of the colored shapes indicates the sample density, the squared shape inside indicates the group mean. PCoA, principal coordinate analysis; RW, refined wheat; WGW, whole grain wheat.

different between the RW and WGW interventions ($P = 0.21$). Both RW and WGW interventions did not change microbial diversity (RW: baseline 11.5 ± 1.38 PD, $\Delta -0.67 \pm 1.66$ PD, $P = 0.19$; WGW: baseline 11.0 ± 1.50 PD, $\Delta -0.09 \pm 1.10$ PD, $P = 0.97$). On the group level, no differences were found in the overall microbiota community composition. Overall, the interventions changed neither microbiota phylogenetic diversity (α-diversity) nor the overall community composition (β-diversity).

The effect of RW and WGW interventions on the microbiota composition

Intervention effects on microbiota abundances were found at the genus level between and within the WGW and RW groups (**Table 2**). Between groups, 4 bacterial taxa were significantly different over time (**Figure 3**), namely *Ruminiclostridium_9* ($P = 0.003$), *Ruminococcaceae_NK4A214_group* ($P = 0.036$), *Lachnospiraceae_UCG-008* ($P = 0.044$), and *Ruminococcaceae_UCG-014* ($P = 0.033$), without

TABLE 2 Fecal bacterial taxa abundances at the genus level at baseline and change after 12 wk of intervention that were found to be significantly different within and/or between the WGW and RW group in middle-aged overweight and obese adults[1]

Bacteria	RW group Baseline relative abundance, %	RW group Δ Relative abundance after 12 wk, %	Within RW P value	Within RW FDR P value	WGW group Baseline relative abundance, %	WGW group Δ Relative abundance after 12 wk, %	Within WGW P value	Within WGW FDR P value	Group comparison P value	Group comparison FDR P value
f_Ruminococcaceae; g_Ruminiclostridium_9	0.20 ± 0.23	−0.081 ± 0.17	0.07	NS[2]	0.065 ± 0.11	0.054 ± 0.14	0.07	NS	0.003	NS
f_Ruminococcaceae; g_Ruminococcaceae_NK4A214_group	0.64 ± 1.3	−0.15 ± 0.49	0.12	NS	0.37 ± 0.56	0.17 ± 0.83	0.11	NS	0.036	NS
f_Lachnospiraceae; g_Lachnospiraceae_UCG-008	0.00 ± 0.00	0.043 ± 0.10	0.18	NS	0.10 ± 0.12	−0.023 ± 0.083	0.19	NS	0.044	NS
f_Ruminococcaceae; g_Ruminococcaceae_UCG-014	1.6 ± 2.1	−0.31 ± 0.78	0.12	NS	2.2 ± 4.6	0.51 ± 4.2	0.13	NS	0.033	NS
f_Lachnospiraceae; g_Dorea	1.7 ± 1.1	−0.72 ± 0.91	0.005	NS	1.6 ± 0.81	−0.018 ± 0.99	0.61	NS	0.055	NS
f_Tannerellaceae; g_Parabacteroides	0.37 ± 0.30	0.60 ± 1.42	0.007	NS	0.45 ± 0.41	0.35 ± 0.97	0.18	NS	0.32	NS
f_Lachnospiraceae; g_[Eubacterium]_ventriosum_group	0.17 ± 0.23	−0.14 ± 0.24	0.044	NS	0.14 ± 0.14	−0.050 ± 0.15	0.71	NS	0.18	NS
f_Peptostreptococcaceae_g_unknown	1.2 ± 2.8	−0.64 ± 1.5	0.024	NS	0.56 ± 0.71	−0.23 ± 0.69	0.18	NS	0.57	NS
f_Ruminococcaceae; g_Ruminococcus_2	1.4 ± 1.3	−0.24 ± 1.5	0.11	NS	2.1 ± 1.8	−1.1 ± 2.1	0.024	NS	0.44	NS
f_Ruminococcaceae; g_Subdoligranulum	6.8 ± 4.7	−1.7 ± 4.6	0.10	NS	6.1 ± 5.3	−1.8 ± 3.7	0.024	NS	0.89	NS
f_Burkholderiaceae; g_Sutterella	0.27 ± 0.44	0.085 ± 0.45	0.31	NS	0.35 ± 0.92	0.30 ± 0.49	0.010	NS	0.46	NS
f_Ruminococcaceae; g_Ruminococcaceae_UCG-005	1.1 ± 1.1	0.38 ± 1.5	0.75	NS	0.91 ± 1.1	0.21 ± 1.0	0.042	NS	0.47	NS

[1] Values are presented as group means ± SD, n = 16 (RW) or n = 21 (WGW). FDR, false discovery rate; RW, refined wheat; WGW, whole grain wheat; Δ, differential change over time.
[2] NS, nonsignificant: P value > 0.05.

significant differences within intervention groups. Not all of the aforementioned bacterial taxa were present in all individuals; in **Supplemental Table 5**, the number of subjects in which the bacterial taxa were detected is shown. Overall, the WGW intervention increased 3 bacterial taxa (within the *Ruminococcaceae* family) and decreased 1 bacterial taxon (within the *Lachnospiraceae* family), whereas the RW intervention mostly decreased these bacteria.

Correlations between the microbiota and liver health parameters

The relative abundances of all bacteria at baseline were then correlated with baseline IHTGs. Significant positive correlations were found between IHTG and *Roseburia* (**Figure 4**, $\rho = 0.38$, $P = 0.025$), *Ruminococcus_2* ($\rho = 0.39$, $P = 0.021$), and *Faecalibacterium* ($\rho = 0.39$, $P = 0.020$). Significant negative correlations were found between IHTGs and *Ruminococcaceae_UCG-010* ($\rho = -0.38$, $P = 0.026$), *Ruminococcaceae_UCG-005* ($\rho = -0.40$, $P = 0.018$), and *Akkermansia* ($\rho = -0.36$, $P = 0.035$). After removal of 2 outliers (18% and 26% IHTG), the correlation between IHTGs and *Ruminococcus_2* was still significant ($P = 0.050$), whereas for the other bacteria there was a trend toward significance (P values between 0.05 and 0.10). After FDR correction, none of these correlations were significant.

Correlations between changes in liver health markers including IHTGs, ALT, AST, GGT, SAA, CRP, and β-hydroxybutyrate with changes in bacterial abundances after 12 wk of intervention were tested (**Supplemental Figure 5**). Change in IHTGs was positively correlated with change in *Ruminococcaceae_NK4A214_group* abundance in both the WGW ($\rho = 0.54$, $P = 0.026$) and RW ($\rho = 0.67$, $P = 0.024$) groups (**Table 3**). Moreover, change in ALT was significantly correlated with changes of 2 bacterial taxa in the RW and 2 in the WGW intervention. Change in AST was correlated with changes of 3 bacterial taxa in the RW group, 1 in the WGW group, and 1 (*Lachnoclostridium*) in both groups. Changes in GGT or SAA were significantly correlated with changes in abundance of 1 bacterial taxon in the RW group and 1 in the WGW group, or 3 bacterial taxa in the WGW group, respectively. Change in CRP was correlated with changes of 3 bacterial taxa in the RW group. Furthermore, change in β-hydroxybutyrate was positively correlated with 3 bacterial taxa in the RW group and 1 (*Streptococcus*) in both groups. After FDR correction, none of these correlations were significant.

Changes in predicted microbial pathways

The intervention effects on predicted microbial pathways were investigated. Between the WGW and the RW interventions, 6 predicted pathways were significantly different over time (**Table 4**), namely hexitol degradation, pantothenate and coenzyme A biosynthesis, acetyl-CoA fermentation to butyrate II, pyruvate fermentation to acetone, aromatic biogenic amine degradation, and L-alanine biosynthesis. Subjects in both groups displayed a variation in response over time for the significantly different predicted pathways (**Figure 5**). Within the WGW group, acetyl-CoA fermentation to butyrate II was significantly increased ($P = 0.017$). Within the RW group, a second pathway related to fermentation was predicted to be decreased, namely pyruvate fermentation to acetate and lactate II ($P = 0.049$; **Table 4**).
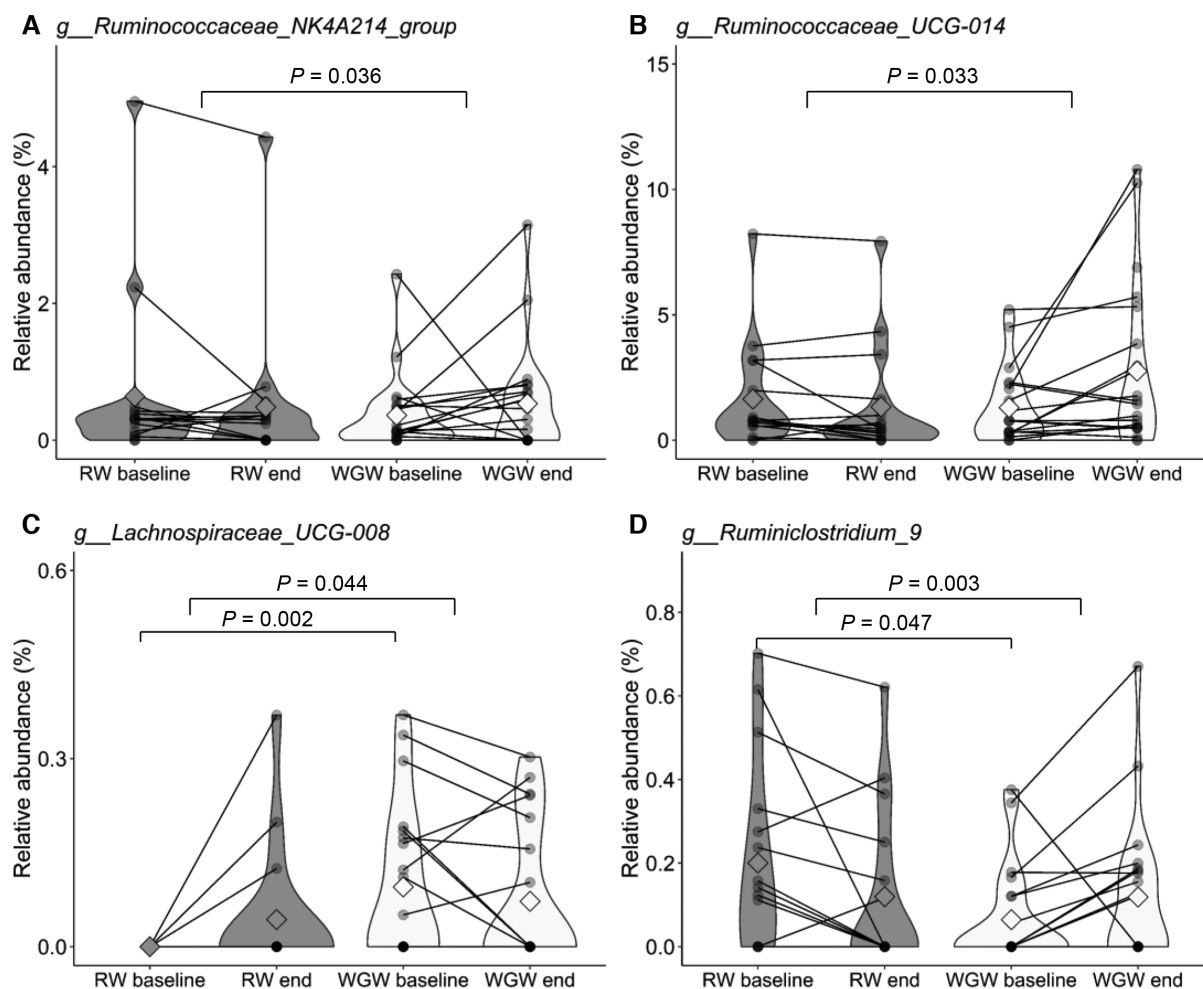
FIGURE 3 Fecal bacterial taxa at the genus level at baseline and after the 12-wk RW or WGW intervention (end) that were found to be significantly different between the groups in middle-aged overweight and obese adults. The relative abundances of (A) *Ruminococcaceae_NK4A214_group*; (B) *Ruminococcaceae_UCG-014*; (C) *Lachnospiraceae_UCG-008*; and (D) *Ruminiclostridium_9* are shown. Data are presented as group mean (the squared shape), *n* = 16 (RW) or *n* = 21 (WGW), and the width of the colored shapes indicates the sample density. Individual paired samples are connected by a line. RW, refined wheat; WGW, whole grain wheat.

