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Dairy Product Intake Alters the Correlations between Circulating Bile Acids and Short-Chain Fatty Acids with the Bacterial Taxa *Roseburia*, *Faecalibacterium*, *Flavonifractor*, and Verrucomicrobia

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Keywords
Dairy intervention · Microbiome composition · Microbial metabolites · Insulin resistance

Abstract
Introduction: Type 2 diabetes (T2D) risk factors are associated with gut microbiota dysregulation that can alter circulating metabolite levels such as bile acids (BAs) and short-chain fatty acids (SCFAs). The objective was to investigate how the high dairy (HD) (≥4 servings/day) product intake compared to adequate dairy (AD) (≤2 servings/day) intake influences the correlations between *Roseburia, Faecalibacterium, Flavonifractor*, as well as Verrucomicrobia and circulating BAs and SCFAs in subjects at risk of T2D. ***Methods:*** In a randomized crossover trial, 10 hyperinsulinemic adults were randomized to HD or AD for 6 weeks separated by a 6-week washout period. Gut microbiota were measured with 16S rRNA-based high-throughput sequencing. BA profiling in plasma was performed by high-performance liquid chromatography-tandem mass spectrometry. Serum SCFAs were determined using headspace gas chromatography. ***Results:*** No significant differences were observed in mean circulating BA or SCFA levels between AD and HD consumption. Verrucomicrobia and *Flavonifractor* showed positive correlations with secondary BAs following HD and AD intake, respectively. Additionally, *Flavonifractor* correlated positively with acetic and propionic acids after HD intake. *Roseburia* correlated positively with primary BAs, propionate, and butyrate after HD intake. *Faecalibacterium* was positively correlated with cholic acid after AD intake and with hexanoic acid after HD intake. ***Conclusion:*** These findings suggest that HD intake may modulate microbiota-metabolite interactions without altering

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circulating metabolite concentrations, highlighting a potential role for dietary patterns in shaping gut-derived metabolic signals in individuals at risk of T2D.

Introduction

Type 2 diabetes (T2D), defined as insulin resistance and impaired insulin secretion, is projected to affect over 700 million individuals by 2045 [1]. The gut microbiota consists of a diverse collection of bacteria, archaea, and eukaryotes that colonize the gastrointestinal tract [2]. The gut microbiota is also responsible for converting bile acids (BAs) and producing small molecules like short-chain fatty acids (SCFAs), which can be absorbed by the intestine [3]. BAs, including primary and secondary BAs, play important roles in emulsifying and solubilizing dietary fats in the small intestine [4]. SCFAs, including straight-chain and branched-chain SCFAs, play essential roles in maintaining gut health, regulating metabolism, and serving as energy sources for colonocytes [5]. Individuals with T2D experience disturbances in their gut bacterial communities, which may lead to altered levels of metabolites, including SCFAs and BAs, in both the gut and the circulation [6].

The gut microbiota can convert primary BAs into secondary BAs, such as deoxycholic acid (DCA) and lithocholic acid (LCA) in the distal ileum and colon [5]. Primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized in the liver from cholesterol. Afterward, more than 95% of BAs are reabsorbed in the terminal ileum, while 5% reach the colon, where gut bacteria convert them into secondary BAs [7]. BAs can then be passively absorbed or reabsorbed, with only about 5% lost in feces daily. Through enterohepatic circulation, BAs are continuously reabsorbed in the intestine and returned to the liver via the portal vein [7]. However, 10–50% of the reabsorbed BAs enter systemic circulation instead of returning directly to the liver [7, 8]. Primary BAs are released into the small intestine, where they facilitate the digestion and absorption of dietary fats [7]. Secondary BAs in both the gut and the blood may exert beneficial effects by regulating glucose homeostasis, modulating immune responses, and reducing intestinal epithelial and hepatic inflammation through farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5) signaling pathways [7].

Certain bacterial taxa can produce SCFAs, including acetic, propionic, butyric, and hexanoic acids, as well as branched-chain SCFAs like isocaproic, isobutyric, and isovaleric acids [9]. Specifically, acetate and propionate are predominantly produced by the Bacteroidetes phylum, whereas butyrate is primarily synthesized by the Bacillota phylum, including *Faecalibacteria*, *Flavonifractor*, *Roseburia*, and *Subdoligranulum*. In the large intestine, 95% of the produced SCFAs are rapidly absorbed by the colonocytes, while the remaining 5% are secreted in the feces [10, 11]. Colonocytes use a portion of SCFAs as an energy source, and the remaining SCFAs are released into the portal vein, leading to higher concentrations in portal blood compared to peripheral blood [10]. Approximately 30–40% of acetate enters the bloodstream and is distributed to peripheral tissues, such as muscle and adipose tissue, where it serves as a substrate for lipid synthesis and energy production [12]. In contrast, less than 10% of butyrate and propionate reach systemic circulation, since they are largely taken up by the liver for gluconeogenesis [13]. SCFAs in the circulation can regulate fat metabolism by activating G-protein-coupled receptors, enhancing lipid oxidation, and inhibiting histone deacetylases (HDACs), hence maintaining the expression of genes involved in insulin sensitivity and glucose homeostasis [14].

Recent studies have demonstrated that modifications in the types and quantities of dairy products consumed can influence the composition and functional activity of the gut microbiota, as well as the metabolites they produce [15]. A systematic literature review in healthy subjects has shown that dairy intake modulates the gut microbiota composition in a manner that may benefit the host [14]. Furthermore, the intake of fermented milk can increase the release of secondary BAs such as LCA and DCA in the small bowel due to the greater demand for BAs in the digestion of dairy-derived fat [16]. Additionally, levels of fecal propionate and butyrate increased after consumption of cheese in healthy individuals [17].

A previous study reported that high dairy (HD) intake modified the abundance of *Roseburia*, *Faecalibacterium*, *Flavonifractor*, and members of the phylum Verrucomicrobia in individuals with hyperinsulinemia [18]. However, the literature remains inconclusive regarding whether changes in specific bacterial taxa following dairy consumption influence circulating metabolite profiles, which may in turn affect risk factors associated with T2D. Notably, correlation analyses with gut microbial taxa may identify potential functional associations. These relationships may reflect microbial activity or host-microbe interactions that are not apparent from absolute metabolite concentrations, thereby offering mechanistic insights beyond group-level comparisons. Thus, the aim of the present study was to identify the correlations between

Roseburia, Faecalibacterium, Flavonifractor, as well as Verrucomicrobia and circulating BAs and SCFAs following HD product intake (≥4 servings/day) compared to adequate dairy (AD) product intake (≤2 servings/day) in subjects at risk of T2D.

Methods and Materials

This sub-analysis was carried out as part of a randomized crossover trial at the CHU de Québec-Université Laval Research Center, spanning from February 2017 to July 2018. The study enrolled 10 adults, comprising 5 men and 5 women, who exhibited hyperinsulinemia. These participants, with a mean ± SD of body mass index (BMI) 31.9 ± 2.9 kg/m^2 and age 58 ± 13 years, successfully completed the study. The inclusion criteria comprised individuals with fasting insulin levels exceeding 90 pmol/L, fasting glucose below 7.0 mmol/L, and glycated hemoglobin (HbA1c) below 6.5%.

Dietary Intervention

Eligible subjects were randomly assigned to either HD product intake or AD for a duration of 6 weeks. Following the initial intervention period and a subsequent 6-week washout period, participants switched to the alternate intervention. During the HD phase, individuals consumed a minimum of four servings of dairy products daily, while in the AD phase, the intake was limited to a maximum of two servings per day, based on the serving sizes recommended by Canada's Food Guide for Healthy Eating (2007) [19]. The range of dairy products included milk, yogurt, cheese, kefir, and cream (with a fat content of ≤ 15%). Ice cream consumption was restricted to three servings per week (one serving equivalent to 125 mL). Not considered in the daily serving count were butter, milk substitutes and derivatives, as well as whipped cream or cream exceeding 15% fat content. Participants were instructed to maintain consistent eating habits, physical activity, and other lifestyle factors throughout the study. Dietary information was collected at each visit using a validated self-administered food frequency questionnaire comprising 91 items and 33 sub-questions, completed via a web platform linked to the Nutrition Data System for Research [20]. The estimation of dietary intake relied on the Canadian Nutrient File 2015 [21].

