Fecal Lactic Acid Bacteria Increased in Adolescents Randomized to Whole-Grain but Not Refined-Grain Foods, whereas Inflammatory Cytokine Production Decreased Equally with Both Interventions1–4

Bobbi Langkamp-Henken,5* Carmelo Nieves Jr,5 Tyler Culpepper,6 Allyson Radford,5 Stephanie-Anne Girard,4 Christine Hughes,4 Mary C. Christman,7 Volker Mai,8 Wendy J. Dahl,5 Thomas Boileau,8 Satya S. Jonnalagadda,8 and Frank Thielecke9

1Department of Food Science and Human Nutrition, 2Department of Microbiology and Cell Science, and 3Department of Statistics, University of Florida, Gainesville, FL; 4General Mills Bell Institute of Health and Nutrition, Minneapolis, MN; and 5Cereal Partners Worldwide, Lausanne, Switzerland

Abstract

The intake of whole-grain (WG) foods by adolescents is reported to be approximately one-third the recommended intake of 48 g/d. This 6-wk randomized interventional study determined the effect of replacing grains within the diet with refined-grain (RG; n = 42) or WG (n = 41) foods/d on gastrointestinal and immune health in adolescents (aged 12.7 ± 0.1 y). A variety of grain-based foods were delivered weekly to participants and their families. Participants were encouraged to eat 3 different kinds of study foods (e.g., bread, cereals, snacks)/d with goals of 0 g/d (RG) and 80 g/d (WG). Stool samples were obtained during the prebaseline and final weeks to measure bifidobacteria and lactic acid bacteria (LAB) using qPCR. Stool frequency was recorded daily. Blood was drawn at baseline and at final visits for immune markers. Across groups, total-grain intake increased by one serving. The intake of WG was similar at baseline (18 ± 3 g) between groups but increased to 60 ± 5 g in the WG group and decreased to 4 ± 1 g in the RG group. Fecal bifidobacteria increased from baseline with both interventions, but LAB increased (P < 0.05) from baseline [2.4 ± 0.2 log10 genome equivalents (eq)] to wk 6 (3.0 ± 0.2 log10 genome eq) in the WG group but not in the RG group (baseline: 2.9 ± 0.2 log10 genome eq; wk 6: 3.0 ± 0.1 log10 genome eq). There was no difference in stool frequency, serum antioxidant potential, or in vitro LPS-stimulated mononuclear cell production of inflammatory cytokines between groups. However, across both groups the number of daily stools tended to increase (P = 0.08) by 0.0034 stools/g WG or by 0.2 stools with 60 g WG, mean antioxidant potential increased by 58%, and mean production of TNF-α, IL-1β, and IL-6 decreased by 24, 22, and 42%, respectively, between baseline and wk 6. Overall, incorporating either WG or RG foods increased serum antioxidant concentrations and decreased inflammatory cytokine production; however, WG study foods had more of an effect on aspects of gastrointestinal health.


Introduction

The 2010 Dietary Guidelines for Americans recommend that at least half of the daily intake of grains derive from whole grains (WG)10

1 Supported by the General Mills Bell Institute of Health and Nutrition. Single-serving whole-grain cereal boxes were provided by Cereal Partners Worldwide.
T. Boileau, S. S. Jonnalagadda, and F. Thielecke are employed by the study sponsors.
3 This trial was registered at clinicaltrials.gov as NCT01094652.
4 Supplemental Figures 1 and 2 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.
5 Abbreviations used: CRP, C-reactive protein; DGGE, denaturing gradient gel electrophoresis; LAB, lactic acid bacteria; OTU, operational taxonomic units; oz-eq, ounce-equivalent; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; RG, refined-grain; sIgA, secretory IgA; WG, whole-grain.
6 To whom correspondence should be addressed. E-mail: henken@ufl.edu.
Participants and Methods

Particpants
Adolescents were recruited from a local middle school (ages 11 to 15 y) with a student population of 970 and where 45% of the student body qualified for free or reduced-fee school meals. A total of 196 adolescents were screened, and 91 assented to participate with the consent of at least one parent. Participants were excluded if they were taking any medications for constipation or diarrhea, receiving antibiotics during the 4 wk prior to randomization, consuming probiotics or >3 servings/wk of yogurt, or had gastrointestinal, kidney, or immune-modulating diseases, diabetes, or any known food allergies. Eight participants were excluded because they did not meet inclusion/exclusion criteria (n = 4) or were no longer interested (n = 4). Eighty-three participants began the 1-wk prebaseline screening and were randomly assigned to either the RG group (n = 42) or the WG group (n = 41). All study procedures were approved by the University of Florida Institutional Review Board and the School Board of Alachua County.

