ORIGINAL RESEARCH

Novel Findings From a Metabolomics Study of Left Ventricular Diastolic Function: The Bogalusa Heart Study

Alexander C. Razavi, MPH; Lydia A. Bazzano, MD, PhD; Jiang He, MD, PhD; Camilo Fernandez, MD, MS; Seamus P. Whelton, MD, MPH; Marie Krousel-Wood, MD, MSPH; Shengxu Li, MD, PhD; Jovia L. Nierenberg, MPH; Mengyao Shi, MPH; Changwei Li, PhD; Xuenan Mi, MS; Jason Kinchen, PhD; Tanika N. Kelly, PhD, MPH

***Background*—**Diastolic dysfunction is one important causal factor for heart failure with preserved ejection fraction, yet the metabolic signature associated with this subclinical phenotype remains unknown.

***Methods and Results*—**Ultra-high-performance liquid chromatography–tandem mass spectroscopy was used to conduct untargeted metabolomic analysis of fasting serum samples in 1050 white and black participants of the BHS (Bogalusa Heart Study). After quality control, 1202 metabolites were individually tested for association with 5 echocardiographic measures of left ventricular diastolic function using multivariable-adjusted linear regression. Measures of left ventricular diastolic function included the ratio of peak early filling velocity to peak late filling velocity, ratio of peak early filling velocity to mitral annular velocity, deceleration time, isovolumic relaxation time, and left atrial maximum volume index (LAVI). Analyses adjusted for multiple cardiovascular disease risk factors and used Bonferroni-corrected alpha thresholds. Eight metabolites robustly associated with left ventricular diastolic function in the overall population and demonstrated consistent associations in white and black study participants. N-formylmethionine (B=0.05; $P=1.50\times10^{-7}$); 1-methylhistidine (B=0.05; $P=1.60\times10^{-7}$); formiminoglutamate (B=0.07; $P=5.60\times10^{-7}$); N2, N5-diacetylornithine (B=0.05; $P=1.30\times10^{-7}$); N-trimethyl 5-aminovalerate (B=0.04; $P=5.10\times10^{-6}$); 5-methylthioadenosine (B=0.04; $P=1.40\times10^{-5}$); and methionine sulfoxide (B=0.04; $P=3.80\times10^{-6}$) were significantly associated with the natural log of the ratio of peak early filling velocity to mitral annular velocity. Butyrylcarnitine (B=3.18; $P=2.10\times10^{-6}$) was significantly associated with isovolumic relaxation time.

***Conclusions*—**The current study identified novel findings of metabolite associations with left ventricular diastolic function, suggesting that the serum metabolome, and its underlying biological pathways, may be implicated in heart failure with preserved ejection fraction pathogenesis. **(*J Am Heart Assoc*. 2020;9:e015118. DOI: 10.1161/JAHA.119.015118.)**

Key Words: biomarker • diastolic function • epidemiology • heart failure • metabolomics

The prevalence of heart failure in the United States is projected to increase by over 40% to affect >8 million individuals by 2030.[1,2] Together with global epidemiologic trends, this estimated rise in prevalence classifies heart failure as the most rapidly increasing form of cardiovascular disease (CVD) in the United States and globally.[3] Among all heart failure cases, heart failure with preserved ejection fraction (HFpEF) is becoming the major disease subtype, presently responsible for at least 50% of all heart failure cases.[4,5] Such data underline a crucial need to characterize and study subclinical HFpEF phenotypes, which may not only allow for early HFpEF risk prediction but also lead to the development of low-risk interventions to delay or prevent heart failure onset, improving quality of life in an aging population.

Left ventricular diastolic dysfunction (LVDD), defined by impaired relaxation, decreased compliance, and/or increased

From the Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA (A.C.R., L.A.B., J.H., C.F., M.K.-W., J.L.N., M.S., X.M., T.N.K.); Department of Medicine, Tulane University School of Medicine, New Orleans, LA (A.C.R., L.A.B., J.H., C.F., M.K.-W.); The Ciccarone Center for the Prevention of Heart Disease, Johns Hopkins University School of Medicine, Baltimore, MD (S.P.W.); Children's Minnesota Research Institute, Children's Hospitals & Clinics of Minnesota, Minneapolis, MN (S.L.); Department of Epidemiology and Biostatistics, University of Georgia College of Public Health, Athens, GA (C.L.); Metabolon, Inc., Durham, NC (J.K.).

Accompanying Tables S1 through S11 and Figures S1 through S7 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.015118

Correspondence to: Tanika N. Kelly, PhD, MPH, Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, 1440 Canal Street, Suite 2000, New Orleans, LA 70112. E-mail: tkelly@tulane.edu

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Clinical Perspective

What Is New?

- The present study is the first population-based, untargeted metabolomic analysis of left ventricular diastolic function in a biracial (35% black, 65% white) community-based sample of middle-aged adults.
- We found that lipid- and amino acid–derived metabolites independently associate with patterns of mitral inflow and left ventricular relaxation in people without heart failure.

What Are the Clinical Implications?

- This hypothesis-generating study serves as an original groundwork to use metabolite biomarkers for the detection of subclinical heart failure in the general population.

left ventricular filling pressures, is one key subclinical precursor for HFpEF.[6] This upstream phenotype represents a potential inflection point on the HFpEF causal disease pathway, as evidence suggests that LVDD is reversible.[7,8] Molecular phenotyping may represent a cost-effective approach for identifying individuals with asymptomatic LVDD, while also implicating novel biological pathways in heart failure pathogenesis. Because metabolites reflect the collective output of both genetic and environmental factors[9] and share a proximal relationship with clinical phenotypes, metabolomics profiling represents a particularly powerful molecular tool for such precision medicine initiatives. Unfortunately, few studies have applied this methodology to the study of LVDD.[10,11] Given the fact that both impaired systemic and myocardial metabolism have been implicated in HFpEF pathogenesis,[12] there is a need to determine the role of systemic metabolites in left ventricular diastolic function (LVDF) to improve heart failure prevention.

The current study used metabolomics profiling and echocardiography to conduct a metabolome-wide association study of LVDF in a cross-sectional analysis of BHS (Bogalusa Heart Study) participants. We hypothesized that serum metabolites, particularly those involved in biological pathways involving fatty acid metabolism, would be associated with diastolic function.

Methods

The authors declare that all supporting data are available within the article and its online supplementary files.

Study Population

The BHS, seated in Bogalusa, Louisiana, is a population-based study examining the natural course of CVD across the life span. Between 1973 and 2016, 7 surveys of children aged 4 to 17 as well as 10 surveys of adults, who had been previously observed as children, were completed. The current BHS cohort includes 1298 participants, born between January 1959 and June 1979, who were examined twice in both childhood and adulthood for CVD risk factors and outcomes. Among these 1298 BHS participants, we selected the 1050 individuals who had echocardiographic, metabolomic, and respective covariable data measured contemporaneously at the most recent BHS study examination taking place from 2013 to 2016 (Figure S1). The current study sample (n=1050) was highly similar to the overall BHS sample (n=1298) with respect to sociodemographic characteristics and traditional CVD risk factors (Table S1). All study participants provided written informed consent at each examination, and study protocols were approved the Institutional Review Board of the Tulane University Health Sciences Center.

Echocardiography Protocol

Two-dimensional and tissue Doppler echocardiography were performed by trained cardiac sonographers at the BHS field office. Participants were placed in a partial left lateral decubitus position for echocardiographic assessment. Ten cycles of each 2-dimensional and Doppler signal were recorded. Echocardiographic recordings were accomplished using an Aplio 300 ultrasound instrument (Toshiba America Medical Systems, Tustin, CA) with a linear array transducer of 7.5 mHz using a standard protocol.[13,14] Diastolic function parameters assessed included early left ventricular filling peak velocity (E), late left ventricular filling peak velocity (A), medial mitral annular velocity (e'), deceleration time of the E wave (DT), isovolumic relaxation time (IVRT), and left atrial maximum volume index.[15] The apical 4-chamber view was used to assess left atrial volume as well as patterns of mitral inflow including, DT, E wave, A wave, and medial e' wave velocities. The E and A wave velocities were assessed using pulsed-wave Doppler echocardiography of transmitral flow at the mitral valve leaflet tips, while medial e' velocity was measured using pulsed-wave tissue Doppler echocardiography of the mitral annulus. The apical 5-chamber view was used to measure IVRT, specifically by placing the sample volume in the left ventricular outflow tract to concurrently evaluate aortic ejection and the onset of mitral inflow. Doppler sample volumes were placed between the mitral leaflet tips to measure DT. Left atrial volume was quantified by observing windows in which the left atrial length and transverse diameter were maximized. Left atrial volume was then divided by body surface area to estimate left atrial maximum volume index. Heart rate was assessed during the echocardiography protocol.

Metabolomic Profiling

Fasting serum samples were obtained between 2013 and 2016 from BHS participants and stored at −80°C prior to metabolite quantification. Metabolomic analysis of serum samples was consequently performed at Metabolon Inc (Durham, NC). Metabolites were quantified using ultra-high-performance liquid chromatography–tandem mass spectroscopy,[16] providing a comprehensive analysis of serum metabolites. Metabolites were identified by automated comparison of ion characteristics to a reference library of chemical standard records that include retention time, molecular weight (m/z), preferred adducts, and in-source fragments, as well as associated mass spectroscopy spectra. The majority of missing data in mass spectroscopy–based metabolomics experiments is due to detection limitations.[17] Thus, if a metabolite value was missing, we assumed that it was below the detection limit and assigned it the lowest detectable value in the data set for the corresponding metabolite. Recent metabolomics studies have employed a similar technique.[18,19]

Untargeted metabolomic analysis yielded 1466 metabolites, including 956 known biochemical compounds. These metabolites belonged to pathways related to amino acids (n=184), lipids (n=408), nucleotides (n=41), peptides (n=35), carbohydrates (n=25), cofactors and vitamins (n=34), xenobiotics (n=220), and energy (n=9). Furthermore, an additional 510 unnamed compounds presently lacking chemical standards were also identified. We excluded metabolites (n=213) detected in <20% of participants or with limited intra-assay reliability, defined by a reliability coefficient <0.30 (n=51) in duplicate samples. After completion of quality control procedures, 1202 metabolites remained for analysis. Among these 1202 metabolites, 167 were missing or under the detection limit in 50% to 80% of the samples. These latter metabolites were categorized and analyzed according to 3 mutually exclusive groups: (1) missing or below the detection limit; (2) below the median of measured values; and (3) greater than or equal to the median.[18,19] For the remaining 1035 metabolites, the minimum observed value of each analyte was imputed for below-the-detection-limit or missing values, with these metabolites examined as continuous variables.

Covariable Assessment

Rigorous protocols were employed to collect clinical data on BHS participants.[20] Validated questionnaires were used to obtain demographic and lifestyle variables, specifically, age, race, sex, cigarette smoking, alcohol consumption, and antihypertensive medications. Fasting measures of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and hemoglobin A_{1c} were collected using standardized methods.[20] Blood pressure was measured in triplicate, while height and weight were measured in duplicate at the time of the physical exam. Weight in kilograms was divided by height in meters squared to calculate body mass index. Serum creatinine was measured using the Kinetic Jaffe method. Serum creatinine was used to calculate estimated glomerular filtration rate via the Chronic Kidney Disease Epidemiology Collaboration equation.[21]

Statistical Analysis

BHS study sample characteristics were presented as means and SDs for continuous variables, and categorical variables were expressed as percentages. The Student *t* test and chi-square test were used to examine differences in normally distributed continuous variables and categorical variables, respectively, between blacks and whites. Normality of distribution was assessed via the Kolmogorov–Smirnov test. Both the E/A and E/e' ratios underwent natural logarithmic transformation to normalize the distribution of these outcome variables before the analysis.

