# *Chronic consumption of probiotics, oats and apples have differential effects on postprandial bile acid profile and cardiometabolic disease risk markers compared to an isocaloric control (cornflakes): a randomized trial*

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Original Research Article

# Chronic consumption of probiotics, oats, and apples has differential effects on postprandial bile acid profile and cardiometabolic disease risk markers compared with an isocaloric control (cornflakes): a randomized trial

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A B S T R A C T

**Background:** Dietary components that impact the gut microbiota may beneficially affect cardiometabolic health, possibly by altered bile acid metabolism. However, impacts of these foods on postprandial bile acids, gut microbiota, and cardiometabolic risk markers are unclear.

**Objectives:** The aim of this study was to determine the chronic effects of probiotics, oats, and apples on postprandial bile acids, gut microbiota, and cardiometabolic health biomarkers.

**Methods:** Using an acute within chronic parallel design, 61 volunteers (mean ± SD: age 52 ± 12 y; BMI 24.8 ± 3.4 kg/m$^2$) were randomly assigned to consume 40 g cornflakes (control), 40 g oats or 2 Renetta Canada apples each with 2 placebo capsules per day or 40 g cornflakes with 2 *Lactobacillus reuteri* capsules (>5 × 10$^9$ CFU) per day, for 8 wk. Fasting and postprandial serum/plasma bile acids and cardiometabolic health biomarkers, fecal bile acids, and gut microbiota composition were determined.

**Results:** At week 0, oats and apples significantly decreased postprandial serum insulin [area under the curve (AUC): 25.6 (17.4, 33.8) and 23.4 (15.4, 31.4) vs. 42.0 (33.7, 50.2) pmol/L × min and incremental AUC (iAUC): 17.8 (11.6, 24.0) and 13.7 (7.7, 19.8) vs. 29.6 (23.3, 35.8) pmol/L × min] and C-peptide responses [AUC: 599 (514, 684) and 550 (467, 632) vs. 750 (665, 835) ng/mL × min], whereas non-esterified fatty acids were increased [AUC 135 (117, 153) vs. 86.3 (67.9, 105) and iAUC 96.2 (78.8, 114) vs. 60 (42.1, 77.9) mmol/L × min] after the apples vs. control ($P \leq 0.05$). Postprandial unconjugated [AUC: predicted means (95% CI) 1469 (1101, 1837) vs. 363 (−28, 754) μmol/L × min and iAUC: 923 (682, 1165) vs. 22.0 (−235, 279) μmol/L × min)] and hydrophobic [iAUC: 1210 (911, 1510) vs. 487 (168, 806) μmol/L × min] bile acid responses were increased after 8 wk probiotic intervention vs. control ($P \leq 0.049$). None of the interventions modulated the gut microbiota.

**Conclusions:** These results support beneficial effects of apples and oats on postprandial glycemia and the ability of the probiotic *Lactobacillus reuteri* to modulate postprandial plasma bile acid profiles compared with control (cornflakes), with no relationship evident between circulating bile acids and cardiometabolic health biomarkers.

*Keywords*: Renetta Canada Apples, probiotic, fiber, polyphenols, gut microbiota, apolipoprotein B, bile acids, glucose, insulin

*Abbreviations:* FGF-19, fibroblast growth factor-19; FXR, farnesoid X receptor; GIP, gastric inhibitory polypeptide; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; PP, pancreatic polypeptide; PWA, pulse wave analysis; PYY, peptide YY; SBP, systolic blood pressure; SFA, saturated fatty acids; TAG, triacylglycerol; TGR5, Takeda G protein-coupled receptor 5.

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## Introduction

Bile acids are potent signaling molecules that have been increasingly recognized as playing an important role in the metabolic health. Primary bile acids [chenodeoxycholic acid (CDCA) and cholic acid (CA) are modified in the intestine by gut microbiota into secondary bile acids which show differential abilities to act as nuclear receptor ligands or inhibitors [1]. Bile acid signaling through the farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), can impact the markers of cardiometabolic health by enhancing cholesterol transport in the gut [2] and increasing the release of incretin hormones [3]. The circulating bile acid concentration and profile have been altered postprandially and is dependent on the food component studied and individual health status [4, 5]. A positive correlation was shown between postprandial taurine or glycine-conjugated CA and CDCA as well as postprandial triacylglycerol (TAG) [4, 6, 7]. Therefore, postmeal bile acid responses may be related to metabolic phenotype, and are the potential biomarkers of cardiometabolic disease risk [4].

Several dietary components, including fiber and plant polyphenols have been reported to influence circulating bile acid profiles, presumably via changes in the gut microbiota, with subsequent positive effects found on the fasting lipid profile in chronic intervention studies [8]. In addition, circulating secondary bile acid concentration, specifically lithocholic acid (LCA) and glycoursodeoxycholic acid (GUDCA), were found to predict plasma total cholesterol (TC) concentrations and the cholesterol-lowering effect of chronic Renetta Canada apple (rich in fiber and polyphenols) intake, but only among women with hypercholesterolemia [9]. Furthermore, many strains of probiotic bacteria carry bile salt hydrolyzing (BSH) activity. These probiotic bacteria hydrolyze and de-conjugate glycine or taurine from the primary bile acid sterol core [10]. Unconjugated bile acids are subsequently subjected to a variety of microbiota mediated transformations to generate secondary bile acids. Probiotics may influence the circulating bile acid profiles, with chronic supplementation of *Lactobacillus reuteri*, a BSH-active strain, having a beneficial effect on lipid profiles while increasing the levels of circulating unconjugated bile acids (11–13). BSH activity may increase the concentrations of hydrophobic, deconjugated bile acids, which are poorly absorbed through passive diffusion and thus excreted in the feces resulting in a net loss of cholesterol [11, 14, 15]. However, studies investigating the effect of gut microbiota modifying foods on postprandial bile acids are limited, with one study in healthy men ($n = 10$) reporting a sharp increase in circulating conjugated bile acids 30 min after consumption of a fiber-rich wheat bread, compared with white pasta [16]. In view of the link between circulating bile acids and host physiology, the CirculAting Bile Acids as Biomarkers of Metabolic Health - Linking microbiotA (CABALA) Diet and Health study evaluated the chronic effects of *L. reuteri*, oats, or polyphenol-rich Renetta Canada apples on postprandial bile acids and cardiometabolic disease risk markers. We hypothesized that the fasting and postprandial bile acid profiles would be changed after the ingestion of oats, apples, and a probiotic compared with a control and associated with benefits on cardiometabolic health.

## Methods

### Participants

Healthy women and men, aged 18–70 y, with a BMI between 20 and 32 kg/m$^2$ and TC <7.5 mmol/L, were recruited from the Reading area between December 2017 and November 2019. Recruitment methods included the display of posters on the University of Reading campus, advertisements on social media (e.g., Facebook and Next door) as well as in local newsletters, and recruitment emails sent to volunteers registered on the Hugh Sinclair Unit of Human Nutrition clinical unit database. Volunteers were asked to complete a medical and lifestyle questionnaire to assess the eligibility before attending the clinical unit within the Department of Food and Nutritional Sciences, University of Reading for a screening appointment after a 12-hour overnight fast. During this visit, written informed consent was obtained before screening measurements including height, weight, and office blood pressure were recorded. A 5-ml blood sample was collected to determine serum glucose and lipid profile as well as markers of liver and kidney function. The full blood count, including measurement of hemoglobin to assess anemia, was determined by the Department of Pathology at the Royal Berkshire Hospital or in-house using the DxH520 hematology analyzer (Beckman Coulter). Individuals with any abnormalities in blood biochemistry were excluded from the study. Other exclusion criteria included pregnancy or breastfeeding, smokers, presence of chronic disease including diabetes, endocrine disease, CVD, gastrointestinal diseases, pancreatic, hepatic or renal diseases, or hypertension (systolic/diastolic blood pressure > 140/90 mm Hg). Volunteers taking medications that could influence study outcomes (e.g., lipid-lowering medications, anti-depressants, and anticoagulants) were also excluded as well as individuals who had been prescribed antibiotics within the preceding 3 mo. Volunteers with food allergies (gluten, dairy, apples) and intolerances (lactose) as well as those individuals who were planning or currently on a weight reducing program were not eligible to participate.

Before commencing the study, volunteers were asked to refrain from consuming probiotic (such as natural yogurt, kefir, and supplements) or prebiotic food supplements or fiber-based laxatives and >1 apple per day for 2 wk. A 4-d diet diary with details of all drinks and food consumed on 3 weekdays and 1 d during the weekend was completed during the week before each study visit. Dietplan7 (Forestfield Software Ltd) was used to determine the macro- and micronutrient content from the diet diaries, using UK food databases. Flavonoid intake was analyzed using the USDA Flavonoid Database (2014).

