Whole Grain Oats Improve Insulin Sensitivity and Plasma Cholesterol Profile and Modify Gut Microbiota Composition in C57BL/6J Mice

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Abstract

Background: Whole grain consumption reduces the risk of major chronic diseases. It is not clear how whole grains exert their beneficial effects.

Objective: The aim was to compare the physiologic effects of whole grain oat (WGO) flour with low bran oat (LBO) flour.

Methods: Two AIN-93G-based diets were formulated with either WGO or LBO flour. Five-week-old male C57BL/6J mice were fed LBO (n = 11) and WGO (n = 13) diets for 8 wk. Cecal microbiota was profiled by pyrosequencing of the 16S ribosomal RNA gene. Data are reported as means ± SEMs or antilogs of the mean (mean – SEM, mean + SEM).

Results: The weight gain was 14.6% less in the WGO group during week 7 (P = 0.04). WGO improved insulin sensitivity as reflected by significantly lower plasma insulin [1500 (1370, 1650) ng/L vs. 2340 (2090, 2620) ng/L; P = 0.006], C-peptide (3980 ± 548 ng/L vs. 7340 ± 1050 ng/L; P = 0.007), and homeostasis model assessment-estimated insulin resistance (21.4 ± 2.3 vs. 34.7 ± 4.9; P = 0.03). Plasma total cholesterol was 9.9% less and non-HDL cholesterol was 11% less in the WGO group. A comparison of relative abundance indicated Prevotellaceae, Lactobacillaceae, and Alcaligenaceae families were 175.5% (P = 0.03), 184.5% (P = 0.01), and 150.0% (P = 0.004), respectively, greater in the WGO group and Clostridiaceae and Lachnospiraceae families were 527% (P = 0.004) and 62.6% (P = 0.01), respectively, greater in the LBO group. Cecal microbiota composition predicts 63.9% variation in plasma insulin and 88.9% variation in plasma non-HDL cholesterol.

Conclusions: In mice, WGOs improved insulin sensitivity and plasma cholesterol profile compared with LBOs, and the effects were associated with the changes in cecal microbiota composition. Increasing WGO consumption may help improve insulin sensitivity and dyslipidemia in chronic diseases.

Keywords: 16S rRNA gene, beta-glucan, HDL cholesterol, chronic disease, gut microbiota, insulin sensitivity, low bran oats, mice, pyrosequencing, whole grain oats

Introduction

A growing body of evidence supports that higher whole grain consumption is associated with a reduced risk of the development of major chronic diseases, including cardiovascular disease, type 2 diabetes, and certain types of cancer (1). Data from 10 prospective cohort studies indicate that the highest whole grain intake is associated with a 21% reduction in cardiovascular disease risk compared with the lowest intake after adjustment of confounding factors (2). Based on data from 6 prospective cohort studies, the group with the highest whole grain intake has an estimated 26% lower risk of development of type 2 diabetes compared with the lowest intake group after multivariate adjustment (2). A meta-analysis of 4 prospective cohort studies indicated that each increment of 3 daily servings of whole grain foods reduces the risk of colorectal cancer by 7–17% (3).

The mechanism by which whole grains exert their beneficial effects is not fully understood. Evidence suggests that dietary fiber from whole grains may be responsible for some of these beneficial effects. β-Glucan, a soluble fiber found in oats and barley, has been extensively studied and has been shown to reduce glycemia (4) and cholesterolemia (5) and alleviate insulin resistance and metabolic syndrome (6). In addition to fiber, whole grains are a source of many bioactive components including minerals, vitamins, and phenolic compounds (7, 8).

As a worldwide staple food, oats contain protein, important minerals, lipids, β-glucan, and various other phytoconstituents (9). Because of its physiologic activities, oats are considered a potential therapeutic agent (9), and a health claim has been approved by the
FDA for lowering plasma cholesterol concentrations and reducing risk of cardiovascular diseases (10). Additional health benefits of whole grain oats (WGOs)\(^6\) may yet be identified.

One potential mechanism for the beneficial effects of oats is through alteration of the gut microbiota. Gut microbiota composition has been associated with various metabolic diseases including obesity and diabetes (11). However, there are only limited data regarding the effects of WGOs on gut microbiota composition. Previous work has shown that oat β-glucan, oat bran, and oat flour increase bifidobacteria in rats (12–14), and oat β-glucan also increases Lactobacillus in rats (12, 13). Furthermore, in vitro fermentation of oat flakes by human fecal microbiota increases both Prevotella (15, 16) and bifidobacteria (15). Much of these effects may be attributable to the relatively large amount of oat fermentable fibers [3–8% (wt:wt)], comprised mainly of β-glucan (6). However, whether the beneficial systemic effects of WGOs are associated with changes in the gut microbiota is currently unknown.

