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Metabolomic Profiling of Cardiac Fibrosis and Steatosis in Women With or at Risk for HIV

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Background: Heart failure is a prevalent disorder whose prognosis remains poor despite advances in treatment. Women with or at risk for HIV may be particularly susceptible, yet the metabolic pathways that promote myocardial disease and heart failure in this context remain incompletely characterized.

Methods: To evaluate the metabolomic signatures of cardiac magnetic resonance measured phenotypes, we used available plasma metabolomic measures from participants in the Women's Interagency HIV Study who underwent cardiac magnetic resonance imaging. Our primary outcomes were myocardial extracellular volume fraction (MECV) and intramyocardial triglyceride content (IMTG). We applied partial least squares and identified the top 10 lipid and polar metabolites associated with MECV and IMTG. We used multivariable linear regression to evaluate these metabolites' individual associations with each phenotype.

Results: The mean age of participants (n = 153) was 53 ± 7, 93% were Black or Hispanic, and 74% were HIV positive. Phenylacetylglutamine, a microbial metabolite, was positively associated with MECV after full adjustment and false discovery rate correction. Three phosphatidylcholine species, N-acetylaspartic acid, and a lysophosphatidylcholine species were inversely associated with IMTG, while prolylglycine, methionine sulfoxide, sphingosine, taurine, and phosphorylcholine were positively associated with this phenotype. We found no evidence of interaction by HIV for the observed associations, but there was effect modification by hepatitis C virus of taurine's and phosphorylcholine's associations with IMTG.

Conclusion: Among women with or at risk for HIV, we related various lipid and polar metabolites to cardiac fibrosis or steatosis, of which phenylacetylglutamine, N-acetylaspartic acid, and prolylglycine are novel. These findings implicate plausible mechanisms that could be targetable for therapeutics.

Key Words: HIV, cardiac dysfunction, metabolomics

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INTRODUCTION

Despite therapeutic advances, heart failure (HF) remains a prevalent disorder whose prognosis, whether for its reduced ejection fraction or preserved ejection fraction (HFpEF) subtype, continues to be poor.[1] The pathophysiology of HFpEF in particular, a subtype especially common in women, is not well understood.[2] Women with HIV receiving antiretroviral therapy have been reported to have a heightened vulnerability to cardiovascular disease (CVD) and HF.[3,4] Female adults with HIV or at risk for HIV come predominantly from socioeconomically disadvantaged race/ethnic groups.[5] This population exhibits a high burden of cardiovascular risk factors that puts them at high risk of cardiac dysfunction and HF.[6] Defining the mechanisms that drive myocardial disease in such vulnerable women may allow development of targeted therapies that could find broader applicability.

Advances in cardiac magnetic resonance imaging (CMR) have make it possible to noninvasively determine the myocardial extracellular volume fraction (MECV), a measure of diffuse myocardial interstitial fibrosis.[7] MECV is a known marker for early stage or established cardiac pathology in various disorders, including HFpEF,[8–10] which has been associated with incident HF, CVD, and mortality.[10,11] Proton spectroscopy is another CMR technique that can be used to determine intramyocardial triglyceride (IMTG) content, a measure of altered myocardial bioenergetics and lipotoxicity.[12–14] Diffuse myocardial interstitial fibrosis and cardiomyocyte lipotoxicity as measured by MECV and IMTG, respectively, are both considered intermediate HF phenotypes.[15–17]

Systemic metabolic dysregulation is a major risk factor for HF, one that contributes to altered myocardial energetics.[18] Yet the metabolic determinants of myocardial disease remain incompletely defined, particularly in high-risk women, such as those with or at risk for HIV. High-throughput metabolomics allows detailed metabolic assessment of hundreds of identified and unidentified metabolites in plasma,[19] offering unprecedented opportunities to investigate pathways involved in the development of HF in at-risk women.[20]

To better define the metabolomic signatures of diffuse myocardial interstitial fibrosis and intramyocardial steatosis, we leveraged available metabolomic measures and CMR as part of 2 separate ancillary studies in the Bronx and Brooklyn sites of the Women's Interagency HIV Study (WIHS). Our aims were to identify specific metabolite profiles associated with elevated MECV and IMTG in susceptible women and to explore whether these differ by HIV status.

METHODS

WIHS is a multicenter longitudinal study of HIV disease in women whose details have been previously described.[5] In brief, women with HIV and sociodemographically similar women without HIV were recruited at 10 US sites. Participants attended semiannual core visits to obtain information on sociodemographic, medical, and lifestyle factors, along with biological samples for storage. Both the parent study and the present ancillary studies received institutional review board approval. All participants provided informed consent.

