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Clinical Research Article

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Plasma Levels of Bile Acids Are Related to Cardiometabolic Risk Factors in Young Adults

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Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; BA, bile acids; BAT, brown adipose tissue; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; CVD, cardiovascular disease; DCA, deoxycholic acid; ^{18}F-FDG, ^{18}F-fluorodeoxyglucose; FMI, fat mass index; FXR, farnesoid X receptor; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid; HDL-C, high-density lipoprotein cholesterol; IL, interleukin; LBM, lean body mass; LMI, lean mass index; PET/CT, positron emission tomography/computed tomography; SUV, standardized uptake value; T2D, type 2 diabetes; TGR5, Takeda G-protein receptor 5.

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Abstract

Context: Bile acids (BA) are known for their role in intestinal lipid absorption and can also play a role as signaling molecules to control energy metabolism. Prior evidence suggests that alterations in circulating BA levels and in the pool of circulating BA are linked to an increased risk of obesity and a higher incidence of type 2 diabetes in middle-aged adults.

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Objective: We aimed to investigate the association between plasma levels of BA with cardiometabolic risk factors in a cohort of well-phenotyped, relatively healthy young adults.

Methods: Body composition, brown adipose tissue, serum classical cardiometabolic risk factors, and a set of 8 plasma BA (including glyco-conjugated forms) in 136 young adults (age 22.1 ± 2.2 years, 67% women) were measured.

Results: Plasma levels of chenodeoxycholic acid (CDCA) and glycoursodeoxycholic acid (GUDCA) were higher in men than in women, although these differences disappeared after adjusting for body fat percentage. Furthermore, cholic acid (CA), CDCA, deoxycholic acid (DCA), and glycodeoxycholic acid (GDCA) levels were positively, yet weakly associated, with lean body mass (LBM) levels, while GDCA and glycolithocholic acid (GLCA) levels were negatively associated with ^{18}F-fluorodeoxyglucose uptake by brown adipose tissue. Interestingly, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), and GUDCA were positively associated with glucose and insulin serum levels, HOMA index, low-density lipoprotein cholesterol, tumor necrosis factor alpha, interleukin (IL)-2, and IL-8 levels, but negatively associated with high-density lipoprotein cholesterol, ApoA1, and adiponectin levels, yet these significant correlations partially disappeared after the inclusion of LBM as a confounder.

Conclusion: Our findings indicate that plasma levels of BA might be sex dependent and are associated with cardiometabolic and inflammatory risk factors in young and relatively healthy adults.

Key Words: biomarkers, cardiometabolic risk, brown adipose tissue, dyslipidemia, insulin resistance

Obesity is a major health problem that increases the risk of cardiometabolic disorders such as dyslipidemia, insulin resistance, hypertension, and diseases such as type 2 diabetes (T2D) and cardiovascular diseases (CVD) (1). Even though CVD usually manifests clinically at middle-age, its onset takes place in the first decades of life (2). In fact, the incidence of CVD is worryingly increasing among the young population (2). Thus, early detection of alterations in the cardiometabolic profile of young individuals may allow the identification of high-risk individuals and treat them adequately (3). For this reason, the discovery and implementation of novel predictive biomarkers may help in detecting the onset of (cardio)metabolic diseases, enabling early prevention and intervention strategies (4).

Bile acids (BA) are synthesized in hepatocytes from cholesterol by enzymes of the cytochrome P450 family and can subsequently be conjugated with glycine (~75%) or taurine (~25%) to form primary BA that are secreted into the bile (5). Due to their emulsifying properties, BA support the intestinal absorption of dietary lipids and fat-soluble vitamins during the digestion process (6). Primary BA can be absorbed by enterocytes present in the ileum to be transported via the portal vein to hepatocytes (7). Alternatively, primary BA can be metabolized by gut microbiota to form secondary BA (8), which can be absorbed in the colon (7). The process in which primary and secondary BA are reabsorbed to again reach the liver is termed enterohepatic circulation. Primary and secondary BA also reach the systemic circulation (9) from where they can reach peripheral tissues and organs and exert signaling functions to regulate glucose and lipid metabolism (10, 11) predominantly mediated by nuclear and G protein-coupled receptors (GPCRs) (12).