Before association analyses, we standardized the concentrations of metabolites by dividing each metabolite by its respective SD. Each metabolite was individually tested for association with the diastolic function parameters using multivariable-adjusted linear regression models. In overall and race-stratified multivariable models, 16 covariables were adjusted for, including age, sex, race (when appropriate), education, cigarette smoking, alcohol drinking, average systolic blood pressure, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, serum triglycerides, hemoglobin A_{1c}, estimated glomerular filtration rate, heart rate, and antihypertensive medications. The analyses were corrected for multiple testing via the Bonferroni approach, with an alpha threshold of 4.16×10^{-5} (0.05/1202) used for determining statistical significance. To further reduce false-positive associations, only findings significant in the overall analysis and in at least 1 race group, while displaying consistent effect direction and nominal significance ($P<0.05$) in the other race group, were reported in the current analysis. We conducted Pearson pairwise correlation tests to assess the relationships between metabolites that achieved significance after Bonferroni correction ($P<4.16\times10^{-5}$).

Weighted correlation network analysis (WGCNA)[22] was employed to discover networks of highly correlated serum metabolites among BHS participants. WCGNA is an unsupervised data reduction technique, but unlike principal components analysis, it allows correlations between components, and thus may more effectively capture biological pathways underlying identified metabolites.[22,23] Previous metabolomic studies have used this data reduction technique.[22,24] In short, an adjacency matrix was generated to represent the

metabolite network based on weighted pairwise-correlations of all metabolites.[25] Densely interconnected metabolites, also referred to as modules, were identified using an unsupervised hierarchical clustering approach.[26] An eigenmetabolite was created for each metabolite module, representing the module's first principal component and can be interpreted as a weighted average value of correlated metabolites. Because there was similar metabolite clustering across ethnic groups, metabolite modules were generated among all study participants using data derived from the 1202 metabolites that passed quality control. To identify biological pathways underlying each module, metabolites most highly correlated with each module's eigenmetabolite were identified (r>0.70). We then used the subpathways associated with such metabolites to describe each module. For weighted correlation network analysis analyses, adjusted LVDF measures were created using the residual values generated by regressing each raw LVDF parameter on age, sex, race (when appropriate), education, cigarette smoking, alcohol drinking, average systolic blood pressure, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, serum triglycerides, hemoglobin A_{1c}, estimated glomerular filtration rate, heart rate, and antihypertensive medications. We then examined the Pearson correlations among each of the 9 metabolite modules and the residual LVDF values, which were independent of the covariable effects. We used a Bonferroni-corrected alpha threshold of 5.56×10^{-3} (0.05/9) to identify significant correlations among the 9 eigenmetabolite modules with the residual of each LVDF phenotype. Analogous to the single-metabolite analysis, modules were considered significant upon achieving significance in the overall population and in at least 1 race group, with nominal significance (P<0.05) and consistent effect direction in the other race group. Statistical analyses were performed in SAS (version 9.4; SAS Institute, Cary, NC) and R (version 3.4.3) software.

Sensitivity Analysis

Four post hoc sensitivity analyses were performed, one of which assessed the relationship of metabolites with diastolic function additionally adjusting for left ventricular mass, a variable hypothesized to be on the causal pathway of diastolic dysfunction. A second sensitivity analysis similarly evaluated the relationship of metabolites with diastolic function, after removing BHS participants (n=12) who self-reported previous myocardial infarction and/or a diagnosis of congestive heart failure. We also performed a sensitivity analysis additionally adjusting for gait speed, one surrogate marker of skeletal muscle breakdown, to help elucidate if significant endogenous metabolites related to myocyte turnover were of skeletal or cardiac origin. Finally, we conducted a sensitivity analysis adjusting for lifetime mean systolic blood pressure, as opposed to the systolic blood pressure measurement at the most recent examination, in a subsample of study participants. Study participants (n=804) who had at least 4 measures of systolic blood pressure from previous BHS exams were included, and these 4 measures were used to calculate a mean lifetime systolic blood pressure, which was included as a covariate in linear regression models.

Results

Table 1 presents the characteristics of the 1050 participants, including information on sociodemographic, lifestyle, medication, metabolic, and hemodynamic factors. The average age of the study cohort was 48 years, composed of 58% women and over one third black individuals. The study sample, on average, was obese (body mass index=31.38±7.80 kg/m^2) and prediabetic (hemoglobin A_{1c}=5.87±1.19), with evidence of dyslipidemia (low-density lipoprotein cholesterol=115.42±35.89 mg/dL), elevated systolic blood pressure (systolic blood pressure=123.32±16.69 mm Hg), and preserved kidney function (estimated glomerular filtration rate=93.34±17.18 mL/min per 1.73 m^2). There were significant differences between blacks and whites with respect to all covariables except for sex and resting heart rate.

LVDF of the study sample is also shown in Table 1. Compared with blacks, whites had statistically significant preserved diastolic function for patterns of mitral inflow, E/A (P=0.0227) and E/e' ratio (P<0.0001), left atrial maximum volume index (P<0.0001), and left ventricular relaxation, via IVRT (P<0.0001). On the other hand, whites had significantly higher values for DT (P=0.0418), compared with blacks.

There were 8 serum metabolites, 7 amino acid derived and 1 lipid derived, that were associated with diastolic function (Figure 1; Table 2). The 7 amino acid–derived metabolites were associated with the log-transformed E/e' ratio phenotype and represented several different biological pathways, including histidine, lysine, methionine, polyamine metabolism, and the urea cycle. Among E/e' ratio–related metabolites, formiminoglutamate (B=0.07; $P=5.60\times10^{-7}$) had the largest effect size, followed by N-formylmethionine (B=0.05; $P=1.50\times10^{-7}$); 1-methylhistidine (B=0.05; $P=1.60\times10^{-7}$); N2, N5-diacetylornithine (B=0.05; $P=1.30\times10^{-7}$); methionine sulfoxide (B=0.04; $P=3.80\times10^{-6}$); 5-methylthioadenosine (B=0.04; $P=1.40\times10^{-7}$); and N-trimethyl 5-amino-valerate (B=0.04; $P=5.10\times10^{-6}$). Butyrylcarnitine, a lipid-derived metabolite, was significantly associated with IVRT (B=3.18; $P=2.10\times10^{-6}$). Of note and with the exception of methionine sulfoxide, all significant associations of metabolites with the log-transformed E/e' ratio were driven by significance in blacks, with nominal significance in whites (Table S2). The reverse of this latter pattern was observed for the diastolic function parameter of IVRT.

Table 1. Characteristics of 1050 Bogalusa Metabolome Study Participants

Variable	All (n=1050)	Whites (n=684)	Blacks (n=366)	*P* Value*
Sociodemographic				
Female, %	57.81	56.14	60.93	0.1668
Post–high school education, %	49.90	56.58	37.43	<0.0001
Age, y, mean (SD)	48.13 (5.32)	48.46 (5.09)	47.52 (5.70)	0.0084
Lifestyle				
Smoking, n (%)				0.0352
Never	529 (50.38)	353 (51.61)	176 (48.09)	
Former	301 (28.67)	204 (29.82)	97 (26.50)	
Current	220 (20.95)	127 (18.57)	93 (25.41)	
Drinking, n (%)				0.0138
Never	116 (11.05)	60 (8.77)	56 (15.30)	
Former	332 (31.62)	219 (32.02)	113 (30.87)	
Current	602 (57.33)	405 (59.21)	197 (53.83)	
Metabolic				
BMI, kg/m^2, mean (SD)	31.38 (7.80)	30.4 (6.94)	33.34 (8.86)	<0.0001
LDL cholesterol, mg/dL, mean (SD)	115.42 (35.89)	117.20 (33.93)	112.08 (39.12)	0.0350
HDL cholesterol, mg/dL, mean (SD)	51.88 (16.30)	50.97 (16.36)	53.57 (16.06)	0.0133
Hemoglobin A_{1c}, %, mean (SD)	5.87 (1.19)	5.72 (0.91)	6.15 (1.54)	<0.0001
Serum triglycerides, mg/dL, mean (SD)	126.76 (68.15)	134.83 (71.48)	111.69 (58.61)	<0.0001
Hemodynamic				
Systolic BP, mm Hg, mean (SD)	123.32 (16.69)	121.13 (14.78)	127.42 (19.14)	<0.0001
Antihypertensive medication, n (%)	359 (34.19)	190 (27.78)	169 (46.17)	<0.001
eGFR, mL/min per 1.73 m^2, mean (SD)	93.34 (17.18)	90.71 (15.02)	98.26 (19.71)	<0.0001
Cardiac				
Resting heart rate, beats/min, mean (SD)	71.67 (11.42)	71.39 (11.09)	72.19 (12.01)	0.2782
E/A ratio (SD)†	1.12 (1.34)	1.14 (1.32)	1.08 (1.35)	0.0227
E/e' ratio (SD)†	7.54 (1.32)	7.24 (1.30)	8.00 (1.36)	<0.0001
Isovolumic relaxation time, ms (SD)	93.49 (22.36)	89.86 (21.26)	100.26 (22.79)	<0.0001
Deceleration time, ms (SD)	186.61 (45.15)	188.81 (44.28)	184.76 (42.72)	0.0418
Left atrial maximum volume index (SD)	28.85 (8.25)	28.10 (7.90)	30.26 (8.70)	<0.0001

BMI indicates body mass index; BP, blood pressure; E/A, peak early filling velocity to peak late filling velocity; E/e', peak early filling velocity to mitral annular velocity eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
**P* value for comparison between white vs. black participants.
†Presented as geometric mean and SD.

Associations of each metabolite with the diastolic function phenotypes, according to super pathway, are displayed in Figures S2 through S6. Such plots are depicted for the overall sample, blacks, and whites for each respective diastolic function outcome. These plots also provide the names of metabolites that were significant for diastolic function parameters in blacks or whites, but not in the overall sample, and thus shed light on potential race-specific associations of metabolites with LVDF.

Several metabolites that met stringent Bonferroni significance-level criteria for their association with E/e' ratio had nominally significant associations with other diastolic function phenotypes (Table 2). N-formylmethionine shared the most robust associations with LVDF, as this amino acid–derived metabolite was significantly associated with the log-transformed E/A ratio (B=0.04; $P=1.30\times10^{-5}$), and had a nominally significant association with left atrial maximum volume index (B=0.58; $P=4.70\times10^{-2}$). Methionine sulfoxide also had nominally significant associations with the log-transformed E/A ratio (B=0.03; $P=1.30\times10^{-3}$) and left atrial maximum volume index (B=0.55; 2.50×10^{-2}), although with

