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Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Impact of Plant-Based Dietary Fibers on Metabolic Homeostasis in High-Fat Diet Mice via Alterations in the Gut Microbiota and Metabolites

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A B S T R A C T

Background: The gut microbiota contributes to metabolic disease, and diet shapes the gut microbiota, emphasizing the need to better understand how diet impacts metabolic disease via gut microbiota alterations. Fiber intake is linked with improvements in metabolic homeostasis in rodents and humans, which is associated with changes in the gut microbiota. However, dietary fiber is extremely heterogeneous, and it is imperative to comprehensively analyze the impact of various plant-based fibers on metabolic homeostasis in an identical setting and compare the impact of alterations in the gut microbiota and bacterially derived metabolites from different fiber sources.

Objectives: The objective of this study was to analyze the impact of different plant-based fibers (pectin, β-glucan, wheat dextrin, resistant starch, and cellulose as a control) on metabolic homeostasis through alterations in the gut microbiota and its metabolites in high-fat diet (HFD)-fed mice.

Methods: HFD-fed mice were supplemented with 5 different fiber types (pectin, β-glucan, wheat dextrin, resistant starch, or cellulose as a control) at 10% (wt/wt) for 18 wk ($n = 12$/group), measuring body weight, adiposity, indirect calorimetry, glucose tolerance, and the gut microbiota and metabolites.

Results: Only β-glucan supplementation during HFD-feeding decreased adiposity and body weight gain and improved glucose tolerance compared with HFD-cellulose, whereas all other fibers had no effect. This was associated with increased energy expenditure and locomotor activity in mice compared with HFD-cellulose. All fibers supplemented into an HFD uniquely shifted the intestinal microbiota and cecal short-chain fatty acids; however, only β-glucan supplementation increased cecal butyrate concentrations. Lastly, all fibers altered the small-intestinal microbiota and portal bile acid composition.

Conclusions: These findings demonstrate that β-glucan consumption is a promising dietary strategy for metabolic disease, possibly via increased energy expenditure through alterations in the gut microbiota and bacterial metabolites in mice.

Keywords: dietary fiber, obesity, gut microbiota, metabolites, metabolic homeostasis

Introduction

Obesity is an epidemic, and its prevalence among United States adults continues to rise dramatically, increasing from 30% to nearly 42% in the past 2 decades. This pervasiveness is associated with increased consumption of the highly palatable Western diet, which is high in dietary fat and sugar but also low in fiber. According to the 2020–2025 USDA Dietary Guidelines

Abbreviations: CDCA, chenodeoxycholic acid; FXR, farnesoid x receptor; GLP-1, glucagon-like peptide-1; HFD, high-fat diet; ITT, insulin tolerance test; OGTT, oral glucose tolerance test; SCFA, short-chain fatty acid; TDCA, taurodeoxycholic acid; TGR5, Takeda G-protein coupled receptor-5; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

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for Americans, >90% of females and 97% of males do not meet the recommended intakes for dietary fiber [1]. Whole grains, fruits, and vegetables, all high in fiber, are underconsumed by >85% of adults [1]. Conversely, obesity-related factors, including bodyweight and adiposity, are decreased with dietary fiber consumption [2–4]. This underscores the importance of determining the potential beneficial impacts of dietary fiber on metabolic homeostasis.

Emerging evidence highlights the importance of the gut microbiota, the collective term for all the microbes residing in the gastrointestinal tract, in metabolic homeostasis. The diet is the primary factor in shaping the gut microbiota [5], and a high-fat diet (HFD), which increases body weight and adiposity and induces glucose dysregulation [6], is associated with alterations in the gut microbiota that recapitulates host phenotype when inoculated into germ-free mice [7]. Conversely, increased fiber consumption improves metabolic dysregulation in rodents and humans and is associated with beneficial shifts in the gut microbiota, including increased abundance of beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Akkermansia* [8–10]. Obesity and insulin resistance are associated with decreased bacterial richness[11], and increased energy intake is inversely correlated with bacterial diversity [12], whereas high-fiber diets have been shown to increase the diversity and richness of the microbiome [13–15]. However, dietary fibers are extremely heterogeneous, and it is still unclear how specific fibers can independently impact the gut microbiota composition to potentially influence human health.

Plant-based fibers vary in their solubility and viscosity, which can differentially affect their function and impact on the host gut microbiota. For example, β-glucan and wheat dextrin are water-soluble fibers that can be easily fermented by gut bacteria, whereas resistant starch is found in many sources, including high-amylose maize, and has mixed solubility properties [16–18]. In a previous study, we found that 10% supplementation of either wheat bran or barley flour, high in wheat dextrin and β-glucan, respectively, in a HFD improved bodyweight and adiposity in rodents, whereas high-amylose maize had no effect. This was associated with improvements in glucose homeostasis and shifts in the gut microbiota, because wheat bran and barley flour increased the relative abundance of beneficial bacterial genera, including *Lachnospiraceae* and *Lactobacillus,* compared with HFD-control [19]. However, whether these improvements were due to the specific fibers within the flours remains unknown. Thus, in the current study, we analyzed the impact of 5 different plant-based fibers supplemented into an HFD at 10% wt/wt in mice: pectin, β-glucan, wheat dextrin, and resistant starch, all of which have demonstrated improvements in metabolic homeostasis and increased abundance of beneficial bacteria in different independent human and rodent studies, although we utilized cellulose as a control. Pectin is a soluble fiber found in fruits and vegetables and decreases bodyweight gain, adiposity, and food intake when supplemented in HFD-fed rodents [20,21]. β-Glucan is a soluble fiber found in oats and barley and is highly viscous, which lengthens its transit time in the small and large intestines, decreases bodyweight, and improves glucose homeostasis in human and rodent studies [16,17]. Wheat dextrin, a less studied soluble fiber with low viscosity, has been shown to decrease body weight and improve insulin sensitivity when supplemented in humans with type 2 diabetes [22,23]. Lastly, resistant starch is an insoluble dietary fiber that has been demonstrated to improve insulin sensitivity in humans with type 2 diabetes and obesity and improve metabolic homeostasis in rodents [24–26]. Although studies have separately investigated the impact of these plant-based fibers on metabolic homeostasis, to our knowledge, no study has directly assessed this in one controlled cohort as done in the current study.

Dietary fiber can be fermented by bacteria, which produce short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, that improve metabolic homeostasis potentially via increased release of colonic gut peptides, such as glucagon-like peptide-1 (GLP-1) [27]. For example, we have previously found that rats with diet-induced obesity exhibit reduced SCFA production, whereas improvements in adiposity and body weight via prebiotic supplementation were associated with increased SCFA production [28]. Likewise, exogenous SCFA administration has been demonstrated to improve body weight, adiposity, and glucose homeostasis in rodents [29,30]. In addition to SCFAs, there are large amounts of bacterially derived metabolites known to impact metabolic homeostasis that could also be impacted by fiber. For example, we have previously found that prebiotic treatment shifts the small-intestinal and portal plasma bile acid composition during HFD-feeding closer toward healthy unpurified diet-fed rats [31]. Primary bile acids, produced in the liver, are secreted and absorbed in the small intestine, where they are deconjugated by gut bacteria and modified into secondary bile acids. Similar to SCFAs, these modifications alter host gut peptide secretion and can impact metabolic homeostasis [32,33]. For example, taurine-conjugated secondary bile acids are ligands for Takeda G protein-coupled receptor 5 (TGR5), a membrane receptor and metabolic regulator, which agonism of this receptor induces secretion of gut peptides GLP-1 and peptide YY. Additionally, primary bile acids are ligands for farnesoid X receptor (FXR), a bile acid nuclear receptor whose expression has contrasting effects in the small intestine and liver on metabolic homeostasis [33–35].

Taken together, despite the large body of evidence that increased dietary fiber improves energy and glucose homeostasis, there is a lack of consistency with how specific fibers impact host metabolism, as well as an absence of complete characterization of the small and large intestinal microbiota and bacterially derived metabolites. Thus, we investigated the impact of different dietary fibers, pectin, β-glucan, wheat dextrin, and resistant starch on energy and glucose homeostasis in one controlled cohort. Given that we previously found that wheat and barley flour, which are high in wheat dextrin and β-glucan, respectively, improved metabolic homeostasis in obese rats and were associated with increased cecal butyrate concentrations, we anticipate that these applied fibers will improve metabolic homeostasis, specifically through shifts in the gut microbiota, with more beneficial, butyrate-producing bacteria.