Anthropometric and Clinical Measurements

Anthropometric and clinical measurements were performed as previously described [18, 22, 23]. Briefly, weight and height were measured to calculate BMI (kg/m^2). Appointments were scheduled at the beginning and end of each intervention period (four visits in total). Serum fasting glucose was measured using hexokinase assay [24], insulin by chemiluminescence immunoassays [25], and homeostatic model assessment of insulin resistance (HOMA-IR) calculated using the standard formula.

Gut Microbiota Analysis

The methodology for gut microbiota analysis has been described in detail in a previous publication [18]. Briefly, stool samples were collected using OMNIgene-GUT kits, stored at −80°C, and DNA was extracted with the PowerMag microbial DNA isolation kit. Gut microbiota data used in the present study were previously generated by 16S rRNA gene sequencing (Illumina Nextera XT protocol on the MiSeq platform, ~30,000 reads per sample) [18].

BA Analysis

Morning fasting plasma samples were collected at four designated time points (before and after AD and HD) [26]. These samples underwent BA analysis using an LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry) system, employing a method adapted by Daniel et al. [27]. Solid-phase extraction involved adding a solution containing formic acid and internal standards to plasma samples, which were then processed using Strata-X SPE columns. BAs were eluted with methanol and evaporated under nitrogen before reconstitution. Analysis was performed using tandem mass spectrometry with an API6500 instrument coupled with UHPLC separation employing a Poroshell 120 EC-C18 column. Chromatography utilized a gradient of water, ammonium acetate, and acetonitrile at specified conditions.

Short-Chain Fatty Acid Analysis

Samples were prepared in a 20-mL screw-cap bottle containing 400 mg of sodium chloride, 71 µL of 85% phosphoric acid, and 910 µL of water. An internal standard, 2-ethylbutyric acid (1.11 mg/mL) sourced from Fisher Scientific (Waltham, MA, USA), was added to the sample in a volume of 19 µL [28]. Subsequently, 50 µL of a serum sample was introduced, followed by gentle shaking for 5 min. Calibration curves were constructed using Volatile Free Acid Mix from Sigma (Oakville, ON, Canada), which included acetic, propionic, butyric, isobutyric, isovaleric, valeric, isocaproic, hexanoic acid, and heptanoic acids. Standard solutions were prepared similarly to samples, adjusting the water content to achieve a total volume of 1 mL.

Sample analysis was performed through headspace injection using a Pal 3 injection system coupled with an Agilent gas chromatograph 7890B and a single quadrupole mass spectrometer 5977B (Santa Clara, CA, USA). Headspace equilibrium was achieved via a 40-min incubation at 85°C with agitation. A sample volume of 1 mL was injected with a split ratio of 3:1 into the injector, which was maintained at 250°C. Helium was used as the carrier gas with a fixed flow rate of 1.75 mL/min. Chromatographic separation was accomplished using an HP-INNOWax column (30 m × 0.25 mm × 0.25 μm) from Agilent following a specified oven program. The initial temperature of 50°C was held for 8 min and then increased to 260°C at a rate of 30°C/min, with this final temperature held for 5 min. Mass spectra were recorded in scan mode (29–200 amu) with an electron impact ion source set at 70 eV.

Statistical Analysis

Prior to conducting statistical analyses, the Shapiro-Wilk test was used to assess the normality of distribution for all quantitative variables. Comparisons between the abundance of bacterial taxa and total or individual BAs in post-AD and post-HD conditions were conducted using paired sample *t* tests and Wilcoxon sign-rank tests. Additionally, adjusted Spearman correlations, accounting for confounding factors such as age, sex, and BMI, were used to assess correlations between BAs or SCFAs and the abundance of bacterial taxa. Data presentation includes arithmetic means ± SD unless otherwise specified. Spearman correlations (adjusted for age, sex, and BMI) were visualized using heatmaps generated in RStudio and Perseus (version 2.0.11).

A post hoc power analysis was conducted using G*Power to evaluate the sample size and the strength of the correlation results. The output revealed a lower critical *r* of 0.241, an upper critical *r* of 0.937, and a power (1-β err prob.) of 0.694, indicating moderate power for detecting correlations. All analyses were performed using SPSS version 26 for macOS, with the significance level set at $p \leq 0.05$.

Results

Study Population Characteristics

Ten adults with hyperinsulinemia, comprising five men and five women, were enrolled in this exploratory sub-study. The complete characteristics of the subjects enrolled in this study, including the differences in dietary intake and glycemic parameters after AD and HD conditions, were reported elsewhere [18]. Briefly, the mean BMI was 31.9 ± 2.9 kg/m2, and the average age was 58 ± 13 years for the subjects. During the AD and HD phases, participants consumed 2.6 ± 1.5 and 6.5 ± 2.0 servings of dairy products, respectively ($p < 0.0001$). No difference was observed in fasting insulin, fasting glucose, and HOMA-IR between the AD and HD phases [18].

Characteristics of Bacterial Taxa, BAs in Plasma, and SCFAs in Serum and Feces after AD and HD Consumption

Previously published results of the current clinical study demonstrated that bacterial taxa composition changed after HD with a decrease in the abundance of *Roseburia* ($p = 0.04$) and members of the phylum Verrucomicrobia ($p = 0.02$) as well as a trend toward an increase in the abundance of *Faecalibacterium* ($p = 0.05$) and *Flavonifractor* ($p = 0.06$) compared with AD [18]. Thus, the current study focused on these bacterial taxa.

No significant difference in the mean levels of individual and total BAs was detected between AD and HD consumption (Table 1). Similarly, there were no significant differences in the mean levels of individual serum and fecal SCFAs between AD and HD consumption (Table 2). The molar ratios of fecal acetate, butyrate, and propionate over total SCFAs were calculated to ascertain if there were any changes in proportion, even if there were no changes in total amounts of fecal SCFAs. No significant differences were observed in any of these ratios between the HD and AD consumption groups (Table 2). Although both fecal and serum SCFAs were measured, it is important to note that fecal SCFAs primarily reflect microbial fermentation activity within the gut, whereas serum SCFAs represent the absorbed fraction available in systemic circulation and may therefore be more relevant for investigating systemic metabolic effects [29, 30].

To explore inter-individual variability, a stacked bar graph of the different circulating metabolites for each participant (individual BAs, total BAs, and serum SCFAs) was generated to visualize changes across the two phases (AD vs. HD). The results showed a lot of variability in the response to HD between subjects for individual BAs and total BAs (shown in Fig. 1) and serum SCFAs (shown in Fig. 2). Spaghetti plots for each individual circulating metabolite were also created to visualize the effect of the intervention sequence (i.e., participants starting with AD or HD) and sex (five males and five females) (online supplementary File; for all online suppl. material, see https://doi.org/10.1159/000550224). The data revealed substantial inter-individual variability, regardless of sex or whether participants began with the AD or HD intervention.