Because changes in predicted fermentation pathways were found, we examined the effect of the interventions on selected genes involved in SCFA production (**Supplemental Table 6**). No significant differences in changes of these predicted genes were observed over time between groups, but butyrate kinase was significantly increased within the WGW group (*P* = 0.038), and phosphate acetyltransferase was significantly decreased within the RW group (*P* = 0.021). Overall, WGW intervention showed a trend toward increased predicted fermentation pathways by the microbiota, whereas RW intervention showed opposite effects.

Discussion

We investigated the effects of 12-wk consumption of RW or WGW products on the gut microbiota composition and predicted microbiota functional pathways in men and women with overweight or obesity. We found significant differences between the WGW and RW intervention for a number of bacterial taxa from carbohydrate-degrading families and on predicted fermentation pathways, with a trend toward increased fermentation to butyrate within the WGW group, and a

trend toward decreased fermentation within the RW group (a graphical overview is provided in **Supplemental Figure 6**). Although WGW consumption is already quite high in The Netherlands, we provided an intervention with 98 g/d WGW, which was higher than the mean habitual WG intake at baseline of participants in both the RW and WGW groups, of 60.1 and 54.4 g/d WG, respectively.

Even though the difference in fiber between the WGW and RW intervention was 10 g/d, we observed only subtle differences between intervention groups over time with respect to microbiota composition and functionality, and no effect on stool consistency. Microbial diversity within samples as calculated previously by the Shannon Index was decreased after RW intervention in Schutte et al. (7), but we did not find a significant difference in microbial diversity changes between groups in a subset of study participants with the phylogenetic diversity index, which takes into account phylogenetic relatedness between bacteria. Twelve weeks of a WGW intervention increased relative abundances of *Ruminiclostridium_9*, *Ruminococcaceae_NK4A214_group*, and *Ruminococcaceae_UCG-014*, and decreased *Lachnospiraceae_UCG-008*, whereas a 12-wk RW intervention decreased abundance, without significant effects within
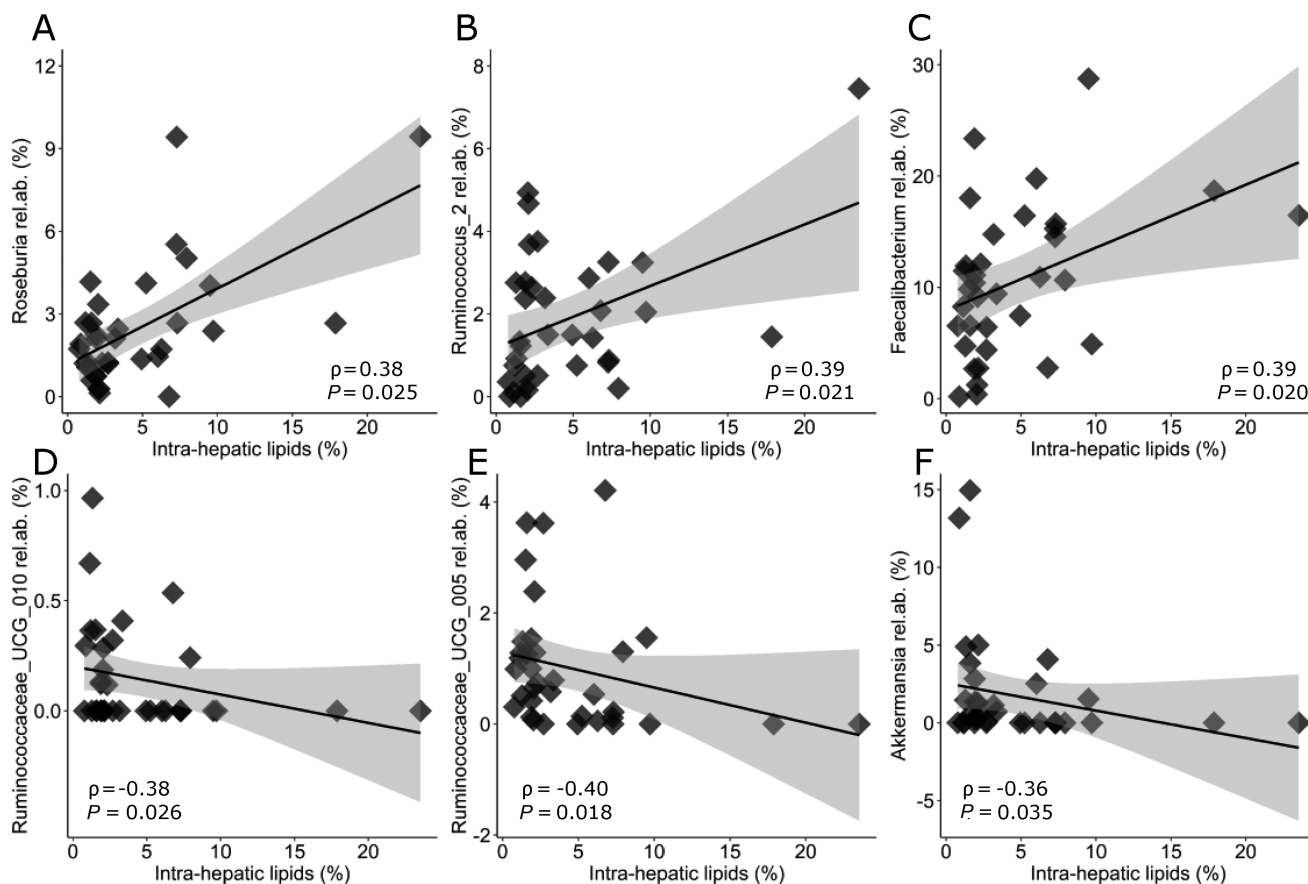
FIGURE 4 Fecal bacterial taxa at the genus level that were found to be significantly correlated with IHTGs at baseline in middle-aged overweight and obese adults. The relative abundances of (A) *Roseburia*; (B) *Ruminococcus_2*; (C) *Faecalibacterium*; (D) *Ruminococcaceae_UCG-010*; (E) *Ruminococcaceae_UCG-005*; and (F) *Akkermansia* are shown, fitted with a linear regression model with a 95% CI. Noncorrected *P* values are shown. Data of *n* = 35 participants are shown, who had both microbiota and IHTG data at baseline available. IHTG, intrahepatic triglyceride.

the groups. *Ruminococcaceae* genera have previously been shown to increase after a resistant starch or nonstarch-polysaccharide diet high in wheat bran mainly comprised of hemicellulose (42, 43). Members of the *Ruminococcaceae* can degrade cellulose and hemicellulose fibers (44–46), also present in WGW, whereas members of the *Lachnospiraceae* are known to ferment a wide variety of fibers (44, 46). In line with our results, Vuholm et al. (47) found an increase in an unassigned genus of the family *Ruminococcaceae* after 6 wk of WGW consumption, in healthy overweight females.

We found the change in relative abundance of *Ruminococcaceae_NK4A214_group* and the intervention-induced change in liver fat correlated in both groups. Several studies showed that *Ruminococcaceae* was lower in the feces of NAFLD patients compared with controls (48–50). However, in this study we found a positive correlation between a change in *Ruminococcaceae_NK4A214_group* and a change in liver fat in both intervention groups, although the RW intervention increased liver fat and decreased abundance of *Ruminococcaceae_NK4A214_group* indicating that the changes relative to each other did not have a strong correlation. At baseline, however, we found a positive correlation between liver fat and *Ruminococcaceae_UCG-010*, and *Ruminococcaceae_UCG-005*. We could not identify any human trials involving these specific bacteria in relation to liver fat. As in many previous dietary trials, the microbiota response to diet was subject-dependent. The microbiota at

the start of intervention of any individual can determine the magnitude of response upon dietary changes (43). Korem et al. (51) found that the gut microbiota composition was person-specific and generally resilient to bread interventions, but their intervention lasted only 1 wk whereas our study encompassed a 12-wk intervention. In contrast to previous findings (17, 18), WGW consumption did not increase *Bifidobacterium* and *Lactobacillus* in our study. This discrepancy could be partly explained by differences in the fermentable fiber composition and fractions in the intervention products. For instance, fructans can have prebiotic activity by inducing specific changes in the composition and/or activity of the microbiota such as *Bifidobacterium* and *Lactobacillus* (52). It has previously been described that the concentration of fructans differs in WGW (53).

Ruminococcaceae and *Lachnospiraceae*, together with *Bacteroidetes*, encompass ∼85% of the total butyrate-producing potential of the gut microbiota (54). Changes in bacterial taxa within the *Ruminococcaceae* might point toward an effect on carbohydrate breakdown, which could fit the predicted changes in SCFA fermentation pathways in feces. WGW increased the predicted relative abundance of fermentation to butyrate, whereas RW lowered the fermentation to butyrate as well as fermentation to acetone. The latter pathway can lead to formation of products such as acetone, butanol, ethanol, acetate, and butyrate. Moreover, a decreased fermentation to acetate and lactate was found within the RW group. Lactate-utilizing bacteria can use acetate and lactate for the

TABLE 3 The significant correlations between changes in fecal bacterial abundances at the genus level, and changes in liver health parameters after 12 wk of an RW or WGW intervention in middle-aged overweight and obese adults[1]

