

Metabolomic signatures of cardiac remodelling and heart failure risk in the community

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Abstract

Aims Heart failure (HF) is associated with several metabolic changes, but it is unknown whether distinct components of the circulating metabolome may be related to cardiac structure and function, and with incident HF in the community.

Methods and results We assayed 217 circulating metabolites in 2336 Framingham Study participants (mean age 55 ± 10 years, 53% women) without HF at baseline. We used linear and Cox regression to relate concentrations of metabolites to left ventricular (LV) diastolic dimension, LV wall thickness, LV ejection fraction, left atrial dimension, LV ventricular mass, and aortic root size cross-sectionally and to incident HF prospectively. Bonferroni-adjusted *P*-values <0.05 denoted statistical significance. Circulating concentrations of kynurenine [$\beta = -0.12$ cm per standard deviation (SD) increment in normalized residual of metabolite, $P = 7.3 \times 10^{-8}$] and amino adipate (-0.11 cm per SD increment, $P = 2.61 \times 10^{-5}$) were associated with left ventricular diastolic dimension, phosphatidylcholine (carbon:double bound = 38:6) with left atrial dimension (0.10 cm per SD increment, $P = 9.7 \times 10^{-6}$), and cholesterol ester (carbon:double bound = 20:5) with left atrial dimension (0.10 cm per SD increment, $P = 1.4 \times 10^{-5}$) in multivariable-adjusted models. During an average follow-up of 15.8 (range 0.02–23.2) years, 113 participants (5%) were diagnosed with HF with reduced ejection fraction and 106 individuals (5%) with HF with preserved ejection fraction. In multivariable analyses, concentrations of phosphatidylcholine (hazard ratio 0.63, $P = 1.3 \times 10^{-5}$) and ornithine (hazard ratio 1.44, $P = 0.00014$) were associated with HF with reduced ejection fraction.

Conclusions Several metabolites, including the vasoactive metabolite kynurenine, were related to cardiac structure and function in our sample. Additional research is warranted to confirm our observations and investigate if these metabolites can risk stratify ambulatory individuals.

Keywords Metabolomics; Echocardiography; Heart failure risk

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Introduction

The heart is the most energy-consuming organ of the human body (per gram of tissue). Consequently, the myocardium is very sensitive to the influence of metabolic alterations, but the relation between the circulating metabolome and heart failure (HF) risk in the community remains unknown. The impact of altered myocardial metabolism on the cardiac

function is evidenced, for instance, in echocardiographic studies of patients with diabetes mellitus, thyroid disorders, and thiamine deficiency.^{1–4} Several metabolic disturbances including obesity,⁵ mitochondrial genetic disorders,⁶ and lipid abnormalities⁷ are also closely linked to the HF risk, but even subtle metabolic variations (e.g. high-normal blood glucose levels) may antedate overt HF by years, underscoring the potential relevance of metabolomics for long-term HF risk

prediction.^{8–11} In the setting of prevalent HF, emerging metabolomic profiling has begun to elucidate specific molecular signatures relating to phenotypic characterization and survival.^{12–14} In the present investigation, we explored the associations of a targeted circulating metabolomic profile with select echocardiographic measures of subclinical cardiac remodelling (cross-sectionally) and with the risk of incident HF in a community-based sample of individuals who were free of overt HF at baseline.

Methods

For the present investigation, we analysed data of the Framingham Offspring cohort.¹⁵ The Framingham Offspring cohort participants were enrolled in 1971 and have been followed up for the onset of cardiovascular disease in examination cycles at approximately 4–6 year intervals. As previously detailed, all participants from Framingham Offspring had blood drawn after an overnight fast at the fifth examination cycle (1991–1995) that was later used for metabolomic profiling.¹⁶ The blood was collected in an ethylenediaminetetraacetic acid medium and was immediately stored at -80°C until assayed. The metabolomic profile of participants was measured between 2009 and 2011 using liquid chromatography with mass spectrometry techniques as detailed previously.^{16–19} In brief, three distinct platforms were applied: the first to measure a variety of amino acids and amines,¹⁶ the second to measure organic acids, nucleotides, and other intermediary metabolites,¹⁹ and the third to measure lipids of varying classes.¹⁸ In the present investigation, we related the measured circulating metabolites to echocardiographic variables in cross-sectional analyses and to the risk of developing HF during follow-up through 31 December 2016.