Table 1. Plasma bile acid concentrations after adequate and high dairy product consumption in 10 subjects

Parameters	AD (mean±SD)	HD (mean±SD)	*p* value
Total BAs (μM/L)			
Total BAs	1,995.76±1,468.29	1,569.37±894.72	0.57
Unconjugated BA	796.65±851.52	732.28±724.98	0.64
Tauro- and glyco-conjugated	1,198.94±1,285.48	836.75±426.07	0.20
Tauro-conjugated	252.05±366.12	138.64±102.73	0.79
Glyco-conjugated	946.89±933.33	698.11±355.14	0.24
Total primary	1,213.83±853.64	820.20±512.13	0.64
Total secondary	772.87±827.88	742.03±501.35	0.64
6a Hydroxylated	8.88±9.97	6.80±5.17	0.28
Total CA	417.81±315.37	298.50±307.41	0.95
Total CDCA	796.02±593.69	521.69±248.76	0.57
Total LCA	17.02±20.25	22.46±15.84	0.95
Total DCA	579.46±831.84	591.84±523.09	0.50
Percentage of unconjugated	38.55±26.80	41.89±18.93	0.33
Percentage of conjugated	61.43±26.80	58.07±18.91	0.33
Percentage of primary	61.65±15.08	52.39±13.08	0.95
Percentage of secondary	37.85±15.19	47.08±13.31	0.95
Ratio total Cato total CDCA	0.61±0.43	0.53±0.31	0.76
Ratio unconjugated CA to unconjugated CDCA	1±0.67	0.73±0.34	0.35
Ratio total primary to total secondary	2.35±2.20	1.31±0.80	0.64
Ratio total nonconjugated to secondary non conjugated	1.71±2.06	0.83±1.13	0.33
Individual BAs			
Cholic acid (CA)	222.43±401.63	155.01±255.37	0.50
Chenodeoxycholic acid (CDCA)	235.43±313.01	176.95±209.12	0.57
Deoxycholic acid (DCA)	307.94±201.87	357.25±302.07	0.72
Hyodeoxycholic acid (HDCA)	1.49±2.21.56	0.52±0.67	0.16
Ursodeoxycholic acid (UDCA)	71.32±98.64	49.31±35.9	0.64
Hyo-cholic acid (HCA)	7.46±5.98	6.42±5.21	0.64
Lithocholic acid (LCA)	13.02±15.91	10.02±6.86	0.85
Glyco-cholic acid (GCA)	98.05±93.52	113.56±52.12	0.44
Glyco-cheno-deoxycholic acid (GCDCA)	220.27±112.84	306.04±144.81	0.13
Glyco-deoxycholic acid (GDCA)	189.36±193.14	204.93±218.44	0.59
Glycoursodeoxycholic acid (GUDCA)	57.47±50.62	77.94±63.16	0.09
Glycolithocholic acid (GLCA)	9.79±11.75	10.98±11.91	0.44
Taurocholic acid (TCA)	47.74±83.47	38.99±42.46	0.72
Tauro-cheno-deoxycholic acid (TCDCA)	66±70.86	60.81±34.76	0.87
Tauroursodeoxycholic acid (TDCA)	44.93±54.71	49.18±47.11	0.76
Tauroursodeoxycholic acid (TUDCA)	2.8±1.05	2.36±1.74	0.28

Table 1 (continued)

Parameters	AD (mean±SD)	HD (mean±SD)	*p* value
Taurolithocholic acid (TLCA)	1.54±2.2	1.16±1.32	0.60
7α-hydroxy-4-cholesten-3-one 177 (7aC4-177)	78.46±77.53	98.7±100.95	0.09

Data presented as mean ± SD. *p* value: comparison between the groups was performed by paired sample *t* tests and Wilcoxon sign-rank tests (post-AD vs. post-HD). $p < 0.05$ was considered significant. AD, adequate intake of dairy products; HD, high intake of dairy products.

Table 2. Serum and fecal short-chain fatty acid concentrations after adequate and high dairy product consumption in 10 subjects

SCFAs, μM	AD (mean±SD)	HD (mean±SD)	*p* value
SCFAs in serum samples			
Total SCFAs	286.98±47.78	303.11±64.33	0.52
Acetic acid	235.57±46.36	251.31±61.81	0.50
Propanoic acid	13.51±2.67	13.25±4.54	0.79
Isobutyric acid	11.82±2.72	12.52±2.47	0.50
Butyric acid	3.94±0.74	4.14±0.97	0.72
Isovaleric acid	2.23±0.62	2.22±0.60	0.95
Valeric acid	3.06±2.35	3.32±2.74	0.33
Isocaproic acid	2.24±0.94	2.14±0.82	0.50
Hexanoic acid	8.38±2.18	8.45±2.63	0.95
Heptanoic acid	6.18±3.44	5.72±1.73	0.79
SCFAs in fecal samples			
Total SCFAs	159.1±176.42	185.2±145.46	0.72
Acetic Acid	136.14±168.48	159.02±137.20	0.76
Propanoic acid	8.60±3.82	10.86±6.73	0.65
Isobutyric acid	1.14±0.54	1.58±1.11	0.70
Butyric acid	9.77±5.98	9.17±4.96	0.94
Isovaleric acid	1.81±1.30	2.66±2.25	0.59
Valeric acid	1.20±0.54	1.54±1.11	0.65
Isocaproic acid	0.07±0.11	0.02±0.02	0.16
Caproic acid	0.22±0.21	0.23±0.25	1.00
Heptanoic acid	0.13±0.16	0.09±0.05	0.88
Molar ratio of acetate[a]	88.10±5.2	89.00±4.80	0.56
Molar ratio of propionate[a]	6.00±2.10	6.30±1.82	0.62
Molar ratio of butyrate[a]	5.90±2.0	5.10±1.65	0.48

Data presented as mean ± SD. *p* value: comparison between the groups was performed by paired sample *t* tests and Wilcoxon sign-rank tests (post-AD vs. post-HD). AD, adequate intake of dairy products; HD, high intake of dairy products. [a]Molar ratios of fecal acetate, propionate, and butyrate were calculated by dividing the molar concentration of each SCFA by the total molar concentration of the three major SCFAs (acetate, propionate, and butyrate). $p < 0.05$ was considered significant.

