Metabolite signatures of heart failure, sleep apnoea, their interaction, and outcomes in the community

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Abstract

Aims Sleep apnoea and congestive heart failure (CHF) commonly co-exist, but their interaction is unclear. Metabolomics may clarify their interaction and relationships to outcome.

Methods and results We assayed 372 circulating metabolites and lipids in 1919 and 1524 participants of the Framingham Heart Study (FHS) (mean age 54 ± 10 years, 53% women) and Women’s Health Initiative (WHI) (mean age 67 ± 7 years), respectively. We used linear and Cox regression to relate plasma concentrations of metabolites and lipids to echocardiographic parameters; CHF and its subtypes heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF); and sleep indices. Adenine dinucleotide phosphate (ADP) associated with left ventricular (LV) fractional shortening; phosphocreatine with LV wall thickness; lysosomal storage molecule sphingomyelin 18:2 with LV mass; and nicotine metabolite cotinine with time spent with an oxygen saturation less than 90% (β = 2.3 min, P = 2.3 × 10⁻⁴). Pro-hypertrophic metabolite hydroxyglutarate partly mediated the association between LV wall thickness and HFpEF. Central sleep apnoea was significantly associated with HFpEF (P = 0.03) but not HFrEF (P = 0.5). There were three significant metabolite canonical variates, one of which conferred protection from cardiovascular death [hazard ratio = 0.3 (0.11, 0.81), P = 0.02].

Conclusions Energetic metabolites were associated with cardiac function; energy- and lipid-storage metabolites with LV wall thickness and mass; plasma levels of nicotine metabolite cotinine were associated with increased time spent with a sleep oxygen saturation less than 90%, a clinically significant marker of outcome, indicating a significant hazard for smokers who have sleep apnoea.

Keywords Framingham; Heart failure; Sleep apnoea; Mortality; Metabolite; Lipid; Biomarker

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Introduction

Nutritional deficiency (e.g. thiamine deficiency), oversupply (e.g. obesity), states of metabolic inflexibility (e.g. insulin resistance and type 2 diabetes), and altered metabolic regulation (e.g. thyroid disorders) all confer structural and functional cardiac phenotypes, underscoring the importance of metabolism in maintaining cardiac performance and integrity.

Heart failure with preserved ejection fraction (HFpEF) has become the most common form of heart failure, yet it remains poorly understood compared with its easily recognized counterpart, HFrEF (reduced ejection fraction). It most commonly manifests in the presence of comorbidities such as the metabolic syndrome. An underappreciated comorbidity is sleep apnoea, the obstructive form of which is more common in this patient population. Central sleep apnoea has long been associated with heart failure, although it is not
clear if it is related more to HFrEF or HFpEF. While severe sleep apnoea confers an increased mortality risk, the impact of interaction with heart failure remains unclear.\textsuperscript{3} Furthermore, there are conflicting data on the impact of moderate sleep apnoea on cardiovascular disease (CVD) and mortality.\textsuperscript{4}

Metabolomics is emerging as a useful tool to uncover molecular signatures of heart failure and outcome.\textsuperscript{5} Therefore, to help generate insight into the potential interaction of HFrEF and/or HFpEF with sleep apnoea, we explored the relationships between metabolites and cardiac structure, function, heart failure, and sleep apnoea indices in ~2000 individuals in the community. We performed validation analyses of congestive heart failure (CHF) associations in 1524 participants in the Women’s Health Initiative (WHI), as women are increasingly recognized to be disproportionately affected by CHF, particularly the HFpEF phenotype.

Methods

Framingham Heart Study

Participants of the Framingham Heart Study (FHS) Offspring cohort were examined.\textsuperscript{6} Of the 3799 participants enrolled, 1919 attended Examination 5 (1991–95; 53% female) and had blood collected and analysed by metabolomics and lipidomics. The corresponding clinical report at Examination 5 was used to obtain blood pressure, body mass index (BMI), age, gender, and so forth. Echocardiography reports from the Offspring cohort were acquired at Examinations 5, 6, and 8. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Women’s Health Initiative

The WHI is a long-term national health study funded by the National Heart, Lung, and Blood Institute, or NHLBI. The original WHI study began in the early 1990s and concluded in 2005, continuing after this as Extension Studies. The blood samples for which the metabolomics was measured were obtained from the substudy Metabolomics of Coronary Heart Disease (CHD) in the WHI. The datasets used for the analyses described in this manuscript were obtained from dbGaP, full details in Supporting Information. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Congestive heart failure diagnosis (heart failure with preserved and reduced ejection fraction)