Taxa (Δ 12 wk)	RW			WGW		
	Spearman ρ	P value	FDR P value	Spearman ρ	P value	FDR P value
IHTG (Δ 12 wk)						
g_Ruminococcaceae_NK4A214_group	0.67	0.024	NS[2]	0.54	0.026	NS
ALT (Δ 12 wk)						
g_Bacteroides	0.30	NS	NS	− 0.44	0.048	NS
g_Bifidobacterium	0.77	0.001	NS	− 0.14	NS	NS
g_Faecalibacterium	− 0.11	NS	NS	− 0.46	0.037	NS
g_Lachnoclostridium	0.60	0.014	NS	− 0.20	NS	NS
AST (Δ 12 wk)						
g_[Eubacterium]_coprostanoligenes_group	0.53	0.034	NS	0.065	NS	NS
g_Butyricicoccus	0.51	0.045	NS	− 0.22	NS	NS
g_Lachnoclostridium	0.51	0.045	NS	− 0.48	0.027	NS
g_Lachnospiraceae_NK4A136_group	0.53	0.036	NS	0.35	NS	NS
g_Ruminiclostridium_6	0.10	NS	NS	0.46	0.036	NS
CRP (Δ 12 wk)						
g_Butyricicoccus	− 0.58	0.025	NS	− 0.052	NS	NS
g_Lachnospiraceae_NK4A136_group	− 0.68	0.007	NS	0.36	NS	NS
g_Parabacteroides	0.54	0.041	NS	0.25	NS	NS
g_Uncultured	− 0.59	0.023	NS	− 0.16	NS	NS
GGT (Δ 12 wk)						
g_[Eubacterium]_coprostanoligenes_group	− 0.21	NS	NS	0.50	0.021	NS
g_Erysipelotrichaceae_UCG-003	0.55	0.028	NS	− 0.071	NS	NS
β-Hydroxybutyrate (Δ 12 wk)						
g_Erysipelotrichaceae_UCG-003	− 0.027	NS	NS	0.67	0.002	NS
g_Lachnospiraceae_ND3007_group	− 0.10	NS	NS	0.48	0.036	NS
g_Ruminococcus_2	0.13	NS	NS	0.46	0.049	NS
g_Streptococcus	0.61	0.011	NS	0.48	0.040	NS
SAA (Δ 12 wk)						
g_Anaerostipes	− 0.23	NS	NS	0.52	0.018	NS
g_Blautia	− 0.29	NS	NS	0.53	0.015	NS
g_Butyricicoccus	− 0.46	NS	NS	0.46	0.036	NS

[1]Values represent the Spearman ρ correlation coefficients, $n = 16$ (RW) or $n = 21$ (WGW). ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; FDR, false discovery rate; GGT, γ-glutamyltransferase; IHTG, intrahepatic triglyceride; RW, refined wheat; SAA, serum amyloid A; WGW, whole grain wheat; Δ, differential change over time.

[2]NS, nonsignificant: P value > 0.05.

production of butyrate (55), indicating that a lowered capacity to produce acetate and lactate potentially leads to a reduced butyrate production. Despite the run-in period, the RW group showed a higher relative abundance for the predicted pyruvate fermentation to acetone pathway and for *Ruminiclostridium_9* compared with the WGW group at baseline. Both were decreased after RW and increased after WGW. Similarly, a higher abundance of *Lachnospiraceae_UCG-008* was observed in the RW group at baseline, which increased after RW and decreased after WGW. Therefore, the differential effects on this pathway and these bacteria between RW or WGW intervention could have been partly caused by regression to the mean. In this study, the effects on microbiota and predicted pathways lost statistical significance after correction for multiple comparisons, which shows that a 12-wk WGW and RW intervention induced subtle changes in gut microbiota. This is not surprising considering the relatively modest modulation of diet, only altering wheat products.

In line with our finding that WGW increased 2 predicted fermentation pathways, Vanegas et al. (20) reported increased concentrations of acetate and total SCFAs in feces after 6 wk of a WG, predominantly wheat, intervention compared with refined grain in middle-aged adults. Moreover, Vuholm

et al. (47) found that a 6-wk RW intervention decreased fecal butyrate concentrations when compared with a WGW intervention in adults with overweight. Additionally, a study in rats showed that a 6-wk WGW intervention increased total SCFAs in colonic content and butyrate in cecum content compared with RW intervention (56). Resistant starch and nonstarch polysaccharide inside the whole-grain matrix were associated with increased production of metabolites (57). Overall, incorporating feasible doses of WGW in the diet favorably affects the gut microbiota phenotype as indicated by the increased predicted potential to produce butyrate. These findings can be explained by the decreased fiber content in the RW intervention products (12, 13), although only a fraction of the 17.6 g/d fiber in the WGW intervention is fermentable. The fermentability of fibers in WGW is relatively low, for instance, when compared with fibers in WG rye (58, 59). Eriksen et al. (60) showed that WG rye resulted in increased *Bifidobacterium*, known to be stimulated by some fermentable fibers, in men with the metabolic syndrome, whereas WG wheat did not.

The reduced potential to produce SCFAs after RW intervention can have implications for health. For instance, SCFAs can decrease intestinal inflammation, as demonstrated by human

TABLE 4 Predicted fecal microbial pathway relative abundance at baseline and change after 12 wk of intervention that were found to be significantly different within and/or between the WGW and RW groups in middle-aged overweight and obese adults[1]

Pathway name	Pathway number	RW group Baseline relative abundance, %	RW group Δ Relative abundance after 12 wk, %	WGW group Baseline relative abundance, %	WGW group Δ Relative abundance after 12 wk, %	Within RW P value	Within RW FDR P value	Within WGW P value	Within WGW FDR P value	Group comparison P value	Group comparison FDR P value
Superpathway of hexitol degradation	HEXITOLDEGSUPER.PWY	0.13 ± 0.049	−0.015 ± 0.056	0.13 ± 0.065	−0.00 ± 0.056	0.70	NS[2]	0.97	NS	0.044	NS
Pantothenate and coenzyme A biosynthesis	PANTOSYN.PWY	0.58 ± 0.057	0.022 ± 0.039	0.55 ± 0.054	0.00 ± 0.034	0.11	NS	0.76	NS	0.022	NS
Acetyl-CoA fermentation to butyrate II	PWY.5676	0.26 ± 0.079	−0.026 ± 0.081	0.23 ± 0.062	0.035 ± 0.059	0.12	NS	0.017	NS	0.031	NS
Pyruvate fermentation to acetone	PWY.6588	0.28 ± 0.12	−0.044 ± 0.12	0.20 ± 0.087	0.017 ± 0.068	0.32	NS	0.18	NS	0.043	NS
Aromatic biogenic amine degradation	PWY.7431	0.010 ± 0.010	0.00 ± 0.010	0.010 ± 0.010	0.0059 ± 0.012	0.30	NS	0.56	NS	0.047	NS
L-Alanine biosynthesis	PWY0.1061	0.36 ± 0.13	−0.067 ± 0.16	0.32 ± 0.10	−0.010 ± 0.12	0.30	NS	0.55	NS	0.041	NS
L-Arginine degradation II	AST.PWY	0.0048 ± 0.010	0.00 ± 0.011	0.010 ± 0.023	−0.00 ± 0.012	0.048	NS	0.23	NS	0.15	NS
L-Lysine biosynthesis I	DAPLYSIESYN.PWY	0.77 ± 0.10	−0.065 ± 0.14	0.75 ± 0.079	−0.030 ± 0.079	0.041	NS	0.15	NS	0.82	NS
(Kdo)2-lipid A biosynthesis	KDO.NAGLIPASYN.PWY	0.024 ± 0.036	−0.010 ± 0.045	0.024 ± 0.045	0.010 ± 0.027	0.043	NS	0.28	NS	0.50	NS
Pyruvate fermentation to acetate and lactate II	PWY.5100	0.94 ± 0.14	−0.049 ± 0.086	0.91 ± 0.084	−0.020 ± 0.071	0.049	NS	0.79	NS	0.13	NS
PreQ0 biosynthesis	PWY.6703	0.32 ± 0.063	0.057 ± 0.077	0.31 ± 0.054	0.021 ± 0.044	0.046	NS	0.82	NS	0.95	NS
Vitamin K-2 biosynthesis	PWY.5845	0.023 ± 0.029	0.00 ± 0.038	0.025 ± 0.039	0.0022 ± 0.025	0.19	NS	0.037	NS	0.52	NS
Superpathway of ubiquinol-8 biosynthesis	UBISYN.PWY	0.025 ± 0.023	0.00 ± 0.022	0.028 ± 0.028	0.0013 ± 0.021	0.61	NS	0.024	NS	1.0	NS

[1]Values are presented as group means ± SD, $n = 16$ (RW) or $n = 21$ (WGW). FDR, false discovery rate; PWY, pathway; RW, refined wheat; WGW, whole grain wheat; Δ, differential change over time.
[2]NS, nonsignificant: P value >0.05.

and animal in vivo studies (61). In addition to local health effects, SCFAs might also influence liver fat through stimulation of hepatic fat oxidation via activation of AMP-activated protein kinase (28, 29). As published previously, in this trial, the 12-wk RW intervention significantly increased liver fat, whereas liver fat did not change in the WGW group (7). Therefore, we hypothesized that RW might increase liver fat content indirectly via decreased cereal fiber fermentation and SCFA production. We found that butyrate-producing (62) *Roseburia* positively correlated with liver fat at baseline. In line with our data, the study of Raman et al. (48) showed a significant overrepresentation of *Roseburia* in the microbiota of NAFLD patients. In contrast, *Roseburia* was decreased in nonobese NAFLD patients compared with healthy controls, and this depletion was linked to increased plasma ALT in 126 nonobese subjects (63). Several of our correlations of other bacteria with liver fat at baseline were in line with previous findings on bacterial composition in NAFLD patients compared with controls (64, 65). This was the case for *Ruminococcus_2* (64) and *Akkermansia* (65), but for *Faecalibacterium* opposite effects were observed (50). Although a previous study showed that ALT and GGT were correlated with changes in fecal bacteria over time in a prospective, cross-sectional study (63), we could not confirm these findings in our correlation analysis. Although correlations do not provide information on causality, these outcomes might be of interest for future studies into the relation between WGW, RW, and liver health.

A limitation of our study is the analysis of microbiota functional metagenomes by PICRUSt2 based on the 16S rRNA sequencing data, instead of using more direct methods, such as metagenomics. Although the quality control indicated that closely related reference genomes were available for the bacteria present in this dataset, indicative for reliable pathway predictions, PICRUSt is subject to inherit biases. Another restraint of our study is that the SCFA concentrations in feces were not measured. Therefore, the predicted pathway findings as well as the potential role of SCFAs for liver health could not be validated in this study. Another limitation of our study is the small sample size of 37 subjects. Because the study was powered to detect changes in plasma cholesterol concentrations and participants had to be excluded due to missing fecal samples or antibiotic use, we might have missed the effects of either RW or WGW on the microbiota or predicted microbiota functionality.

The strengths of our study include the long study duration of 16 wk in total, and the good compliance with the diet based upon alkylresorcinol data (7). Therefore, our findings could show more long-term changes rather than acute effects. Comparisons with outcomes of existing literature about WGW, RW, and microbiota composition revealed few similarities with our trial (17, 18, 19, 24, 47). Some previous trials could have missed the subtle effects of WGW and RW on the microbiota because only a few selected bacterial taxa were targeted in these studies via, for example, qPCR (17, 18, 24), whereas the fecal microbiota is a highly complex community with multiple species present. The use of 16S rRNA sequencing provided important insights into the effect of WGW and RW on microbiota as a whole, as well as effects on predicted microbial community functionality.

