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Circulating bile acids are related to an adverse cardiometabolic profile and higher blood pressure in middle-aged adults

Víctor Manuel López-Espinosa
viclespi@correo.ugr.es

Hospital Universitario Virgen de las Nieves
Carmen Rodríguez-García
University of Almería
Lucas Jurado-Fasoli
University of Granada
Francisco J. Amaro-Gahete
University of Granada
Francisco J. Osuna-Prieto
Hospital Universitari Joan XXIII de Tarragona

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Abstract

Elevated circulating bile acid (BA) levels have been associated with cardiometabolic risk; however, the contribution of body composition and sex-related differences to these associations is poorly understood. In the current study, we examined the associations of circulating BAs with body composition and cardiometabolic risk markers, including sex- related differences, in 72 sedentary middle-aged adults (39 women, 40-65 years) from the FIT-AGEING study (NCT03334357). Circulating BA concentrations were quantified by liquid chromatography–tandem mass spectrometry, body composition was assessed by dual- energy X-ray absorptiometry, and cardiometabolic risk factors were analyzed using standard methods. Men exhibited higher BA concentrations than women, and these differences were largely explained by variation in lean mass and visceral adiposity. Total and secondary BAs were positively associated with whole-body adiposity and visceral fat (ρ=0.21–0.42), whereas glycolithocholic acid was inversely associated with lean mass (ρ=–0.38). BA species were associated with GPT, GGT, total cholesterol, and blood pressure after adjustment for body composition (β=0.211-0.437). After sex stratification, BA levels were associated with general and visceral adiposity in women (ρ=0.33-0.51), whereas in men these associations were restricted to visceral fat (ρ=0.34-0.45). Associations between BA levels and blood pressure remained significant only in men, in whom total and individual secondary BAs remained independently associated with systolic and diastolic blood pressure (β=0.167-0.564) after adjustment for visceral adiposity. These findings indicate that circulating BA profiles exhibit sex differences in middle-aged adults that are partially explained by body composition and are associated with an adverse cardiometabolic profile, particularly higher blood pressure.

Key points

- Circulating bile acids are related to adiposity in sedentary middle-aged adults.
- Sex differences in circulating bile acids are partly explained by body composition.
- Circulating bile acids are linked to adverse cardiometabolic profiles.
- In men, secondary bile acids remain associated with blood pressure.

INTRODUCTION

Cardiometabolic diseases, including obesity, type 2 diabetes, and metabolic dysfunction–associated fatty liver disease, remain leading causes of morbidity and mortality worldwide [1, 2]. Identifying early biological pathways and biomarkers that contribute to cardiometabolic risk in this population is therefore critical for improving prevention strategies [3].

In this context, bile acids (BAs) have emerged as key endocrine and metabolic signaling molecules linking hepatic and intestinal physiology with systemic metabolic regulation [4]. Primary BAs are synthesized from cholesterol in hepatocytes, conjugated with glycine or taurine, and secreted into the duodenum through the biliary tract [5]. Gut microbial enzymes subsequently convert primary into secondary BAs, expanding the diversity of the circulating BA pool [6]. While most BAs undergo enterohepatic recycling [7, 8], a fraction enters the systemic circulation, where they act as signaling molecules regulating glucose, lipid, and energy metabolism through receptors such as the Farnesoid X receptor (FXR) and the Takeda G-protein–coupled receptor (TGR5) [9–11]. In the ileum, BAs activate intestinal FXR, inducing the secretion of fibroblast growth factor 19 (FGF19), which travels via the portal circulation to the liver to suppress BAs, forming a critical endocrine feedback loop known as the intestinal FXR-FGF19 axis [12].

Consistent with these endocrine functions, alterations in circulating BA concentrations and composition have been associated with cardiometabolic disorders, including obesity, hypertension, and metabolic dysfunction–associated fatty liver disease [13]. Experimental and clinical evidence also suggests that BA signaling may influence blood pressure regulation through renal and vascular pathways [14]. However, most studies have been conducted in clinical populations with established metabolic disease, and the role of body composition and sex in shaping circulating BA profiles and their cardiometabolic associations remains poorly characterized [15, 16].

Here, we examined the associations of fasting circulating BAs with body composition and cardiometabolic risk markers in middle-aged adults. Characterizing BA signatures in this relatively healthy population may improve early risk stratification and provide insight into biological pathways linking BA metabolism with cardiometabolic health.