Echocardiographic measures

During the fifth examination cycle, all participants also underwent standard transthoracic echocardiography with Doppler colour flow imaging. The left ventricular diastolic dimension (LVDD) and systolic dimension (LVSD) were measured from the parasternal short-axis view at the level just above the papillary muscles. Left ventricular fractional shortening was calculated as $(\text{LVDD} - \text{LVSD}) / \text{LVDD}$ and may be used as a proxy for left ventricular ejection fraction (LVEF). Aortic root dimension, left atrial dimension (LAD), and left ventricular wall thickness (the sum of septum and lateral wall thickness in end diastole) were also measured from M-mode frames. LAD was measured in the end systole. Left ventricular mass was calculated based on the formula of Devereux and Reichek.²⁰

Heart failure definition

Heart failure was defined according to the established Framingham Heart Study (FHS) epidemiological criteria, which require at least two major, or one major plus two minor criteria, as outlined elsewhere.²¹ All participants have been under continuous surveillance for incident HF that is adjudicated by reviewing hospitalization charts and by participant history and physical examinations during the FHS clinic visits. For all HF events, the final adjudication was undertaken by an expert study committee comprising at least three FHS physicians, of whom at least two were cardiologists. We defined HF with preserved ejection fraction (HFpEF) as HF with $\text{LVEF} \geq 50\%$ and HF with reduced ejection fraction (HFrEF) as HF with $\text{LVEF} < 50\%$ at the time of HF diagnosis.

Statistical analyses

Both echocardiographic variables and metabolites were regressed first on age, sex, height, and weight to obtain residuals, which were then rank-based inverse normal transformed for all subsequent analyses. Echocardiography variables (dependent variables) were related to metabolites (independent variables) using age-adjusted and sex-adjusted linear regression models. In secondary models, we additionally adjusted for weight, height, prevalent myocardial infarction, current smoking, diabetes, total and high-density lipoprotein cholesterol levels, treatment for hypertension, and systolic blood pressure values. As sensitivity, we included estimated glomerular filtration rates (eGFR) in the models; this metabolite was not included in primary analyses because it may be along the causal pathway of some metabolites.²²

To model associations of metabolites with prospective risk of HFpEF and HFrEF, we used Cox regression after confirming that the assumption of proportionality of hazards was met. Two separate models were run, one for each of HFpEF and HFrEF. First, we adjusted for age and sex. Second, we additionally adjusted for weight, height, prevalent myocardial infarction, current smoking, diabetes, total and high-density lipoprotein cholesterol levels, treatment for hypertension, and systolic blood pressure values. As sensitivity, we also adjusted for eGFR. Participants were censored when they died or at the end of follow-up. For HFrEF models, participants were censored if they developed HFpEF (and vice versa for HFpEF). We used sandwich estimators to obtain robust standard errors accounting for familial relatedness among participants.

All statistical analyses were performed using R software Version 3.4.3 (R Foundation, Vienna, Austria). Two-sided P -values < 0.05 were considered statistically significant after Bonferroni correction. For the echocardiographic variables, this corresponded to $P = 3.8 \times 10^{-5}$ (217 metabolites \times 6 echocardiography variables, i.e. 1302 tests, $\alpha = 0.05/$

$1302 = 3.8 \times 10^{-5}$). For HFpEF and HFrEF, this corresponded to $P = 2.3 \times 10^{-4}$ (i.e. $\alpha = 0.05/217$ tests). In addition, we used a less stringent false discovery rate (FDR) of $q \leq 0.10$, as an exploratory hypothesis-generating analysis because a Bonferroni may be overly conservative, especially when correlated variables are analysed.