Experimental design
Participants were enrolled in this 6-wk, randomized, controlled, parallel-arm study over a 3-wk period in February 2010. In the week prior to randomization, one stool sample and 2 targeted 24-h recalls were collected, and height (portable stadiometer), weight (digital scale), and birth date were obtained to determine BMI percentile-for-age. On the morning of randomization, baseline blood and saliva samples were obtained. Participants were stratified on the basis of prebaseline weight category (<95th BMI percentile-for-age and BMI <30 kg/m²; ≥95th percentile-for-age or BMI ≥30 kg/m²) and randomly assigned to the RG or WG groups via sealed envelope. The randomization scheme was generated by the statistician (M.C.C.) who did not have contact with study participants. A grocery bag of a variety of grain-based foods was picked up at the school or delivered to the participants’ homes each week. Over the intervention period, 6 targeted dietary recalls were obtained from participants. Each day, participants recorded the number of bowel movements and scored gastrointestinal symptoms (i.e., stomach pain, bloating, and gas) on a scale of 0 (no symptoms) to 10 (extreme). On school days, participants turned in questionnaires and picked up study snacks. During the final week of the study a stool sample was obtained, and on the final day of the study blood and saliva samples were obtained.

Grain-based foods and administration protocol
Four rotating weekly food packages containing ~10 different grain-based foods (e.g., cereal, pasta, rice, bread, pancake mix, snack foods) were provided to participants and their families. Two single-serving snack packs (i.e., WG cereal provided by Cereal Partners Worldwide) or 100-kcal cookie packs were distributed to study participants on school days. The study foods were primarily wheat-based but also included oats, rice, and corn. One serving of the study foods provided 0 g (RG group) or 16 ± 1 g (WG group) of whole grains. Participants were told to eat the study foods in place of grains consumed as part of their typical diet, and they were encouraged to eat 3 different kinds of study foods each day with the goal of 5 oz-eq of grains/d.

Targeted diet recalls and daily questionnaires
Multipass, targeted 24-h recalls were obtained primarily before school. If participants were unavailable before school, recalls were obtained during lunch or after school. Due to time constraints, a targeted diet recall was used. For these recalls, trained study coordinators asked participants to list all of the foods and beverages they consumed in the previous 24 h. Detailed information was obtained only for grain products, fruit, vegetables, and legumes. Serving containers and pictures of foods, including study foods, were used to help determine serving size and type of grain. The school food service director provided lists of WG foods served with school meals. Nutrient intake analyses were completed by using Food Processor software (version 10.8.0.0; ESHA Research). Information on WG content of foods was obtained from the food label or directly from the manufacturer. When this information could not be obtained, the mean WG content from similar foods was used.

Sample collection and laboratory analyses
Stool samples were collected by participants in their home or in the school clinic by using a commode specimen collection system (Fisher Scientific). Samples were immediately placed on ice and picked up by study staff. Stool samples were mixed, divided into aliquots, and frozen at −70°C within 6 h of defecation. On blood-draw days, participants

FIGURE 1 Participant flow through recruitment, allocation, and analysis.

Preliminary screen (n=196)

- Self-excluded (n=105)
- 10 Healthy adolescents were enrolled in this 6-wk, randomized, controlled, parallel-
  experimental design study to determine the effect of incorporating refined-grain (RG) or whole-grain (WG) foods into the usual diets of healthy adolescents on gastrointestinal and immune health. Adolescents were encouraged to eat at least 3 different foods/d to meet recommendations for total grain intake (1), with a goal of at least 5 ounce-equivalents (oz-eq) of RG or WG foods/d, where an ounce equivalent is defined as the amount of grains equivalent to that found in a 28-g serving of regular bread.
reported to the school clinic from 0700 to 0900 where saliva and blood samples were collected. Participants were asked to rinse their mouths with water, wait 10 min, and then pass as much saliva as possible through a straw into a collection tube for 2 min. The unstimulated saliva samples were maintained on ice until centrifuged for 15 min at 1500 g at 4°C. Samples were stored at −70°C until analysis. Blood was collected via venepuncture into serum separator (5 mL) and sodium-heparin (10 mL) tubes. Serum was stored at −70°C until assayed for C-reactive protein (CRP; ALPCO Immunoassays), TG (L-Type TG; Wako Diagnostics), and total antioxidant potential (Cayman Chemical) by using commercially available kits. sIgA was measured in salivaary and fecal samples via ELISA by using a commercially available kit (ALPCO Immunoassays).