Congestive heart failure reports were acquired from the Framingham database, performed in 2013 and 2016. Participants deemed to have CHF (either hospitalized or not hospitalized) or to meet the Framingham criteria of CHF (two major criteria or one major and two minor) were identified, and the date of CHF onset was identified. CHF diagnosed at the time of Examination 5 (1991–95) were classified as prevalent CHF, while CHF diagnosed from Examination 6 onwards (1995–2016) were classified as incident CHF for the purposes of correlation to metabolites and lipids. All CHF diagnoses were further identified as HFpEF or HFrEF based on ejection fraction measurements obtained from echocardiography performed closest to the date of diagnosis/onset of CHF. CHF diagnosis in conjunction with ejection fraction of <50%, or graded as mild, moderate, or severe systolic dysfunction, was classified as HFrEF, while ejection fraction of ≥50%, or graded as normal or borderline systolic dysfunction, was graded as HFpEF.

In the WHI, CHF heart failure was defined as a constellation of symptoms and physical signs. Only a hospitalization involving new or worsening CHF was a WHI outcome. A comprehensive description of CHF diagnostic methodology is available elsewhere.\textsuperscript{7} Sufficient echocardiographic data were not available in WHI for a study of CHF subtype.

Metabolomics and lipidomics profiling

Metabolomic and lipidomic profiles of the Offspring cohort at Examination 5 were acquired from the Framingham database. Plasma samples were collected in EDTA coated tubes after an overnight fast, spun at 2000 rcf at 4°C to remove red blood cells, and the plasma stored at −80°C. Metabolomics and lipidomics were acquired as previously described,\textsuperscript{8} described in Supporting Information.

Statistical analysis

All 113 metabolites and 104 lipids were regressed against incident CHF, HFpEF, HFrEF, and echocardiographic, and sleep parameters using age, gender, and BMI adjusted regression.

Linear, logistic, and ordinal logistic regression were used for continuous, two-class, and ordinal measures, respectively. A Cox proportional hazard regression model was used to establish association of sleep parameters and death due to CVD as well as overall death. Models were adjusted for age, gender, and BMI. For any significant metabolite–sleep association, mediation analysis was performed to identify mediation impact of metabolites. Canonical correlation analysis (CCA) was performed to understand the joint relationship between the set of metabolites and sleep parameters, HFpEF, and HFrEF.
Results

Congestive heart failure diagnosis and its subtypes

Of the 1919 FHS participants studied, 175 had definite CHF according to the Framingham criteria: 7 with prevalent CHF (4 HFpEF and 3 HFrEF) and 152 with incident CHF (131 HFpEF and 21 HFrEF). Due to the small number of prevalent CHF, we focused on incident CHF. Baseline characteristics are provided in Table 1. In the WHI, there were 1295 women with metabolomics data at baseline, 269 of whom had CHF (baseline—prevalent). After the baseline metabolomics samples were taken, there were 10 new diagnoses of CHF before

### Table 1 Baseline characteristics

|                | Men (907) | Women (1012) |
|----------------|-----------|--------------|
| **Age**        | 54 (10)   | 54 (9)       |
| **BMI**        | 28.2 (4)  | 26.6 (5)     |
| **Systolic blood pressure (mmHg)** | 129 (18) | 123 (19)     |
| **Diastolic blood pressure (mmHg)** | 77 (10)  | 73 (10)      |
| **Heart rate (b.p.m.)** | 63 (11)  | 67 (10)      |
| **Currently smoking (N)** | 165 (18%) | 184 (18%)    |
| **Use of antihypertensive medications (N)** | 194 (21%) | 151 (15%)    |
| **Diabetes (N)** | 65 (7%)   | 45 (4%)      |
| **Use of statins (N)** | 80 (9%)  | 54 (5%)      |
| **Fasting blood glucose level (mg/dL)** | 103 (26) | 97 (24)      |
| **Total cholesterol (mg/dL)** | 202 (35) | 208 (38)     |
| **Triglycerides (mg/dL)** | 161 (136) | 136 (114)    |
| **HDL cholesterol (mg/dL)** | 43 (11)  | 56 (16)      |
| **LDL cholesterol (mg/dL)** | 129 (32) | 125 (34)     |

