Journal of the American Heart Association

ORIGINAL RESEARCH

Metabolomic Profiles, Ideal Cardiovascular Health, and Risk of Heart Failure and Atrial Fibrillation: Insights From the Framingham Heart Study

Yi Li, MA*; Ayana Gray, BA*; Liying Xue, MS; Melissa G. Farb, PhD; Nir Ayalon, MD; Charlotte Andersson, MD, PhD; Darae Ko, MD, MSc; Emelia J. Benjamin, MD, ScM; Daniel Levy, MD; Ramachandran S. Vasan, MD; Martin G. Larson, SD; Jian Rong, PhD; Vanessa Xanthakis, PhD†; Chunyu Liu, PhD†; Jessica L. Fetterman, PhD† Deepa M. Gopal, MD, MS†

BACKGROUND: The American Heart Association's framework "ideal cardiovascular health" (CVH) focuses on modifiable risk factors to reduce cardiovascular disease (CVD). Metabolomics provides important pathobiological insights into risk factors and CVD development. We hypothesized that metabolomic signatures associate with CVH status, and that metabolites, at least partially, mediate the association of CVH score with atrial fibrillation (AF) and heart failure (HF).

METHODS AND RESULTS: We studied 3056 adults in the FHS (Framingham Heart Study) cohort to evaluate CVH score and incident outcomes of AF and HF. Metabolomics data were available in 2059 participants; mediation analysis was performed to evaluate the mediation of metabolites in the association of CVH score and incident AF and HF. In the smaller cohort (mean age, 54 years; 53% women), CVH score was associated with 144 metabolites, with 64 metabolites shared across key cardiometabolic components (body mass index, blood pressure, and fasting blood glucose) of the CVH score. In mediation analyses, 3 metabolites (glycerol, cholesterol ester 16:1, and phosphatidylcholine 32:1) mediated the association of CVH score with incident AF. Seven metabolites (glycerol, isocitrate, asparagine, glutamine, indole-3-proprionate, phosphatidylcholine C36:4, and lysophosphatidylcholine 18:2), partly mediated the association between CVH score and incident HF in multivariable-adjusted models.

CONCLUSIONS: Most metabolites that associated with CVH score were shared the most among 3 cardiometabolic components. Three main pathways: (1) alanine, glutamine, and glutamate metabolism; (2) citric acid cycle metabolism; and (3) glycerolipid metabolism mediated CVH score with HF. Metabolomics provides insights into how ideal CVH status contributes to the development of AF and HF.

Key Words: atrial fibrillation ■ CVH score ■ heart failure ■ mediation analysis ■ metabolomics

In 2010, the American Heart Association (AHA) reframed the approach to reduce cardiovascular disease (CVD) by introducing the concept of ideal cardiovascular health (CVH), a positive, holistic approach to health.[1] Contrary to conceptualizing CVD development in terms of a binary presence or absence of risk

Correspondence to: Deepa M. Gopal, MD, MS, Boston University Chobanian & Avedisian School of Medicine, 72 E. Concord St – Suite 812D, Boston, MA 02118. Email: dmgopal@bu.eduand Jessica L. Fetterman, PhD, Boston University Chobanian & Avedisian School of Medicine, 600 Albany St, W-602A, Boston, MA, 02118. Email: jefetter@bu.edu

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.122.028022

*Y. Li and A. Gray are co-first authors.

†V. Xanthakis, C. Liu, J. L. Fetterman, and D. M. Gopal share senior authorship.

For Sources of Funding and Disclosures, see page 12.

This manuscript was sent to Pamela N. Peterson, MD, Deputy Editor, for review by expert referees, editorial decision, and final disposition.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In a cohort of 2059 Framingham Heart Study participants with ideal cardiovascular health (CVH) metrics and metabolomics, 144 metabolites associated with CVH score; most of the significant metabolites were lipid species and shared by 3 key cardiometabolic components of the CVH score (body mass index, blood pressure, and fasting blood glucose).
- In mediation analyses with multivariable-adjusted models, several lipid metabolites (glycerol, cholesterol ester 16:1, phosphatidylcholine 32:1) partially mediated the association of CVH score and incident atrial fibrillation.
- Glycerol, isocitrate, asparagine, glutamine, indole-3-proprionate, phosphatidylcholine C36:4, and lysophosphatidylcholine 18:2 partly mediated the association between CVH score and incident HF.

What Are the Clinical Implications?

- The circulating metabolome may lend insight on key alterations in metabolic pathways with different CVH scores and possible biology of benefit observed when modifiable risk factors, and in turn, CVH scores, are optimized in individuals with mitigation in atrial fibrillation and heart failure risk.
- Validation of metabolomic signatures in well-phenotyped cohorts may reveal novel metabolic pathways in complex diseases, such as heart failure, to help direct future mechanistic studies, biomarkers, and therapeutics.

Nonstandard Abbreviations and Acronyms

CE	cholesterol ester
CVH	cardiovascular health

factors, the AHA CVH score provides a platform for a non-zero-sum approach in decreasing CVD. The AHA CVH score empowers both patients and clinicians alike to actively optimize CVH components over the course of a lifetime to not only mitigate CVD development, but also to enhance primordial prevention.[1,2]

The construct of ideal CVH is composed of 7 components: ideal body mass index (BMI), nonsmoking, physical activity at goal levels, healthy diet consistent with current guideline recommendations, untreated and controlled total cholesterol, blood pressure, and the absence of diabetes. Individuals with higher CVH scores[3–6] have lower rates of CVD and decreased all-cause mortality.[7] Importantly, ideal CVH associates with lower incident HF[8–10] and AF.[11–13]

The biologic underpinnings of the association of ideal CVH with lower risk of HF and AF are not well-defined. Metabolomics offers a unique reflection of gene and protein functional activity and provides important pathobiological insights into CVD risk factors and development.[14] Whether circulating metabolites relate to ideal CVH status and mediate the association between ideal CVH and AF and HF has not been fully elucidated. Accordingly, we hypothesized that metabolomic signatures (lipid and nonlipid metabolites) associate with the CVH score, and that metabolites partly mediate the association of CVH score with AF and HF.

METHODS

All participants gave written informed consent, and all protocols were approved by the Institutional Review Board of Boston University Medical Center. All data and materials have been made publicly available at the Biologic Specimen and Data Repository Information Coordinating Center repository and can be accessed at https://biolincc.nhlbi.nih.gov/studies/framcohort/. Code used for analysis is available upon reasonable request and for collaboration and reproducibility purposes.

Study Sample

The study design and methodology for the Framingham Offspring Study has previously been described.[15] Of the 3799 participants in the Offspring cohort who attended a routine fifth examination cycle (1991–1995), referred to as the baseline for the present investigation, 2 sample sets were used for the analysis (Figure 1). For the analysis evaluating the CVH components and incident outcomes of AF and HF, participants with missing CVH metrics (n=525), prevalent AF or HF (n=57), and lacking follow-up time (n=161) were excluded, yielding a base sample of 3056 available for analysis (sample 1). For the second sample, profiling of polar positive-charge, polar negative-charge, and lipid metabolites were not available in 997 individuals, yielding a total sample of 2059 (sample 2) included for all analyses with metabolites and mediation.