Peripheral blood mononuclear cells (PBMC) were obtained by diluting whole blood with an equal part of saline and. a density gradient (Lympholyte-H; Cedarlane Labs). The PBMC were plated at 1.0 × 10⁶ cells/L in Roswell Park Memorial Institute -1640 complete media with 10% autologous serum, stimulated with LPS (20 mg/mL, lot 079K4111; Sigma) or phytohemagglutinin (PHA; 20 mg/mL, Sigma), and incubated for 48 h at 37°C with 95% humidity and 5% CO₂. Culture supernatants were collected and stored at −80°C until cytokine production was quantified by using a magnetic multiplexing assay (Group I; Bio-Rad). IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-15, and IL-17A concentrations were measured in PHA-stimulated cultures; and TNF-α, IL-1β, IL-6, and IL-10 were measured in LPS-stimulated cultures.

Microbiota analysis

**Denaturing gradient gel electrophoresis analysis.** Bacterial genomic DNA was isolated from fecal samples by using the QiAmp DNA Stool kit (Qiagen) with an initial bead beating step (23). Denaturing gradient gel electrophoresis (DGGE) profiles were generated as an initial quality control for DNA extraction and as a crude tool for determining diversity. A 487-bp fragment from the V6 to V8 region of the bacterial rDNA gene (27) was amplified by using barcoded pyrosequencing primer set targeting the V1–V3 region of the bacterial 16S rRNA gene (28). The PCR reactions included an initial melting step at 95°C for 2 min and 30 cycles at 95°C for 30 s, at 55°C for 30 s, and at 68°C for 1 min. Reactions included 1 mmol/L magnesium chloride and 4% formamide. Sequences were obtained from one full 454 pyrosequencing run. The reads were subjected to an initial quality control step for DNA extraction and as a crude tool for determining diversity. A 487-bp fragment from the V6 to V8 region of the bacterial rDNA gene (27) was amplified by using barcoded pyrosequencing primer set targeting the V1–V3 region of the bacterial 16S rRNA gene (28). The PCR reactions included an initial melting step at 95°C for 2 min and 30 cycles at 95°C for 30 s, at 55°C for 30 s, and at 68°C for 1 min. Reactions included 1 mmol/L magnesium chloride and 4% formamide. Sequences were obtained from one full 454 titanium plate. Sequence reads with a low-quality score or a length of <150 nucleotides were removed from the analysis. Sequence reads were binned by using ESPRIT (28) into operational taxonomic units (OTU) at the 98% similarity level. The QIIME package was used to calculate the following: 1) Chao rarefaction diversity, which estimates how many OTU are present in a sample, and 2) UniFrac distances (30), which allow for a comparison of the distribution of OTU among samples.

**Statistical analyses**

Categorical data were compared by using the chi-square statistic. Gastrointestinal symptoms were reported by very few participants so data were reduced to 2 × 2 tables (i.e., diet group by the absence or presence of the symptoms) with participants as a covariate. The mean number of daily stools across all study days for each participant was regressed on the mean grams of WG consumed during the intervention. One observation for a participant on a single day with >10 stools was removed. Race/ethnicity and baseline weight category on the basis of BMI percentile-for-age were included in the model; however, they were not significant so they were removed. The effect of mean dietary fiber intake during the intervention on mean daily stool frequency during the same period was examined as well. The mean daily stool frequency data were transformed (square root) to achieve approximate normality. The original model included the baseline weight category, but there was no effect of weight category either with or without interactions so it was dropped from the model. The final model was a simple regression of mean daily stool number on mean fiber intake during the intervention.