To study HIV determinants of cardiac fibrosis and steatosis, an ancillary study performed contrast-enhanced CMR at 3 WIHS sites, Bronx, Brooklyn, and San Francisco. This study focuses on participants from the Bronx and Brooklyn sites, in whom metabolomic measures were obtained for a separate ancillary study on metabolomics and gut microbiome.[21] Contrast-enhanced CMR was conducted using Philips 3.0T scanners at Einstein-Montefiore (Bronx, NY) for both the Bronx and Brooklyn participants. This was first performed in a pilot stage (n = 36 women; 2014–2016) followed by the main funded study (n = 230; 2016–2018). The main study, which involved intravenous administration of 0.2 mmol/kg gadoterate meglumine, used an updated T1 mapping protocol. Therefore, for MECV, only participants from the main study were included, whereas for IMTG, which used the same MR spectroscopy technique in both the pilot and main study stages, participants were included from each stage. Exclusions for contrast-enhanced CMR were weight >159 kg, sagittal abdominal diameter >32 cm, claustrophobia, metal device or implant, pregnancy or lactation, estimated glomerular filtration (eGFR) < 45 mL/minute/1.73 m^2, allergy to gadolinium, uncontrolled asthma, or severe environmental allergies. Standard steady-state free precession cine MR images were acquired in 2-chamber and 4-chamber long-axis and multiple short-axis images to obtain left ventricular and right ventricular mass and volume parameters. Modified Look–Locker inversion recovery sequence[22] was used to obtain T1 maps before and 20 minutes after contrast administration, as described before.[23] MECV, a measure of diffuse myocardial interstitial fibrosis, was calculated using participants' hematocrits as previously described.[24] The proton MR spectroscopy technique was used for positioning an approximately 8 mL voxel within the interventricular septum using 4-chamber and short-axis cine images.[12,13] The spectra were obtained at the mid-to-late diastolic phase using ECG gating. Spectra were analyzed using MATLAB and the Advanced Method for Accurate, Robust, and Efficient Spectral fitting algorithm in Java-based magnetic resonance user interface.[25] The ratio of the fat peak to water peak was used to calculate the IMTG content.[26]

For the omics ancillary study, participants willing to provide stool for gut microbiome assessment underwent concurrent blood metabolomic measurements. Fasting plasma stored at −80°C since collection (2017–2019) was sent to the Broad Institute (Cambridge, MA) for performance of untargeted metabolomics by tandem liquid chromatography/mass spectrometry.[21] Two separate methods were performed to measure lipid and polar metabolites in each sample, as previously described.[27] Raw data from Orbitrap mass spectrometers were processed using Progenesis QI software (NonLinear Dynamics, Durham, NC) for feature alignment, untargeted signal detection, and signal integration. Metabolites were quantified using area under the curve of the peaks. The targeted processing of a subset of known metabolites was conducted using TraceFinder software (version 3.1; Thermo Fisher Scientific; Waltham, MA).

Covariates for analysis were from the study visit corresponding to the CMR assessment. Height and weight were measured using standardized approaches. Smoking status, alcohol use, history of injection drug use and heroin

or cocaine use, menopausal status, and history of myocardial infarction (MI) and HF were based on a standard questionnaire. Heavy alcohol use was defined as >7 drinks per week. Hypertension, diabetes, and dyslipidemia were defined as previously reported.[28] HIV-seronegative participants received testing for HIV by immunoassay at every core visit and, if positive, confirmation by repeat immunoassay. Plasma HIV RNA was measured by commercial immunoassay (detection limit: 20 copies/mL). Hepatitis C virus (HCV) seropositivity and RNA levels were determined with commercial assays and HCV treatment from participant self-report. Positive HCV status was defined as a history of positive HCV RNA or antibody. eGFR was calculated using a validated equation.[29]

The outcomes of interest, MECV and IMTG, were prespecified as primary phenotypes for the CMR ancillary study. Comparisons applied the Mann–Whitney U or Kruskal–Wallis test for continuous variables and the χ^2 or Fisher exact test for categorical variables. Correlation coefficients were computed by the Spearman method.

The current analyses focus on the identified metabolites from the untargeted metabolomic assessment for the 2 platforms involved, namely lipid and polar. We decided a priori to drop metabolites with ≥20% missing vales. None of the metabolites had a coefficient of variation ≥30% for the pooled plasma sample during data quality check procedures. For metabolites with <20% missing values, assuming that such values were attributable to very low abundance, we imputed the missing values as 0.5 times the value of the smallest observation for that metabolite. Because the untargeted metabolomic outputs are not in absolute units but relative levels,[30] we applied rank-based inverse normal transformation to all metabolite measures for normality.[31,32] This procedure gives a mean of zero with a unit SD. MECV was normally distributed, but IMTG was right-skewed and converted to its natural logarithm for analysis. We used the supervised technique of partial least squares (PLS) with 10-fold cross-validation to derive principal components of the metabolites. We arranged the metabolites in the first principal component computed for each platform by decreasing magnitude of their loading coefficients. The PLS procedure was repeated for each of the 4 pairings of platform and CMR phenotype, namely lipid metabolites~MECV, polar metabolites~MECV, lipid metabolites~IMTG, and polar metabolites~IMTG. It was a priori decided to evaluate the adjusted associations with phenotypes for only the top 10 metabolites in each pairing. Then, using linear regression, we sequentially adjusted for covariates to examine associations per SD increment of each metabolite with MECV and IMTG. Covariates were selected based on reported associations from previous literature and known biology. Model 1 adjusted for age, race/ethnicity, and site. Model 2 additionally adjusted for body mass index (BMI), injection drug use, heroin or cocaine