AHA Cardiovascular Health Score

A CVH score was constructed for each participant by assigning a score of 0 (poor status), 1 (intermediate status), or 2 (ideal status) to each of the 7 AHA CVH metrics (Table S1).[3,16] Every individual was assigned a CVH score ranging from a minimum value of 0 (poor CVH) to maximum of 14 (ideal CVH) by summing the scores of each metric. BMI, blood pressure, cholesterol, fasting blood glucose, and self-reported smoking

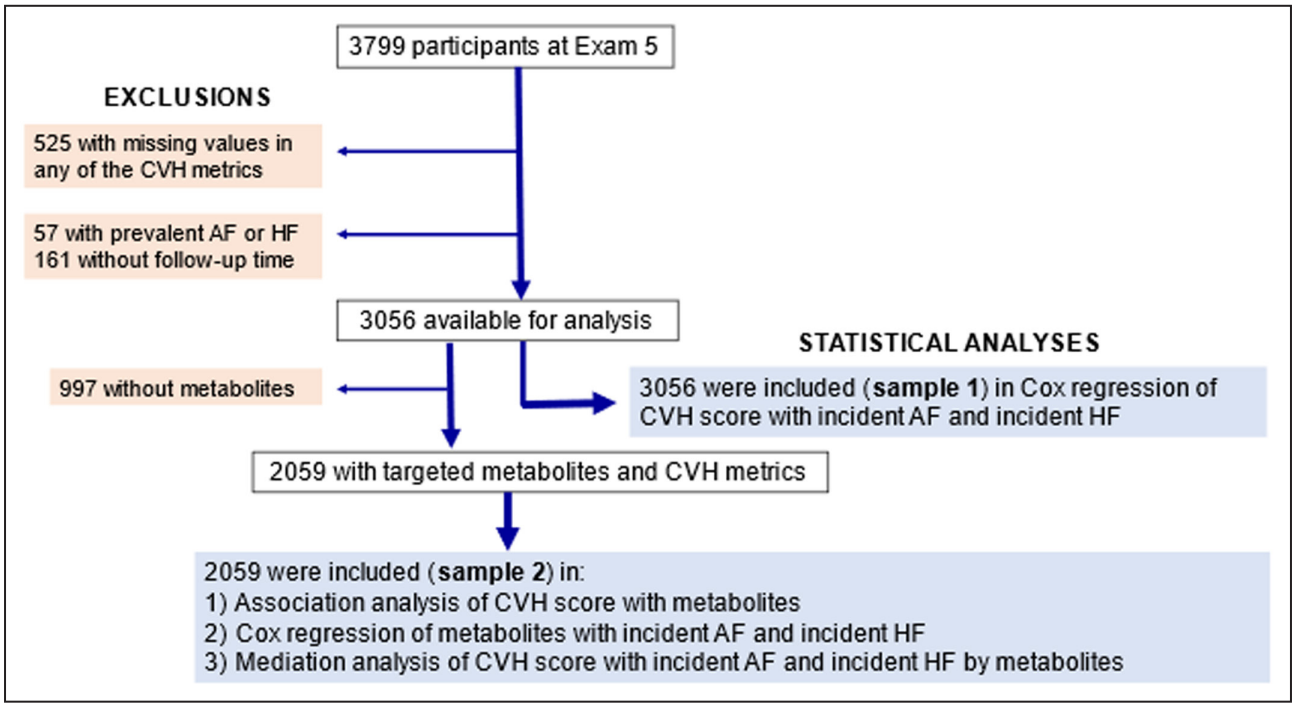


Figure 1. Cohort flow diagram and sample sizes for cardiovascular health score and metabolite analyses.
In FHS (Framingham Heart Study), 3799 Generation 2 participants completed Exam 5. A total of 743 participants were excluded because of missing data related to cardiovascular health metrics, presence of prevalent atrial fibrillation or heart failure, or without follow-up time. A total of 3056 participants were included in the cardiovascular health score and incident atrial fibrillation and heart failure Cox regression models (sample 1). An additional 997 individuals did not have metabolomic data available and were excluded from the metabolomics analysis; 2059 were ultimately used for statistical analyses with metabolomic data (sample 2). AF indicates atrial fibrillation; CVH, cardiovascular health; and HF, heart failure.

status were measured at routine FHS (Framingham Heart Study) visits. As previously published, ideal diet and physical activity metrics proposed by the AHA guidelines were aligned with the FHS physical activity index and food frequency questionnaire administered at the fifth examination to create the diet and physical activity components of the CVH score.[6,9]

Outcome Events

Incident HF was determined by a physician adjudication panel who reviewed all inpatient, outpatient, and FHS history and physical examinations, applying the well-established FHS epidemiological criteria for HF.[17] Incident HF events included participants hospitalized for HF and those meeting FHS HF criteria without hospitalization. HF with preserved ejection fraction (HFpEF) was defined with left ventricular ejection fraction ≥50% and HF with reduced ejection fraction (HFrEF) with left ventricular ejection fraction <50% at the time of HF diagnosis.

During FHS examination visits, 12-lead ECGs were obtained, in addition to physical examinations and standardized questionnaires. A physician adjudication panel reviewed outpatient records, inpatient admissions, and ECGs (with outside ECGs sought in individuals with high suspicion of heart rhythm disorder) and the diagnosis of AF (or atrial flutter) was made if at least 2 FHS cardiologists verified the rhythm abnormality on a collected ECG (including Holter ECG, telemetry, or other monitoring platform).

Metabolomics

Plasma samples, collected in EDTA at the fifth examination following an overnight fast, were immediately processed, and stored at −80 °C until assayed. Liquid chromatography with tandem mass spectrometry platform and hydrophilic interaction chromatography method was performed for targeted metabolite profiling as previously described.[18–20] Negatively charged polar metabolites (ie, organic acids, bile acids, and sugars), positively charged polar metabolites (ie, amino acids, urea cycle intermediates, nucleotides), and lipid metabolite species (ie, cholesterol esters, diacylglycerols, lysophosphatidylcholines, lysophosphatidylethanolamines, phosphatidylcholines, syphingomyelins, triacylglycerols) were profiled.[18–21] Lipid metabolite nomenclature was notated with the first number indicating the total number of carbons in the lipid acyl chain and the number following the colon denoting the number of double bonds. Five metabolites with missing values

in greater than 1000 participants were excluded; thus, 212 metabolites were used in the analysis.