### Study design

The CABALA Diet and Health study had an acute within chronic, randomly assigned, controlled, 4 arm parallel dietary intervention design. A minimization program (minim) was used to randomize participants to 1 of the 4 interventions, stratified by age (18–54 y or 55–70 y), sex (male or female), BMI (19–21.9 kg/m$^2$ or 22–32 kg/m$^2$), and TC concentration (≤5.4 mmol/L or 5.5–7.5 mmol/L). This randomization approach was employed to balance the levels of potential confounding variables between the 4 intervention groups [17, 18]. The minim algorithm is a dynamic allocation method and has been used in similar studies to randomly allocate participants to the treatment groups [19, 20]. Participants attended the Hugh Sinclair clinical unit for acute study days on 2 occasions, at the beginning and end of the 8-wk intervention period. The control and intervention arms consisted of 1) Cornflakes (Kellogg's) (40 g) with 180 mL semi-skimmed milk and 2 placebo capsules (UAS Laboratories) (Control); 2) Cornflakes (Kellogg's) (40 g) with 180 mL semi-skimmed milk and 2 probiotic capsules containing the BSH positive strain of *L. reuteri* NCIMB 30242 (UAS Laboratories, >2.5 billion CFU per capsule); 3) Jumbo whole rolled oats (Quaker, PepsiCo) (40 g) with 180 mL semi-skimmed milk and 2 placebo capsules; or 4) 2 Renetta Canada apples (Melinda SCA) and 2 placebo capsules, consumed daily for breakfast (Table 1). Compliance

was monitored using 4-d food diaries (before and after intervention) and daily consumption records. Subjects were asked to return any unused probiotic/placebo capsules at the end of the study and remaining capsules were counted to confirm compliance. The test meals provided at the week 0 and week 8 study visits were representative of the assigned dietary intervention served with a high-fat chocolate drink and were matched for energy, fat content and composition, and carbohydrate as well as protein content (Table 1).

On the day before the study visits, participants were asked to refrain from consuming alcohol and excessive exercise. They were provided with a standard, low fiber/low fat meal to be consumed on the evening before, and water (either tap or bottled mineral water) was allowed ad libitum during the 12-hour overnight fast. On the morning of each visit, participants were asked to provide a fecal sample for bile acid and microbial analysis. On arrival at the Hugh Sinclair Unit of Human Nutrition, body weight and composition were measured using a Tanita BC-418 digital scale (Tanita) before the participants were taken to a temperature controlled clinical room, cannulated in the forearm, and 2 fasting blood samples were taken. The assigned breakfast test meal was provided and consumed within 20 min. Blood samples were then drawn at 20, 40, 60, 80, 100, 120, 150, 180, 210, 240, 300, and 360 min after the start of the test meal for measurement of circulating bile acids (primary outcome measure), lipids, glucose, insulin, and gut hormones. Blood pressure and pulse wave analysis (PWA) measurements were performed before the test meal (0 min) and at 120, 240, and 360 min after the test breakfast using the Mobil-O-Graph Ambulatory Blood Pressure Monitor (IEM GmbH). After the first acute study visit, participants were provided with their assigned study foods. As the apples required storage under dark conditions at 4 °C, those participants assigned to the apple intervention were provided with enough apples for 2 wk and returned bi-monthly to collect additional apples. All sample and data analyses were performed blinded using a code which was broken only after completion of all sample and statistical analyses.

## Intervention foods

Renetta Canada apples from Val di Non, Trentino, Italy (Melinda SCA), were chosen for the study as they are polyphenol-rich and have previously been reported to have prebiotic properties [9, 13]. Apples were transported from Trento, Italy and stored under dark conditions at 4 °C from the time of delivery to consumption by the participants. The phenolic acid concentration of the apples was measured during storage and between batches. The nutritional composition and polyphenol content of the apples were analyzed by the laboratory of Fondazione Edmund Mach. *L. reuteri* was chosen as the probiotic as it has been previously reported to reduce plasma low density lipoprotein-cholesterol (LDL-C) and TC [12]. The probiotic NCIMB 30242 capsules (UAS Laboratories) contained >2.5 billion CFU per capsule [28% (wt/wt)] of *L. reuteri*, as well as 69% (wt/wt) microcrystalline cellulose, 1% (wt/wt) silica, and 2% (wt/wt) magnesium stearate. The placebo capsules (UAS Laboratories), contained 97% (wt/wt) microcrystalline cellulose, 1% (wt/wt) silica, and 2% (wt/wt) magnesium stearate. All capsules were stored at 4 °C. Jumbo whole rolled oats were chosen owing to their hypocholesterolemic effects and prebiotic activity [21, 22] (Pepsico), and Cornflakes (Kellogg's) were stored at room temperature. The nutrient content of the high-fat chocolate drink was adjusted by altering the amounts of oils, sugar, and micellar casein (Supplementary Table 1) so that all breakfast test meals provided 50-g fat, 85-g carbohydrate, and 20-g protein (Table 1).

## Research ethics

The CABALA Diet and Health study was given a favorable ethical opinion for conduct by the University of Reading Research Ethics Committee (UREC Project No. 17/47) and was registered at clinicaltrials.gov (reference ID: NCT03369548). The study was conducted in line with the guidelines set by the Declaration of Helsinki (2013). Subjects gave written informed consent before taking part in the study.

## Blood, urine, and fecal sample collection

Blood samples were collected into serum separator, EDTA, or lithium heparin (LH) vacutainer tubes (Greiner Bio-One Ltd), with 200 kallikrein inhibiting units (KIU) aprotinin (Sigma-Aldrich) added to the EDTA tube immediately after blood collection for gut peptide measurements. EDTA and LH tubes were placed on ice immediately after collection and before centrifugation at 1750 × *g* for 15 min at 4 °C to separate plasma. Serum separator tubes were stored upright at room temperature for 30 min after collection and before being centrifuged at 1750 × *g* for 15 min at room temperature. Plasma and serum were then aliquoted into cryogenic (polypropylene) vials for storage at −80 °C and −20 °C, respectively.

Fecal samples were collected on the morning of the study visit. Volunteers were provided with collection kits comprising 1L plastic lidded pot (Aw Gregory), Seward stomacher 400 circulator bags, and 2.5L anaerobic jar (Fisher Scientific). The sample was collected into the bag and placed inside the lidded pot which was stored in the anaerobic jar with an Oxoid AnaeroGen sachet (Fisher Scientific). On receipt, the whole stool sample was weighed before a 20-g sample was added to a clean stomacher bag and homogenized for 2 mins before being aliquoted and stored in sterile Eppendorf tubes at −80°C.

**TABLE 1**
Nutritional composition of the intervention foods and test breakfasts

| Energy or Nutrient | Intervention foods | | | | Test breakfast composition | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Probiotic | Oats | Apples | Control | Probiotic | Oats | Apples |
| Energy MJ (kcal) | 0.98 (233) | 0.98 (233) | 0.97 (232) | 0.98 (234) | 3.6 (852) | 3.6 (852) | 3.6 (864) | 3.5 (845) |
| CHO (g) | 44.3 | 44.3 | 33.7 | 63.5 | 86.0 | 86.0 | 88.1 | 88.8 |
| Sugars (g) | 11.7 | 11.7 | 8.9 | 44.0 | 46.6 | 46.6 | 63.1 | 69.0 |
| Fiber (g) | 1.2 | 1.2 | 3.6 | 8.5 | 1.4 | 1.4 | 3.6 | 8.5 |
| Protein (g) | 9.3 | 9.3 | 10.5 | 1.1 | 19.2 | 19.2 | 19.8 | 19.3 |
| Fat (g) | 3.4 | 3.4 | 6.3 | 0.7 | 50.3 | 50.3 | 49.6 | 50.1 |
| SFA (g) | 2.0 | 2.0 | 2.5 | — | 20.8 | 20.8 | 20.5 | 20.5 |
| MUFA (g) | 0.8 | 0.8 | 0.7 | — | 18.2 | 18.2 | 16.8 | 18.0 |
| PUFA (g) | 0.2 | 0.2 | 0.1 | — | 8.6 | 8.6 | 7.2 | 8.4 |

CHO, carbohydrate..

## Circulating bile acids

Plasma bile acids were quantified following the validated method previously reported by Ulaszewska et al. [23], in 2019. Briefly, 50 µL of plasma was loaded onto an Ostro 96-well plate (Waters SPA), together with internal standard solution consisting of a known amount of unconjugated (Spectra 2000 s.r.l.), glycine-conjugated (Cabru s.r.l.) and taurine-conjugated (Sigma-Aldrich) bile acids labeled with deuterium and hippuric acid d5 (0.15 µM–0.3 µM), and followed by elution with 150 µL of cold acetonitrile with 1% of formic acid (Sigma-Aldrich) and filtration using a positive pressure-96 manifold (Waters). Samples were dried completely at room temperature with a gentle stream of nitrogen using a Techne Dr-block DB 3D (Cole-Parmer). Samples were then re-dissolved with 100 µL of water: methanol (1:1 V/V).

Chromatographic separation of the compounds was conducted using ultra-HPLC (UHPLC) UltiMate 3000 (Dionex) coupled with a Triple Quad 5500 (AB Sciex) mass spectrometer (injected volume from prepared sample, 5 µL). Isotopic dilution method was used for bile acid quantification and raw data were processed using AB Sciex Analyst 1.6.1 software. Individual bile acids were classified according to their type and characteristics with 7 classes being used for statistical analysis; primary, secondary, conjugated, unconjugated, hydrophobic, hydrophilic, and total (Supplementary Table 2).

For determining fecal bile acids, analysis was performed as previously reported by Joyce et al. [24] and Pereira et al. [25] in 2014. Briefly, fecal material was spiked with deuterated internal standards (D4 cholic acid, D4 chenodeoxycholic acid, D4 deoxycholic acid), and extracts of the mixture were used for Ultra Performance Liquid Chromatography Mass Spectrometry analysis. Each analyte was identified according to its mass and retention time. Standard curves were performed, for bile acids from 1 mg/mL stock solutions in 50% methanol to concentration ranges between 0.0064 and 20 µg/mL, using known bile acids listed in Supplementary Table 3. Sample bile acids were quantified according to the standard curve and concentrations normalized to the deuterated internal standards. MassLynx and TargetLynx TM application by WatersR were used for sample data processing and quantity determination of each compound against known standard curves.