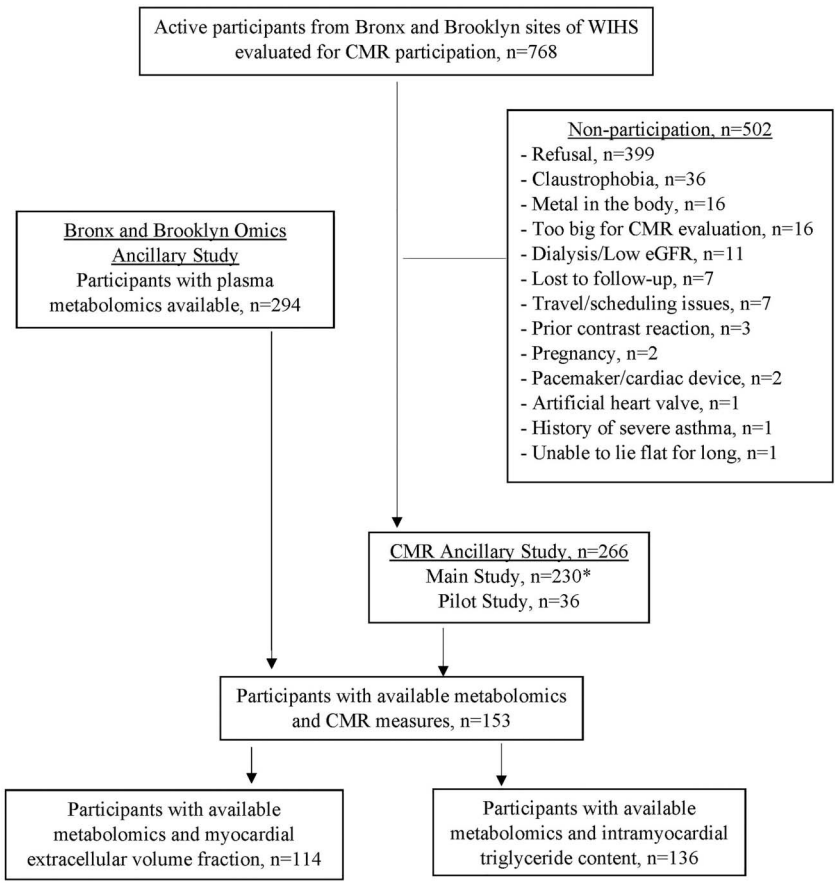

FIGURE 1. Flowchart of study sample. *n = 207 received 0.2 mmol/kg gadoterate meglumine intravenously.

TABLE 1. Characteristics of the Study Sample

	Overall Study Sample (n=153)	Women with HIV (n=114)	Women without HIV (n=39)	*P*
Age, yr	53 (49, 58)	53 (49, 58)	54 (49, 58)	0.868
Race/ethnicity, n (%)				0.014
Non-Hispanic White	7 (4.6)	7 (6.1)	0 (0.0)	
Hispanic	40 (26.1)	35 (30.7)	5 (12.8)	
Non-Hispanic Black	102 (66.7)	68 (59.6)	34 (87.2)	
Other	4 (2.6)	4 (3.5)	0 (0.0)	
Recruitment wave, n (%)				0.759
1994–95	73 (47.7)	54 (47.4)	19 (48.7)	
2001–02	59 (38.6)	43 (37.7)	16 (41.0)	
2011–12	21 (13.7)	17 (14.9)	4 (10.3)	
Site, n (%)				1.000
Bronx	94 (61.4)	70 (61.4)	24 (61.5)	
Brooklyn	59 (38.6)	44 (38.6)	15 (38.5)	
Education, n (%)				0.543
No schooling	1 (0.7)	1 (0.9)	0 (0.0)	
Grades 1–6	2 (1.3)	2 (1.8)	0 (0.0)	
Grades 7–11	59 (38.6)	48 (42.1)	11 (28.2)	
Completed high school	49 (32.0)	33 (28.9)	16 (41.0)	
Some college	37 (24.2)	26 (22.8)	11 (28.2)	
Completed 4 years of college	5 (3.3)	4 (3.5)	1 (2.6)	
Annual household income				0.209
$6000 or less	15 (9.8)	10 (8.8)	5 (12.8)	
$6001 to $12,000	62 (40.5)	49 (43.0)	13 (33.3)	
$12,001 to $18,000	28 (18.3)	22 (19.3)	6 (15.4)	
$18,001 to $24,000	9 (5.9)	9 (7.9)	0 (0.0)	
$24,001 to $30,000	8 (5.2)	4 (3.5)	4 (10.3)	
$30,001 to $36,000	8 (5.2)	5 (4.4)	3 (7.7)	
$36,001 to $75,000	16 (10.5)	10 (8.8)	6 (15.4)	
$75,001 and above	7 (4.6)	5 (4.4)	2 (5.1)	
BMI, kg/m^2	29.3 (25.2, 34.6)	29.4 (25.0, 34.8)	28.3 (25.2, 33.1)	0.377
Current smoker, n (%)	67 (43.8)	43 (37.7)	24 (61.5)	0.016
Heavy alcohol use, n (%)	9 (5.9)	5 (4.4)	4 (10.3)	0.234
History of injection drug use, n (%)	23 (15.0)	18 (15.8)	5 (12.8)	0.798
History of heroin or cocaine use, n (%)	96 (62.7)	70 (61.4)	26 (66.7)	0.693
Menopause status, n (%)				0.582
Premenopausal	25 (16.3)	17 (14.9)	8 (20.5)	
Perimenopausal	20 (13.1)	14 (12.3)	6 (15.4)	
Postmenopausal	108 (70.6)	83 (72.8)	25 (64.1)	
Hypertension, n (%)	130 (85.0)	96 (84.2)	34 (87.2)	0.851
Diabetes, n (%)	52 (34.0)	41 (36.0)	11 (28.2)	0.492
Dyslipidemia, n (%)	135 (88.2)	101 (88.6)	34 (87.2)	1.000
History of lipid-lowering therapy, n (%)	60 (39.2)	44 (38.6)	16 (41.0)	0.938
History of myocardial infarction, n (%)	11 (7.2)	5 (4.4)	6 (15.4)	0.032
History of heart failure, n (%)	1 (0.7)	0 (0.0)	1 (2.6)	0.255
History of HCV seropositivity, n (%)	38 (24.8)	30 (26.3)	8 (20.5)	0.611
Detailed HCV status at CMR visit*, n (%)				0.774
Seronegative	117 (77.0)	86 (76.1)	31 (79.5)	
Spontaneously cleared	5 (3.3)	4 (3.5)	1 (2.6)	
Cleared with treatment	14 (9.2)	12 (10.6)	2 (5.1)	
Actively viremic	16 (10.5)	11 (9.7)	5 (12.8)	
eGFR, mL/min/1.73 m^2	90.5 (74.1, 101.2)	85.1 (69.2, 98.8)	95.6 (84.5, 110.1)	0.003
MECV, %	26.4 (24.6, 28.2)	26.2 (24.6, 28.1)	26.4 (24.2, 28.5)	0.895
IMTG, %	0.5 (0.3, 0.8)	0.5 (0.3, 0.9)	0.5 (0.3, 0.8)	0.436