**Echocardiographic parameters**

| Parameter                                      | Men (907) | Women (1012) |
|------------------------------------------------|-----------|--------------|
| **Left atrial dimension (cm)**                | 4.0 (0.5) | 3.5 (0.4)    |
| **Left ventricular diastolic dimension (cm)** | 5.0 (0.4) | 4.6 (0.4)    |
| **Left ventricular systolic dimension (cm)**  | 3.2 (0.5) | 2.8 (0.4)    |
| **Fractional shortening (%)**                | 36 (7)    | 37 (6)       |
| **Left ventricular wall thickness (cm)**      | 2.0 (0.2) | 1.8 (0.2)    |
| **Aortic root dimension (cm)**               | 3.4 (0.3) | 3.0 (0.3)    |
| **Interventricular septal thickness diastole (cm)** | 1.0 (0.1) | 0.9 (0.1)   |
| **Left ventricular posterior wall thickness diastole (cm)** | 1.0 (0.1) | 0.9 (0.1)   |

**FHS—sleep parameters**

| Parameter               | Men (198) | Women (192) |
|-------------------------|-----------|-------------|
| **Obstructive sleep apnoea** | 121       | 66          |
| Mild                    | 72 (60%)  | 42 (64%)    |
| Moderate                | 34 (28%)  | 24 (36%)    |
| Severe                  | 15 (12%)  | 0 (0%)      |
| **Central sleep apnoea** | 92        | 46          |
| Mild                    | 67 (73%)  | 36 (78%)    |
| Moderate                | 18 (20%)  | 9 (20%)     |
| Severe                  | 7 (8%)    | 1 (2%)      |

**WHI**

| Parameter               | Men (907) | Women (1012) |
|-------------------------|-----------|--------------|
| **Age**                 | 67 (7)    |              |
| **BMI**                 | 28.5 (6)  |              |
| **Systolic blood pressure (mmHg)** | 131 (18) |              |
| **Diastolic blood pressure (mmHg)** | 75 (9)    |              |
| **Smoking**             |           |              |
| Past smoker             | 602 (39.5%) |             |
| Current smoker          | 153 (10%) |              |
| **Hypertension**        |           |              |
| Untreated hypertension  | 139 (9%)  |              |
| Treated hypertension    | 454 (29.7%) |             |
| **Diabetes**            |           |              |
| Ever                    | 154 (10%) |              |
| Current                 | 115 (7.5%) |             |
| Treated                 | 118 (8%)  |              |
| **Congestive heart failure (CHF)** | | |
| CHF at baseline         | 269 (17.5%) |             |
| New CHF before Timepoint 2 | 10 (0.006%) |             |
| New CHF after Timepoint 2 | 132 (9%)   |              |

BMI, body mass index; FHS, Framingham Heart Study; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WHI, Women’s Health Initiative.
samples were taken again for metabolomics (second timepoint). At the second metabolomics sampling, 1295 samples were again available for metabolomics profiling, 279 of whom had CHF (second timepoint–prevalent). After the second metabolomics sampling, there were 132 incident cases of CHF (second timepoint–incident).

**Metabolite and lipid associations with prevalent cardiac structure and function in Framingham Heart Study**

A total of 1870 individuals with metabolomic data also had echocardiographic data available at Examination 5. A total of 113 metabolites and 104 lipids (Supporting Information, Table S1) were profiled and then associated. After multivariable adjustment, cross-sectional association analysis of prevalent echocardiographic parameters at Exam 5 revealed 104 significant metabolite–echocardiographic parameter associations at nominal significance levels and 20 at false discovery rate (FDR)-adjusted threshold (Table 2). Remarkably, energetic metabolite adenine dinucleotide phosphate (ADP) was positively associated (β = 0.04, P = 1.3 × 10⁻⁴) with fractional shortening, indicating that this circulating energy metabolite can report on cardiac systolic function, an energy-intensive process (Figure 1). As reported previously, kynurenine was inversely associated with left ventricular (LV) end-diastolic dimension (β = −0.7, P = 8.98 × 10⁻⁵), as were branched chain amino acids and cardiac energy substrates leucine (β = −0.7, P = 8.98 × 10⁻⁵) and valine (β = −1.0, P = 3.1 × 10⁻⁴); glycine was positively associated (β = 0.54, P = 8.98 × 10⁻⁵). LV end-systolic dimension was inversely associated with niacinamide (β = −0.34, P = 2.2 × 10⁻⁴), the precursor of nicotinamide adenine dinucleotide, known to decrease with cardiac hypertrophy and cardiac dilatation.⁹ Inositol was significantly associated with aortic dimension (β = 0.4, P = 4.3 × 10⁻⁴), which may reflect the relationship of inositol and inositol-3-phosphate receptors with hypertension,¹⁰ a known cause of aortic dilatation.