Interestingly, several studies have shown significant differences in BA amount between individuals, in particular, higher circulating BA levels in obese than in lean females (13), and between the sexes, namely, higher circulating BA levels in males than in females (13, 14). Several observational studies have shown that higher levels and alterations in the pool of circulating BA are linked to an increased risk of obesity and a higher incidence of T2D (15). On the contrary, it has been reported that lower serum levels of total BA were associated with higher presence and severity of coronary artery disease and myocardial infarction (16). Bearing that in mind, a recent meta-analysis concluded that circulating BA levels are not associated with adiposity but with higher fecal BA excretion (17). Some studies have shown a link between BA and altered glucose metabolism, with levels of some circulating BA being positively associated with insulin resistance (18, 19). This observational evidence has led to interventions targeting BA metabolism through the use of BA sequestrants (20). Crucially, some

of the improvements in glucose metabolism observed after bariatric surgery in T2D individuals might be related to changes in the pool of circulating BA (21). Despite these findings, the relationship between plasma BA with cardiometabolic risk factors in young adults, a population where the incidence of CVD is rapidly increasing, remains to be elucidated.

In the present study, we aimed to investigate the association between plasma levels of BA and cardiometabolic risk factors in a well-phenotyped cohort of relatively healthy young adults.

Methods

Study Population

This study was performed using baseline measurements from the ACTIBATE study that primarily aimed at investigating the effect of exercise on brown adipose tissue (BAT) activity (ClinicalTrials.gov ID: NCT02365129) (22). The study included 136 young healthy adults (45 male and 91 female participants), as shown in Table 1. All participants were recruited via advertisements in electronic media and leaflets, and all gave their written informed before enrollment. The inclusion criteria were: age from 18 to 25 years; being sedentary, that is, less than 20 minutes of moderate or vigorous physical activity on < 3 days/week (self-reported); not smoking; stable body weight over the past 3 months (changes < 3 kg); not presenting any cardiometabolic disease (eg, hypertension or diabetes); not taking any medication that might affect cardiovascular function; having no history of cancer among first-degree relatives. The study protocol and design were approved by the Human Research Ethics Committee of the University of Granada (n°924) and the Servicio Andaluz de Salud, in adherence with the Declaration of Helsinki (2013).

Cardiometabolic Risk Factors

Anthropometry and body composition

Waist circumference was measured in the minimum perimeter with a measuring tape (at 1-mm precision), at the end of a normal breath expiration, with the arms relaxed on both sides of the body. When the minimum perimeter could not be detected, such as in people who were overweight or obese, the measurements were taken just 2 cm above the umbilicus, following a horizontal plane. Body weight and height were measured using a SECA model 799 electronic column scale and a stadiometer (SECA, Hamburg, Germany). Lean body mass (LBM), body fat mass, and visceral adipose tissue were determined with a Hologic Discovery Wi dual-energy x-ray absorptiometer (DXA) (Hologic, Marlborough, MA, USA). Body mass index

Table 1. Age, anthropometry, body composition and cardiometabolic profile of the subjects included in the study (n = 136)

	All (n)	Mean	SD	Men (n)	Mean	SD	Women (n)	Mean	SD	*P* value (sex)
Age, y	136	22.1	2.2	45	22.3	2.3	91	21.9	2.2	0.319
ANTHROPOMETRY AND BODY COMPOSITION										
Waist circumference, cm	130	81.0	13.8	43	89.9	15.2	87	76.5	10.5	**<0.001**
BMI, kg/m^2	136	24.9	4.6	45	26.8	5.5	91	23.9	3.7	**0.002**
LBM, kg	136	41.8	9.7	45	52.8	7.2	91	36.3	5.0	**<0.001**
LMI, kg/m^2	136	14.7	2.4	45	17.2	2.1	91	13.5	1.4	**<0.001**
FMI, kg/m^2	136	8.8	3.0	45	8.1	3.6	91	9.1	2.7	0.094
Body fat mass, kg	136	24.7	8.8	45	24.8	11.0	91	24.6	7.5	0.884
Body fat percentage, %	136	35.5	7.6	45	29.7	7.6	91	38.3	5.9	**<0.001**
VAT mass, g	136	336	174	45	418	176	91	296	159	**<0.001**
CLINICAL PARAMETERS										
Glucose, mg/dL	132	87.6	6.6	43	88.9	7.4	89	87.0	6.1	0.134
Insulin, µIU/mL	132	8.3	4.9	43	9.1	6.4	89	8.0	4.0	0.638
Total cholesterol, mg/dL	132	165	32	43	160	31	89	168	33	0.183
HDL-C, mg/dL	132	53	11	43	46	7	89	56	11	**<0.001**
LDL-C, mg/dL	132	96	25	43	97	26	89	96	25	0.990
Triglycerides, mg/dL	132	83	45	43	88	47	89	80	43	0.313
HOMA index	132	1.8	1.2	43	2.1	1.6	89	1.7	1.0	0.562
C-reactive protein, mg/L	132	2.4	3.4	43	2.1	2.3	89	2.5	3.8	0.937
Systolic pressure, mm Hg	134	117	12	44	125	11	90	113	10	**<0.001**
Diastolic pressure, mm Hg	134	70.9	7.6	44	72.2	9.2	90	70.3	6.7	0.185