METHODS

Study population

Seventy-two sedentary middle-aged adults (40–65 years, 39 women) from the FIT-AGEING study (ClinicalTrials.gov: NCT03334357; registered November 7, 2017) with valid BA determinations were included. Participants were recruited through social media. Inclusion criteria were sedentary lifestyle (<20 min of moderate physical activity on <3 days/week during the previous 3 months), absence of acute or chronic disease, and stable body weight (<5 kg change during the previous 6 months). Exclusion criteria included pregnancy or lactation, chronic medication use, and any condition that could interfere with or be aggravated by exercise. The study was approved by the Ethics Committee on Human Research of the University of Granada and Servicio Andaluz de Salud (0838-N-2017), and all participants provided written informed consent.

Anthropometry and body composition

Waist circumference, body weight, and height were assessed using standardized procedures. Lean Body Mass (LBM), Body Fat Mass (BFM), and Visceral Adipose Tissue (VAT) were measured by dual-energy X-ray absorptiometry using a Discovery Wi device (Hologic Inc., Bedford, MA, USA). Subsequently, Lean Mass Index (LMI) and Fat Mass Index (FMI) were calculated by dividing body weight, LBM, and FM (in kg) by the square of the height (in m^2), respectively. Body fat percentage was calculated as the body fat mass divided by total body mass ×100.

Blood sampling and cardiometabolic markers

Fasting blood samples were collected between 8:30 and 10:00 AM after an overnight fast (≥12 h). Participants were instructed to avoid alcohol, caffeine, medications, and exercise before sampling.

Circulating cardiometabolic risk factors were determined in plasma [i.e., glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), GPT, and GGT]. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [Total cholesterol – HDL-C – (TG/5)]. Insulin sensitivity was estimated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [17] and the quantitative insulin sensitivity check index (QUICKI) [18]. Fat Liver Index (FLI) was calculated as a validated proxy indicator of metabolic dysfunction–associated fatty liver disease [19]. A detailed description can be found in the **Supplemental Material.**

Blood pressure was determined in the right arm after a 30-min rest in a supine position, using an Omron® HEM 705 CP automatic monitor (Omron Health-Care Co., Kyoto, Japan), following the recommendations of the European Society of Cardiology [20]. Three measurements were taken 1 min apart, and the mean value was calculated.

Determination of plasma bile acid levels

Primary BAs, [*i.e.,* cholic acid (CA), chenodeoxycholic acid (CDCA), glycocholic acid (GCA), and glycochenodeoxycholic acid (GCDCA)], as well as secondary BAs, [*i.e.,* glycodeoxycholic acid (GDCA), deoxycholic acid (DCA), glycolithocholic acid (GLCA), and glycoursodeoxycholic acid (GUDCA),] were determined in plasma samples using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. BAs were grouped as primary and secondary according to their biosynthetic origin. **Table S1** lists the targeted BA. Detailed information on sample preparation, LC–MS/MS, and data preprocessing and quality control of BAs is provided in the **Supplemental Material**.

Statistical analysis

Categorical and continuous variables were used to describe the clinical characteristics of the study participants. Normality was assessed with both Q-Q graphs and using the Shapiro–Wilk test. The mean ± standard deviation (SD) was used to represent normally distributed variables, while the median and interquartile range (IQR) were used to represent non-normally distributed variables.

Differences in phenotypic, cardiometabolic parameters, and plasma BA levels between men and women were also examined using independent t-tests. Due to the non-normal distribution of BA concentrations, values were log10-transformed prior to these analyses. As body composition parameters differed between sexes, we performed analyses of covariance (ANCOVA) to examine whether sex differences in plasma BA levels persisted after adjusting for body composition parameters. Sex interactions were tested by including a sex × BA interaction term in regression models; no significant interaction was observed (P interaction>0.1). Therefore, pooled analyses adjusted for sex were performed as the primary analyses. However, given the observed differences in body composition and association patterns by sex, additional sex-stratified analyses were performed to further characterize these relationships.

Associations between circulating BA levels and cardiometabolic outcomes were initially explored using rank-based partial correlations (Spearman partial correlations), given the non-normal distribution of BA concentrations, adjusted for age and sex (or age only in sex-stratified analyses). Multiple testing was accounted for using the Benjamini–Hochberg false discovery rate (FDR), and associations with $q < 0.15$ were carried forward to multivariable regression analyses.

Multiple linear regression analyses were subsequently conducted including only variables identified as significant in these exploratory bivariate analyses, in order to assess their independent associations with plasma BA levels. Log10 transformations were applied selectively to BA variables when required to ensure approximate normality of standardized residuals, and model assumptions were subsequently verified. In the pooled-sex analyses, models were adjusted for age and sex, and additionally for BMI, LMI, and VAT mass, as these were the body composition parameters most strongly associated with BA levels and considered the main confounders. In men-only analyses, models were adjusted for age and VAT mass, as VAT mass was the only body composition parameter significantly associated with BA levels in this group. Standardized β coefficients and adjusted R^2 values are reported. A two-sided $P<0.05$ was considered statistically significant.