This study’s objective was to compare the effects of WGO flour vs. low bran oat (LBO) flour on metabolic phenotypes and cecal microbiota composition with the use of diets matched for total protein, carbohydrates, fat, and insoluble fiber.

**Methods**

**Animals.** The protocol was approved by the Institutional Animal Care and Use Committee at Utah State University. Twenty-six 5-wk-old male C57BL/6J mice were purchased from Charles River (Wilmington, Massachusetts). Mice were individually housed in polycarbonate cages and kept on a 12:12 light:dark cycle (lights on at 07:00 and off at 19:00) under constant temperature (24°C ± 1°C) and controlled humidity (60% ± 10%). After 1 wk of acclimatization, mice were randomized to LBO (n = 13) flour or WGO (n = 13) flour-supplemented diet based on AIN-93G formulation (17). Mice consumed ad libitum with free access to water for 8 wk. Coprophagy was allowed. Food intake and body weight were measured weekly. Immediately before experimental diets, during week 4, and during week 8, body composition was estimated by NMR (EchoMRI Analyzer; Echo Medical Systems LLC). During weeks 2 and 6, cumulative food intake was measured over a 7-d period. The food efficiency ratio was calculated as change in body weight/cumulative food intake. Two mice from the LBO group died during the experiment because of unknown causes.

**Diets.** The compositions of diets appear in *Supplemental Table 1*. LBO and WGO flours were provided by General Mills. The flours were analyzed for macronutrient and FA content by General Mills. Flours were incorporated into the diets at concentrations to provide 32% of energy as available carbohydrate. Protein concentrations in the diets were matched by adding wheat gluten to the LBO diet. Fat and linoleic acid concentrations in the diets were matched by reducing the amount of soybean oil in the WGO diet. Insoluble fiber concentrations in the diets were matched by adding cellulose to the LBO diet. Diets were prepared by Research Diets and were stored at 4°C.

**Sampling protocol.** For logistical reasons, mice were divided into 3 cohorts (each cohort containing 3–5 mice from each diet group), with each cohort killed on separate consecutive days. On each collection day, food was removed at 08:00 and the mice were killed between 10:00 and 12:00. Mice were injected intraperitonally with Nembutal (150 mg/kg body weight) and exsanguinated via cardiac puncture. The liver, heart, kidneys, retroperitoneal fat pads, epididymal fat pads, and cecum were carefully dissected and immediately weighed. Ceca contents were expressed into cryovials, and the empty ceca were reweighed. All samples were immediately frozen in liquid nitrogen and were stored at −80°C until further analysis.

**Chemical analyses.** Blood was collected and immediately placed into tubes containing 50 μL of a protease inhibitor cocktail to prevent degradation of protease-sensitive hormones. The protease inhibitor cocktail contained 1 μmol Pefabloc SC (Sigma), 10 μL Protease Inhibitor Cocktail (Sigma), and 10 μL dipeptidyl peptidase-4 inhibitor (DPP IV Inhibitor; Millipore) per milliliter of blood. Blood was centrifuged, the volume of plasma was recorded, and the plasma was immediately frozen in liquid nitrogen. Blood glucose concentrations were measured using a portable glucometer (OneTouch Ultra; LifeScan, Inc.). Plasma total cholesterol, HDL cholesterol and TG, and hepatic TG were measured enzymatically using commercial reagents (Infinity Total Cholesterol and Infinity Triglycerides, Thermo Fisher Scientific, Inc.; HDL Cholesterol Precipitating Reagent Set, Pointe Scientific, Inc.). Plasma non-HDL cholesterol was calculated. Plasma hormones and cytokines were measured using multiplex bead-based reagent kits (Millipore) on a BioPlex 200 (BioRad). Plasma measurements were adjusted for the dilution associated with the addition of the protease inhibitor cocktail. In instances where isolated analyte values were below the detectible limits of an assay, a value equal to one-half of the lowest detectible value was substituted for the missing value. In cases where all values were below the lowest value on the standard curve, the raw fluorescent intensity was used for analysis.

**Intraperitoneal glucose tolerance tests.** Intraperitoneal glucose tolerance tests (IGTTs) were conducted during week 6 of the study. Mice were divided into 3 cohorts with each cohort tested on separate consecutive days. At time \(t = 0\), 1.5 mg of glucose/kg of body weight (as sterile 20% glucose solution) was injected intraperitoneally. Immediately before the glucose injection and then at \(t = 15\), 30, 60, and 90 min, blood glucose concentrations were measured from tail vein blood. Data were analyzed in terms of absolute glucose concentrations at each time point, change in glucose concentrations from \(t = 0\), and as the total AUC. Data analysis included the test cohort in the model.