In conclusion, we demonstrated that a 12-wk 98 g/d WGW intervention increased relative abundances of a number of bacterial taxa that are involved in carbohydrate degradation and SCFA production and predicted fermentation pathways, whereas an RW intervention decreased abundance of these bacteria or predicted fermentation capacity, pointing toward a
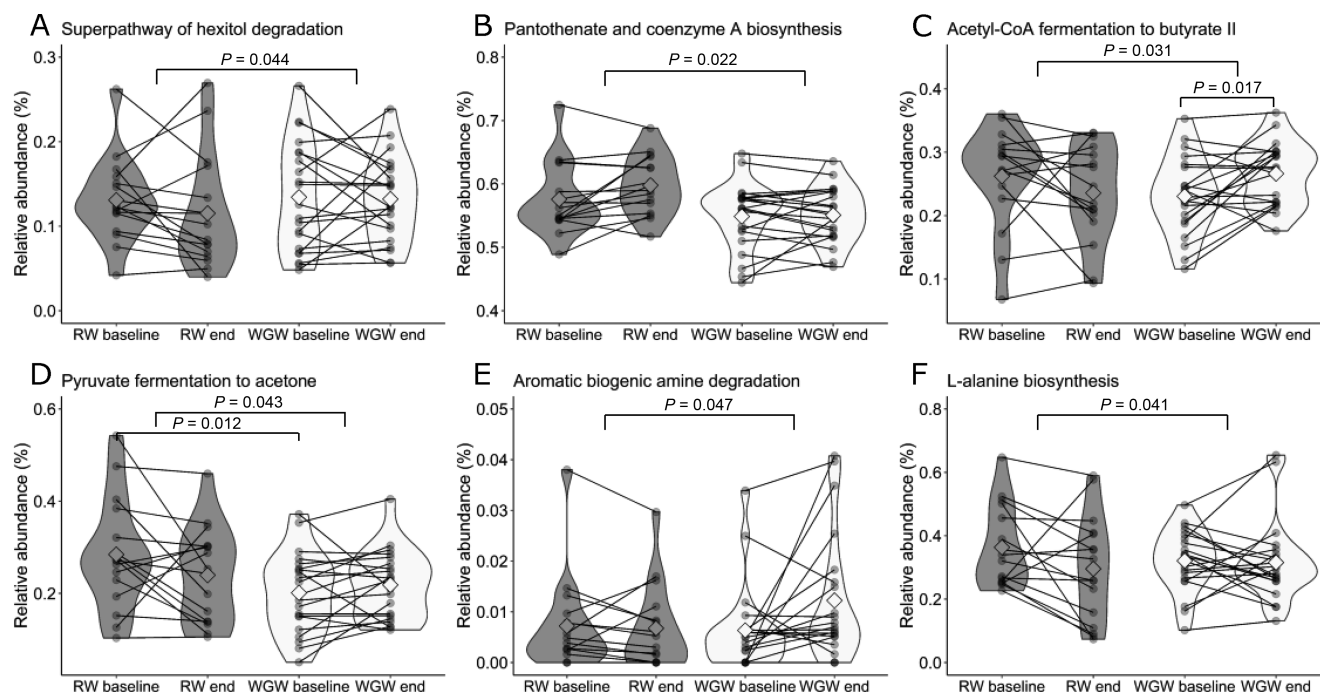
FIGURE 5 Predicted fecal microbial pathway relative abundance at baseline and after 12 wk of RW or WGW intervention that were found to be significantly different between the groups in middle-aged overweight and obese adults. The relative abundances of the predicted pathways (A) superpathway of hexitol degradation; (B) pantothenate and coenzyme A biosynthesis; (C) acetyl-CoA fermentation to butyrate II; (D) pyruvate fermentation to acetone; (E) aromatic biogenic amine degradation; and (F) L-alanine biosynthesis are shown. Data are presented as group mean (the squared shape), $n = 16$ (RW) or $n = 21$ (WGW), and the width of the colored shapes indicates the sample density. Individual paired samples are connected by a line. RW, refined wheat; WGW, whole grain wheat.

less healthy gut microbiota phenotype. The difference in fiber intake during the WGW intervention (17.6 g/d fiber) compared with the RW intervention (7.2 g/d fiber) likely resulted in differences in predicted bacterial fermentation capacity. This may be one of the mechanisms underlying the significant increases of liver fat observed with RW intervention. Potential health effects of replacement of RW by WGW via modulation of the microbiota, and consequent protective effects on metabolic organs such as the liver, deserve further investigation.

References

1. de Munter JS, Hu FB, Spiegelman D, Franz M, van Dam RM. Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. PLoS Med. 2007;4(8):e261.

2. Truswell A. Cereal grains and coronary heart disease. Eur J Clin Nutr 2002;56(1):1.

3. Sahyoun NR, Jacques PF, Zhang XL, Juan W, McKeown NM. Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. Am J Clin Nutr 2006;83(1):124–31.

4. Ross AB, Godin J-P, Minehira K, Kirwan JP. Increasing whole grain intake as part of prevention and treatment of nonalcoholic fatty liver disease. Int J Endocrinol 2013;2013:585876.

5. Bartłomiej S, Justyna R-K, Ewa N. Bioactive compounds in cereal grains—occurrence, structure, technological significance and nutritional benefits—a review. Food Sci Technol Int 2012;18(6):559–68.

6. McKevith B. Nutritional aspects of cereals. Nutr Bull 2004;29(2):111–42.

7. Schutte S, Esser D, Hoevenaars FP, Hooiveld GJ, Priebe MG, Vonk RJ, Wopereis S, Afman LA. A 12-wk whole-grain wheat intervention protects against hepatic fat: the Graandioos study, a randomized trial in overweight subjects. Am J Clin Nutr 2018;108(6):1264–74.

8. Kirwan JP, Malin SK, Scelsi AR, Kullman EL, Navaneethan SD, Pagadala MR, Haus JM, Filion J, Godin JP, Kochhar S, et al. A whole-grain diet reduces cardiovascular risk factors in overweight and obese adults: a randomized controlled trial. J Nutr 2016;146(11):2244–51.

9. Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, Mutch W, Wahle K, Horgan G, Thies F. Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: a randomized controlled trial. Am J Clin Nutr 2010;92(4):733–40.

10. Holländer PL, Ross AB, Kristensen M. Whole-grain and blood lipid changes in apparently healthy adults: a systematic review and meta-analysis of randomized controlled studies. Am J Clin Nutr 2015;102(3):556–72.

11. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev 2010;23(1):65–134.

12. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. Cell Host Microbe 2018;23(6):705–15.

13. Holscher HD. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut microbes 2017;8(2):172–84.

14. Kortman GA, Dutilh BE, Maathuis AJ, Engelke UF, Boekhorst J, Keegan KP, Nielsen FG, Betley J, Weir JC, Kingsbury Z. Microbial metabolism shifts towards an adverse profile with supplementary iron in

the TIM-2 in vitro model of the human colon. Front Microbiol 2016;6: 1481.

15. Fehlbaum S, Prudence K, Kieboom J, Heerikhuisen M, van den Broek T, Schuren F, Steinert R, Raederstorff D. In vitro fermentation of selected prebiotics and their effects on the composition and activity of the adult gut microbiota. IJMS 2018;19(10):3097.

16. Jefferson A, Adolphus K. The effects of intact cereal grain fibers, including wheat bran on the gut microbiota composition of healthy adults: a systematic review. Front Nutr 2019;6(33).

17. Christensen EG, Licht TR, Kristensen M, Bahl MI. Bifidogenic effect of whole-grain wheat during a 12-week energy-restricted dietary intervention in postmenopausal women. Eur J Clin Nutr 2013;67(12):1316–21.

18. Costabile A, Klinder A, Fava F, Napolitano A, Fogliano V, Leonard C, Gibson GR, Tuohy KM. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br J Nutr 2008;99(1):110–20.

19. Vitaglione P, Mennella I, Ferracane R, Rivellese AA, Giacco R, Ercolini D, Gibbons SM, La Storia A, Gilbert JA, Jonnalagadda S, et al. Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. Am J Clin Nutr 2015;101(2):251–61.

20. Vanegas SM, Meydani M, Barnett JB, Goldin B, Kane A, Rasmussen H, Brown C, Vangay P, Knights D, Jonnalagadda S. Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. Am J Clin Nutr 2017;105(3):635–50.

21. Lappi J, Salojärvi J, Kolehmainen M, Mykkänen H, Poutanen K, de Vos WM, Salonen A. 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. J Nutr 2013;143(5):648–55.

22. Ampatzoglou A, Atwal KK, Maidens CM, Williams CL, Ross AB, Thielecke F, Jonnalagadda SS, Kennedy OB, Yaqoob P. Increased whole grain consumption does not affect blood biochemistry, body composition, or gut microbiology in healthy, low-habitual whole grain consumers. J Nutr 2015;145(2):215–21.

23. Cooper DN, Kable ME, Marco ML, De Leon A, Rust B, Baker JE, Horn W, Burnett D, Keim NL. The effects of moderate whole grain consumption on fasting glucose and lipids, gastrointestinal symptoms, and microbiota. Nutrients 2017;9(2):173.

24. Bird AR, Vuaran MS, King RA, Noakes M, Keogh J, Morell MK, Topping DL. Wholegrain foods made from a novel high-amylose barley variety (Himalaya 292) improve indices of bowel health in human subjects. Br J Nutr 2008;99(5):1032–40.

25. Slavin J. Why whole grains are protective: biological mechanisms. Proc Nutr Soc 2003;62(1):129–34.

26. Boets E, Gomand SV, Deroover L, Preston T, Vermeulen K, Preter V, Hamer HM, den Mooter G, Vuyst L, Courtin CM. Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. J Physiol 2017;595(2): 541–55.

27. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015;11(10):577.

28. den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, Oosterveer MH, Jonker JW, Groen AK, Reijngoud D-J. Short-chain fatty acids protect against high-fat diet-induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. Diabetes 2015;64(7):2398–408.

29. Mollica MP, Raso GM, Cavaliere G, Trinchese G, De Filippo C, Aceto S, Prisco M, Pirozzi C, Di Guida F, Lama A. Butyrate regulates liver mitochondrial function, efficiency, and dynamic, in insulin resistant obese mice. Diabetes 2017;66(5):1405–18.

30. Kobyliak N, Abenavoli L, Mykhalchyshyn G, Kononenko L, Boccuto L, Kyriienko D, Dynnyk O. A multi-strain probiotic reduces the fatty liver index, cytokines and aminotransferase levels in NAFLD patients: evidence from a randomized clinical trial. J Gastrointestin Liver Dis 2018;27(1):41–9.

31. Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, Mastrojeni S, Malaguarnera G, Mistretta A, Li Volti G, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci 2012;57(2):545–53.

32. Manzhalii E, Virchenko O, Falalyeyeva T, Beregova T, Stremmel W. Treatment efficacy of a probiotic preparation for non-alcoholic steatohepatitis: a pilot trial. J Dig Dis 2017;18(12):698–703.

33. Aller R, De Luis DA, Izaola O, Conde R, Gonzalez Sagrado M, Primo D, De La Fuente B, Gonzalez J. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci 2011;15(9):1090–5.

34. Hoevenaars FPM, Esser D, Schutte S, Priebe MG, Vonk RJ, van den Brink WJ, van der Kamp JW, Stroeve JHM, Afman LA, Wopereis S. Whole grain wheat consumption affects postprandial inflammatory response in a randomized controlled trial in overweight and obese adults with mild hypercholesterolemia in the Graandioos study. J Nutr 2019;149(12):2133–44.

35. Ramiro-Garcia J, Hermes GD, Giatsis C, Sipkema D, Zoetendal EG, Schaap PJ, Smidt H. NG-Tax, a highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes. F1000Res 2018;5:1791.