Statistical Analysis

A multistep analytic plan was used to test the hypothesis that metabolites mediate the association between ideal CVH and incident AF and HF (Figure 2). We assigned the CVH score for each participant from 0 to 14 (as described above). For primary analyses, the CVH score was used as a continuous variable. For metabolomic analyses, each metabolite measurement was natural logarithmically transformed, centered to mean zero, and scaled using an SD of 1. Metabolite values beyond ±4 SDs were set at ±4 SD. Using this approach, all metabolites were normally distributed.

Association of CVH Score and Incident AF and HF (Path A)

For the first step, we investigated the association of CVH score with incident AF and incident HF in Cox proportional hazard regression models, adjusting for age and sex, after confirming the assumption of proportionality of hazards was met (separate model for each outcome). As the relations of CVH score with incident HF in FHS[9] and with incident AF in other cohorts[13,22] have been previously reported, we additionally modeled individual components of the CVH score to identify which of the individual components contribute to the greatest magnitude of association of CVH score with AF and HF. In exploratory analyses, the association of CVH score with HF subtypes (HFpEF and HFrEF) in Cox regression models (as described above) were also evaluated.

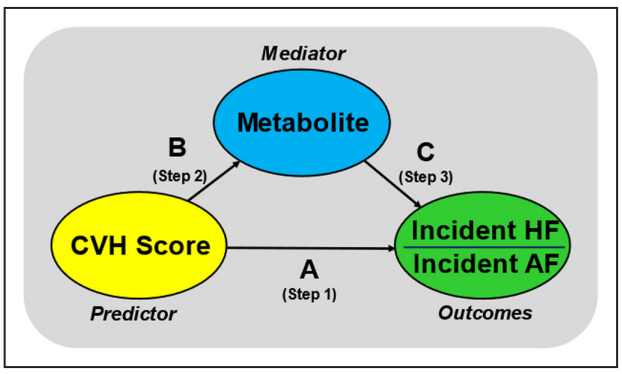


Figure 2. Statistical approach with a path diagram of the mediation analysis.
One-way regression models were used to evaluate the relation between cardiovascular health score (predictor) and incident atrial fibrillation or heart failure (outcomes) (**A**); cardiovascular health score and metabolite (**B**); and metabolites and incident atrial fibrillation and heart failure (**C**). Significant variables in all 3 steps for each of the outcomes were then used in mediation analysis to determine whether the metabolite mediates the association between cardiovascular health score and incident atrial fibrillation or heart failure. AF indicates atrial fibrillation; CVH, cardiovascular health; and HF, heart failure.

Association of CVH Score and Metabolites (Path B)

In the second step, we evaluated the cross-sectional association between CVH score (independent variable) and each of the 212 metabolites (dependent variable) in separate age-, sex-, estimated glomerular filtration rate (eGFR)-, and sibship-adjusted linear mixed models. In addition, we assessed the association of metabolites and each of the seven CVH components using each component score as a continuous variable. We characterized the overlap of metabolites and CVH components. Multiple hypothesis testing was accounted for using the false discovery rate (FDR) adjusted methodology of Benjamini and Hochberg[23] with a P value ≤0.05 considered significant. To gain insight into metabolic pathways associated with the CVH score components, we performed a pathway enrichment analysis of the metabolites associated with CVH score using MetaboAnalyst v5.[24]

Association of Metabolites and Incident AF and HF (Path C)

For the third step, we investigated the association of the significant metabolites from the previous step (Path B) with the association of incident AF and HF (separate model for each outcome), adjusting for age-, sex-, and eGFR, using Cox proportional hazards regression. Multiple testing was applied in this step with FDR adjusted P value ≤0.05 considered significant. Similar models were constructed for HFrEF and HFpEF. eGFR was included in all models with metabolite data as known renal function has been shown to decline over time in the FHS cohort.[25] Further, based upon sensitivity analyses, we chose to include baseline renal function as a covariate between metabolites and the outcomes of interest.

Mediation Analysis

For our final step, mediation analysis was used to examine whether metabolites mediate the association of CVH score with incident AF and HF. Only variables that were statistically significant in the 3-way associations described in paths A–C (ie, all 3 associations were significant at P<0.05) were included in mediation analysis (Figure 2).[26] The application of VanderWeele's approach to mediation analysis was used (PROC CAUSALMED procedure, SAS 9.4).[27,28] The hazard ratio for total effect, natural indirect effect, and natural direct effect were calculated. All mediation analyses included age, sex, and eGFR as covariates.

Analyses were performed using R version 4.0.3 (The R Foundation for Statistical Computing) and SAS software version 9.4 (SAS Institute, Cary, NC).

RESULTS

The baseline characteristics of sample 1, the larger sample, are shown in Table 1. Participants were middle-aged to older adults with a mean age of 54 years with women comprising 54% of the cohort. The prevalence of obesity was 24% and prevalence of hypertension was 53%. Sample 2, the smaller cohort used for metabolite and mediation analysis, had similar baseline characteristics (see Table S2). The mean CVH score for sample 1 by sex was 9±2 for women and 8±2 for men with only 25% of women and 13% of men classifying as "optimal" (CVH score >10; Figure S1). Except for the physical activity component, women had a higher percentage of ideal CVH components, particularly in ideal cardiometabolic domains (BMI, blood pressure, and fasting blood glucose). The only 2 components that achieved greater than 50% of individuals classifying as ideal were fasting blood glucose and smoking status (Figure S2).

Association of CVH Score and Score Components With AF and HF Risk

In 3056 participants (sample 1), 554 individuals developed AF (median follow-up of 18.3 years) and 185 individuals developed HF (median follow-up of 18.4 years). In age- and sex-adjusted models, each unit increase in CVH score decreased the hazard ratio (HR) for AF incidence by 9% (HR, 0.91 [95% CI 0.86–0.97]). Additionally, each unit increase in CVH score resulted in a 23% reduction in HR for HF incidence (HR, 0.77 [95% CI 0.68–0.89]). A greater number of ideal cardiometabolic CVH components (ideal BMI, blood pressure, fasting blood glucose) was associated with a lower proportion of incident AF and HF events (Figure 3).