Immune and biochemical markers of health and fecal samples were obtained at baseline and after the 6-wk intervention (final time period). Because obesity is known to be associated with markers of inflammation, baseline weight category was included in all analyses. Data were transformed by taking the natural log (fecal sIgA and LAB, LPS-induced IL-10, and serum CRP and TG) or square root (fecal bifidobacteria; PHA-induced IFN-γ and IL-10; LPS-induced TNF-α, IL-1β, and IL-6) to correct for a skewed distribution and analyzed as a function of diet group (RG or WG) and time period (baseline and final study period), baseline weight category (<85th, 85th–94th, and ≥95th percentiles-for-age), and all interactions. Data were analyzed by using mixed-effects linear models with participant as a random blocking factor. Nonsignificant effects were hierarchically removed to obtain the final model. Differences between means were tested by using the Tukey-Kramer adjustment to control the experiment-wise error rate.

Serum TG were obtained at baseline and after the 6-wk intervention. Serum TG data were transformed by square root to correct for unequal variances and skewed distributions. Data were analyzed as a function of total-grain intake in ounce equivalents (as the means of the prebaseline and intervention periods), baseline weight category, and study period. Participant was included as a random blocking factor to account for the paired data. Nonsignificant effects were removed hierarchically to obtain the final model. Diet, immune, and biochemical analyses were conducted by using SAS version 9.2 (SAS Institute). Unless stated otherwise, values are means ± SEM, and significance is denoted at P < 0.05.

For DGGE profile analysis, background was subtracted from each lane and the profiles were subjected to Gauss modeling. The similarity matrix was calculated on the basis of the Dice correlation coefficient. Phyllogenetic trees were generated by using Ward’s clustering method. Shannon diversity indices were calculated for each sample by using the total number of bands per sample and their relative intensities.

Results

All 83 participants who were randomly assigned to an intervention completed the 6-wk study, and no study-related adverse events were reported. No significant between-group differences were found in gender, age, grade in school, race/ethnicity, or baseline BMI percentile-for-age (Table 1). Intake of WG was not different between groups prebaseline. During the intervention, participants in the RG group decreased (P < 0.05) and those in the WG group increased (P < 0.0001) their daily intake of WG, and participants in the WG group consumed 3 g more fiber/d (P < 0.05; Table 1). All participants (i.e., RG and WG groups) increased their total-grain intake by 1.1 ± 0.4 oz-equivalent across both diet groups mean nutrient intakes of folate (456 ± 32 vs. 531 ± 36 µg/d DFE), thiamin (1.17 ± 0.06 vs. 1.51 ± 0.05 mg/d),
riboflavin (1.00 ± 0.06 vs. 1.36 ± 0.05 mg/d), niacin (11.8 ± 0.7 vs. 16.5 ± 0.6 mg/d), vitamin B-12 (1.82 ± 0.19 vs. 3.40 ± 0.28 μg/d), vitamin B-6 (1.02 ± 0.07 vs. 1.64 ± 0.09 mg/d), vitamin E (1.68 ± 0.15 vs. 2.62 ± 0.21 mg/d), iron (11.6 ± 0.7 vs. 14.7 ± 0.6 mg/d), zinc (4.12 ± 0.33 vs. 5.21 ± 0.33 mg/d), and calcium (296 ± 17 vs. 360 ± 19 mg/d) from all targeted foods increased (P < 0.05) between the prebaseline and intervention periods. There were no changes in fruit or vegetable intake for either group and no changes in vitamins A or C with the interventions (data not shown).

**Gastrointestinal function.** A total of 3374 daily questionnaires were completed by participants. Stomach ache or pain, flatus, and bloating were reported on 25, 35, and 19% of the participant days, respectively. There was no effect of diet group on these gastrointestinal symptoms.

Participants reported 0, 1, 2, 3, 4, and 5 daily stools on 25, 49, 19, 5, 2, and 0.2% of the days, respectively. There was no difference between groups in the number of daily stools. Because RG participants also consumed WG, the relationship between intake of WG and the number of daily stools in general was examined. Across both diet groups, the estimated model for the mean number of daily stools over the study period was stool n = 0.99 + 0.00343 (g WG). This trend implies that for each gram of whole grain the mean number of stools tended to increase by 0.99 + 0.0034 (g WG). There was no effect of diet group on LPS-stimulated IL-1, IL-6, and IL-10 all decreased (P < 0.05) between baseline and the final study days in both diet groups. Across time periods, the concentration of TNF-α was significantly greater in cell cultures from participants in an obese versus healthy baseline weight category. No effects of diet group, baseline weight category, or time period were noted on LPS-induced concentrations of IL-8 (data not shown).