Data are presented as mean and SD. *P* values are obtained from analyses of the variance after $\log_{10}$ transformation of all blood parameters.
Abbreviations: ATP III, National Cholesterol Education Program Adult Treatment Panel III; BMI, body mass index; FMI, fat mass index; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low-density lipoprotein cholesterol; LMI, lean mass index; VAT, visceral adipose tissue.

(BMI), lean mass index (LMI), and fat mass index (FMI) were calculated by dividing body weight, LBM, and body fat mass (in kg) by the square of the height (in cm), respectively. Body fat percentage was calculated as the body fat mass divided by the total body mass and multiplied by 100.

Brown adipose tissue assessment

Activation of BAT was assessed after a personalized cooling protocol for each participant, carried out on 2 independent days and extensively described elsewhere (23). Briefly, participants were first exposed for 30 minutes in a warm room to allow for acclimation, before the transfer to a mild-cold room. Participants then wore a cooling vest (Polar Products Inc., Stow, OH, USA) and the water temperature was slightly decreased until they reached a shivering state. After 48 to 72 hours, the participants went to the hospital where they were exposed to the same cooling protocol for 2 hours at ~4 °C above their shivering threshold. After 1 hour of cold exposure, a bolus of ~185 MBq of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) was injected, and a positron emission tomography/computed tomography (PET/CT) scan (Siemens Biograph 16 PET/CT, Siemens Healthcare, Erlangen, Germany) was performed 2 hours later. The ^{18}F-FDG-PET/CT scans were analyzed using the open-source Beth Israel plugin for the FIJI program (24). The determination of BAT volume and ^{18}F-FDG uptake was based on an individualized standardized uptake value (SUV) of [1.2/ (LBM/body mass)] (25), with a Hounsfield Unit range from −190 to −10. The BAT volume was determined as the number of pixels in this range with an SUV value above the SUV threshold. The BAT activity was determined as the mean SUV (SUVmean), that is, the mean quantity of ^{18}F-FDG in the above same pixels, and the peak SUV (SUVpeak), that is, the mean of the 3 highest ^{18}F-FDG uptake values in 3 pixels within a volume of <1 cm^3. BAT radiodensity was calculated as a proxy of the fat content (26). We also quantified the ^{18}F-FDG uptake in the descending aorta (as reference tissue), subcutaneous white adipose tissue in the dorsocervical and tricipital areas, as well as in several skeletal muscles (paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps braquis muscles at both the left and right sides of the body). Lastly, we grouped different skeletal muscles for these analyses as previously described (27).

Blood parameters

Blood samples were collected from the antecubital vein at baseline after at least 10 hours of fasting in the morning (8:00 to 9:00 AM) (22). Samples were collected in Vacutainer Tubes and immediately centrifuged to obtain serum (Vacutainer SST II Advance tubes) and plasma (Vacutainer Hemogard tubes, containing potassium salt of EDTA as an anticoagulant), of which aliquots were stored at −80 °C until analyses. Serum samples were used for the assessment of blood cardiovascular risk factors (except for leptin and adiponectin, which were determined in plasma), while plasma samples were used for BA quantitation. A detailed description of all the parameter determinations is listed in the Supplementary Information (28).

Determination of Plasma Bile Acid Levels

Primary BA, namely, cholic acid (CA), chenodeoxycholic acid (CDCA), glycocholic acid (GCA), and glycochenodeoxycholic acid (GCDCA), as well as secondary BA, specifically, glycodeoxycholic acid (GDCA), deoxycholic acid (DCA), glycolithocholic acid (GLCA), and glycoursodeoxycholic acid (GUDCA), were assessed in plasma samples using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. Supplementary Table 1 (28) lists the targeted BA. Full description of BA determinations is listed in Supplementary Information (28).