Methods

Mice

All mice were housed and maintained in accordance with the University of Arizona Institutional Animal Care and Use Committee. Ten-week male C57BL/6J mice (Jackson Laboratory) were group-housed in a single room randomly arranged on the

rack ($n = 3$ per cage) on a 12-h light/dark cycle with ad libitum access to an unpurified diet and were acclimated for 2 wk. At 12 wk of age, mice (body weight: 25.7 +/- 1.3 g; $n = 12$ per group) were switched and maintained on diets that were based on a high-fat, high-sucrose diet containing 10% (wt/wt) of various dietary fibers (Figure 1A, Supplemental Table 1): cellulose fiber as control (HFD, Research Diets D20091002), or β-glucan (Yestimun; Research Diets D21092907), pectin (Genu β; Research Diets D21092909), wheat dextrin (Benefiber product #: 00886790218302; Research Diets D21092910), or resistant starch (MSPrebiotic; Research Diets D21092911). The diets were macronutrient and kilocalorie matched as best as possible, based on macronutrient and fiber analysis for each fiber (Medallion Labs; Supplemental Table 2). Thus, specific types of macronutrients (casein, sucrose, and lard) varied slightly in amounts due to differences in purity of each fiber supplement to account for each diet to be macronutrient and calorically matched. Cellulose was used as a control, because this fiber has been shown to have no impact on metabolic homeostasis and the gut microbiota [36, 37]. Body weight was recorded every week and body fat percentage was measured every 2 wk by EchoMRI-1100 (EchoMRI). After 18 wk on the diet, mice were 5 h food-deprived and deeply anesthetized with isoflurane. Portal plasma was collected and immediately stored at –20°C for GLP-1 and bile acid analysis. Contents from the cecum and small intestine were collected in equal weights, with an mean of 75–100 mg for the cecal contents and 30 mg for the small-intestinal contents, and snap-frozen in liquid nitrogen for microbiota and SCFA analysis.

Metabolic cages and indirect calorimetry

Halfway through the experiment, at 10 wk on the diets, mice ($n = 8$ per group/ randomly assigned) were single-housed in Promethion core metabolic monitoring cages housed in an environmental chamber maintained at 22°C and 40% humidity on a 12-h light/dark cycle. Mice were given 48 h to acclimate, and then the last 24 h measurements were recorded and analyzed. Indirect calorimetry was utilized to measure respiratory exchange ratio and energy expenditure (Weir equation), and food intake and total locomotor activity (X,Y,Z infrared beams) were continuously monitored. Data were converted using the ExpeData and Macro Interpreter program and analyzed with GraphPad Prism Software.

Glucose and insulin tolerance tests

Following 18 wk on the diet, mice were 4 h food deprived for oral glucose tolerance test (OGTT), and several days later, they were 4 h food-deprived for insulin tolerance tests (ITTs). For OGTT, basal blood glucose was taken via the tail vein, and mice were gavaged with 45% glucose (1.5 g/kg body weight). Blood glucose measurements were taken from the tail vein at 15, 30, 60, 90, and 120-min time points post gavage via glucometer, with a 15-min sample of blood taken for insulin measurement via ELISA. For ITT, basal blood glucose was taken from the tail vein via a glucometer, and mice were injected intraperitoneally with insulin (0.5 U/kg body weight). Blood glucose was measured from the tail vein at 30, 60, 90, and 120-min time points after insulin injection.

Cecal and small-intestinal microbiota analysis

The hypervariable V4 region of the 16S rRNA gene was amplified from each cecal and small-intestinal sample using barcoded forward (515F) and reverse (806R) primers, as previously described [38]. Amplified PCR products were pooled at equimolar concentration into a sequencing library and purified utilizing an UltraClean polymerase chain reaction cleanup kit (Qiagen) and sequenced on an Illumina MiSeq (Illumina), at the PANDA Core for Genomics and Microbiome Research at the Steele Children's Research Center, University of Arizona. Reads were demultiplexed using idemp (https://github.com/yhwu/idemp). Quality filtering, denoising, and paired-end read merging were performed with the DADA2 R pipeline v 1.24.0 [39]. Taxonomic identification was utilized via the Ribosomal Database Project against the SILVA database v 138 [40]. α and β-diversity metrics were calculated on rarefied data (76061 reads for cecum and 33400 for small intestine samples). Significant differences in α diversity were tested with a nonparametric Kruskal–Wallis test. Compositional differences between sample groups were tested using a permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities calculated on rarefied data using the vegan R package v 2.6-4 [41]. Nonmetric multidimensional scaling ordinations were used to visualize such differences. Kruskal–Wallis, followed by Dunn's test, was further used to identify differential abundance between experimental groups at the genus level.

Biochemical analysis

Plasma glucose concentration was measured with a glucometer. Plasma insulin concentration was measured by ELISA (Cat. # 80-INSMS-E01; Alpco). Portal GLP-1 concentrations were measured using GLP-1 (Active) ELISA (Cat. # 242 EGLP-35K; MilliporeSigma).

SCFA analysis

Bead-beating homogenization and subsequent measurement of SCFA concentrations via the GC-MS platform were utilized, as previously described [42,43]. SCFA concentrations were measured and presented in nanograms per milligram of cecal contents analyzed.

Bile acid analysis

Portal plasma bile acids were analyzed at the University of Arizona Cancer Center Analytical Chemistry Shared Resource via liquid-liquid extraction utilizing ethyl acetate, as previously described [31].

Bile salt hydrolase activity assay

Bile salt hydrolase (BSH) activity was measured as reported previously [44]. Small-intestinal contents were collected (there was enough starting material for control-HFD, β-glucan-HFD and wheat-dextrin-HFD groups only), and 10 mg matter was mixed with 240 μL Dulbecco's Modified Eagle Medium (Sigma-Aldrich) containing taurine-conjugated bile acids, including taurocholic acid, tauroursodeoxycholic acid (TUDCA), and taurodeoxycholic acid (TDCA) (each 1 μg/reaction; all from Sigma-Aldrich). A control 0 min sample was collected, and the rest of the reaction

was incubated in a 37°C water bath for 30 min. Afterward, the 0 min and 30 min samples were centrifuged down, and the supernatant was collected, evaporated, and re-dissolved in 100 µL methanol. The solution was transferred into an HPLC vial. Bile acids analysis was conducted in positive modus using an LCMS-8040 liquid chromatography-mass spectrometer (Shimadzu Corporation) with an Atlantis T3 3 µm column (2.1 × 150 mm; Waters). The column temperature was 30°C. Water with 0.1% formic acid and 20 mmol/L ammonium acetate served as solvent A. Solvent B contained acetonitrile/methanol (3/1, vol/vol) with 0.1% formic acid and 20 mmol/L ammonium acetate. The solvent gradient was 30% B for 5 min, followed by 100% B at 25 min and 30% B for 10 min for re-equilibration. The BSH activity was depicted as the change in the concentration of taurine-conjugated bile acids between the 30 min and control 0 min time points. The values were expressed as percentage (%) difference between the time points.

Statistical analysis

Statistical analyses were completed using GraphPad Prism 9 software (GraphPad Software). Body weight, adiposity, and lean mass were analyzed by one-way repeated measures ANOVA followed by Tukey's post hoc multiple comparisons test. OGTT and ITTs were analyzed by using two-way ANOVA with multiple comparisons with Tukey's post hoc multiple comparisons test. Metabolic cage data, insulin/GLP-1 ELISAs, AUC, SCFAs, and bile acids were analyzed using a one-way ANOVA with multiple comparisons followed by Tukey's post hoc multiple comparisons test. $P < 0.05$ was considered significant. Data presented as mean ± SEM.

Results

β-glucan supplementation in HFD-feeding reduced body weight gain and adiposity in mice

HFD-fed mice supplemented with 10% β-glucan gained significantly less weight over 18 wk on diet compared with cellulose mice, whereas there were no reductions in body weight for mice supplemented with pectin, wheat dextrin, or resistant starch (Figure 1B). This decrease in body weight was due to a reduction in fat mass, because β-glucan-HFD had significantly decreased adiposity and maintained lean mass over time compared with the cellulose group (Figure 1C and D). There was no effect of any other fiber supplements on adiposity or lean mass compared with the cellulose group (Figure 1D). These findings indicate that β-glucan supplementation during HFD-feeding reduces the development of obesity.