Serum CRP and antioxidant potential were measured as indicators of inflammatory status (Table 2). There was no effect of diet group on either of these indicators. Serum CRP concentrations were lowest for participants at a healthy baseline weight and were progressively higher for those who were overweight or obese. Serum CRP did not change during the intervention. Serum antioxidant potential was not different between diet groups or among the baseline weight categories; however, it increased between baseline and wk 6 in both RG and WG groups. Serum TG was measured as a way to account for changes in serum lipids that may affect total antioxidant potential. Although no associations between serum TG and antioxidant potential were observed, serum TG was altered by the interventions. There was a 3-way interaction between diet group, baseline weight category, and study day (i.e., time period effect, P = 0.0437; Table 2), although there were no differences between individual means. There was also a relationship between total-grain intake, baseline weight category, and TG. Baseline weight category strongly influenced how total-grain intake modulated serum TG (P = 0.0004; Fig. 2). For participants at a healthy baseline weight, there was no change in serum TG related to intake of total grains, but for participants in the overweight or obese category, there was a large drop in serum TG at higher intakes of total grain. This effect was similar for participants who were obese or overweight (P = 0.11) and different from that observed in participants who were in the healthy-weight category (P = 0.0003).

**Microbiota.** The success of DNA extraction in all samples was confirmed by DGGE, but no distinct profiles or individual bands for either diet group or time period were revealed. Using the