Statistical analyses were performed using SPSS (v. 22.0, IBM SPSS Statistics, IBM). Figures and correlations were generated in R (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

Results

The characteristics of the participants are presented in **Table 1**. Compared with women (n = 39), men (n = 33) showed higher waist circumference (+14%), BMI (+11%), LBM (+37%), LMI (+25%), and VAT (+36%), and lower body fat percentage (−29%, all P ≤ 0.001). Men also exhibited higher creatinine (+22%), GGT (+30%), GPT (+37%), and FLI (+48%), as well as higher systolic (+10%) and diastolic (+9%) blood pressure (all P ≤ 0.004). No additional cardiometabolic markers differed significantly between sexes (all P ≥ 0.271).

Circulating bile acid levels are higher in men than in women

Total (+11%), primary (+21%), and secondary (+12%) BAs were higher in men than in women (all P ≤ 0.008, **Table 2**). Except for GDCA (P = 0.052), men showed higher levels of all individual BA species, including CA (+62%), CDCA (+56%), GCA (+47%), GCDCA (+34%), DCA (+38%), GDCA (+33%), GLCA (+29%), and GUDCA (+22%) (all P ≤ 0.031). Adjustment for BMI, LMI, and VAT substantially attenuated most sex differences in circulating BA levels, whereas these differences were largely unchanged after adjustment for FMI (**Table 2**).

Higher circulating bile acid levels were positively associated with an unfavorable body composition profile

In the whole cohort, total and secondary BAs were positively associated with indices of adiposity, including BMI, FMI, and VAT mass (ρ = 0.26–0.42) (**Fig. 1**). CA and DCA showed similar positive associations with BMI, FMI, and VAT (ρ = 0.22–0.47). CDCA was also positively correlated with VAT (ρ = 0.22) and BMI (ρ = 0.29), while DCA correlated with FM (ρ = 0.22) (**Fig. 1**). In contrast to the overall pattern, GLCA showed an inverse association with indices of lean mass (−0.24 to −0.38) (**Fig. 1**).

Sex-stratified analyses reveal distinct body composition–related association patterns

Sex-stratified analyses revealed distinct patterns. In men, circulating BAs were primarily associated with visceral adiposity, with total and secondary BAs as well as CA, CDCA, and DCA correlating with VAT mass (ρ = 0.34–0.45) (**Fig. S1**). In contrast, women showed broader associations between circulating BAs and overall adiposity. Total and secondary BAs, as well as CA, CDCA, and DCA were positively associated with BMI (0.42 ≤ ρ ≤ 0.51) (**Fig. S2**). Secondary BAs were also positively associated with FMI (ρ=0.36), DCA with FMI and VAT mass (ρ=0.35-0.38), CA with LMI (ρ=0.33), and CDCA with FM and FMI (ρ=0.33-0.34). Interestingly, GLCA was negatively associated with lean body mass (ρ=-0.44)

Elevated circulating bile acid levels are associated with an adverse cardiometabolic profile and higher blood pressure

Several BA species were positively associated with liver-related markers. Conjugated BAs, including GCA, GCDCA, and GUDCA, correlated positively with GPT and GGT (ρ = 0.28–0.34) (**Fig. 2**). Total and secondary BAs were also associated with the fatty liver index (both ρ = 0.33) (**Fig. 2**). Regarding lipid profile parameters, GCDCA and GUDCA showed positive correlations with total cholesterol (ρ = 0.29–0.32), while CDCA was positively associated with LDL-C (ρ = 0.27). In contrast, GUDCA showed an inverse association with HDL cholesterol (ρ = −0.24) (**Fig. 2**).

The associations between circulating bile acids and cardiometabolic risk markers are more pronounced in men

Sex-stratified analyses revealed a markedly stronger association pattern in men. In this group, total and secondary BAs correlated positively with GPT, FLI, and both systolic and diastolic blood pressure (ρ = 0.35–0.56) **(Fig. S3).** At the individual BA level, DCA showed consistent associations with GPT, FLI, and both systolic and diastolic blood pressure (ρ = 0.39–0.52) **(Fig. S3)**, while GLCA was positively associated with systolic and diastolic blood pressure (ρ = 0.45–0.50). Additional positive associations were observed between GCDCA and GDCA and diastolic blood pressure (ρ = 0.36–0.48) (**Fig. S3**). In contrast, women showed minimal cardiometabolic associations. The only significant correlation observed was between GUDCA and creatinine (ρ = 0.39) (**Fig. S4**).