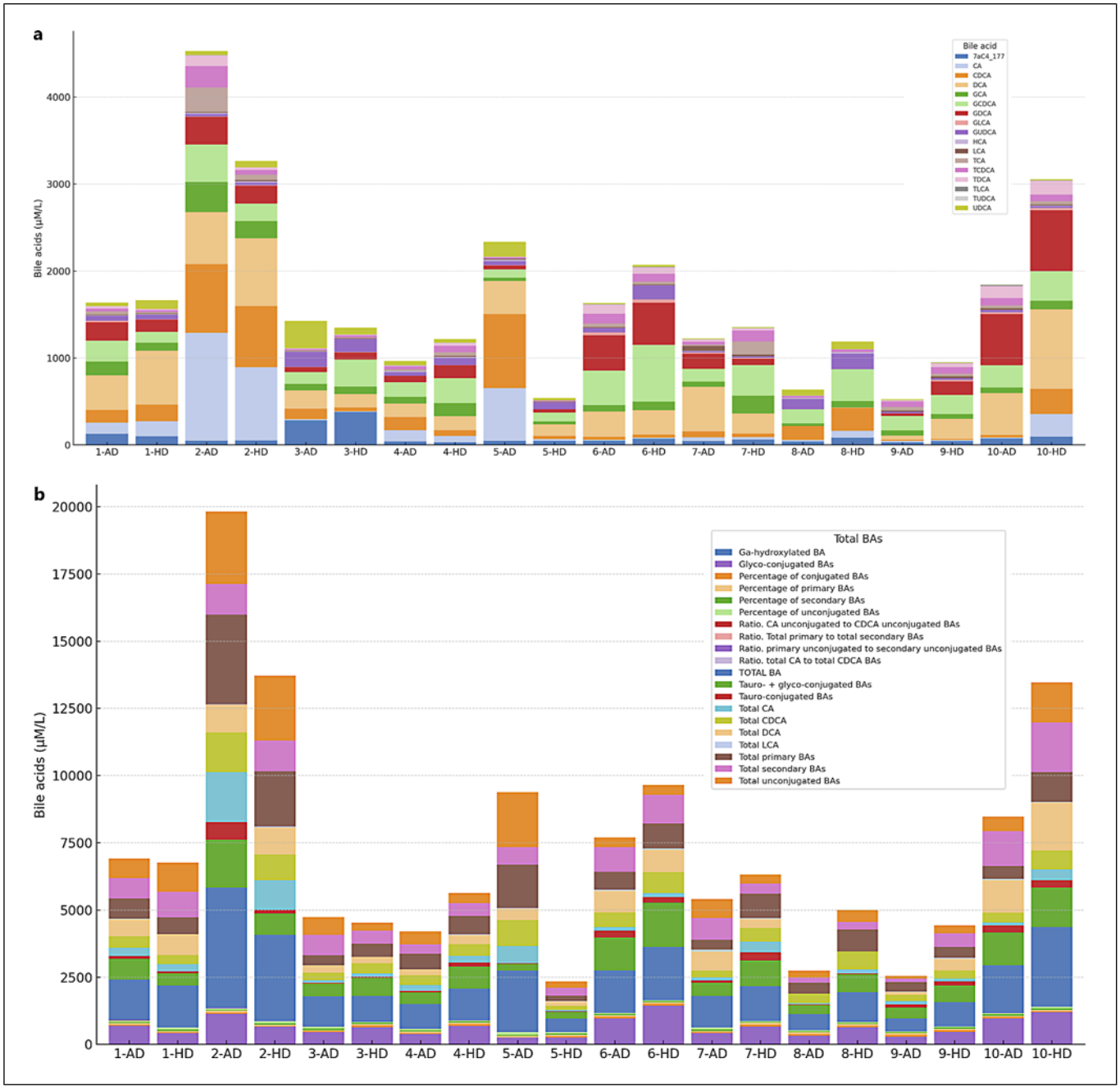


Fig. 1. Inter-individual variability of individual and total BAs in plasma after AD and HD intakes. **a** Stacked bar graph showing the inter-individual variability of individual BAs in plasma after AD and HD intakes. Each color represents an individual BA, and the height of each bar corresponds to the total concentration of BAs (μmol/L) for each participant under the two conditions. **b** Stacked bar graph showing the inter-individual variability of total BAs in plasma after AD and HD intakes. Each color represents a total BA, and the height of each bar corresponds to the total concentration of BAs (μmol/L) for each participant under the two conditions. LCA, lithocholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; TLCA, taurolithocholate; TDCA, taurodeoxycholic acid; UDCA, ursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TCA, taurocholic acid; CA, cholic acid; TCDCA, taurochenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CDCA, chenodeoxycholic acid; GCA, glycocholic acid; 7aC4-177, 7α-hydroxy-4-cholesten-3-one 177, a BA precursor.

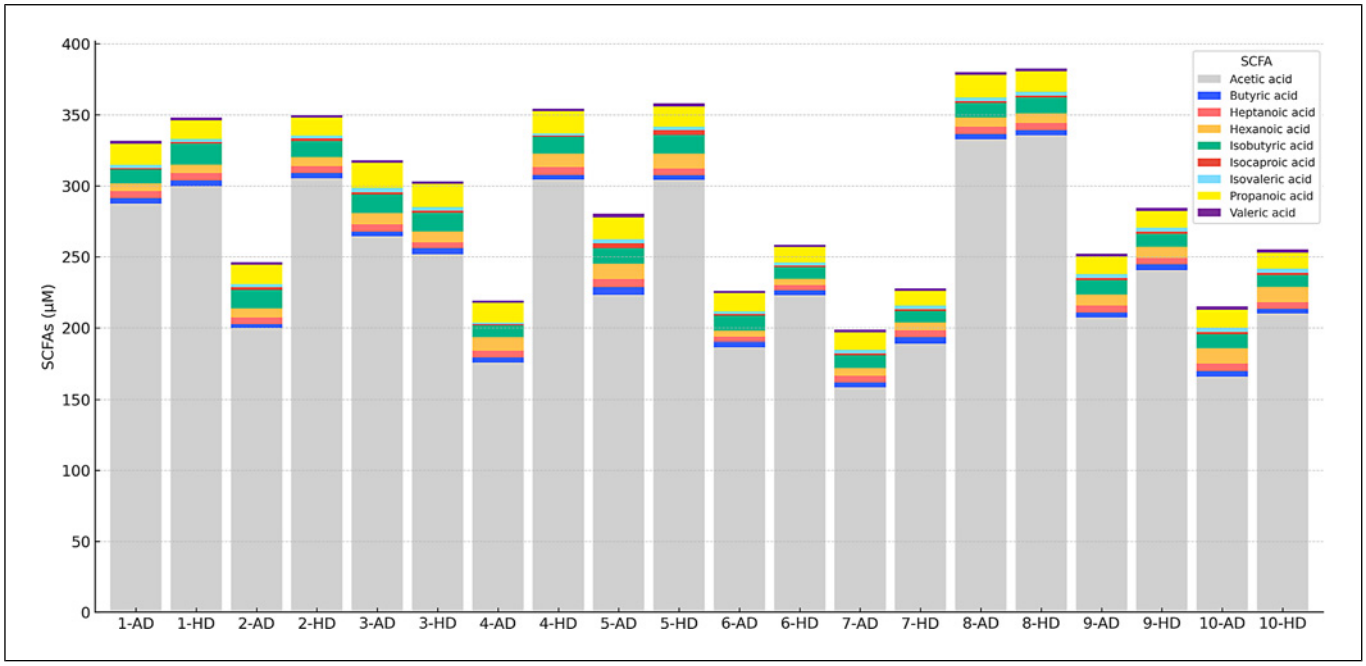
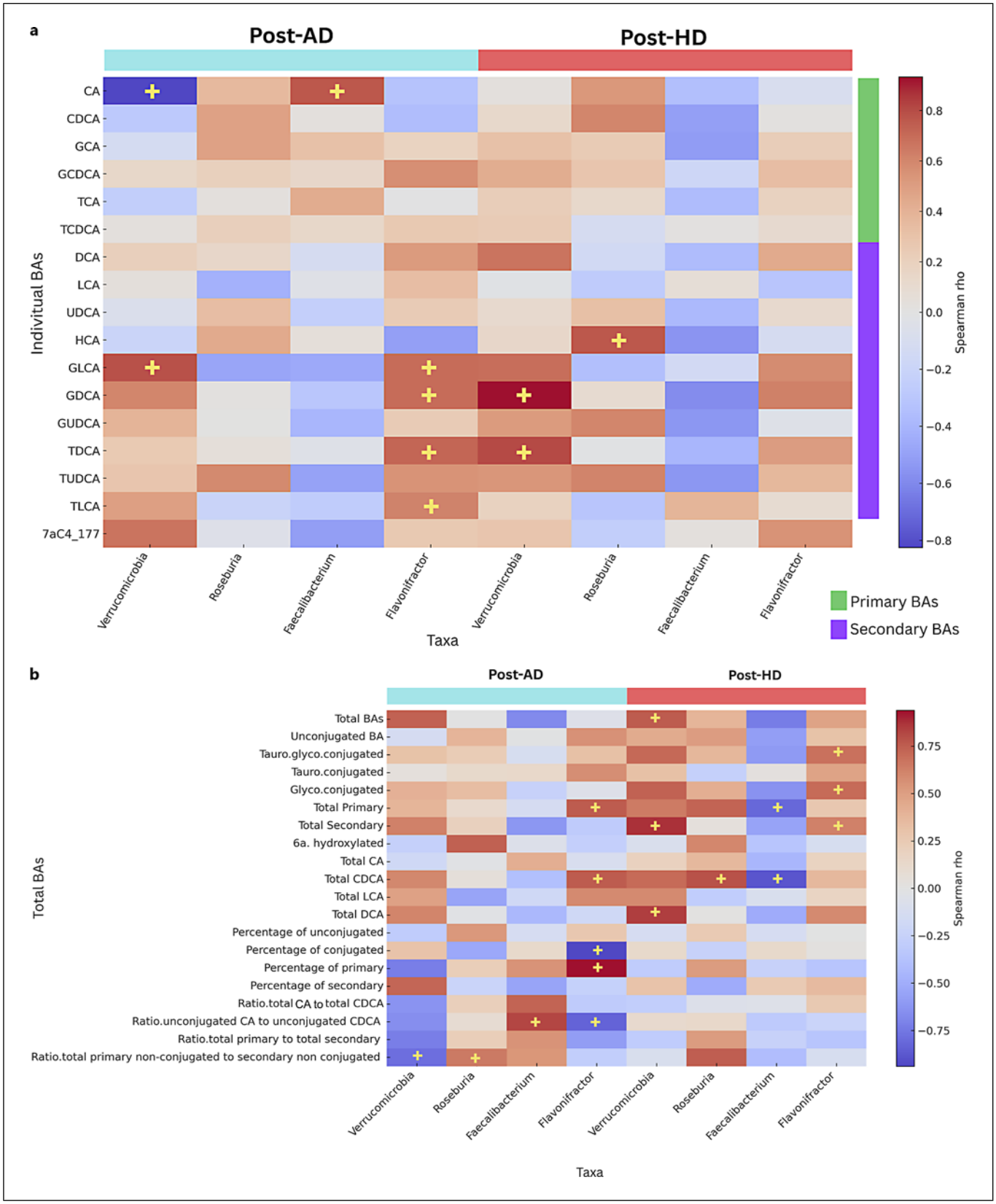