### TABLE 2 Participant characteristics and whole-grain and fiber intake

<table>
<thead>
<tr>
<th></th>
<th>Refined-grain (n = 42)</th>
<th>Whole-grain (n = 41)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F), n</td>
<td>25/17</td>
<td>23/18</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>12.7 ± 0.2</td>
<td>12.6 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Grade in school, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sixth</td>
<td>19 (45)</td>
<td>22 (54)</td>
<td></td>
</tr>
<tr>
<td>Seventh</td>
<td>15 (36)</td>
<td>9 (22)</td>
<td></td>
</tr>
<tr>
<td>Eighth</td>
<td>8 (19)</td>
<td>10 (24)</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity, n (%)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>White</td>
<td>18 (43)</td>
<td>20 (49)</td>
<td></td>
</tr>
<tr>
<td>Black/African American</td>
<td>14 (33)</td>
<td>12 (29)</td>
<td></td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>6 (14)</td>
<td>5 (12)</td>
<td></td>
</tr>
<tr>
<td>Mixed/other</td>
<td>4 (10)</td>
<td>4 (10)</td>
<td></td>
</tr>
<tr>
<td>BMI percentiles, n (%)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>&lt;85th (healthy weight)</td>
<td>23 (55)</td>
<td>23 (56)</td>
<td></td>
</tr>
<tr>
<td>85th–94th (overweight)</td>
<td>10 (24)</td>
<td>6 (15)</td>
<td></td>
</tr>
<tr>
<td>≥95th (obese)</td>
<td>9 (21)</td>
<td>12 (29)</td>
<td></td>
</tr>
<tr>
<td>Whole-grain intake, g/d</td>
<td>18.4 ± 4.2</td>
<td>17.7 ± 3.4</td>
<td>&lt;0.0001 4</td>
</tr>
<tr>
<td>Prebaseline</td>
<td>3.6 ± 0.7</td>
<td>60.4 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>Fiber intake, g/d</td>
<td>12.1 ± 1.2</td>
<td>10.7 ± 0.9</td>
<td>0.0083 4</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM or n (%).
2 The "other" category consisted of Asians (n = 2), mixed white/African American (n = 4), and other (n = 2). Participants who self-defined themselves as white and American Indian/Alaskan native (n = 2) or white and native Hawaiian/Pacific Islander (n = 1) were classified as white. The participant who self-classified as Hispanic and American Indian/Alaskan native was classified as Hispanic.
3 Diet recalls were not available for one participant during the prebaseline period (n = 40).
4 P for the interaction between diet group and time period. There was no main effect of baseline weight category.
Salivary sIgA, n | B: 35/F: 36 | B: 37/F: 39 | D×T: NS
Baseline, μg/min | 531 ± 52 | 593 ± 66 | D: NS
Final, μg/min | 649 ± 61 | 609 ± 46 | T: NS
Fecal sIgA, n | B: 30/F: 34 | B: 28/F: 34 | D×T: NS
Baseline, mg/g wet wt | 1.21 ± 0.25 | 1.14 ± 0.25 | D: NS
Final, mg/g wet wt | 1.48 ± 0.38 | 1.50 ± 0.31 | T: NS
PHA-stimulated PBMC
IL-2 production, n | 36 | 32 | D×T: NS
Baseline, ng/μL | 560 ± 98 | 567 ± 111 | D: NS
Final, ng/μL | 751 ± 116 | 882 ± 130 | T: 0.0008
IL-10 production, n | 36 | 32 | D×T: NS
Baseline, ng/μL | 125 ± 32 | 137 ± 23 | D: NS
Final, ng/μL | 75 ± 13 | 69 ± 11 | T: 0.0313
LPS-stimulated PBMC
TNF-α, n | B: 39/F: 41 | B: 37/F: 39 | D×T: NS
Baseline, pg/mL | 1.47 ± 0.24 | 1.30 ± 0.16 | D: NS
Final, pg/mL | 1.16 ± 0.16 | 0.95 ± 0.16 | T: 0.0038
IL-1β, n | 37 | 34 | D×T: NS
Baseline, pg/mL | 3.79 ± 0.44 | 3.54 ± 0.51 | D: NS
Final, pg/mL | 2.22 ± 0.30 | 3.54 ± 0.33 | T: 0.0083
IL-6 production, n | B: 25/F: 30 | B: 26/F: 27 | D×T: NS
Baseline, pg/mL | 19.2 ± 2.8 | 15.3 ± 2.4 | D: NS
Final, pg/mL | 9.8 ± 1.6 | 10.1 ± 2.1 | T: 0.0026
IL-10 production, n | B: 39/F: 41 | B: 37/F: 39 | D×T: NS
Baseline, pg/mL | 217 ± 39 | 189 ± 25 | D: NS
Final, pg/mL | 106 ± 17 | 141 ± 32 | T: 0.0002
Serum CRP, n | 39 | 39 | D×T: NS
Baseline, mg/dL | 0.6 ± 0.1 | 1.1 ± 0.3 | D: NS
Final, mg/dL | 0.5 ± 0.1 | 1.0 ± 0.2 | T: 0.24
Serum antioxidant potential, n | 42 | 40 | D×T: NS
Baseline, Trolox eq mmol/L | 1.83 ± 0.05 | 1.89 ± 0.07 | D: NS
Final, Trolox eq mmol/L | 3.03 ± 0.15 | 2.98 ± 0.16 | T: <0.0001
Serum TG†, n | 42 | 40 | D×T×W: 0.0437
Baseline, mmol/L | 0.81 ± 0.07 | 0.88 ± 0.09 | D×T×W: 0.0437
Final, mmol/L | 0.70 ± 0.05 | 0.84 ± 0.09 | W: 0.05
Fecal profile, genome eq
Bifidobacteria, n | B: 31/F: 35 | B: 31/F: 35 | D×T: NS
Baseline, log10 | 4.7 ± 0.1 | 4.3 ± 0.2 | D: NS
Final, log10 | 4.9 ± 0.2 | 4.8 ± 0.2 | T: <0.0001
Lactic acid bacteria, n | B: 28/F: 37 | B: 28/F: 37 | D×T: 0.0237
Baseline, log10 | 2.9 ± 0.2 | 2.4 ± 0.2 | D: NS
Final, log10 | 3.0 ± 0.1 | 3.0 ± 0.2* | T: 0.0008

Shannon-Weiner and Simpson diversity indices, no significant differences in community diversity were detected. Profiles within participants remained stable across time periods, suggesting that the intervention did not considerably perturb microbiota profiles (data not shown).

LAB and bifidobacteria were measured via qPCR. There was a significant (P < 0.05) diet group by time period interaction; LAB increased (P = 0.0002) between baseline and final time periods in stools from participants in the WG group. LAB genome equivalents trended toward being higher (P = 0.08) at baseline in the stools from the RG participants but did not change with the intervention. There was no difference in bifidobacteria between diet groups; however, there was a significant increase (P < 0.0001) in bifidobacteria in both diet groups. There was no effect of baseline weight category on LAB or bifidobacteria (Table 2).
FIGURE 2  Predicted mean serum TG by total-grain intake of adolescents regardless of diet intervention group or time point. Predicted mean serum TG concentrations were dependent on the interaction of baseline weight category based on BMI percentile-for-age (i.e., healthy weight (<85th percentile), overweight (85th–94th percentile), or obese (≥95th percentile)) and total-grain intake. The relationship between serum TG and total-grain intake did not differ before or after the intervention, and therefore the effect of time point was removed from the model. An ounce-equivalent of grains is defined as the amount of grains equivalent to that found in a 29-g serving of regular bread. Values are means and upper and lower 95% CI. To convert mmol/L to mg/dL, divide by 0.01129.

