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Associations between fecal bile acids, neutral sterols, and serum lipids in the KORA FF4 study

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HIGHLIGHTS

- Fecal bile acids are positively associated with serum lipids and dyslipidemia.
- Associations are most consistent with serum triglycerides or hypertriglyceridemia.
- Elevated fecal bile acids may be a response to the development of dyslipidemia.

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ABSTRACT

Background and aims: Dyslipidemia is a major risk factor for cardiovascular disease, the leading cause of preventable death worldwide. As a result, a full understanding of the factors influencing dyslipidemia is urgently necessary. Bile acids have been recognized as regulators of lipid metabolism, and neutral sterols may influence serum lipid levels. Therefore, this analysis was conducted to better understand the relationship between bile acids, neutral sterols, and dyslipidemia.
Methods: We examined cross-sectional associations between selected fecal metabolites and serum lipids or markers of dyslipidemia in 1387 participants of the KORA FF4 study using linear and logistic regression models.
Results: We found positive associations between fecal bile acids and serum high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-c), total cholesterol, triglycerides and markers of dyslipidemia, though associations were seen most consistently with triglycerides and hypertriglyceridemia. We also found positive associations between fecal cholesterol and serum LDL-c, total cholesterol, triglycerides, hypertriglyceridemia and high serum total cholesterol, though only associations with triglycerides or hypertriglyceridemia remained significant after applying the Bonferroni correction. Unexpectedly, several fecal plant sterols were positively associated with serum lipids and the associated markers of dyslipidemia. However, many of these associations were no longer statistically significant after adjusting for multiple testing.
Conclusions: Our results provide insight into the role that bile acids may play in the development or progression of dyslipidemia. However, further confirmation of these results is warranted. Longitudinal and experimental studies are necessary to clarify the mechanisms behind these associations and to determine causality.

Abbreviations: CAD, coronary artery disease; CVD, cardiovascular disease; ESI, electrospray ionization; FXR, farnesoid X receptor; HDL-c, high-density lipoprotein cholesterol; HILIC, hydrophilic interaction liquid chromatography; KORA, Cooperative Health Research in the Region of Augsburg; LDL-c, low-density lipoprotein cholesterol; UPLC-MS/MS, ultra high-performance liquid chromatography/tandem accurate mass spectrometry; VLDL, very low density lipoprotein

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1. Introduction

Cardiovascular disease (CVD) is the leading cause of preventable death worldwide, with an estimated 17.9 million deaths attributed to CVD worldwide in 2015 alone [1,2]. Dyslipidemia, characterized by high serum low density lipoprotein cholesterol (LDL-c), total cholesterol, or triglycerides, or low serum high density lipoprotein cholesterol (HDL-c), is a major risk factor for CVD [3]. Substantial evidence demonstrates that maintaining optimal serum lipid levels, especially in regards to LDL-c, reduces the risk of CVD and cardiovascular events [4]. As a result, the prevention and treatment of dyslipidemia is of major importance.

It has recently been recognized that bile acids are not only necessary for the digestion of lipids, but are also important regulators of lipid metabolism [5]. Through the activation of farnesoid X receptor (FXR) in the liver and intestine, bile acids can lead to decreased very low density lipoprotein (VLDL) and HDL-c levels, increased LDL-c levels, decreased bile acid absorption and increased bile acid secretion and conjugation [6]. The conversion of cholesterol to bile acids themselves is also a major source of elimination of excess cholesterol from the body [7], and several lipid-lowering medications reduce serum lipid levels by wasting cholesterol either through increasing cholesterol or bile acid waste, or inhibiting cholesterol synthesis [8].

Plant and animal sterols (subsequently referred to collectively as neutral sterols) have also been recognized as having an impact on serum lipids levels. Cholesterol, the major animal sterol, is closely intertwined with the pathogenesis of dyslipidemia and CVD as a major atherogenic component of blood [4,9]. Conversely, the cholesterol-lowering effect of phytosterols has been well demonstrated in the literature, an effect which is achieved primarily by competitive inhibition of the absorption of cholesterol in the intestine [10].

Both bile acids and neutral sterols likely play a role in the development of dyslipidemia, but these relationships have mainly been examined in select populations and remain poorly understood. The purpose of this analysis is to examine the cross-sectional association between fecal metabolites, serum lipids and markers of dyslipidemia in a large, population-based cohort.

2. Patients and methods

2.1. Study population

The present analysis was carried out in participants of the Cooperative Health Research in the Region of Augsburg (KORA) FF4 study. Conducted between 2013 and 2014, KORA FF4 is the second follow-up survey of KORA S4 (1999–2001), one of four population-based health surveys (S1–S4) conducted in the southern German city of Augsburg and its two surrounding counties. KORA FF4 included 2279 participants aged 38–88. Fecal metabolite data were available for a subsample of 1410 individuals, selected based on stool sample quality and storage conditions. For the analysis, additional participants were excluded if they had missing covariate data (n = 11) or were not fasted before the blood draw (n = 7). Another five participants who reported taking antibiotics in the previous four weeks during the interview, but who did not report this on the stool sample collection questionnaire were also excluded from the analysis.