Results

Baseline characteristics of our study sample are shown in *Table 1*. In total, 2343 participants (mean age 55 ± 10 years, 53% women) had available metabolomics data. Of these, 7 (0.3%) were excluded from further analyses because of prevalent HF. The remaining 2336 individuals were followed up for an average of 15.8 years (min–max 0.02–23.2 years). During this period, 219 (9.4%) participants were diagnosed with HF: 113 (5%) of whom had HFrEF and 106 (5%) had HFpEF. The average time to HF onset was 12.6 years (p25–p75 8.9–17.4 years). Characteristics of the HF cases are available in Supporting Information, *Table S1*.

Table 1 Baseline characteristics of the study sample

	Men (n = 1109)	Women (n = 1227)
Age (years)	55 (10)	55 (10)
Weight (kg)	87 (14)	70 (14)
Height (m)	175 (7)	161 (6)
Total cholesterol concentration (mg/dL)	202 (35)	209 (38)
High-density lipoprotein cholesterol concentration (mg/dL)	43 (11)	56 (15)
Low-density lipoprotein cholesterol concentration (mg/dL)	129 (31)	126 (34)
Very-low-density lipoprotein cholesterol concentration (mg/dL)	26 (12)	25 (11)
Systolic blood pressure (mmHg)	129 (18)	124 (20)
Prevalent myocardial infarction (N)	44 (4%)	6 (0.5%)
Current smoking (N)	196 (18%)	218 (18%)
Diabetes (N)	93 (8%)	63 (5%)
Use of antihypertensive medications (N)	237 (21%)	206 (17%)
Education		
No high school degree	57 (5%)	71 (6%)
High school degree	378 (31%)	299 (27%)
Some college degree	394 (32%)	252 (23%)
College degree	348 (28%)	447 (40%)
Echocardiographic variables		
Left atrial dimension (cm)	4.0 (0.5)	3.6 (0.5)
Left ventricular diastolic dimension (cm)	5.0 (0.4)	4.6 (0.4)
Left ventricular systolic dimension (cm)	3.2 (0.5)	2.9 (0.4)
Fractional shortening	0.36 (0.07)	0.38 (0.06)
Left ventricular wall thickness (cm)	2.0 (0.3)	1.8 (0.2)
Aortic root dimension (cm)	3.4 (0.3)	3.0 (0.3)
Left ventricular mass (g)	184 (37)	140 (29)

Numbers in parentheses refer to standard deviations unless specified.

Echocardiographic traits

At an FDR $q < 0.10$, 36 metabolites were associated with echocardiographic variables in age-adjusted and sex-adjusted models (*Figure 1*). Of these, 24 metabolites remained statistically significant after multivariable adjustment. Using the more stringent Bonferroni-adjusted threshold of $P < 3.8 \times 10^{-5}$, three metabolites were significantly associated with echocardiographic traits in sex-adjusted and age-adjusted models: kynurenine with LVDD [β estimate -0.11 cm for each 1 standard deviation (SD) increment of normalized residual, $P = 1.3 \times 10^{-5}$], diacylglycerol (carbon number:double bond number = 36:1) with LVDD (β estimate -0.11 cm per SD increment, $P = 1.9 \times 10^{-5}$), and leucine with LVDD (β estimate -0.09 cm per SD increment, $P = 3.2 \times 10^{-5}$). After multivariable adjustment, the association of kynurenine with LVDD remained statistically significant per the Bonferroni-corrected threshold (-0.12 cm per SD increment, $P = 7.3 \times 10^{-8}$). Upon additional adjustment for eGFR, estimate remained similar [β estimate -0.10 , standard error (SE) 0.028, $P = 0.00030$].