(*continued on next page*)

TABLE 1. (*Continued*) Characteristics of the Study Sample

	Overall Study Sample (n=153)	Women with HIV (n=114)	Women without HIV (n=39)	*P*
LVEF, %	56 (53, 59)	56 (53, 60)	56 (53, 59.0)	0.328
LVMI, g/m^2	43.6 (38.8, 48.5)	43.5 (38.4, 48.5)	43.8 (41.0, 48.1)	0.320
HIV-specific factors				
ART use, n (%)	NA	112 (98.2)	NA	NA
CCR5 antagonist	NA	1 (0.9)	NA	NA
EI	NA	1 (0.9)	NA	NA
INSTI	NA	67 (58.8)	NA	NA
NNRTI	NA	41 (36.0)	NA	NA
NRTI	NA	102 (89.5)	NA	NA
PI	NA	46 (40.4)	NA	NA
Detectable viral load, n (%)	NA	26 (22.8)	NA	NA
CD4 count, cells/μL	NA	665 (489, 854)	NA	NA
Nadir CD4 count, cells/μL	NA	172 (84, 267)	NA	NA

*Data missing in 1 participant. Median (IQR) for continuous variables. Frequency (%) for categorical variables.

ART, antiretroviral therapy; CCR5, C–C motif chemokine receptor 5; EI, entry inhibitor; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NA, not applicable; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

use, smoking status, heavy alcohol use, HIV status, and HCV status. Model 3 additionally adjusted for hypertension, diabetes, dyslipidemia, history of MI, history of HF, and eGFR. To account for multiple testing, false discovery rate (FDR) adjusted q was calculated using the Benjamini–Hochberg procedure[33] for n = 40 study-wide tests. We also tested for interaction by HIV and HCV status. In sensitivity analyses, we examined the impact of additionally adjusting for education and income, interval between plasma collection and CMR, or menopausal status in Model 3 or of excluding non-Hispanic White participants or those with a history of MI or HF. Analyses used SAS 9.4 (Cary, NC) and R 4.0.5 (Vienna, Austria). Statistical significance was set at $P < 0.05$ or $q < 0.05$, as appropriate.

RESULTS

Details on selection of the study sample are shown in Figure 1. As described in Table S1, Supplemental Digital Content, http://links.lww.com/QAI/B979, women in our sample more frequently came from the Bronx and had a history of current smoking, drug use, and hypertension, compared with women who did not undergo CMR. The characteristics of the sample overall, and by HIV status, are presented in Table 1. Women with HIV were less often of non-Hispanic Black race/ethnicity, included fewer current smokers, and exhibited lower eGFR compared with women with HIV. There was no difference in CMR phenotypes by HIV status.

Relationships between covariates and primary cardiac phenotypes are presented in Table 2. BMI was modestly negatively correlated, whereas eGFR tended to be modestly positively correlated, with MECV. Current smokers had higher MECV than nonsmokers. There were no significant associations between covariates and IMTG.

The median (interquartile range) interval between plasma collection and CMR was 0.7 (0.3, 1.2) years. A total of 375 unique metabolites (209 lipid and 166 polar) measurable in ≥80% of the sample were selected. Because untargeted metabolomics involve semiparametric methods, we focused on the statistical significance and the directionality of the associations rather than their magnitude. Figure 2 presents a scatterplot of MECV and IMTG, showing that the 2 were not correlated.

Figure 3 shows metabolites significantly associated with MECV and IMTG on sequential adjustment and FDR correction. Table S2, Supplemental Digital Content, http://links.lww.com/QAI/B980, presents the top 10 lipid and polar metabolites selected by the PLS procedure in relation to MECV, as well as corresponding linear regression analyses. Although 2 phosphatidylethanolamine (PE) species and 2 phosphatidylcholine (PC) species were significantly associated with MECV on FDR correction in the minimally adjusted model (Model 1), none of the top 10 lipid metabolites were significantly associated with this phenotype in the fully adjusted model (Model 3). For the top 10 polar metabolites, 4 met the FDR significance threshold in the minimally adjusted model, namely deoxyuridine, phenylacetylglutamine (PAG), kynurenic acid, and leucine. On additional adjustment, however, only PAG showed a significant FDR-corrected association—a positive one—with MECV in the main model.