Statistical Analysis

Categorical and continuous variables were used to describe the clinical characteristics of the study participants. Since plasma BA levels, serum cardiometabolic, and inflammatory risk factors did not follow a normal distribution, we $\log_{10}$-transformed the variables to achieve normal distribution. We subsequently evaluated whether plasma BA levels were different between men and women using analyses of variance (ANOVA). Since body composition parameters differed between men and women, we next investigated whether observed sex differences in plasma BA levels persisted after adjusting for body composition parameters using analyses of covariance (ANCOVA). Since the association between plasma BA levels and cardiometabolic risk factors followed a similar direction in men and women separately (no statistical interaction, Supplementary Figure 1) (28), these analyses were also performed including both sexes together (all $P > 0.05$). Pearson correlations between plasma BA levels with cardiometabolic and inflammatory risk factors were performed using R software (R Studio, Boston, MA, USA). Multiple linear regressions were conducted to determine the association of plasma BA levels with cardiometabolic risk factors after adjusting for LBM and body fat percentage. BA correlation plots were built using the R package "corrplot". Supplementary Figures 1 and 2 (28) were built using the GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA). ANOVAs, ANCOVAs, and chi-square tests were performed with SPSS (v. 22.0, IBM SPSS Statistics, IBM), with a level of significance set at $P < 0.05$.

Results

The characteristics of the participants are shown in Table 1. Compared with women (n = 91), men (n = 45) had lower body fat percentage (–23%), lower serum HDL-C (–18%), and higher SBP (+11%) (all $P < 0.001$, Table 1). We did not find significant differences in the other cardiometabolic parameters (all $P \geq 0.05$, Table 1).

Plasma Levels of Chenodeoxycholic Acid and Glycoursodeoxycholic Acid Are Higher in Men Than in Women

Plasma BA levels were similar between men and women (Table 2), except for CDCA and GUDCA, which were higher in men (+40% and +31%, respectively). Based on the body composition differences observed between sexes (Table 1) and to explore to what extent the different body components could be contributing to plasma levels of BA, we repeated these analyses including the BMI, LMI, and body fat mass as confounders, and the differences for CDCA and GUDCA persisted (data not shown). Intriguingly, the differences in plasma CDCA and GUDCA levels between men and women only disappeared when adjusting for body fat percentage ($P = 0.119$ and $P = 0.141$, respectively, Table 2).

Plasma Levels of Bile Acids Are Positively Associated With the Amount of Lean Body Mass and Negatively Associated With Brown Adipose Tissue Levels

We found that plasma levels of CA, GCA, CDCA, GCDCA, DCA, and GDCA were positively correlated with LBM and LMI ($0.17 \leq r \leq 0.20$; $P \leq 0.049$, Fig. 1), whereas only plasma levels of GUDCA were negatively correlated with body fat percentage ($r = -0.22$; $P = 0.011$, Fig. 1). It has been shown that a single dose of CDCA (15 mg/kg) increases human BAT ^{18}F-FDG uptake (29), a thermogenic tissue associated with improved cardiometabolic health (30). In the present study, plasma levels of CDCA were not related to any BAT-related outcomes (all $P \geq 0.05$, Fig. 1), while plasma levels of GDCA and GLCA were negatively correlated with BAT volume ($r = -0.23$; $P = 0.009$ and $r = -0.25$; $P = 0.003$, respectively), BAT SUV mean ($r = -0.22$; $P = 0.012$ and $r = -0.26$; $P = 0.003$, respectively) and BAT SUV peak ($r = -0.17$; $P = 0.048$ and $r = -0.21$; $P = 0.017$, respectively), but not with BAT radiodensity (all $P \geq 0.05$; Fig. 1). We studied the correlation between plasma levels of BA with ^{18}F-FDG uptake by other tissues (Supplementary Figure 2) (28). No associations were found between plasma BA levels and ^{18}F-FDG uptake by skeletal muscle, subcutaneous adipose tissue at the dorsocervical or tricipital areas, or descending aorta as reference tissue (Supplementary Figure 2) (28), suggesting that plasma levels of GDCA and GLCA are solely related to BAT ^{18}F-FDG uptake.