**Hepatic gene expression.** RNA was isolated from liver samples and the expression levels for genes involved in FA oxidation, FA/TG synthesis, glucose uptake, and gluconeogenesis were estimated using a bead-based multiplex assay (QuantiGene; Panomics, Affymetrix) designed for a Luminex type of analytical platform (BioPlex 200; BioRad). Gene expression data were normalized relative to 3 housekeeping genes (hypoxanthine phosphoribosyltransferase 1, peptidylprolyl isomerase B, and Gapdh).

**Hepatic and intestinal microarray analyses.** Hepatic RNA samples from successive groups of 3 mice (based on their final body weight ranking) were pooled for microarray analysis (providing 4 pools from each diet group). Successful hepatitis RNA pools from 2 diet groups were reverse labeled with Cy3 and Cy5, pooled, and applied to a Mouse GE 4 × 4K v2 microarray (Agilent). RNA samples from intestinal mucosa were arranged similarly as hepatic RNA samples. The microarrays were run at the University of Utah Genomics Core. The lowest normalized expression data files in text format were obtained from Agilent Feature Extraction. Data quality was checked by principal component analysis. The data were imported into GeneSpring GX 12.6 (Agilent). Each 2-color channel was split into 2 individual single-color channels, and the new channels were treated as single color samples. Quantile normalization was performed, and the data were baseline transformed to the mean of all samples. The data were then filtered to keep detected entities with a well above background signal. Paired \(t\) tests were performed with a \(P\) cutoff of 0.05 and no multiple-test correction. A fold change cutoff of 1.1 was applied, and the gene list was subjected to pathway analysis through the Simple Experiment Analysis feature using curated WikiPathways (157 analysis pathways and 32 other pathways). Significant pathways (\(P < 0.001\)) were selected.

**Fecal microbiota analysis.** DNA was isolated from cecal samples (\(n = 11\) for LBO group or \(n = 13\) for WGO group). The V1 + V2 region of the
bacterial 16S ribosomal RNA (rRNA) gene was amplified using tag-encoded primers for pyrosequencing (Roche 454 GS FLX; Roche). The V1 forward primer was 5’-AGAGTTTGATCCTGGCTCAG (BSF8) and the V2 reverse primer was 5’-CTCGTCCCGTCTCAG (BSR357). Microbiota sequences were processed through QIIME 1.8.0 (QIIME Team) (18). After quality filtering and sample assignment, the sequences were denoised using denoise_wrapper.py. The denoised sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity against a reference GreenGenes OTU database (gg_13_8_otus) (19) using the open-reference OTU picking approach with UCLUST (20). The most abundant sequences were made with Ribosomal Database Project Classifier (22) using the default lanemask file and the chimeras were removed. On diversity analysis, the chimera-filtered representative sequences were further subjected to OTU abundance quality filtering (OTUs were discarded with a number of sequences <0.005% of the total number of sequences). Unweighted and weighted UniFrac distances were then generated.

**Statistical analysis.** Statistical analyses were conducted using Microsoft Excel 2010 (Microsoft Corp.), JMP 11.0.0 (SAS Institute, Inc.), and R 3.02 (R Foundation). Because some analytes or gene expression levels may be sensitive to the length of food deprivation and/or specific environmental conditions on the date of termination, both the date and the time of sample collection were initially included in the statistical model. If the P-values for these potential covariates were <0.1 in the initial model, they were retained in the final model, otherwise they were removed. Data with normal distributions are presented as means ± SEMs. Data with non-normal distributions were log transformed before analysis. For easy interpretation, rather than presenting the log transformed data, the data are presented as the antilogs of the mean (mean – SEM, mean + SEM). A value of P < 0.05 was considered statistically significant.

The filtered OTU table (>0.005% abundance) was subjected to redundancy analysis (RDA) by CANOCO (version 5) for Windows (Microcomputer Power) according to the manufacturer’s instructions (25). Key OTUs were identified as having at least 15% of the variability in their values explained by the first axis (26). The key OTUs were subjected to linear discriminant analysis effect size (LEfSe) analysis (27). Threshold on the logarithmic linear discriminant analysis score for discriminative features was 4.0 (a threshold of 2 generated too many differential features). The α-value for the factorial Kruskal-Wallis test among classes was 0.05. The strategy for multiclass analysis was all-against-all. Partial least squares (PLS) regression was performed with JMP 11.0.0 (SAS Institute, Inc.) to assess the relation between the gut microbiota composition and host phenotypes (28). The predictors and responses were centered and scaled to have mean 0 and SD 1. The PLS model was verified by leave-one-out cross-validation. The nonparametric Kendall’s rank correlation matrix between the key OTUs and host phenotypes were generated to create a heat map. Spearman correlations between unweighted UniFrac distances and selected phenotype variables were generated and plotted as a network.