After preprocessing, 387,933 16S rRNA sequences were retained, with a mean of 3043 sequences/sample and a mean length of 498 nucleotides. Sequence readings were binned by using ESPRIT-Tree into 11722 OTU at the 98% similarity level. Chao1-based rarefaction curves did not significantly differ between diet groups or across time periods, suggesting that overall diversity did not change during the study period. To evaluate changes in overall microbiota composition, principal coordinates analysis based on the unweighted UniFrac metric was performed. No distinct clustering by time period or diet group (data not shown) was detected.

When the prevalence of individual OTU were compared at the 98% similarity level, 21 OTU were detected to be significantly more prevalent and 8 OTU significantly less prevalent at the final time period (Supplemental Fig. 1), suggesting a treatment effect in both groups. When the effects of WG consumption on individual OTU were examined, 5 OTU showed a strong positive correlation whereas 3 OTU were negatively associated with WG consumption (Supplemental Fig. 2).

Discussion

The purpose of this study was to determine the effect on gastrointestinal and immune health of replacing foods in the diets of adolescents with RG or WG foods and encouraging them to consume at least 3 different study foods/d. With the intervention, total-grain intake increased across diet groups, and adolescents in the WG group met the current dietary recommendations of a minimum of 48 g WG/d (1). We anticipated that the added fiber, resistant starch, and oligosaccharides provided by the WG would improve bowel habits and alter the intestinal microbiota. Although we did not observe a difference in the number of daily stools between groups, across groups a small increase in daily stools from a mean of 1 with no WG to a mean of 1.2 with an intake of 60 g WG was observed. Similarly, based on a predictive model, participants across both diet groups would have a mean of 0.54 stools/d with no fiber intake, suggesting that intake of fiber from grains, fruit, vegetables, and legumes was more strongly associated with stool output than WG intake alone. A greater increase in stool frequency may be expected in populations experiencing constipation: Haack et al. (31) suggested that fiber increases stool frequency mostly in individuals with an output of <1 stool/d. In our study population, few participants had <1 stool/d.

Dietary fiber represents one of the main sources of nutrients for the gut microbiota. In this study, the difference in total dietary fiber intake was small between the 2 groups because WG foods are not necessarily good sources of fiber; thus, it is not surprising that we did not observe significant differences in overall microbiota diversity. However, the data showed a variety of bacterial signatures that were affected by the intervention. Differences in microbiota composition including higher amounts of total fecal bacteria might have contributed to changes in stool frequency. In this study total stool output over multiple days was not collected, which would be required to determine differences in total fecal bacteria output. The bacterial signatures affected by the interventions likely represent bacteria best adapted to the changes in the gut environment associated with the added grains. Bifidobacteria and LAB, often considered beneficial for the host’s health, increased with both WG and RG foods and with WG foods, respectively. Although the baseline genome equivalents of LAB were higher in participants in the RG group, there was no difference between the diet groups at the final time period. It is plausible that an effect of RG on LAB was not observed due to the higher baseline genome equivalents in the RG group.

Fecal and salivary sIgA were measured as indicators of mucosal immune function. Low concentrations of sIgA are thought to be related to higher rates of upper respiratory infections and decreased clearance of pathogens from the intestine (32–34). Neither fecal nor salivary sIgA was influenced by intake of WG; however, both were dependent on baseline weight category. Participants who were at a healthy weight had higher concentrations of fecal sIgA compared with those who were obese, and salivary sIgA concentrations increased between baseline and final time periods only in participants who were at a healthy weight. Although limited, studies in normal-weight versus overweight children show differences in circulating concentrations of immunoglobulin and numbers of leukocytes (35,36). The authors suggested that the increased number of circulating immune cells observed in overweight and obese children indicates the presence of a low-level systemic inflammation (36). Our data are consistent with that hypothesis because both serum CRP and LPS-stimulated TNF-α concentrations were significantly higher in obese participants.