The Association of Plasma Levels of Bile Acids With Cardiometabolic and Inflammatory Risk Factors Partially Disappear When Adjusting for Lean Body Mass

We found that plasma levels of GCA and GCDCA were positively correlated with the concentration of serum alkaline phosphatase, glucose, and insulin, as well as the HOMA index ($0.17 \leq r \leq 0.26$; $P < 0.05$, Fig. 2), while only plasma levels of GUDCA were positively correlated with serum glucose levels ($r = 0.19$; $P = 0.031$, Fig. 2). On the other hand, plasma levels of GCA, GCDCA, and GUDCA were negatively correlated with the serum HDL-C and ApoA1 levels ($-0.34 \leq r \leq -0.20$; $P < 0.05$, Fig. 2). Furthermore, plasma levels of GCA and GCDCA were

Table 2. Comparison of plasma levels of primary and secondary bile acids between men and women (n = 133)

		Men (n=43)		Women (n=90)		P	P_1
	Bile Acid	Mean	SD	Mean	SD		
PRIMARY (CA)	CA	36.5	61.7	23.5	40.8	0.131	0.255
	GCA	2.8	3.3	2.0	1.9	0.136	0.230
SECONDARY (CA)	DCA	21.7	18.2	16.9	19.2	0.075	0.221
	GDCA	3.0	2.4	2.0	2.1	0.067	0.288
PRIMARY (CDCA)	CDCA	0.7	0.9	0.5	0.7	**0.039**	0.119
	GCDCA	7.3	5.4	5.4	3.8	0.072	0.303
SECONDARY (CDCA)	GLCA	5.1	4.6	4.6	4.3	0.374	0.976
	GUDCA	21.3	17.1	16.2	20.3	**0.010**	0.141

Data are presented as mean and SD. P values are derived from analyses of variance (ANOVA). P_1 values are derived from the analyses of covariance adjusting for body fat percentage. Plasma bile acids levels were $\log_{10}$ transformed.

Abbreviations: CA, cholic acid; GCA, glycocholic acid; CDCA, chenodeoxycholic acid; glycochenodeoxycholic acid. DCA, deoxycholic acid; GDCA, glycodeoxycholic acid, GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid.

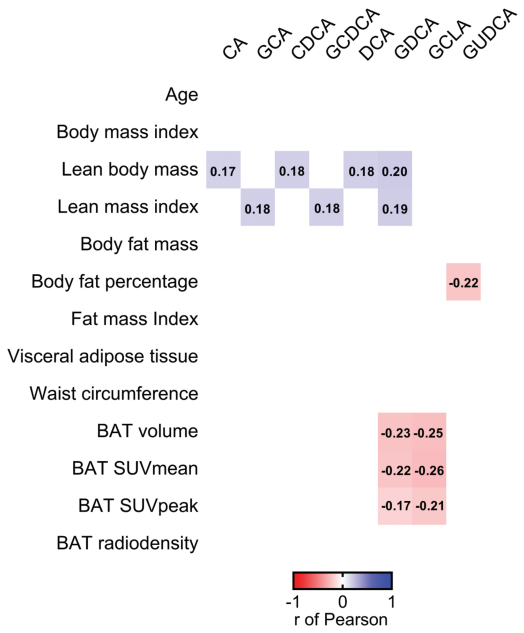

Figure 1. Correlations between plasma levels of bile acids with body composition and brown adipose tissue parameters in young adults (n = 133). Every colored box represents a significant correlation coefficient (all $P < 0.05$), whereas invisible (white) boxes represent nonsignificant correlations. Values within the boxes express the *r* of Pearson coefficient. Bile acids concentration values were $\log_{10}$ transformed. Abbreviations: BAT, brown adipose tissue; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid; SUV, standardized uptake value.