**Results**

**Effects of WGO flour on organ weights and cecum contents.** Weight gain tended to be less in the WGO group than in the LBO group (P = 0.09) with the difference substantial at week 7 (P = 0.04; Figure 1). At the end of the study, there were no significant differences in body weight, weight gain, fat gain, lean mass gain, food intake, or food efficiency ratio between groups (Supplemental Table 2). No difference was found in relative weight as a percentage of body weight for liver, kidney, heart, retroperitoneal fat, epididymal fat, and cecum (Supplemental Table 3). The WGO group had 20% higher cecum contents compared with the LBO group (P = 0.04; Supplemental Table 3).

**WGO flour increased insulin sensitivity.** Plasma glucose in the WGO group was less than that in the LBO group at 15 and 30 min after the glucose challenge during IGTT (P < 0.05; Figure 2). Plasma insulin, HOMA-IR, C-peptide, leptin, and resistin concentrations in the WGO group were less than those in the LBO group (P < 0.05; Table 1). Plasma gastric inhibitory peptide (GIP) in the WGO group was also less than that in the LBO group (P = 0.04; Table 1). The intake of WGO did not affect plasma glucagon, adiponectin, ghrelin, active amylin, glucagon-like peptide 1, peptide YY (PYY), and pancreatic polypeptide (Table 1).

**WGO flour reduced plasma non-HDL cholesterol but did not affect systemic inflammation.** Plasma non-HDL cholesterol in the WGO group was 11% less than that in the LBO group (P = 0.009; Table 1). No difference in plasma HDL cholesterol or TG was observed (Table 1). No difference was observed in plasma IL-6, TNF-α, and monocyte chemoattractant protein 1 (MCP-1) between groups (Supplemental Table 4).

**Hepatic TG and gene expression.** Hepatic TG concentrations in the WGO group were less than that in the LBO group (3.37 [2.90, 3.91] mg/g vs. 5.00 [4.45, 5.61] mg/g of liver).

**Blood glucose increments of male C57BL/6J mice fed a WGO or LBO diet for 8 wk at 15 and 30 min during IGTT were significantly lower in the WGO group than in the LBO group. The inset shows the AUC. Values are means ± SEMs; n = 11 for LBO diet and n = 13 for WGO diet. *Different from WGO diet at that time (P < 0.05).**
TABLE 1  Plasma glucose, insulin, hormones, and lipids in male C57BL/6J mice fed LBO or WGO diet for 8 wk1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LBO</th>
<th>WGO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>261 ± 21</td>
<td>253 ± 18</td>
<td>0.77</td>
</tr>
<tr>
<td>Insulin, ng/L</td>
<td>2340 (2030, 2620)</td>
<td>1500 (1370, 1650)</td>
<td>0.0062</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>34.7 ± 4.9</td>
<td>21.4 ± 2.3</td>
<td>0.03</td>
</tr>
<tr>
<td>C-peptide, ng/L</td>
<td>7340 ± 1050</td>
<td>3890 ± 548</td>
<td>0.007</td>
</tr>
<tr>
<td>Glucagon, ng/L</td>
<td>27.9 (21.6, 36.2)</td>
<td>31.9 (26.3, 38.8)</td>
<td>0.392</td>
</tr>
<tr>
<td>Leptin, ng/L</td>
<td>7580 (6420, 8960)</td>
<td>4370 (3700, 5180)</td>
<td>0.03</td>
</tr>
<tr>
<td>Adiponectin, μg/L</td>
<td>14.1 ± 0.6</td>
<td>12.8 ± 0.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Ghrelin, ng/L</td>
<td>65.1 (52.8, 80.2)</td>
<td>73.0 (58.9, 90.5)</td>
<td>0.702</td>
</tr>
<tr>
<td>Amylin (active), ng/L</td>
<td>209 ± 17</td>
<td>179 ± 17</td>
<td>0.04</td>
</tr>
<tr>
<td>GIP, ng/L</td>
<td>210 (171, 258)</td>
<td>122 (105, 143)</td>
<td>0.012</td>
</tr>
<tr>
<td>Glucagon-like peptide 1, ng/L</td>
<td>198 ± 36</td>
<td>210 ± 31</td>
<td>0.692</td>
</tr>
<tr>
<td>Pancreatic polypeptide, ng/L</td>
<td>11.4 (8.6, 21.1)</td>
<td>14.4 (8.3, 25.1)</td>
<td>0.792</td>
</tr>
<tr>
<td>PYY, ng/L</td>
<td>162 (145, 181)</td>
<td>178 (159, 202)</td>
<td>0.082</td>
</tr>
<tr>
<td>Resistin, μg/L</td>
<td>29.3 ± 1.9</td>
<td>23.7 ± 1.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>333 ± 13</td>
<td>300 ± 13</td>
<td>0.0092</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>56.3 ± 1.8</td>
<td>55.3 ± 1.4</td>
<td>0.22</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mg/dL</td>
<td>275 ± 12</td>
<td>244 ± 12</td>
<td>0.0092</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>64.2 (58.8, 70.2)</td>
<td>55.3 (51.7, 59.2)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

1 Data are means ± SEMs or the antilogs of the mean (mean – SEM, mean + SEM), n = 11 for LBO diet or n = 13 for WGO diet. GIP, gastric inhibitory peptide; LBO, low bran oat; PYY, peptide YY; WGO, whole grain oat.
2 Length of fasting included in model.
3 Day of dissection included in model.