**Metabolite and lipid associations with incident cardiac structure and function in Framingham Heart Study**

A total of 1711 and 1698 individuals with metabolomic data also had incident echocardiographic data at Examinations 6 and 8, respectively. We also determined associations of metabolites and lipids with incident cardiac structural and functional parameters, for potential insight into early pathogenic changes underpinning cardiac remodelling. There were 440 metabolite–incident echocardiographic associations at nominal significance, with 51 meeting FDR-adjusted significance (top 20 metabolite and lipid associations shown in Table 3). The strongest metabolite–cardiac structure association was phosphocreatine, the phosphorylated creatine molecule that serves as a reserve of high-energy phosphates, and was inversely associated with incident LV wall thickness (β = −0.3, P = 7.2 × 10⁻⁷); this relationship was previously seen using magnetic resonance spectroscopy.¹¹ Cotinine was associated with several incident cardiac structural and functional parameters including positive associations with wall motion, heart

### Table 2 Framingham Heart Study: Metabolites and lipids associated with prevalent cardiac structure/function

| Parameter     | Metabolite/lipid | β coefficient | R²   | P value | Adj P value (FDR) |
|---------------|------------------|---------------|------|---------|-------------------|
| LVIDd (cm)    | Kynurenine       | −0.741        | 0.231| 9.0E-05 | 0.005             |
| LVIDd (cm)    | Leucine          | −1.007        | 0.231| 9.1E-05 | 0.005             |
| LVIDd (cm)    | Glycine          | 0.538         | 0.230| 2.4E-04 | 0.007             |
| LVIDd (cm)    | Aminoadipate     | −0.524        | 0.230| 2.6E-04 | 0.007             |
| LVIDd (cm)    | Valine           | −1.011        | 0.230| 3.1E-04 | 0.007             |
| LVFS (%)      | C38_6_PC         | 0.963         | 0.359| 1.3E-04 | 0.007             |
| LVIDd (cm)    | C58_9_TAG        | 0.474         | 0.359| 1.7E-04 | 0.007             |
| LVIDs (cm)    | C58_6_TAG        | 0.450         | 0.359| 1.9E-04 | 0.007             |
| LVIDs (cm)    | C58_12_TAG       | −0.181        | 0.133| 8.2E-05 | 0.009             |
| LVIDs (cm)    | C58_8_TAG        | 0.505         | 0.358| 4.9E-04 | 0.013             |
| LVIDs (cm)    | ADP              | 0.040         | 0.023| 1.3E-04 | 0.015             |
| LVIDs (cm)    | Isoleucine       | −0.724        | 0.229| 7.7E-04 | 0.015             |
| LVIDs (cm)    | C56_8_TAG        | 0.449         | 0.358| 8.1E-04 | 0.016             |
| LVIDs (cm)    | C20_5_CE         | 0.311         | 0.358| 9.1E-04 | 0.016             |
| LVIDs (cm)    | Niacinamide      | −0.338        | 0.132| 2.2E-04 | 0.023             |
| LVIDs (cm)    | Alpha-ketoglutarate | −0.311   | 0.131| 7.5E-04 | 0.023             |
| LVIDs (cm)    | Aminoadipate     | −0.514        | 0.131| 7.6E-04 | 0.023             |
| LVIDs (cm)    | ADP              | −0.214        | 0.131| 9.6E-04 | 0.023             |
| LVWtd (cm)    | Kynurenine       | −0.653        | 0.130| 1.2E-03 | 0.023             |
| IVSd (cm)     | Lactate          | −0.724        | 0.130| 1.4E-03 | 0.023             |

ADP, adenine dinucleotide phosphate; FDR, false discovery rate; IVSd, interventricular septum; LVWtd, left ventricular wall thickness; LVIDd, left ventricular internal dimension; LVWTd, left ventricular internal diameter, systole; LVFS, left ventricular fractional shortening; LVIDs, left ventricular internal diameter, diastole; LVWtd, left ventricular wall thickness, diastole.