## Biochemical analysis

Serum lipid concentrations [TC, high density lipoprotein-cholesterol (HDL-C), TAG, and NEFAs], CRP, and glucose were determined using the Daytona Plus clinical chemistry analyzer (Randox Laboratories), using kits supplied by Randox Laboratories. LDL-C was estimated by using the Friedewald formula [26]. Non–HDL-C, a predictor for coronary atherosclerosis risk, was calculated by subtracting the HDL-C from the TC concentration [27]. Atherogenic risk ratios (TC:HDL-C and LDL-C:HDL-C) were also calculated [28, 29].

For analysis of serum insulin an ELISA kit (Crystal Chem) was used, following the manufacturer's instructions. A human metabolic hormone magnetic bead panel kit (Millipore Corporation) analyzed plasma concentrations of gut peptides [glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), ghrelin, C-peptide, pancreatic polypeptide (PP), and PYY] using the Luminex 200 with xPONENT software version 4.2 (Invitrogen, Thermo Fisher Scientific). An ELISA kit was used for the determination of IL-18, with TNF-α and IL-6 measured using high sensitivity Quantikine ELISA kits according to the manufacturer's instructions (Bio-Techne). Human fibroblast growth factor-19 (FGF-19) was measured using the ELISA kit from Protein Simple (Bio-Techne) and the Ella automated Simple Plex instrument (Protein Simple, Bio-Techne). Briefly, plasma samples were centrifuged at 4 °C for 10 min (16,000 x g) and the supernatant (50 µL) used for analysis, following the manufacturer instructions.

## 16S gut microbial analysis

Total DNA extraction from fecal samples (250 mg, wet weight) was performed using the FastDNA SPIN Kit for Feces (MP Biomedicals), following the manufacturer's instructions. Samples were subjected to PCR amplification, using the specific bacterial primer set 341F (5' CCTACGGGNGGCWGCAG 3') [30] and 806R (5' GACTACNVGGGTWTCTAATCC 3') [31] with overhang Illumina adapters. PCR amplification of each sample was performed using 25 µL reactions with 0.2 µM of each primer with a GeneAmp PCR System 9700 (Thermo Fisher Scientific) and the following steps: melting step: 95 °C for 3 min (1 cycle); annealing step: 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds (25 cycles); extension step: 72 °C for 5 min (1 cycle). The PCR products were checked on 1.5 % agarose gel and cleaned from free primers and primer dimer, using the Agencourt AMPure XP system (Beckman Coulter), following the manufacturer's instructions. Subsequently, dual indices and Illumina sequencing adapters Nextera XT Index Primer (Illumina), were attached by 7 cycles PCR (16S Metagenomic Sequencing Library Preparation, Illumina). The final libraries, after purification by the Agencourt AMPure XP system (Beckman), were analyzed on a Typestation 2200 platform (Agilent Technologies) and quantified using the Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific). Finally, all the libraries were pooled in an equimolar way, in a final amplicon library and analyzed on the Typestation 2200 platform. Barcoded libraries were sequenced on the llumina MiSeq (PE300) platform (MiSeq Control Software 2.0.5 and Real-Time Analysis software 1.16.18).

## Illumina data analysis

Reads were processed using the QIIME2 pipeline [32], and samples were rarefied to 29,000 sequences using the phyloseq R package [33]. For each treatment and time point taxonomic richness, Chao1 estimates, and Shannon diversity were calculated. Differences in microbial composition among treatments for each time point was assessed by PERMANOVA with 999 permutations based on weighted and unweighted UniFrac distances, and Bray-Curtis dissimilarity index within the vegan package in R [34].

## Statistics

The primary outcome for this study was the changes in postprandial circulating plasma bile acids. Secondary outcomes included changes in fasting plasma bile acids, cholesterol (TC, LDL-C and HDL-C) and inflammatory markers, fecal bile acids, gut microbiota, fasting and postprandial FGF-19, serum lipids (TAG and NEFA), glucose, insulin, and gut peptides, as well as blood pressure and arterial stiffness.

The sample size was calculated to provide 80% statistical power (MGH Biostatistics Software) based on an average (±SD) change in postprandial bile acids after the consumption of fiber-rich bakery products in healthy male volunteers [16]. On the basis of an average change in total bile acids of 1.1 µmol/L and an SD of 0.95 between the control and intervention foods, a sample size of 64 participants ($n = 16$/group) was sufficient for the study and allowed for a 10% drop-out rate.

Statistical analyses for clinical outcomes were conducted using PROC MIXED in SAS 9.4 statistical software (SAS Institute Inc). All outcomes were modeled on a logarithmic scale, adjusted for the prognostics age, sex, and BMI. Data were back-transformed to the

original scale for presentation in tables and charts. Subject characteristics between treatment groups at baseline were analyzed with an F-Test, using post model fitting manipulation of results.

For fasting measures, the change in concentration from baseline (pre-intervention visit) to week 8 was calculated using logarithmically transformed data before being statistically modeled, using the calculation: log week 8 concentration – log week 0 concentration = Δ (change from week 0). For the change in fasting data, the effect of group was evaluated by a marginal linear mixed model with a compound symmetry structure to account for the correlation between repeated measures recorded within the same subject. Finally, participant was fitted as a random effect [35]. Where there was significant effect, post hoc analysis was performed on the different intervention arms compared with control only.

Summary measures of postprandial responses were calculated for week 0 and week 8, including area under the curve [(AUC) calculated by the trapezoidal rule] and incremental area under the curve [(iAUC) calculated by AUC – (fasting concentration × time {min})]. The iAUC summary measure gave an indication of the change in postprandial responses while accounting for the fasting concentration. The time interval for AUC and iAUC was 360 min. As the postprandial NEFA time course profile represents an inverse bell shape, the AUC and iAUC were calculated from 80 min (estimated time for NEFA to reach minimum concentration, *C*min) to 360 min. For all outcomes, peak concentration reached after the test meal (*C*max) was calculated (for NEFA, this was calculated over 80–360 min). For NEFA, an additional summary measure, percentage NEFA suppression from 0 min was also calculated. Data from week 0 were used for the analysis of the acute effects only, of the intervention test meals and a test meal × time effect (for postprandial time course profiles) and test meal effect (for postprandial summary data) was also evaluated by a marginal linear mixed model with the same parameters as the fasting data. Data from week 0 and week 8 were used in the analysis of acute within chronic effects of the intervention foods with the group × visit (for postprandial summary data) effect also being evaluated by a marginal linear mixed model. For all outcomes, $P < 0.05$ was considered statistically significant.

For the fecal bile acids data tabulated quantities were subjected to multivariate analysis, Principal Component Analysis (PCA) and Heat plots analysis using R Studio Version 1.2.5033 and IBM SPSS Version 22.0.0.1. For the gut microbiota data, all statistical analyses were performed using R (R: A language and environment for statistical computing, https://www.r-project.org/). Wilcoxon-rank sum test was used for the comparison of relative abundances of microbial taxa between groups, and the resulting *P* values were corrected using Benjamini & Hochberg correction to account for multiple testing at all the taxonomic levels.

## Results

Sixty-three volunteers started the study, 2 participants dropped out owing to reported hypertension and ill health unrelated to the intervention, during the postprandial study day, with 61 (15 men and 46 women) completing the study (Figure 1). The mean ± SD age of these 61 participants was 52 ± 12 y; BMI 24.8 ± 3.4 kg/m$^2$; and TC 5.10 ± 0.87 mmol/L. Table 2 shows the baseline characteristics of the volunteers assigned to the different intervention foods, including habitual dietary intakes, blood pressure, and anthropometrics. The baseline blood biochemistry of the volunteers assigned to the different intervention groups are shown in Table 3. All subjects had ≥80% compliance to the study interventions across the duration of the trial, as confirmed by completion of daily tick sheets and counting of unused probiotic/placebo capsules.

### Impact of chronic consumption of interventions on outcomes in the fasted state

There were no significant differences in the change in fasting plasma or fecal bile acids or plasma FGF-19 after 8 wk of intervention

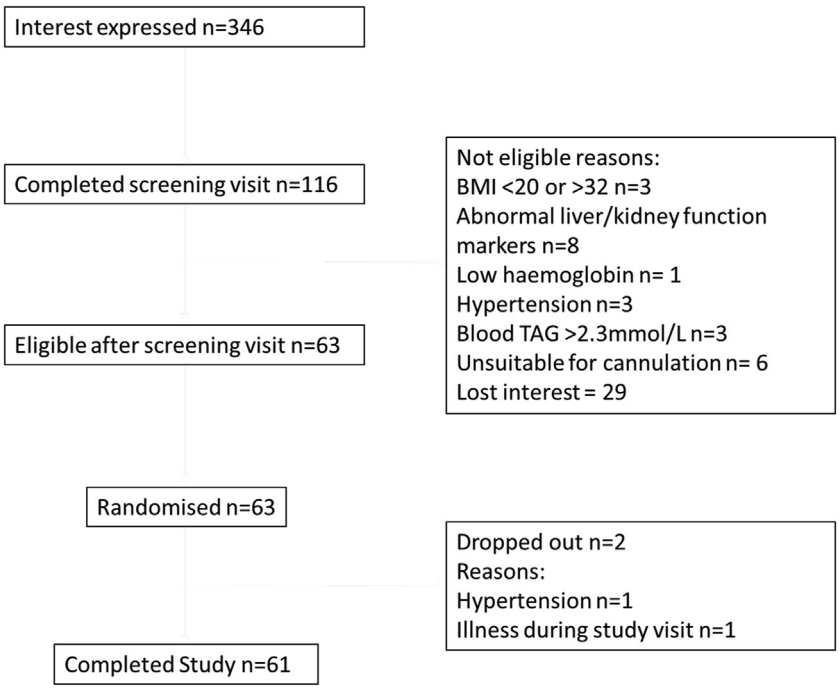


**Figure 1.** Participant flow diagram of the circulating bile acids as biomarkers of metabolic health - Linking microbiotA, Diet and Health (CABALA study).