The final study population included in the present analysis consisted of 1387 individuals. This study was conducted in accordance with the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the Bavarian Chamber of Physicians in Munich. All participants gave their written, informed consent.

2.2. Collection and processing of biosamples

Blood samples were obtained on the study visit day by trained examiners according to standardized procedures. HDL-c, LDL-c, total cholesterol and triglycerides were measured in serum using enzymatic, colorimetric methods from either Siemens (Siemens Healthcare Diagnostics Inc., Newark, USA) or Cobas (Roche Diagnostics GmbH, Mannheim, Germany), as the assays and instruments were changed during the study. A calibration between the two methods was performed using 122 samples from KORA FF4. In these participants, measurements were made with both instruments, and an appropriate formula was developed to calibrate the Roche measurements to the Siemens measurements. Further detail on the calibration process has been given elsewhere [11]. The samples measured by Siemens were assessed using LDLC, HDLC, CHOL, and TRIG Flex reagent cartridge assays on a Dimension Vista 1500 instrument. The samples measured by Roche were assessed using LDL_C, HDLC3, CHOL2 and TRIGL assays on a Cobas c 701/702 instrument. Continuous serum lipid levels were given in mmol/l. Cut-off levels for binary serum lipid variables were classified based on the 2003 National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III Guidelines as follows: HDL-c $<$ 1.03 mmol/l in men and $<$ 1.29 mmol/l in women, LDL-c $\geq$ 3.36 mmol/l, total cholesterol $\geq$ 5.17 mmol/l, and triglycerides $\geq$ 1.69 mmol/l [12]. Conservative cut-offs were chosen because they are used in this analysis for statistical purposes only, rather than for therapeutic decision-making.

Stool samples were collected at home. Participants received a stool collection kit by mail several days before the study center visit and were instructed on how to collect a hygienic and uncontaminated sample using gloves, a toilet bowl cover, and the stool collector. A questionnaire regarding details about the sample and collection process was provided. The stool sample was to be taken on the day of their visit to the study center if possible. A spoonful from two different areas of the stool specimen was filled into a collection tube and kept refrigerated inside a sealed bag until transport to the study center. Samples exposed to room temperature for a period of 3 h or more, or from participants who took antibiotics within the previous two months or who did not follow instructions were excluded. Stool samples were stored at −80 °C.

The frozen samples (weight between 136 and 143 mg) were placed into pre-cooled 2 ml homogenization tubes (Precellys Ceramic Kit 1.4 mm, 50 × 2,0 ml tubes, Peqlab) and diluted to a concentration of 12.5 µl/mg sample with pre-cooled water. Samples were homogenized in a Precellys 24 homogenizer (PEQLAB Biotechnology GmbH, Germany), equipped with a cooling unit 3 times for 20 s at 6500 rpm, with 15 s intervals between homogenization steps. An aliquot of 450 µl was transferred to a 0.5 ml Eppendorf tube and used for dry mass determination, and a 100 µl aliquot of the homogenate was pipetted onto a 2 ml 96-deep well plate. Additionally, six wells were filled with a human stool reference (Seralab, West Sussex, UK), and one well was filled with a human plasma reference (Seralab, West Sussex, UK), which were used to evaluate process variability. A further six wells were filled with water as process blanks. The samples in the deep well plate were extracted with 475 µl methanol, containing four recovery standards to monitor the extraction efficiency. After centrifugation, the supernatant was split into 4 aliquots in two 96-well microplates.

Sample extracts were dried on a TurboVap 96 (Zymark) and reconstituted with 80 µl of solvent. The solvent was compatible with each of the 4 methods and also contained internal standards which additionally served as retention reference markers. Two of the four aliquots were analyzed by reverse phase ultra high-performance liquid chromatography/tandem accurate mass spectrometry (RP/UPLC-MS/MS) with positive ion mode electrospray ionization (ESI). The first was optimized for hydrophilic compounds, and was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1 × 100 mm, 1.7 µm) with water and methanol containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). The second was optimized for hydrophobic compounds. It was eluted with water, methanol, acetonitrile, 0.05% PFPA, and 0.01% FA. The third aliquot was analyzed by RP/UPLC-MS/MS with negative ion mode ESI, and was eluted from the column using

water and methanol with 6.5 mM Ammonium Bicarbonate at a pH of 8. The final aliquot was analyzed by hydrophilic interaction liquid chromatography (HILIC)/UPLC-MS/MS with negative ion mode ESI. It was eluted from a HILIC column (Waters UPLC BEH Amide 2.1 × 150 mm, 1.7 μm) with water and acetonitrile with 10 mM ammonium formate at a pH of 10.8.

Liquid handling was performed on an automated MicroLab STAR® robot (Hamilton Bonaduz AG, Bonaduz, Switzerland). All metabolite analysis was performed using a Waters ACQUITY UPLC and Thermo Scientific Q-Exactive high-resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer (35,000 mass resolution). The analysis alternated between MS and data-dependent MSn scans using dynamic exclusion, with a scan range between 80 and 1000 *m/z*. The metabolite concentrations were extracted and profiled by Metabolon, Inc (Durham, NC, USA).