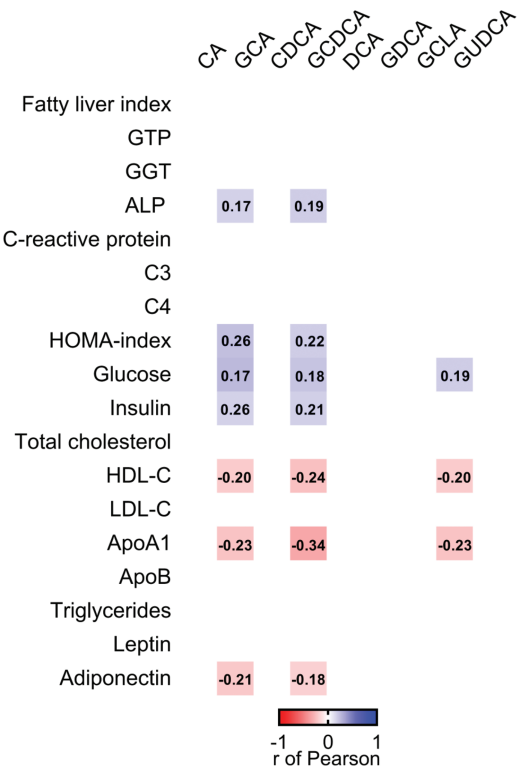

Figure 2. Correlations between plasma levels of bile acids concentrations with cardiometabolic risk factors in young adults (n = 133). Every colored box represents a significant correlation coefficient (all $P < 0.05$), whereas invisible (white) boxes represent nonsignificant correlations. Values within the boxes represent the *r* of Pearson coefficient. All blood parameters were $\log_{10}$ transformed. Abbreviations: ALP, alkaline phosphatase; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GGT, gamma-glutamyl transferase; GLCA, glycolithocholic acid; GTP, phosphoglycerate kinase; GUDCA, glycoursodeoxycholic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

negatively correlated with plasma levels of adiponectin ($r = -0.21$; $P = 0.018$ and $r = -0.18$; $P = 0.045$, respectively, Fig. 2). Additionally, we also found that plasma levels of GCA were positively correlated with plasma levels of the pro-inflammatory cytokines interleukin (IL)-8 and tumor necrosis factor alpha ($r = 0.23$; $P = 0.015$ and $r = 0.21$; $P = 0.027$), whereas the plasma levels of GDCA were positively related with IL-2 and IL-8 ($r = 0.22$; $P = 0.025$ and $r = 0.21$; $P = 0.048$) (Figure S3) (28). Since circulating BA are weakly correlated with LBM in this cohort (Fig. 1), we performed a sensitivity analysis, which showed that most of the significant correlations between plasma BA levels and cardiometabolic risk factors disappeared after adjusting for LBM (Table S2) (28), while all significant correlations remained when body fat percentage was included in the model. The correlations between plasma levels of BA and the inflammatory risk factors persisted after adjusting for LBM and body fat percentage (data not shown)

Discussion

Here, we aimed to investigate whether plasma levels of BA were related to cardiometabolic risk factors in a well-phenotyped cohort of relatively healthy young adults. The findings indicate that CDCA and GUDCA levels were higher in men than in women, but these differences disappeared after adjusting for body fat percentage. We also observed that plasma BA levels were not related to adiposity; however, CA, CDCA, DCA, and GDCA levels were positively related to LBM. Furthermore, levels of GDCA and GLCA were negatively related to ^{18}F-FDG-uptake by BAT. Interestingly, plasma levels of GCA, GCDCA, and GUDCA were weakly related to adverse cardiometabolic and inflammatory profiles, albeit these associations partially disappeared when LBM was included as a confounder in the model.

Plasma levels of CDCA and GUDCA were higher in men than in women, which is in concurrence with other studies (13, 14). A study that included individuals with a wide age range (20-70 years) showed that men had higher

serum concentrations of CA, CDCA, UDCA, and GUDCA than women (13), while another study in older adults (53 ± 15 years) found that serum CA and CDCA levels were higher in men than women (14). In our study, the differences in plasma levels of CDCA and GUDCA disappeared when adjusting for body fat percentage. However, none of the aforementioned studies considered the differences in body fat composition between men and women in their analyses. Several possible hypotheses have been proposed to explain the higher BA levels in men compared to women, such as differences in (i) sex hormone levels (eg, estrogen and progesterone); (ii) medication (eg, statins); (iii) total cholesterol levels; or (iv) body fat distribution (13). Of special interest are the differences in levels of estrogen and progesterone in women, as their circulating levels exhibit significant variations during the premenopausal, menopause, and postmenopausal stages (31). Since the group of women included in the aforementioned studies significantly differed in menopause stages (13, 14), our results might be not comparable to cohorts with women within different menopause stages. Future studies should include estrogen and progesterone as potential confounders in their analyses, as well as other factors that can have an impact on them (eg, the use of contraceptive pills (32)). Furthermore, it is well known that the body fat distribution between men and women is different. Women generally have a larger body fat percentage as they are more likely to accumulate fat subcutaneously and on their lower extremities than men (33). Ideally, to study whether plasma levels of BA are different between men and women, both groups should be matched in terms of body fat composition, which was not the case (Table 1) in our study. Therefore, we adjusted the analysis for body composition outcomes (ie, BMI, LBM, and body fat percentage), which showed that body fat percentage could be partially explaining the differences observed in plasma levels of CDCA and GUDCA between men and women.