Plasma insulin levels were not significantly different between groups. Glucagon and C-peptide levels were increased with WGO diet compared with LBO diet. Plasma glucose was higher in the WGO group compared with the LBO group. Leptin levels were lower in the WGO group compared with the LBO group.

Hepatic expression levels for genes involved in FA oxidation, FA/TG synthesis, gluconeogenesis, and glucose uptake were not significantly different between groups (Supplemental Table 5).

**Affected pathways identified by microarray analysis.**

Principal component analysis revealed that replicates within one group clustered together and separately from arrays in the other group, which indicated good data quality. When a P-value of 0.001 was used as a cutoff, WGOs significantly affected 3 pathways in the liver and 1 pathway in the intestinal mucosa compared with LBO (Table 2). WGOs upregulated the transcription of 7 of the 8 differentially regulated genes in the insulin signaling pathway. WGOs upregulated all 5 differentially regulated genes in the TGF-β signaling pathway and 4 of 6 differentially regulated genes in the adipogenesis pathway in the liver. WGOs upregulated the transcription of all 6 differentially regulated genes in the cell cycle pathway in the intestinal mucosa (Table 2).

**Overall compositional changes of gut microbiota.**

Of 1,081,757 reads from 24 samples with an average of 45,073 reads per sample (±14,380 SD), 1,234 species-level OTUs were identified using 97% as a homology cutoff value. Of these, 596 OTUs were selected with an abundance >0.005% of the total number of sequences for downstream analysis. Unweighted UniFrac distances of cecal microbiota indicated that LBO and WGO groups were well separated (Supplemental Figure 1). Among the 596 OTUs, 549 were Firmicutes and 34 were Bacteroidetes, which accounted for >99% of the bacteria. The abundance of Firmicutes (55.8% ± 3.6% vs. 48.5% ± 2.8% for LBOs vs. WGOs, respectively; P = 0.054) and Bacteroidetes (43.8% ± 3.6% vs. 50.6% ± 2.8% for LBOs vs. WGOs, respectively; P = 0.064) were not significantly different between groups. The WGO group had 1.8 times more Lactobacillales than the LBO group (1.04% ± 0.25% vs. 0.37% ± 0.12%; P = 0.01). Prevotellaceae, Lactobacillaceae, and Alcaligenaceae families in the WGO group were 175.5% (P = 0.03), 184.5% (P = 0.01), and 150.0% (P = 0.004), respectively, greater than those in the LBO group. Clostridiaceae and Lachnospiraceae families in the LBO group were 527.1% (P = 0.004) and 62.6% (P = 0.01), respectively, greater than those in the WGO group. The RDA revealed 124 OTUs that had at least 15% of the variability in their values explained by the first axis of RDA. The list of 124 OTUs was subjected to LEfSe analysis. A cladogram representation of gut microbiota significantly affected by diet was generated using LEfSe analysis (Figure 3A). The most differentially affected taxa between the 2 groups are shown in Figure 3B. Bacteroidetes-Prevotellaceae, Firmicutes-Lactobacillaceae, and Proteobacteria-Sutterellaceae genera were greater in the WGO group whereas Firmicutes-Clostridiaceae and Firmicutes-Lachnospiraceae families were greater in the LBO group.

**Nonparametric correlation between gut microbiota composition and host phenotypes.** Nonparametric Kendall’s correlations between relative abundance of OTUs and relevant phenotype variables are shown in Figure 4. The selected phenotype variables formed 3 clusters. The correlations between the left cluster (liver TG, leptin, adiposity and body weight) and OTUs were generally characterized as being positive for Clostridiales but negative for Bacteroidetes (Figure 4). The right cluster (C-peptide, insulin, GIP, and resistin) were negatively correlated with Coriobacteriales and Bacteroidales but positively correlated with Lachnospiraceae, Clostridiaceae, and Ruminococcaceae (Figure 4). There were strong negative correlations between insulin/C-peptide and OTUs in the Bacteroidetes group (Figure 4). Nonparametric Spearman correlation analysis also showed that unweighted UniFrac principle coordinates 1 and 2 were negatively correlated with insulin, C-peptide, leptin, total cholesterol, and non-HDL cholesterol (Supplemental Figure 2).