**TABLE 2**
Characteristics and dietary intakes of the participants assigned to the different test meals

| Parameters | Control ($n = 15$) | Probiotic ($n = 15$) | Oats ($n = 15$) | Apples ($n = 16$) |
|---|---|---|---|---|
| Age (y) | 49 ± 4 | 53 ± 2 | 50 ± 3 | 55 ± 4 |
| Sex (M/F) | 5/10 | 4/11 | 3/12 | 3/13 |
| BMI, kg/m$^2$ | 24.9 (23.1, 26.8) | 24.5 (22.6, 26.4) | 25.3 (23.4, 27.3) | 25.7 (23.8, 27.6) |
| Body fat, % | 26.6 (24.6, 28.5) | 28.6 (26.7, 30.5) | 26.9 (24.9, 28.8) | 27.2 (25.2, 29.1) |
| SBP, mm Hg | 125 (119, 131) | 124 (117, 131) | 118 (111, 125) | 121 (115, 128) |
| DBP, mm Hg | 81 (77, 85) | 82 (77, 87) | 77 (72, 81) | 77 (73, 82) |
| Dietary intakes | | | | |
| Energy, kcal | 2173 (1863, 2483) | 1911 (1600, 2222) | 1834 (1514, 2154) | 2081 (1769, 2393) |
| Energy, MJ | 9.1 (7.8, 10.4) | 8.0 (6.7, 9.3) | 7.7 (6.3, 9.0) | 8.7 (7.4, 10.0) |
| Carbohydrates, %TE | 42.4 (37.9, 46.9) | 43.4 (38.9, 47.9) | 39.9 (35.2, 44.5) | 46.1 (41.5, 50.6) |
| Total sugar, %TE | 17.8 (14.9, 20.7) | 18.2 (15.3, 21.1) | 16.0 (13.1, 19.0) | 17.8 (14.9, 20.7) |
| Fiber (AOAC), g | 23.4 (18.4, 28.4) | 19.7 (14.7, 24.7) | 19.1 (14.0, 24.3) | 27.2 (22.2, 32.2) |
| Fat, %TE | 35.8 (32.4, 39.2) | 38.6 (35.2, 42.1) | 39.4 (35.9, 42.9) | 36.8 (33.4, 40.2) |
| SFA, %TE | 12.0 (10.1, 13.9) | 15.0 (13.1, 16.9) | 13.3 (11.4, 15.2) | 12.1 (10.2, 13.9) |
| PUFA, %TE | 4.9 (4.1, 5.7) | 4.4 (3.6, 5.2) | 4.5 (3.6, 5.3) | 4.7 (3.8, 5.5) |
| MUFA, %TE | 11.3 (9.5, 13.1) | 13.0 (11.2, 14.8) | 13.7 (11.8, 15.5) | 12.4 (10.7, 14.2) |
| Protein, %TE | 16.9 (15.2, 18.6) | 15.3 (13.7, 17.0) | 16.3 (14.6, 18.0) | 15.8 (14.1, 17.5) |
| Total flavonoids, mg | 184.4 (78.9, 290.0) | 158.4 (52.5, 264.3) | 160.8 (52.3, 269.3) | 184.7 (79.1, 290.2) |

Values for age are means ± SEM. For all other outcomes, values are predicted means with 95% CIs estimated with a joint mixed-effect linear model, adjusted for BMI, age, and sex; the outcome BMI was adjusted only for age and sex. The model was built using log-transformed variables and the results were back-transformed to the original scale for the reader's convenience. For control group, $n = 15$; probiotic group, $n = 15$; oats group, $n = 15$; and apples group, $n = 16$. DBP, diastolic blood pressure; F, female; M, male; SBP, systolic blood pressure; TE, total energy.

compared with the control (Table 3). Heat plots and PCA of fecal bile acids, between treatment groups and visits did not show any distinct differences (Figure 2).

There were also no significant differences in the changes in fasting lipids, glucose, insulin, or inflammatory markers after any of the interventions compared with the control. There was a group effect for change in 2 of the fasting gut peptides studied, C-peptide and GIP ($P \leq 0.05$) although post hoc analysis did not reveal any significant differences between any of the intervention groups compared with the control (Table 4). There was also a trend for group effect for change in fasting serum apoB between weeks 0 and 8 ($P = 0.056$) with a greater reduction in concentration evident after the consumption of apples for 8 wk compared with the control ($P = 0.037$) (Table 4). Fasting blood pressure and measures of PWA were not significantly altered by any of the interventions compared with the control over the 8-wk period (Supplementary Table 4).

There were no significant differences between any of the intervention groups and the control for any of the of the gut bacteria taxa at genus level or for the change in microbial alpha diversity between weeks 0 and 8 (Supplementary Tables 5–7).

## Acute (postprandial) response

### *Baseline (before intervention) postprandial response*

At week 0, there was a significant test meal × time interaction for the postprandial plasma total and hydrophobic bile acids response ($P = 0.003$ and 0.001, respectively), with a higher concentration at 240 min after the probiotic than control test meal ($P = 0.019$ and 0.015, respectively) (Figure 3). There was also a 95% higher *C*max for hydrophobic bile acids after the probiotic compared with the control test meal at week 0 ($P = 0.026$) (Supplementary Table 8). A significant test meal × time interaction for unconjugated bile acids ($P < 0.0001$) was also found with significantly greater concentrations between 120 and 360 min ($P < 0.05$) after the probiotic test meal associated with a higher *C*max, AUC, and iAUC compared with control test meal ($P \leq 0.047$) (Supplementary Table 8). The time course profile was also significant for secondary bile acids (test meal × time interaction $P = 0.007$), with higher concentrations between 180 and 240 min ($P < 0.05$) after the probiotic compared with the control test meal (Figure 3). There were no significant test meal × time interactions for hydrophilic or conjugated bile acids. There was a significant test meal × time interaction for primary bile acids ($P = 0.003$); however, post hoc tests failed to reveal significant differences between the interventions compared with the controls. There were no significant acute effects of the apple or oat test meals on circulating bile acid concentrations at week 0 compared with the control (Supplementary Table 8).

There were significant test meal × time interactions for the glucose and insulin responses at week 0 ($P < 0.0001$) with lower glucose concentrations evident between 40 and 150 min following the apples test meal ($P \leq 0.042$) and between 60 and 150 min following the oats test meal ($P \leq 0.046$) compared with the control (Figure 4). A 19% reduced *C*max for glucose was also observed after the apples compared with the control test meal ($P = 0.004$) (SupplementSupplementary Table 9). In addition, lower insulin concentrations were found between 40 and 150 min after consumption of the Renetta Canada apples ($P \leq 0.042$) and between 60 and 150 min after the oats test meal ($P \leq 0.046$) than with the control test meal (Figure 4). This was reflected in the AUC and iAUC, which were reduced by 39% and 40% after the oats test meal ($P = 0.020$, $P = 0.029$) and by 44% and 64% after the apples test meal ($P = 0.006$, $P = 0.002$), respectively, compared with the control. The *C*max insulin concentration reached after the apple test meal was also lower ($P = 0.025$) compared with the control test meal (Supplementary Table 9). There was no acute effect of the probiotic containing test meal on the postprandial glucose or insulin responses at week 0. The postprandial C-peptide responses after the different test meals at week 0 are shown in Figure 4. There was a significant test meal × time interaction evident for C-peptide ($P = 0.001$), with reduced concentrations between 40 and 180 min after the apples test meal ($P \leq 0.042$) and at 60 min and between 120 and 180 min after the oats test meal ($P \leq 0.049$) compared with the control at week 0 (Figure 4). The AUC was also reduced after the apples

**TABLE 3**
Fasting plasma and fecal bile acid and FGF-19 concentrations in healthy participants before and after consumption of the control, apples, oats, or probiotic intervention for 8 wk