Phytochemicals and antioxidants in WG foods may modulate the inflammatory state (37,38); however, we did not observe differences in the production of inflammatory cytokines between diet groups with autologous serum in LPS-stimulated cultures. This could be due to the types of WG foods provided, which varied in their phenolic acid content and bioaccessibility (39,40). In addition, foods provided to the RG group, such as white bread and pasta, are known sources of phenolic acids (40,41). Miller et al. (41) reported that the mean antioxidant activities of white bread and white-rice cereal (bran and germ removed) were 1200 and 1300 Trolox equivalents/100 g, respectively, compared with WG bread, which provided 2000 Trolox equivalents/100 g.

It was surprising to observe decreased in vitro concentrations of the proinflammatory cytokines with both dietary interventions...
(i.e., WG and RG foods). This effect was not related to total fiber or total-grain intake (data not shown). Others have observed diet-induced changes in in vitro cytokine production, which they attributed to changes in plasma and cell membrane fatty acids. For example, 1 wk of supplementation with a medical food containing protein, fish oil, and specific oligosaccharides decreased the ratio of (n-6):(n-3) PUFA in plasma and white blood cells and increased production of IL-1β, TNF-α, and IL-6 in ex vivo LPS-stimulated whole-blood cultures (42). Kalogeropoulos et al. (43) showed that plasma concentrations of SFA were positively correlated with serum concentrations of IL-6 and TNF-α. Although it is unknown whether the composition of dietary fat or concentrations of plasma fatty acids changed with the introduction of WG or RG foods, serum TG concentrations decreased and total antioxidant capacity increased with both interventions, and autologous serum was used in the in vitro cultures.

For both the WG and RG groups, concentrations of IL-2 increased and IL-10 decreased in PBMC cultures stimulated with the T cell mitogen. It is possible that the interventions or other unmeasured lifestyle changes altered the percentages of circulating lymphocytes to monocytes. Changes in diet, exercise, and levels of stress have been shown to modulate cytokine production as well as leukocyte percentages (44–46).

Another possible explanation for the observed changes in mitogen-induced cytokine production and salivary and fecal sIgA with the WG and RG interventions is that the overall quality of the diet improved. Enriched grains fortified with folic acid are still an important part of a balanced diet. In fact, a recent systematic review concluded that consuming up to 50% of all grains as “core” refined grains (i.e., those without significant added sugar, fat, and sodium) is not associated with disease risk (47). Mean folate, thiamin, riboflavin, niacin, iron, zinc, calcium, and vitamins B-12, B-6, and E from the targeted foods increased from prebaseline levels across both diet groups. It is possible that increased micronutrient intakes observed in both groups contributed to the increase in total antioxidant capacity and decreased production of inflammatory cytokines (48).

There were a number of limitations associated with this study. Due to time constraints, multipass, targeted 24-h recalls were obtained. This method decreased participant burden and allowed investigators to obtain 8 dietary recalls from each participant including for those who rode the school bus or who did not have the flexibility of arriving at school earlier on the mornings of their recalls; however, it did not provide dietary intake information from protein or dairy foods or solid fats and added sugars. This information would have been helpful to determine whether intake of added sugars and solid fats decreased when total-grain intake increased and whether these changes altered serum TG and markers of immune function.

Most studies of inflammatory cytokines and health outcomes measure circulating cytokines from the plasma (49). This study took the novel approach of measuring in vitro mitogen-induced cytokine production because it was not anticipated that there would be measurable concentrations of cytokines in the plasma of healthy adolescents. By completing the in vitro mitogen stimulation studies, measurable differences across all baseline weight categories were observed. Although these differences were attributed to the dietary interventions, it is possible that they could be a result of unmeasured lifestyle changes adopted by the participants and his or her family as a result of almost daily contact with study coordinators. Future studies may need to include a nonintervention control group.

In summary, adolescents and their families were given a grocery bag of grain-based foods each week and asked to replace the grains in their kitchen with the study foods. Participants were encouraged to eat 3 different kinds of study foods each day with the goal of 5 oz-eq of RG or WG foods/d. Both groups successfully incorporated a variety of study foods into their diets and increased their total grain and related micronutrient intake. Whereas WG study foods improved aspects of gastrointestinal function to a greater extent than did RG foods, both interventions equally increased serum antioxidant concentrations and decreased inflammatory cytokine production. These data give support to the Dietary Guidelines for Americans (1), which recommend that Americans eat a minimum of 48 g WG/d and include enriched and fortified grains as an important source of micronutrients. Future studies need to examine health outcomes related to dietary intake of WG or nutrient-dense RG as part of a complete diet.

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Literature Cited