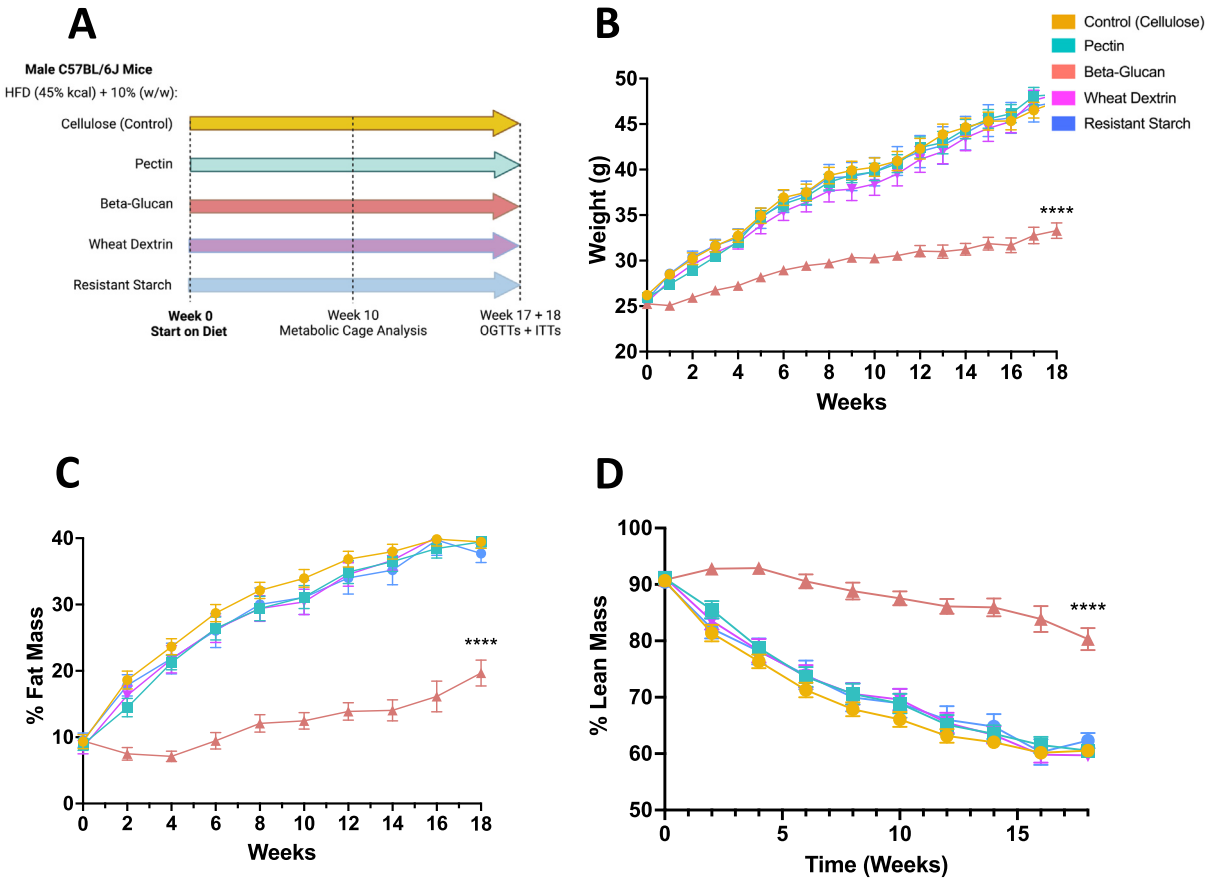

FIGURE 1. (A) Timeline of experimental design. (B) Bodyweight over time in HFD-control ($n = 12$), HFD-pectin ($n = 12$), HFD-β glucan ($n = 12$), HFD-wheat dextrin ($n = 12$), and HFD-resistant starch ($n = 12$) mice. (C) % Fat mass over time. (D) % Lean mass over time. All data are presented as mean +/- SEM, assessed by 1-way ANOVA with multiple comparisons. ANOVA, analysis of variance; HFD, high-fat diet; ITT, insulin tolerance test; OGTT, oral glucose tolerance test; SEM, standard error of the mean.

β-glucan supplementation in HFD-feeding significantly improved energy expenditure

To determine if plant-based fibers impact energy regulation, mice were placed in metabolic cages at 10 wk of dietary intervention. All dietary fiber groups had no significant differences in total caloric intake compared with control (Figure 2A). As such, there were no differences in meal size in all dietary fiber groups (Supplemental Figure 1B; Figure 2B); however, mice with β-glucan supplementation exhibited significantly increased number of meals in the total 24-h period compared with control

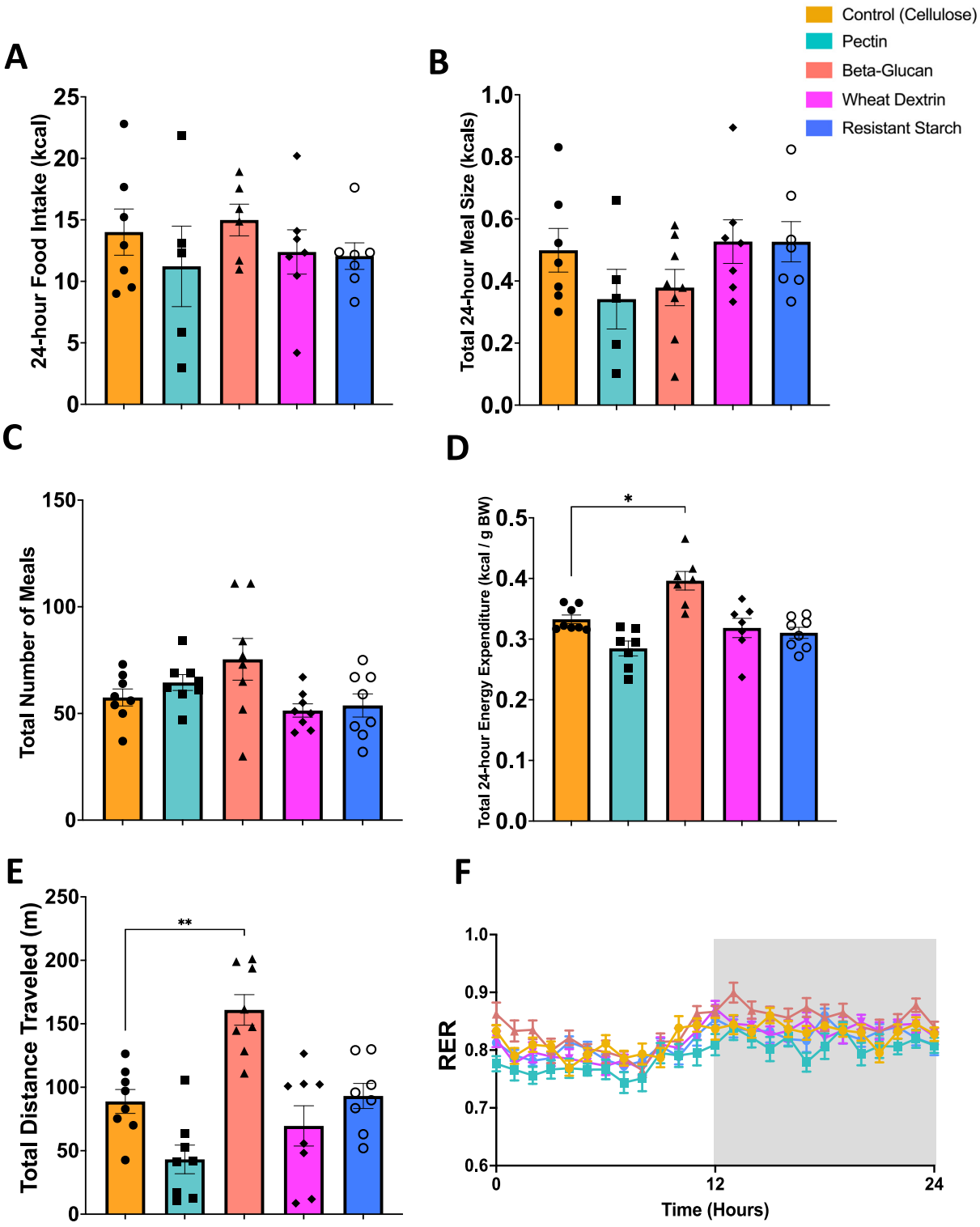

FIGURE 2. (A) Food intake in total 24-h period. (B) Meal size (kcals), (C) number of meals, (D) energy expenditure (kcals/g bodyweight), (E) distance traveled in the cage (meters) and (F) respiratory exchange ratio (RER) in total 24-h period. All data are presented as mean +/- SEM (n = 12/group). *P < 0.05, **P < 0.01, ***P < 0.001, assessed by 1-way ANOVA with multiple comparisons. ANOVA, analysis of variance; SEM, standard error of the mean.

(Figure 2C). There were no differences in energy expenditure in the light cycle for all fiber supplementation groups; however, only β-glucan-HFD significantly increased total energy expenditure in the dark cycle and total 24-h period compared with control (Supplemental Figure 1D; Figure 2D). There was no difference in total energy expenditure in the pectin-HFD, wheat dextrin-HFD, and resistant starch-HFD groups (Figure 2D). Similar to energy expenditure, only β-glucan-HFD mice exhibited increased distance traveled in the cages in the dark cycle and total 24-h period compared with control mice (Supplemental Figure 1E; Figure 2E). No significant differences were observed in all the groups for the respiratory exchange ratio (Figure 2F).