The metabolites were compared against Metabolon's biochemical reference library for identification based on retention index, mass (± 10 ppm), and MS/MS forward and reverse scores. A total of 1262 metabolites were measured, 1140 of which could be identified. Of these metabolites, 248 were excluded due to a coefficient of variation over run days of more than 0.25. A further 85 metabolites were excluded due to missing coefficient of variation values. Two samples were classified as outliers due to a metabolite mean that was greater than four times the standard deviation of the metabolite. Samples were also corrected for sample weight.

2.3. Assessment of covariables

Potential confounders were selected based on existing literature on fecal metabolites and serum lipids or dyslipidemia [13–15]. Age (years), sex (M/F), waist circumference (cm), physical activity (active ≥ 1 h per week in summer and winter/inactive), smoking (current/ex-/never smoker), lipid-lowering medication use (yes/no), alcohol intake (self-reported, g/d) and education (< 13 years and ≥13 years) were chosen as covariables for the present analysis. Waist circumference was measured at the study center in standardized fashion by trained examiners. Physical activity, smoking status, alcohol intake, medication use, and education level were assessed during a face-to-face interview conducted by trained investigators. More details about the assessment of the variables has been outlined previously [11,16,17].

2.4. Statistical analysis

Primary and secondary bile acids, as well as plant and animal sterols were selected from the total set of 807 available metabolites. This subset of metabolites was selected based on existing literature regarding bile acids, neutral sterols, and serum lipids [6,10]. Any of the selected metabolites with more than 25% of observations missing were excluded from the analysis (n = 5). Remaining missing values were imputed with the minimum observed value for each metabolite, as it was determined that values were most likely missing due to metabolite concentrations below the limit of detection rather than due to technical errors or random missing. The sums of the primary bile acids, secondary bile acids, plant sterols, and animal sterols were also calculated and included as variables in the analysis. Metabolites were log transformed prior to analysis, as the distributions were heavily skewed.

The participants' baseline characteristics were described according to sex and were given as median and 25%, 75% or percentage and number. Significant differences in each characteristic between men and women were evaluated using the Mann-Whitney *U* test for continuous variables and the Chi-squared test for categorical variables. The median and 25%, 75% percentiles, minimum and maximum values, and number of missing values before imputation were calculated for each metabolite. A linear regression model was fitted for each metabolite and each of the four continuous outcome variables (serum HDL-c, LDL-c, total cholesterol and triglycerides) in order to evaluate associations between fecal metabolites and serum lipid levels. A logistic regression model was fitted for each metabolite and each of the four binary outcome variables (low HDL-c, high LDL-c, high total cholesterol and high triglycerides) in order to examine relationships between fecal metabolites and dyslipidemia. All models were adjusted for age, sex, education, smoking, physical activity, waist circumference, alcohol intake and use of lipid-lowering medication. Results of the regression models were

Table 1
Characteristics of the study population by sex.

	Men (n = 703)		Women (n = 684)		p^a
Continuous characteristics	Median	25%, 75%	Median	25%, 75%	
Age (years)	60	49, 70	59	49, 69	0.692
BMI (kg/m^2)	27.32	25.21, 30.53	26.58	23.67, 30.39	< 0.001
Waist circumference (cm)	101.20	94.1, 108.9	90.65	80.30, 100.5	< 0.001
HDL-c (mmol/l)	1.45	1.21, 1.76	1.85	1.55, 2.17	< 0.001
LDL-c (mmol/l)	3.44	2.87, 4.07	3.41	2.79, 4.08	0.588
Total cholesterol (mmol/l)	5.45	4.81, 6.05	5.62	4.99, 6.41	< 0.001
Triglycerides (mmol/l)	1.29	0.93, 1.81	1.10	0.83, 1.44	< 0.001
Alcohol (g/d)	15.86	2.86, 34.29	2.86	0, 13.92	< 0.001
Categorical characteristics	%	n	%	n	p^a
Education (≥13 y)	41.4	291	30.4	208	< 0.001
Physical activity (active)	56.3	396	61.0	417	0.090
Smoking (current smoker)	17.8	125	13.6	93	< 0.001
Smoking (ex-smoker)	42.1	296	29.4	201	.
Smoking (never smoker)	40.1	282	57.0	390	.
HDL-c < 1.03 mmol/l in men, < 1.29 mmol/l in women	31.4	221	10.2	70	< 0.001
LDL-c ≥ 3.36 mmol/l	54.5	383	52.9	362	0.598
Total cholesterol ≥5.17 mmol/l	62.0	436	68.3	467	0.017
Triglycerides ≥1.69 mmol/l	28.6	201	16.1	110	< 0.001
Elevated LDL-c and triglycerides	16.8	118	11.1	76	0.003
Elevated LDL-c and triglycerides, low HDL-c	9.2	65	4.2	29	< 0.001
Lipid-lowering medication (yes)	18.5	130	14.2	97	0.036

LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.
[a] *p*-values for differences between men and women calculated using Man-Whitney *U* test for continuous variables and Chi-squared test for categorical variables.