To identify the key CVH components explaining the association of CVH score with incident AF and HF (in separate models for each outcome), we investigated the association of each CVH component score with AF and HF outcomes as a continuous variable with the hazard ratio reflecting per unit increase in category score in age- and sex-adjusted models (Table S3). The largest reductions in AF hazard ratios were observed when 3 cardiometabolic components (BMI, blood pressure, and fasting blood glucose) of the CVH score were ideal. Similarly, ideal scores for BMI, blood pressure, and fasting blood glucose conferred the lowest hazard ratios for HF development. Ideal smoking status and active physical lifestyle also reduced incident HF risk but to a lesser extent than ideal BMI, blood pressure, and fasting blood glucose. Ideal total cholesterol was not associated with either incident AF or incident HF development when evaluated as an individual component. As 3 of the 4 classic cardiometabolic traits were

Table 1. Clinical Characteristics of FHS Participants With Cardiovascular Health Data

Characteristic	Women (n=1649)	Men (n=1407)
Age, y	54 (10)	55 (10)
Body mass index, kg/m^2	27 (6)	28 (4)
Systolic blood pressure, mm Hg	124 (20)	129 (17)
Diastolic blood pressure, mm Hg	73 (10)	77 (10)
Antihypertensive medications, %	274 (17)	269 (19)
Total cholesterol, mg/dL	208 (38)	202 (35)
HDL, mg/dL	57 (15)	43 (11)
Fasting glucose, mg/dL	97 (25)	104 (30)
Diabetes medication, %	38 (2)	58 (4)
eGFR, mL/min per 1.73 m^2	68 (15)	75 (16)
Smoking, %	318 (19)	251 (18)
CVH score metrics		
CVH score	9 (2)	8 (2)
CVH category frequency, %		
Smoke		
Poor	318 (19)	251 (18)
Intermediate	0 (0)	0 (0)
Ideal	1331 (81)	1156 (82)
Body mass index		
Poor	352 (21)	378 (27)
Intermediate	533 (32)	727 (52)
Ideal	764 (46)	302 (22)
Blood pressure		
Poor	346 (21)	357 (25)
Intermediate	614 (37)	683 (49)
Ideal	689 (42)	367 (26)
Total cholesterol		
Poor	305 (19)	176 (13)
Intermediate	650 (39)	598 (43)
Ideal	694 (42)	633 (45)
Fasting blood glucose		
Poor	75 (5)	113 (8)
Intermediate	333 (20)	466 (33)
Ideal	1241 (75)	828 (59)
Physical activity		
Poor	354 (22)	223 (16)
Intermediate	742 (45)	511 (36)
Ideal	553 (34)	673 (48)
Diet		
Poor	559 (34)	650 (46)
Intermediate	1015 (62)	709 (50)
Ideal	75 (5)	48 (3)

Continuous variables represent mean (SD); categorical variables represent number (percentage). CVH indicates cardiovascular health; eGFR, estimated glomerular filtration rate; FHS, Framingham Heart Study; and HDL, high-density lipoprotein.

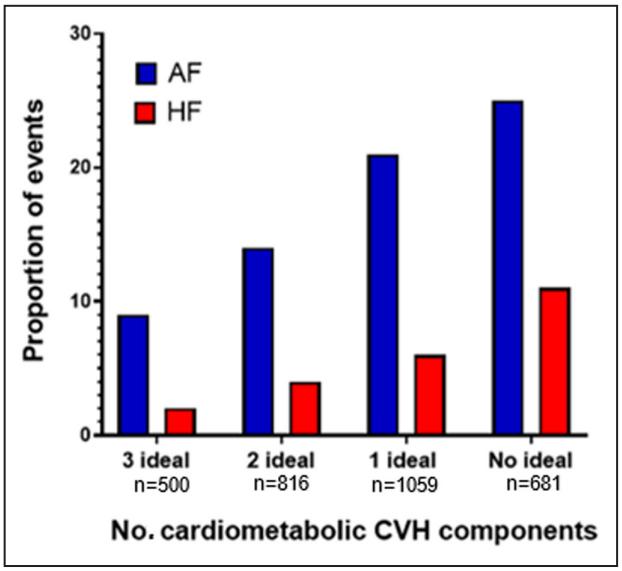


Figure 3. Number of ideal cardiometabolic cardiovascular health components and proportion of atrial fibrillation and heart failure events.
Participants with a greater number of ideal cardiometabolic components (cardiometabolic cardiovascular health components defined using blood pressure, obesity, and fasting blood glucose metrics) was associated with a lower proportion of incident atrial fibrillation and heart failure events. AF indicates atrial fibrillation; CVH, cardiovascular health; and HF, heart failure.

significantly associated with incident AF and HF, we restricted the "cardiometabolic components" to include BMI, blood pressure and fasting blood glucose from this point forward in this analysis.

A sensitivity analysis was performed to evaluate the presence of interim myocardial infarction with incident HF. Of the 132 participants with incident HF development during the follow-up of this study, 20 participants had prevalent coronary artery disease with a history of myocardial infarction. 51 participants had interim myocardial infarctions (defined as any new myocardial infarction event from baseline visit to end of follow-up); 20 were diagnosed with HFpEF and 24 with HFrEF (7 individuals had missing myocardial infarction data). From Sample 1 (n=3056), 78 observations had missing data, leaving a total of n=2978 participants with 165 HF events to evaluate interim myocardial infarction as covariate in the models of CVH score with incident HF in age- and sex-adjusted models. Adjustment with interim myocardial infarction in Cox proportional hazard models showed a similar association of CVH score (per 1-unit increase) with incident HF (HR, 0.83 [95% CI 0.77–0.89], *P* value <0.001) compared to analyses without interim myocardial infarction adjustment (Table S3). Thus, further multivariable analyses did not include interim myocardial infarction as it did not significantly affect the association between CVH score and incident HF.

Associations Between CVH Score and Score Components With Metabolites

In sample 2 (n=2059), using separate age-, sex-, eGFR-, and random effect-adjusted linear mixed models, CVH score was associated with 144 of the 212 metabolites, using an FDR-adjusted *P* value of 0.05. The number of metabolites that were associated with total CVH score and each of the CVH components are shown in Table S4. Three of four cardiometabolic CVH components (ideal BMI, blood pressure, fasting blood glucose) shared the greatest number of overlapping metabolites (n=67) with 64 of these metabolites overlapping with total CVH score (41 lipids, 64% of total metabolite panel; Figure 4A; Figure S3). The metabolites associated with these 3 cardiometabolic components of the CVH score are enriched in amino acid metabolism, particularly glucose-alanine cycle, alanine metabolism, and lactose degradation (Figure 4B). In contrast, the CVH components smoking, physical activity, and diet had the fewest number of metabolites overlapping with the CVH score (Figure S4) and these 3 components shared no significant metabolites with each other (Figure S3).

We then evaluated the lipid metabolites separately to assess the relations between the type of lipid, carbon length, and saturation to total CVH score. Overall, most metabolites that related to CVH score were lipids (72%). A trend of increasing carbon length and less saturation (more double bonds) was noted across several of the lipid species, specifically cholesterol esters, lysophosphatidylcholines, and phosphatidylcholines, and associated with higher CVH scores. The relations of carbon length and saturation with CVH score was less defined in triacylglycerols, syphingomyelins, and diacylglycerols (Figure 4C; Figure S5).

