Exopolysaccharide-Producing Probiotic Lactobacilli Reduce Serum Cholesterol and Modify Enteric Microbiota in ApoE-Deficient Mice1,2

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Abstract

Background: Probiotic bacteria have been associated with a reduction in cardiovascular disease risk, a leading cause of death and disability. Objectives: The aim of this study was to assess the impact of dietary administration of exopolysaccharide-producing probiotic Lactobacillus cultures on lipid metabolism and gut microbiota in apolipoprotein E (apoE)-deficient mice. Methods: First, we examined lipid metabolism in response to dietary supplementation with recombinant β-glucan-producing Lactobacillus paracasei National Food Biotechnology Centre (NFBC) 338 expressing the glycosyltransferase (Gtf) gene from Pediococcus parvulus 2.6 (GTF), and naturally exopolysaccharide-producing Lactobacillus mucosae Dairy Product Culture Collection (DPC) 6426 compared with the non-β-glucan-producing isogenic control strain Lactobacillus paracasei/NFBC 338 (PNZ) and placebo (15% wt:vol trehalose). Second, we examined the effects on the gut microbiota of dietary administration of DPC 6426 compared with placebo. Probiotic Lactobacillus strains at 1 × 10^9 colony-forming units/d per animal were administered to apoE−/− mice fed a high-fat (60% fat)/high-cholesterol (2% wt:wt) diet for 12 wk. At the end of the study, aortic plaque development and serum, liver, and fecal variables involved in lipid metabolism were analyzed, and culture-independent microbial analyses of cecal content were performed. Results: Total cholesterol was reduced in serum (P < 0.001; −33–50%) and liver (P < 0.05; −30%) and serum triglyceride concentrations were reduced (P < 0.05; −15–25%) in mice supplemented with GTF or DPC 6426 compared with the PNZ or placebo group, respectively. In addition, dietary intervention with GTF led to increased amounts of fecal cholesterol excretion (P < 0.05) compared with all other groups. Compositional sequencing of the gut microbiota revealed a greater prevalence of Porphyromonadaceae (P = 0.001) and Prevotellaceae (P = 0.001) in the DPC 6426 group and lower proportions of Clostridiaceae (P < 0.05), Peptococcaceae (P < 0.001), and Staphylococcaceae (P < 0.01) compared with the placebo group. Conclusion: Ingestion of exopolysaccharide-producing lactobacilli resulted in seemingly favorable improvements in lipid metabolism, which were associated with changes in the gut microbiota of mice. J Nutr 2014;144:1956–62.

Keywords: probiotics, lactobacilli, exopolysaccharide, cholesterol, gut microbiota, lipid metabolism

Introduction

Cardiovascular disease (CVD)7 (1) is a leading cause of death and disability globally (1). The human intestinal microbiota has been implicated in the development of obesity (2), insulin resistance (3), and more recently, CVD risk (4, 5). Patients with symptomatic atherosclerosis were shown to harbor an altered gut metagenome compared with healthy controls (6), whereas a

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link between intestinal microbial production of proatherosclerotic metabolites from dietary phospholipids and atherosclerosis risk was also recently reported (4). Moreover, a mechanistic link has been established between changes in intestinal microbiota (via oral treatment with vancomycin in rats) and myocardial infarction. Intestinal microbial metabolism of L-carnitine, abundant in red meat, to proatherogenic trimethylamine-N-oxide (TMAO) has recently been postulated as a mechanism by which red meat ingestion may be linked to CVD risk (7). In a study by Wang et al. (4), it was also reported that dietary administration of a probiotic mixture comprising *Lactobacillus plantarum* (Lp299y) and *Bifidobacterium lactis* (Bi-07) was cardioprotective.