Associations between circulating bile acids and cardiometabolic markers persist after adjustment for body composition

Given the consistent associations between circulating BAs and body composition, we evaluated whether relationships with cardiometabolic markers were independent of adiposity and lean body mass. Multiple linear regression models adjusted for BMI, LMI, and VAT showed that several BA species remained associated with liver-related markers. In particular, GCDCA and GCA were consistently associated with GGT and GPT across all models (β = 0.265–0.437, P ≤ 0.016), while GUDCA remained positively associated with GGT and total cholesterol (β = 0.222–0.274, P ≤ 0.043) (**Table 3**). In addition, total BAs remained positively associated with systolic and diastolic blood pressure after all adjustments (β = 0.258–0.316, P ≤ 0.046). Associations between total BA, secondary BAs, DCA and FLI were observed only in models adjusted for LMI (β = 0.211–0.255, P ≤ 0.038) (**Table 3**).

When analyses were restricted to men and adjusted for VAT, most associations with metabolic markers were attenuated. However, associations between circulating BAs and blood pressure remained significant. Total BAs were positively associated with diastolic blood pressure (β = 0.503, P = 0.01), whereas secondary BAs were associated with both systolic and diastolic blood pressure (β = 0.410–0.564, P ≤ 0.028) (Table S2). At the individual BA level, these associations were mainly driven by DCA, GDCA, and GLCA, which were positively associated with diastolic blood pressure (β = 0.476–0.534, P ≤ 0.013) (**Table S2**; **Fig. S5**).

DISCUSSION

Our findings indicate that circulating BA levels are higher in men than in women and are associated with adiposity and a less favorable cardiometabolic profile in middle-aged adults. Sex differences were partially attenuated after adjustment for body composition, suggesting that dimorphism is partly driven by differences in lean mass and visceral adiposity. Associations between circulating BAs and cardiometabolic risk markers, particularly liver

enzymes and blood pressure, remained significant after adjustment and were mainly driven by secondary BA species, being more evident in men. These findings indicate that cardiometabolic associations depend not only on total BA concentrations but also on BA composition, with more hydrophobic species such as DCA emerging as key players. These patterns are consistent with differential activation of BA-responsive pathways, potentially involving FXR signaling and TGR5 signaling, although causality cannot be inferred. Together, these results suggest that circulating BA profiling may have potential value for early cardiometabolic risk stratification.

Higher circulating levels of several BA species in men have been reported previously and appear to be consistent across different populations, largely independent of demographic and environmental factors [21–24]. It is widely accepted that sex hormones modulate hepatic BA synthesis and enterohepatic cycling [25, 26], potentially contributing to sex-dependent BA patterns. In particular, estrogen signaling suppresses CYP7A1 expression, reducing BA synthesis primarily via modulation of FXR [27]. However, the extent to which these differences are influenced by sex-related distinctions in body composition has been poorly investigated in middle-aged populations. In our cohort, several sex-related differences in circulating BA levels were attenuated after adjustment for LMI and VAT mass, but not after adjustment for FMI. Consistent with these observations, sex-stratified analyses showed that in men higher BA levels were predominantly associated with visceral adiposity, whereas in women BA levels were associated with general adiposity. Given that men typically exhibit higher levels of visceral adipose tissue [28, 29], this depot may partly explain sex-specific BA profiles. In this sense, VAT differs metabolically from subcutaneous adipose tissue by exhibiting greater lipolytic activity and direct portal drainage [30, 31]. Furthermore, obesity and excess VAT have been associated with increased BA synthesis and altered enterohepatic transport [32–34]. These findings highlight the importance of considering body fat distribution, particularly visceral adiposity, when interpreting sex-related differences in circulating BA profiles.

Furthermore, the attenuation of sex differences after adjustment for LMI also suggests a potential contribution of lean mass to circulating BA dynamics in humans, and the inverse association observed between GLCA and lean mass indices suggests a potential interaction between circulating BA signaling and skeletal muscle metabolism. Emerging evidence indicates that BAs can interact with skeletal muscle through receptors such as TGR5, influencing intracellular energy expenditure and mitochondrial activity, which may indirectly affect systemic BA turnover and metabolic signaling [35–37]. In this context, variation in muscle mass between men and women could contribute to differences in circulating BA profiles through effects on whole-body metabolic demand and substrate utilization. Therefore, our findings suggest that part of the differences in circulating BA levels between men and women are likely influenced by differences in body composition, particularly visceral adiposity and lean mass.