| Bile Acids (BA) | Control (95% CI) ($n$ = 15) | | Probiotic (95% CI) ($n$ = 15) | | Oats (95% CI) ($n$ = 15) | | Apples (95% CI) ($n$ = 16) | | $P$ value (Δ) |
|---|---|---|---|---|---|---|---|---|---|
| | Week 0 | Week 8 | Week 0 | Week 8 | Week 0 | Week 8 | Week 0 | Week 8 | |
| Plasma | | | | | | | | | |
| Total BAs, μM | 1.20 (0.87, 1.64) | 1.42 (1.03, 1.94) | 1.55 (1.13, 2.13) | 1.71 (1.24, 2.34) | 1.44 (1.04, 1.99) | 1.49 (1.07, 2.06) | 1.59 (1.15, 2.18) | 1.94 (1.41, 2.67) | 0.638 |
| Conjugated BAs, μM | 0.49 (0.34, 0.69) | 0.66 (0.46, 0.93) | 0.73 (0.52, 1.04) | 0.62 (0.44, 0.88) | 0.68 (0.47, 0.98) | 0.71 (0.49, 1.02) | 0.81 (0.57, 1.15) | 0.98 (0.69, 1.40) | 0.156 |
| Unconjugated BAs, μM | 0.64 (0.44, 0.92) | 0.68 (0.47, 0.99) | 0.69 (0.48, 1.00) | 0.99 (0.68, 1.43) | 0.64 (0.44, 0.93) | 0.61 (0.42, 0.89) | 0.69 (0.48, 0.99) | 0.83 (0.57, 1.20) | 0.191 |
| Primary BAs, μM | 0.50 (0.33, 0.78) | 0.62 (0.40, 0.96) | 0.54 (0.35, 0.83) | 0.62 (0.40, 0.96) | 0.54 (0.35, 0.85) | 0.58 (0.37, 0.90) | 0.55 (0.36, 0.86) | 0.81 (0.52, 1.25) | 0.542 |
| Secondary BAs, μM | 0.65 (0.50, 0.85) | 0.74 (0.56, 0.96) | 0.97 (0.74, 1.27) | 0.99 (0.76, 1.30) | 0.82 (0.62, 1.08) | 0.84 (0.63, 1.10) | 0.95 (0.73, 1.25) | 0.99 (0.76, 1.30) | 0.960 |
| Hydrophobic BAs, μM | 0.71 (0.52, 0.97) | 0.86 (0.63, 1.17) | 0.94 (0.69, 1.29) | 0.97 (0.71, 1.33) | 0.84 (0.61, 1.16) | 0.86 (0.62, 1.18) | 0.96 (0.70, 1.32) | 1.14 (0.84, 1.57) | 0.600 |
| Hydrophilic BAs, μM | 0.45 (0.31, 0.67) | 0.52 (0.35, 0.77) | 0.57 (0.39, 0.84) | 0.67 (0.45, 0.99) | 0.54 (0.36, 0.81) | 0.57 (0.38, 0.85) | 0.58 (0.4, 0.86) | 0.73 (0.49, 1.07) | 0.790 |
| FGF-19, pg/mL | 114 (86.6, 151) | 165 (125, 218) | 102 (76.9, 134) | 127 (96.4, 168) | 126 (95.2, 168) | 108 (81.4, 144) | 114 (85.6, 152) | 221 (168, 291) | 0.144 |
| Fecal | | | | | | | | | |
| Total BAs, pg/mL | 270 (178, 411) | 287 (189, 436) | 352 (231, 536) | 400 (262, 609) | 273 (177, 420) | 246 (160, 378) | 233 (153, 355) | 219 (144, 333) | 0.900 |
| Conjugated BAs, mg/dL | 5.70 (3.49, 9.31) | 5.33 (3.26, 8.71) | 6.8 (4.15, 11.1) | 5.7 (3.48, 9.33) | 5.18 (3.12, 8.58) | 3.64 (2.20, 6.04) | 7.18 (4.39, 11.7) | 9.46 (5.78, 15.5) | 0.053 |
| Unconjugated BAs, mmol/L | 252 (164, 388) | 279 (181, 429) | 341 (221, 526) | 389 (252, 600) | 265 (170, 413) | 240 (154, 374) | 221 (143, 340) | 201 (130, 309) | 0.842 |
| Secondary BAs, pg/mL | 249 (161, 386) | 274 (177, 423) | 341 (220, 528) | 388 (251, 601) | 262 (167, 410) | 237 (151, 371) | 211 (137, 327) | 184 (119, 284) | 0.942 |
| Primary BAs, pg/mL | 8.76 (5.21, 14.7) | 7.8 (4.63, 13.1) | 8.88 (5.27, 15.0) | 9.18 (5.45, 15.5) | 8.58 (5.02, 14.7) | 5.81 (3.40, 9.92) | 13.2 (7.84, 22.2) | 14.9 (8.88, 25.2) | 0.135 |
| Hydrophobic BAs, pg/mL | 258 (169, 395) | 280 (183, 429) | 344 (225, 528) | 393 (256, 602) | 268 (173, 415) | 242 (156, 374) | 220 (144, 337) | 200 (131, 306) | 0.918 |
| Hydrophilic BAs, pg/mL | 4.6 (2.62, 8.07) | 4.02 (2.29, 7.05) | 5.02 (2.86, 8.83) | 5.19 (2.95, 9.13) | 4.2 (2.35, 7.49) | 2.79 (1.56, 4.97) | 7.07 (4.03, 12.4) | 9.15 (5.21, 16.1) | 0.089 |

Values are predicted means with 95% CIs estimated with a linear mixed model, adjusted for BMI, age, and sex. The $P$ value (Δ) denotes a group effect for change over the 8-wk intervention period calculated as log week 8 – log baseline = Δ, evaluated by a marginal linear mixed model with post hoc analysis of differences between groups, with Bonferroni adjustment. $P \leq 0.05$ was deemed significant.
BA, bile acid; FGF-19, fibroblast growth factor 19.

and oats containing test meals compared with the control ($P = 0.004$ and 0.045, respectively), with a reduction in iAUC and *C*max for the C-peptide response evident after the apple ($P = 0.004$ and 0.0001 respectively) relative to the control test meal (Supplementary Table 9). The iAUC for PYY was also reduced after the apples compared with the control test meal ($P = 0.002$). There were no acute effects of any of the test meals on the postprandial ghrelin, GIP, GLP-1, or PP responses at week 0 (Supplementary Table 9).

The NEFA response was also modulated by the different test meals at week 0, with a significant test meal × time interaction evident ($P < 0.0001$) and higher NEFA concentrations between 80 and 240 mins after the apples test meal ($P \leq 0.027$) and the oats test meal between 40 and 60 mins ($P \leq 0.043$) and at 180 mins ($P = 0.0003$) than after the control meal (Figure 4). The *C*min ($P = 0.027$), AUC ($P = 0.001$), and iAUC ($P = 0.015$) for the postprandial NEFA response were high, ~47%, 56%, and 60%, respectively, after the apples compared with the control test meal (Supplementary Table 9). There were no significant acute effects of the oats or probiotics containing test meals on any of the summary measures for the postprandial NEFA response at week 0. There were no significant differences between test meals for any of the postprandial summary measures (AUC, iAUC, and *C*max) for the TAG response at week 0 (Supplementary Table 9).

Acute FGF-19 responses were also not different after the intervention test meals compared with the control at week 0 (Table 3). There were also no significant differences in postprandial blood pressure or indices of pulse wave velocity between the test meals containing the different intervention foods at week 0 (Supplementary Table 4).

### *Acute (postprandial) within chronic response*

The summary measures for postprandial circulating bile acids at week 0 and week 8 are shown in Supplementary Table 8. There was a significant group × visit interaction for the summary measures for circulating unconjugated bile acids ($P = 0.041$), with a significantly higher AUC, iAUC, and *C*max after the probiotic than after the control intervention/test meal ($P \leq 0.002$). There was also a significant group × visit interaction for *C*max for secondary bile acids ($P = 0.029$) which were also significantly increased after the probiotic compared with the control intervention/test meal ($P = 0.016$). A significant group × week interaction was also evident for hydrophobic bile acids iAUC ($P = 0.049$), with a significantly higher iAUC after the probiotic than after the control intervention/test meal ($P = 0.018$).

The summary measures for postprandial clinical outcomes at week 0 and week 8 are shown in Supplementary Table 9. There were no significant group × visit effects for postprandial summary measures AUC, iAUC, or *C*max for fibroblast growth factor 19 (FGF-19), lipid, glucose, insulin, or gut hormone responses, between baseline and week 8 except for TAG iAUC ($P = 0.032$), and a nonsignificant trend for PYY *C*max ($P = 0.057$). However, post hoc analysis of the differences between groups, with Bonferroni adjustment did not reveal significant differences between any of the intervention groups compared with the control for TAG or PYY (Supplementary Table 9). Blood pressure and measures of PWA were not significantly altered after the 8-wk intervention period by any of the interventions compared with the control (Supplementary Table 4).

## Discussion

Findings from our study have shown that compared with the control, bile acids were changed postprandially with the probiotic test meal leading to significantly higher concentrations of total, secondary,