β-glucan supplementation significantly improved glucose tolerance and insulin sensitivity in HFD-fed mice

After 18 wk on a diet, only β-glucan supplementation improved oral glucose tolerance in mice at 30, 60, 90, and 120 min postoral glucose gavage compared with HFD-cellulose, with no effect of any other fibers (Figure 3A). As such, only β-glucan-supplemented mice exhibited a reduced AUC in the OGTT compared to control mice (Figure 3B). Furthermore, only β-glucan-supplemented mice exhibited significantly reduced plasma insulin concentrations post 15-min oral glucose gavage (Figure 3C). β-glucan supplementation, but no other fibers, improved insulin sensitivity in mice during the ITT at 30, 60, 90, and 120 min, along with an increase in AUC, compared with control (Figure 3D and E). Previous studies have shown that specific dietary fibers promote the production of the incretin GLP-1, which regulates glucose homeostasis [45]; however, there were no significant differences in 5 h food-deprived portal active GLP-1 concentrations at 18 wk on the diets (Figure 3F).

Dietary fiber supplementation altered the cecal microbiota and SCFA composition

After 18 wk on a diet, β-glucan, resistant starch, and wheat dextrin supplementation in HFD-fed mice decreased amplicon sequencing variants (ASV) richness (α-diversity) in the cecum of mice compared to control mice (Figure 4A). Cecal β-diversity was significantly influenced by dietary fibers (PERMANOVA R^2

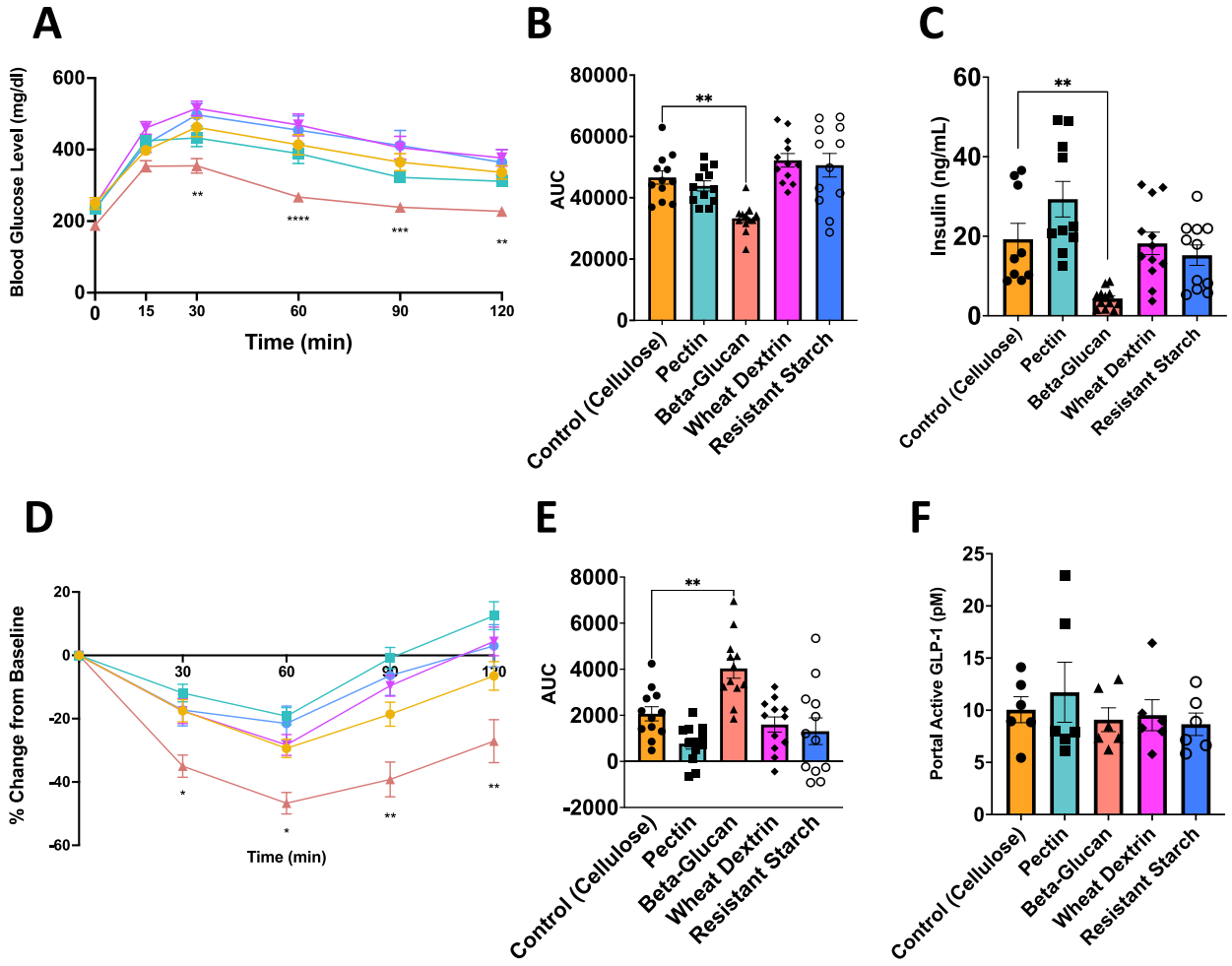

FIGURE 3. (A) Oral glucose tolerance test and (B) AUC at 17 wk on diet. (C) Plasma insulin at 17 wk on diet. (D) Insulin tolerance test and (E) AUC at 18 wk on diet. (F) Portal plasma active GLP-1 (pM). All data are presented as mean +/- SEM ($n = 12$/group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, as assessed by 2-way ANOVA with multiple comparisons with Tukey's post hoc (A, D), or 1-way ANOVA with multiple comparisons followed by Tukey's post hoc (B, C, E, F). ANOVA, analysis of variance; AUC, area under curve; GLP-1, glucagon-like peptide-1; SEM, standard error of the mean; SEM, standard error of the mean.