Metabolite Associations With Incident AF and HF

Using Cox proportional hazard models, we evaluated the association of the CVH score-related 144 metabolites with incident AF and HF, adjusting for age, sex, and eGFR. Two long-chain fatty acid species, cholesterol ester 16:1 and phosphatidylcholine 32:1, associated with higher AF risk (HR, 1.26 [95% CI, 1.12–1.42], FDR-adjusted *P* value 0.022; HR, 1.24 [95% CI, 1.10–1.39], FDR-adjusted *P* value 0.037; respectively) per unit increase of log-transformed metabolite. Glycerol levels were associated with AF development (HR, 1.29 [95% CI, 1.11–1.49], FDR-adjusted *P* value 0.037; Figure 5A; Table S5). Eight metabolites, including 6 nonlipid metabolites (glycerol, isocitrate, asparagine, glutamine, α-hydroxybutyrate, and indole-3-propionate), and 2 lipid metabolites (phosphatidylcholine-A 36:4, lysophosphatidylcholine 18:2), were associated with incident HF (Figure 5B; Table S5).

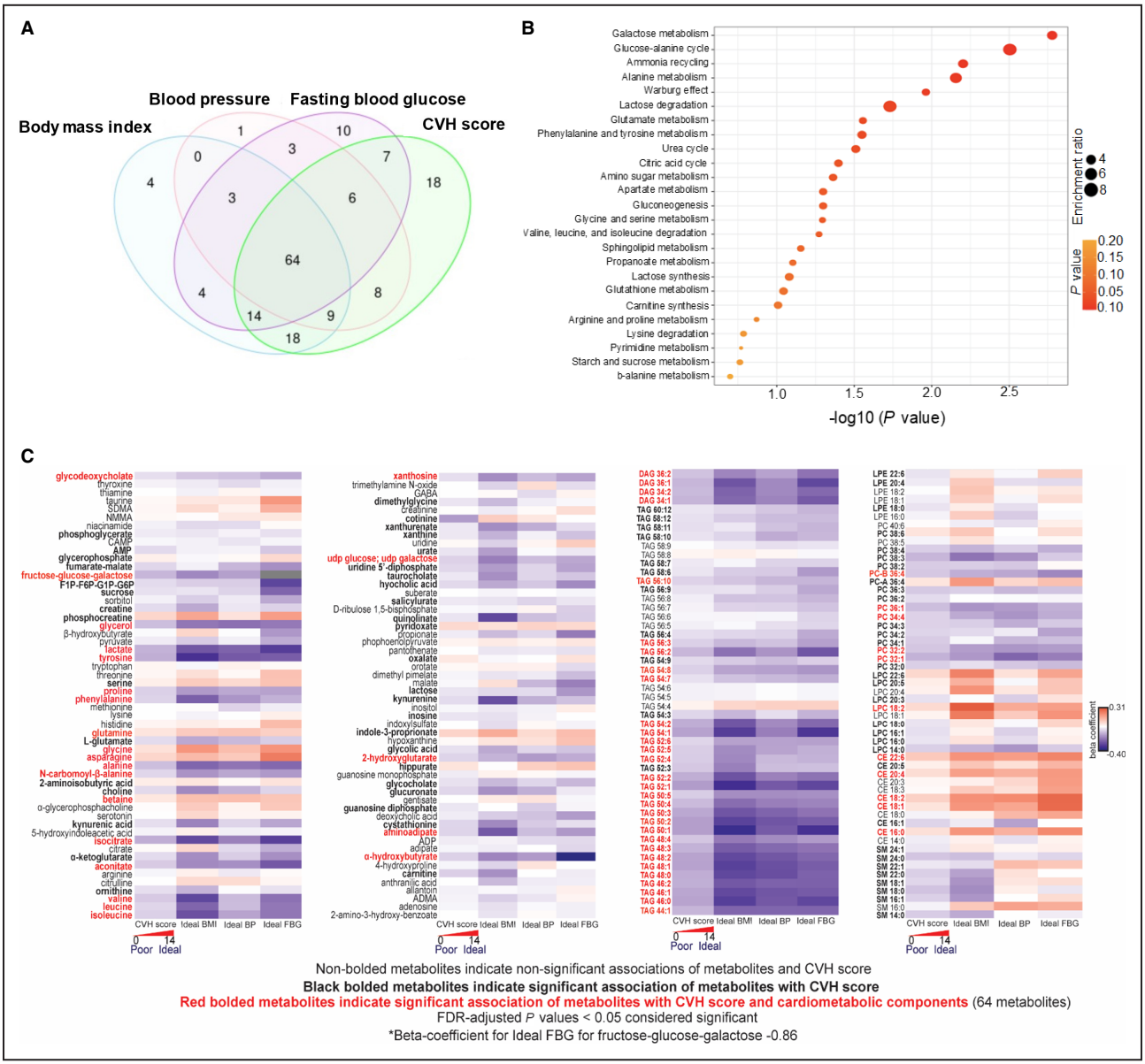


Figure 4. Overlap of metabolites associated with cardiovascular health (CVH) score and cardiometabolic CVH components. Of the 144 metabolites associated with the CVH score in multivariate models (false discovery rate adjusted *P* value ≤0.05), 64 metabolites were shared across the cardiometabolic components of the CVH score (body mass index, blood pressure, and fasting blood glucose) and composite CVH score (**A**). Pathway analysis of the 23 non-lipid metabolites (of the 64 shared metabolites of the CVH score and cardiometabolic components) indicate that the glucose-alanine, alanine, and lactose degradation pathways are enriched (**B**). Of the 64 shared metabolites between the CVH score and ideal cardiometabolic components (body mass index, blood pressure, fasting blood glucose), 23 were non-lipid metabolites and 41 were lipid metabolites (cholesterol ester, diacylglycerol, lysophosphatidylcholine, phosphatidylcholine, triacylglycerol) (**C**). BMI indicates body mass index; BP, blood pressure; CVH, cardiovascular health; FBG, fasting blood glucose; and FDR, false discovery rate.

Metabolite Associations in HFpEF Versus HFrEF

HF is a heterogeneous syndrome with differing pathobiology and mechanisms underpinning the 2 main HF subtypes, HFpEF and HFrEF. Consequently, metabolic associations may be diluted by grouping all HF cases together and hence, in exploratory analyses, we evaluated the association of CVH score and metabolites with HF subtypes. In sample 1, a total of 72 HFpEF and 74 HFrEF cases were observed (Figure S6). Total CVH score was inversely associated with HFpEF risk (HR, 0.75 [95% CI 0.67–0.84], *P*<0.0001) and HFrEF risk (HR, 0.77 [95% CI 0.68–0.86], *P*<0.0001) in age- and sex-adjusted models. Restricting our analysis to the 144 metabolites significantly associated with CVH score, 13 metabolites related to HFrEF: 10 non-lipid metabolites (isocitrate,