Lipid denoted as #carbon_#double bonds_class. CE, cholesterol ester; PC, phosphatidylcholine; TAG, triacylglycerol.
rate, mitral valve E-point septal separation, and LV internal diameter and negative associations with LV ejection fraction and LV fractional shortening. Plasma glucose was associated with incident left atrial internal dimension ($\beta = 3$, $P = 9.4 \times 10^{-5}$). Ornithine was inversely associated with LV percentage fractional shortening ($\beta = -7.11$, $P = 1 \times 10^{-4}$). Glutamine was inversely associated with heart rate ($\beta = -13$, $P = 1 \times 10^{-4}$). As previously reported for prevalent association,\textsuperscript{5} we also found asparagine to be associated with LV internal dimension.

There were 579 lipid–incident echocardiographic associations at nominal significance, with 59 reaching FDR-adjusted significance (Table 3 shows top 20 metabolite and lipid associations). Sphingomyelin 18:2 was the top associated lipid, showing an inverse association with LV mass ($\beta = -70.1$ mg per SD increment, $P = 2.3 \times 10^{-5}$), which may report on the association of sphingolipids to lysosomal storage and cardiac hypertrophy in metabolic disease.\textsuperscript{12}

**Metabolite and lipid associations with incident heart failure with preserved ejection fraction**

We subsequently performed analyses on CHF subtypes: HFpEF and HFrEF. There were 11 metabolites associated with incident HFpEF after multivariable adjustment (age, gender, and BMI) at nominal significance ($P < 0.05$) (Supporting Information, Table S3). The top associated metabolite with incident HFpEF was ornithine ($\beta = 3.6$, $P = 2.8 \times 10^{-3}$), which as above, may reflect perturbed arginine and nitric oxide metabolism.\textsuperscript{13} The second most associated was glycerol ($\beta = 2.3$, $P = 5.7 \times 10^{-3}$). Not widely appreciated, glycerol is a significant cardiac energy substrate that serves as a key regulator of cardiomyocyte lipid metabolism and energy balance.\textsuperscript{14}

The top associated metabolite with incident CHF was ornithine ($\beta = 3.6$, $P = 2.8 \times 10^{-3}$), consistent with previous reports of elevated plasma ornithine in CHF patients, possibly reflecting perturbed arginine and nitric oxide metabolism.\textsuperscript{13} The second most associated was glyceral ($\beta = 2.3$, $P = 5.7 \times 10^{-3}$). Not widely appreciated, glyceral is a significant cardiac energy substrate that serves as a key regulator of cardiomyocyte lipid metabolism and energy balance.\textsuperscript{14}
dimethylglycine ($\beta = 2.1$, $P = 0.01$), followed by nicotine metabolite cotinine ($\beta = 0.35$, $P = 0.02$).

After multivariable adjustment, only one lipid species, phosphatidylcholine 36:4 ($\beta = -6.2$, $P = 0.001$), was associated with incident HFrEF, an inverse association (Supporting Information, Table S3).

### Metabolite and lipid associations with incident heart failure with reduced ejection fraction

We found only one metabolite associated with incident HFrEF after multivariable adjustment, the nicotine metabolite cotinine ($\beta = 0.6$, $P = 0.045$) (Supporting Information, Table S4). Aetiologically, this makes sense, as the most common cause of HFrEF is ischaemic heart disease that is causally linked to smoking.

### Metabolite–congestive heart failure associations in the Women’s Health Initiative

As the most common form of CHF has higher prevalence in women, we next examined metabolite–CHF associations in ~1300 female participants of the WHI. There were 100 metabolite/lipid-prevalent CHF associations at FDR-adjusted significance (top 20 shown in Supporting Information, Table S5). There were several baseline metabolite–prevalent CHF associations in the WHI that validated associations seen in FHS. For example, at FHR-adjusted significance after adjustment for age and BMI, choline ($\beta = 3.8$, $P = 1.3 \times 10^{-5}$), dimethylglycine ($\beta = 2.1$, $P = 9.2 \times 10^{-6}$), and ornithine ($\beta = 1.4$, $P = 6.7 \times 10^{-3}$) were all significantly associated with prevalent CHF in WHI; the choline and dimethylglycine associations persisted at Timepoint 2. Therefore, amongst the validated associations, choline and its one-carbon metabolite dimethylglycine had the most significant associations with CHF and warrant further investigations as to underlying pathology.