The top 10 lipid and polar metabolites for IMTG are presented in Table S3, Supplemental Digital Content, http://links.lww.com/QAI/B981. Of the lipid metabolites, 3 PC species, 2 PE species, and 1 lysophosphatidylcholine (LPC) species were significantly associated with IMTG after FDR correction in the minimally adjusted model. After full adjustment, only the 3 PC species, each having a different carbon-bond configuration, were significantly associated—and that inversely—with IMTG on FDR correction. For the polar metabolites, there were 8 metabolites that exhibited FDR-corrected significant associations with IMTG. After full adjustment, 7 remained significantly associated with this phenotype after FDR correction. These included N-acetylaspartic acid and 1 LPC species, which showed inverse associations, as well as prolylglycine, methionine sulfoxide, sphingosine, taurine, and phosphorylcholine, which exhibited

TABLE 2. Associations of Covariates With Cardiac Magnetic Resonance Imaging Phenotypes

	Myocardial Extracellular Volume Fraction (%), n = 114		Intramyocardial Triglyceride Content (%), n = 136	
Variables	**Spearman ρ/Median (IQR)**	***P***	**Spearman ρ/Median (IQR)**	***P***
Age, yr	−0.07	0.436	0.01	0.936
Race/ethnicity		0.713		0.272
Non-Hispanic White	25.5 (24.4, 26.5)		0.44 (0.40, 0.76)	
Hispanic	26.4 (24.6, 28.4)		0.65 (0.37, 0.95)	
Non-Hispanic Black	26.7 (24.6, 28.2)		0.56 (0.47, 0.71)	
Other	24.9 (23.9, 25.9)		0.63 (0.46, 0.80)	
Site		0.859		0.838
Bronx	26.6 (24.6, 28.1)		0.50 (0.34, 0.88)	
Brooklyn	26.7 (24.8, 28.3)		0.53 (0.31, 0.75)	
Education		0.324		0.149
No schooling	26.9 (26.9, 26.9)		0.19 (0.19, 0.19)	
Grades 1–6	27.3 (26.9, 27.7)		1.01 (1.01, 1.01)	
Grades 7–11	26.7 (25.2, 28.4)		0.53 (0.28, 0.76)	
Completed high school	25.8 (24.0, 28.0)		0.47 (0.27, 0.72)	
Some college	25.5 (24.2, 28.3)		0.66 (0.39, 0.89)	
Completed 4 years of college	28.3 (28.2, 29.5)		0.41 (0.26, 0.50)	
Annual household income		0.101		0.947
$6000 or less	26.7 (26.4, 28.8)		0.76 (0.48, 0.92)	
$6001 to $12,000	26.7 (25.1, 28.4)		0.51 (0.25, 0.81)	
$12,001 to $18,000	24.8 (23.8, 27.1)		0.50 (0.35, 0.93)	
$18,001 to $24,000	25.5 (24.7, 26.2)		0.53 (0.36, 0.74)	
$24,001 to $30,000	27.0 (24.0, 27.6)		0.48 (0.36, 0.74)	
$30,001 to $36,000	28.4 (26.7, 30.7)		0.59 (0.38, 0.70)	
$36,001 to $75,000	25.3 (23.8, 27.9)		0.49 (0.32, 0.75)	
$75,001 and above	28.3 (26.2, 28.7)		0.41 (0.38, 0.90)	
BMI, kg/m^2	−0.20	0.031	0.04	0.690
Current smoker		0.023		0.512
Yes	27.3 (25.2, 29.3)		0.55 (0.30, 0.83)	
No	26.3 (24.3, 27.6)		0.49 (0.31, 0.75)	
Heavy alcohol use		0.402		0.557
Yes	27.3 (26.0, 29.0)		0.38 (0.26, 0.81)	
No	26.7 (24.6, 28.1)		0.52 (0.31, 0.82)	
History of injection drug use		0.813		0.274
Yes	26.7 (24.8, 28.3)		0.49 (0.28, 0.63)	
No	26.7 (24.6, 28.2)		0.54 (0.31, 0.85)	
History of heroin or cocaine use		0.101		0.497
Yes	26.9 (24.8, 28.7)		0.51 (0.24, 0.83)	
No	25.7 (24.1, 27.3)		0.52 (0.35, 0.80)	
Menopause status		0.197		0.551
Premenopausal	26.9 (26.3, 28.4)		0.47 (0.33, 0.80)	
Perimenopausal	26.4 (24.3, 29.1)		0.65 (0.38, 0.90)	
Postmenopausal	25.7 (24.4, 27.8)		0.51 (0.30, 0.81)	
Hypertension		0.189		0.056
Yes	26.5 (24.4, 28.1)		0.51 (0.30, 0.80)	
No	27.1 (25.7, 28.5)		0.75 (0.43, 1.03)	
Diabetes		0.534		0.602
Yes	26.8 (24.7, 28.9)		0.48 (0.30, 0.78)	
No	26.7 (24.4, 28.0)		0.53 (0.31, 0.85)	
Dyslipidemia		0.766		0.420
Yes	26.8 (24.6, 28.3)		0.51 (0.31, 0.81)	
No	26.6 (25.3, 27.1)		0.69 (0.35, 0.89)	

(*continued on next page*)

TABLE 2. (*Continued*) Associations of Covariates With Cardiac Magnetic Resonance Imaging Phenotypes