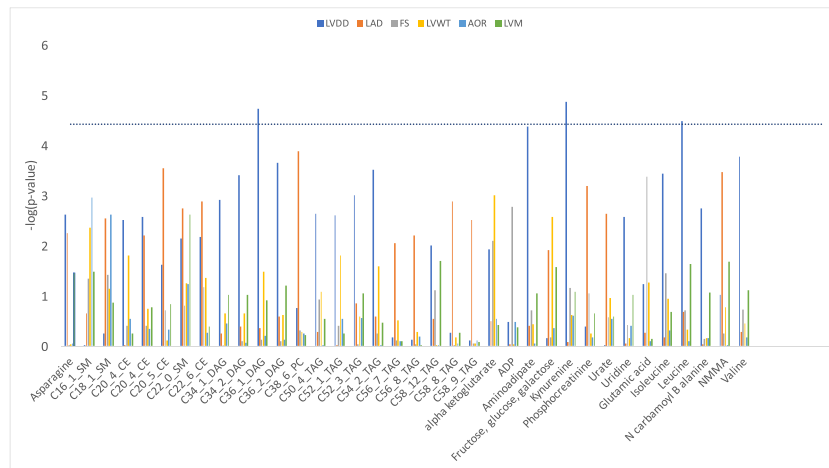
In addition, in multivariable models, amino adipate was associated with LVDD (β estimate -0.11 cm per SD increment, $P = 2.61 \times 10^{-5}$), phosphatidylcholine (carbon:double bond = 38:6) with LAD (β estimate 0.10 cm per SD increment, $P = 9.7 \times 10^{-6}$), and cholesterol ester (carbon:double bond = 20:5) with LAD (0.10 cm per SD increment, $P = 1.4 \times 10^{-5}$). After additional adjustment for eGFR, estimates were similar: $\beta -0.10$, SE 0.028, $P = 0.00037$, for amino adipate and LVDD; β 0.11, SE 0.025, $P = 1.7e - 5$, for phosphatidylcholine (carbon:double bond = 38:6) and LAD; and β 0.094, SE 0.025, $P = 0.00017$, for cholesterol ester (carbon:double bond = 20:5) and LAD.

Heart failure

In age-adjusted and sex-adjusted models, cotinine (hazard ratio 1.61 per SD increment normalized residual, $P = 8.4 \times 10^{-5}$) and hydroxybutyrate (hazard ratio 1.49 per SD increment, $P = 0.00017$) were significantly associated with incident HFpEF after Bonferroni correction. Upon multivariable adjustment, none of these (or other) metabolites remained, however, significantly associated with HFpEF risk (*Table 2*).

Sixteen metabolites were associated with HFrEF at FDR $q \leq 0.10$, and nine remained statistically significant in multivariable-adjusted models (*Table 2*). Phosphatidylcholine (carbon:double bond = 36:4; hazard ratio 0.64 per SD increment, $P = 1.4 \times 10^{-5}$), isocitrate (hazard ratio 1.57 per SD increment, $P = 2.9 \times 10^{-5}$), and lysophosphatidylcholine (carbon:double bond = 18:2; hazard ratio 0.67 per SD increment, $P = 6.6 \times 10^{-5}$) were associated with incident HFrEF in age-adjusted and sex-adjusted models, and cotinine was

Figure 1 Association of selected metabolites and echocardiographic measures. Only variables that were significant at a false discovery rate level of ≤ 0.10 in one or more of the echocardiographic measures are presented. The y axis presents $-\log(P\text{-value})$. Dotted line represents a Bonferroni-corrected P -value of < 0.05 . AOR, aortic root; FS, fractional shortening; LAD, left atrial dimension; LVDD, left ventricular diastolic dimension; LVM, left ventricular mass; LVWT, left ventricular wall thickness. Metabolite abbreviations: SM, sphingomyelin; CE, cholesterol ester; DAG, diacylglycerol; TAG, triacylglycerol. C stands for carbon:double bond; for example, C16_1_SM is sphingomyelin (carbon:double bond = 16:1).