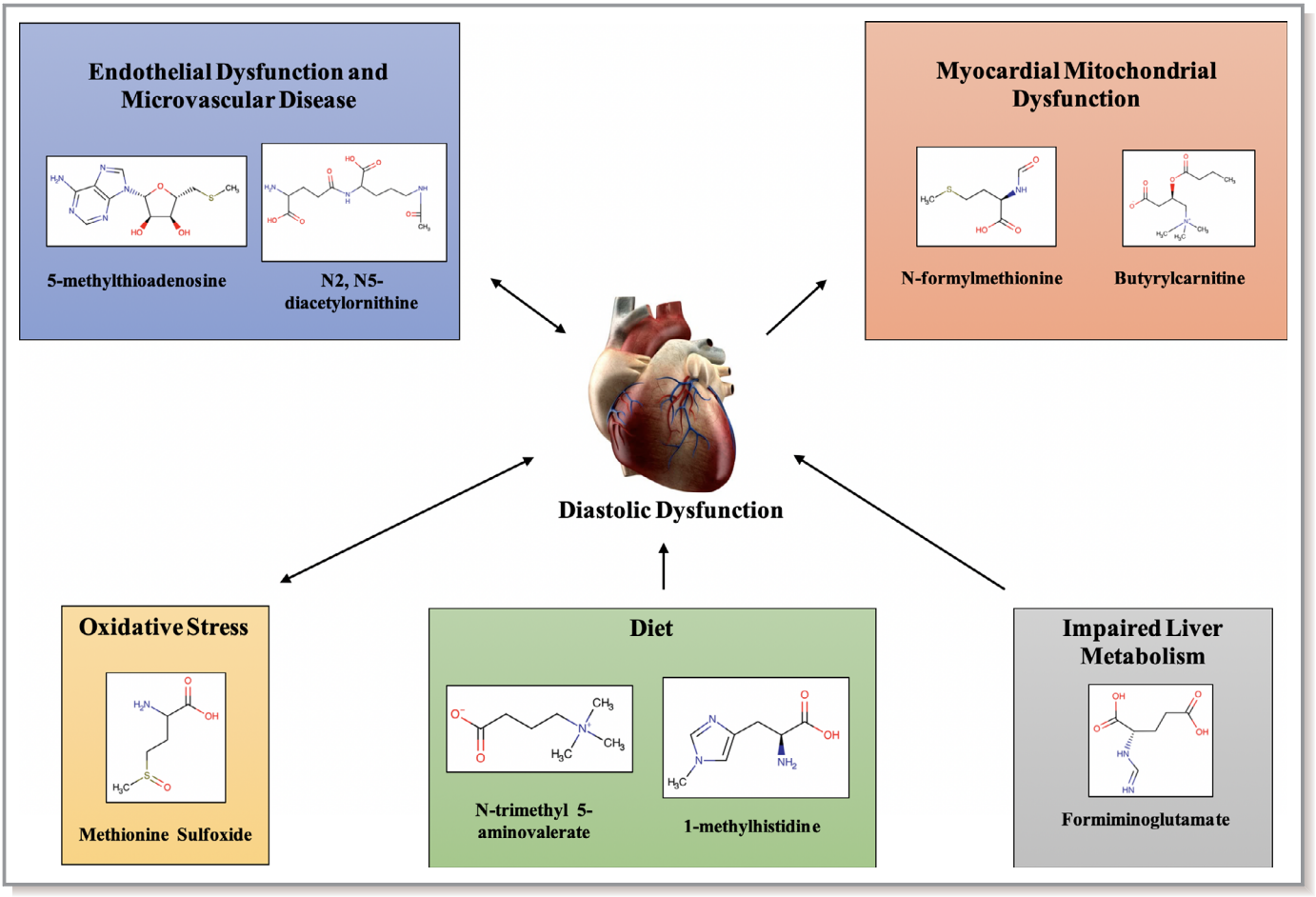

Figure 1. Proposed biological relationships between identified metabolites and left ventricular diastolic dysfunction. Arrows indicate proposed direction(s) of biological relationships.

lower parameter estimates when compared to N-formylmethionine. Finally, 3 metabolites, formiminoglutamate, N-trimethyl 5-amino-valerate, and N-trimethyl 5-amino-valerate, demonstrated nominally significant associations with both IVRT and the log-transformed E/A ratio.

Significant metabolites demonstrated modest to moderate intercorrelation (Figure 2). The highest pairwise correlation coefficients were observed between N-formylmethionine and 1-methylhistidine (r=0.64) and between N-formylmethionine and 5-methylthioadenosine (r=0.53). With respect to biological pathways, the 9 metabolite modules identified among study participants are presented in Tables S3 through S7. A module containing metabolites involved in primary and secondary bile acid metabolism demonstrated a significant positive correlation with E/e' in the overall population (r=0.16; P<0.0001) and in blacks (r=0.27; P<0.0001), with a consistent effect direction in whites (r=0.06; P=0.09) (Table S3). Figure S7 presents the Pearson correlations of the 8 metabolites that were most highly correlated with the primary and secondary bile acid metabolism eigenmetabolite value (r>0.70).

Results from sensitivity analyses are presented in Table S8, excluding individuals with self-report myocardial infarction or congestive heart failure; Table S9, adding left ventricular mass to the multivariable regression model; Table S10, adding gait speed to the multivariable regression model; and Table S11, adjusting for lifetime mean systolic blood pressure. Beta estimates and P values in all 3 sensitivity analyses were consistent with primary study results depicted in Table 2. N-formylmethionine maintained its effect size with the log-transformed E/e' phenotype when excluding individuals with self-report myocardial infarction or congestive heart failure (B=0.05; $P=2.60\times10^{-7}$), adjusting for mean lifetime systolic blood pressure (B=0.04; $P=4.10\times10^{-4}$), and when adding left ventricular mass (B=0.05 $P=4.80\times10^{-6}$) or gait speed (B=0.04; $P=3.90\times10^{-5}$) to the multivariable regression model. For each standardized unit increase in butyrylcarnitine, we observed a significant 3-ms higher IVRT across all sensitivity analyses, except for the sensitivity analysis that controlled for mean lifetime systolic blood pressure.

Table 2. Novel Metabolites Significantly Associated With Echocardiographic Measures of Diastolic Function

Metabolite	Sub-Pathway	Ln E/e' Ratio		Ln E/A Ratio		IVRT (ms)		DT (ms)		LAVI (mL/m^2)	
		β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Amino acid											
1-methylhistidine	Histidine metabolism	0.05 (0.01)	1.60E-07*	0.03 (0.01)	1.10E-03†	1.41 (0.75)	6.10E-02	1.35 (1.63)	4.10E-01	0.40 (0.32)	2.20E-01
Formiminoglutamate	Histidine metabolism	0.07 (0.01)	5.60E-07*	0.03 (0.01)	4.50E-02†	2.68 (1.13)	1.90E-02†	0.94 (2.46)	7.00E-01	−0.09 (0.47)	8.40E-01
5-methylthioadenosine	Polyamine metabolism	0.04 (0.01)	1.40E-05*	0.03 (0.01)	1.10E-03†	0.10 (0.74)	8.90E-01	2.18 (1.60)	1.70E-01	0.26 (0.28)	3.60E-01
N-trimethyl 5-amino-valerate	Lysine metabolism	0.04 (0.01)	5.10E-06*	0.02 (0.01)	2.00E-02†	1.91 (0.75)	1.10E-02†	0.15 (1.62)	9.30E-01	0.19 (0.28)	5.00E-01
N2, N5-diacetylornithine	Urea cycle; arginine and proline metabolism	0.05 (0.01)	1.30E-07*	0.03 (0.01)	8.50E-04†	1.62 (0.70)	2.10E-02†	1.67 (1.51)	2.70E-01	0.16 (0.31)	6.00E-01
N-formylmethionine	Methionine, cysteine, SAM, and taurine metabolism	0.05 (0.01)	1.50E-07*	0.04 (0.01)	1.30E-05*	1.00 (0.78)	2.00E-01	2.10 (1.69)	2.10E-01	0.58 (0.29)	4.70E-02†
Methionine sulfoxide	Methionine, cysteine, SAM, and taurine metabolism	0.04 (0.01)	3.80E-06*	0.03 (0.01)	1.30E-03†	0.55 (0.67)	4.10E-01	0.90 (1.44)	5.30E-01	0.55 (0.24)	2.50E-02†
Lipid											
Butyrylcarnitine	Fatty acid metabolism, BCAA metabolism	0.03 (0.01)	1.40E-04†	−0.01 (0.01)	2.60E-01	3.18 (0.67)	2.10E-06*	−1.69 (1.46)	2.50E-01	−0.33 (0.25)	1.80E-01

Adjusted for age, race, sex, education, smoking, alcohol drinking, antihypertensive medications, heart rate, estimated glomerular filtration rate, body mass index, systolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, serum triglycerides, and hemoglobin A_{1c}. E indicates peak velocity blood flow in early diastole due to gravity; e', medial mitral annular velocity; IVRT, isovolumic relaxation time; Ln, natural logarithm; SAM, s-adenosylmethionine.

*Statistically significant *P* value (*P*<4.16E-05).

†Nominally significant *P* value (*P*<0.05).

Discussion

While the increasing availability of noninvasive imaging methods has allowed for the assessment of LVDF in broader populations, the biochemical profile associated with this upstream heart failure (HF) phenotype remains largely unexplored. To address this critical gap in CVD prevention knowledge, we conducted one of the first epidemiologic studies that assesses the role of serum metabolites in LVDF, via an untargeted approach. Formiminoglutamate; 1-methylhistidine; N2, N5-diacetylornithine; N-trimethyl 5-aminovalerate; N-formylmethionine; 5-methylthioadenosine; and methionine sulfoxide were positively associated with the E/e' ratio, while butyrylcarnitine had a significant positive association with IVRT. In addition to single-metabolite analyses, a network of correlated metabolites involved in primary and secondary bile metabolism was associated with the E/e' ratio. These results build on previous smaller, targeted metabolomic studies of diastolic function[10,11] and suggest that a higher concentration of both lipid and amino acid–derived metabolites, particularly those associated with mitochondrial impairment, may serve as early biomarkers for preclinical LVDD. Given the diagnostic and therapeutic challenges associated with HFpEF,[27–29] findings from the current study may serve as a platform to develop more precise biological assays and risk stratification protocols for HFpEF.

Findings from our analysis suggest that amino acid–derived metabolites hold a close relationship with the E/e' ratio, one indicator of left ventricular filling.[30] The E/e' ratio has emerged as one promising echocardiographic parameter to help facilitate the diagnosis of HFpEF,[31] as evident by its presence in the H_2FpEF (F=filling pressure, Doppler echocardiographic E/e' >9 equates to 1 point) score clinically recommended to differentiate cardiac versus noncardiac causes of heart failure.[32] While IVRT has not been incorporated into diagnostic scoring criteria for HFpEF, increases in this cardiac time interval have been independently associated with risk of future ischemic heart disease, peripheral artery disease, and stroke.[33] A similar relationship has also been described for increases in E/e' and the risk of incident primary cardiac events.[34] Below, we discuss the relevance of each of the 8 robustly identified metabolites in LVDD and outline potential pathophysiological mechanisms underlying such associations by referencing previous work in this research space.

Butyrylcarnitine and N-Formylmethionine: Mitochondrial Impairment

We speculate that mitochondrial uncoupling is driving metabolic alterations in subclinical HF, such that asymptomatic

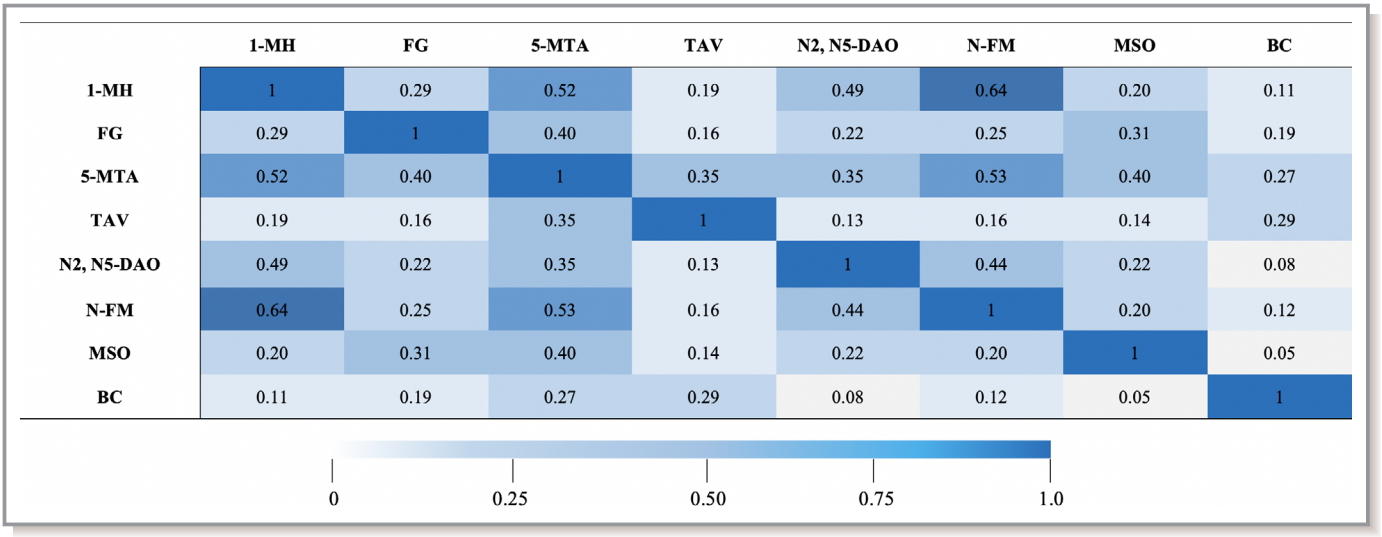

Figure 2. Heat map displaying pairwise correlation coefficients for metabolites significantly associated left ventricular diastolic function. 1-MH indicates 1-methylhistidine; 5-MTA, 5-methylthioadenosine; BC, butyrylcarnitine; FG, formiminoglutamate; MSO, methionine sulfoxide; N2, N5-DAO, N2, N5-diacetylornithine; N-FM, N-formylmethionine; TAV, N-trimethyl 5-aminovalerate.