Several dietary fibers were shown to significantly decrease serum cholesterol concentrations and thereby reduce risk of CVD (8). β-Glucan is a polysaccharide found in yeast, mushrooms, oats, barley, and bacteria; and β-glucan from whole oats has been recommended by the U.S. FDA (9) as a safe and practical dietary approach for blood cholesterol reduction. Cholesterol-lowering effects of oats, barley, and yeast are well established (10); and β-glucan from oats and yeast has been shown to improve lipid profiles (11). Some lactic acid bacteria produce exopolysaccharides, a wide diversity of carbohydrates varying in structure and monosaccharide composition, which, when ingested, may confer health benefits, including cardioprotective effects such as hypocholesterolemic, antioxidant, and immunomodulatory activities, in addition to prebiotic, antitumor effects, and affect gastrointestinal health (12–15). However, there are little data available from in vivo studies in humans and animal models and in vitro studies that demonstrate beneficial effects of bacterial β-glucan, and exopolysaccharides. In an animal model, the ingestion of bacterial β-glucan led to increased concentrations of serum HDL cholesterol and decreased TG and total cholesterol (TC) concentrations (16). Interestingly, the ingestion of isolated exopolysaccharides enhanced serum cholesterol metabolism (17), reduced TC in serum, LDL-cholesterol concentrations in the liver (18), and size of atherosclerotic lesions in rabbits (19). In a human study, the ingestion of nondairy, oat-based foods containing the β-glucan–producing strain *Pediococcus parvulus* 2.6 resulted in decreased serum cholesterol concentrations (20).

We previously reported the functional expression of the pediococcal glycosyltransferase gene (*Gtf*) in *Lactobacillus paracasei* National Food Biotechnology Center (NFBC) 338 (21) resulted in β-glucan production. The objectives of the present study were as follows: 1) to compare the impact of dietary supplementation of the β-glucan–producing recombinant *L. paracasei* NFBC 338 (GTF) with its non-β-glucan-producing isogenic control (PNZ) and a placebo control on lipid metabolism in apolipoprotein E (apoE)–deficient mice fed a high-fat/high-cholesterol diet for 12 wk, 2) to compare the impact of dietary supplementation of the exopolysaccharides-producing *Lactobacillus mucosae* Dairy Product Culture Collection (DPC) 6426 (DPC 6426) with a placebo control on lipid metabolism in apoE-deficient mice fed a high-fat/high-cholesterol diet for 12 wk, and 3) to investigate the impact on the gut microbial community of the host after ingestion of DPC 6426.

**Methods**

**Experimental animals and diet.** All mouse studies were undertaken in accordance with the Department of Health and Children of the Irish Government. A license and permission for the study were obtained from the Department of Health, Ireland. Male ApoE<sup>−/−</sup>/J mice, which develop atherosclerotic lesions similar to the process of atherogenesis in humans (22), that were between 10 and 12 wk old at the beginning of the experiment were obtained from JAX (The Jackson Laboratory through Charles River Laboratories International). The mice were caged either individually, in pairs, or in groups of 4–6 per cage. Group housing differences were distributed into the different feeding groups. All mice consumed a basal diet (23) ad libitum for 2 wk to stabilize all metabolic conditions, with free access to water at all times. After the acclimatization period, mice were divided into 4 groups (*n* = 9) and fed a high-fat (60% kcal from fat), high-cholesterol (2% wt:wt) diet (23). Mice were subjected to the following daily dietary interventions for 12 wk: the GTF group were administered −10<sup>9</sup> CFU/(mouse · d) of β-glucan–producing *L. paracasei* NFBC 338 expressing the Gtf gene from *P. parvulus* 2.6 in drinking water, the PNZ group were administered −10<sup>9</sup> CFU/(mouse · d) of *L. paracasei* NFBC 338 in drinking water, the DPC 6426 group were administered −10<sup>9</sup> CFU/(mouse · d) of DPC 6426 in drinking water, and the placebo group were administered resuspended freeze-dried powder containing trehalose (15% wt:vol) as a placebo control in drinking water. Mice were housed in an isolator station, exposed to a 12-h light/dark cycle, and maintained at a constant temperature of 25°C. Body weight and food intake were monitored weekly. After 12 wk of being fed experimental diets, serum was collected by retro-orbital blood sampling from mice not deprived of food and stored at 4°C for 30 min, followed by centrifugation at 10,000 × *g* for 5 min, and divided into aliquots and stored at −20°C until processing and mice were killed by cervical dislocation. Tissues were removed, blotted dry, weighed, and frozen in liquid nitrogen and stored at −80°C until use.