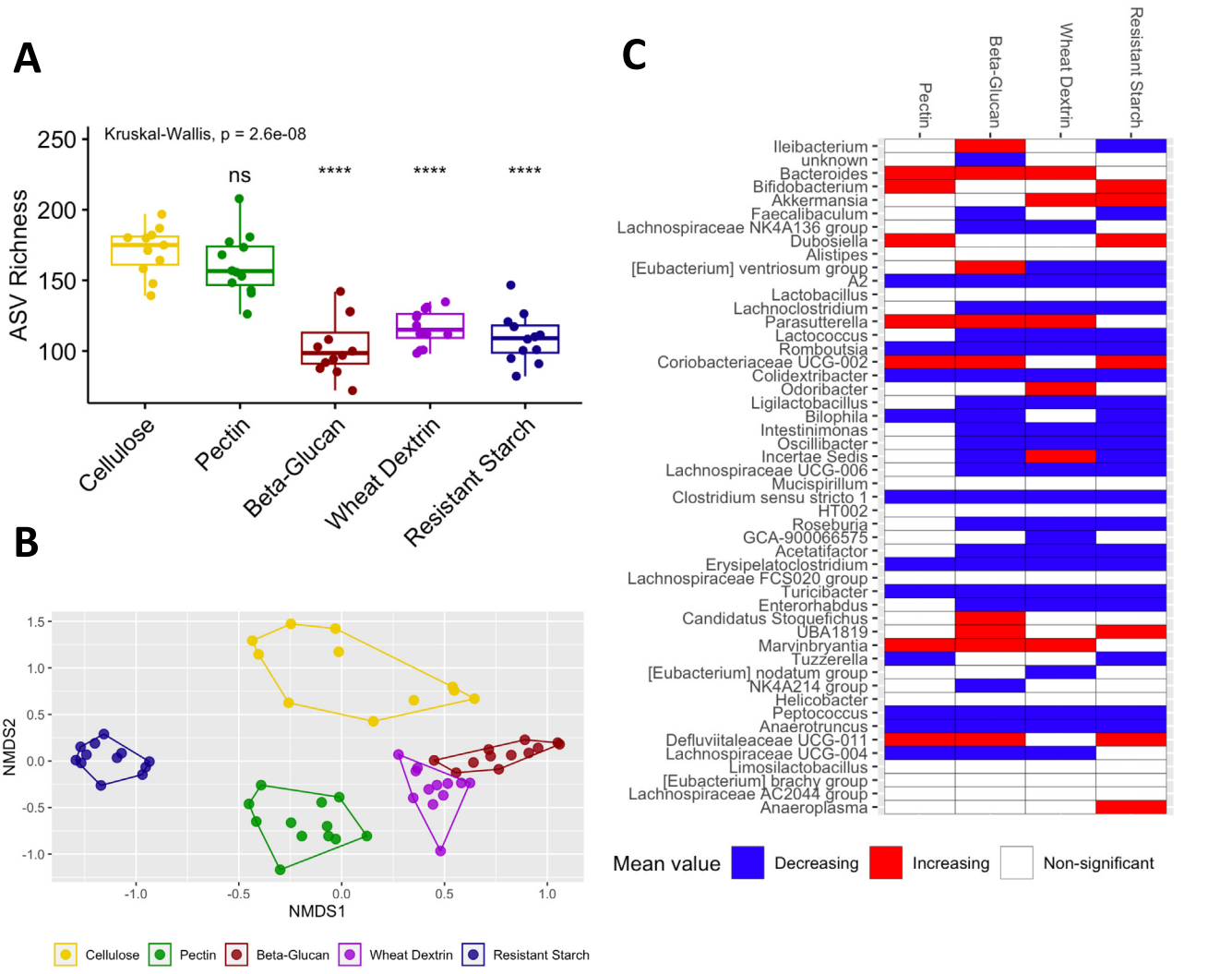

FIGURE 4. Cecum samples. (A) α-diversity index, ASV richness. *P* values indicate Wilcoxon comparisons to cellulose as a reference. (B) Nonmetric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity. (C) Heatmap showing significant changes of 50 most abundant bacterial genera in HFD-β glucan ($n = 12$), HFD-pectin ($n = 12$), HFD-resistant starch ($n = 12$), and HFD-wheat dextrin ($n = 12$) treatments compared with cellulose ($n = 12$). Significance was assessed with a Wilcoxon test on relative abundances (FDR adjusted $P < 0.05$). White cells indicate nonsignificant changes. Genera are ordered by mean relative abundance (most abundant on top). HFD, high-fat diet; ASV, amplicon sequencing variants; FDR, false discovery rate.

$= 0.67$, $P < 0.001$) (Figure 4B), with all pairwise comparisons showing significant differences (Supplemental Table 3). Differential and relative abundance analysis at the genus level revealed many alterations in the cecal microbiota composition of each dietary fiber group compared with cellulose-HFD-control; for example, β-glucan-HFD increased *Ileibacterium* relative abundance, whereas β-glucan-HFD, pectin-HFD and wheat dextrin-HFD increased *Bacteroides,* pectin-HFD and resistant starch-HFD increased *Bifidobacterium,* and resistant starch-HFD and wheat dextrin-HFD increased *Akkermansia* relative abundance (Supplemental Figure 2; Figure 4C).

As we identified changes in the cecal microbiota composition with all dietary fiber supplementations, we examined the cecal SCFA composition upon fiber fermentation. Resistant starch-HFD had significantly increased total SCFAs compared to the control, with no difference in the other fiber groups (Figure 5A). Cecal acetate concentrations were significantly increased in pectin-HFD or resistant starch-HFD groups compared with control-HFD mice, whereas cecal propionate concentrations were increased in pectin-HFD, β-glucan-HFD, and wheat dextrin-HFD groups compared with control (Figure 5B and C). Only β-glucan-HFD increased cecal butyrate concentrations in mice compared to the control (Figure 5D). Lastly, the proportion of propionate and butyrate was only significantly different in β-glucan-supplemented mice compared with control mice (Figure 5E).

Spearman correlation analysis revealed a negative correlation between *Ileibacterium* relative abundance and adiposity and bodyweight at 18 wk of diet (Supplemental Figure 3). Cecal acetate concentrations were positively correlated with *Akkermansia*, *Bifidobacterium,* and *Dubosiella* and negatively correlated with *Bilophila* and *Ileibacterium* relative abundance (Supplemental Table 4). Cecal propionate concentrations were positively correlated with *Bacteroides* and *Parasutterella* relative abundance and negatively correlated with *Lachnospiraceae, Ligilactobacillus*, and *Romboutsia* relative abundance (Supplemental Table 4).

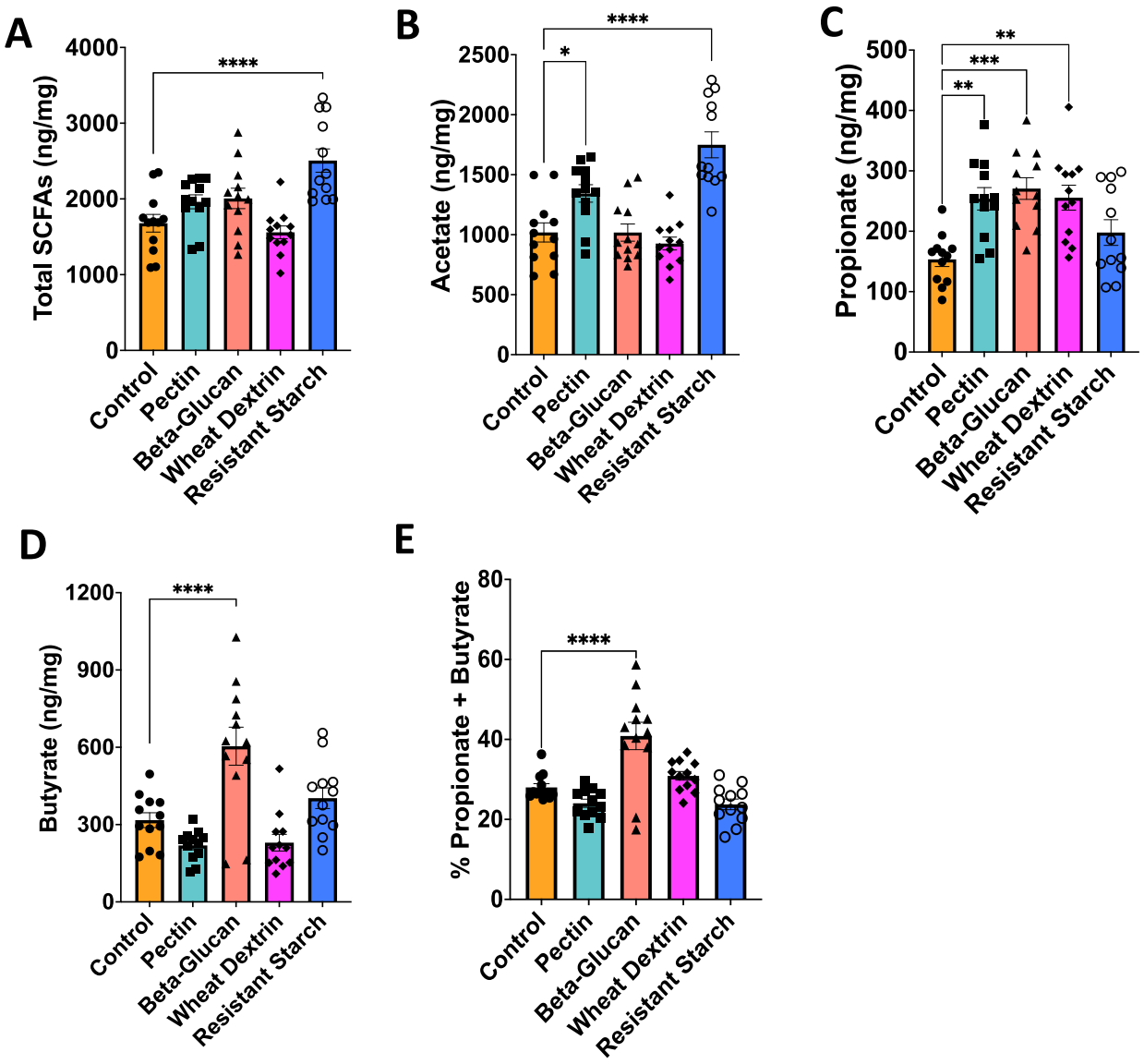

FIGURE 5. (A) Total SCFAs in the cecum of 5-h fasted mice ($n = 12$) after 18 weeks on diet. (B) Acetate, (C) propionate, (D) butyrate, and (E) proportion of propionate and butyrate (ng/mg) in the cecum of 5-h fasted mice ($n = 12$) after 18 wk on diet. All data are presented as mean +/- SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, as assessed by 1-way ANOVA with multiple comparisons followed by Tukey's post hoc. ANOVA, analysis of variance; SCFA, short-chain fatty acid; SEM, standard error of the mean.

Cecal butyrate concentrations were negatively correlated with *Bacteroides, Bilophila, Faecalibaculum, Lachnospiraceae,* and *Roseburia* relative abundance (Supplemental Table 4). Total SCFAs were positively correlated with *Bifidobacterium* and *Dubosiella* and negatively correlated with *Parasutterella* relative abundance (Supplemental Table 4).