LVDD is associated with mitochondrial impairment and leads to elevations in serum butyrylcarnitine and N-formylmethionine. In the present study, butyrylcarnitine and N-formylmethionine were positively associated with IVRT and E/e', respectively. Butyrylcarnitine is a member of the acylcarnitine family, a group of metabolites functioning in fatty acid metabolism, particularly in mitochondrial fatty acid beta oxidation.[35] Likewise, N-formylmethionine is an initiator of protein synthesis in the mitochondria of all eukaryotic cells.[36] Unlike other tissues and organs, fatty acids produce between 70% and 90% of ATP needed for heart function and metabolism.[37,38] Heart failure pathophysiology attributable to mitochondrial dysfunction is associated with both impaired fatty acid and glucose oxidation.[37–39] Our results support previous work in this research space, which has demonstrated that individuals with HF have a higher venous concentration of mitochondrial-dependent metabolites, succinate, 3-hydroxybutyrate, and acetone, compared with controls.[40] Notably, succinate, 3-hydroxybutyrate, and acetone share a positive association of myocardial energy metabolism, further underlining the central role of altered mitochondrial metabolism in HF.[40]

Mitochondrial impairment may also trigger apoptosis, causing cardiac myocyte N-formylmethionine to leak into circulation. These hypotheses are in part supported by research demonstrating that individuals with HFpEF have higher systemic concentrations of acylcarnitines compared with age-matched non–heart failure controls.[41,42] Similarly, a 4-component metabolite panel, including butyrylcarnitine, was found to better predict heart failure prognosis in individuals with stage B and stage C, when compared with brain natriuretic peptide.[43] Systemic N-formylmethionine release may occur after myocardial infarction,[44] suggesting that ischemia may be one contributing factor in cardiomyocyte mitochondrial impairment in the setting of LVDD. These latter results, together with the findings of the present study, highlight the potential diagnostic value of serum butyrylcarnitine and N-formylmethionine in LVDD and HFpEF.

1-Methylhistidine and N-Trimethyl 5-Aminovalerate: Diet

Exogenous environmental factors, specifically diet, have significant effects on the human metabolome.[45] Our findings demonstrated that 2 diet-associated metabolites, 1-methylhistidine and N-trimethyl 5-aminovalerate, were positively correlated with the E/e' ratio. Derived from dietary anserine sources, 1-methylhistidine has been reported as a marker of meat consumption, specifically red meat and chicken. Several previous studies have found robust linear relationships between meat intake and urinary 1-methylhistidine.[46,47] On the other hand, external evidence suggests that N-trimethyl 5-aminovalerate may be a metabolite indicator of dairy milk intake.[48,49] Importantly, restriction of both dairy and red meat consumption, via the Dietary Approaches to Stop Hypertension and Mediterranean diets, are associated with a reduced risk of developing heart failure.[50–52] While systolic blood pressure reduction may be the primary mechanism of the protective HF effects of these 2 diets, it is also important to consider other CVD risk factors in the setting of our metabolite findings. For example, N-trimethyl 5-aminovalerate positively associates with abdominal adiposity and intramuscular adiposity,[53] risk factors for insulin resistance, type 2 diabetes mellitus, and coronary microvascular dysfunction. Notably, type 2 diabetes

mellitus is a common comorbid disease that is present in up to one half of all patients with HFpEF.[54] Thus, our work serves as preliminary biological evidence that may help explain the potential benefits of animal protein restriction in atherosclerosis and HF risk reduction. However, future longitudinal studies that combine nutritional and metabolome data with diastolic function are undoubtedly required to understand and test such hypotheses and biological phenomena.

N2, N5-Diacetylornithine and 5-Methylthioadenosine: Endothelial Dysfunction

N2, N5-diacetylornithine is a metabolite by-product of the urea cycle, an important biological pathway to consider in the setting of HFpEF and diastolic function. Compared with individuals with HF with reduced ejection fraction, those with HFpEF have a higher chronic kidney disease–associated mortality rate.[54] Urea-to-creatinine ratio, and urinary albumin-to-creatinine ratio, biochemical indices of renal function, associate with parameters of LVDD.[55] Although BHS participants in the current study had preserved kidney function, it is possible that N2, N5-diacetylornithine levels begin to accumulate due to urea cycle perturbations in subclinical renal disease, which may be accompanied by systemic microvascular and endothelial dysfunction.[56]

Findings from our study lead to the postulation that subclinical LVDD attributable to underlying endothelial dysfunction is associated with impaired nitric oxide synthesis and may lead to an elevation of systemic polyamines particularly, 5-methylthioadenosine. Present in all mammalian tissues, 5-methylthioadenosine is a polyamine and sulfur-based nucleoside.[57] The 5-methylthioadenosine/methionine and polyamine pathways have previously been implicated in heart failure among a sample of 515 individuals.[43] Additionally, animal models of ventricular hypertrophy and aortic stenosis demonstrate increases in systemic polyamines after tissue remodeling.[58,59] Nitric oxide, a vasodilatory and protective metabolite in CVD, inhibits polyamine production.[60]

Methionine Sulfoxide: Oxidative Stress

Methionine sulfoxide reductase is a powerful antioxidant that reduces methionine sulfoxide[61] and has been implicated in vascular disease and cardiac ischemia.[62] We observed a significant positive association between methionine sulfoxide and left ventricular filling. Methionine oxide results from the oxidation of methionine and is produced from activated neutrophils. Present in both the cytoplasm and mitochondria, methionine sulfoxide may be released from cardiac myocyte turnover in the presence of LVDD or systemic endothelial dysfunction.

Formiminoglutamate: Impaired Liver Metabolism

Formiminoglutamate is an intermediate of histidine metabolism, and high levels of this metabolite have been traditionally used to identify vitamin B_{12} and/or folate deficiency, as well as underlying liver disease.[63,64] Such nutritional deficiencies have been hypothesized to contribute to anemia observed in patients with congestive HF, yet scarce data exist. Megaloblastic anemia caused by vitamin B_{12} and/or folate deficiency are relatively rare in heart failure[65,66]; therefore, the positive relationship identified between formiminoglutamate and E/e' may point more toward co-occurrence of subclinical liver disease, as opposed to anemia, in diastolic dysfunction and HF. Folate and vitamin B_{12} deficiency do not currently predict prognosis in heart failure[66]; therefore, our results should be considered only as hypothesis-generating observations and exploratory in nature. Furthermore, though our analyses adjusted for alcohol intake in BHS participants, regular alcohol consumption associates with inferior diastolic function[67] and leads to higher urinary excretion of formiminoglutamate.[64]

In addition to single-metabolite analyses, aggregation approaches also provided novel insights. Among metabolites with a significant relationship with LVDF, N-formylmethionine and 1-methylhistidine, as well as N-formylmethionine and 5-methylthioadenosine demonstrated moderate pairwise correlation. These results suggest that there may be an interaction between exogenous and endogenous pathways that influences the role of metabolites in diastolic dysfunction. Likewise, a correlated network of metabolites involved in primary and secondary bile acid metabolism significantly was associated with E/e' in the overall population and in blacks and had a consistent effect direction in whites. In addition to implicating the role of bile acid metabolites, such as glycohyocholate and taurocholate, with E/e', our results may further underline the importance of E/e' and left ventricular filling in the natural course of diastolic dysfunction and HFpEF. Our findings are consistent with and build on current knowledge regarding the role of bile acids in HF. The ratio of secondary to primary bile acids is increased in individuals with HF and this pattern has been associated with a reduced HF survival time.[68] Likewise, an increased level of serum bile acids, together with diastolic dysfunction has been well described in cirrhotic cardiomyopathy.[69]

Previous Mechanistic Studies: Branched Chain Amino Acid Metabolism

Much of the existing literature regarding the metabolomics of HF has assumed a biomarker-based approach, assessing the ability of metabolites to diagnose and predict disease prognosis. The identification of biomarkers associated with HF phenotypes, however, has also provided valuable mechanistic information on the pathophysiology of HFpEF. On the

whole, impaired fatty acid and glucose oxidation, urea cycle dysfunction, and anabolic-catabolic imbalance are main pathophysiological processes underlying HF.[70] Ventricular filling is an energy-intensive process,[71] and recent mechanistic studies have implicated branched chain amino acids (BCAAs), leucine, isoleucine, and valine, in the modulation of left ventricular function.[72–75] Branched-chain keto acid accumulation in cardiomyocytes has been documented in mitochondrial targeted 2C-type serine/threonine protein phosphatase knockout mice[75] as well as in humans with dilated cardiomyopathy.[74] On the other hand, animal models also suggest that supplementation with BCAA may improve cardiomyocyte survival and preserve ventricular function at middle age.[73] Thus, while a broad physiological range of circulating BCAA concentrations may exist, these amino acids may become detrimental when BCAA catabolism is impaired in subclinical or overt HF. Hypertrophic hearts in particular exhibit a reduction in mitochondrial targeted 2C-type serine/threonine protein phosphatase mRNA and protein expression,[75] and future longitudinal omics studies of mitochondrial targeted 2C-type serine/threonine protein phosphatase and LVDD may be valuable to further elucidate the biological relationships of BCAA metabolism, mitochondrial function, and diastolic function. We found that butyrylcarnitine, a lipid metabolite involved in BCAA metabolism, had a positive association with IVRT; an association that warrants further investigation.

Conclusions

This study had several important strengths. To our knowledge, this is one of the first metabolome-wide association studies of LVDF. Furthermore, identified metabolites demonstrated consistent associations with diastolic function across race groups and in sensitivity analyses adjusting for left ventricular mass and gait speed and excluding individuals with self-reported myocardial infarction and/or congestive HF. On the other hand, our study was not without limitations. Its cross-sectional design prohibits temporal inferences. Nevertheless, metabolites reported here may serve as biomarkers for LVDD, making them particularly relevant for future metabolomics studies involving HFpEF. Furthermore, to minimize false-positive findings, only metabolites that demonstrated consistent associations with diastolic function across race groups were reported here. Therefore, our study may miss race-specific metabolite–diastolic function associations. To address this limitation, we reported all significant race-specific metabolites in Figures S2 through S4, to inform future studies and research regarding the potential effect modifying role of race on the association of metabolites and LVDF.

In conclusion, we have observed that lipid- and amino acid–derived metabolites associate with LVDF in a racially diverse cohort of middle-aged adults. Butyrylcarnitine was positively correlated with left ventricular relaxation, while N-formylmethionine; formiminoglutamate; N2, N5-diacetylornithine; N-trimethyl 5-aminovalerate; 5-methylthioadenosine; and methionine sulfoxide had positive associations with left ventricular filling pressure. Such metabolites may serve as early biomarkers for subclinical HFpEF in the general population. Future replication and longitudinal studies are required to further understand the role of serum metabolites in LVDF and progression to HFpEF.

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Disclosures

Dr Kinchen, who contributed to metabolomics data collection, is employed by Metabolon Inc and did not participate in the study design, statistical analysis, or scientific interpretation of the current research. The remaining authors have no disclosures to report.

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SUPPLEMENTAL MATERIAL

Table S1. Comparison of Bogalusa Heart Study Samples.