**Preparation and administration of lactobacilli.** Cholamphenicol-resistant and recombinant cultures of *L. paracasei* NFBC 338 were activated and propagated 3 times in de Man, Rogosa, and Sharpe (MRS) medium (Difco Laboratories) containing 5 mg/L chloramphenicol (Sigma–Aldrich) at 37°C anaerobically (21). Rifampicin-resistant variants of *L. mucosae* DPC 6426 (originally isolated from bovine feces at Teagasc, Moorepark Food Research Centre) were activated and propagated 3 times in MRS containing 5 mg/L rifampicin (Sigma–Aldrich) at 37°C anaerobically. Fermentation of *L. mucosae* DPC 6426 was undertaken in MRS broth containing 5% (wt:vol) sucrose (Sigma–Aldrich) at 37°C anaerobically. Fermentation of *L. mucosae* DPC 6426 was performed in MRS broth containing 5 mg/L rifampicin (Sigma–Aldrich) at 37°C anaerobically for 20 h and 18 h for both *L. paracasei* NFBC 338 (GTF and PNZ) cultures. After fermentation, the cultures were washed twice in PBS (Sigma–Aldrich) and resuspended at a concentration of ~2 × 10<sup>10</sup> cells/mL in 15% (wt:vol) trehalose (Sigma–Aldrich). Aliquots were freeze-dried (VirTis AdVantage Freeze Dryer; SP Industries) as outlined by Wall et al. (24). Viable cells in freeze-dried powders (produced weekly) containing *L. paracasei* NFBC 338 and *L. mucosae* DPC 6426 were confirmed weekly for every batch of powder produced and involved enumeration of the strains on MRS agar supplemented with appropriate antibiotic after anaerobic incubation for 72 h at 37°C. Individual mice consumed ~1 × 10<sup>9</sup> live microorganisms. This was achieved by resuspending appropriate quantities of freeze-dried powder in the water that mice consumed ad libitum.

**Microbial analysis.** Fresh fecal samples were taken from experimental mice weekly and analyzed for the presence of β-glucan–producing and non-β-glucan–producing *L. paracasei* NFBC 338 and exopolysaccharide-producing *L. mucosae* DPC 6426. Microbial analysis of the fecal samples involved enumeration of the strains on MRS agar supplemented with appropriate antibiotic after anaerobic incubation for 72 h at 37°C. This was achieved by resuspending appropriate quantities of freeze-dried powder in the water that mice consumed ad libitum.

**Preparation and analysis of aortas.** The whole length of the aorta was removed and fixed in a 4% (wt:vol) paraformaldehyde (Sigma–Aldrich) solution in PBS. Quantification of the “en face” plaque area was performed as previously described (25, 26) and inspected under the microscope for quantification of atherosclerotic lesions. Lipid-containing plaque area was determined as a percentage of Oil Red O-stained area by using AxioVision 4.6 (Carl Zeiss Vision) or NIH Image J software.

**Measurement of serum variables.** A commercially available murine ELISA kit (Quantikine; R&D Systems) was used to quantify serum cholesterol.
soluble vascular cell adhesion molecule 1 (sVCAM-1) following the manufacturer's protocol. Serum β-glucan concentrations were determined with Glucatell, a 1,3-β-D-glucan detection kit (Associates of Cape Cod Inc.) for mice that received β-glucan–producing and non-β-glucan-producing L. paracasei NFBC 338. Serum TC was determined by using the EnzyChrom Cholesterol Assay kit (ECCH-100; BioAssay Systems). Determination of HDL cholesterol in serum samples was undertaken by using the EnzyChrom HDL and LDL/VLDL Assay kit (EHDL-100; BioAssay Systems). TGs in serum were determined by using the LabAssay Triglyceride kit (Wako Diagnostics). All samples and standards were analyzed in duplicate.