	Myocardial Extracellular Volume Fraction (%), n = 114		Intramyocardial Triglyceride Content (%), n = 136	
Variables	**Spearman ρ/Median (IQR)**	***P***	**Spearman ρ/Median (IQR)**	***P***
History of lipid-lowering therapy		0.952		0.541
Yes	26.7 (24.3, 28.6)		0.52 (0.32, 0.82)	
No	26.3 (25.0, 28.0)		0.52 (0.29, 0.82)	
History of myocardial infarction		0.137		0.868
Yes	28.8 (26.2, 30.1)		0.50 (0.26, 0.79)	
No	26.7 (24.6, 28.0)		0.52 (0.31, 0.82)	
History of heart failure		0.136		0.990
Yes	22.6 (22.6, 22.6)		0.52 (0.52, 0.52)	
No	26.7 (24.6, 28.3)		0.52 (0.31, 0.83)	
HIV status		0.898		0.437
Positive	26.7 (24.6, 28.1)		0.53 (0.31, 0.88)	
Negative	26.8 (24.2, 28.5)		0.50 (0.32, 0.78)	
HCV status		0.707		0.534
Positive	25.7 (24.6, 29.3)		0.48 (0.33, 0.79)	
Negative	26.8 (24.6, 28.1)		0.56 (0.31, 0.83)	
eGFR, mL/min/1.73 m^2	0.18	0.051	−0.06	0.509
LVEF, %	0	0.998	0.07	0.478
LVMI, g/m^2	0.06	0.556	−0.06	0.543

IQR, interquartile range; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index.

positive associations with IMTG. Characteristics obtained from the Human Metabolome Database[34] for these metabolites and those significantly associated with MECV are presented in Table S4, Supplemental Digital Content, http://links.lww.com/QAI/B982.

Sensitivity analyses adding education and income, the interval between plasma collection and CMR, or menopausal status to Model 3 covariates did not meaningfully affect the documented associations. Similarly, exclusion of non-Hispanic White participants or those with a history of MI or HF did not alter the findings. Addition of cross-product terms for metabolites and HIV status in the models revealed no evidence of interaction for any of the metabolites with significant associations (all $P \geq 0.215$). There was evidence of interaction by HCV status for 2 metabolites in relation to IMTG, wherein associations were limited to the HCV-negative group for taurine (HCV negative: β = 0.286, $P = 0.008$; HCV positive: β = −0.102, $P = 0.601$; $p_{interaction} = 0.004$) and phosphorylcholine (HCV negative: β = 0.234, $P = 0.030$; HCV positive: β = 0.099, $P = 0.598$; $p_{interaction} = 0.016$).

DISCUSSION

In this cohort of mostly middle-aged women with or at risk for HIV, who had low socioeconomic position, and were

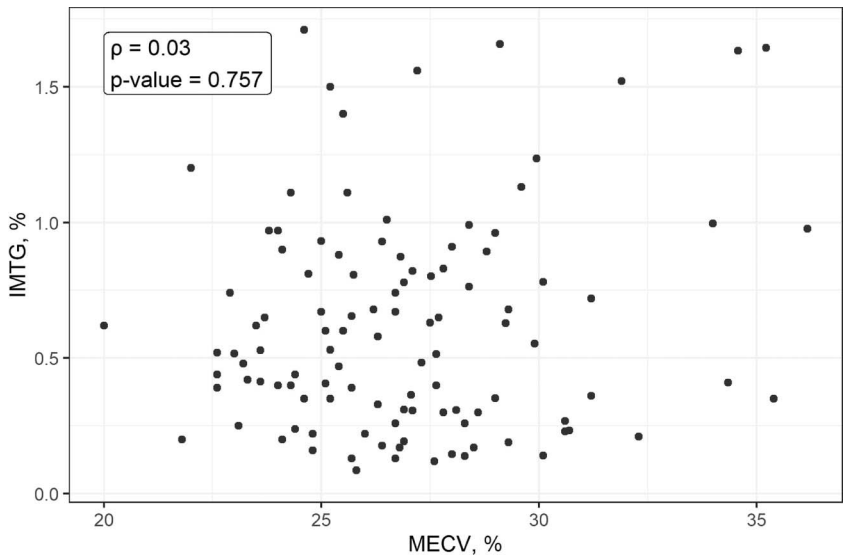

FIGURE 2. Scatter plot for MECV and IMTG.