Table 2
Descriptive statistics for selected fecal metabolite concentrations (area counts) from the KORA FF4 study population (n = 1387), untransformed and before imputation.

Metabolite	Median	25%, 75%	Min.	Max.	NA
Primary bile acids					
Cholate	0.051	0.01, 0.213	0.0002	64.810	42
Glycochenodeoxycholate	0.052	0.024, 0.129	0.0026	12.835	83
Glycocholate	0.052	0.021, 0.152	0.0015	13.097	14
Secondary bile acids					
12-dehydrocholate	0.053	0.017, 0.234	0.0015	61.283	240
3b-hydroxy-5-cholenoic acid	0.053	0.031, 0.090	0.0009	1.066	169
6-oxolithocholate	0.052	0.030, 0.090	0.0015	0.739	207
7,12-diketolithocholate	0.050	0.023, 0.134	0.0014	23.395	291
7-ketodeoxycholate	0.054	0.019, 0.226	0.0006	53.246	147
Dehydrolithocholate	0.051	0.024, 0.092	0.0003	0.465	18
Deoxycholate	0.053	0.020, 0.111	0.0002	1.809	25
Glycodeoxycholate	0.052	0.022, 0.202	0.0007	11.315	122
Glycolithocholate sulfate	0.053	0.021, 0.153	0.0015	20.000	97
Glycoursodeoxycholate	0.056	0.025, 0.134	0.0030	6.540	252
Hyocholate	0.052	0.028, 0.106	0.0012	2.646	129
Isoursodeoxycholate	0.051	0.023, 0.138	0.0006	8.105	6
Lithocholate	0.051	0.031, 0.083	0.0010	0.723	14
Ursocholate	0.052	0.020, 0.201	0.0021	54.447	8
Ursodeoxycholate	0.049	0.022, 0.127	0.0008	6.654	22
Plant sterols					
Stigmasterol	0.051	0.033, 0.078	0.0023	0.663	63
Sitostanol	0.052	0.029, 0.079	0.0006	0.646	105
Beta-sitosterol	0.052	0.030, 0.103	0.0015	0.932	4
Ergosterol	0.055	0.025, 0.119	0.0011	13.529	59
Campesterol	0.051	0.028, 0.104	0.0012	1.139	16
Animal sterols					
Coprostanol	0.053	0.030, 0.085	0.0005	0.530	65
Cholesterol	0.055	0.025, 0.143	0.0023	1.507	3

NA, number of missing values.

additionally adjusted for multiple testing using the Bonferroni correction ($0.05/29 = p < 0.0017$). All analyses were performed using RStudio version 1.1.447 with R version 3.5.0.

3. Results

The 1387 participants included in the present analysis were 50.7% male (n = 703) and 49.3% female (n = 684). The characteristics of the study population according to sex are presented in Table 1.

The characteristics of the fecal metabolites included in this analysis are displayed in Table 2. The metabolites ranged from 0.2% to 21.0% missing values before imputation.

Linear associations between the selected fecal metabolites and serum lipid levels are presented in Table 3, while risk estimates for dyslipidemia based on fecal metabolite concentrations are listed in Table 4. The primary bile acids as well as their sum were positively associated with serum triglycerides (Table 3) and hypertriglyceridemia (Table 4). Nearly all primary bile acids and their sum were positively associated with serum total cholesterol and elevated total serum cholesterol (Tables 3 and 4, respectively). None of the primary bile acids were associated with serum HDL-c or low HDL-c levels, and only fecal cholate and glycocholate concentrations were positively associated with serum LDL-c (Table 3) and having elevated serum LDL-c (Table 4). Mainly the associations between primary bile acids and serum triglycerides or hypertriglyceridemia remained significant after correction with the Bonferroni method.

Most secondary bile acids, as well as their sum, were positively associated with both serum triglycerides (Table 3) and hypertriglyceridemia (Table 4), and nearly all of these associations remained statistically significant after adjusting for multiple testing. Eight of 15 secondary bile acids were significantly inversely associated with serum HDL-c, and one additional secondary bile acid was positively associated with having low HDL-c levels. Only one of these associations remained significant after adjustment for multiple testing. Five of the secondary bile acids were significantly positively associated with serum LDL-c levels, while only three of these were significantly associated with having elevated LDL-c levels. Only two of these associations remained significant after correction with the Bonferroni method. Similar results were seen for total serum cholesterol levels (Tables 3 and 4).

Fecal plant sterol concentrations were not significantly associated with serum HDL-c levels or with having low HDL-c. Sitostanol was inversely associated with serum triglycerides and hypertriglyceridemia. However, several other plant sterols were positively associated with serum LDL-c, total cholesterol, or triglycerides, or the respective markers of dyslipidemia. Most associations with plant sterols were no longer significant after adjusting for multiple testing. Fecal cholesterol was positively associated with serum LDL-c, total cholesterol, serum triglycerides and high total cholesterol and hypertriglyceridemia. Coprostanol was inversely associated with serum triglycerides and hypertriglyceridemia, although the association with hypertriglyceridemia was no longer significant after correction for multiple testing.