The leave-one-out cross-validated PLS model revealed that the gut bacterial species (124 OTUs) were only significantly correlated with glucose, insulin, and leptin. There was a strong negative correlation between C-peptide and OTUs in the Ruminococcaceae (Figure 4). There were strong negative correlations between insulin/C-peptide and OTUs in the Bacteroidetes group (Figure 4). Nonparametric Spearman correlation analysis also showed that unweighted UniFrac principle coordinates 1 and 2 were negatively correlated with insulin, C-peptide, leptin, total cholesterol, and non-HDL cholesterol (Supplemental Figure 2).

**TABLE 2** Gene expression pathways significantly affected by WGO diet vs. LBO diet in the liver and intestinal mucosa of male C57BL/6J mice fed LBO or WGO diet for 8 wk1

<table>
<thead>
<tr>
<th>Matched/pathway</th>
<th>WGO ↑/↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathway in liver</td>
<td></td>
</tr>
<tr>
<td>Mm_Insulin_Signaling_WP65_71726</td>
<td>0.0006</td>
</tr>
<tr>
<td>Mm_TGF_Beta_Signaling_Pathway_WP113_69818</td>
<td>0.0008</td>
</tr>
<tr>
<td>Mm_Adipogenesis_WP447_72045</td>
<td>0.0009</td>
</tr>
<tr>
<td>Pathway in intestinal mucosa</td>
<td></td>
</tr>
<tr>
<td>Mm_Cell_cycle_WP190_71755</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

1 LBO, low bran oat; WGO, whole grain oat; ↑, upregulated; ↓, downregulated.

Non-HDL cholesterol, mg/dL | 275 ± 12 | 55.3 ± 1.4 | 0.0092 |

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associated with 18 variables of over 100 phenotype variables measured. Results from the PLS model are presented in Table 3. Among those 18 variables, relative weight of cecum content as a percentage of body weight, non-HDL cholesterol, total cholesterol, insulin, C-peptide, resistin, PYY, and GIP were significantly different between the 2 groups. Insulin, C-peptide, GIP, and resistin formed a cluster (Figure 4) and together they also fit a PLS model (Table 3). Weight gain, final body lean mass, relative weight of mesenteric fat as a percentage of body weight, cecum content, HDL cholesterol, amylin, glucagon-like peptide 1, and hepatic expression of carnitine palmitoyltransferase 1A (Cpt1a), Ppard, and Pparg were also correlated with gut microbiota composition but were not significantly different between the 2 groups (Supplemental Table 6).

Discussion

The overall goal of the current study was to identify potential novel health benefits of WGOs in C57BL/6J mice. Previous preclinical and clinical studies show favorable effects of whole oats or oat fractions (including β-glucans) on plasma lipid concentrations (reduction in LDL cholesterol) and insulin sensitivity (5, 6). In this study, we similarly showed improvements in plasma cholesterol concentrations and insulin sensitivity by whole oats in mice. We also observed significant improvements in the metabolic profiles of mice fed WGO flour compared with animals fed LBO flour. In contrast, no significant effects were observed for indices of systemic inflammation. Finally, WGOs additionally upregulated the transcription of several genes in the hepatic insulin signaling pathway and modified the gut microbiota composition, which was associated with many of the endpoints that were significantly different between the 2 diet groups.

When whole foods with complex compositions are studied, the design of an appropriate control diet remains a challenge and ultimately influences any conclusions that can be derived from the study. In the current study, the 2 diets had equivalent amounts of carbohydrates, total protein, fat, linoleic acid, and insoluble fiber. Remaining differences in soluble fiber, specific proteins, FA profiles, and phytochemicals including phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides (7) between the 2 diets would presumably drive any differences in the measured phenotypes. Of these differences, it is likely that
the increased content of soluble fiber in WGO (1.4% wt/wt more soluble fiber, mainly β-glucan) contributed to many of the observed effects of the WGO diet.

A potential mechanism for β-glucan inhibition of food intake and weight gain is through its gel-forming effect (29, 30), which may delay gastric emptying (31) and reduce or delay the