In the WHI, the strongest three prevalent associations, adjusted for age and BMI, at both baseline and the second timepoint had a diabetic/insulin resistance signature (Supporting Information, Tables S5 and S6). At baseline, glucose ($\beta = 6.7$, $P = 2.9 \times 10^{-9}$), C4-acylcarnitine ($\beta = 1.3$, $P = 1.8 \times 10^{-7}$), and isoleucine ($\beta = 4.9$, $P = 2.2 \times 10^{-7}$) were the top 3 associations, with similar association metrics at the second timepoint. Unsurprisingly, creatinine was significantly associated with prevalent CHF at both timepoints in WHI, reporting on the known association between reduced renal function and CHF, with impaired renal function conferring a worse outcome in CHF.16 There were no second timepoint metabolite–incident CHF associations after FDR adjustment in the WHI.
all CHF cases in Examinations 5 and 6 with the closest measured metabolites (Exam 5) and BNP (Exam 6). We then performed receiver-operator characteristic (ROC) curve analysis to obtain an approximate comparison of the ability of metabolites and BNP to distinguish between CHF and no CHF. As can be seen in Supporting Information, Figure S1, the top performing metabolite ornithine [area under the curve (AUC) = 0.77] had almost equal discriminatory capacity for CHF as gold standard biomarker BNP (AUC 0.78).

Using metabolite association, we next performed a metabolite mediation analysis between HFrEF and risk factors known to be associated with HFrEF (hypertension and diabetes) and between HFrEF and structural characteristics (left atrial size and LV wall thickness). Although no metabolites appeared to mediate the hypertension association with HFrEF, two metabolites mediated the association of diabetes with HFrEF: ornithine 3.4% \((P < 0.05)\) and asparagine 5% \((P < 0.05)\) (Figure 1C; Supporting Information, Table S7).

We next determined if any metabolites mediated the relationship between cardiac remodelling changes and HFrEF, results summarized in Supporting Information, Table S8. Intriguingly, the α-amino acid asparagine used in the biosynthesis of proteins partly mediated several cardiac structural parameters. Asparagine negatively mediated the association between left atrial dimension in systole and HFrEF by −4.8% \((P = 0.004)\) and in diastole by −3.7% \((P = 0.012)\), positively mediated the association between LV systolic wall thickness and HFrEF by 4.2% \((P = 0.036)\) and LV posterior wall thickness by 4.8% \((P = 0.02)\). Hydroxyglutarate mediated LV systolic wall thickness–HFrEF by 5% \((P < 0.01)\) and LV posterior wall thickness–HFrEF by 3.6% \((P < 0.05)\) (Supporting Information, Table S8). This is intriguing, as elevations in this enzyme are seen in α-2-hydroxyglutarate acidaemia syndrome that includes cardiac hypertrophy as part of the syndrome, and preclinical work demonstrated it leads to up-regulation of pro-hypertrophic genes\(^{17,18}\) and post-translational modifications in cardiac hypertrophy.\(^{17,18}\)

**Metabolite signatures of sleep apnoea indices and their relationship to heart failure and mortality**

As per Table 1, there were 390 individuals (198 male and 192 female) with sleep study data at Exam 5. We first determined the association between sleep study indices including obstructive-apnoea hypopnoea index (obAHI), central-AHI (cAHI), average \(O_2\) saturation, minimum \(O_2\) saturation, and time spent with \(O_2\) saturation below 90% and the following outcomes: CHF; HFrEF; HFrEF and death due to any cause; and death due to CVD. The only significant association was between cAHI and CHF \((P = 0.03)\) and between cAHI and HFrEF \((P = 0.031)\) (Supporting Information, Table S9), and sleep indices were not associated with HFrEF, overall mortality, or mortality from CVD. We next performed a mediation analysis and found that glycine \((P = 0.01)\) and ribose \((P = 0.02)\) significantly mediated the association between central sleep apnoea and HFrEF (Supporting Information, Table S10). Ribose is an intermediate in the pentose phosphate pathway, implicating this pathway as a mediator. Glycine, inversely associated with insulin resistance, may confer protection in both diseases.

We next performed association of metabolites with sleep apnoea parameters. We also examined the association of metabolites with average oxygen saturation, minimum oxygen saturation, and time spent with oxygen saturations less than 90%, the latter being one of the strongest predictors of cardiovascular outcome.\(^{19}\) Cotinine, the nicotine metabolite, was the strongest associated metabolite with each parameter, and each SD increase in cotinine was associated with 2.3 min spent with an oxygen saturation less than 90% \((β = 2.3 \text{ min}, P = 2.3 \times 10^{-4})\) (Table 4). To our knowledge, this is the first quantitative report of the relationship between plasma concentrations of a nicotine metabolite with oxygen saturations in people with sleep apnoea.