Variable		Full BHS Sample (n=1,298)	Current Study Sample (n=1,050)
Sociodemographic			
Black, %		34.10	34.86
Female, %		58.86	57.81
Post-high school education, %		49.03	49.90
Age, y, mean (SD)		48.15 (5.24)	48.13 (5.32)
Lifestyle			
Smoking, %			
	Never	50.04	50.38
	Former	29.74	28.67
	Current	19.72	20.95
Drinking, %			
	Never	12.39	116 (11.05)
	Former	31.84	332 (31.62)
	Current	55.77	602 (57.33)
Metabolic			
BMI, kg/m2, mean (SD)		31.44 (7.80)	31.38 (7.80)
LDL Cholesterol, mg/dL, mean (SD)		114.53 (35.49)	115.42 (35.89)
HDL Cholesterol, mg/dL, mean (SD)		51.44 (16.29)	51.88 (16.30)
Hemoglobin A1c, %, mean (SD)		5.91 (1.25)	5.87 (1.19)
Hemodynamic			
eGFR, mL/min/1.73m2, mean (SD)		93.73 (16.90)	93.34 (17.18)
Systolic BP, mmHg, mean (SD)		123.64 (17.27)	123.32 (16.69)
Anti-hypertensive medication, (%)		35.05	34.19

BMI = body mass index; BP = blood pressure; eGFR = estimated glomerular filtration rate; HDL = high density lipoprotein cholesterol; LDL = low density lipoprotein cholesterol; mmHg = millimeters of Mercury; SD = standard deviation.

Table S2. Novel Metabolites Significantly Associated with Echocardiographic Measures of Diastolic Function, Stratified by Race.

Metabolite	Sub-Pathway	Ln E/e' Ratio		Ln E/A Ratio		IVRT (ms)		DT (ms)		LAVI (mL/m2)	
		Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
White (n=684)											
Amino Acid											
1-methylhistidine	Histidine Metabolism	0.03 (0.01)	2.30E-02†	0.01 (0.01)	4.20E-01	1.69 (1.12)	1.30E-01	6.13 (2.41)	1.10E-02†	0.64 (0.40)	1.10E-01
Formiminoglutamate	Histidine Metabolism	0.04 (0.02)	2.10E-02†	-4.0E-03 (0.02)	8.30E-01	2.99 (1.56)	5.60E-02	7.39 (3.36)	2.80E-02†	0.13 (0.56)	8.10E-01
5-methylthioadenosine	Poylamine Metabolism	0.02 (0.01)	3.50E-02†	0.02 (0.01)	1.00E-01	0.53 (0.96)	5.80E-01	1.37 (2.06)	5.10E-01	0.53 (0.34)	1.20E-01
N-trimethyl 5-amino-valerate	Lysine Metabolism	0.02 (0.01)	4.50E-02†	4.5E-03 (0.01)	6.60E-01	2.08 (0.87)	1.70E-02†	-0.26 (1.88)	8.90E-01	0.19 (0.31)	5.40E-01
N2, N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	0.04 (0.02)	2.40E-02†	0.02 (0.02)	1.60E-01	1.27 (1.44)	3.80E-01	2.91 (3.11)	3.50E-01	0.83 (0.52)	1.10E-01
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.03 (0.01)	2.00E-02†	0.04 (0.01)	2.50E-03†	0.59 (1.04)	5.70E-01	3.85 (2.24)	8.60E-02	0.58 (0.37)	1.20E-01
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.05 (0.01)	2.10E-05*	0.04 (0.01)	3.50E-04†	1.07 (0.91)	2.40E-01	1.10 (1.97)	5.80E-01	0.82 (0.33)	1.10E-02†
Lipid											
Butyrylcarnitine	Fatty Acid Metabolism, BCAA Metabolism	0.03 (0.01)	6.60E-04†	-0.01 (0.01)	1.40E-01	2.93 (0.69)	2.40E-05*	-2.17 (1.50)	1.50E-01	-0.26 (0.25)	3.00E-01
Black (n=366)											
Amino Acid											
1-methylhistidine	Histidine Metabolism	0.07 (0.01)	9.10E-07*	0.05 (0.01)	3.70E-04†	1.00 (1.06)	3.50E-01	-1.66 (2.28)	4.70E-01	0.04 (0.55)	9.40E-01
Formiminoglutamate	Histidine Metabolism	0.10 (0.02)	2.20E-05*	0.06 (0.02)	4.00E-03†	2.31 (1.72)	1.80E-01	-5.88 (3.70)	1.10E-01	-0.32 (0.86)	7.10E-01
5-methylthioadenosine	Poylamine Metabolism	0.07 (0.02)	1.40E-05*	0.05 (0.02)	1.00E-03†	0.04 (1.22)	9.70E-01	3.30 (2.62)	2.10E-01	0.13 (0.51)	7.90E-01
N-trimethyl 5-amino-valerate	Lysine Metabolism	0.10 (0.02)	1.30E-07*	0.07 (0.02)	3.20E-04†	1.59 (1.48)	2.80E-01	0.50 (3.19)	8.80E-01	0.21 (0.62)	1.30E-01
N2, N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	0.05 (0.01)	6.40E-06*	0.03 (0.01)	4.60E-03†	1.53 (0.84)	6.70E-02	1.81 (1.80)	3.20E-01	-0.05 (0.40)	8.90E-01
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.08 (0.02)	1.50E-07*	0.05 (0.02)	1.80E-03†	1.50 (1.22)	2.20E-01	1.04 (2.63)	2.20E-01	0.65 (0.47)	6.90E-01
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.03 (0.01)	3.40E-02†	0.02 (0.01)	2.30E-01	-0.13 (1.01)	9.00E-01	0.11 (2.18)	9.60E-01	0.28 (0.38)	4.60E-01
Lipid											
Butyrylcarnitine	Fatty Acid Metabolism, BCAA Metabolism	0.09 (0.03)	4.70E-03†	0.04 (0.03)	1.80E-01	5.79 (2.48)	2.00E-02†	1.68 (5.38)	7.60E-01	-0.65 (1.01)	5.20E-01

A = peak velocity blood flow in late diastole; E = peak velocity blood flow in early diastole; e'=medial mitral annular velocity; IVRT = isovolumic relaxation time; Ln = natural logarithm; SAM=s-adenosylmethionine; SE = standard error.

* = statistically significant p-value (p<4.16E-05); † = statistically significant p-value (p<0.05)

Adjusted for age, sex, education, smoking, alcohol drinking, antihypertensive medications, heart rate, eGFR, BMI, SBP, LDL-C, HDL-C, serum triglycerides, and HbA1c.

Table S3. Correlations of nine metabolite modules from WGCNA analysis with the E/e' ratio.

Sub-pathways for metabolites most highly correlated with each module (r > 0.7)	Overall r (P-value)	White r (P-value)	Black r (P-value)
Lysophospholipid; Phosphatidylethanolamine; Phosphatidylcholine; Moonoacylglycerol; Diacylglycerol	0.05 (0.09)	0.08 (0.04)	0.03 (0.63)
Fatty acid metabolism (acyl choline); Lysophospholipid	0.01 (0.84)	0.04 (0.29)	-0.04 (0.46)
Long chain fatty acid; Polyunsaturated fatty acid (n3 and n6); Fatty acid, monohydroxy; Fatty Acid, branched; Medium chain fatty acid; Endocannabinoid	0.01 (0.69)	-0.02 (0.61)	0.05 (0.34)
Sphingolipid metabolism	-0.03 (0.34)	-0.04 (0.25)	-0.01 (0.87)
Primary bile acid metabolism; Secondary bile acid metabolism	0.16 (<0.0001)	0.06 (0.09)	0.27 (<0.0001)
Methionine, cysteine, SAM and taurine, polyamine metabolism; Tryptophan, tyrosine, lysine, histidine metabolism, and other amino acid; Amino-sugar, pentose metabolism; Purine and pyrimidine metabolism, Acetylated peptides; Mevalonate metabolism, fatty acid, dicarboxylate, and androgenic steroids, Food component, benzoate metabolism, and other xenobiotics	0.08 (0.01)	0.03 (0.37)	0.11 (0.03)
Food component/Plant	0.08 (0.01)	0.05 (0.15)	0.11 (0.03)
Androgenic steroids	-0.04 (0.23)	0.00 (0.92)	-0.08 (0.13)
Androgenic steroids; Pregnenolone steroids; Progestin steroids	-0.05 (0.14)	-0.03 (0.44)	-0.05 (0.33)

E = peak velocity blood flow in early diastole; e' = medial mitral annular velocity; r = correlation coefficient; SAM=s-adenosylmethionine.

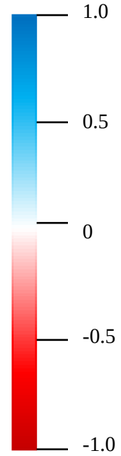


Table S4. Correlations of nine metabolite modules from WGCNA analysis with the E/A ratio.

Sub-pathways for metabolites most highly correlated with each module (r > 0.7)	Overall r (P-value)	White r (P-value)	Black r (P-value)
Lysophospholipid; Phosphatidylethanolamine; Phosphatidylcholine; Moonoacylglycerol; Diacylglycerol	0.04 (0.25)	0.04 (0.25)	0.02 (0.67)
Fatty acid metabolism (acyl choline); Lysophospholipid	0.02 (0.49)	0.02 (0.53)	0.02 (0.74)
Long chain fatty acid; Polyunsaturated fatty acid (n3 and n6); Fatty acid, monohydroxy; Fatty Acid, branched; Medium chain fatty acid; Endocannabinoid	-0.05 (0.07)	-0.09 (0.01)	-0.01 (0.90)
Sphingolipid metabolism	-0.02 (0.58)	-0.03 (0.44)	0.01 (0.80)
Primary bile acid metabolism; Secondary bile acid metabolism	0.04 (0.16)	0.01 (0.89)	0.10 (0.05)
Methionine, cysteine, SAM and taurine, polyamine metabolism; Tryptophan, tyrosine, lysine, histidine metabolism, and other amino acid; Amino-sugar, pentose metabolism; Purine and pyrimidine metabolism, Acetylated peptides; Mevalonate metabolism, fatty acid, dicarboxylate, and androgenic steroids, Food component, benzoate metabolism, and other xenobiotics	0.05 (0.11)	0.04 (0.31)	0.06 (0.28)
Food component/Plant	0.05 (0.11)	0.03 (0.45)	0.07 (0.17)
Androgenic steroids	-0.01 (0.65)	0.00 (0.99)	-0.04 (0.49)
Androgenic steroids; Pregnenolone steroids; Progestin steroids	-0.04 (0.17)	-0.06 (0.09)	-0.01 (0.91)

A = peak velocity blood flow in late diastole; E = peak velocity blood flow in early diastole; r = correlation coefficient; SAM= s-adenosylmethionine.

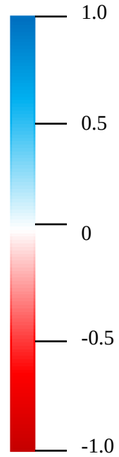


Table S5. Correlations of nine metabolites modules from WGCNA analysis with isovolumic relaxation time (IVRT).