FIGURE 4 Nonparametric Kendall’s rank correlations between relative abundance of OTUs and relevant phenotype variables in male C57BL/6J mice fed a WGO or LBO diet for 8 wk. OTUs within the Clostridiales family were largely positively correlated with the phenotypes in the body weight, adiposity, leptin, liver TG cluster, and the C-peptide, insulin, GIP, resistin cluster. OTUs within the families of S24-7 and Prevotellaceae were negatively correlated with both aforementioned clusters (n = 24). f., family; g., genus; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; IGTT, intraperitoneal glucose tolerance test; LBO, low bran oat; NR, new reference; o., order; OTU, operational taxonomic unit; s., species; uni., unidentified; WGO, whole grain oat.
Although the terminal glucose values were artificially high, both at 12:00, during which the mice may have had a glucose peak (43). Nembutal injection at 150 mg/kg body weight. Therefore, the blood was withdrawn a few minutes after injection. The blood glucose returns to baseline after 60 min in C57BL/6J mice (42). The blood glucose by about 60 mg/dL at 15 min after the injection butal injection. Nembutal at 50 mg/kg body weight increases hyperglycemia relative to the time 0 values obtained during fasting. Oat products also decrease fasting glucose and glycosylated serum protein in both humans and animal models. Addition of oat bran to the diet reduces the postprandial PYY 3–36 release may be under neural control and that further release of PYY 3–36 may be caused by the nutrients appearing in the distal gut (36). A difference in dietary β-glucan above 3.5% results in a significantly higher plasma PYY. It is thought that the initial postprandial PYY 3–36 release may be under neural control and that further release of PYY 3–36 may be caused by the nutrients appearing in the distal gut (36). A difference in dietary β-glucan above 3.5% results in a significantly higher plasma PYY (35). The small difference (1.4%) in β-glucan between the LBO and WGO diets could account for the observed small but nonsignificant (P = 0.08) increase in plasma PYY in the current study.

Our observations of improvements in several markers of insulin sensitivity in mice are consistent with previous observations in both humans and animal models. Addition of oat bran to the diet reduces the postprandial insulin responses in both normolipidemic men (37) and subjects with non–insulin-dependent diabetes (38). Oat β-glucan decreases both postprandial glucose and insulin responses in normal-weight subjects (39). Oat products also decrease fasting glucose and glycosylated serum protein in streptozotocin-induced diabetic male Kun Ming mice (40). The presence of intact oat cell-wall materials in the small intestine has been suggested as a key factor that influences the rate at which glucose, maltose, and maltotriose are released (41).

At the conclusion of the study, we noticed a marked hyperglycemia relative to the time 0 values obtained during the IGTT in both groups. Several factors may have contributed to the observed hyperglycemia. The first factor was the Nembutal injection. Nembutal at 50 mg/kg body weight increases blood glucose by about 60 mg/dL at 15 min after the injection and blood glucose returns to baseline after 60 min in C57BL/6J mice (42). The blood was withdrawn a few minutes after Nembutal injection at 150 mg/kg body weight. Therefore, the increase of blood glucose concentration by Nembutal could be over 60 mg/dL. Second, the fasting (2–4 h) was shorter than usual (4–6 h). Finally, the mice were killed between 10:00 and 12:00, during which the mice may have had a glucose peak (43). Although the terminal glucose values were artificially high, both diet groups were treated identically, suggesting that comparisons across diet groups may still be valid.

The observed reduction in non-HDL cholesterol in our study is well documented. The cholesterol-lowering activities of oats have been attributed to the β-glucan fractions in humans (5) and animals (44–46). Several mechanisms have been proposed for the hypocholesterolemic effect of oat fractions and/or β-glucan including 1) increasing viscosity of intestinal contents and forming an unstirred layer over intestinal mucosa, which in turn result in reduced absorption of dietary cholesterol and reabsorption of bile acids (47); 2) increasing binding of luminal bile acids by β-glucan thereby decreasing enterohepatic circulation of bile acids with subsequent increased hepatic uptake of circulating cholesterol for bile acids synthesis (48, 49); 3) reducing hepatic cholesterol synthesis secondary to improved insulin sensitivity (49, 50); and 4) inhibiting hepatic cholesterol synthesis by acetate (11) and propionate (12) produced by colonic bacteria fermentation of soluble fibers. The design of our study could not differentiate between the aforementioned mechanisms.

This is one of the first studies exploring the effect of WGO flour on gut microbiota using 16S rRNA gene pyrosequencing. Others (51) have used more targeted approaches through the use of PCR-based methods to estimate the abundance of specific taxonomic groups of gut microbiota. In the current study, we chose to use a more global approach to understand the effects of WGOs on overall gut microbiota composition. We recognize that the specific microbiota composition derived from next-generation sequencing analysis critically depends on both the 16S rRNA hypervariable region chosen for amplification and the specific primers used (52, 53). The V1-V2 hypervariable region chosen for the current study has been extensively used in previous studies (54–56). The primers used in the current study were selected because of their performance in capturing taxonomic information based on in silico analyses (53). However, the primers used to amplify V1-V2 hypervariable region of the 16S rRNA gene have limitations, specifically when it comes to amplification of bifidobacteria species (57). Nonetheless, between-group comparisons remain valid for those species that are successfully amplified, given that the same primer pairs were used in both groups.

In comparison with those in the LBO group, Prevotella and Sutterella were higher while Clostridiaceae and Lachnospiraceae were lower in the WGO group. In vitro fermentation of β-glucan fractions from oats by human fecal microbiota increases in the Prevotella group (16), and long-term dietary fiber intake is positively correlated with relative Prevotella abundance (58), which is consistent with our findings. In general, fecal Prevotella abundance is higher in humans that consume more carbohydrates and/or plant-based diets relative to Western diets (56, 59, 60). The physiologic relevance of WGO effects on Prevotella, Sutterella, Lachnospiraceae, and Clostridiaceae awaits further investigation.