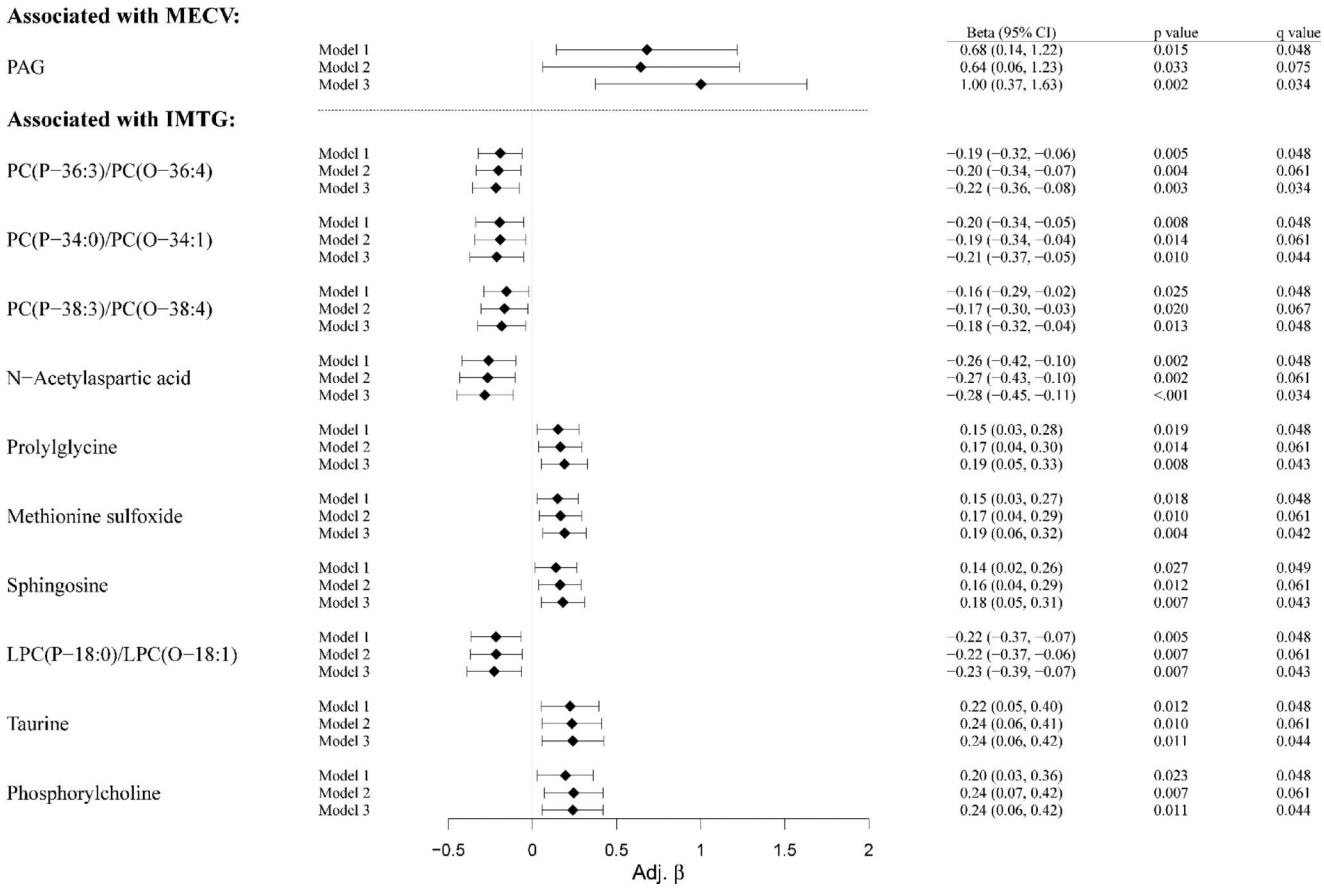

FIGURE 3. Risk estimates for associations of metabolites with MECV and log IMTG. Beta is per SD increment of each metabolite. Model 1 adjusts for age, race/ethnicity, and site. Model 2 adjusts for Model 1 covariates, BMI, injection drug use, heroin or cocaine use, smoking status, heavy alcohol use, HIV status, and HCV status. Model 3 adjusts for Model 2 covariates, history of MI, history of HF, hypertension, diabetes, dyslipidemia, and eGFR. CI=Confidence interval; PAG=Phenylacetylglutamine; PC=Phosphatidylcholine; SD=Standard deviation.

predominantly of non-Hispanic Black and Hispanic race/ethnicity, we evaluated the associations of plasma metabolomic species with CMR-determined intermediate phenotypes. We found that 1 polar metabolite was associated with MECV, while 3 lipid and 7 polar metabolites were associated with IMTG. Specifically, PAG was positively associated with MECV, as were prolylglycine, methionine sulfoxide, sphingosine, taurine, and phosphorylcholine with IMTG, while 3 PC species, 1 LPC species, and N-acetylaspartic acid were inversely associated with IMTG.

Several previous studies have used CMR to evaluate subclinical cardiac disease and dysfunction in people with HIV from high-income countries, documenting higher MECV and/or IMTG than in HIV-negative comparison groups.[35–39] Such reports have included studies of small samples of women[37] but have predominantly focused on men. Notably, these investigations have often drawn on healthy volunteers as controls or on comparators otherwise lacking the adverse behavioral (ie, smoking, alcohol, and drug use) and clinical risk factor profiles enriched in people with HIV.[35–37,39] By contrast, women without HIV in our sample showed a similarly high risk factor burden as those with HIV in crude comparisons, and cardiac phenotypic measures were likewise comparable in the 2 HIV serostatus groups. Our analyses did not detect evidence of effect modification by HIV in any of our metabolite associations with cardiac phenotypes, although only one-quarter of participants were HIV negative. Although detailed assessment of HIV in relation to cardiac phenotypes is beyond the scope of this report, it is probable that the closer risk factor profiles of the HIV serostatus groups led to more comparable metabolite CMR phenotype signatures between the 2.

Previous metabolomic studies of cardiac dysfunction or HF have focused almost entirely on general populations.[40,41] There are few data on metabolomic determinants of myocardial disease or dysfunction among people with HIV. To the best of our knowledge, this is the first study examining metabolomic profiles associated with CMR-determined MECV and IMTG, intermediate cardiac phenotypes for HF, in a cohort of women with or at risk for HIV.

PAG, a compound generated by gut microbes during metabolism of phenylalanine, has been associated with increased risk of atherosclerotic CVD.[42] PAG has been found to bind G-protein–coupled receptors, including adrenergic

receptors, leading to adverse CVD phenotypes. Engagement of such adrenergic receptors was shown to promote platelet hyperactivation, a potential mechanism underlying its link with atherothrombotic events.[42] Similar interaction of PAG with cardiac adrenoreceptors could account for the novel relationship documented here with diffuse myocardial interstitial fibrosis.