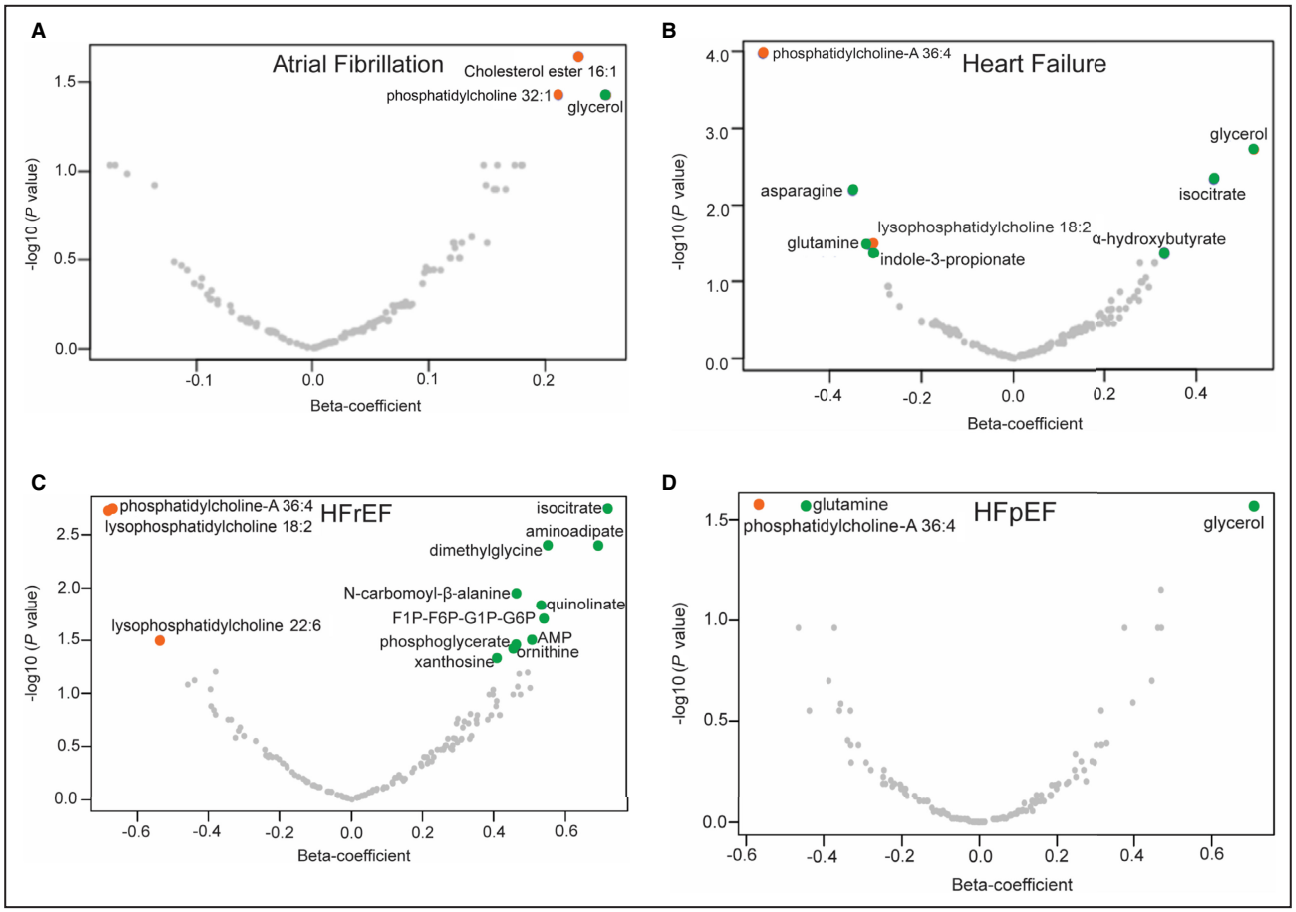


Figure 5. Metabolomic profile of incident atrial fibrillation, incident heart failure, and heart failure subtypes.
A series of volcano plots (**A** through **D**) depicting significant metabolites with atrial fibrillation, heart failure, and heart failure subtypes. Orange indicates significant lipid metabolites; green indicates significant non-lipid metabolites. All analyses are age-, sex-, and estimated glomerular filtration rate-adjusted models using a significance of 5% false discovery rate level. HFrEF indicates heart failure with reduced ejection fraction; and HFpEF, heart failure with preserved ejection fraction.

aminoadipate, dimethylglycine, carbamoylalanine, quinolinate, intermediates of glycolysis and pentose phosphate pathway [fructose-1-phosphate; fructose-6-phosphate; glucose-1-phosphate; glucose-6-phosphate], adenosine monophosphate, phosphoglycerate, ornithine, and xanthosine) and 3 lipid metabolites (phosphatidylcholine-A 36:4, lysophosphatidylcholine 18:2, and lysophosphatidylcholine 22:6) (Figure 5C; Table S6). Interestingly, 3 metabolites were associated with HFpEF risk in fully adjusted models: 2 non-lipid metabolites (glycerol, glutamine) and 1 lipid metabolite (phosphatidylcholine-A 36:4; Figure 5D; Table S6).

Metabolites as a Mediator Between CVH Score and Incident AF and HF

All variables that fulfilled the assumption of a 3-way association between CVH score, metabolites, and each of the outcomes (incident AF or incident HF) were entered into formal mediation analyses with Cox models. All 3 metabolites that were significantly associated with incident AF, glycerol, cholesterol ester 16:1, and phosphatidylcholine 32:1 were retained in mediation analysis. Seven metabolites (5 non-lipids: glycerol, isocitrate, asparagine, glutamine, indole-3-propionate; 2 lipid species: phosphatidylcholine-A 36:4, lysophosphatidylcholine 18:2) significantly mediated the association of CVH score with incident HF; α-hydroxybutyrate was not significant in FDR-adjusted analyses (Table 2). Pathway analysis of these metabolites highlights 3 main pathways: (1) alanine, glutamine, and glutamate metabolism; (2) citric acid cycle metabolism; and (3) glycerolipid metabolism (Figure S7).

In secondary and exploratory analyses, we evaluated metabolites as a mediator between CVH score and HF subtypes. Glycerol (24%), glutamine (5%), and phosphatidylcholine-A 36:4 (5%) mediated the association of CVH score with HFpEF (Table 3). Multiple metabolites, including metabolites in the citric acid cycle, urea metabolism, and long-chain fatty acids species were significant mediators of HFrEF (Table 3).