Fig. 2. Stacked bar graph illustrating the inter-individual variability of serum SCFAs after AD and HD intakes. Each color represents a specific SCFA, and the height of each bar indicates the total concentration (μM) for each participant under the two conditions.

Correlations between Bacterial Taxa and Circulating BAs after AD and HD Consumption

Spearman correlation analyses were performed on all the bacterial taxa previously quantified in the 10 hyperinsulinemic subjects [18]. To account for potential confounding effects, correlations were adjusted for age, BMI, and sex. Diagrams showing only the significant positive (in red) and negative (in blue) correlations between the microbial taxa and individual BAs, total BAs (including conjugated and unconjugated) and serum or fecal SCFAs were generated. The results are shown in online supplementary Figures 1–3. Overall, when the diagrams after AD and after HD were compared, the concentrations of metabolites significantly correlating positively or negatively with the abundance of bacteria totally differed for each individual bacterial taxon.

Since a previous study showed changes in the abundance of *Roseburia, Faecalibacterium, Flavonifractor,* and Verrucomicrobia between AD and HD consumption [18], heatmaps were generated to explore the correlations between these bacterial taxa, BAs, and SCFAs following adjustments for age, sex, and BMI. The correlations between the bacterial taxa whose abundance was altered between AD and HD groups and the different types of BAs are shown in Figure 3. After AD consumption, the abundance of Verrucomicrobia showed a positive correlation with glycolithocholic acid (GLCA) ($r = 0.78$, $p = 0.03$) and a negative correlation with CA ($r = -0.82$, $p = 0.02$) (shown in Fig. 3a). The abundance of Verrucomicrobia also showed a negative correlation with the ratio of primary to secondary conjugated BAs ($r = -0.78$, $p = 0.03$) (shown in Fig. 3b). Conversely, *Roseburia* exhibited a positive correlation with the ratio of primary to secondary conjugated BAs ($r = 0.53$, $p = 0.04$) (shown in Fig. 3b). *Faecalibacterium* exhibited positive correlations with CA ($r = 0.76$, $p = 0.04$) (shown in Fig. 3a) and the ratio of CA to unconjugated CDCA ($r = 0.82$, $p = 0.02$) (shown in Fig. 3b). Finally, the abundance of *Flavonifractor* showed significant positive correlations with taurolithocholic acid ($r = 0.72$, $p = 0.02$), glycodeoxycholic acid (GDCA) ($r = 0.69$, $p = 0.02$), GLCA ($r = 0.69$, $p = 0.02$), tauroursodeoxycholic acid (TDCA) ($r = 0.72$, $p = 0.01$), total CDCA ($r = 0.75$, $p = 0.01$), total primary BAs ($r = 0.76$, $p = 0.01$), and the percentage of primary ($r = 0.94$, $p < 0.001$) (shown in Fig. 3a). Conversely, *Flavonifractor* exhibited a negative correlation with the ratio of unconjugated CA to unconjugated CDCA ($r = -0.94$, $p = 0.001$) and the percentage of conjugated ($r = -0.83$, $p = 0.003$) (shown in Fig. 3b).



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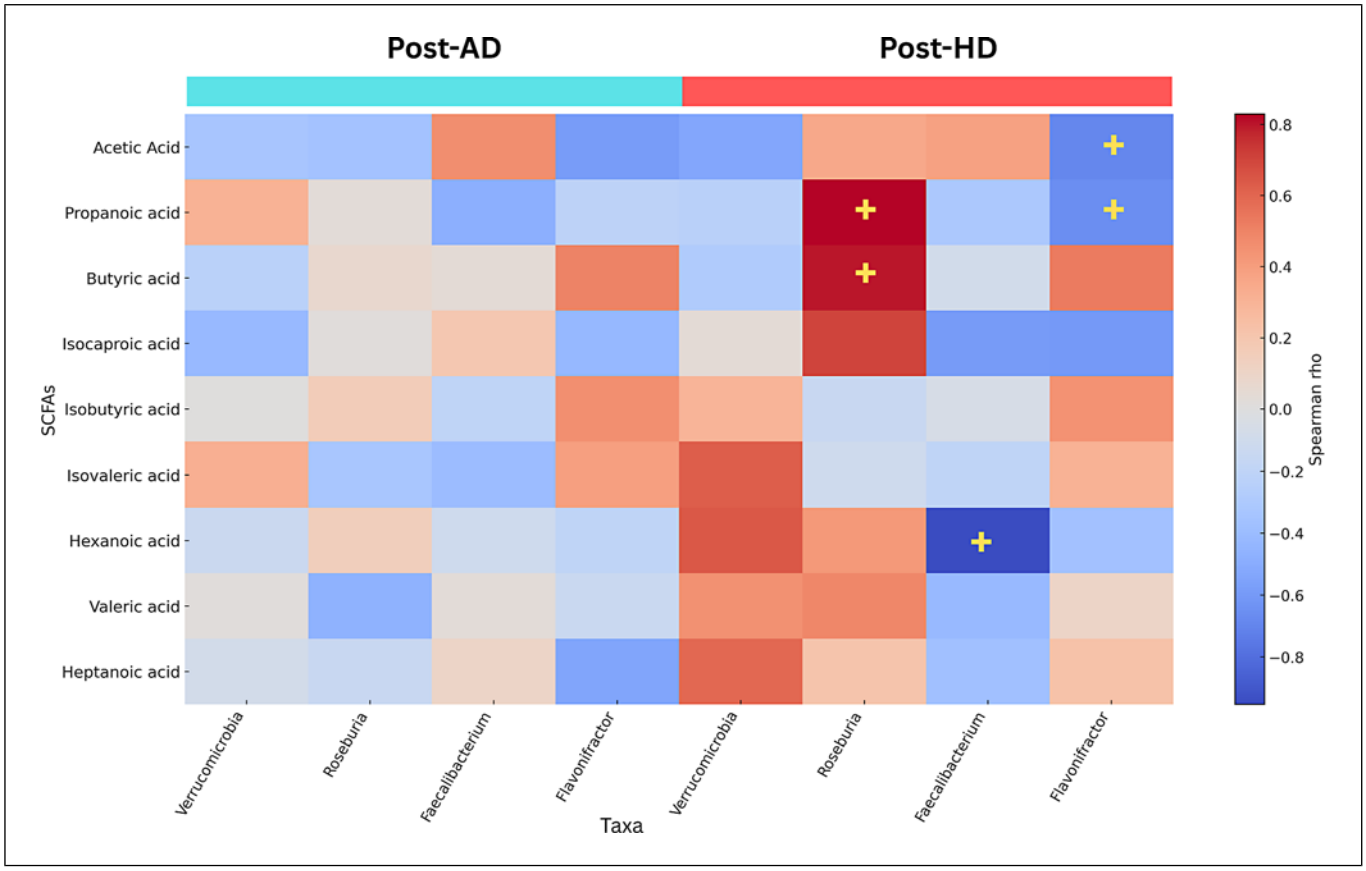


Fig. 4. Correlations between taxa and SCFAs after AD and HD consumption; +: Spearman correlation p value <0.05.