Three PC species from the lipid metabolites were found to be inversely associated with IMTG, as was the LPC species [LPC(P-18:0)/LPC(O-18:1)] captured by the polar platform. A previous metabolomic study found the levels of various PC and LPC species, including those identified here, to be significantly lower in patients with HFpEF compared with controls in unadjusted analyses.[41] Because increased IMTG, a measure of cardiomyocyte lipotoxicity, has been linked with HFpEF,[43] our results are consistent with previous findings.[41] The association with intramyocardial steatosis of lower PC levels, a marker of shift in choline metabolism toward less PC production,[41] is of interest as choline deficiency has been reported to cause cardiac dysfunction in animal models.[44]

N-acetylaspartic acid, a derivative of aspartic acid, has been implicated in experimental studies in mitochondrial energy expenditure, energy metabolism, and metabolic adaptation in brown adipose tissue.[45,46] This led to the proposition that components of the N-acetylaspartate pathway could be promising targets for antiobesity therapies. The inverse association between N-acetylaspartic acid and IMTG is notable in this context, newly relating this pathway to intramyocardial steatosis in women at high risk of CVD. Pending independent replication, this finding raises the possibility that therapeutic manipulation could have favorable effects on myocardial energetics and afford protection against HF onset in susceptible individuals.

Prolylglycine, a dipeptide product of protein degradation, is a bioactive signaling molecule that has been shown to promote insulin-like growth factor-1 (IGF-1) expression and secretion in mouse models.[47] A recent Mendelian randomization study found evidence that high IGF-1 levels may be causally associated with type 2 diabetes and coronary artery disease.[48] The positive association between prolylglycine and IMTG in our study is novel and was observed after adjustment for diabetes, but its underpinnings will require further investigation.

Methionine sulfoxide, a byproduct of oxidative stress that may modulate signal transduction pathways,[49] has been implicated in experimental models of myocardial injury[50] and was linked to abnormal diastology in a general population sample.[51] Taurine, a sulfur amino acid involved in bile acid and taurine metabolism pathways, has shown increased circulating levels in patients with HF as compared with controls, signaling increased protein turnover.[52] Our findings indicate that these relationships also hold for IMTG, with exploratory analyses of interaction raising questions about a counteracting effect of HCV. Because IMTG is closely associated with cardiac dysfunction,[36] these results should motivate further investigation as to whether related pathways may be viable targets for HF prevention.

Sphingosine is a 1,2-aminoalcohol involved in sphingolipid metabolism pathways and a precursor of sphingosine-1-phosphate.[53] Sphingolipids are both structural compounds and signaling molecules, and sphingosine-1-phosphate has been documented to have favorable effects on CVD.[53] Circulating sphingosine levels have not been linked to subclinical or clinical myocardial disease. How their positive association with IMTG, if confirmed, ties in with sphingosine-1-phosphate levels or other sphingolipid pathways will warrant additional study.

Finally, a previous study found that a metabolite score combining plasma metabolites from the choline pathway was associated with an increased risk of CVD.[54] The score included measures of 5 metabolites from the choline pathway including phosphorylcholine. The positive association in our study between phosphorylcholine and IMTG and the possible modifying effect of HCV suggest new avenues for investigation into the cardiometabolic consequences of this metabolite and associated pathways.

We recognize the following limitations. Although we subjected our analyses to FDR correction, our novel findings will require independent replication. The present cross-sectional, observational design cannot demonstrate causality, inference for which can be strengthened by longitudinal designs and Mendelian randomization approaches. Nor can the possibility of residual confounding be excluded. Circulating metabolites identified in these analyses reflect whole-body metabolism and are not necessarily cardiac specific. Plasma metabolomics was not concurrent and often post-dated CMR assessment, yet additional adjustment for the intervening period did not have an appreciable impact on the effect estimates. Given the low prevalence of self-reported HF, the likelihood that HF drove the metabolomic alterations, rather than vice versa, is low, but this will require testing in prospective studies. Our sample size is modest such that we lacked power to properly evaluate differences by HIV status or to examine metabolite profiles in subgroups defined by risk factor levels or medications. We lacked information on diet and physical activity, and their influence on metabolite levels or their relationships with cardiac phenotypes could not be evaluated. Such evaluations will require larger samples. The negative correlation between BMI and MECV suggests overdosing of gadolinium contrast relative to blood volume in this sample with prevalent obesity,[55] although our models adjusted for BMI. High dosing has a downward impact on MECV values, which along with their dependence on scanner, sequence, and timing characteristics, as emphasized by CMR guidelines, precludes comparability with ranges reported elsewhere. Finally, our study focuses on women with or at risk for HIV from predominantly race/ethnic minority groups and are not necessarily generalizable to other settings.

In conclusion, we identified 1 polar metabolite, PAG, to be positively associated with MECV. We also identified 3 phosphatidylcholine species with varying carbon-bond configurations, N-acetylaspartic acid, and 1 lysophosphatidylcholine species to be inversely associated with IMTG. We also identified prolylglycine, methionine sulfoxide, sphingosine, taurine, and phosphorylcholine to be positively associated with IMTG. The associations of PAG, methionine sulfoxide, taurine, phosphorylcholine and phosphatidylcholine, and sphingosine pathways with different cardiac phenotypes, although not with CMR-derived MECV and IMTG, were

previously known. The associations of PAG, N-acetylaspartic acid, and prolylglycine with CMR phenotypes are novel in nature. Taken together, our findings suggest new and plausible metabolic intermediates and pathways that may play a role in the development of diffuse myocardial interstitial fibrosis or intramyocardial steatosis. These results will require confirmation but suggest potential targets for manipulation in the search for effective approaches to HF prevention and treatment.

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