Dietary fiber supplementation altered the small-intestinal microbiota and bile acid composition in HFD-fed mice

Only β-glucan significantly decreased small-intestinal α-diversity compared to the control-HFD, whereas all other dietary groups had no significant effect compared with the control-HFD (Figure 6A). Similar to the cecal microbiota composition, differences in fiber intake significantly explain a large portion of community variation (PERMANOVA $R^2 = 0.58$, $P < 0.001$, Figure 6B). In paired comparisons, only β-glucan-HFD and wheat dextrin-HFD were less dissimilar when compared with all other pairs (Supplemental Table 5; Figure 6B). β-glucan-HFD increased small-intestinal *Ileibacterium* and *Parasutterella,* and decreased *Bilophila* relative abundance at the genus level compared to control-HFD. Pectin-HFD increased *Blautia, Bifidobacterium,* and *Dubosiella* and decreased *Romboutsia* relative abundance compared with control-HFD. Resistant starch-HFD increased *Bifidobacterium* and *Dubosiella* and decreased *Ileibacterium* relative abundance compared with control-HFD. Wheat dextrin-HFD increased *Blautia, Parasutterella,* and *Akkermansia* and decreased *Romboutsia* relative abundance compared with control-HFD (Supplemental Figure 4; Figure 6C).

As we have identified changes in the small-intestinal microbiota composition, we next wanted to identify if there were changes in small-intestinal bile acids given their interaction. There were no differences in total bile acid concentrations, as

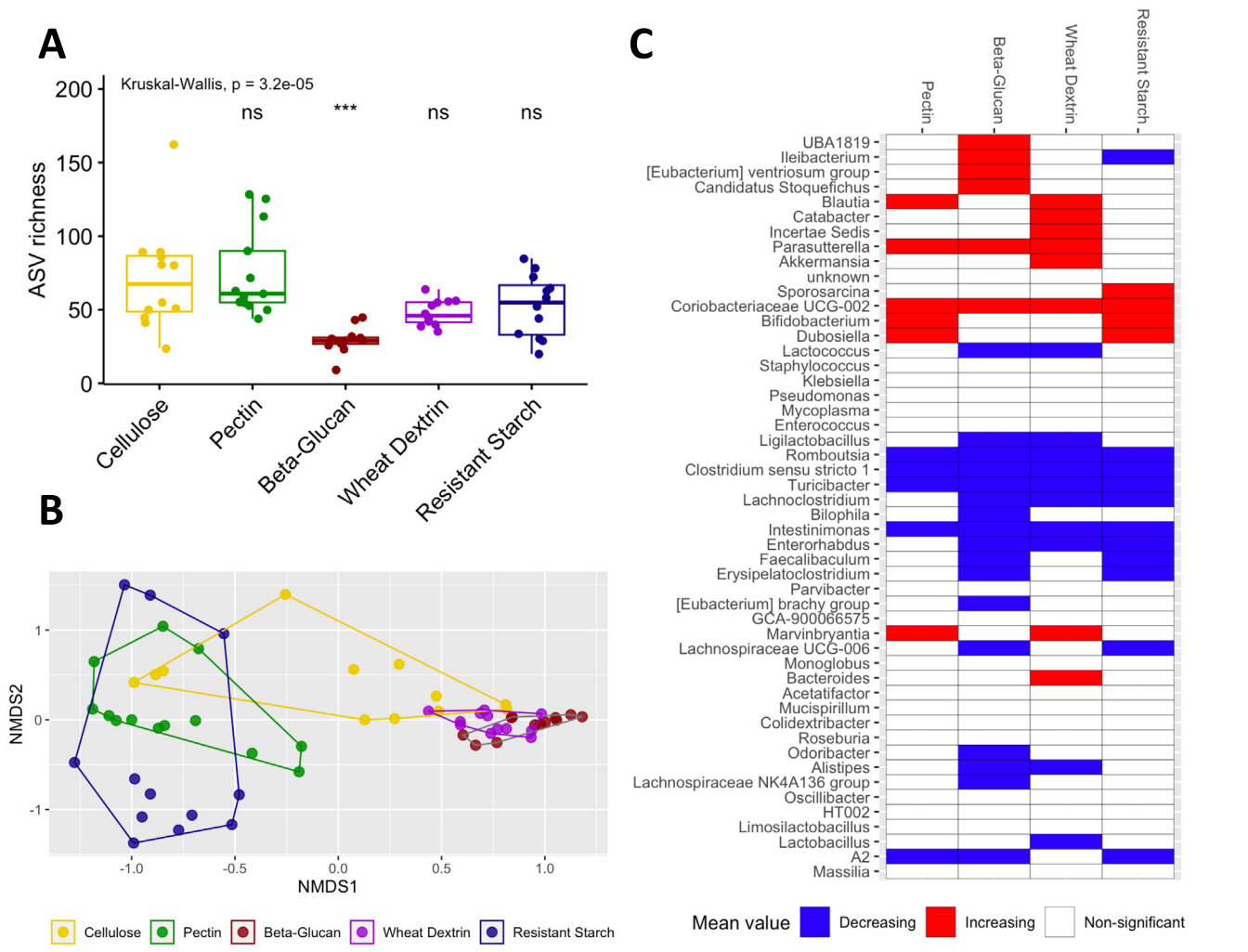

FIGURE 6. Small intestine samples. (A) α-diversity index, ASV richness. *P* values indicate Wilcoxon comparisons to cellulose as a reference. (B) Nonmetric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity. (C) Heatmap showing significant changes of 50 most abundant bacterial genera in HFD-β glucan ($n = 12$), HFD-pectin ($n = 12$), HFD-resistant starch ($n = 12$), and HFD-wheat dextrin treatments compared with cellulose ($n = 12$). Significance was assessed with a Wilcoxon test on relative abundances (FDR adjusted $P < 0.05$). White cells indicate nonsignificant changes. Genera are ordered by mean relative abundance (most abundant on top). HFD, high-fat diet; ASV, amplicon sequencing variants; FDR, false discovery rate.

well as the proportion of either primary or secondary bile acids across all groups compared with the control-HFD (Figure 7A and B). β-Glucan-HFD and wheat dextrin-HFD significantly increased the proportion of total unconjugated bile acids and decreased the proportion of total conjugated bile acids and taurine-conjugated bile acids compared with control-HFD, with no effect in pectin-HFD and resistant starch-HFD groups (Supplemental Figure 5A; Figure 7C). When examining individual bile acids, all fiber supplementations increased primary bile acid chenodeoxycholic acid (CDCA) compared with control-HFD. Additionally, wheat-dextrin-HFD and resistant starch-HFD increased cholic acid compared with control-HFD. Only wheat dextrin-HFD increased α-muricholic acid compared with control. β-Glucan-HFD and wheat dextrin-HFD both significantly increased small-intestinal β-muricholic acid (β-MCA) and ursodeoxycholic acid (UDCA), whereas resistant starch-HFD decreased UDCA, TUDCA, and glycine-β-muricholic acid (GbMCA) compared with control-HFD (Figure 7D). Lastly, small-intestinal contents collected from mice were used to measure BSH activity. Compared with contents from cellulose-HFD mice, incubation with contents from β-glucan-HFD mice resulted in a significantly decreased amount of TUDCA and TDCA, as exhibited via a suppression from baseline for each individual mouse, whereas there was a similar nonsignificant trend with wheat dextrin-HFD mice small-intestinal contents (Supplemental Figure 5B and C). However, there were no differences between the groups in suppression of starting taurocholic acid following incubation (Supplemental Figure 5D).

Small-intestinal *Akkermansia* and *Parasutterella* relative abundance was positively correlated with primary bile acids α-muricholic acid, β-MCA, cholic acid, and CDCA concentrations in mice compared to control-HFD. *Bifidobacterium* was negatively correlated with β-MCA and GbMCA compared with control-HFD. *Akkermansia, Ileibacterium,* and *Parasutterella* relative abundance were positively correlated with β-MCA concentrations compared with control-HFD (Supplemental Figure 6).