4. Discussion

4.1. Fecal bile acids and serum lipids

The significant positive associations seen in this study between fecal bile acids and serum lipid levels and/or dyslipidemia are consistent with the results of a study conducted by Briones et al., which reported increased excretion of bile acids in both diabetic and non-diabetic participants with hypertriglyceridemia [18].

Conversely, a number of studies have found lower fecal bile acid levels or reduced bile acid production in patients with coronary artery disease (CAD) than in people without CAD, leading to the hypothesis that impaired excretion of excess cholesterol as bile acids may be a factor in the development of CAD [15,19–22]. In contrast, we found positive associations between fecal bile acids and serum LDL-c, total cholesterol, triglycerides, or markers of dyslipidemia. A further study by Harchaoui et al. found lower bile acid excretion in subjects with familial hypoalphalipoproteinemia [23]; however, we found a positive association between two bile acids and low HDL-c levels, and inverse associations between several secondary bile acids and serum HDL-c levels. This discrepancy may be due to the fact that our study sample was selected from the general population rather than participants with a genetic predisposition, or that we evaluated associations with dyslipidemia or serum lipids rather than CAD. It is possible that impaired bile acid excretion is a factor in whether patients with dyslipidemia progress to develop CAD or not. Indeed, while Charach and colleagues (2011 and 2018) found that bile acid excretion was decreased in patients with CAD, a positive association between bile acid excretion and plasma triglycerides in non-CAD patients was seen [19,20].

Bile acids are known to regulate lipid metabolism, mainly through their role as agonists of FXR [6,24]. When activated by high bile acid concentrations, FXR in the intestine raises LDL-c levels by increasing cholesterol absorption. FXR activation in the liver also increases plasma LDL-c by suppressing the conversion of cholesterol to bile acids, leading to decreased LDL receptor activity and increased hepatic cholesterol levels. FXR also increases HDL-c clearance, leading to lowered plasma HDL-c levels. Additionally, the production of triglyceride-rich VLDL is decreased and lipoprotein lipase activity is increased with FXR activation. Both intestinal and hepatic FXR inhibit lipogenesis and decrease levels of lipoprotein (a), a pro-atherogenic lipoprotein, and hepatic FXR may simultaneously increase plasma LDL-c while decreasing plasma triglycerides. FXR also regulates a number of genes involved in triglyceride metabolism [6,24]. The associations seen in this study between bile acid levels and plasma lipids could potentially be the result of these actions of FXR, making this receptor a promising target for the treatment of metabolic diseases/abnormalities. Indeed, medications such as FXR-agonists and bile acid sequestrants are already under development

Table 3
Fully adjusted associations[a] between fecal metabolite concentrations (area counts) and serum HDL-c, LDL-c, total cholesterol, and triglyceride levels (n = 1387).