borderline statistically significant in the same models (hazard ratio 1.49 per SD increment, $P = 0.00023$). After additional adjustment, phosphatidylcholine (carbon:double bond = 36:4; hazard ratio 0.63 per SD increment, $P = 1.3 \times 10^{-5}$) and ornithine (hazard ratio 1.44 per SD increment, $P = 0.00014$) were observed to be significantly associated with incident HFrEF. Additional adjustment for eGFR did not change these associations [hazard ratio 0.56 per SD increment, $P = 7.8 \times 10^{-7}$, for phosphatidylcholine (carbon:double bond = 36:4), and hazard ratio 1.47 per SD increment, $P = 0.00032$, for ornithine].

Validation of echocardiographic associations

During their first examination cycle (2002–2005), the FHS Third Generation cohort ($N = 4095$; mean age 40 years \pm standard deviation 9 years; 53% women)²³ underwent echocardiography and had certain metabolites measured in a similar platform. We sought to replicate our observations with $FDR\ q < 0.10$ based on a common set of metabolites across the two cohorts. Levels of α -ketoglutarate, asparagine, carnosine, dimethylglycine, glutamic acid, glutamine, hydroxybutyrate, isoleucine, kynurenine, lactate, leucine, niacinamide, N^G -monomethyl-L-arginine, ornithine, phosphocreatine, quinolate, uridine, uric acid, valine, and xanthurenate were available in both generations. Of these, nine metabolites were associated with at least one of the echocardiographic traits in age-adjusted and sex-adjusted models (Table 3). Of note, we confirmed a strong association between kynurenine and LVDD in the FHS Third Generation sample.

Discussion

In this discovery effort using a high-throughput mass spectrometry-based targeted metabolomics panel in the Framingham Offspring cohort, we identified a set of metabolites associated with adverse cardiac remodelling and antedating the onset of HF by over a decade.

Metabolites associated with echocardiographic indices

Higher levels of circulating kynurenine were associated with lower LVDD (an endophenotype of HF risk)²⁴ in both the FHS Offspring and Third Generation cohorts.²⁵ Kynurenine is derived from the amino acid tryptophan after it has been metabolized via the kynurenine pathway. Kynurenine is a neuroactive molecule that is implicated in many mental disorders (partly through its effects on the N -methyl-D-aspartate receptor signalling system).²⁶ It is also important in the setting of cytokine signalling and inflammation and has been associated with atherosclerosis.²⁷ Kynurenine acid levels, the downstream product of tryptophan metabolism in the kynurenine pathway, have previously shown to be increased in various emergencies such as cardiac arrest, sepsis, and stroke.²⁸ Higher ratios of kynurenine to tryptophan levels were recently reported to be associated with increased cardiovascular and all-cause mortality in patients with stable coronary artery disease.²⁹ At the molecular level, kynurenine can also cause vascular relaxation via the $K_{v}7$ ion channels mediated by the adenylate and soluble guanylate cyclase pathways.^{25,30}