Table 2. Metabolites as a Mediator Between CVH Score and Incident AF and HF

	Total effect		Direct effect		Indirect effect=mediated				Sample	Event
							FDR	%		
Metabolites	HR	95% CI	HR	95% CI	HR	95% CI	*P* value	Mediated	Size	(n)
Incident AF										
Glycerol	0.92	0.86–0.98	0.94	0.88–1.00	0.97	0.96–0.99	0.009	25†	1561	276
Cholesterol ester 16:1	0.92	0.87–0.97	0.93	0.88–0.98	0.98	0.97–0.99	0.009	15†	1476	296
Phosphatidylcholine 32:1	0.92	0.86–0.97	0.93	0.88–0.99	0.97	0.96–0.99	0.009	24†	1476	296
Incident HF										
Glycerol	0.78	0.70–0.86	0.81	0.72–0.90	0.96	0.93–0.98	0.008	15*	1561	91
Isocitrate	0.77	0.69–0.85	0.80	0.72–0.89	0.95	0.92–0.98	0.008	15*	1474	91
Asparagine	0.75	0.68–0.82	0.77	0.70–0.84	0.97	0.96–0.99	0.008	7*	1765	111
Glutamine	0.75	0.68–0.83	0.76	0.69–0.84	0.98	0.97–0.99	0.029	4*	1765	111
α-hydroxybutyrate	0.79	0.71–0.87	0.80	0.72–0.88	0.98	0.96–1.00	0.074	6	1474	91
Indole-3-proprionate	0.78	0.70–0.86	0.80	0.72–0.88	0.98	0.96–0.99	0.043	7†	1474	91
Phosphatidylcholine-A 36:4	0.78	0.70–0.86	0.80	0.72–0.88	0.97	0.96–0.99	0.008	9*	1476	91
Lysophosphatidylcholine 18:2	0.78	0.70–0.86	0.80	0.72–0.88	0.98	0.96–0.99	0.029	6*	1487	91

Values are hazard ratios (HR), 95% CI, and *P* values. Each hazard ratio change represents the change in log-metabolite per 1-SD increase in metabolite in the association of cardiovascular health score and incident atrial fibrillation and heart failure. AF indicates atrial fibrillation; CVH, cardiovascular health; and HF, heart failure. Lipid nomenclature: the first number indicates the carbon length, and the subsequent number after the colon indicates the number of double bonds.

*$P<0.05$.

†$P<0.10$.

DISCUSSION

Higher CVH scores associate with reduced cardiovascular events, strokes, cardiovascular mortality, and all-cause mortality in several prospective cohort studies.[3–7,29] Our investigation extends the known association of CVH score with incident AF in other cohorts to the FHS. Using targeted metabolomics, we identified 144 metabolites associated with CVH score, with the greatest number of metabolites shared among 3 cardiometabolic components: BMI, blood pressure, and fasting blood glucose. Among the metabolites associated with CVH score, 3 metabolites partly mediated the association between CVH score and incident AF, and 7 metabolites partly mediated the association between CVH score and incident HF. In exploratory analyses, we found that the metabolites mediating the association of CVH score with incident HFpEF differed from the metabolites mediating the association of CVH score with incident HFrEF.

Association of CVH Score With AF and HF

Several studies have shown a lower CVH score associates with increased risk of AF[11,13,22] and this present investigation extends similar findings in FHS. Our findings build on the concept of approaching CVD risk as a fluid entity and active optimization of CVD risk factors throughout an individual's lifetime decreases cardiovascular risk. More recent studies have found that not only elevated risk factor burden, but also the addition of genetic predisposition elevates AF risk with lower risk factor burden associating with later AF onset after adjustment for polygenic risk.[29,30] Similarly, ideal CVH associates with lower incident HF.[8–10] Collectively, these findings strengthen the argument for clinicians and patients alike to continually work together to strategize and implement lifestyle and health behaviors, alongside effective therapeutics, to address modifiable CVD risk factors to mitigate both AF and HF risk.

Metabolites and CVH Score

FHS investigators previously evaluated the association of metabolites to CVH score restricting their analyses to 12 metabolites that concomitantly associated with longevity; of the 12 metabolites, the lowest tertiles of isocitrate and aconitate were associated with higher CVH scores.[31] We extended these prior findings by fully investigating the entire targeted platform of 212 metabolites measured in FHS. We found 144 metabolites associated with CVH score. Evaluating the intersection of each of the 3 key cardiometabolic components (BMI, blood pressure, fasting blood glucose) and total CVH score, we noted 64 shared metabolites with a lipid species majority composed of long-chain triacylglycerols (fatty-acid tails of ≥22 carbons). Consistent with prior studies associating metabolites with obesity and insulin resistance,[18,32–35] we observed both shorter carbon length triacylglycerols with a lower number of double-bonds (ie, more saturation with hydrogen ions) and diacylglycerols presence overall

Table 3. Metabolites as a Mediator Between CVH Score and HF Subtypes

	Total effect		Direct effect		Indirect effect=mediated					
							FDR	%	Sample	Event
Metabolites	HR	95% CI	HR	95% CI	HR	95% CI	*P* value	Mediated	Size	(n)
HFpEF										
Glycerol	0.79	0.66–0.92	0.84	0.69–0.98	0.94	0.90–0.98	0.011	24†	1506	36
Glutamine	0.72	0.62–0.83	0.74	0.63–0.84	0.98	0.96–0.99	0.018	5*	1702	48
Phosphatidylcholine-A 36:4	0.70	0.59–0.81	0.72	0.60–0.82	0.97	0.95–0.99	0.018	5*	1424	39
HFrEF										
Isocitrate	0.78	0.65–0.92	0.85	0.70–1.00	0.92	0.87–0.96	0.007	32†	1418	35
Aminoadipate	0.77	0.64–0.91	0.81	0.66–0.95	0.95	0.93–0.98	0.012	15†	1418	35
Dimethylglycine	0.73	0.61–0.84	0.74	0.63–0.86	0.97	0.96–0.99	0.021	6*	1697	43
N-carbamoyl-beta-alanine	0.73	0.62–0.85	0.75	0.63–0.87	0.97	0.96–0.99	0.021	7*	1697	43
Quinolinate	0.77	0.64–0.91	0.79	0.65–0.93	0.98	0.96–0.99	0.026	7†	1418	35
Glycolysis/PPP metabolites‡	0.77	0.63–0.90	0.79	0.65–0.93	0.97	0.95–0.99	0.021	9†	1418	35
AMP	0.78	0.65–0.91	0.80	0.66–0.94	0.97	0.95–0.99	0.024	10†	1418	35
Phosphoglycerate	0.79	0.66–0.92	0.80	0.66–0.94	0.98	0.97–1.00	0.06	5*	1418	35
Ornithine	0.73	0.61–0.84	0.74	0.63–0.86	0.97	0.96–0.99	0.028	6†	1697	43
Xanthosine	0.73	0.61–0.84	0.74	0.63–0.86	0.98	0.96–0.99	0.028	5†	1697	43
Phosphatidylcholine-A 36:4	0.77	0.64–0.90	0.79	0.65–0.93	0.98	0.96–0.99	0.023	9†	1420	35
Lysophosphatidylcholine 18:2	0.78	0.65–0.91	0.81	0.67–0.95	0.96	0.93–0.98	0.012	14†	1420	35
Lysophosphatidylcholine 22:6	0.77	0.64–0.91	0.80	0.66–0.94	0.96	0.93–0.99	0.021	13†	1420	35

Values are hazard ratios, 95% CI, and *P* values. Each hazard ratio change represents the change in log-metabolite per 1-SD increase in metabolite in the association of cardiovascular health score and heart failure subtypes. AF indicates atrial fibrillation; CVH, cardiovascular health; FDR, false discovery rate; HF, heart failure; HFpEF, HF with preserved ejection fraction; HFrEF, HF with reduced ejection fraction; HR, hazard ratio; and PPP, pentose phosphate pathway. Lipid nomenclature: the first number indicates the carbon length, and the subsequent number after the colon indicates the number of double bonds.