Metabolite	Serum HDL-c (mmol/l)			Serum LDL-c (mmol/l)			Serum total cholesterol (mmol/l)			Serum triglycerides (mmol/l)		
	Est.	SE	*p*	Est.	SE	*p*	Est.	SE	*p*	Est.	SE	*p*
Primary bile acids												
SUM	−0.006	0.006	0.298	0.019	0.013	0.140	**0.030**	**0.014**	**0.037**	**0.047**	**0.011**	**0.000***
Cholate	−0.007	0.005	0.168	**0.022**	**0.010**	**0.035**	**0.032**	**0.012**	**0.006**	**0.058**	**0.009**	**0.000***
Glycochenodeoxycholate	−0.005	0.007	0.520	0.023	0.015	0.142	**0.039**	**0.017**	**0.024**	**0.056**	**0.014**	**0.000***
Glycocholate	−0.003	0.007	0.603	**0.039**	**0.014**	**0.005**	**0.053**	**0.016**	**0.001***	**0.054**	**0.013**	**0.000***
Secondary bile acids												
SUM	−0.010	0.008	0.198	0.024	0.017	0.154	0.033	0.019	0.076	**0.051**	**0.015**	**0.001***
12-dehydrocholate	**−0.009**	**0.005**	**0.046**	0.013	0.010	0.169	0.019	0.011	0.080	**0.044**	**0.009**	**0.000***
3b-hydroxy-5-cholenoic acid	−0.009	0.007	0.166	−0.003	0.015	0.831	−0.003	0.016	0.861	0.020	0.013	0.139
6-oxolithocholate	−0.006	0.007	0.397	0.026	0.015	0.092	0.017	0.017	0.315	−0.020	0.014	0.149
7,12-diketolithocholate	**−0.011**	**0.005**	**0.043**	0.009	0.012	0.420	0.012	0.013	0.360	**0.049**	**0.010**	**0.000***
7-ketodeoxycholate	**−0.011**	**0.005**	**0.014**	0.028	0.010	**0.004**	**0.032**	**0.011**	**0.004**	**0.057**	**0.009**	**0.000***
Dehydrolithocholate	−0.006	0.009	0.530	0.000	0.019	0.990	−0.016	0.021	0.443	−0.026	0.017	0.133
Deoxycholate	−0.014	0.007	0.059	−0.003	0.016	0.870	0.000	0.017	0.980	**0.050**	**0.014**	**0.000***
Glycodeoxycholate	0.000	0.006	0.946	−0.001	0.012	0.956	0.012	0.014	0.395	**0.040**	**0.011**	**0.000***
Glycolithocholate sulfate	−0.004	0.006	0.492	0.022	0.013	0.106	**0.036**	**0.015**	**0.017**	**0.057**	**0.012**	**0.000***
Glycoursodeoxycholate	**−0.013**	**0.006**	**0.040**	0.053	0.014	**0.000***	**0.066**	**0.015**	**0.000***	**0.073**	**0.012**	**0.000***
Hyocholate	−0.008	0.007	0.225	0.038	0.015	**0.011**	**0.044**	**0.017**	**0.009**	**0.055**	**0.013**	**0.000***
Isoursodeoxycholate	**−0.024**	**0.008**	**0.002**	0.037	0.016	**0.022**	**0.040**	**0.018**	**0.028**	**0.080**	**0.015**	**0.000***
Lithocholate	**−0.025**	**0.012**	**0.035**	0.010	0.026	0.711	−0.009	0.029	0.741	0.034	0.023	0.144
Ursocholate	**−0.020**	**0.006**	**0.002**	0.034	0.013	**0.010**	**0.038**	**0.015**	**0.011**	**0.076**	**0.012**	**0.000***
Ursodeoxycholate	**−0.026**	**0.007**	**0.000***	0.017	0.015	0.271	0.021	0.017	0.219	**0.085**	**0.014**	**0.000***
Plant sterols												
SUM	0.002	0.011	0.821	**0.061**	**0.023**	**0.009**	**0.057**	**0.026**	**0.029**	−0.027	0.021	0.191
Stigmasterol	−0.002	0.011	0.888	0.043	0.024	0.076	0.052	0.027	0.056	0.039	0.022	0.077
Sitostanol	0.003	0.007	0.717	0.006	0.016	0.697	−0.016	0.018	0.371	**−0.085**	**0.014**	**0.000***
Beta-sitosterol	−0.006	0.012	0.602	**0.078**	**0.026**	**0.002**	**0.100**	**0.029**	**0.001***	**0.084**	**0.023**	**0.000***
Ergosterol	0.001	0.007	0.891	**0.045**	**0.016**	**0.005**	**0.052**	**0.018**	**0.004**	0.025	0.014	0.081
Campesterol	−0.005	0.011	0.619	**0.061**	**0.023**	**0.008**	**0.078**	**0.026**	**0.003**	**0.071**	**0.021**	**0.001***
Animal sterols												
SUM	−0.017	0.010	0.096	0.030	0.022	0.173	0.013	0.024	0.600	−0.020	0.020	0.318
Coprostanol	−0.007	0.008	0.395	0.008	0.017	0.656	−0.022	0.019	0.254	**−0.079**	**0.015**	**0.000***
Cholesterol	−0.014	0.009	0.124	**0.048**	**0.020**	**0.016**	**0.060**	**0.022**	**0.007**	**0.079**	**0.018**	**0.000***

LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.
[a] Adjusted for age, sex, education, smoking, physical activity, waist circumference, alcohol intake, and lipid-lowering medication use. $p < 0.05$ in bold.
*Statistically significant after adjustment with Bonferroni correction ($p < 0.0017$).

or in use [24]. However, as FXR activation has both metabolically positive and negative effects, it is clear that further studies are required both to fully understand the effects of the interplay between bile acids and lipid metabolism, as well as to determine if FXR activation or suppression is desirable for the treatment of metabolic diseases [6].

Another potential mediator of associations between bile acids and serum lipid levels is the gut microbiome. The gut microbiome plays an important role in bile acid metabolism by converting primary bile acids into numerous secondary bile acids, as well as other compounds that may act as signaling molecules [25,26]. Bile acids in turn modulate the composition of the gut microbiome [27,28]. Indeed, several studies have shown the ability of bile-modifying gut bacteria to modulate lipid metabolism, mainly by increasing bile acid excretion and therefore production [26]. Additionally, probiotics, including strains known to modify bile acids, have been demonstrated to improve lipid profiles in patients with hypercholesterolemia, hypertriglyceridemia, and in the critically ill [29–32]. Another recent study including 893 participants investigated associations between bacterial taxa and serum lipid levels, among other outcomes, and determined that the gut microbiome may be responsible for approximately 5% of variation in serum triglycerides and HDL-c levels [33]. However, longitudinal and intervention studies will be necessary to further examine to what extent the microbiome affects bile acid and lipid metabolism, and whether it may be another viable target for the modulation of cardiovascular and/or metabolic disease.