Overall, higher circulating BA levels were associated with a more adverse cardiometabolic profile, particularly in relation to markers of liver function. In this context, the observed associations with liver enzymes and FLI support the potential relevance of circulating BAs as indicators of altered hepatic metabolic function [38]. These findings suggest that, even in middle-aged adults, circulating BA profiles may reflect early metabolic disturbances related to hepatic dysfunction. Mechanistically, chronic BA elevation may impair FXR/TGR5 signaling through receptor desensitization, thereby promoting steatosis and metabolic stress [39–41]. In line with this, DCA was the only BA species associated with FLI in our cohort, aligning with its established hepatotoxic effects and enrichment in advanced stages of liver disease [42–44]. Microbial transformations such as the conversion of primary into secondary BAs can substantially modify BA composition and downstream receptor signaling [45]. Increased DCA levels are thought to reflect alterations in gut microbial BA metabolism, particularly enhanced bacterial 7α-dehydroxylation activity, which has been linked to the expansion of DCA-producing taxa such as *Lachnospiraceae* and *Ruminococcaceae* [46]. Beyond their role as indicators of hepatic metabolic alterations, these findings also support the concept that BAs may act as systemic metabolic regulators with potential implications for broader cardiometabolic processes.

Importantly, many of the observed BA–risk associations were strongly influenced by body composition, highlighting the central role of adiposity and lean mass in shaping circulating BA profiles and their metabolic correlates. Although most BA–risk associations weakened after adjusting for body composition, relationships between some BA species and hepatic enzymes, total cholesterol, and blood pressure remained after further adjustment for BMI, LMI and VAT. Notably, after sex stratification, cardiometabolic associations persisted exclusively in men, even though visceral fat represented the adiposity compartment most closely linked to BA levels in men. Total and secondary BAs, particularly DCA, GDCA, and GLCA, remained associated with both systolic and diastolic blood pressure independently of visceral adiposity, indicating a sex-specific contribution of secondary BA signaling to hemodynamic regulation. This interpretation is supported by experimental and clinical evidence linking BAs to blood pressure control through FXR/TGR5-mediated effects on renal sodium handling, vascular tone, and inflammatory pathways [14, 47–49]. Consistent with this concept, elevated circulating BA levels in humans have been linked to an increased risk of hypertension [21], supporting a model in which circulating BAs exert regulatory effects on renal and vascular physiology that may influence blood pressure regulation in humans.

Limitations

First, the cross-sectional design of the study precludes causal inference. Second, the study cohort consisted predominantly of relatively healthy middle-aged adults, which may limit the generalizability of our findings to younger or older populations, as well as to individuals with established cardiometabolic disease. Finally, our BA profiling focused primarily on glycine-conjugated species and did not include taurine-conjugated BAs. Although taurine conjugates are generally less abundant in human circulation and are not consistently captured in targeted BA profiling approaches [50], their omission may have limited a more comprehensive characterization of the circulating BA pool.

CONCLUSION

Circulating BA profiles exhibit marked sex differences in middle-aged adults that are partly explained by variation in body composition and are associated with a less favorable cardiometabolic profile, particularly higher blood pressure levels. These associations are primarily driven by secondary BA species and were largely restricted to men, suggesting a possible sex-specific contribution of BA signaling to cardiometabolic risk.

Clinical Implications

From a clinical perspective, profiling specific circulating BAs may complement conventional cardiometabolic assessment and could be integrated into metabolomics-based risk stratification approaches, enabling earlier identification of individuals at increased cardiometabolic risk, even before overt disease develops [16]. In this context, interventions known to influence BA metabolism, such as physical activity and dietary composition [51], as well as emerging pharmacological approaches targeting FXR or TGR5 signaling, could represent promising avenues to modulate BA pathways and potentially improve metabolic health, including blood pressure regulation [52]. Further longitudinal and interventional studies are required to determine whether BA-based risk stratification or targeted modulation of BA signaling ultimately translates into measurable clinical benefit.

Declarations

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Author contributions

VML-E: Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. LJ-F: Writing – review & editing. CR-G: Writing – review & editing. FJA-G: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. FJO-P: Conceptualization, Formal analysis, Data curation, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. The authors declare that all data were generated in-house and that no paper mill was used.

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Competing interests
The authors declare that they have no competing interests.

Ethics approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee on Human Research of the University of Granada and Servicio Andaluz de Salud (0838-N-2017).

Consent to participate

Written informed consent was obtained from all individual participants included in the study.

Data availability

The data underlying this article are available from the corresponding author on reasonable request. Data are not publicly available due to ethical restrictions.

Clinical trial registration

This cross-sectional analysis used baseline data from the FIT-AGEING study. The parent trial was registered at ClinicalTrials.gov (NCT03334357; registered November 7, 2017).

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Tables

Table 1. Clinical and body composition characteristics of the study participants.