**Metabolite signatures sleep parameters and association of signature with congestive heart failure and mortality**

We next sought to derive baseline metabolite signatures of heart failure subtype and five indices of sleep (average \(O_2\) saturation, minimum \(O_2\) saturation, time spent with \(O_2\) saturation below 90%, obAHI, and cAHI). Figure 2A shows that the first three metabolite variates were significant. Figure 2B shows correlations of these three metabolite variates with HFrEF, HFrEF, and the five sleep indices. Metabolite Variate 1 was equally and inversely associated with both HFrEF and HFrEF. Metabolite Variate 2 was most strongly inversely associated with HFrEF. Metabolite Variate 3 was most strongly positively associated with HFrEF. Figure 2C illustrates the associations of each metabolite variate with overall mortality and CVD mortality.

**Discussion**

Although there have been previous efforts to identify circulating metabolite biomarkers of sleep apnoea,\(^{20}\) to our knowledge, we are the first to use metabolites to determine the relationship between sleep disordered breathing, cardiac remodelling, heart failure risk, mediation of risk, and canonical variates related to outcome.

We confirm several previous metabolite associations with cardiac remodelling parameters and heart failure. Increased α-ketoglutarate correlated with increased LV wall thickness,
while increased asparagine demonstrated an association with increased LV diastolic dimension.\textsuperscript{5,13} Higher levels of orni- thine, which has a role in the urea cycle, were associated with CHF.\textsuperscript{5} We report an intriguing inverse association between baseline levels of phosphocreatine, the major cardiac reservoir of high-energy phosphates, and future LV wall thickness. Likewise, we found a strong inverse association between baseline (lysosomal storage) sphingomyelins and future LV wall thickness. This may suggest that individuals storing more energy and lipids in LV myocardium at baseline are most prone to subsequent loss of LV wall thickness/mass; however, this needs further study to con-

Central sleep apnoea is known to be associated with CHF, but the association with HfPEF rather than HFrEF was unexpected. Ribose partly mediated this association. Ribose is an intermediate in the pentose phosphate pathway and is a critical component of adenosine and essential for maintaining ATP levels. In a clinical trial, administration of ribose improved diastolic function and quality of life in CHF patients.\textsuperscript{2} The relationship to diastolic, rather than systolic, function is unclear but is consistent with the relationship we found to HfPEF rather than HFrEF.

Our mediation analyses offer insight into the cardiac re-modelling process in HfPEF. Both asparagine and 2-hydroxyglutarate partly mediated the association of LV wall thickness with HfPEF. LV wall thickening is a cardinal feature of HfPEF, and changes in the proteogenic amino acid asparagine may relate to the structural composition of a thickening heart. 2-Hydroxyglutarate is associated with cardiac hypertrophy and activates several processes that are pro-hypertrophic including glyco- gen biosynthesis, histone modification, and protein acetylation.\textsuperscript{17,18} Myocardial hypoxia from sleep apnoea likely serves as the stimulus for up-regulation of 2-hydroxyglutarate in this context.\textsuperscript{22}

Choline and its one-carbon metabolite dimethylglycine were both associated with CHF in both FHS and WHI. The associations in WHI were much more significant, which likely represents the closer temporal relationship of metabolite sampling to CHF diagnosis in this cohort. Although both metabolites are reported risk factors for CVD\textsuperscript{23} or CV mortality, neither have been previously shown to have direct associations with CHF. However, preclinical work has shown that mice ingesting diets supplemented with choline had worse myocardial fibrosis and worsened haemodynamic and ana- tomic parameters after trans-aortic constriction (TAC).\textsuperscript{25} Choline is a precursor of trimethylamine-N-oxide (TMAO), and there are several reports of clinical associations between TMAO and CHF\textsuperscript{26} and diastolic dysfunction\textsuperscript{27}; one study even demonstrated greater predictive utility of TMAO compared with BNP in HFrEF patients.\textsuperscript{28}