Table 2 Associations of various metabolites with HFpEF and HFrEF risks: top hits

Metabolite	Estimated β	SE	Robust SE	Hazard ratio	P-value	FDR q
HFpEF, age-adjusted and sex-adjusted						
Cotinine	0.48	0.14	0.12	1.61	8.43e-05	0.02
β -Hydroxybutyrate	0.40	0.11	0.11	1.49	0.00017	0.02
HFrEF, age-adjusted and sex-adjusted						
C36_4_PC_A	-0.44	0.12	0.10	0.64	1.44e-05	0.003
Isocitrate	0.45	0.10	0.11	1.57	2.93e-05	0.003
C18_2_LPC	-0.40	0.10	0.10	0.67	6.61e-05	0.005
Cotinine	0.40	0.12	0.11	1.49	0.00023	0.01
Ornithine	0.27	0.10	0.09	1.32	0.0017	0.07
ADMA	0.29	0.09	0.09	1.34	0.0020	0.07
Choline	0.25	0.09	0.08	1.28	0.0023	0.07
5-HIAA	0.23	0.09	0.08	1.26	0.0027	0.07
N-carbamoyl- β -alanine	0.30	0.09	0.10	1.34	0.0032	0.07
Aconitate	0.32	0.10	0.11	1.38	0.0032	0.07
Lactate	0.34	0.10	0.12	1.40	0.0036	0.07
C18_2_CE	-0.26	0.10	0.09	0.77	0.0046	0.08
Dimethylglycine	0.28	0.09	0.10	1.32	0.0054	0.09
Sucrose	0.35	0.12	0.13	1.42	0.0056	0.09
C14_0_SM	-0.26	0.11	0.09	0.77	0.0062	0.09
C56_4_TAG	0.28	0.10	0.11	1.33	0.0072	0.10
HFrEF, multivariable-adjusted						
C36_4_PC_A	-0.45	0.12	0.10	0.63	1.29e-05	0.003
Ornithine	0.36	0.10	0.10	1.44	0.00014	0.01
C18_2_LPC	-0.40	0.11	0.11	0.67	0.00027	0.02
ADMA	0.37	0.09	0.11	1.45	0.00048	0.03
Isocitrate	0.36	0.11	0.10	1.43	0.00068	0.03
Quinolate	0.33	0.10	0.10	1.39	0.00093	0.03
Cotinine	0.57	0.20	0.17	1.78	0.00099	0.03
Dimethylglycine	0.31	0.09	0.10	1.36	0.0020	0.06
Trimethylamine	0.27	0.09	0.09	1.31	0.0038	0.09
N-oxide						

5-HIAA, 5-hydroxyindoleacetic acid; ADMA, asymmetric dimethylarginine; FDR, false discovery rate; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; SE, standard error. Metabolite abbreviations: SM, sphingomyelin; CE, cholesterol ester; DAG, diacylglycerol; TAG, triacylglycerol. C stands for carbon:double bond; for example, C16_1_SM is sphingomyelin (carbon:double bond = 16:1).

K_v7 potassium channels are present in both the myocardium (K_v7.1 subtype) and the arteries (K_v7.4 and K_v7.5), and kynurenine has just emerged as a potentially important HF biomarker. For instance, blood kynurenine levels have been

reported to be inversely correlated with functional capacity, renal and liver function, and the risk of death in HF patients.³¹ In our study, blood levels of kynurenine were inversely correlated with LVDD, which may be counterintuitive given its association to worsened status in HF patients. However, higher kynurenine levels were also associated with lower fractional shortening in the Third Generation cohort. These observations may both be consistent with a negative impact of kynurenine on the Frank–Starling curve (i.e. lowered preload from peripheral vasodilation can result in lower end-diastolic dimensions and a lower fractional shortening). In agreement with such a hypothesis, kynurenine has been suggested to be contributing to hypotension in patients with sepsis.³² The mechanisms by which kynurenine is associated to LVDD, fractional shortening, and potentially HF warrant more investigations, however (because it may relate to lifestyle-related factors as well as be markers of adverse subclinical processes).

We observed that higher blood levels of diacylglycerol were associated with lower LVDD. Biological underpinnings of this association are unclear. Diacylglycerol is the backbone of phosphatidic acid, which can be converted to phosphatidylinositol, an important metabolite in several signalling pathways regulating cardiac contractility. It is also a major building block for phosphatidylcholine, which may be implicated in cardiac remodelling and HF.³³ Finally, we observed that circulating leucine levels were inversely associated with LVDD. This observation may be consistent with at least one prior study reporting that greater leucine intake was associated with lower blood pressures and lower carotid–femoral pulse wave velocities in women.³⁴ A leucine-rich diet was also recently shown to attenuate HF risk in rats with cancer-associated cachexia, but the mechanisms remain unclear.³⁵

Metabolites associated with heart failure risk

In our study, lower levels of circulating phosphatidylcholine (carbon:double bond = 36:4) and lysophosphatidylcholine