Sub-pathways for metabolites most highly correlated with each module (r > 0.7)	Overall r (P-value)	White r (P-value)	Black r (P-value)
Lysophospholipid; Phosphatidylethanolamine; Phosphatidylcholine; Moonoacylglycerol; Diacylglycerol	0.01 (0.77)	0.01 (0.85)	0.00 (0.98)
Fatty acid metabolism (acyl choline); Lysophospholipid	-0.02 (0.54)	-0.02 (0.67)	-0.05 (0.38)
Long chain fatty acid; Polyunsaturated fatty acid (n3 and n6); Fatty acid, monohydroxy; Fatty Acid, branched; Medium chain fatty acid; Endocannabinoid	-0.03 (0.28)	-0.01 (0.72)	-0.07 (0.20)
Sphingolipid metabolism	0.01 (0.74)	0.00 (0.95)	0.02 (0.66)
Primary bile acid metabolism; Secondary bile acid metabolism	-0.01 (0.63)	-0.03 (0.43)	0.01 (0.88)
Methionine, cysteine, SAM and taurine, polyamine metabolism; Tryptophan, tyrosine, lysine, histidine metabolism, and other amino acid; Aminosugar, pentose metabolism; Purine and pyrimidine metabolism, Acetylated peptides; Mevalonate metabolism, fatty acid, dicarboxylate, and androgenic steroids, Food component, benzoate metabolism, and other xenobiotics	0.02 (0.45)	0.03 (0.48)	0.01 (0.81)
Food component/Plant	0.00 (0.95)	0.00 (0.92)	0.01 (0.81)
Androgenic steroids	0.01 (0.74)	0.03 (0.41)	-0.01 (0.81)
Androgenic steroids; Pregnenolone steroids; Progestin steroids	-0.02 (0.49)	0.00 (0.89)	0.04 (0.42)

r = correlation coefficient; SAM=s-adenosylmethionine

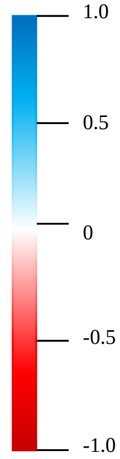


Table S6. Correlations of nine metabolite modules from WGCNA analysis with deceleration time (DT).

Sub-pathways for metabolites most highly correlated with each module (r > 0.7)	Overall r (P-value)	White r (P-value)	Black r (P-value)
Lysophospholipid; Phosphatidylethanolamine; Phosphatidylcholine; Moonoacylglycerol; Diacylglycerol	0.01 (0.71)	0.00 (0.94)	0.04 (0.46)
Fatty acid metabolism (acyl choline); Lysophospholipid	0.04 (0.22)	0.03 (0.43)	0.05 (0.35)
Long chain fatty acid; Polyunsaturated fatty acid (n3 and n6); Fatty acid, monohydroxy; Fatty Acid, branched; Medium chain fatty acid; Endocannabinoid	0.02 (0.47)	0.00 (0.99)	0.06 (0.24)
Sphingolipid metabolism	-0.02 (0.45)	-0.05 (0.16)	0.05 (0.36)
Primary bile acid metabolism; Secondary bile acid metabolism	0.05 (0.12)	0.06 (0.11)	0.01 (0.80)
Methionine, cysteine, SAM and taurine, polyamine metabolism; Tryptophan, tyrosine, lysine, histidine metabolism, and other amino acid; Aminosugar, pentose metabolism; Purine and pyrimidine metabolism, Acetylated peptides; Mevalonate metabolism, fatty acid, dicarboxylate, and androgenic steroids, Food component, benzoate metabolism, and other xenobiotics	0.02 (0.46)	0.06 (0.10)	0.01 (0.90)
Food component/Plant	-0.07 (0.82)	-0.01 (0.87)	-0.01 (0.85)
Androgenic steroids	-0.01 (0.78)	-0.01 (0.76)	0.02 (0.74)
Androgenic steroids; Pregnenolone steroids; Progestin steroids	0.02 (0.46)	-0.02 (0.68)	0.07 (0.19)

r = correlation coefficient; SAM=s-adenosylmethionine

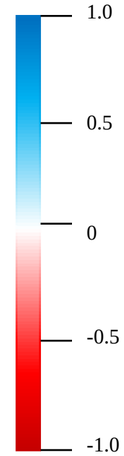


Table S7. Correlations of nine metabolite modules with left atrial maximum volume index (LAVI).

Sub-pathways for metabolites most highly correlated with each module (r > 0.7)	Overall r (P)	White r (P)	Black r (P)
Lysophospholipid; Phosphatidylethanolamine; Phosphatidylcholine; Moonoacylglycerol; Diacylglycerol	0.04 (0.18)	0.05 (0.21)	0.04 (0.48)
Fatty acid metabolism (acyl choline); Lysophospholipid	0.03 (0.28)	0.06 (0.14)	-0.01 (0.87)
Long chain fatty acid; Polyunsaturated fatty acid (n3 and n6); Fatty acid, monohydroxy; Fatty Acid, branched; Medium chain fatty acid; Endocannabinoid	0.01 (0.91)	0.08 (0.03)	-0.12 (0.02)
Sphingolipid metabolism	-0.03 (0.38)	0.01 (0.89)	-0.08 (0.15)
Primary bile acid metabolism; Secondary bile acid metabolism	0.04 (0.17)	0.08 (0.04)	-0.02 (0.75)
Methionine, cysteine, SAM and taurine, polyamine metabolism; Tryptophan, tyrosine, lysine, histidine metabolism, and other amino acid; Aminosugar, pentose metabolism; Purine and pyrimidine metabolism, Acetylated peptides; Mevalonate metabolism, fatty acid, dicarboxylate, and androgenic steroids, Food component, benzoate metabolism, and other xenobiotics	0.00 (0.98)	0.03 (0.42)	-0.02 (0.69)
Food component/Plant	-0.03 (0.42)	0.01 (0.89)	-0.07 (0.18)
Androgenic steroids	-0.05 (0.10)	-0.05 (0.17)	-0.03 (0.34)
Androgenic steroids; Pregnenolone steroids; Progestin steroids	-0.03 (0.39)	-0.02 (0.60)	-0.02 (0.66)

r = correlation coefficient; SAM=s-adenosylmethionine

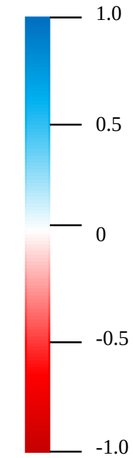


Table S8. Novel Metabolites Significantly Associated with Echocardiographic Measures of Diastolic Function in 1,035 BHS Participants, Excluding Individuals with CHF or MI .

Metabolite	Sub-Pathway	Ln E/e' Ratio		Ln E/A Ratio		IVRT (ms)		DT (ms)		LAVI (mL/m2)	
		Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Amino Acid											
1-methylhistidine	Histidine Metabolism	0.05 (0.01)	1.50E-07*	0.03 (0.01)	1.70E-03†	1.40 (0.76)	6.50E-02	1.24 (1.64)	4.50E-01	0.39 (0.32)	2.30E-01
Formiminoglutamate	Histidine Metabolism	0.05 (0.01)	4.60E-07*	0.04 (0.01)	4.10E-02†	1.03 (0.79)	2.00E-02†	2.04 (1.71)	6.90E-01	0.58 (0.29)	9.70E-01
5-methylthioadenosine	Poylamine Metabolism	0.04 (0.01)	3.20E-05*	0.03 (0.01)	2.30E-03†	0.17 (0.75)	8.20E-01	2.08 (1.62)	2.00E-01	0.26 (0.28)	3.70E-01
N-trimethyl 5-amino-valerate	Lysine Metabolism	0.04 (0.01)	4.30E-06*	0.02 (0.01)	2.20E-02†	1.84 (0.75)	1.50E-02†	0.06 (1.63)	9.70E-01	0.19 (0.28)	5.00E-01
N2, N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	0.05 (0.01)	2.60E-07*	0.02 (0.01)	1.70E-03†	1.68 (0.71)	1.70E-02†	1.48 (1.53)	3.30E-01	0.12 (0.31)	7.00E-01
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.05 (0.01)	2.60E-07*	0.04 (0.01)	2.40E-05*	1.04 (0.80)	2.00E-01	2.04 (1.71)	2.30E-01	0.58 (0.29)	4.90E-02*
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.04 (0.01)	7.10E-06*	0.03 (0.01)	1.70E-03†	0.62 (0.67)	3.60E-01	0.80 (1.46)	5.80E-01	0.56 (0.24)	2.30E-02†
Lipid											
Butyrylcarnitine	Fatty Acid Metabolism, BCAA Metabolism	0.03 (0.01)	3.00E-01	0.00 (0.01)	1.10E-04†	3.27 (0.67)	1.30E-06*	-1.57 (1.47)	2.90E-01	-0.34 (0.25)	1.70E-01

A = peak velocity blood flow in late diastole; E = peak velocity blood flow in early diastole; e'=medial mitral annular velocity; IVRT = isovolumic relaxation time; Ln = natural logarithm; SAM=s-adenosylmethionine; SE = standard error. * = statistically significant p-value (p<4.16E-05); † = nominally significant p-value (p<0.05)

1. Adjusted for age, race, sex, education, smoking, alcohol drinking, antihypertensive medications, heart rate, eGFR, BMI, SBP, LDL-C, HDL-C, serum triglycerides, and HbA1c.

Table S9. Novel Metabolites Significantly Associated with Echocardiographic Measures of Diastolic Function in 1,050 BHS Participants, Additionally Adjusting for Left Ventricular Mass .

Metabolite	Sub-Pathway	Ln E/e' Ratio		Ln E/A Ratio		IVRT (ms)		DT (ms)		LAVI (mL/m2)	
		Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Amino Acid											
1-methylhistidine	Histidine Metabolism	0.04 (0.01)	3.40E-06*	0.03 (0.01)	7.50E-04†	0.64 (0.73)	3.80E-01	0.90 (1.65)	5.90E-01	0.14 (0.32)	6.70E-01
Formiminoglutamate	Histidine Metabolism	0.06 (0.01)	4.80E-06*	0.03 (0.01)	5.10E-02	1.96 (1.09)	7.20E-02	0.85 (2.48)	7.30E-01	-0.28 (0.46)	5.40E-01
5-methylthioadenosine	Poylamine Metabolism	0.04 (0.01)	1.00E-03†	0.03 (0.01)	4.40E-05†	-0.31 (0.71)	6.60E-01	2.07 (1.61)	2.00E-01	0.16 (0.28)	5.60E-01
N-trimethyl 5-amino-valerate	Lysine Metabolism	0.04 (0.01)	3.10E-02†	0.02 (0.01)	2.90E-05*	1.56 (0.71)	2.90E-02†	0.14 (1.63)	9.30E-01	0.07 (0.27)	8.00E-01
N2, N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	0.04 (0.01)	4.70E-04†	0.03 (0.01)	3.80E-07*	1.25 (0.67)	6.20E-02	1.60 (1.52)	3.00E-01	0.07 (0.30)	8.10E-01
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.05 (0.01)	4.80E-06*	0.05 (0.01)	2.00E-06*	0.06 (0.76)	9.40E-01	1.77 (1.73)	3.10E-01	0.31 (0.29)	2.80E-01
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.04 (0.01)	1.00E-03†	0.03 (0.01)	7.50E-06*	0.33 (0.64)	6.00E-01	0.91 (1.45)	5.30E-01	0.50 (0.24)	3.70E-02†
Lipid											
Butyrylcarnitine	Fatty Acid Metabolism, BCAA Metabolism	0.03 (0.01)	2.00E-01	-0.01 (0.01)	3.70E-04†	3.05 (0.64)	2.20E-06*	-1.91 (1.47)	1.90E-01	-0.41 (0.24)	8.70E-02

A = peak velocity blood flow in late diastole; E = peak velocity blood flow in early diastole; e'=medial mitral annular velocity; IVRT = isovolumic relaxation time; Ln = natural logarithm; SAM=s-adenosylmethionine; SE = standard error.
* = statistically significant p-value (p<4.16E-05); † = nominally significant p-value (p<0.05).
Adjusted for age, race, sex, education, smoking, alcohol drinking, antihypertensive medications, heart rate, eGFR, BMI, SBP, LDL-C, HDL-C, serum triglycerides, HbA1c, and left ventricular mass.

Table S10. Novel Metabolites Significantly Associated with Echocardiographic Measures of Diastolic Function in 1,050 BHS Participants, Additionally Adjusting for Gait Speed.