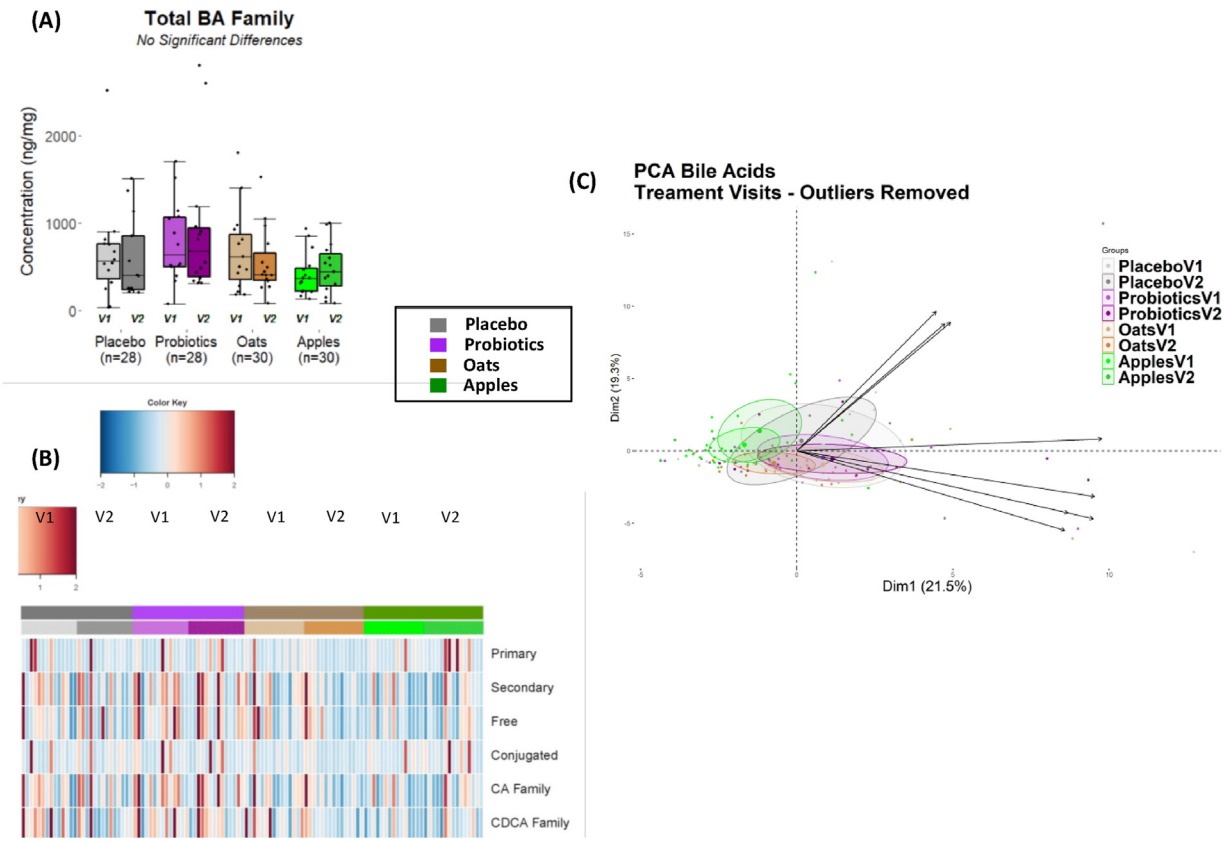


**Figure 2.** Total Fecal Bile Acid Concentration, between visits, for each treatment. (A) Boxplot showing the total bile acid concentration of each dietary treatment for week 0 and week 8. (B) Un-clustered heat plot of bile acid families and (C) Principal Component Analysis showing no distinct differences between treatments/visits.

hydrophobic, and unconjugated bile acids at week 0 and after 8-wk intervention in the case of unconjugated, hydrophobic, and secondary bile acids. However, there was no effect on fasting bile acids. The observed increases in postprandial bile acids, evident ~120 min after the consumption of *L. reuteri*. are likely because of the increased deconjugation of bile acids in the small intestine after bacterial production of BSH enzymes, resulting in increased abundances of circulating unconjugated and hydrophobic bile acid species [36]. In contrast to previous studies reporting beneficial effects of chronic *L. reuteri* ($4 \times 109$ CFU/d) supplementation on fasting LDL-C, TC, apoB, and CRP concentrations in conjunction with increased concentrations of circulating unconjugated bile acids [11, 12], we did not observe changes in bile acids to be related to any beneficial effects on the cardiometabolic health or inflammatory markers. Previous studies have focused on hypercholesterolemic subjects (LDL-C >3.4 mmol/L), which may have resulted in a greater magnitude of lipid-lowering effects, a recognized phenomenon. Some species of secondary bile acids also have cytotoxic effects and have been shown to increase LDL-C concentrations [37]. The detrimental effects may be correlated to hydrophobicity, with the more hydrophobic bile acids exerting negative effects and the more hydrophilic bile acids having cardioprotective effects [38].

Our results confirm previous observations of a significant beneficial effect of Renetta Canada apple and oat consumption on postprandial glycemia, with a reduction in glucose and insulin concentrations compared with those after consumption of the control meal. Insulin is capable of systemic NEFA inhibition because of the inhibitory effects on intracellular TAG lipolysis in adipose tissue [39]. Reduced NEFA suppression after the consumption of apples test meal may be due to improved glycemic control, with a lower C-peptide response indicative of reduced insulin secretion, in agreement with previous studies on apple polyphenols [40]. Oat β-glucans have also been implicated in reduced postprandial glycemic responses and improved glycemic control in patients with type 2 diabetes mellitus [41–43]. Furthermore, studies have shown beneficial effects of apples and their derivatives on postprandial glycemia and insulinemia [40, 44–46]. Apples contain between 66 (Fuji variety) to 212 (Renetta Canada variety) mg/100 g total polyphenols and are a rich source of fiber (approximately 2–3 g/100 g) mainly in the form of pectin [47]. These bioactive components may be responsible for the beneficial effects on host health, with apple flesh and peel as well as polyphenols such as quercetin, proanthocyanins, and chlorogenic acid having been shown to inhibit digestive enzymes, α-amylase, and α-glucosidases in vitro, potentially delaying the digestion of starch and disaccharides and reducing glucose absorption [48–51]. Additionally apple polyphenols may inhibit glucose transporter (SGLT1 and GLUT2) action in the small intestine to reduce glucose uptake which may also be responsible for the beneficial effects on postprandial glycemic control [52]. Oats are also a rich source of bioactive phytochemicals including avenanthramides and a range of phenolic acids, particularly ferulic acid [53]. In vitro studies have revealed inhibitory effects of ferulic acid on starch digestion via interactions with amylopectin leading to the formation of cross-linked feruloyl amylopectin which may be related to suppression of glucose and insulin responses observed in vivo [54]. In contrast to previous studies showing inhibitory effects of apple polyphenol supplementation [40, 46] and commercial apple juice [45] on GIP and GLP-1, we

**TABLE 4**
Fasting serum/plasma lipid, glucose, insulin, gut hormone, and inflammatory marker concentrations in healthy participants before and after control, apples, oats, or probiotic intervention for 8 wks

| Biochemical characteristics | Control (95% CI) ($n = 15$) | | Probiotic (95% CI) ($n = 15$) | | Oats (95% CI) ($n = 15$) | | Apples (95% CI) ($n = 16$) | | $P$ value (Δ) |
|---|---|---|---|---|---|---|---|---|---|
| | Week 0 | Week 8 | Week 0 | Week 8 | Week 0 | Week 8 | Week 0 | Week 8 | |
| TC, mmol/L | 5.05 (4.65, 5.49) | 5.18 (4.76, 5.63) | 5.18 (4.77, 5.64) | 5.25 (4.83, 5.71) | 4.82 (4.42, 5.25) | 4.78 (4.39, 5.21) | 4.66 (4.28, 5.07) | 4.59 (4.22, 4.99) | 0.546 |
| LDL-C, mmol/L | 2.76 (3.49, 5.49) | 2.84 (3.58, 5.63) | 2.82 (3.57, 5.64) | 2.84 (3.60, 5.71) | 2.42 (3.08, 5.25) | 2.40 (3.06, 5.21) | 2.54 (3.21, 5.07) | 2.45 (3.10, 4.99) | 0.153 |
| HDL-C, mmol/L | 1.35 (1.24, 1.47) | 1.36 (1.25, 1.48) | 1.45 (1.33, 1.58) | 1.47 (1.35, 1.60) | 1.57 (1.44, 1.71) | 1.58 (1.45, 1.72) | 1.30 (1.19, 1.42) | 1.31 (1.20, 1.43) | 0.887 |
| Non–HDL-C, mmol/L | 1.30 (1.19, 1.41) | 1.32 (1.21, 1.43) | 1.30 (1.19, 1.41) | 1.31 (1.20, 1.43) | 1.15 (1.04, 1.26) | 1.13 (1.02, 1.25) | 1.20 (1.09, 1.31) | 1.18 (1.07, 1.29) | 0.135 |
| LDL-C:HDL-C ratio | 0.84 (0.70, 0.97) | 0.85 (0.72, 0.99) | 0.79 (0.65, 0.92) | 0.78 (0.64, 0.91) | 0.56 (0.42, 0.7) | 0.54 (0.40, 0.68) | 0.79 (0.65, 0.92) | 0.74 (0.61, 0.88) | 0.401 |
| TC:HDL-C ratio | 1.32 (1.23, 1.41) | 1.34 (1.25, 1.43) | 1.27 (1.18, 1.37) | 1.27 (1.18, 1.37) | 1.12 (1.03, 1.22) | 1.11 (1.01, 1.20) | 1.28 (1.18, 1.37) | 1.25 (1.16, 1.35) | 0.304 |
| TAG, mmol/L | 1.16 (0.97, 1.37) | 1.23 (1.03, 1.46) | 1.03 (0.87, 1.22) | 1.07 (0.90, 1.27) | 0.87 (0.73, 1.04) | 0.85 (0.71, 1.01) | 1.00 (0.84, 1.19) | 1.02 (0.86, 1.21) | 0.233 |
| NEFA, mmol/L | 0.55 (0.44, 0.68) | 0.56 (0.45, 0.69) | 0.61 (0.49, 0.76) | 0.63 (0.50, 0.79) | 0.47 (0.37, 0.59) | 0.50 (0.40, 0.62) | 0.64 (0.51, 0.80) | 0.52 (0.42, 0.65) | 0.253 |
| apoB, g/L | 0.95 (0.85, 1.06) | 0.99 (0.89, 1.10) | 0.97 (0.87, 1.08) | 0.99 (0.89, 1.10) | 0.86 (0.77, 0.96) | 0.85 (0.76, 0.95) | 0.91 (0.82, 1.01) | 0.87 (0.78, 0.97) | 0.056* |
| Glucose, mmol/L | 4.84 (4.67, 5.02) | 4.93 (4.75, 5.11) | 5.06 (4.87, 5.26) | 5.07 (4.88, 5.27) | 5.11 (4.93, 5.30) | 5.15 (4.96, 5.34) | 5.11 (4.93, 5.30) | 5.19 (5.00, 5.38) | 0.606 |
| Insulin, pmol/L | 28.5 (20.5, 39.7) | 28.9 (20.7, 40.2) | 24.6 (17.6, 34.3) | 27.9 (20.0, 38.9) | 17.3 (12.3, 24.3) | 18.9 (13.5, 26.7) | 17.3 (12.4, 24.2) | 21.6 (15.5, 30.2) | 0.722 |
| C-peptide, ng/mL | 0.97 (0.84, 1.12) | 0.97 (0.84, 1.12) | 0.86 (0.75, 1.00) | 0.97 (0.84, 1.13) | 0.75 (0.65, 0.87) | 0.73 (0.62, 0.84) | 0.82 (0.71, 0.95) | 0.92 (0.79, 1.06) | 0.043 |
| Ghrelin, pg/mL | 54.6 (30.3, 98.5) | 50.0 (27.7, 90.1) | 33.2 (18.3, 59.9) | 39.8 (22.0, 72.0) | 56.8 (31.0, 104) | 66.8 (36.4, 123) | 46.8 (25.9, 84.5) | 38.3 (21.2, 69.1) | 0.654 |
| GLP-1, ng/mL | 1.17 (0.50, 2.71) | 1.33 (0.57, 3.08) | 2.09 (0.89, 4.87) | 3.29 (1.41, 7.68) | 1.19 (0.50, 2.84) | 2.02 (0.85, 4.83) | 3.66 (1.57, 8.53) | 5.37 (2.30, 12.5) | 0.532 |
| GIP, ng/mL | 33.1 (25.2, 43.5) | 34.0 (25.9, 44.7) | 30.7 (23.4, 40.3) | 47.0 (35.8, 61.8) | 38.6 (29.1, 51.1) | 34.1 (25.8, 45.1) | 36.1 (27.5, 47.4) | 47.1 (35.9, 61.9) | 0.029 |
| PP, ng/mL | 34.7 (22.0, 54.9) | 32.4 (20.5, 51.2) | 41.1 (26.0, 65.2) | 56.5 (35.7, 89.5) | 37.5 (23.4, 60.2) | 31.5 (19.6, 50.5) | 54.4 (34.3, 86.1) | 67.2 (42.4, 107) | 0.138 |
| PYY, ng/mL | 38.3 (27.5, 53.5) | 37.4 (26.8, 52.2) | 51.5 (36.8, 72.0) | 50.7 (36.3, 71.0) | 41.1 (29.1, 58.1) | 38.3 (27.1, 54.2) | 62.3 (44.5, 87.2) | 62.9 (44.9, 88.1) | 0.998 |
| IL-18, pg/mL | 5.00 (4.76, 5.24) | 4.99 (4.75, 5.23) | 5.32 (5.08, 5.56) | 5.33 (5.09, 5.57) | 5.15 (4.90, 5.39) | 5.14 (4.89, 5.39) | 5.28 (5.04, 5.52) | 5.32 (5.08, 5.56) | 0.768 |
| IL-6, mg/dL | 0.20 (−0.05, 0.45) | 0.29 (0.04, 0.54) | 0.47 (0.22, 0.72) | 0.47 (0.22, 0.72) | 0.26 (0.01, 0.52) | 0.18 (−0.07, 0.44) | 0.79 (0.53, 1.04) | 0.78 (0.53, 1.03) | 0.080 |
| CRP, mg/L | 0.62 (0.38, 0.99) | 0.71 (0.44, 1.14) | 1.07 (0.66, 1.73) | 1.00 (0.62, 1.62) | 0.73 (0.45, 1.2) | 0.63 (0.39, 1.04) | 1.16 (0.72, 1.88) | 1.07 (0.66, 1.73) | 0.190 |
| TNFα, pg/mL | −0.14 (−0.27, −0.01) | −0.14 (−0.27, −0.01) | −0.06 (−0.19, 0.08) | −0.02 (−0.15, 0.12) | −0.16 (−0.3, −0.02) | −0.19 (−0.33, −0.06) | −0.09 (−0.22, 0.04) | −0.02 (−0.15, 0.12) | 0.550 |