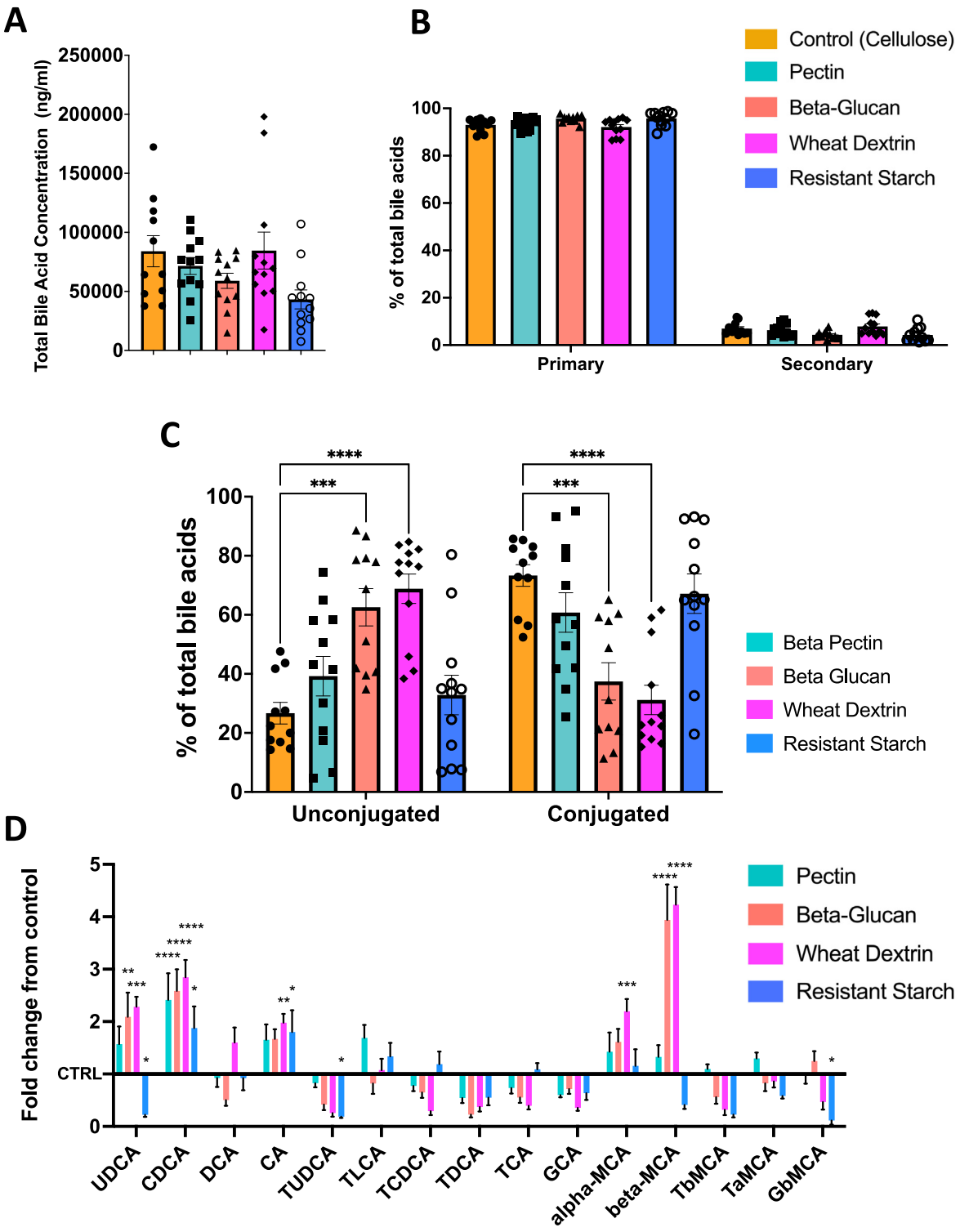

FIGURE 7. (A) Total portal plasma bile acids in mice ($n = 12$) after 5-h fast 18 wk on diet. (B) Primary and secondary bile acids and (C) unconjugated and conjugated bile acids are presented as a percentage of total bile acids. Bile acid composition (each bile acid calculated as a percentage of total bile acids) in the portal plasma, presented as fold change from HFD-control. All data are presented as mean +/- SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, as assessed by 1-way ANOVA with multiple comparisons followed by Tukey's post hoc. α-MCA, α-muricholic acid; ANOVA, analysis of variance; β-MCA, β-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; GbMCA, glycine-β-muricholic acid; HFD, high-fat diet; SEM, standard error of the mean; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; TaMCA, tauro-muricholic Acid; TbMCA, tauro-B-muricholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid.

Discussion

Dietary fiber consumption improves body weight and adiposity and is associated with shifts in the gut microbiota composition in rodents and humans [37,46,47]. Despite various studies that have separately investigated the impact of plant-based fibers on metabolic homeostasis, there is no study that has investigated the role of various fibers in one cohort. Similar to previous studies, we demonstrate that β-glucan supplementation in HFD-feeding attenuates body weight gain and adiposity compared to a control-HFD [48]. There were no differences in bodyweight or adiposity with pectin supplementation, despite recent findings that have displayed contrasting evidence with 10% pectin supplementation in HFD-fed mice [49]. Previous studies demonstrate that improvements in bodyweight and adiposity with pectin supplementation in rodents utilized an apple-sourced pectin, which is more viscous compared with the sugar beet pulp pectin used in this study [21,49–51]. Therefore, a more comprehensive analysis comparing the impact of differing viscosities of pectins on bodyweight and adiposity is needed, as viscosity is known to slow nutrient absorption in the intestine [52,53]. Interestingly, we also observed no differences in bodyweight and adiposity with wheat dextrin supplementation, despite our previous study, which demonstrated improvements in body composition with wheat bran supplementation, which is high in wheat dextrin, in HFD-fed rats [19]. This suggests that there may be more components to the wheat bran diet, which may contribute to the observed improvements in bodyweight and adiposity. Moreover, wheat bran contains other components, including β-glucan and arabinoxylan, which this study (regarding β-glucan) and other previous studies have demonstrated improvements in bodyweight and glucose homeostasis [54–56]. We also observed no changes in bodyweight or adiposity with resistant starch supplementation, which was also evident in a recent study, where there was no difference in bodyweight gain in mice supplemented with either 5%, 15%, or 25% potato-resistant starch in HFD-fed mice [57]. Both the previous study and this current study utilized resistant starch (RS)-2; however, resistant starch (RS)-3 supplementation in HFD-feeding attenuates bodyweight gain in obese mice [58]. These contrasting findings may be due to the composition of the resistant starch, as (RS)-2 is indigestible by enzymes in the small intestine, and (RS)-3 is a type of RS that is formed through a process called retro-degradation, where its structure becomes less soluble after heating and dissolving in water and cooling [59]. Moreover, the structure of (RS)-3 may increase its resistance to digestion and thus increase its capacity for bacterial fermentation compared with (RS)-2 [60].

The Western diet, which is high in fat and sugar and low in dietary fiber, is associated with the development of obesity, where energy intake exceeds energy expenditure. Dietary fiber can improve metabolic homeostasis via changes in energy expenditure, although this mechanism needs to be further elucidated. In our previous findings, barley flour supplementation also increased energy expenditure in the dark cycle compared to control-HFD, which was associated with reduced adiposity [19]. In line with these previous findings, only β-glucan supplementation in HFD-feeding increased energy expenditure compared to control-HFD, whereas the other fiber groups had no effect compared to control-HFD [61]. Dietary fiber can also reduce energy intake to induce weight loss [45]. However, in the current study, none of the fibers supplemented into a HFD reduced food intake compared to the control. It should be noted that our metabolic cage analyses were performed at around 10 wk of treatment, similar to when we saw changes in our previous study [19]; however, β-glucan supplementation resulted in reduced body weight and adiposity much earlier. Therefore, early reductions in food intake may have caused some weight loss, although we observed no differences in food intake between cages (data not shown), and this is in line with our previous findings where rats on a HFD supplemented with barley flour (high in β-glucan) exhibited reduced body weight and adiposity, which was associated with increased energy expenditure and no change in food intake. However, future studies should examine energy intake and expenditure at earlier timepoints.

All dietary fiber groups significantly and uniquely shifted the cecal and ileal microbiota compared with the control. Specifically, there was an increased relative abundance of *Ileibacterium* observed in both the cecum and small intestine in the β-glucan-HFD group only. Interestingly, a recent study in mice reported an increase in the abundance of fecal *Ileibacterium valens* upon supplementation of a *trans*-10, *cis*-12 conjugated linoleic acid, which resulted in weight loss by increasing lipid oxidation and energy expenditure [62]. β-glucan supplementation similarly resulted in weight loss that was associated with increased energy expenditure, highlighting a potential therapeutic role of *Ileibacterium*. β-glucan supplementation in HFD-feeding also increased cecal and ileal relative abundance of *Parasutterella* compared to control-HFD, which, abundance of this bacterial genera in the gut is associated with weight loss and bile acid metabolism upon dietary fiber treatment composed of barley and beet pulp in recent studies [63–65]. Wheat dextrin-HFD had increased *Akkermansia* cecal and small-intestinal relative abundance, which has been associated with reduced bodyweight and reduced plasma glucose concentrations after an OGTT [10]. Pectin-HFD and resistant starch-HFD increased *Bifidobacterium* relative abundance in both the cecum and small intestine, which, in previous findings and in our recent studies, its small-intestinal abundance is associated with reductions in food intake and meal size [66,67]. These observations on the increased abundance of bacteria, such as *Akkermansia* and *Bifidiobacterium*, generally recognized as beneficial to host metabolic homeostasis, in dietary fiber supplementation that had no overall differences in body weight or glucose tolerance highlight that the phenotypical shifts likely require changes both in the gut microbiota composition and functional output (i.e., metabolite production).