Lipid extraction from feces and liver. Livers and feces were stored at −80°C before analysis. Liver and fecal lipids were extracted with chloroform/methanol 2:1 (vol:vol) according to the method of Folch et al. (27). The extracted fat samples were dried and dissolved in assay buffer. Liver cholesterol and TG and fecal cholesterol concentrations were determined by using the same kits as used for blood lipid analysis. Extraction of total bile acids from feces was performed as described by Van der Meer et al. (28). Dried samples were reconstituted in 1 mL assay buffer, and total bile acid concentrations were determined by using the Colorimetric Total Bile Acids Assay kit (Diazyme Laboratories). Samples were analyzed in duplicate.

RNA preparation and real-time fluorescence monitoring RT-PCR. Total RNA was extracted from liver samples by using the Qiagen RNase mini kit according to the manufacturer’s protocol. Total RNA was reverse-transcribed into cDNA by using the cDNA Synthesis kit (Bioline) according to the manufacturer’s protocol. To quantify mRNA expression, PCR was performed by using a fluorescence temperature cycler (LightCycler System; Roche Diagnostics). The oligonucleotide primers for β-actin, cholesterol 7α-hydroxylase (Cyp7a1) and 3-hydroxy-3-methylglutaryl-CoA enzyme (Hmg-coa) were designed on the basis of published nucleotide sequences for named genes in apoe-deficient mice (29). The oligonucleotide primers for FA synthase (fas; 5′ TCCACTCTTATGGTCGCTG 3′, 5′ TTCGTCCTGTCACTGTCCC 3′), sterol regulatory element-binding protein 1c (Srebp-c1; 5′ CTCCAGCTCTACAAACCCACAGC, 5′ AGAGGAGGCCAGAGCAGAGAA 3′, and cluster of differentiation 36 (Cd36; 5′ TGGTCCTAGGCCGCTTCC 3′, 5′ TTTCCACACTCCTTCTGTCA 3′) were newly designed on the basis of gene sequences available on the gene network service at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Basic relative quantification of expression was determined by using the comparative 2−ΔΔCt method.

Culture-independent microbial analysis. Cecal content was used for analysis of the microbial community composition. Total bacterial DNA was extracted from the cecal content of 2 groups of mice that were supplemented with either DPC 6426 or the placebo control by using the QIAamp DNA stool mini kit (Qiagen), according to the manufacturer’s instructions. The 16S ribosomal RNA (rRNA) bacterial gene amplicons flanking the V4–V5 region were generated with a view to high-throughput sequence reads by using the Roche genome sequencer FLX platform at the Teagasc 454 sequencing facility. Amplicons were generated by using one forward primer, F1 (5′-AYTGGGYDTAAGNGN), and a combination of 4 reverse primers, R1 (5′-TACCGGRGGTCTAATCC), R2 (5′-TACCAGAGTATCTAATTC), R3 (5′-TACDGGMTTCTAATC), and R4 (5′-TACNVGGGTATCTAATC). PCR was undertaken as previously described (30), and PCR products were cleaned by using an AMPure kit (Beckman Coulter Genomics) as per the manufacturer’s protocol. Emulsion-based clonal amplification was completed as part of the 454-pyrosequencing process. Raw sequencing reads were quality trimmed as previously described (31). The SILVA 16S rRNA (version 106) database was used to BLAST (Basic Local Alignment Search Tool) the trimmed fasta sequence files using default parameters. BLAST output was parsed by using MEGAN (32), which assigns reads to National Center for Biotechnology Information taxonomies by using the Lowest Common Ancestor algorithm. A bit score of 86 was selected as previously used for 16S RNA sequencing data (33). Clustering of sequence reads into operational taxonomical units at a 97% identity level was achieved by using Qiime. The ChimeraSlayer program was used to remove chimeras from aligned operational taxonomical units and the FastTreeMP tool generated a phylogenetic tree (34–36). α Diversity indices (Shannon diversity, Chao1, Simpson, Phylogenetic diversity, and observed species) and rarefaction curves were generated with the use of Qiime. β Diversities were also calculated on the sequence reads on the basis of weighted and unweighted UniFrac and bray curtis distance matrices; subsequently, principal coordinate analysis was implemented.