Metabolite	Sub-Pathway	Ln E/e' Ratio		Ln E/A Ratio		IVRT (ms)		DT (ms)		LAVI (mL/m2)	
		Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Amino Acid											
1-methylhistidine	Histidine Metabolism	0.05 (0.01)	5.70E-07*	0.03 (0.01)	2.50E-03†	1.60 (0.76)	3.70E-02†	1.33 (1.66)	4.20E-01	0.36 (0.33)	2.70E-01
Formiminoglutamate	Histidine Metabolism	0.07 (0.01)	2.20E-06*	0.03 (0.01)	3.10E-02†	2.81 (1.21)	2.00E-02†	2.08 (2.62)	4.30E-01	-0.06 (0.51)	9.10E-01
5-methylthioadenosine	Poylamine Metabolism	0.04 (0.01)	2.10E-04†	0.03 (0.01)	5.30E-03†	0.39 (0.79)	6.20E-01	1.60 (1.70)	3.50E-01	0.07 (0.30)	8.10E-01
N-trimethyl 5-amino-valerate	Lysine Metabolism	0.05 (0.01)	1.50E-05*	0.02 (0.01)	1.40E-02†	1.76 (0.71)	8.50E-03†	1.09 (1.55)	9.30E-01	0.03 (0.32)	6.00E-01
N2, N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	0.05 (0.01)	1.00E-07*	0.02 (0.01)	4.20E-03†	1.76 (0.71)	1.40E-02†	1.09 (1.55)	4.80E-01	0.03 (0.32)	9.30E-01
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.04 (0.01)	3.90E-05*	0.04 (0.01)	2.90E-04†	1.30 (0.82)	1.10E-01	2.12 (1.77)	2.30E-01	0.48 (0.30)	1.10E-01
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.03 (0.01)	2.40E-04†	0.03 (0.01)	3.20E-03†	0.60 (0.74)	4.20E-01	0.52 (1.60)	7.50E-01	0.35 (0.27)	1.90E-01
Lipid											
Butyrylcarnitine	Fatty Acid Metabolism, BCAA Metabolism	0.03 (0.01)	1.80E-03†	-0.02 (0.01)	6.80E-02	3.44 (0.71)	1.30E-06*	-2.02 (1.55)	1.90E-01	-0.42 (0.26)	1.10E-01

A = peak velocity blood flow in late diastole; E = peak velocity blood flow in early diastole; e'=medial mitral annular velocity; IVRT = isovolumic relaxation time; Ln = natural logarithm; SAM=s-adenosylmethionine; SE = standard error.
* = statistically significant p-value (p<4.16E-05); † = nominally significant p-value (p<0.05).
Adjusted for age, race, sex, education, smoking, alcohol drinking, antihypertensive medications, heart rate, eGFR, BMI, SBP, LDL-C, HDL-C, serum triglycerides, and HbA1c.

Table S11. Novel Metabolites Significantly Associated with Echocardiographic Measures of Diastolic Function in 804 BHS Participants, Adjusting for Mean Lifetime Systolic Blood Pressure.

Metabolite	Sub-Pathway	Ln E/e' Ratio		Ln E/A Ratio		IVRT (ms)		DT (ms)		LAVI (mL/m$_2$)	
		Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Amino Acid											
1-methylhistidine	Histidine Metabolism	0.02 (0.01)	5.50E-02	0.01 (0.01)	3.20E-01	-0.80 (1.06)	4.50E-01	5.05 (2.33)	3.10E-02†	0.27 (0.38)	4.90E-01
Formiminoglutamate	Histidine Metabolism	0.04 (0.02)	3.40E-02†	-1.3E-03 (0.02)	9.40E-01	1.58 (1.57)	3.20E-01	1.43 (3.47)	6.80E-01	-0.14 (0.57)	8.10E-01
5-methylthioadenosine	Poylamine Metabolism	0.03 (0.01)	1.00E-02†	0.02 (0.01)	5.50E-02†	-0.48 (0.90)	5.90E-01	4.15 (1.99)	3.70E-02†	0.39 (0.33)	2.30E-01
N-trimethyl 5-amino-valerate	Lysine Metabolism	0.03 (0.01)	1.10E-02†	0.01 (0.01)	2.30E-01	0.97 (0.90)	2.80E-01	0.72 (1.98)	7.20E-01	0.23 (0.33)	4.80E-01
N2, N5-diacetylornithine	Urea cycle; Arginine and Proline Metabolism	0.01 (0.01)	2.90E-01	0.01 (0.01)	4.40E-01	-0.11 (0.97)	9.10E-01	5.66 (2.12)	7.80E-03†	-0.28 (0.35)	4.20E-01
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	0.04 (0.01)	4.10E-04†	0.03 (0.01)	4.20E-03†	0.50 (0.95)	6.00E-01	5.41 (2.09)	1.00E-02†	0.51 (0.34)	1.40E-01
Methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	0.03 (0.01)	9.50E-04†	0.02 (0.01)	3.90E-02†	0.74 (0.85)	3.80E-01	1.07 (1.87)	5.70E-01	0.54 (0.31)	7.70E-02
Lipid											
Butyrylcarnitine	Fatty Acid Metabolism, BCAA Metabolism	0.02 (0.01)	6.50E-02†	-0.01 (0.01)	4.20E-01	1.28 (0.83)	1.20E-01	-0.65 (1.83)	7.20E-01	-0.27 (0.31)	3.70E-01

A = peak velocity blood flow in late diastole; E = peak velocity blood flow in early diastole; e'=medial mitral annular velocity; IVRT = isovolumic relaxation time; Ln = natural logarithm; SAM=s-adenosylmethionine; SE = standard error.
* = statistically significant p-value (p<4.16E-05); † = nominally significant p-value (p<0.05).
Adjusted for age, race, sex, education, smoking, alcohol drinking, antihypertensive medications, heart rate, eGFR, BMI, mean lifetime systolic blood pressure, LDL-C, HDL-C, serum triglycerides, and HbA1c.

Figure S1. Flow diagram of study participant inclusion.

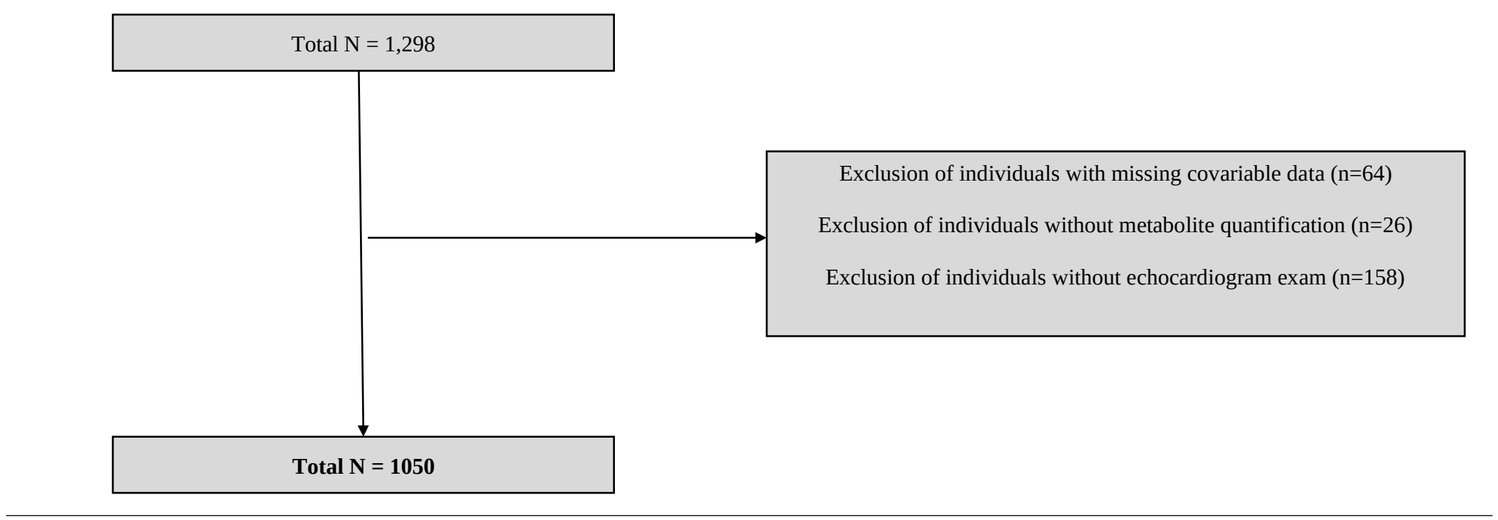
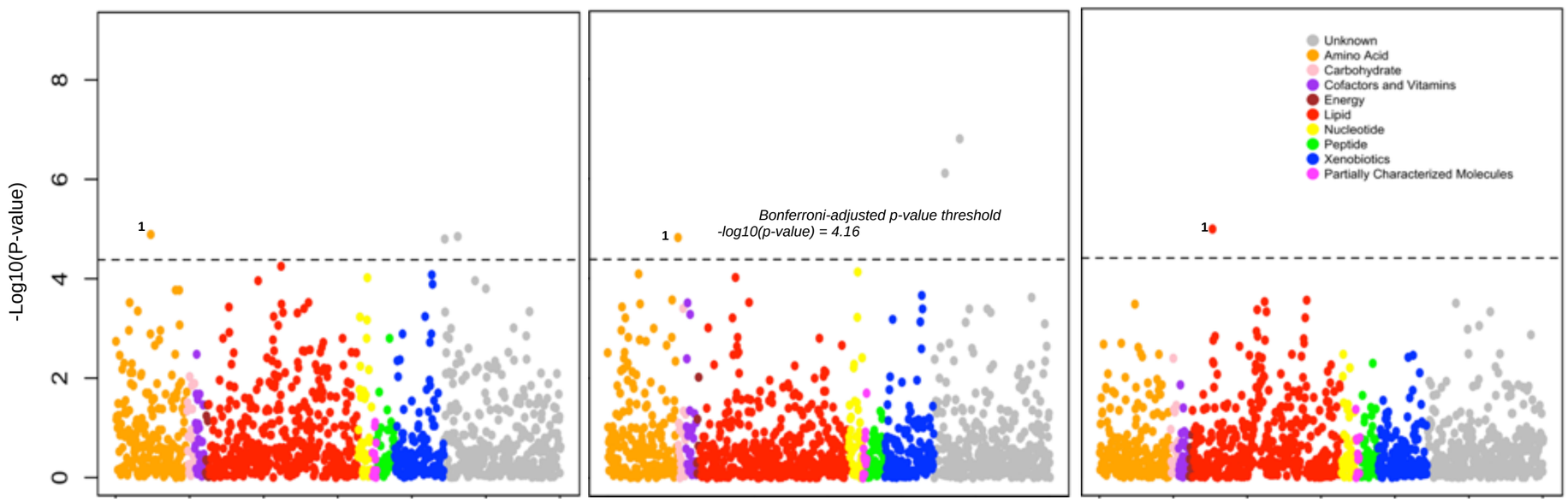


Figure 2S. -Log10(P) probability plot of the multivariable-adjusted association of serum metabolites with the natural logarithm of the E/A ratio: all participants (left), African-Americans (middle), Whites (right)



1. N-formylmethionine

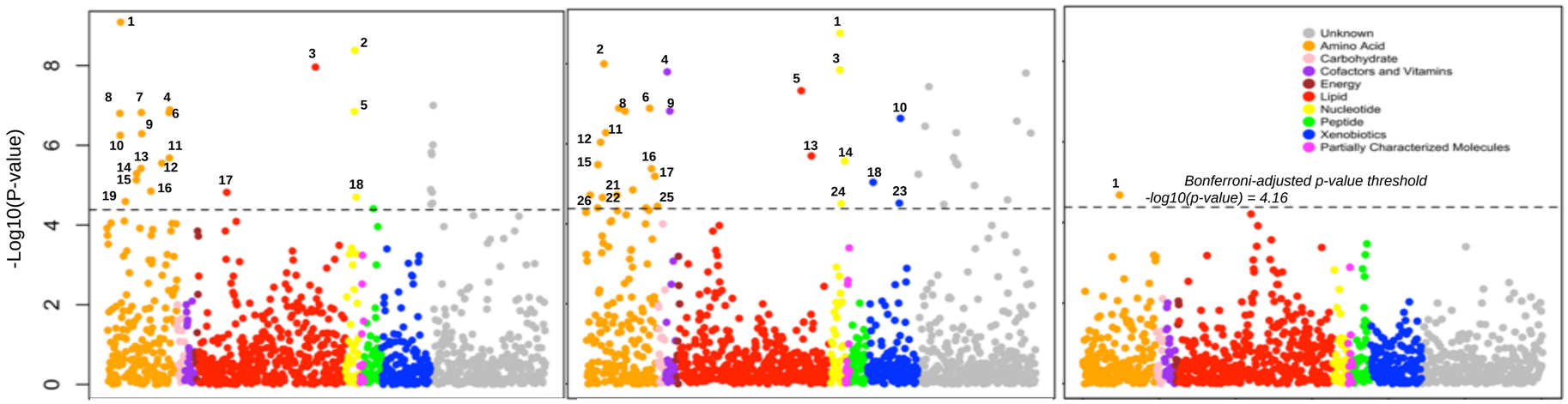
*2 significant unnamed metabolites

1. Urea

*2 significant unnamed metabolites

1. N-oleolytaurine

Figure S3. -Log10(P) probability plot of the multivariable-adjusted association of serum metabolites with the natural logarithm of the E/e' ratio: all particpants (left), African-Americans (middle), Whites (right)