Table 3 Significant associations of metabolites and various echocardiographic variables (at age-adjusted and sex-adjusted $P < 0.05$) in the Third Generation cohort

Metabolite	Significant echocardiographic outcomes
α -Ketoglutarate	LV wall thickness (β estimate 0.089 cm, $P = 0.0055$)
Asparagine	LVDD (β estimate 0.10 cm, $P = 0.0018$)
Dimethylglycine	LV wall thickness (β estimate 0.085 cm, $P = 0.0076$)
Glutamic acid	LV thickness (β estimate 0.099 g, $P = 0.0020$)
Hydroxybutyrate	Fractional shortening (β estimate 0.068%-units, $P = 0.036$)
Kynurenine	LVDD (β estimate -0.10 cm, $P = 0.0020$)
Lactate	LVDD (β estimate -0.12 cm, $P = 0.00067$)
Quinolate	LVDD (β estimate -0.082 cm, $P = 0.012$)
Uric acid	LV wall thickness (β estimate 0.11 cm, $P = 0.00081$)
	LVDD (β estimate -0.080 cm, $P = 0.014$)
	Fractional shortening (β estimate -0.064%-units, $P = 0.047$)
	LV mass (β estimate 0.081 g, $P = 0.032$)
	LV wall thickness (β estimate 0.099 cm, $P = 0.0033$)

LV, left ventricular; LVDD, left ventricular diastolic dimension. Estimates are per 1 SD increment of normalized residual metabolite.

(carbon:double bond = 18:2) were associated with greater risk of HF_rEF. Much research has suggested that, in some individuals, the gut microbiome can metabolize phosphatidylcholine into trimethylamine *N*-oxide before it is absorbed in the intestines.³⁶ High levels of trimethylamine *N*-oxide are known to promote atherosclerosis and thrombosis.^{37,38} Recent research has also suggested that higher trimethylamine *N*-oxide levels may contribute to increased HF risk. For instance, mice fed with a diet rich in trimethylamine *N*-oxide developed greater cardiac dilation, cardiac hypertrophy, lower LVEF, and more cardiac fibrosis after transaortic binding than mice fed with a control diet.³⁹ In humans with systolic HF, higher levels of trimethylamine *N*-oxide have been associated with diabetes, greater New York Heart Association class, higher levels of pro-BNP, worse diastolic function, and greater mortality risk.⁴⁰ In our analyses, higher trimethylamine *N*-oxide levels were indeed associated with greater HF_rEF risk, although it did not reach our Bonferroni-adjusted statistical significance level ($P = 0.0038$ in multivariable-adjusted models). Because the correlation coefficient between phosphatidylcholine (carbon:double bond = 36:4) and trimethylamine *N*-oxide levels was negligible (data not shown), the observed association of higher levels of phosphatidylcholine with lowered risk of HF_rEF is, however, unlikely to be explained by altered gut intestinal metabolism. To the best of our knowledge, an association of circulating blood levels of phosphatidylcholine (carbon:double bond = 36:4) with cardiovascular disease has not been reported previously, and further studies are warranted to confirm our observations. Other possible mechanistic explanations may include a direct effect of lysophosphatidylcholine on the microvascular endothelial signalling and environment,⁴¹ its influence on the vasculature and thrombosis as a component of oxidized low-density lipoprotein,⁴² stimulation of cholesterol synthesis in the liver, and an effect on macrophages, B-cells, and other inflammatory signalling pathways.⁴³

The amino acid ornithine was positively associated with incident HF_rEF in multivariable-adjusted models. Ornithine is not proteinogenic but has an important role in the urea cycle; L-arginine is degraded to ornithine and urea, and ornithine can be recycled to form new L-arginine. L-Arginine is the substrate for endothelial nitric oxide synthesis, which may explain the observed association. In this context, dysregulated arginine metabolism and consequent elevated levels of asymmetric dimethylarginine have been noted in advanced systolic HF previously.⁴⁴ In our study, higher asymmetric dimethylarginine levels were associated with greater HF_rEF, but not HF_pEF risk in multivariable-adjusted models, although the association did not meet the Bonferroni-corrected P -value ($P = 0.00048$).