Select bacterial strains of the genus Lactobacillus and Bifidobacterium are hypothesized to confer numerous health benefits, including gastrointestinal (61) and cardiovascular (62) health, as well as weight management (63). In this study, no sequences belonging to the genus Bifidobacterium were identified in mice, likely because of the choice of primers as previously noted. However, Lactobacillus in the WGO group was more than 2 times that in the LBO group. Lactobacillus (measured by plate counting) in male SD rats fed oat β-glucan is greater than that in the control fed no oat β-glucan (12, 13). Additionally, Lactobacillus in BioBreeding rats fed high-viscosity barley β-glucan at 5.54% of

### TABLE 3 Prediction of host phenotypes in male C57BL/6J mice fed LBO or WGO diet for 8 wk using leave-one-out cross-validated PLS regression components of the RDA-identified key cecal microbiota OTUs

<table>
<thead>
<tr>
<th>Phenotype variable</th>
<th>Factors, n</th>
<th>Variation by X, %</th>
<th>Variation by Y, %</th>
<th>VIP &gt; 0.8, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum contents/body weight × 100</td>
<td>1</td>
<td>18.4</td>
<td>59.4</td>
<td>50</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>2</td>
<td>25.0</td>
<td>88.9</td>
<td>39</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5</td>
<td>42.8</td>
<td>99.8</td>
<td>44</td>
</tr>
<tr>
<td>Insulin</td>
<td>1</td>
<td>17.9</td>
<td>63.9</td>
<td>62</td>
</tr>
<tr>
<td>C-peptide</td>
<td>1</td>
<td>18.2</td>
<td>58.9</td>
<td>66</td>
</tr>
<tr>
<td>Resistin</td>
<td>1</td>
<td>18.2</td>
<td>49.7</td>
<td>64</td>
</tr>
<tr>
<td>PYY</td>
<td>2</td>
<td>20.4</td>
<td>90.3</td>
<td>56</td>
</tr>
<tr>
<td>GIP, C-peptide</td>
<td>2</td>
<td>27.5</td>
<td>89.3</td>
<td>45</td>
</tr>
<tr>
<td>Insulin, C-peptide, GIP, Resistin</td>
<td>2</td>
<td>26.9</td>
<td>62.3</td>
<td>61</td>
</tr>
</tbody>
</table>

1 GIP, gastric inhibitory peptide; LBO, low bran oat; OTU, operational taxonomic unit; PLS, partial least squares; PYY, peptide YY; RDA, redundancy analysis; VIP, variable importance for projection; WGO, whole grain oat.

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the diet is greater than that in animals fed cellulose. In contrast, in Wistar rats, oat product–based diets containing 3–9.2% β-glucan do not affect Lactobacillus as revealed by plate counting (14). The effect of soluble fiber on Lactobacillus does not appear to be restricted to β-glucan. Lactobacillus in men consuming soluble corn fiber is greater than that in the control consuming no supplemental fiber or polydextrose, as estimated by 454 pyrosequencing (64).

Of interest, changes in the gut microbiota composition were associated with improvements in insulin sensitivity and blood non-HDL cholesterol concentrations, the well-documented beneficial effects of oat-soluble fibers. Plasma insulin and C-peptide were strongly negatively correlated with key OTUs in the Bacteroidetes group of the gut microbiota in mice. Although there were far fewer key OTUs in the Bacteroidetes group than in the Firmicutes group (34 vs. 549), combined, they comprised a similar percentage of the bacteria as did the key OTUs from the Firmicutes group (47.5% vs. 51.8%). The overall gut microbiota composition predicted 88.9%, 63.9%, and 58.9% variation in plasma non-HDL cholesterol, insulin, and C-peptide, respectively. The gut microbiota composition also predicted differences in plasma resistin, PYY, and GIP. Our data suggest that WGOs may affect insulin sensitivity, plasma non-HDL cholesterol, and hepatic lipid metabolism through modification of gut microbiota. Although we speculate that most of these effects are caused by the increased presence of β-glucan in WGOs we cannot discount the additional effects of other WGO components.

The findings from this study further confirm the beneficial effect of WGOs in increasing insulin sensitivity and decreasing non-HDL cholesterol and suggest a potential additional benefit in terms of favorably modifying the gut microbiota composition in mice. Our study also suggests that the favorable effects of WGOs on insulin sensitivity and lipid concentrations are mediated through a new mechanism involving changes in gut microbiota composition in mice.

Acknowledgments
ML designed the research; NH and ML conducted the research; GR conducted the sequencing; ALZ analyzed the data and ML designed the research; NH and ML conducted the research; ALZ analyzed the data and ML designed the research.

References