	All (n)	Mean		SD	Men (n=33)	Mean		SD	Women (n=39)	Mean		SD	P value (sex)
Age (y)	72	53.6	±	5.1		54.4	±	5.1		53.0	±	5.0	0.265
ANTHROPOMETRY AND BODY COMPOSITION													
Waist circumference (cm)	72	94.8	±	11.7	33	102.6	±	8.9	39	88.2	±	9.7	**<0.001**
BMI (kg/m^2)	72	26.7	±	3.8	33	28.3	±	3.7	39	25.3	±	3.3	**<0.001**
LBM (kg)	72	43.1	±	11.6	33	53.8	±	6.4	39	34.1	±	5.8	**<0.001**
LMI (kg/m^2)	72	15.2	±	2.9	33	17.5	±	1.9	39	13.2	±	1.8	**<0.001**
FMI (kg/m^2)	72	10.8	±	3.1	33	10.0	±	3.2	39	11.4	±	2.9	0.058
BFM (kg)	72	29.9	±	8.3	33	30.6	±	9.6	39	29.2	±	7.1	0.501
BFP (%)	72	40.0	±	9.0	33	34.6	±	7.8	39	44.5	±	7.4	**<0.001**
VAT mass (g)	72	786	±	383	33	975	±	384	39	626	±	303	**<0.001**
CARDIOMETABOLIC RISK FACTORS													
Glucose (mg/dL)	72	93.6	±	11.3	33	95.0	±	13.6	39	92.4	±	8.8	0.415
Insulin (µIU/mL)	72	8.1	±	5.6	33	8.7	±	6.7	39	7.6	±	4.6	0.917
HOMA IR	72	1.9	±	1.7	33	2.1	±	2.1	39	1.8	±	1.1	0.561
Total cholesterol (mg/dL)	72	207.2	±	33.6	33	203.2	±	37.1	39	210.6	±	30.5	0.271
HDL-C (mg/dL)	72	59.5	±	12.6	33	56.2	±	12.4	39	62.3	±	12.2	**0.034**
LDL-C (mg/dL)	72	126.4	±	29.5	33	127.5	±	32.3	39	125.5	±	27.2	0.870
Triglycerides (mg/dL)	72	133.1	±	63.7	33	141.5	±	77.5	39	126.1	±	49.1	0.581
Creatinine (mg/dL)	72	0.8	±	0.2	33	0.9	±	0.1	39	0.7	±	0.1	**<0.001**
GGT (U/L)	72	34.4	±	23.4	33	41.1	±	23.7	39	28.7	±	21.9	**0.004**
GPT (U/L)	72	23.3	±	12.6	33	29.2	±	13.8	39	18.3	±	9.0	**<0.001**
Fatty liver index	69	49.5	±	26.3	32	66.6	±	20.0	37	34.8	±	21.9	**<0.001**
Systolic blood pressure (mm Hg)	68	126.9	±	15.6	30	134.5	±	13.7	38	121.0	±	14.5	**<0.001**
Diastolic blood pressure (mm Hg)	68	81.1	±	11.7	30	85.5	±	11.1	38	77.7	±	11.1	**<0.001**

Data are presented as mean and standard deviation (SD). *P* values are obtained from an independent t-test. BFM: body fat mass; BFP: body fat percentage; BMI: body mass index; FMI: fat mass index; GGT: gamma-glutamyl transferase; GPT: glutamic-pyruvic transaminase; HDL-C: high density lipoprotein-cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; LDL-C: low density lipoprotein-cholesterol; LMI: lean mass index; VAT: visceral adipose tissue.

Table 2. Comparison of plasma levels of total, primary, and secondary bile acids between middle-aged men and women.

		All (n=72)		Men (n=33)		Women (n=39)			BMI	LMI	VAT	FMI
	Bile Acid	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**P**	P_1	P_2	P_3	P_4
GROUPED BA	Total BA	70.9	64.5	83.7	97.4	55.8	55.0	**0.003**	0.008	0.277	0.074	**<0.001**
	Primary BA	13.3	20.9	19.6	27.5	8.3	13.5	**0.006**	0.046	0.101	0.052	**0.003**
	Secondary BA	51.8	57.5	63.6	77.1	42.3	47.1	**0.008**	0.175	0.295	0.139	**0.001**
PRIMARY BA	CA	0.9	6.4	1.7	8.6	0.7	1.3	**0.010**	0.134	0.410	0.143	**0.004**
	CDCA	0.9	2.5	1.4	3.7	0.6	1.4	**0.020**	0.186	0.682	0.220	**0.008**
	GCA	5.2	6.7	6.1	14.0	4.2	4.0	**0.006**	**0.022**	**0.019**	**0.024**	**0.004**
	GCDCA	2.3	3.3	3.1	14.0	4.25	4.0	**0.025**	0.063	0.160	0.068	**0.023**
SECONDARY BA	DCA	40.5	48.4	44.1	64.7	32.3	48.8	**0.009**	0.250	0.470	0.194	**0.001**
	GDCA	1.0	1.5	1.1	2.62	0.79	1.1	0.052	0.232	0.386	0.149	**0.029**
	GLCA	12.5	11.4	13.1	10.4	11.8	10.9	**0.031**	0.059	**0.017**	0.133	**0.020**
	GUDCA	0.10	0.10	0.12	0.12	0.08	0.04	**0.031**	0.089	0.062	0.233	**0.020**