1. N-acetyl-1-methylhistidine
2. Orotate
3. Glycohenodeoxycholate sulfate
4. N2, N5-diacetylornithine
5. Orotidine
6. N-acetylhomocitrulline
7. N-formylmethionine
8. 1-methylhistidine
9. Cystathionine
10. Formiminoglutamate
11. Homocitrulline
12. 3-(4-hydroxyphenyl)-lactate
13. Methionine sulfoxide
14. N-trimethyl 5-aminovalerate
15. N2-acetyllysine
16. 5-methylthioadenosine
17. Dihomo-linolenoylcarnitine (20:3n3 or 6)
18. Pseudouridine
19. 2-methylbutyrylcarnitine

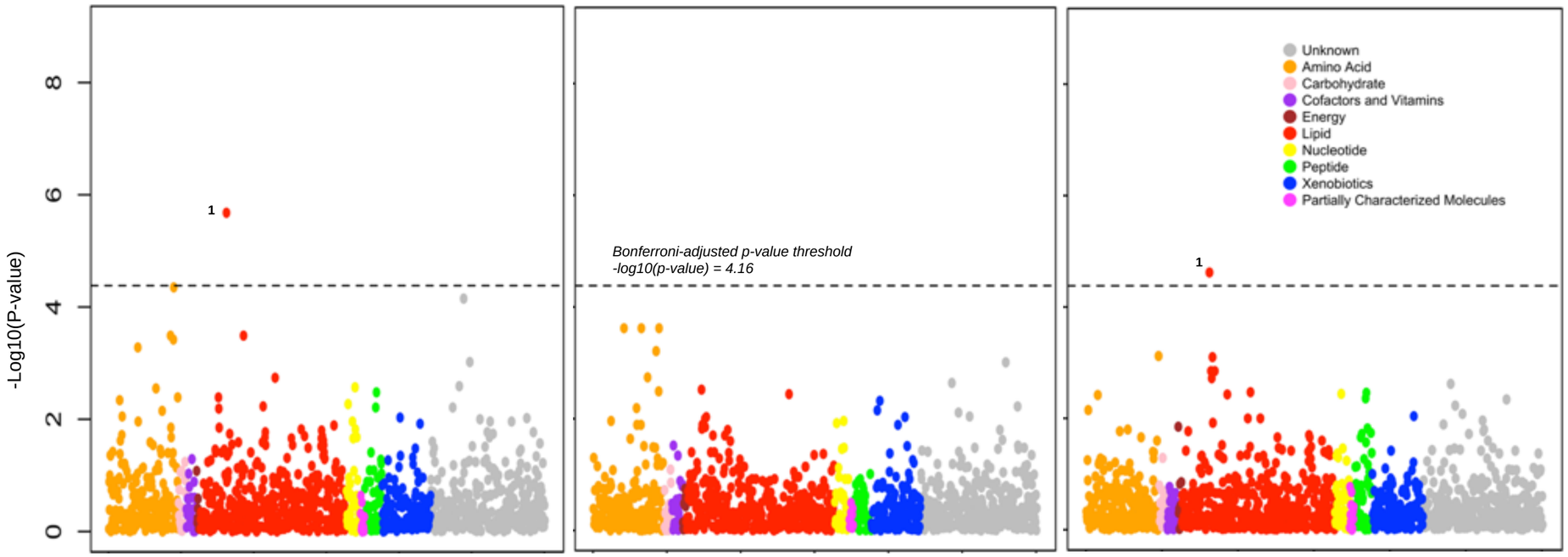
*8 significant unnamed metabolites

1. Orotate
2. N-acetyl-1-methylhistidine
3. Orotidine
4. I-urobilinogen
5. Glycohenodeoxycholate sulfate
6. N-trimethyl 5-aminovalerate
7. N-acetylhomocitrulline
8. N-formylmethionine
9. N1-methyl-2-pyridone-5-carboxamide
10. Sucralose
11. N-carbamoylvaline
12. 1-methylhistidine
13. Taurocholenate sulfate
14. Pseudouridine
15. Dimethylglycine
16. Homocitrulline
17. N2, N5-diacetylornithine
18. N-methylpipecolate
19. 5-methylthioadenosine
20. N-acetylglutamine
21. N2-acetyllysine
22. Formiminoglutamate
23. Homostachydrine
24. 5,6-dihydrothymine
25. Urea
26. Betaine

*13 significant unnamed metabolites

1. N-acetylaspartate

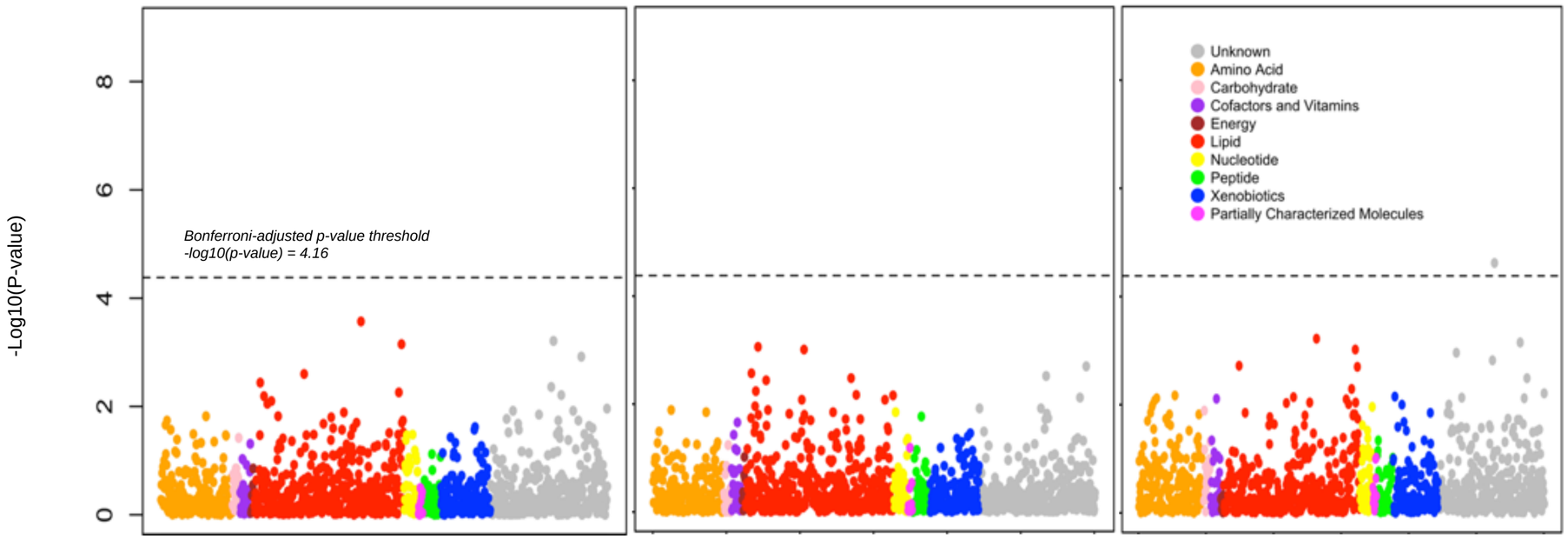
Figure S4. -Log10(P) probability plot of the multivariable-adjusted association of serum metabolites with isovolumic relaxation time: all particpants (left), African-Americans (middle), Whites (right)



1. Butyrylcarnitine

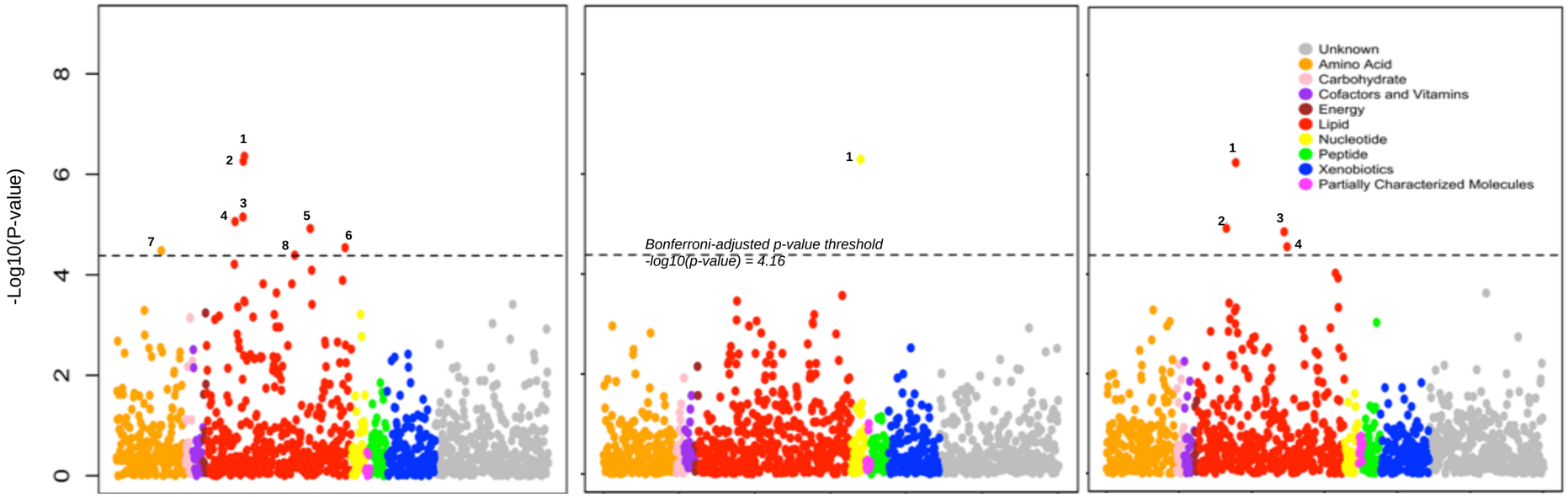
1. Butyrylcarnitine

Figure S5. -Log10(P) probability plot of the multivariable-adjusted association of serum metabolites with deceleration time: all participants (left), African-Americans (middle), Whites (right)



*1 unnamed metabolite

Figure S6. -Log10(P) probability plot of the multivariable-adjusted association of serum metabolites with left atrial volume index: All pariticipants (left), African-Americans (middle), Whites (right)



1. Dihomo-linoleoylcarnitine (C20:2)
2. Ximenoylcarnitine (C26:1)
3. Cerotoylcarnitine (C26)
4. Palmitoylcarnitine (C16)
5. 1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)
6. N-palmitoyl-sphinganine (d18:0/16:0)
7. N-acetylputrescine
8. 1-palmitoyl-2-stearoyl-GPC (16:0/18:0)

1. Orotate

1. N-acetylaspartate
2. N-oleoyltaurine
3. N-acetylasparagine
4. N-carbamoylalanine

Figure S7. Heat map displaying pairwise correlation coefficients for the network of metabolites underlying primary and secondary bile acid metabolism pathways.