Statistical analysis. Data are presented as per-group means ± SEMs. Statistical analysis was performed by ANOVA, and data were analyzed by 1-factor ANOVA followed by Tukey’s multiple-comparison test. Differences were considered significant at P < 0.05 unless otherwise stated.

Results

Weekly quantification of fecal bacterial numbers of individual confirmed gastrointestinal transit and recovery of administrated strains. There was no significant difference in fluid intake between the groups and all mice received expected doses (1 × 105 CFU/d). Recovery of GTF was 1 × 106 CFU/g feces, for PNZ was 3 × 106 CFU/g feces, and for DPC 6426 was 2 × 107 CFU/g feces.

Body mass, relative organ, and tissue weights of experimental mice. There were no significant differences in food intake between groups (data not shown). As expected, body weight increased gradually over time for all groups and

| TABLE 1 Serum profile of mice after administration of live polysaccharide-producing lactobacilli/placebo and a high-fat/high-cholesterol diet for 12 wk1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | β-Glucan, mg/L  | sVCAM-1, mg/L   | TC, mg/L         | HDL cholesterol, mg/L | Ratio HDL-C/TC  | TG, mg/L        |
| GTF             | 5.4 × 10−4 ± 1.1 × 10−4 | 0.53 ± 0.1 | 1700 ± 100.0 | 500.0 ± 120.0 | 0.10 ± 0.00 | 2600 ± 90.00 |
| PNZ             | 5.8 × 10−4 ± 1.2 × 10−4 | 0.53 ± 0.1 | 3100 ± 180.0 | 700.0 ± 100.0 | 0.20 ± 0.10 | 3100 ± 890.0 |
| DPC 6426       | —               | 0.53 ± 0.1 | 2100 ± 130.0 | 500.0 ± 100.0 | 0.40 ± 0.10 | 3000 ± 160.0 |
| P               | —               | 1.16 ± 0.1 | 3900 ± 260.0 | 800.0 ± 200.0 | 0.10 ± 0.00 | 3500 ± 140.0 |

1 Values are means ± SEMs, n = 9/group. Labeled means in a column with superscripts without a common letter differ, P < 0.05. DPC 6426, Lactobacillus mucosae Dairy Product Culture Collection 6426, GTF, Lactobacillus paracasei National Food Biotechnology Center 338 expressing the glycosyltransferase (GTG gene; HDL-C, HDL cholesterol; P, placebo control; PNZ, isogenic control strain Lactobacillus paracasei National Food Biotechnology Center 338; sVCAM-1, soluble vascular cell adhesion molecule 1; TC, total cholesterol.
there were no significant differences in final change in body mass between groups. There were no significant differences in the masses of various adipose tissues (subcutaneous adipose tissue, epididymal adipose tissue, and mesenteric adipose tissue), liver, and cecum (data not shown).

**Quantification of atherosclerotic lesions.** All 4 experimental groups developed atherosclerotic lesions after 12 wk (data not shown). However, there were no statistical differences observed for plaque area and plaque density (data not shown).

**Effect of dietary administration of L. paracasei NFBC 338 on β-glucan concentration in serum.** No differences in serum β-glucan concentrations were found after ingestion of GTF and PNZ (Table 1).

**Effect of administered lactobacilli on sVCAM-1 in serum.** All 3 groups that received lactobacilli (GTF, PNZ, and DPC 6426) had significantly decreased serum concentrations of sVCAM-1 compared with the placebo control group, which received no lactobacilli (Table 1).