36. Poncheewin W, Hermes GDA, van Dam JCJ, Koehorst JJ, Smidt H, Schaap PJ. NG-Tax 2.0: a semantic framework for high-throughput amplicon analysis. Front Genet 2019;10:1366.

37. Team RC. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2007.

38. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8(4):e61217.

39. Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-Baeza Y, Birmingham A, et al. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 2017;5(1):27.

40. Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin P, O'Hara R, Simpson G, Solymos P. vegan: Community Ecology Package. R package version 2.5-2. R Foundation; 2018.

41. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814.

42. Salonen A, Lahti L, Salojärvi J, Holtrop G, Korpela K, Duncan SH, Date P, Farquharson F, Johnstone AM, Lobley GE, et al. Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. ISME J 2014;8:2218.

43. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, Brown D, Stares MD, Scott P, Bergerat A, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J 2011;5:220.

44. Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB. Bergey's manual of systematic bacteriology. Vol. 3. The Firmicutes: Springer Science & Business Media; 2011.

45. Robert C, Bernalier-Donadille A. The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose-degrading bacteria in methane-excreting subjects. FEMS Microbiol Ecol 2003;46(1): 81–9.

46. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes 2012;3(4):289–306.

47. Vuholm S, Nielsen DS, Iversen KN, Suhr J, Westermann P, Krych L, Andersen JR, Kristensen M. Whole-grain rye and wheat affect some markers of gut health without altering the fecal microbiota in healthy overweight adults: a 6-week randomized trial. J Nutr 2017;147(11):2067–75.

48. Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2013;11(7):868–75.e3.

49. Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J, Liu Y. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015;5(1):8096.

50. Iino C, Endo T, Mikami K, Hasegawa T, Kimura M, Sawada N, Nakaji S, Fukuda S. Significant decrease in Faecalibacterium among gut microbiota in nonalcoholic fatty liver disease: a large BMI- and sex-matched population study. Hepatol Int 2019;13(6):748–56.

51. Korem T, Zeevi D, Zmora N, Weissbrod O, Bar N, Lotan-Pompan M, Avnit-Sagi T, Kosower N, Malka G, Rein M. Bread affects clinical parameters and induces gut microbiome-associated personal glycemic responses. Cell Metab 2017;25(6):1243.

52. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 2017;66(11): 1968–74.

53. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 2017;9(9):1021.

54. Vital M, Karch A, Pieper DH. Colonic butyrate-producing communities in humans: an overview using omics data. mSystems 2017;2(6):e00130–17.

55. Muñoz-Tamayo R, Laroche B, Walter E, Doré J, Duncan SH, Flint HJ, Leclerc M. Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. FEMS Microbiol Ecol 2011;76(3):615–24.

56. Han F, Wang Y, Han Y, Zhao J, Han F, Song G, Jiang P, Miao H. Effects of whole-grain rice and wheat on composition of gut microbiota and short-chain fatty acids in rats. J Agric Food Chem 2018;66(25):6326–35.

57. Rose DJ. Impact of whole grains on the gut microbiota: the next frontier for oats? Br J Nutr 2014;112(S2):S44–S9.

58. Andersson AA, Andersson R, Piironen V, Lampi A-M, Nyström L, Boros D, Fraś A, Gebruers K, Courtin CM, Delcour JA. Contents of dietary fibre components and their relation to associated bioactive components in whole grain wheat samples from the HEALTHGRAIN diversity screen. Food Chem 2013;136(3-4):1243–8.

59. Andersson R, Fransson G, Tietjen M, Aman P. Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. J Agric Food Chem 2009;57(5):2004–8.

60. Eriksen AK, Brunius C, Mazidi M, Hellström PM, Risérus U, Iversen KN, Fristedt R, Sun L, Huang Y, Nørskov NP, et al. Effects of whole-grain wheat, rye, and lignan supplementation on cardiometabolic risk factors in men with metabolic syndrome: a randomized crossover trial. Am J Clin Nutr 2020;111(4):864–76.

61. Bach Knudsen KE, Lærke HN, Hedemann MS, Nielsen TS, Ingerslev AK, Gundelund Nielsen DS, Theil PK, Purup S, Hald S, Schioldan AG, et al. Impact of diet-modulated butyrate production on intestinal barrier function and inflammation. Nutrients 2018;10(10):1499.

62. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 2009;294(1):1–8.

63. Wang B, Jiang X, Cao M, Ge J, Bao Q, Tang L, Chen Y, Li L. Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. Sci Rep 2016;6:32002.

64. Del Chierico F, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, et al. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. Hepatology 2017;65(2):451–64.

65. Nistal E, Sáenz de Miera LE, Ballesteros Pomar M, Sánchez-Campos S, García-Mediavilla MV, Álvarez-Cuenllas B, Linares P, Olcoz JL, Arias-Loste MT, García-Lobo JM, et al. An altered fecal microbiota profile in patients with non-alcoholic fatty liver disease (NAFLD) associated with obesity. Rev Esp Enferm Dig 2019;111(4):275–82.

Supplementary Data

Minor changes in the composition and function of the gut microbiota during a 12-week whole grain wheat or refined wheat intervention correlate with liver fat in overweight and obese adults, van Trijp et al., Online Supplementary Material

Supplemental Methods

Prediction of functional potential of the microbiota

PICRUSt2 analysis was performed on the sequencing dataset to predict the microbial gene content. Amplicon sequence variants (ASV) were used as input for PICRUSt2, and aligned to reference sequences using HMMER software to identify homologous nucleotide sequences using hidden Markov models (1). The aligned amplicon sequence variants were placed into a reference tree using phylogenetic placement algorithms (EPA-NG) (2) and GAPPA (3). Nearest-sequenced taxon index (NSTI) values for each ASV were calculated, which correspond to the branch length of the tree from the placed ASV to the nearest reference 16S sequence. The NSTI values default cut-off is 2. For bacteria without available genome sequences, the sequenced relative was used as reference. The tree of ASVs and reference sequences was combined with information about gene family copy numbers per ASV, to predict the gene content per ASV after normalizing by 16S copy number using castor (4). The read depth per ASV was divided by the predicted 16S copy numbers to control for variation in 16S copy numbers across bacteria. Afterwards, the predicted gene content per ASV was combined with the ASV known abundances input to determine gene family abundance per sample. These predicted sample gene family profiles were used as input, together with a map of gene families to pathways, to infer pathway abundances using a minimal set of pathways (MinPath, (5)). Per sample, predicted pathways, EC numbers, and KEGG orthology (KO) metagenomes were reconstructed for the dataset. Finally, EC numbers are re-grouped to MetaCyc reactions to predict pathway abundances and coverages (6). For more information about the PICRUSt2 workflow we refer to the PICRUSt website: https://github.com/picrust/picrust2.

Markers for short chain fatty acid production

As markers for acetate production, the enzymes acetate kinase (K00925) (7), phosphate acetyltransferase (K13788), and putative phosphotransacetylase (K15024) were selected. As markers for propionate production, methylmalonyl-CoA decarboxylase (K11264), propionaldehyde dehydrogenase (K13922), propionate CoA-transferase (K01026) (8), and propionyl-CoA synthetase (K01908) were selected. As markers for butyrate production, butyrate kinase (K00929) and butyryl-coA:acetate CoA-transferase (α subunit: K01034, β subunit: K01035) were selected (9, 10). For lactate production, L-lactate dehydrogenase (K00016) and D-lactate dehydrogenase (K03778) were selected (11).