After HD consumption, Verrucomicrobia abundance showed positive correlations with GDCA (r = 0.93, p = 0.002), TDCA (r = 0.80, p = 0.04), total BAs (r = 0.76, p = 0.04), total secondary BAs (r = 0.87, p = 0.01), and total DCA (r = 0.84, p = 0.01) (shown in Fig. 3a and b). *Roseburia* abundance was correlated positively with hyocholic acid (HCA) (r = 0.76, p = 0.04) and total CDCA (r = 0.79, p = 0.03). *Faecalibacterium* displayed a negative correlation with total primary BAs (r = −0.81, p = 0.02) and total CDCA (r = −0.88, p = 0.009) (shown in Fig. 3b). The abundance of *Flavonifractor* showed significant positive correlations with total tauro- and glyco-conjugated BAs (r = 0.69, p = 0.02), glyco-conjugated BAs (r = 0.71, p = 0.02), and total secondary BAs (r = 0.63, p = 0.04) (shown in Fig. 3b).

Correlations between Taxa of Bacteria and SCFAs in Serum after AD and HD Consumption

SCFAs in serum levels represent the fraction of SCFAs that have been absorbed into the bloodstream and are available to tissues throughout the body. These levels are influenced by absorption efficiency, host metabolism, and other systemic factors, and may not directly parallel fecal levels. After AD, there were no correlations between *Roseburia, Faecalibacterium, Flavonifractor,* Verrucomicrobia

Fig. 3. Heatmaps of correlative assessments between taxa and BAs (individual and total) (+: Spearman correlation p value <0.05). **a** Heatmap of correlations between bacterial taxa and individual BAs. **b** Heatmaps of correlations between bacterial taxa and total BAs. LCA, lithocholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; TLCA, taurolithocholate; TDCA, taurodeoxycholic acid; UDCA, ursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TCA, taurocholic acid; CA, cholic acid; TCDCA, taurochenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CDCA, chenodeoxycholic acid; GCA, glycocholic acid; 7aC4-177, 7α-hydroxy-4-cholesten-3-one 177, a BA precursor.

bacterial taxa, and serum SCFAs. After HD, *Faecalibacterium* was negatively correlated with hexanoic acid ($r = -0.92$, $p < 0.001$), and *Roseburia* showed a positive correlation with propionic ($r = 0.673$, $p = 0.03$) and butyric acids ($r = 0.673$, $p = 0.03$) measured in the bloodstream. Further, the abundance of *Flavonifractor* showed significant inverse correlations with acetic acid ($r = 0.68$, $p = 0.02$) and propanoic acid ($r = 0.66$, $p = 0.03$) (shown in Fig. 4).

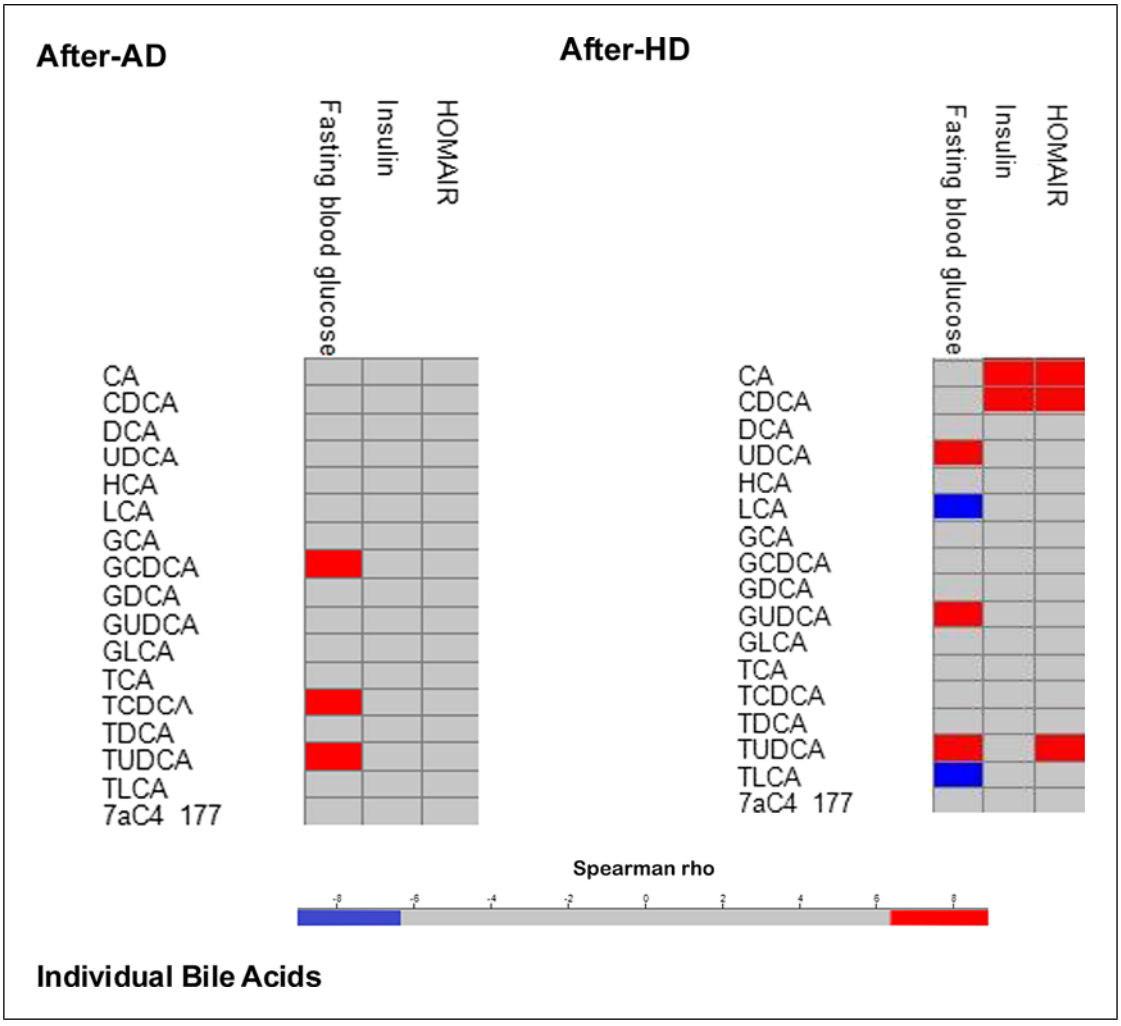
Correlations between Glycemic Parameters and Circulating BAs as well as SCFAs after AD and HD Consumption

Since a previous study indicated that the *Flavonifractor* correlated inversely with the levels of insulin and HOMA-IR [18], the next step of this study was to find the metabolites that correlated with these parameters. Diagrams showing only the significant Spearman correlations between glycemic parameters (fasting blood glucose, fasting insulin, and HOMA-IR) and circulating BAs, as well as serum SCFA levels after AD and HD intake are presented in Figure 5. After AD intake, fasting blood glucose was positively correlated with GCDCA, TCDCA, and TUDCA. Fasting insulin correlated negatively with acetic and isocaproic acids. After HD intake, fasting blood glucose showed negative correlations with taurolithocholic acid and LCA but positive correlations with UDCA, GUDCA, and TCDCA. Fasting insulin correlated positively with CA, CDCA, total BAs and unconjugated BAs, total CA, and the ratio of primary to secondary nonconjugated BAs. HOMA-IR correlated positively with CA, CDCA, TUDCA, total BAs and unconjugated BAs, glyco-conjugated BAs, total primary BAs, 6α-hydroxylated BAs, total CA, total CDCA, and the ratio of primary to secondary nonconjugated BAs.