**Serum lipid profiles.** Total serum cholesterol concentration was decreased (by ~50%) for the GTF and DPC 6426 group compared with the placebo (P < 0.01) and PNZ (P < 0.01) control group (Table 1). In addition, total serum cholesterol was significantly lower for GTF and DPC 6426 groups (P < 0.01) than the PNZ group and, furthermore, the PNZ group was significantly lower (P < 0.01) when compared with the placebo group. There were no significant differences in serum HDL-cholesterol concentrations between the GTF and DPC 6426 groups after 12 wk of dietary intervention (Table 1). Furthermore, the ratio of HDL cholesterol to TC was not significantly altered in any of the groups (Table 1). The serum TG concentration was significantly lower in the GTF (P < 0.001) and DPC 6426 (P < 0.05) groups than the placebo group (P < 0.05; Table 1). However, there was no significant difference between the GTF group and the PNZ group (Table 1).

**Liver lipids.** Liver lipid concentrations were significantly decreased for the GTF (P < 0.01) and DPC 6426 (P < 0.01) groups compared with PNZ and placebo groups (Figure 1A), which relates to the findings of fatty livers (data not shown). TG accumulation in the liver was similar for the PNZ group and the placebo group and was significantly decreased (P < 0.05) for both GTF and DPC 6426 groups (Figure 1B). Hepatic total cholesterol concentrations were similar between GTF, DPC 6426, and placebo groups but were found to be increased for the PNZ group relative to the GTF and DPC 6426 groups (P < 0.05; Figure 1C).

**Total bile acid and cholesterol in feces.** Dietary intervention with GTF led to increased fecal cholesterol excretion compared with all other groups (P < 0.05; Figure 1D). Furthermore, there was no significant difference between fecal bile acid excretions among the groups (data not shown).

**Expression of hepatic key enzymes involved in fat and cholesterol metabolism.** The mRNA expression of Cyp7a1 was significantly increased in the GTF group compared with all other groups (P < 0.05; Figure 2). In addition, the mRNA expression of Cyp7a1 was significantly decreased (P < 0.05) in the GTF and DPC 6426 groups compared with the PNZ and placebo control groups (Figure 2). There were no significant differences in mRNA expression of either Hmg-coA, Srebp-1c, or Fas (Figure 3).

**Effects on gut microbiota composition after dietary intervention with DPC 6426.** Bioinformatic analysis of 16S rRNA sequence data, generated for DPC 6426 and placebo

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**FIGURE 1** Liver profile and fecal cholesterol excretion in mice after administration of live polysaccharide-producing lactobacilli/placebo and high-fat/high-cholesterol diets for 12 wk. (A) Total liver lipids; (B) liver TGs; (C) total liver cholesterol; and (D) total fecal cholesterol excretion. Values are means ± SEMs, n = 9/group. Labeled means without a common letter differ, P < 0.05. DPC 6426, Lactobacillus mucosae Dairy Product Culture Collection 6426; GTF, Lactobacillus paracasei National Food Biotechnology Center 338 expressing the glycosyltransferase (Gtf gene); P, placebo control; PNZ, isogenic control strain Lactobacillus paracasei National Food Biotechnology Center 338.

**FIGURE 2** Relative gene expression of hepatic mRNA in mice after administration of live polysaccharide-producing lactobacilli/placebo and high-fat/high-cholesterol diets for 12 wk. Values are means ± SEMs, n = 9/group. Labeled means for each enzyme/protein without a common letter differ, P < 0.05. Cyp7a1, cholesterol 7α-hydroxylase; DPC 6426, Lactobacillus mucosae Dairy Product Culture Collection 6426; Fas, FA synthase; GTF, Lactobacillus paracasei National Food Biotechnology Center 338 expressing the glycosyltransferase (Gtf gene); Hmg-coA, 3-hydroxy-3-methylglutaryl coenzyme A; P, placebo control; PNZ, isogenic control strain Lactobacillus paracasei National Food Biotechnology Center 338; Srebp-1c, sterol regulatory element-binding protein 1.
groups, revealed a total of 306,282 reads, averaging at 18,017 reads per mouse. Rarefaction curves for each data set were parallel or approaching parallel with the x axis, demonstrating that the depth of sequencing was sufficient (data not shown). At the 97% similarity level, the Shannon Index indicated good overall biodiversity within the samples with a mean value of 4.3 (data not shown). Chao1 (an indicator of species richness) also indicated a sufficient level of overall diversity with a mean value of 703 (data not shown). Taxonomy-based analysis at the family and genus levels but remained similar at the phylum level compared with placebo controls. Dietary intervention with DPC 6426 resulted in significantly less Clostridiales