Supplemental Results

Technical information about the microbiota and predicted pathway datasets

The number of detected ASVs did not increase with higher sample reads (**Supplemental Figure 3A**). Correlation coefficients of the 23 technical duplicates were 0.97±0.027 (Supplemental Figure 3B), and the mean number of reads per sample was 37399±10216. The microbiota functionality predictions were reliable, because the weighted NSTI for the samples in the dataset was 0.089±0.048 (Supplemental Figure 3C), without differences between intervention groups (Supplemental Figure 3D). This suggests that closely related reference genomes were available for the ASVs in this dataset. Correlation coefficients of the predicted gene content in the 23 technical duplicate pairs were 0.99±0.0013 (Supplemental Figure 3E).
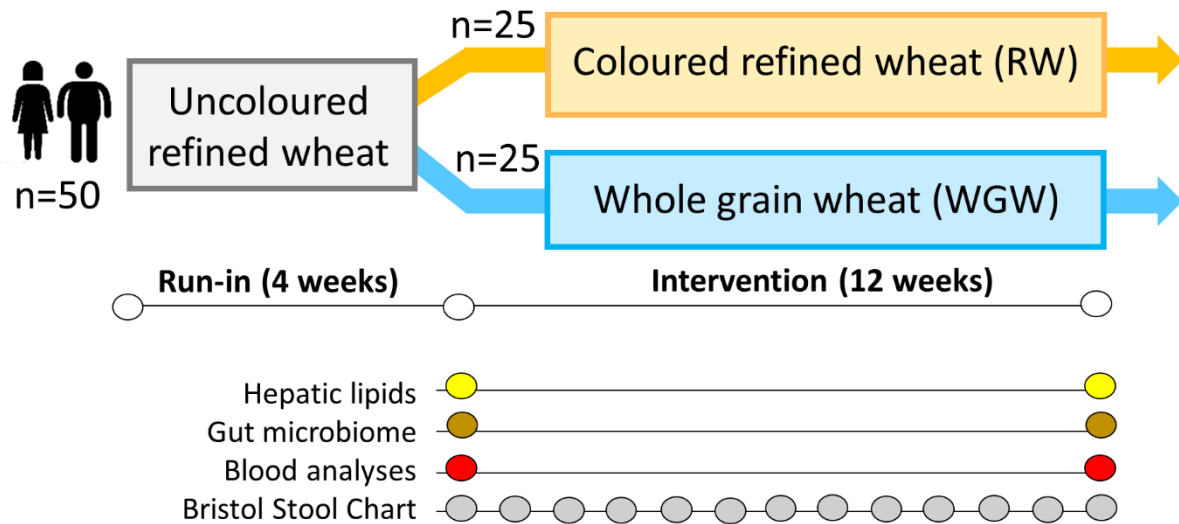
Supplemental Figure 1. The study design of the randomized, controlled, double-blind parallel trial. After 4 weeks run-in with RW products, subjects (n=50) were randomly assigned to the WGW (n=25) or RW (n=25) intervention group. Before and after the intervention hepatic lipids were measured, and feces and blood was collected. Subjects weekly recorded stool consistency using The Bristol Stool chart.
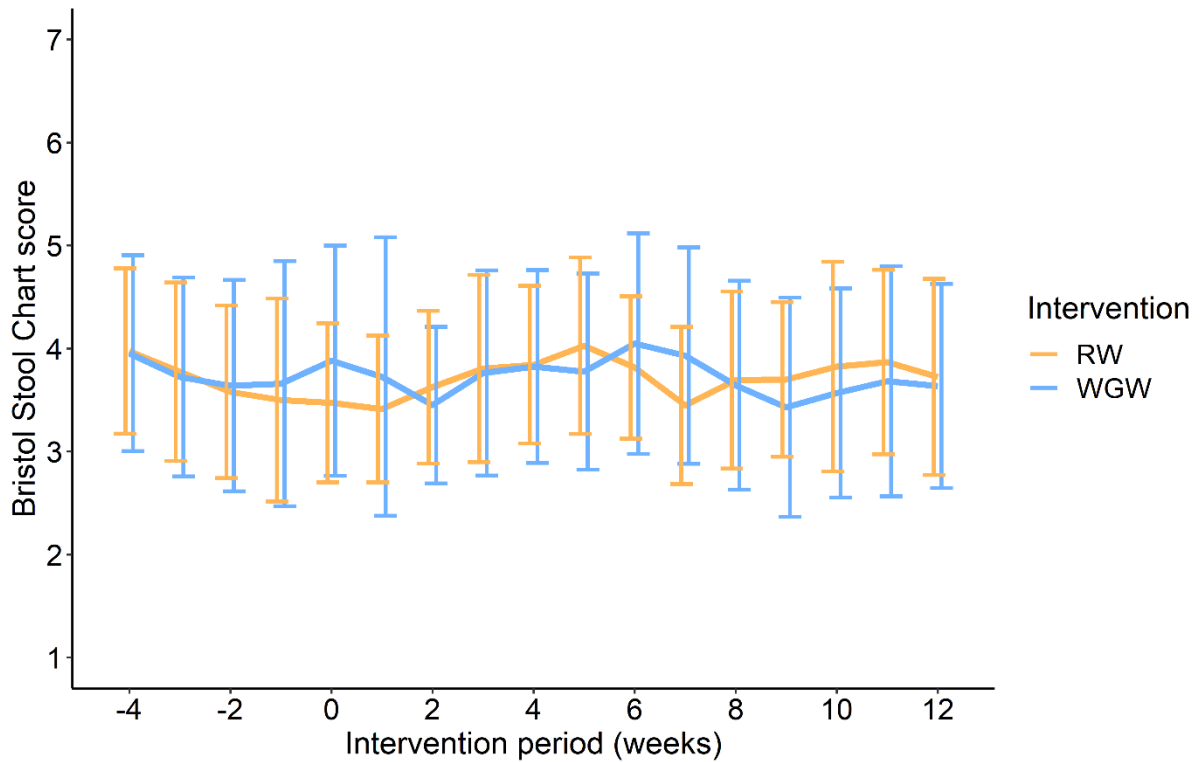
Supplemental Figure 2. The stool consistency of middle-aged overweight and obese adults during the run-in period and the 12 week of RW or WGW intervention. Data is presented as group means ± SD, n=20 (RW) or n=22 (WGW), after exclusion of antibiotic users. The Bristol Stool Chart score describes 7 types of stools ranging from 1: hard/lumpy to 7: watery without solid pieces. RW, refined wheat; WGW, whole grain wheat.
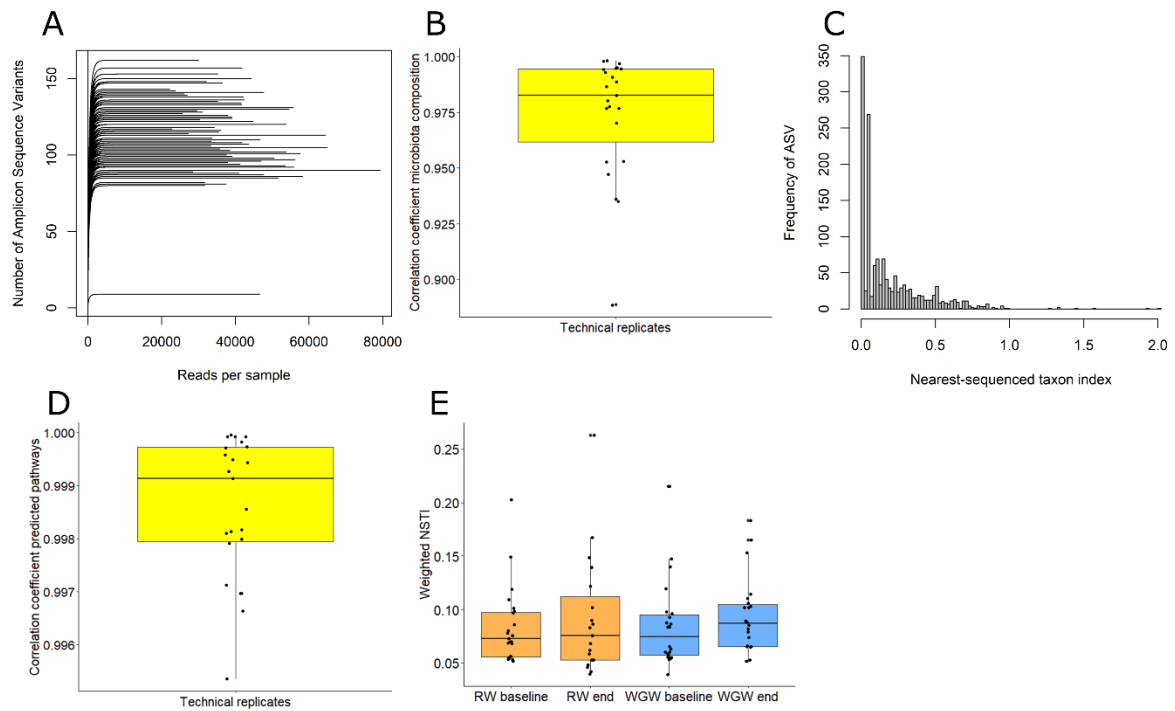
Supplemental Figure 3. The quality characteristics of the ASV and PICRUSt2 datasets. (**a**) The rarefaction curve of the sequenced samples; (**b**) the correlation coefficients of the relative microbiota composition on genus level between the samples within one technical replicate pair; (**c**) the NSTI score and the frequency of all ASVs; (**d**) the correlation coefficients of predicted pathways between the samples within one technical replicate pair; and (**e**) the weighted NSTI score at baseline and after the 12 week of intervention (end) in the RW and WGW group. Data in Figure B, D, and E is presented through their quartiles, n=16 (RW) or n=21 (WGW). ASV, amplicon sequence variant; NSTI, nearest sequenced taxon index; RW, refined wheat; WGW, whole grain wheat.

Supplementary Data
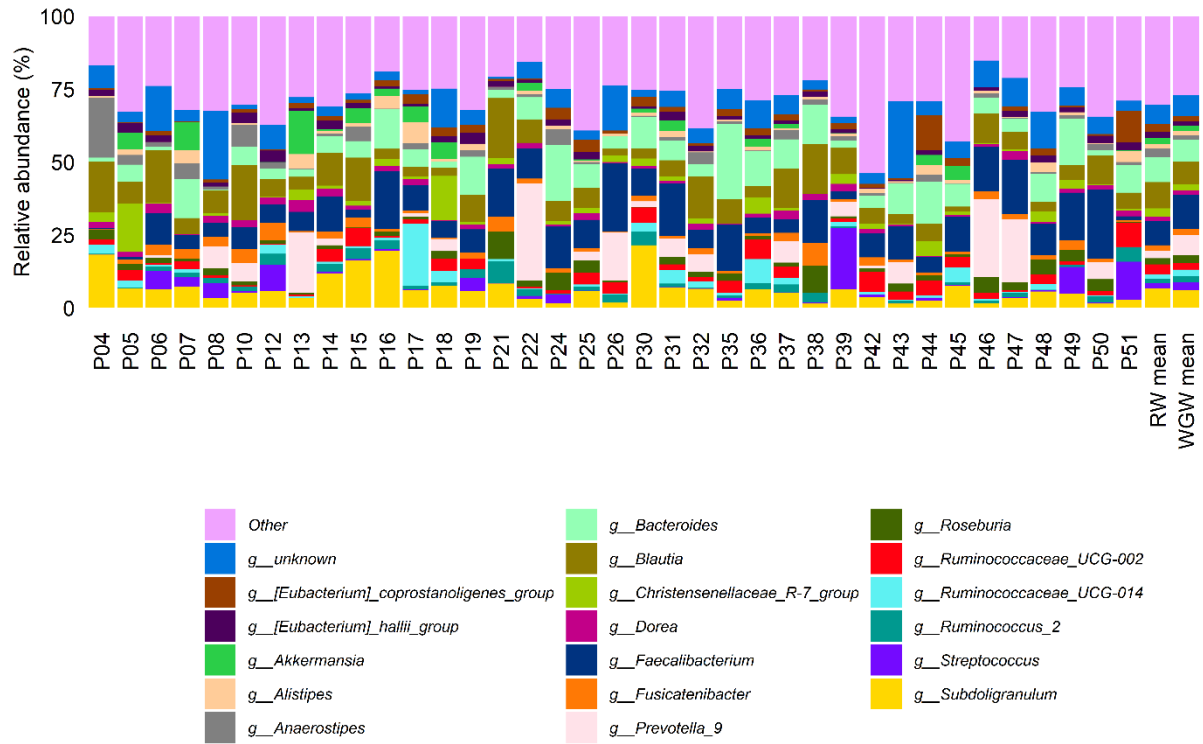
Supplemental Figure 4. The relative microbiota abundance of the top 20 taxa on genus level at baseline for every middle-aged overweight or obese adult, and the group means for the RW (n=16) and WGW (n=21) group. RW, refined wheat; WGW, whole grain wheat.
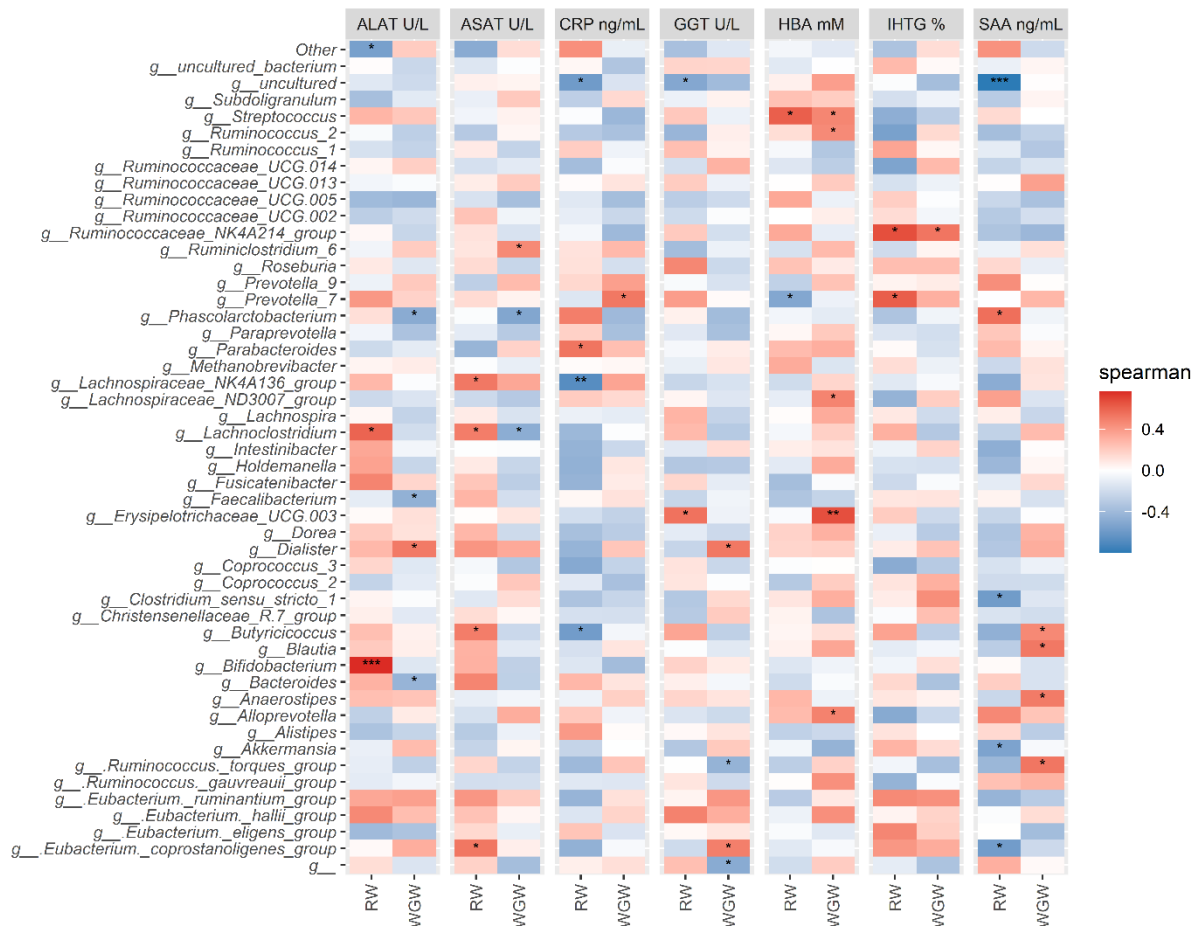
Supplemental Figure 5. Correlations between changes in bacteria abundances at genus level, and changes in liver health markers upon 12 weeks of RW or WGW intervention in middle-aged overweight and obese adults. Colors represent the Spearman rho correlation coefficients, n=16 (RW) or n=21 (WGW), with positive correlations in red and negative correlations in blue. *P-value<0.05, **P-value<0.01, ***P-value<0.001. ALAT, alanine transaminase; ASAT, aspartate transaminase; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; b-HBA, beta-hydroxybutyrate; IHTG, intrahepatic triglycerides; RW, refined wheat; SAA, serum amyloid A; WGW, whole grain wheat.