Discussion

The results of the present study showed that the correlations between specific bacterial taxa and BAs as well as SCFAs are altered even though the levels of BAs and SCFAs in the bloodstream were unchanged in subjects at risk of T2D. Studies have demonstrated that the fasting samples reflect the baseline stability of BAs and SCFAs concentrations in circulation in healthy subjects [31, 32]. Specifically, BAs undergo efficient enterohepatic circulation, maintaining low and stable concentrations in peripheral blood [8]. Serum BAs also remain relatively constant between fasting and postprandial states in healthy individuals [33]. Although studies in mice have shown that high-fat diets alter BA composition in blood, human studies remain inconclusive [34, 35]. Likewise, plasma SCFAs did not increase after high-SCFA intake, as most SCFAs are metabolized by colonocytes or hepatocytes during the first pass [31, 35]. In contrast, serum SCFAs and BAs were higher in T2D patients, likely due to increased intestinal permeability caused by hyperglycemia [6]. For example, a study demonstrated that serum total SCFAs, including acetate and propionate, as well as BAs such as CA, DCA, GDCA, and GCA, were increased in T2D patients compared to healthy controls [6]. In addition, SCFAs and BAs may be excessively absorbed from the leaky gut in T2D, allowing these metabolites to enter the circulation [6]. Since the subjects in the current study had prediabetes, it is possible that the intestinal barrier had not yet been compromised by hyperglycemia or other mechanisms associated with T2D. Overall, these findings suggest that BA and SCFA levels remain stable following dairy consumption in individuals at risk of T2D, similar to observations in healthy populations.

Although linear correlation analysis may oversimplify the complexity of biological systems, the Spearman correlation performed on all quantified bacterial taxa in this study underscores the intricate interactions between gut microbiota and systemic metabolites. This complexity is further illustrated by the number and types of metabolites that significantly correlate, either positively or negatively, with the abundance of individual bacterial taxa, which differed markedly between HD and AD consumption. By focusing on bacterial taxa that exhibited changes in their abundance after HD and AD intakes, the results also show that the number of BAs and SCFAs that were correlated with *Roseburia, Faecalibacterium, Flavonifractor*, as well as Verrucomicrobia changed after HD compared to AD intake. The Verrucomicrobia phylum includes *Akkermansia muciniphila*, a bacterium that typically represents ~3% of the gut microbiota in healthy adults. *A. muciniphila* promotes mucus layer renewal and produces SCFAs, such as acetate, during mucin degradation, which are associated with improved intestinal barrier function [36]. In this study, both Verrucomicrobia and *Flavonifractor* correlated positively with total secondary BAs. Secondary BAs regulate lipid metabolism by activating receptors like FXR and TGR5 [37], as well as enhancing insulin sensitivity by inhibiting gluconeogenesis and stimulating glucagon-like peptide-1 (GLP-1), which improves glucose control and insulin response [38]. Both Verrucomicrobia and *Flavonifractor* correlated positively with GLCA after AD. Although not significant, there was still a positive correlation after HD (shown in Fig. 3a). GLCA



(Figure continued on next page.)

is formed by the glycine conjugation of LCA, a microbial metabolite of primary BAs. In addition to the role of GLCA in lipid digestion and taxa modulation, GLCA can signal through BA receptors such as FXR and TGR5, which is suggested to downregulate the transcription of pro-inflammatory cytokines. GDCA also correlated positively with Verrucomicrobia abundance. GDCA is a glycine-conjugated form of the DCA [39]. GDCA in immune cells inhibits macrophage migration and reduces the secretion of pro-inflammatory cytokines and chemokines [40]. TDCA, which also correlated with Verrucomicrobia after HD, is a taurine-conjugated form of DCA that has the potential to enhance glucose regulation by stimulating GLP-1 secretion and supports gastric health by mitigating inflammation [41]. Although further studies are needed to assess the precise inflammatory status of the subjects, the positive correlations observed between the abundance of Verrucomicrobia or *Flavonifractor* and various secondary BAs after AD or HD intakes may suggest a beneficial effect.

The abundance of several bacterial taxa was also correlated with primary BAs. After AD intake, the abundance of *Faecalibacterium* was positively correlated with CA levels; oppositely, the abundance of Verrucomicrobia was negatively correlated with CA levels. CA can act as a signaling molecule that activates the FXR receptor to regulate lipids, glucose, and energy metabolism [40]. Furthermore, after HD intake, the abundance of *Roseburia* was positively correlated with primary BAs, HCA, and CDCA. Previously, a cohort study found that serum HCA levels were inversely correlated with fasting and post-load blood glucose levels, as well as