Values are predicted means with 95% CIs estimated with a linear mixed model, adjusted for BMI, age and sex. The $P$ value (Δ) denotes a group effect for change over 8 wk intervention period calculated as log week 8 – log baseline = Δ, evaluated by a marginal linear mixed model with post hoc analysis of differences between groups, with Bonferroni adjustment. $P \leq 0.05$ was deemed significant.
apoB, apolipoprotein B, CRP, C-reactive protein; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; HDL-C, high density lipoprotein cholesterol; IL-6, interleukin-6, IL-18, interleukin-18; LDL-C, low density lipoprotein cholesterol; NEFA; nonesterified fatty acid; PP, pancreatic polypeptide; PYY, peptide YY; TAG, triacylglycerol; TC, total cholesterol; TNFα, tumor necrosis factor α.

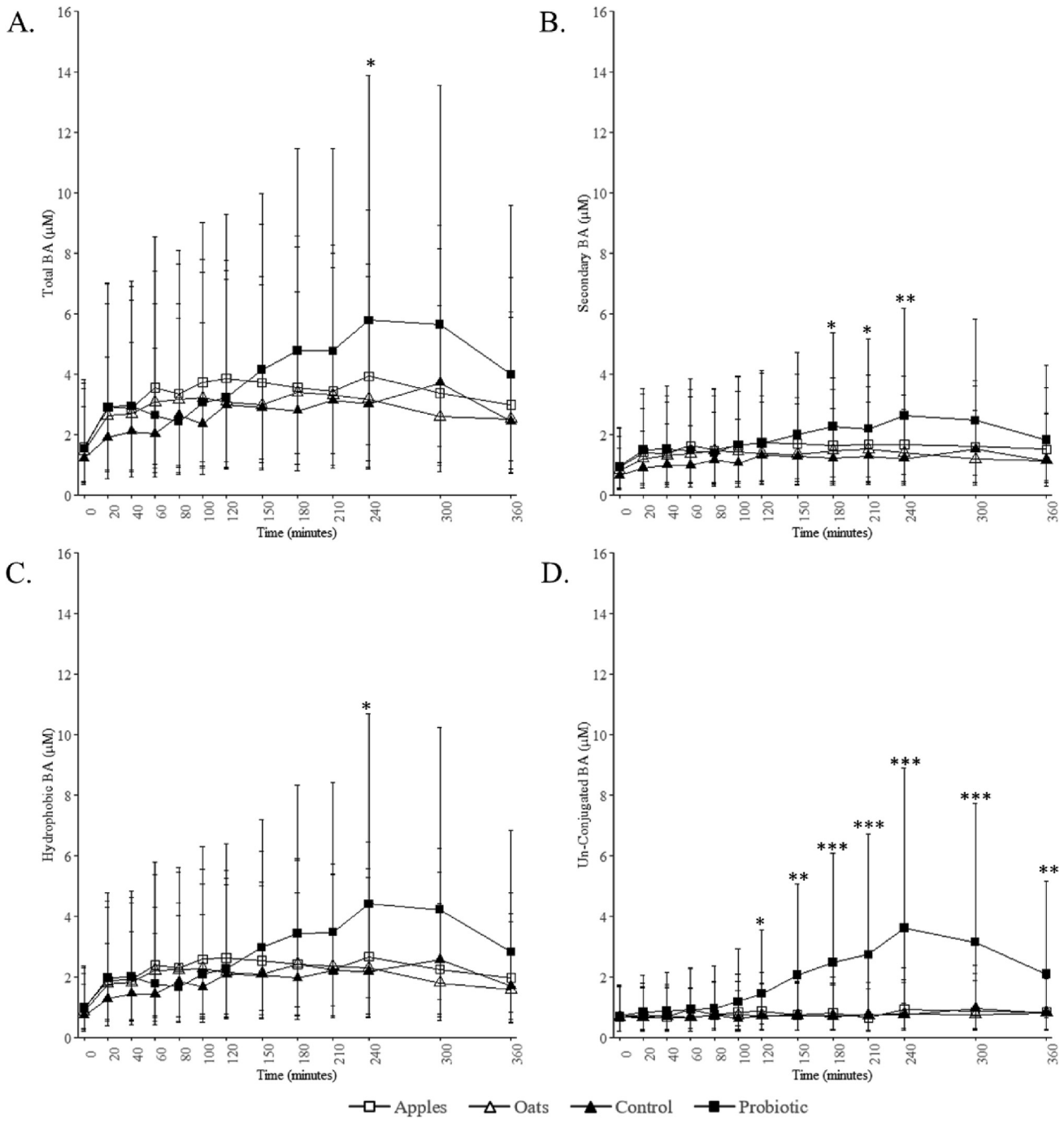


**Figure 3.** Postprandial time course profiles for the total (A), secondary (B), hydrophobic (C) and unconjugated (D) plasma bile acid (BA) concentrations in healthy participants following control ($n = 15$), apples ($n = 16$), oats ($n = 15$) or probiotic ($n = 15$) test meals at week 0. Values are predicted means with 95% CIs estimated with a joint mixed-effect linear model, adjusted for BMI, age and sex, the model was built using log-transformed variables and the results were back-transformed to the original scale for the reader's convenience. Statistical significance was also evaluated by a marginal linear mixed model with post hoc analysis of differences between groups, with Bonferroni adjustment, $P \leq 0.05$ was deemed significant. There was an overall intervention × time effect for total ($P = 0.003$), secondary ($P = 0.007$), hydrophobic ($P = 0.001$) and unconjugated bile acids ($P < 0.0001$). Post hoc analysis of time point differences in change from baseline: control compared with probiotic; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

found no impact on these incretin hormones in this study. One recent study reported increased postprandial GLP-1 concentrations after pectin intake in mice [55]; therefore, it is possible that pectin in Renetta Canada apples counteracted any polyphenol mediated increase in GIP/GLP-1 concentrations. There are some inconsistencies in the effects of oats or oat β-glucans on gut peptide secretion which may be due to differences in dose and gel forming capability or length of β-glucans [56–60] or an effect of the food matrix, as delivery and absorption of carbohydrates, as well as other nutrients from the test meal in the small intestine is dependent on gastric emptying and viscosity of the gut contents [61, 62].