Supplementary Data

Supplemental Table 1. Nutritional composition of the RW and WGW intervention products[1].

	Bread		RTEC	
	RW	**WGW**	**RW**	**WGW**
Energy, kJ	1066	983	1586	1524
Moisture, g	35.4	38.7	3.4	3.7
Ash, g	1.3	1.8	1.8	2.5
Carbohydrates, g	49.3	39.2	78.5	69.7
Fiber[2], g	3.3	6.8	3.9	10.8
Protein, g	9.1	11.3	10.9	11.2
Fat, g	1.6	2.1	1.5	2.1
Sodium, mg	0.45	0.46	0.51	0.49
Iron, mg	0.92	2.1	1.3	3.9
Magnesium, mg	19	62	31	120
Zinc, mg	0.67	1.5	0.89	2.4
Folate, µg	17	28	14	38
Vitamin B1, mg	0.06	0.17	<0.1	<0.1
Vitamin B3, mg	0.47	0.76	1.1	2.6
Vitamin B6, mg	<0.1	<0.1	<0.1	<0.1

[1]Values are per 100 g of product. [2]Determined using AOAC985.29. Reproduced with permission from Schutte et al. (12). RTEC, ready-to-eat cereal; RW, refined wheat; WGW, whole-grain wheat.

Supplemental Table 2. Habitual daily intake of (macro)nutrients and whole grains of middle-aged overweight and obese subjects in the RW or WGW group[1].

Nutrients (g/d)	RW group	WGW group	P-value
Megajoules, MJ/day	10.3 ± 2.36	10.5 ± 2.89	0.80
Total carbohydrates[2]	246 ± 59.6	279 ± 85.5	0.19
Mono- and disaccharides	101 ± 29.8	127 ± 41.8	0.04
Polysaccharides	145 ± 35.8	152 ± 57.8	0.64
Fiber[3]	25.4 ± 6.50	26.3 ± 7.20	0.68
Whole grains	60.1 ± 59.2	54.4 ± 38.0	0.73
Total protein	95.9 ± 18.4	92.5 ± 18.0	0.58
Protein from plant sources	40.6 ± 10.6	39.5 ± 11.5	0.77
Protein from animal sources	55.2 ± 10.5	53.0 ± 10.3	0.52
Total fat	105 ± 32.1	101 ± 34.5	0.70
Saturated fatty acids	38.1 ± 12.5	37.5 ± 12.1	0.89
Monounsaturated fatty acids	37.4 ± 11.7	35.1 ± 11.9	0.56
Polyunsaturated fatty acids	20.9 ± 8.30	19.7 ± 9.50	0.70
Linoleic acid	17.5 ± 7.30	16.2 ± 7.80	0.60
Trans fatty acids	1.67 ± 0.577	1.82 ± 0.688	0.51
Cholesterol	285 ± 77.6	242 ± 106	0.18
Alcohol[4]	12.5 ± 11.2	7.60 ± 9.90	0.08

[1]Values are presented as group means ± SD, n=16 (RW) or n=21 (WGW), [2]Dietary fiber is not included in the total carbohydrates, [3]Include high molecular weight fibers (*e.g.* cellulose, resistant starch, cereal β-glucan, guar gum, and certain xylans), insoluble fibers in water (*e.g.* cellulose, resistant starch, and certain xylans), fibers soluble in water and precipitated by 78% ethanol (*e.g.* cereal β-glucan, guar gum, and certain xylans). Excluded are low molecular weight fibers (*e.g.* fructan, GOS, Polydextrose®, and resistant maltodextrins), and non-resistant starch, [4]Non-parametric distribution, evaluated with the Mann-Whitney U test. RW, refined wheat; WGW, whole grain wheat.

Supplementary Data

Supplemental Table 3. Liver health parameters at baseline and change after 12 weeks of RW or WGW intervention in middle-aged overweight and obese adults[1] in a subset of the total study population[2].

	RW group		WGW group		Within RW	Within WGW	Group comparison
	Baseline	Change after 12 wk	Baseline	Change after 12 wk	P-value	P-value	P-value
IHTG, %	3.6 ± 2.5	1.8 ± 1.6	5.5 ± 6.4	-0.26 ± 3.0	0.003	0.93	0.033
CRP, µg/mL	2.9 ± 3.1	0.20 ± 1.9	4.8 ± 7.3	-3.0 ± 6.7	0.49	0.038	0.06
SAA, µg/mL	2.1 ± 1.9	0.70 ± 2.2	7.2 ± 16	-5.7 ± 15	0.64	0.016	0.042
GGT, U/L	19 ± 13	0.82 ± 4.9	21 ± 13	-0.43 ± 4.9	0.47	0.77	0.56
AST, U/L	19 ± 5.6	-0.18 ± 4.0	19 ± 4.7	-0.91 ± 4.2	0.98	0.27	0.57
ALT, U/L	31 ± 9.4	0.088 ± 9.3	36 ± 12	-2.8 ± 8.3	0.78	0.24	0.39
Beta-hydroxybutyrate, mmol/L	0.30 ± 0.20	-0.058 ± 0.36	0.20 ± 0.20	0.11 ± 0.17	0.06	0.012	0.003

[1]Values are presented as group means $\pm$ SD, n=16 (RW) or n=21 (WGW), and for IHTG n=11 (RW) or n=17 (WGW). [2]The total study population is described in this reference: Schutte S, et al. A 12 week whole grain wheat intervention protects against hepatic fat; the Graandioos study, a randomized trial in overweight subjects. Am J Clin Nutr. 2018;108:1264–74. ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; IHTG, intrahepatic triglycerides; RW, refined wheat; SAA, serum amyloid A; WGW, whole grain wheat.

Supplemental Table 4. Gut bacteria and predicated pathways that were found to be significantly different between the RW and WGW group at baseline in middle-aged overweight and obese adults[1].

	RW group		WGW group		Group comparison	
	Number of subjects in which detected (% of total)	Relative abundance at baseline (%)	Number of subjects in which detected (% of total)	Relative abundance at baseline (%)	P-value	FDR P-value
Genus						
Dialister	5 (31)	0.40 ± 0.88	13 (62)	1.5 ± 2.2	0.046	NS
[Eubacterium]_hallii_group	16 (100)	2.2 ± 1.0	18 (86)	1.4 ± 0.97	0.038	NS
[Eubacterium]_coprostanoligenes_group	16 (100)	2.6 ± 2.7	21 (100)	1.8 ± 2.3	0.047	NS
Ruminiclostridium_9	10 (63)	0.20 ± 0.23	7 (33)	0.065 ± 0.11	0.047	NS
Lachnospiraceae_UCG-008	0 (0)	0.00 ± 0.00	10 (48)	0.10 ± 0.12	0.002	NS
Lachnospiraceae_NK4A136_group	16 (100)	0.65 ± 0.44	20 (85)	1.9 ± 1.9	0.035	NS
f__Porphyromonadaceae_uncultured genus	3 (19)	0.051 ± 0.14	0 (0)	0.00 ± 0.00	0.045	NS
Predicted pathways						
L-arginine degradation	16 (100)	0.076 ± 0.044	21 (100)	0.060 ± 0.041	0.020	NS
superpathway of adenosylcobalamin salvage from cobinamide I	16 (100)	0.69 ± 0.077	21 (100)	0.73 ± 0.10	0.040	NS
superpathway of tetrahydrofolate biosynthesis and salvage	16 (100)	0.57 ± 0.052	21 (100)	0.53 ± 0.050	0.044	NS
heterolactic fermentation	16 (100)	0.11 ± 0.044	21 (100)	0.088 ± 0.043	0.003	NS
Glucose fermentation to lactate II/Bifidobacterium shunt	16 (100)	0.15 ± 0.066	21 (100)	0.12 ± 0.062	0.011	NS
ppGpp biosynthesis	16 (100)	0.016 ± 0.022	21 (100)	0.019 ± 0.041	0.050	NS

Supplementary Data

adenosylcobalamin biosynthesis from adenosylcobinamide-GDP I	16 (100)	0.66 ± 0.079	21 (100)	0.70 ± 0.10	0.049	NS
superpathway of adenosylcobalamin salvage from cobinamide II	16 (100)	0.67 ± 0.080	21 (100)	0.70 ± 0.10	0.041	NS
pyruvate fermentation to acetone	16 (100)	0.28 ± 0.12	21 (100)	0.20 ± 0.087	0.012	NS
UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate biosynthesis	12 (75)	0.010 ± 0.013	8 (38)	0.0015 ± 0.0024	0.032	NS
guanosine ribonucleotides de novo biosynthesis	16 (100)	0.83 ± 0.057	21 (100)	0.82 ± 0.062	0.033	NS

[1]Values are presented as group means ± SD, or as the number of subjects in which the bacteria was detected and the percentage of total, n=16 (RW) or n=21 (WGW). [2]NS = P-value>0.05. FDR, false discovery rate; RW, refined wheat; WGW, whole grain wheat.

Supplementary Data

Supplemental Table 5. The number of middle-aged obese or overweight subjects in which the significant gut bacteria were detected at baseline and after 12 weeks of RW or WGW intervention[1].