![Figure 3](https://academic.oup.com/jn/article-abstract/144/12/1956/4575074)

**FIGURE 3** Phylum-level distributions of the microbial communities in the cecal contents, expressed as a percentage of total population of assignable tags, in mice after administration of DPC 6426 (n = 9) or a placebo control (n = 9) and a high-fat/high-cholesterol diet for 12 wk. Labeled means for each phylum without a common letter differ, P < 0.05. DPC 6426, *Lactobacillus mucosae* Dairy Product Culture Collection 6426; P, placebo control.

![Figure 4](https://academic.oup.com/jn/article-abstract/144/12/1956/4575074)

**FIGURE 4** Family-level taxonomic distribution of the microbial communities in cecal contents, expressed as a percentage of total tags assignable at the family level, in mice after administration of DPC 6426 (n = 9) or a placebo control (n = 9) and a high-fat/high-cholesterol diet for 12 wk. *Significant difference, P < 0.05. DPC 6426, *Lactobacillus mucosae* Dairy Product Culture Collection 6426; P, placebo control.

The present study shows that lipid metabolism in high-fat/high-cholesterol-fed apoE-deficient mice is positively influenced by oral administration of GTF and DPC 6426 compared with PNZ and placebo controls, respectively. We showed that dietary intervention with GTF and DPC 6426 are both associated with significantly decreased serum cholesterol (by ~57% and ~47%, respectively), compared with the placebo control. Hong et al. (16) reported similar results for dietary bacterial β-glucan, which decreased serum total cholesterol in Sprague-Dawley rats. In our study, administration of PNZ was associated with significantly decreased TC concentration in serum (by ~32%) compared with the placebo control. These data suggest that the hypocholesterolemic effect seen for both exopolysaccharides-producing bacteria is not solely attributable to the β-glucan and exopolysaccharides but to the lactobacilli used in this study in general. This is consistent with evidence for the cholesterol-reducing abilities of other lactobacilli previously reported (38). In addition, both GTF and DPC 6426 are further associated with prevention of hypercholesterolemia by a decreased hepatic accumulation of cholesterol. However, only GTF led to the induction of hepatic Cyp7a1 mRNA levels, indicating that cholesterol homeostasis is regulated differently for GTF and DPC 6426. In some species, the excess consumption of cholesterol results in a homeostatic response that includes the induction of Cyp7a1 mRNA levels (39). This would suggest that DPC 6426 might prevent intestinal absorption of dietary cholesterol. On the contrary, we did not find significantly increased fecal cholesterol for DPC 6426 but we did for GTF. This finding is important because it indicates that the hypocholesterolemic effect of DPC 6426 and GTF might not be solely caused by a decrease in cholesterol absorption in the host, but by modifying cholesterol homeostasis through synthesis and conjugation of cholesterol to bile acids. Our study also shows that hepatic lipid contents are decreased by dietary intervention with GTF and DPC 6426 in the apoE mouse model but not by PNZ and the suppression of hepatic Cd36 mRNA levels in mice that received either GTF or DPC 6426, indicating that the uptake of FAs from circulation and intracellular transport of long-chain FAs was also altered. Furthermore, dietary intervention with GTF and DPC 6426 resulted in significantly less Clostridiales, Bacteroidetes, and Firmicutes, whereas these families were not found in the placebo group (Figure 4). At the genus level, there were a few populations present only in the cecal microbiota of the placebo group (Figure 5); i.e., *Coproccocus* (P < 0.001), *Eubacterium* (P < 0.001), and *Erysipelotrichales Incertae Sedis* (P < 0.001). Furthermore, dietary administration of DPC 6426 was associated with the presence of *Porphyromonadaceae* and *Prevotellaceae*, whereas these families were not found in the placebo group (Figure 4). At the phylum level, the mouse gut microbiota was dominated by *Firmicutes* (Figure 3), which is in agreement with previous findings (37). The most abundant families were *Peptostreptococcaceae*, *Ruminococcaceae*, and *Lachnospiraceae* (Figure 4); and the most abundant genera were *Ruminococcaceae Incertae Sedis*, *Peptostreptococcaceae Incertae Sedis*, *Lachnospiraceae Incertae Sedis*, *Clostridium*, *Blautia*, and *Anaerotruncus* (Figure 5). The composition of the microbiota of cecal content of mice supplemented with DPC 6426 was significantly altered at the genus level after dietary administration of DPC 6426 included a significant increase in cecal Allobaculum (P < 0.05), *Lactobacillus mucosae* Dairy Product Culture Collection 6426; *P*, placebo control.
in significantly decreased serum TG concentrations compared with PNZ and placebo controls. This apparent discrepancy might provide a novel mechanism for how exopolysaccharides-producing lactobacilli could affect lipid metabolism. We posit that the ingestion of GTF and DPC 6426 alters cholesterol homeostasis/lipid metabolism because of their production of β-glucan/exopolysaccharides (21), although the mechanism need to be further elucidated.