In addition to shifts in the distal gut microbiota composition, we identified changes in the metabolites, specifically SCFAs, with increased propionate in the pectin-HFD, wheat dextrin-HFD, and β-glucan-HFD groups, and only β-glucan displayed increased concentrations of butyrate in the cecum. Interestingly, in our previous study, the weight loss and reduced adiposity following supplementation with barley and wheat flour were associated with increased cecal butyrate concentrations compared to the HFD-control rats that were not observed in any of the other flours that failed to increase butyrate concentrations, highlighting that increasing endogenous butyrate concentrations may be necessary for weight loss effects [19]. Butyrate induces thermogenesis and adipocyte browning in mice [68]. Moreover,

numerous studies demonstrate that exogenous administration of SCFAs, mainly propionate and butyrate, decreases body weight gain, possibly via increased energy expenditure [29,30,69,70]. In addition, butyrate reproducibly improves metabolic homeostasis and has been shown to improve intestinal inflammation and activate hepatic lipid oxidation and brown adipose tissue thermogenesis [68,71]. As mentioned previously, *trans*-10, *cis*-12 conjugated linoleic acid supplementation resulted in weight loss via increased energy expenditure and this was associated with increased abundance of *Ileibacterium.* This was associated with increased butyrate concentrations, further highlighting that the effectiveness of β-glucan supplementation might be due to an *Ileibacterium*-butyrate mechanism that increases energy expenditure. Conversely, resistant starch-HFD or pectin-HFD groups had no increase in butyrate concentrations but exhibited increased cecal acetate. There is contrasting evidence on the impact of acetate on metabolic homeostasis, and in general, increases in acetate and decreases in butyrate concentrations are associated with impairments in energy and glucose metabolism [28,72].

In addition to weight loss, β-glucan supplementation improved glucose and insulin sensitivity. Although it is possible that this was secondary to reductions in adiposity, it may be due to changes in the small-intestinal microbial metabolites. For example, it has previously been shown that nutrient infusion in the upper small intestine and ileum improves glucose tolerance in the unpurified diet but not in HFD-fed rats. Furthermore, transplantation of healthy microbiota in HFD-fed rats restores the ability to sense glucose and further increases glucose tolerance. In addition, healthy microbiota upper small-intestinal transplant lowers small-intestinal Taurochenodeoxycholic acid (TCDCA) concentrations, which is an FXR agonist, and increased TCDCA concentrations are associated with insulin resistance and elevated blood glucose concentrations [73]. FXR plays an important role in glucose homeostasis and lipogenesis, and more specifically, bile acid synthesis is regulated by small-intestinal FXR [74]. In the same study, TCDCA infusion into the small intestine increases FXR expression, and further, small-intestinal FXR inhibition enhances glucoregulation and nutrient sensing in the small intestine of HFD-fed rats. These findings demonstrate the role of bile acid signaling in the regulation of glucose homeostasis through changes in the small-intestinal microbiota [75]. In the current study, all treatment groups had decreased concentrations of TCDCA, which is associated with healthy microbiota [75,76]. Moreover, in type 2 diabetes humans, TCDCA and TDCA concentrations are elevated and positively linked with worsened glucose homeostasis[73]. Notably, TCDCA concentrations are decreased in the β-glucan-HFD and wheat-dextrin-HFD groups, and TDCA concentrations are decreased in all dietary fiber groups, but not significantly, although β-glucan-HFD displayed a trend toward significance with TDCA ($P = 0.0595$). Interestingly, only either β-glucan-HFD or wheat dextrin-HFD groups displayed increased concentrations of portal plasma CDCA, a potent FXR ligand. Despite prior studies that demonstrate the role of small-intestinal FXR activation in altering nutrient sensing and glucoregulatory mechanisms, some studies have demonstrated that FXR knockout in the liver in mice display worsened glucose homeostasis, suggesting opposing roles for FXR activation in the liver compared with the small intestine [77]. Moreover, liver FXR induces transcriptional activation of small heterodimer partners, which suppresses hepatic gluconeogenesis and lipid synthesis and can improve glucose homeostasis [33]. Thus, a greater understanding of the role of β-glucan supplementation on FXR transcriptional activity and the activation of small heterodimer partners is needed. Bile acids also affect host metabolism by targeting not only FXR but also TGR5, a nuclear receptor that is activated by secondary bile acids and plays an important role in regulating lipid metabolism and energy expenditure [78]. In this study, β-glucan and wheat dextrin, but not RS or pectin supplementation groups, displayed increased concentrations of UDCA, a TGR5 ligand that has shown to improve energy homeostasis through reducing free fatty acids and triglycerides and increase adipose tissue browning in mice [79,80]. Only β-glucan-HFD had the lowest bodyweight and adiposity over time, along with increased energy expenditure compared with control-HFD, which may be due to the changes in the bile acid pool through the gut microbiota that are driving these improvements in energy expenditure, although future studies are needed to further explore this mechanism.

Primary bile acids, derived from cholesterol in the liver, are conjugated with glycine or taurine and released postprandially into the small intestine. In the intestine, they are then deconjugated and converted to secondary bile acids by gut bacteria containing bile salt hydrolase enzymes, and a large proportion is then reabsorbed into the portal circulation and recycled back into the liver [32]. In this study, groups receiving either β-glucan or wheat dextrin supplementation displayed higher concentrations of β-MCA and lower concentrations of tauro-β-MCA in the portal plasma. There was also an increase in the proportion of unconjugated bile acids and a decrease in conjugated bile acids. Lastly, there was an increase in the suppression of tauro-conjugated bile acids, possibly indicating increased gut microbial bile acid deconjugation via BSH activity with either β-glucan or wheat dextrin supplementation. In line with this, we found that BSH activity, specifically for TUDCA and TDCA, was significantly increased in small-intestinal contents collected from HFD-fed mice supplemented with β-glucan. Therefore, it is possible that shifts in specific bacteria with BSH enzymes targeting these specific bile acids are unique toward β-glucan supplementation [44]. Although there were no significant changes observed in body weight, adiposity, glucose tolerance, or energy homeostasis within the wheat dextrin supplementation group, this suggests that the presence of various bacterial-derived metabolites, mainly both butyrate and bile acids, might play a role in improving metabolic homeostasis. However, further studies may be needed to further explore this mechanism.

One limitation of our study was in the generation of the diets due to the varying purity of the fiber supplements. Optimally, fibers would have been pure fiber with no other ingredients; however, this was not possible. As such, several ingredients, including casein, lard, and sucrose, had to be adjusted to account for the protein, fat, and carbohydrates, respectively, in each fiber supplement to ensure that the overall macronutrient composition and kilocalorie content was nearly identical across the diets. This does create a challenge when interpreting the data, and future studies can attempt to address this with the development of more pure fiber sources to rule out any nonfiber impact. Nonetheless, although there was a decrease in sucrose content in the β-glucan group compared with the control (12% compared with 19% of sucrose in diet), it is unlikely this is driving

improvements in energy homeostasis in the β-glucan-supplemented mice. Several studies conducted in male C57BL/6J mice have revealed feeding a HFD containing either a high or low-sucrose content resulted in similar increases in bodyweight and adiposity compared to mice on a normal control diet. Importantly, there was no difference observed in these metabolic parameters between the mice fed a high-fat-low-sucrose diet or high-fat-high-sucrose diet, suggesting that the fat, rather than the sucrose concentrations, primarily contribute to the obesogenic effects observed [81,82]. Additionally, in an extensive study on the impact of dietary components on energy homeostasis [83], it was demonstrated that only increased dietary fat content was associated with increased energy intake and adiposity. Additionally, varying the sucrose concentrations (5%, 10%, 15%, 20%, 25%, and 30%) in a HFD (41%) had no impact on body weight, adiposity, or energy expenditure in C57BL/6J mice; thus, we do not believe the slight reduction in sucrose concentrations in the β-glucan diet had any impact on our observed outcomes.

Overall, this study investigates the role of various plant-derived dietary fibers, pectin, β-glucan, wheat dextrin, and RS, on metabolic homeostasis. Importantly, β-glucan supplementation in HFD-feeding significantly improved bodyweight, adiposity, energy, and glucose homeostasis, along with increased concentrations of butyrate and changes in bile acid metabolism compared to control-HFD. These data demonstrate the therapeutic potential of β-glucan to attenuate body weight gain and adiposity and improve glucose homeostasis and insulin sensitivity in HFD-fed mice. These findings enhance the current understanding of the potential mechanisms through which β-glucan may be employing its beneficial effects, particularly by identifying gut microbiota-mediated mechanisms upon dietary fiber supplementation.

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Author contributions

The authors' responsibilities were as follows – EJH, FAD, TM: designed the research; EJH, RKM, SNW, TM, HRW, MP, AA-L, AK, KD, HG, GS, DL: conducted the research; EJH, FAD: analyzed the data; EJH, FAD: wrote the paper; EJH: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Conflict of interest

FD reports financial support was provided by National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Environmental Health Sciences, and National

Institute of Food and Agriculture. SW reports financial support was provided by

National Institute of Food and Agriculture. RM reports financial support was provided by

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Data availability

The gut microbiota datasets supporting the conclusions of this manuscript are available in the SRA Repository (Accession Number: BioProject ID PRJNA1066829).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.05.003.

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