Genus	RW group		WGW group	
	Baseline	At 12 weeks	Baseline	At 12 weeks
	Number of subjects in which detected (% of total)	Number of subjects in which detected (% of total)	Number of subjects in which detected (% of total)	Number of subjects in which detected (% of total)
f_Ruminococcaceae; g_Ruminiclostridium_9	10 (63)	6 (38)	7 (33)	10 (48)
f_Ruminococcaceae; g_Ruminococcaceae_NK4A214_group	13 (81)	9 (56)	16 (76)	12 (57)
f_Lachnospiraceae; g_Lachnospiraceae_UCG-008	0 (0)	3 (19)	10 (48)	7 (33)
f_Ruminococcaceae; g__Ruminococcaceae_UCG-014	14 (88)	12 (75)	18 (86)	19 (90)
f_Tannerellaceae; g_Parabacteroides	12 (75)	14 (88)	21 (100)	15 (71)
f_Lachnospiraceae; g_Dorea	16 (100)	15 (94)	18 (86)	21 (100)
f_Lachnospiraceae; g__[Eubacterium]_ventriosum_group	8 (50)	3 (19)	13 (62)	12 (57)
f__Peptostreptococcaceae_g__unknown	14 (88)	8 (50)	14 (67)	9 (43)
f_Ruminococcaceae; g_Ruminococcus_2	14 (88)	13 (81)	21 (100)	17 (81)
f_Ruminococcaceae; g_Subdoligranulum	16 (100)	16 (100)	21 (100)	21 (100)
f_Burkholderiaceae; g_Sutterella	7 (44)	8 (50)	8 (38)	12 (57)
f_Ruminococcaceae; g__Ruminococcaceae_UCG-005	14 (88)	13 (81)	17 (81)	20 (95)

[1]Values are presented as the number of subjects in which the bacteria was detected and the percentage of total, n=16 (RW) or n=21 (WGW). RW, refined wheat; WGW, whole grain wheat.

Supplementary Data

Supplemental Table 6. The selected predicted genes involved in SCFA production at baseline and after 12 weeks of RW or WGW intervention in the feces of in middle-aged overweight and obese adults[1].

Microbial predicted gene	KO number	RW group		WGW group		Within RW	Within WGW	Group comparison
		Baseline relative abundance (%)	Δ Relative abundance after 12wk (%)	Baseline relative abundance (%)	Δ Relative abundance after 12wk (%)	P-value[2]	P-value[2]	P-value[2]
Acetate production								
Acetate kinase	K00925	7.95E-02 ± 4.28E-03	-1.09E-03 ± 3.61E-03	7.88E-02 ± 4.06E-03	-3.42E-04 ± 4.11E-03	0.35	0.68	0.39
Phosphate acetyltransferase	K13788	1.48E-03 ± 1.38E-03	-1.76E-04 ± 1.71E-03	1.50E-03 ± 1.92E-03	1.50E-03 ± 1.59E-03	0.021	0.29	0.41
Putative phosphotransacetylase	K15024	3.13E-02 ± 7.70E-03	-2.52E-03 ± 8.59E-03	3.28E-02 ± 7.53E-03	-1.96E-03 ± 7.89E-03	0.56	0.71	0.66
Propionate production								
Propionyl-coA synthetase	K01908	5.01E-04 ± 9.66E-04	1.16E-04 ± 1.27E-03	6.42E-04 ± 1.51E-03	1.37E-04 ± 2.13E-03	0.17	0.96	0.27
Propionate coA-transferase	K01026	1.15E-02 ± 4.86E-03	-1.81E-03 ± 4.66E-03	9.04E-03 ± 3.42E-03	9.34E-04 ± 4.39E-03	0.63	0.37	0.37
Propionaldehyde dehydrogenase	K13922	6.44E-03 ± 3.60E-03	-1.75E-03 ± 4.49E-03	5.58E-03 ± 3.15E-03	-6.24E-04 ± 2.82E-03	0.60	0.66	0.96
Methylmalonyl-CoA decarboxylase	K11264	4.65E-04 ± 9.55E-04	-6.56E+01 ± 1.18E-03	6.19E-04 ± 1.48E-03	6.19E-04 ± 2.06E-03	0.09	0.51	0.16
Butyrate production								
Butyrate kinase	K00929	1.80E-02 ± 7.75E-03	2.18E-03 ± 8.02E-03	1.61E-02 ± 4.84E-03	1.49E-03 ± 7.25E-03	0.71	0.038	0.96
Acetate CoA-transferase α subunit	K01034	9.51E-03 ± 4.65E-03	-1.35E-03 ± 4.63E-03	6.45E-03 ± 3.30E-03	7.53E-04 ± 2.79E-03	0.21	1.0	0.75
Acetate CoA-transferase β subunit	K01035	9.59E-03 ± 4.69E-03	-1.27E-03 ± 4.70E-03	6.51E-03 ± 3.33E-03	7.66E-04 ± 2.83E-03	0.43	1.0	0.59
Lactate production								

Supplementary Data

L-lactate dehydrogenase	K00016	7.10E-02 ± 1.32E-02	-7.09E-03 ± 1.24E-02	6.95E-02 ± 1.18E-02	-4.24E-03 ± 1.26E-02	0.39	0.81	0.63
D-lactate dehydrogenase	K03778	3.01E-02 ± 7.56E-03	-2.63E-03 ± 8.23E-03	3.10E-02 ± 8.73E-03	3.34E-04 ± 9.76E-03	0.96	0.81	0.24

[1]Values are presented as group means ± SD, n=16 (RW) or n=21 (WGW). [2]P-values without FDR correction. KO, KEGG ortholog; RW, refined wheat; WGW, whole grain wheat.
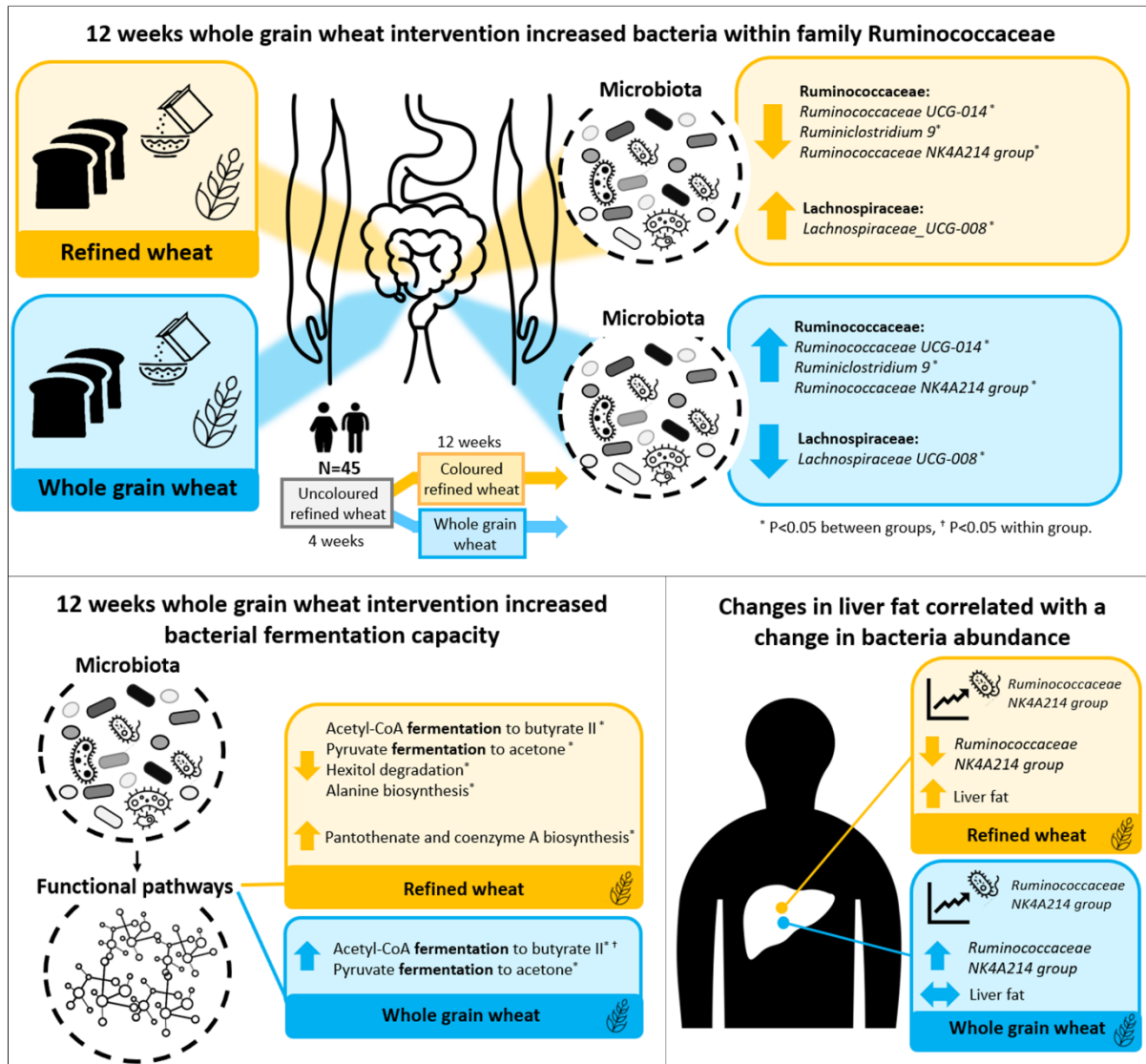
Supplemental Figure 6. A graphical overview of the main findings in this study.

Supplementary Data

Supplemental references

1.   Institute CCHHM. HMMER: biosequence analysis using profile hidden Markov models. Available from: http://hmmer.org/.
2.   Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, Stamatakis A. EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences. *Syst Biol*. 2018;68(2):365-9.
3.   Czech L, Barbera P, Stamatakis A. Genesis and Gappa: Processing, Analyzing and Visualizing Phylogenetic (Placement) Data. *bioRxiv*. 2019;647958.
4.   Louca S, Doebeli M. Efficient comparative phylogenetics on large trees. *Bioinformatics*. 2017;34(6):1053-5.
5.   Ye Y, Doak TG. A Parsimony Approach to Biological Pathway Reconstruction/Inference for Genomes and Metagenomes. *PLoS Comput Biol*. 2009;5(8):e1000465.
6.   Caspi R, Altman T, Dreher K, Fulcher CA, Subhraveti P, Keseler IM, Kothari A, Krummenacker M, Latendresse M, Mueller LA. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Res*. 2011;40(D1):D742-D53.
7.   Wolfe AJ. The acetate switch. *Microbiol Mol Biol Rev*. 2005;69(1):12-50.
8.   Reichardt N, Duncan SH, Young P, Belenguer A, Leitch CM, Scott KP, Flint HJ, Louis P. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME J*. 2014;8(6):1323.
9.   Pryde SE, Duncan SH, Hold GL, Stewart CS, Flint HJ. The microbiology of butyrate formation in the human colon. *FEMS Microbiol Lett*. 2002;217(2):133-9.
10.   Vital M, Howe AC, Tiedje JM. Revealing the bacterial butyrate synthesis pathways by analyzing (meta) genomic data. *MBio*. 2014;5(2):e00889-14.
11.   Juturu V, Wu JC. Microbial production of lactic acid: the latest development. *Crit Rev Biotechnol*. 2016;36(6):967-77.
12.   Schutte S, Esser D, Hoevenaars FP, Hooiveld GJ, Priebe MG, Vonk RJ, Wopereis S, Afman LA. A 12-wk whole-grain wheat intervention protects against hepatic fat: the Graandioos study, a randomized trial in overweight subjects. *Am J Clin Nutr*. 2018;108(6):1264-74.