We observed that plasma levels of BA were not related to adiposity levels (Fig. 1). Accordingly, a recent meta-analysis, including 42 studies in humans, concluded that there are no significant differences in serum or plasma BA levels between obese and lean individuals. This meta-analysis study demonstrated that BA excretion in the feces was higher in obese individuals, suggesting that BA removal and/or production was higher upon obesity conditions (17). Therefore, future studies should include the measurement of BA both in blood and feces to allow the estimation of BA removal.

In this study, we found that plasma levels of CDCA and CA were not related to BAT, whereas plasma levels of GDCA and GLCA were negatively correlated with BAT. Preclinical studies have shown a link between BA and BAT activation, but findings were controversial (29, 34-36). Injection of CA in mice increased energy expenditure via the BA receptor Takeda G-protein receptor 5 (TGR5) present in BAT and skeletal muscle (34). Another study showed that the cardiometabolic benefits of the injection of CA were independent of BAT activation in diet-induced obese mice (35). Alternatively, mice fed with CDCA showed an increase in the uncoupling protein 1 (UCP1) gene expression levels in BAT, the molecular hallmark of BAT activity (36). A human study showed that the injection of CDCA (15 mg/kg) for 2 days increased BAT ^{18}F-FDG uptake and energy expenditure in women (29), suggesting that CDCA injection enhances human cardiometabolic health via BAT activation. However, most of the studies that investigated the effect of BA on BAT metabolism used oral administration of BA (34-36), making the comparison between those findings and the findings of the current study impossible. Whether there exists a link between circulating CA, CDCA, GDCA, and GLCA and BAT activity at baseline conditions (which probably will differ from the response to a BA acute administration) remains to be further explored in humans.

Our results reveal that plasma levels of BA (GCA and GCDCA) are related to an adverse cardiometabolic and inflammatory profiles. However, when LBM was included as a confounder, many of these associations disappeared. Plasma levels of CA, DCA, and CDCA have been reported to be positively associated with homeostatic model assessment (HOMA) index and insulin levels in T2D patients (18). Similar to our study, plasma GCA, GDCA, and GUDCA levels were positively associated with HOMA index in a cohort of healthy adults (19). Additionally, it is known that BA can act as pro-inflammatory molecules when they are dysregulated (37) and can drive the expression of pro-inflammatory genes in hepatocytes (38), although this phenomenon should be further investigated. Curiously, none of these studies investigated whether those significant correlations were independent of body composition parameters. Preclinical studies have shown that BA can modulate insulin secretion, glucose homeostasis, and immune response via activation of the TGR5 and the farnesoid X receptor (FXR) (34, 39), supporting that the activation of these receptors by BA may be a possible therapy for combating cardiometabolic diseases. Additionally, our results suggest that the relationship of GCA and GCDCA with an adverse cardiometabolic profile could be driven by LBM. Since skeletal muscle represents approximately 40% of the total body mass in humans and is linked to lower insulin resistance (40) and expresses both FXR and TGR5 receptors (41), further research is needed to understand the physiological relevance of this association between plasma levels of BA and LBM.

Limitations

Our study suffers from an inherent limitation of a cross-sectional design and, thus, no causality can be established. The study population included young and relatively healthy adults; therefore, these findings may not be extrapolatable to even younger, older, or unhealthy people. We did not measure estrogen or progesterone levels in women, nor did we register whether they were taking contraception pills. Moreover, we only analyzed glycine-conjugated BA but not taurine-conjugated BA. Additionally, the ^{18}F-FDG-PET/CT scan was static (a limitation in the estimation of cold-induced BAT metabolic activity (42)), and while ^{18}F-FDG uptake is the most commonly used method, it also suffers from limitations in the assessment of BAT metabolic activity (43).

Conclusion

Our study reveals that plasma levels of BA might be sex dependent. Moreover, the findings indicate that plasma levels of BA are associated with cardiometabolic and inflammatory risk factors in young and relatively healthy adults. Further prospective studies are needed to confirm these results and to unveil the putative role of circulating BA in the onset and development of cardiometabolic diseases.

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