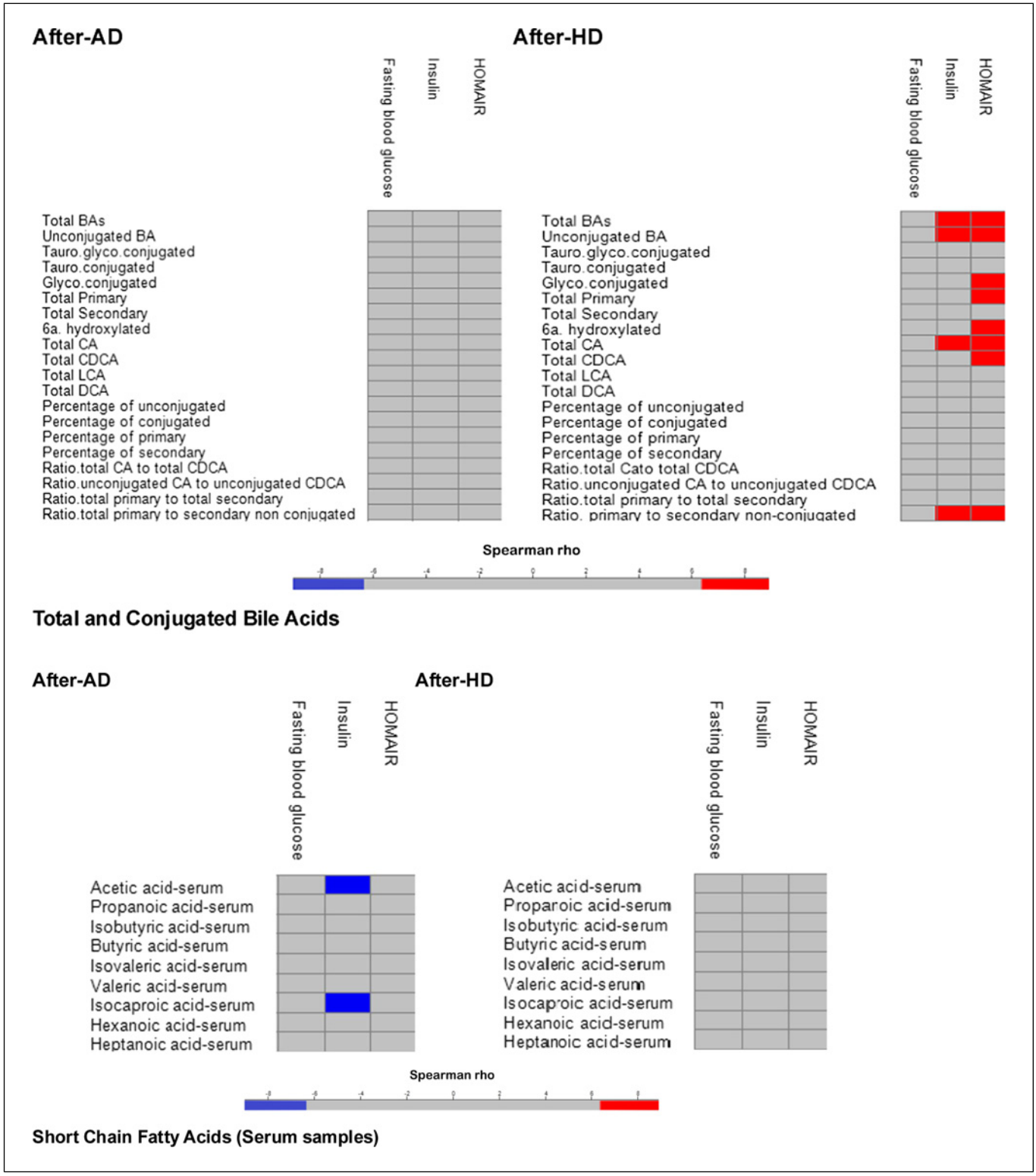


Fig. 5. Correlations between glycemic parameters (fasting blood glucose, fasting insulin, and HOMA-IR) and circulating individual and total BAs, as well as serum SCFA levels after AD and HD consumption. LCA, lithocholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; TLCA, taurolithocholate; TDCA, taurodeoxycholic acid; UDCA, ursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; GUDCA, glycoursodeoxycholic acid; TCA, taurocholic acid; CA, cholic acid; TCDCA, taurochenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CDCA, chenodeoxycholic acid; GCA, glycocholic acid; 7aC4-177, 7α-hydroxy-4-cholesten-3-one 177, a BA precursor.

hemoglobin A1c [40]. Additionally, circulating HCA levels improved serum fasting GLP-1 secretion and glucose homeostasis in prediabetic and newly diagnosed diabetic groups [42]. The present study revealed that the number of primary and secondary BAs that exhibited either positive or negative correlations with glycemic parameters, including fasting blood glucose, insulin, and HOMA-IR, was increased fivefold in response to HD consumption compared to an AD treatment. Increased BAs secretion induced by HD consumption may enhance bile flow from the gallbladder, thereby altering the circulating pool of primary and secondary BAs that activate FXR and TGR5 receptors involved in glucose homeostasis and insulin sensitivity [16]. Thus, these findings suggest that the correlations between *Roseburia,* Verrucomicrobia, or *Flavonifractor* and BAs after dairy intake may influence glucose metabolism.

The abundances of *Roseburia* and *Flavonifractor*, both belonging to the Bacillota phylum [43], were positively associated with propionic acid. Additionally, *Roseburia* showed a positive correlation with butyric acid, whereas *Flavonifractor* was positively correlated with acetic acid after HD intake [44]. *Roseburia* plays a crucial role in maintaining intestinal health by producing SCFAs, particularly acetate, propionate, and butyrate. Butyrate is primarily generated in the colon, where it serves as a major energy source for colonocytes and exerts anti-inflammatory effects by modulating cytokine production in the intestinal mucosa [45]. A smaller fraction of butyrate can translocate into the circulation via the portal vein, where it may further modulate hepatic and systemic immune responses. Studies have identified an interaction between butyrate and immune cell receptors [46]. In addition, propanoic and butyric acids have been associated with enhanced β-cell function and glucose-stimulated insulin secretion that provide protection for the human islets through the direct inhibition of apoptosis induced by inflammatory cytokines [47]. Furthermore, acetic acid, the most abundant SCFA in the colon, serves as an energy source for colonocytes, regulates appetite and insulin sensitivity through PYY and GLP-1 stimulation, and contributes to immune balance via T regulatory cell differentiation [48]. Previous studies have reported positive correlations between *Flavonifractor* and acetic, propionic, and butyric acids. These associations may be explained by its ability to degrade dietary flavonoids, generating metabolites that serve as substrates for other gut microbes involved in SCFA production [44, 49]. In sum, the abundance of *Roseburia* and *Flavonifractor* show a positive correlation with SCFAs in serum after HD intake.

The abundance of *Faecalibacterium* showed a positive correlation with hexanoic acid after HD intake. *Faecalibacterium* is another genus of anaerobic, butyrate-producing bacterium within the Bacillota phylum, primarily represented by *Faecalibacterium prausnitzii* [50]. Hexanoic acid has been found to reduce the expression and activity of fatty acid synthase, an enzyme that is stimulated by insulin and tri-iodothyronine [51]. A recent study analyzing fecal volatile organic compounds and gut microbiota profiles in Alzheimer's disease reported that hexanoic acid abundance increased in patients at advanced stages (GDS-5) and was positively associated with members of the phylum Bacillota, particularly *Ruminococcus* and *Blautia* [52]. However, up to now, no study in the literature has examined the correlation between *Faecalibacterium* and hexanoic acid. Thus, the physiological meaning of the association between *Faecalibacterium* and hexanoic acid after HD intake remains unknown.

Some limitations exist that should be considered when interpreting the results. First, the study is of exploratory nature with a small sample size, therefore, limiting its statistical power to detect the significant differences in correlation analysis. Post hoc power analyses of mean differences in SCFAs and BAs between AD and HD conditions indicated low statistical power with the current sample size (n = 10). Projections suggest that a paired design with approximately 15–30 participants would be required to achieve 80% power for metabolites showing the strongest effects (e.g., GCDCA, %Primary, and %Secondary BAs), while metabolites with smaller effect sizes, including most SCFAs, would require larger sample sizes. Therefore, although our correlation analyses reached moderate power (1-β = 0.694), the study remains underpowered for detecting between-condition differences in metabolite concentrations. Furthermore, reducing inter-individual variability by controlling additional parameters (e.g., fasting status and exclusion of recent antibiotic/probiotic use) would improve statistical power. These measures could lower the required sample size by decreasing variability across individuals. Nevertheless, the results presented in this study can be examined in a larger cohort and in all populations.

In conclusion, HD intake, compared to AD, was associated with distinct relationships between bacterial taxa and circulating metabolites, without altering overall metabolite concentrations in individuals at risk for T2D. Further research is needed to determine whether HD consumption confers benefits through gut-derived metabolites entering the bloodstream, potentially improving inflammatory status or glycemic homeostasis in those at higher risk of T2D.

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Statement of Ethics

The study was approved by the Ethics Committee at the CHU de Québec-Université Laval Research Center (2017–3228 and 2022–6092) and adhered to the ethical principles outlined in the Declaration of Helsinki. Thorough information about the trial's goals and procedures was communicated to all patients, who were then presented with consent forms that they signed. Additionally, the trial was officially registered on ClinicalTrials.gov (accessed on 24 September 2023) with the identifier NCT02961179.

Conflict of Interest Statement

Iwona Rudkowska was a member of the journal's Editorial Board at the time of submission.

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Author Contributions

Conceptualization and methodology: M.L. and I.R.; laboratory analyses: K.G. and J.F.B.; bile analysis: O.B. and J.T.; resources: O.B., M.L., and I.R.; data analysis: A.M., M.L., and I.R.; writing – original draft preparation: A.M., M.L., and I.R.; all authors were involved in reviewing and editing the manuscript; M. Lebel and I.R. were responsible for supervision and funding acquisition.

Data Availability Statement

The data that support the findings of this study are not publicly available due to confidentiality and privacy; the data involve sensitive information about individuals, such as medical or personal data; it is crucial to protect the privacy of participants. Additional inquiries can be contacting to the corresponding authors: iwona.rudkowska@crchudequebec.ulaval.ca, michel.lebel@crchudequebec.ulaval.ca.

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