That nicotine metabolite cotinine was signi- ficantly associated with several sleep study indices was unsurprising, but the strong association with time spent with oxygen saturation less than 90%, a major predictor of cardiovascular outcome,\textsuperscript{19} is an important finding. We found that each SD increment in cotinine levels was associated with a 2.3 min increase in time spent with oxygen saturations below 90%. To our knowledge, we are the first to quantify the effect of smoking on sleep apnoea. This result underscores the risk of smoking in those with sleep apnoea, a very common disorder estimated to affect up to one-third of the adult population or 1 billion people worldwide.\textsuperscript{29}
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Figure 2  (A) Of the 10 identified canonical variates (dimensions), three were significant. (B) Metabolite Variate 1 was inversely associated with HFrEF and HFpEF; Metabolite Variate 2 was inversely associated with HFrEF; and Metabolite Variate 3 was most positively associated with HFpEF. (C) Metabolite Variate 1 was significantly protective for cardiovascular death. Av O₂ sat, average oxygen saturation; cAHI, apnoea–hypopnoea index, central sleep apnoea; CI, confidence interval; CV, cardiovascular; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; Min O₂ sat, minimum oxygen saturation; obAHI, apnoea–hypopnoea index, obstructive sleep apnoea; T < 90, time spent with oxygen saturation less than 90%.

| ID  | Wilk’s Lambda Statistic | F-approximation | Numerator degrees of freedom for F-approximation | Denominator degrees of freedom for F-approximation | P value |
|-----|-------------------------|-----------------|--------------------------------------------------|--------------------------------------------------|---------|
| 1 to 10 | 0.004 | 1.805 | 1130 | 2684 | 0.0e+00 |
| 2 to 10 | 0.017 | 1.387 | 1008 | 2422 | 1.4e-10 |
| 3 to 10 | 0.047 | 1.139 | 888 | 2159 | 9.6e-03 |
| 4 to 10 | 0.089 | 1.019 | 770 | 1894 | 3.8e-01 |

| T<90 | obAHI | min O2 sat | HFrEF | HFpEF | cAHI | Av O2 sat |
|------|-------|------------|-------|-------|------|-----------|

| Metabolite Variate 1 | Overall death HR (95%CI) | P value | CV death HR (95%CI) | P value |
|----------------------|--------------------------|---------|-------------------|---------|
| Metabolite Variate 1 | 0.12 (0.01, 1.76) | 0.12 | 0.30 (0.11, 0.81) | 0.02 |
| Metabolite Variate 2 | 1.10 (0.27, 4.41) | 0.89 | 0.98 (0.45, 2.14) | 0.96 |
| Metabolite Variate 3 | 0.70 (0.23, 2.14) | 0.53 | 1.13 (0.62, 2.04) | 0.69 |
Contract HHSN268201300008C. The datasets used for the analyses described in this manuscript were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap through dbGaP accession phs000200.v11.p3.

**Conflict of interest**

The authors report no conflicts of interest.

**Author contributions**

J.F.O.S. conceived and designed the study, interpreted the results, and wrote the manuscript. S.D. and J.Y. designed the statistical plans and performed the statistical analyses with Y.W. and wrote the manuscript. D.L., A.W., and M.I. retrieved and organized the relevant data from the databases and wrote the manuscript. Y.C.K. gave intellectual input to the sleep study data and reviewed the manuscript. K.C. and M.F. wrote the manuscript. I.W. gave intellectual input on heart failure data. P.C. gave intellectual input on sleep data and reviewed the manuscript. H.D. retrieved and analysed sleep data. P.C. gave intellectual input on the metabolomic analysis and interpretation. S.L. gave intellectual input to the heart failure results and interpretation and wrote the manuscript. H.D. retrieved and analysed sleep data. P.C. gave intellectual input on sleep data and reviewed the manuscript. I.W. gave intellectual input on heart failure—sleep interaction and reviewed the manuscript. K.C. and M.F. gave intellectual oversight to the sleep study data and offered interpretation. All authors reviewed the manuscript and have approved the final version of the manuscript.

**Supporting information**

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

**Figure S1.** Receiver-operator characteristic curve of ornithine and BNP reveals similar capacity to distinguish between presence and absence of CHF.

**Table S1.** List of all Metabolites and Lipids.

**Table S2.** FHS: Metabolites and lipids associated with incident CHF.

**Table S3.** FHS: Metabolites and lipids associated with incident HFrEF.

**Table S4.** FHS: Metabolites and lipids associated with incident HFrEF.

**Table S5.** WHI: Metabolites associated with prevalent CHF at baseline.

**Table S6.** WHI: Metabolites associated with prevalent CHF at 2nd timepoint.

**Table S7.** Summary of mediation analyses of risk factors with HFrEF.

**Table S8.** Summary of mediation analyses of cardiac remodeling with HFrEF.

**Table S9.** Association of Sleep Apnoea Parameters with CHF and Mortality Outcomes.

**Table S10.** Summary of mediation analysis of sleep apnoea with CHF and HFrEF.

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