4.2. Fecal neutral sterols and serum lipids

After adjustment for multiple testing, positive associations between fecal cholesterol and serum triglycerides or hypertriglyceridemia remained statistically significant. This may again reflect a high excretion of cholesterol in the feces in response to high serum lipid levels. This is also the strategy of several classes of lipid-lowering medications, which seek to waste cholesterol through mechanisms such as decreased absorption or increased excretion in the form of bile acids [8,34]. Our results are consistent with the findings of Briones et al., who found increased excretion of fecal sterols and bile acids in patients with hypertriglyceridemia, and which is consistent with our findings regarding fecal bile acids as well [18].

Also consistent with the literature related to bile acids, Rajaratnam et al. reported an inverse relationship between fecal sterol excretion and CAD, and Harchaoui et al. found reduced fecal steroid excretion in persons with familial hypoalphalipoproteinemia [23]. In comparison, we did not see significant associations between fecal steroids and serum

Table 4
Fully adjusted associations[a] between fecal metabolite concentrations (area counts) and markers of dyslipidemia (n = 1387).

Metabolite	Low serum HDL-c		High serum LDL-c		High serum total cholesterol		High serum triglycerides	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Primary bile acids								
SUM	0.99	0.91–1.07	1.05	0.98–1.11	1.06	1.00–1.14	**1.14**	**1.06–1.22***
Cholate	0.97	0.91–1.03	1.05	1.00–1.11	**1.06**	**1.01–1.12**	**1.15**	**1.08–1.22***
Glycochenodeoxycholate	1.01	0.91–1.11	1.04	0.97–1.13	1.07	0.99–1.16	**1.15**	**1.05–1.25***
Glycocholate	1.02	0.93–1.11	**1.08**	**1.01–1.16**	**1.12**	**1.04–1.20**	**1.17**	**1.08–1.27***
Secondary bile acids								
SUM	1.04	0.93–1.14	1.04	0.95–1.13	1.06	0.97–1.15	**1.14**	**1.04–1.25**
12-dehydrocholate	0.96	0.90–1.02	1.02	0.97–1.07	1.02	0.97–1.08	**1.10**	**1.04–1.16***
3b-hydroxy-5-cholenoic acid	**1.10**	**1.00–1.21**	0.98	0.92–1.06	0.99	0.92–1.07	1.03	0.95–1.12
6-oxolithocholate	1.00	0.91–1.11	1.07	0.99–1.15	1.02	0.94–1.10	0.97	0.89–1.06
7,12-diketolithocholate	1.04	0.96–1.11	1.00	0.94–1.06	0.98	0.92–1.04	**1.16**	**1.08–1.24***
7-ketodeoxycholate	1.00	0.94–1.06	**1.05**	**1.00–1.11**	**1.05**	**1.00–1.11**	**1.15**	**1.08–1.21***
Dehydrolithocholate	1.12	1.00–1.27	0.99	0.90–1.09	0.92	0.83–1.01	1.02	0.92–1.14
Deoxycholate	1.02	0.93–1.13	0.98	0.91–1.06	1.01	0.93–1.09	**1.26**	**1.14–1.40***
Glycodeoxycholate	1.01	0.93–1.09	0.98	0.93–1.05	1.03	0.97–1.10	**1.13**	**1.05–1.22***
Glycolithocholate sulfate	1.01	0.93–1.09	1.02	0.95–1.09	1.07	1.00–1.15	**1.14**	**1.06–1.24***
Glycoursodeoxycholate	1.05	0.96–1.14	**1.13**	**1.05–1.21***	**1.14**	**1.06–1.22***	**1.21**	**1.12–1.31***
Hyocholate	1.05	0.96–1.16	1.07	0.99–1.15	**1.09**	**1.01–1.18**	**1.16**	**1.06–1.27***
Isoursodeoxycholate	1.08	0.97–1.19	1.08	0.99–1.17	**1.10**	**1.01–1.19**	**1.24**	**1.13–1.36***
Lithocholate	1.17	0.99–1.38	1.01	0.89–1.15	0.96	0.84–1.09	**1.28**	**1.09–1.50***
Ursocholate	1.02	0.94–1.10	**1.08**	**1.01–1.16**	**1.10**	**1.03–1.19**	**1.20**	**1.12–1.29***
Ursodeoxycholate	1.08	0.98–1.19	1.02	0.95–1.10	1.06	0.98–1.15	**1.30**	**1.19–1.43***
Plant sterols								
SUM	0.99	0.86–1.14	1.11	0.99–1.24	1.11	0.98–1.25	0.92	0.80–1.05
Stigmasterol	0.98	0.84–1.14	1.04	0.92–1.17	1.12	0.99–1.27	0.99	0.86–1.14
Sitostanol	1.00	0.91–1.11	1.02	0.94–1.10	0.97	0.89–1.05	**0.86**	**0.79–0.93***
Beta-sitosterol	1.07	0.91–1.25	1.11	0.98–1.26	**1.20**	**1.05–1.37**	**1.19**	**1.03–1.38**
Ergosterol	1.03	0.93–1.14	1.06	0.98–1.15	1.08	0.99–1.17	1.05	0.96–1.15
Campesterol	1.03	0.89–1.19	**1.12**	**1.00–1.26**	**1.20**	**1.06–1.35**	**1.19**	**1.04–1.36**
Animal sterols								
SUM	1.09	0.95–1.25	1.06	0.96–1.18	0.99	0.89–1.11	1.00	0.89–1.13
Coprostanol	1.02	0.92–1.13	1.03	0.95–1.12	0.94	0.86–1.03	**0.89**	**0.81–0.98**
Cholesterol	1.08	0.96–1.22	1.10	0.99–1.21	**1.13**	**1.02–1.25**	**1.21**	**1.08–1.35***

LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.
[a]Adjusted for age, sex, education, smoking, physical activity, waist circumference, alcohol intake, and lipid-lowering medication use. $p < 0.05$ in bold.
* Statistically significant after adjustment with Bonferroni correction ($p < 0.0017$).

HDL-c. Again, we hypothesize that this discrepancy may be a result of examining a population with CAD versus a sample from the general population.

Coprostanol was significantly inversely related to triglycerides and hypertriglyceridemia in our study population. However, the association with hypertriglyceridemia was no longer significant after the Bonferroni correction. As coprostanol is produced from cholesterol via bacterial activity, it is possible that interpersonal variations in coprostanol-producing bacteria are a reason for the contrasting association between fecal cholesterol vs. coprostanol and serum lipids. It has been demonstrated that there is high interpersonal variation in cholesterol-to-coprostanol conversion rates [35,36]. However, the sum of cholesterol and coprostanol was not significantly associated with serum lipids or any marker of dyslipidemia.

Our results regarding plant sterols and dyslipidemia were unexpected. We found mostly positive associations between fecal plant sterol concentrations and serum LDL-c, total cholesterol, and triglycerides. Several plant sterols were also positively associated with having elevated LDL-c, elevated total cholesterol, and hypertriglyceridemia; however, many of these associations did not remain statistically significant after correction for multiple testing. Only sitostanol was inversely associated with hypertriglyceridemia and serum triglycerides, and this association did remain significant after the Bonferroni correction. The lipid-lowering effects of phytosterols have been well-documented in the literature, though these effects are typically only seen at phytosterol intakes of 1.5–2 g/d [37,38]. Phytosterol intake is not available in KORA FF4, but it is reasonable to assume an average intake of between 200 and 400 mg/day, which is typical for a Western diet [39,40]. As a result of this assumed low phytosterol intake, a significant effect of phytosterol consumption on serum lipids would not be expected, although several epidemiological studies have associated dietary phytosterol intake with lower cholesterol levels [38]. One explanation for the unexpected positive associations between fecal phytosterols and serum lipids seen in this study may be related to the isolated nature of the stool samples, which may not accurately depict a person's average phytosterol intake and could be responsible for the contradictory results.

4.3. Strengths and weaknesses

The strengths of this study include the large number of participants with fecal metabolite data available. While several studies have

evaluated the relationship between fecal bile acids or neutral sterols and atherosclerosis, these studies were all conducted in selected populations with limited sample sizes [15,18–22]. Our study is the first to our knowledge to assess the relationship between fecal bile acids and serum lipids or dyslipidemia in a large sample selected from the general population. Furthermore, the use of Metabolon's large, high-quality metabolite analysis and identification platform, as well as the availability of individual bile acid and neutral sterol concentrations are also strengths. As the large majority of metabolites included in this analysis had less than 10% missing values, we do not expect the imputation to have had a strong influence on our results or conclusions. Indeed, the associations with metabolites containing few missings *versus* those with more than 10% missings are similar. However, the fact that only one stool sample was available for each participant may limit the representativeness of the samples [41]. Additionally, the cross-sectional nature of this study means that these results cannot be used to infer causality. Longitudinal and experimental studies will be necessary to clarify and confirm our findings.

4.4. Conclusions

In conclusion, we found positive associations between fecal bile acids and markers of dyslipidemia and serum lipids, especially serum triglycerides and hypertriglyceridemia. Similarly, we also found positive associations between fecal cholesterol and hypertriglyceridemia. Our results provide insight into the role that bile acids and neutral sterols play in metabolic/cardiovascular health and suggest that bile acids may play a role in the development or progression of dyslipidemia. However, bile acid signaling and control of lipid metabolism is not fully understood. Longitudinal and experimental studies are necessary to clarify the mechanisms behind these associations and to determine if increased fecal bile acid and cholesterol excretion is truly a compensatory response to existing elevated serum lipid levels.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

J.L. and T.A.B. formulated the research question and designed the study; C.M., A.P., A.A., J.A., and H.G. conducted research and/or provided essential materials; T.A.B. analyzed data, performed statistical analysis, interpreted results, and drafted the manuscript; N.W. contributed to statistical analysis and interpretation. All authors have read and approved the final manuscript.

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