Similar to previous acute human studies on whole apple and apple polyphenols [40, 63] as well as oat β-glucans, we did not find a significant effect on TAG except at 360 min [64]. However, a trend for a

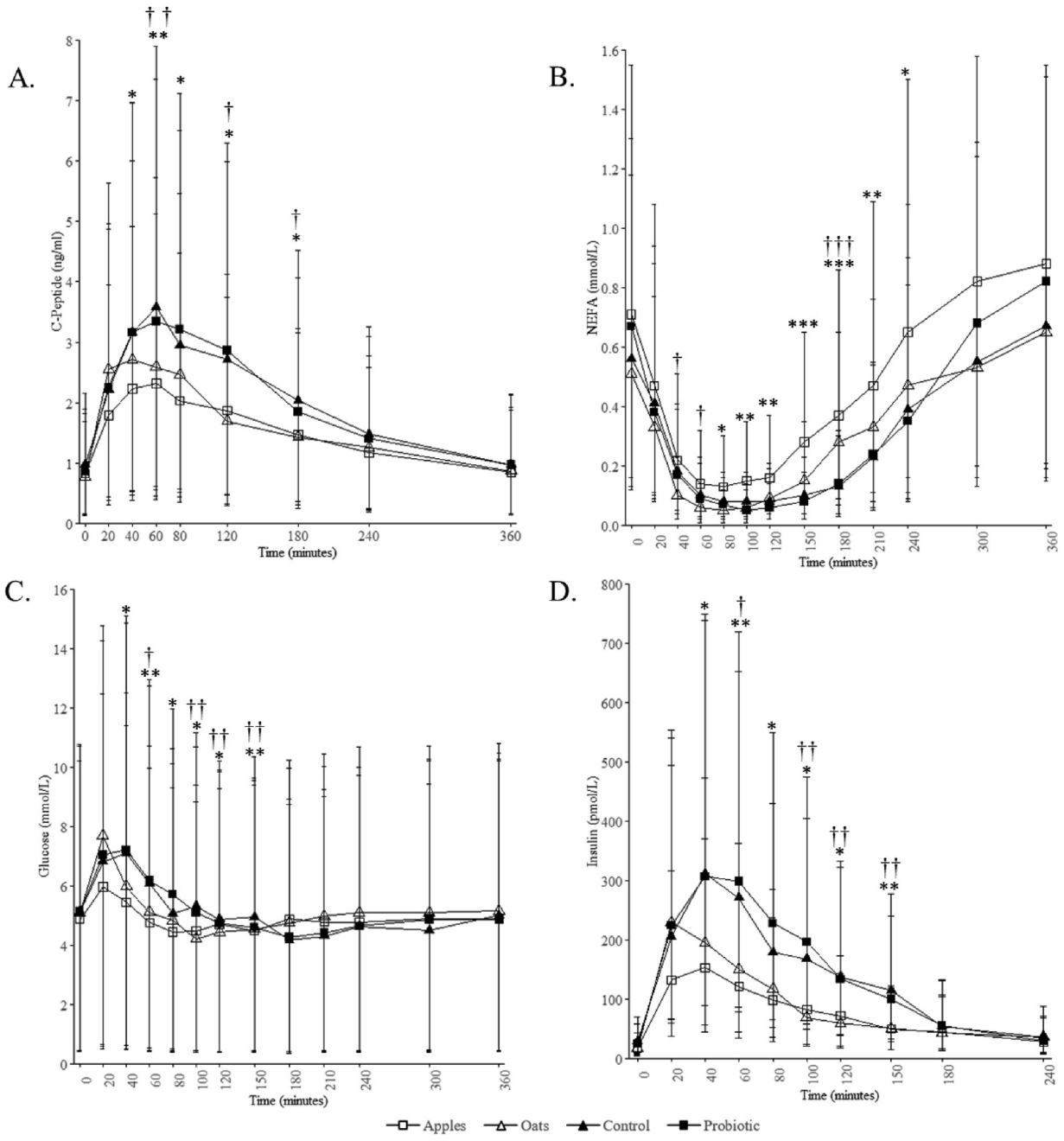


**Figure 4.** Time courses of postprandial C-Peptide (A), NEFA B), glucose (C) and insulin (D) in healthy participants following control ($n = 15$), apples *(n* = 16), oats ($n = 15$) or probiotic ($n = 15$) test meals at week 0. Values are predicted means with 95% CIs estimated with a joint mixed-effect linear model, adjusted for BMI, age and sex, the model was built using log-transformed variables and the results were back-transformed to the original scale for the reader's convenience. For control group $n = 15$, probiotic group $n = 15$, oats group $n = 15$, apples group $n = 16$. Statistical significance was also evaluated by a marginal linear mixed model with post hoc analysis of differences between groups, with Bonferroni adjustment, $P \leq 0.05$ was deemed significant. There was an overall intervention × time effect for C-Peptide ($P = 0.001$), NEFA ($P < 0.0001$), glucose ($P < 0.0001$) and insulin ($P < 0.0001$). Post hoc analysis of time point differences in change from baseline, with Bonferroni adjustment: control compared with apple; $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$; control compared with oats; $\dagger P \leq 0.05$, $\dagger\dagger P \leq 0.01$, $\dagger\dagger\dagger P \leq 0.001$.

significant effect of Renetta Canada apples on fasting apoB concentrations, an indicator of LDL particle number, was apparent compared with the control intervention. Although previous studies investigating consumption of whole [65] and dried apple [66, 67] or apple juice [68] have not found significant effects on apoB, significant reductions in fasting LDL-C have been observed after the consumption of 2 Renetta Canada apples per day [9]. Furthermore, in vitro studies have shown inhibition of intestinal apoB synthesis in apple polyphenol treated intestinal cells [69, 70]. Although previous studies have reported beneficial effects of oats on lipid profiles and inflammatory markers [71–73], in this study there were no significant effects. In a systematic review, intakes of between 57–137 g/d of oat cereal were required to see beneficial effects [74]; therefore, increased intakes might be required to elicit effects in normocholesterolemic subjects [75].

Circulating bile acid profiles have previously been related to the markers of cardiometabolic disease risk including the fasting lipid profile (76–78), possibly via microbial metabolism in the gut, resulting in more hydrophobic secondary bile acids which are excreted in the stool, resulting in a net loss of cholesterol [8]. The results from this study did not find significant differences in fasting or postprandial FGF-19, a marker of FXR activation [79], following the different interventions, which may be due to the high-fat test meal because lipid ingestion does not appear to induce FGF-19 despite the rise in plasma bile acid concentrations [80]. The lack of effect of apples or oats on postprandial bile acids or FGF-19 in this study may be due to the lack of significant effects on the gut microbiota. Our results are consistent with previous studies on effects of oatmeal porridge [81], *L. reuteri* [82, 83], and apples [84] on the gut microbiota. Previous studies have also indicated the presence of responders and non-responders within the population, with the composition of gut microbiota at baseline, namely greater diversity and abundance of Euryarchaeota, strongly influencing the metabolic responses to probiotic supplementation [83]; therefore, further subgroup analysis of the study group may be of interest. There were limited effects on fasting outcomes in this study; therefore, it is possible that the consumption of a high-fat meal is required to "stress" the system and elicit the release and conversion of bile acids for the absorption and metabolism of the study foods.

This study benefited from a randomly assigned, controlled, parallel design and the use of whole foods to mimic typical consumption of the dietary components being studied. In addition, a wide range of bile acid species were determined, providing a novel evaluation of circulating bile acid profiles. A placebo supplement was used for comparison with the probiotic, although the study lacked a true food placebo. The study subjects were healthy adults and therefore results may not be transferrable to other population subgroups. Although sufficiently powered to observe changes in postprandial bile acids, the subject group may not have been large enough to detect changes in cardiometabolic health biomarkers or gut microbiota composition. For the main metabolic outcomes, fasting TC and LDL-C, we calculated a power of 44% and 59% to detect differences between the oats, apples, and probiotic interventions and the controls. There was also a greater proportion of women (75%), although the groups were balanced for sex to mitigate the effect on the measured outcomes.

In conclusion, both acute and chronic consumption of the probiotic led to significant increases in postprandial circulating bile acids with little impact evident on markers of cardiometabolic health compared with the control. The beneficial effects on postprandial insulin and glucose responses following high-fat test meals containing apples or oats relative to the control were not associated with changes in bile acids suggesting that alternative mechanisms may operate to improve postprandial glycemic control.

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The authors' responsibilities were as follows—JAL, CC, SAJ, KGT and KGJ: conceived and designed the study; R-AGP, SA and CC: conducted the human clinical study. R-AGP, SA and KGJ performed the clinical laboratory analysis. R-AGP performed data analysis and wrote the manuscript; AM and KGT: performed the plasma bile acid and gut microbiota analysis and wrote the methods and produced figures for these outcomes; KQ and SAJ extracted, performed the analysis, wrote the methods, conducted PCA and produced the figures for fecal bile acids. KGJ and JAL share equal senior authorship; they are co-last authors. JAL and KGJ: coordinated the project and funding; JAL and KGJ: had overall responsibility for the manuscript contents; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

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## Conflict of interest

The authors report no conflicts of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2022.10.013.

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