A few metabolites that are directly implicated in myocardial energetics were also identified in our investigation as associated with the long-term risk of HF: higher concentrations

of isocitrate with increased HF_rEF risk and higher concentrations of hydroxybutyrate with increased HF_pEF risk. Isocitrate is an important metabolite of the mitochondrial citric acid cycle and of major importance in the production of ATP molecules, but the exact mechanism underlying our observations between isocitrate concentrations and HF_rEF is not known. Hydroxybutyrate is an important ketone body for energy production in humans. A recent interventional study demonstrated that infusion of hydroxybutyrate enhanced cardiac blood flow and lowered the myocardial glucose uptake when infused in healthy individuals.⁴⁵ At the same time, however, the heart rate was increased by 25%, which may lead to increased long-term risk of HF.⁴⁶ One of the more common subphenotypes of HF_pEF includes insulin resistance and the metabolic syndrome, in which ketone body levels are increased^{47,48}; this could also be one of the reasons underlying our observed associations. In addition, high levels of ketone bodies may be a marker of subclinical myocardial dysfunction, because HF patients have well-recognized higher levels of circulating ketone bodies than controls and greater myocardial utilization of ketone bodies for energy production.^{49–51} Yet this latter hypothesis may not be fully supported by our data, where higher levels of hydroxybutyrate were associated with greater fractional shortening in the FHS Third Generation sample (*Table 3*).

Cotinine, a metabolite of nicotine, was also associated with HF risk in our community-based sample. This association may reflect concomitant smoking and was attenuated upon adjustment for the same.⁵² Current smokers were recently shown to have greater left ventricular mass and higher risk of incident HF, compared with never smokers, while prior smokers had a risk intermediate between the two other groups in the Jackson Heart Study.⁵³ Also, smokeless tobacco has also been associated with accelerated atherosclerosis and HF.^{54–56}

Strengths and limitations

The major strengths of our investigation include the well-phenotyped population of the FHS and the agnostic strategy relating a large panel of metabolites to both echocardiographic indices and HF risk. Limitations include the echocardiographic measures, which did not include the most sensitive measures of left ventricular function (including tissue Doppler, speckle tracking, and strain). The diagnostic criteria for HF_pEF relied on a clinical examination rather than echocardiographic variables, which may have influenced the sensitivity and specificity of the diagnosis.⁵⁷ The sample was moderate sized, and the number of incident HF events was limited and occurred on average more than 10 years later than the obtained metabolites, which may have weakened the associations between metabolites and HF risk; it may also explain why plasma kynurenine concentrations were

associated with echocardiographical traits cross-sectionally (with LVDD and fractional shortening), but not with incident HF prospectively. Blood metabolites are to some extent dependent on dietary habits and possibly other environmental factors, which can vary widely between different populations and countries. In this context, most participants in our study were of European ancestry, and the majority belonged to the middle class, which may have influenced the measured metabolite levels and potentially limited variability in the circulating metabolome. More studies, including studies of ethnically diverse populations, as well as independent replication, are warranted.

Conclusions and clinical implications

In summary, we identified several metabolites (cotinine, hydroxybutyrate, phosphatidylcholine, lysophosphatidylcholine, isocitrate, ornithine, kynurenine, diacylglycerol, and leucine) that were associated with cardiac function cross-sectionally or HF risk prospectively. Additional research is needed to confirm if these metabolites can be used as risk stratification tools in ambulatory patients (especially HFpEF risk, as fewer significant metabolites were associated with HFpEF risk). Further, mechanistic studies are warranted to determine if associations are causal and, if so, whether dietary interventions can modify the long-term risk of HF.

Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Characteristics of HF cases.

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