In this study, all mouse groups exhibited atherosclerosis to a certain degree, a finding that is in agreement with the animal model (40). However, studies addressing the effect of lactobacilli on atherosclerosis are limited. Ingested Lactobacillus acidophilus American Type Culture Collection 4356 was recently associated with a decrease in atherosclerotic lesion size by reducing vascular oxidative stress and mitigating the inflammatory response rather than by regulating cholesterol homeostasis (41). Although we did not observe any significant atheroprotective effect after consumption of lactobacilli nor associated changes in the HDL cholesterol concentration or HDL cholesterol:TC ratio, we found that serum sVCAM-1 significantly decreased in all Lactobacillus-supplemented groups, indicating that dietary intervention with lactobacilli may positively influence the inflammation process of atherosclerosis (42, 43).

Our results indicate that dietary intervention with DPC 6426 has an effect on the composition of the murine gut microbiota and that administration of this single strain can play a role in the modulation of the gut microbiota composition in vivo. Indeed, the microbiota was linked to complex disease phenotypes such as obesity and insulin resistance (44, 45). Moreover, after chronic l-carnitine supplementation, an increase in Prevotella was recently linked to a microbiota-dependent trimethylamine production and subsequent hepatic TMAO production in the host, leading to proatherosclerosis phenotypes by impairing reverse cholesterol transport, whereas an increase in Bacteroidetes led to less trimethylamine and TMAO production (7). Surprisingly, we found that dietary intervention with DPC 6426 led to an apparent enrichment in Prevotellaceae and Prevotella at taxa and genus levels, which were both absent in the cecum of placebo mice. However, we did not see any reverse effect on cholesterol homeostasis and atherosclerosis as seen before. We accept that pyrosequencing of the 16S rRNA genes does not provide quantitative population data, but it provides an overview of the effects of dietary intervention with DPC 6426 on the entire microbial population.

In conclusion, we show that dietary intervention with a β-glucan-producing probiotic strain results in modulation of lipid metabolism in a mouse model of atherosclerosis. We also show that β-glucan production by a probiotic strain conferred an advantage in terms of reduced serum cholesterol and TG concentrations and reduced hepatic lipid concentrations compared with the use of a non-β-glucan-producing isogenic control strain. Furthermore, dietary intervention with exopolysaccharides-producing DPC 6426 resulted in modulation of lipid metabolism in the mouse model of atherosclerosis, including significantly lower serum cholesterol and TG concentrations, and was associated with changes in the gut microbial community. These results confirm that dietary intervention with exopolysaccharides-producing (probiotic) strains is a feasible strategy for managing lipid metabolism.

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