Data are presented as median and interquartile range (IQR). *P* values correspond to the crude comparison between men and women (independent T-test); P_1 values are derived from the analyses of covariance (ANCOVA) adjusting for BMI; P_2 adjusting for LMI; P_3 adjusting for VAT and P_4 adjusting for FMI. BA: bile acid; BMI: body mass index; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; FMI: fat mass index; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GLCA: glycolithocholic acid; GUDCA: glycoursodeoxycholic acid; LMI: lean mass index; VAT: visceral adipose tissue.

Table 3. Association between plasma levels of bile acids and cardiometabolic risk factors that were significant in the previous correlation analyses in middle-aged adults (n=72).

		Model 1: adjusted by BMI + age + sex			Model 2: adjusted by LMI + age + sex			Model 3: adjusted by VAT mass + age + sex		
Dependent variable	Predictor	Standardized β	adjusted R^2	P value	Standardized β	adjusted R^2	P value	Standardized β	adjusted R^2	P value
Creatinine	CA	0.095	0.480	0.300	0.102	0.477	0.267	0.123	0.460	0.185
GPT	GCA	**0.304**	**0.242**	**0.007**	**0.308**	**0.242**	**0.005**	**0.306**	**0.242**	**0.006**
	GCDCA	**0.265**	**0.224**	**0.016**	**0.271**	**0.221**	**0.014**	**0.269**	**0.222**	**0.015**
	GUDCA	0.154	0.211	0.168	**0.219**	**0.230**	**0.046**	**0.218**	**0.230**	**0.050**
GGT	GCA	**0.327**	**0.146**	**0.006**	**0.299**	**0.132**	**0.011**	**0.306**	**0.134**	**0.009**
	GCDCA	**0.437**	**0.234**	**<0.001**	**0.427**	**0.228**	**<0.001**	**0.431**	**0.228**	**<0.001**
	GUDCA	**0.273**	**0.182**	**0.017**	**0.274**	**0.184**	**0.016**	**0.273**	**0.181**	**0.018**
Total cholesterol	GCDCA	**0.231**	**0.227**	**0.035**	**0.217**	**0.302**	**0.036**	0.212	0.211	0.054
	GUDCA	**0.252**	**0.235**	**0.023**	**0.222**	**0.303**	**0.036**	**0.230**	**0.216**	**0.043**
HDL-C	GUDCA	-0.151	0.245	0.165	-0.104	0.321	0.309	-0.127	0.221	0.256
LDL-C	CDCA	0.170	0.276	0.136	0.167	0.292	0.132	0.135	0.266	0.231
FLI	Total BA	-0.020	0.734	0.785	**0.225**	**0.405**	**0.033**	0.105	0.681	0.176
	Secondary BA	-0.300	0.739	0.678	**0.214**	**0.415**	**0.034**	0.089	0.683	0.233
	DCA	-0.037	0.739	0.606	**0.211**	**0.413**	**0.038**	0.109	0.687	0.140
Systolic blood pressure	Total BA	**0.264**	**0.228**	**0.046**	**0.276**	**0.228**	**0.025**	**0.274**	**0.227**	**0.030**
Diastolic blood pressure	Total BA	**0.316**	**0.162**	**0.023**	**0.312**	**0.170**	**0.015**	**0.258**	**0.204**	**0.036**

Standardized *β corresponds to the standardized regression coefficient.* R^2, and *P* values were obtained from multiple linear regression analysis between plasma levels of bile acids adjusted by sex and age, and: Model 1: adjusted for body mass index (BMI); Model 2: adjusted for lean mass index (LMI). Model 3: adjusted for visceral adipose tissue (VAT). BA: bile acids; BMI: body mass index; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; FLI: fatty liver index; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GGT: gamma-glutamyl transferase; GLCA: glycolithocholic acid; GPT: glutamate pyruvate transaminase; GUDCA: glycoursodeoxycholic acid; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol

Figures

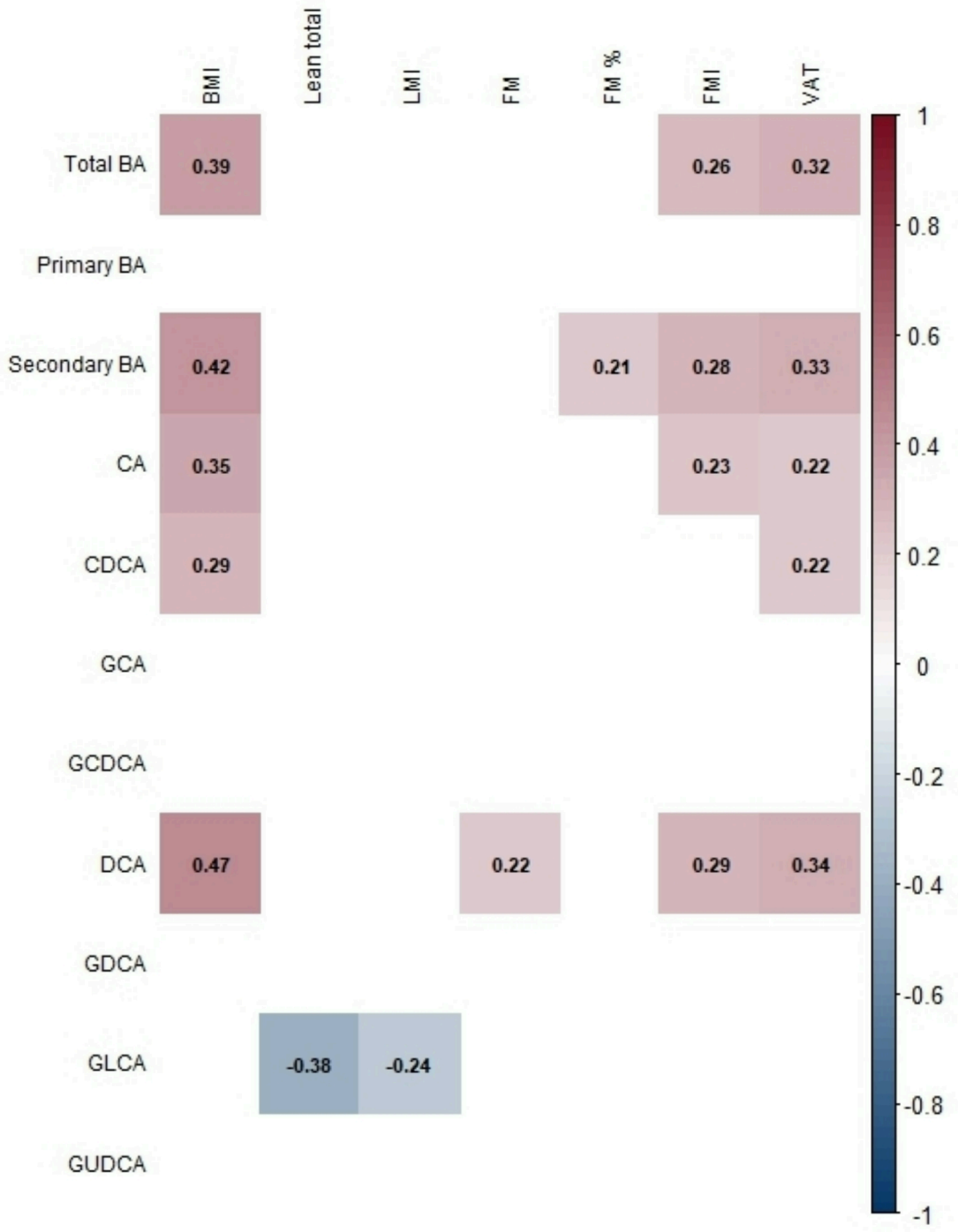


Figure 1

Values within the boxes represent significant Spearman's rho coefficients after adjusting for multiple comparisons using the Benjamini-Hochberg False Discovery Rate (FDR < 0.15). BA: bile acid; BMI: body mass index, CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; FM: fat mass; FMI: fat mass index; FFM: free fat mass; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GLCA: glycolithocholic acid; GUDCA: glycoursodeoxycholic acid; VAT: visceral adipose tissue.



Figure 2

Values within the boxes represent significant Spearman's rho coefficients after adjusting for multiple comparisons using the Benjamini-Hochberg False Discovery Rate (FDR, q < 0.15). BA: bile acid; BP: blood pressure; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; FLI: fat liver index; GGT: gamma-glutamyl transferase; GLCA: glycolithocholic acid; GPT:glutamic-pyruvic transaminase; GUDCA: glycoursodeoxycholic acid; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; Chol: cholesterol.

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