*$P<0.05$.

†$P<0.10$.

‡Glycolysis and pentose phosphate pathway intermediates include fructose-1-phosphate, fructose-6-phosphate, glucose-1-phosphate, and glucose-6-phosphate.

associated with lower CVH score and lower number of ideal cardiometabolic components. In our study, select citric acid cycle metabolites (citrate, aconitate, isocitrate, α-ketoglutarate, fumarate-malate) were inversely related with a higher CVH score, further supporting the importance of a functional citric acid cycle for CVH. This finding is consistent with prior studies associating elevated citric acid cycle metabolites with insulin resistance, obesity, and blood pressure.[18,36,37]

Metabolic Mediators of CVH Score and AF and HF

In our mediation analyses, we found that lipid species, specifically higher metabolite levels of glycerol, cholesterol ester 16:1, and phosphatidylcholine 32:1, were associated with higher risk of incident AF. To date, a limited number of studies have evaluated the relationship between circulating metabolites and AF.[38–44] In the community-based ARIC (Atherosclerosis Risk in Community) Study, glycolithocholate sulfate and glycocholenate sulfate (bile acid metabolites) in Black individuals[38] and pseudouridine and uridine (pyrimidine metabolism), and acisoga (polyamine metabolism) were associated with incident AF.[39] However, a prior FHS study found no association of AF risk with the 217 metabolites in the sample studied with 156 AF events with 10 years of follow-up.[44] The current study had greater statistical power with 388 AF events with >18 years of follow-up (sample 2 analysis) and we restricted our analyses to metabolites that were significantly associated with CVH score. Congruent with our results, lipid metabolites have been consistently noted with lower levels of free fatty acid species (monounsaturated, polysaturated, and unsaturated),[41] elevated phosphatidylcholine species[41] and 9-decenoylcarnitine (oxidative metabolite with a fatty acid esterified to a carnitine molecule),[43] and lower lysophosphatidylcholine 20:3[40] conferring greater AF risk.

Seven metabolites (5 non-lipids: glycerol, isocitrate, asparagine, glutamine, indole-3-propionate; 2 lipid species: phosphatidylcholine-A 36:4, lysophosphatidylcholine 18:2) mediated the association of CVH score with incident HF in the present study. Pathway analysis of these metabolites highlights 3 main pathways: (1) alanine, glutamine, and glutamate metabolism; (2)

citric acid cycle metabolism; and (3) glycerolipid metabolism. Glutamine is the most abundant amino acid in the body with important roles in energy metabolism, nitrogen, and carbon donation for growth-promoting pathways, and mitochondrial function.[45–47] The mediation of CVH score and incident HF by glutamine may be reflective of impaired citric acid cycle as glutamine can enter citric acid cycle via conversion to glutamate with subsequent conversion to α-ketoglutarate, or donate nitrogen for amino acid production (including aspartate to asparagine).[48] Impairments in mitochondrial function (ie, fatty acid and glucose oxidation impairments, lower mitochondrial respiration) in both human and animal studies have been previously reported in HF[49–54] and may contribute to citric acid cycle metabolic aberrations.[55] A recent elegant study, with comprehensive fuel phenotyping in preserved and reduced ejection fraction, showed that despite high citric acid cycle utilization, the heart released high levels of citric acid cycle intermediates into the circulation.[56] As citrate is an inhibitor of glycolysis and fatty acid oxidation,[57] and other citric acid cycle metabolites may serve as signaling molecules in cardiac remodeling[58]; further delineation of the circulating metabolome and cardiac phenotype in clinical HF is necessary. Lastly, glycerolipids emerged in our mediation analysis with HF risk. Our findings extend 2 prior lipidomic evaluations—one associating lipids with CVD events (not inclusive of HF) and the other a case–control study with HF that found that elevated phosphatidylcholine 32:0 levels conferred HF risk.[59,60] Although this finding is directionally opposite to our findings of higher phosphatidylcholine-A 36:4 associated with decreased HF risk, it is likely that the length and saturation (number of double bonds) of lipids is of crucial importance.

Limitations

We acknowledge several limitations in the present study. Our sample cohorts are comprised of white, middle-aged participants with European ancestry and our findings do require external validation across different age groups, ancestry, and existing risk factors. The FHS population is predominately of working or middle class with little variation in the socioeconomic structure; therefore, we did not adjust for socioeconomic status in our models. We acknowledge this as a limitation of this analysis as social determinants of health contribute to both AF and HF risk.[61–63] The diet and physical activity metrics used a food questionnaire and formula for summating physical activity differs from the one proposed by the AHA for CVH score but are closely approximated and similar to prior FHS publications.[6,9,31] Diet and physical activity metrics are self-reported and misclassification may exist. The lack of major associations of diet, physical activity, and smoking with metabolites may be attributable to measurement artifact in this study and should not be regarded as less important metrics of the CVH score. Our CVH score and metabolomics analysis are obtained from a single examination, are cross-sectional, and not over time. Such single-point assessment may underestimate the association with AF and HF risk because of regression attenuation. The directionality of whether CVH score drives metabolite levels or vice versa requires additional investigation as our study is associational. The metabolomics data available consisted of a targeted panel, and hence, not all metabolites and pathways that are relevant to the outcomes of interest may be represented. Distinct lipid metabolites between circulating plasma lipid species and atrial myocardium exist,[64] and importantly, the circulating metabolome may not reflect the tissue level metabolome.

CONCLUSIONS

Our study highlights the presence that even 1 ideal cardiometabolic component of the CVH score (ideal BMI, blood pressure, or fasting glucose) reduced AF and HF risk. Importantly, these 3 cardiometabolic components contributed the greatest number of metabolites that associated with CVH score. Among the metabolites associated with CVH score, several lipid metabolites mediated the association between CVH score and incident AF. Several key metabolites emerged in mediation analysis with incident HF, with different metabolites associated with HF subtypes in exploratory analyses. Although our study was observational and requiring validation, our findings indicate that metabolites may provide insights into the association of modifiable risk factors and CVD development. Further, metabolomics may provide unique biomarker signatures to track lifestyle and therapeutic interventions targeted to optimizing the CVH score to decrease CVD risk.

ARTICLE INFORMATION

Received November 26, 2022; accepted April 13, 2023.

Affiliations

Department of Biostatistics, School of Public Health, Boston University, Boston, MA (Y.L.); Harvard University, Cambridge, MA (A.G.); Evans Department of Medicine and Whitaker Cardiovascular Institute, Boston University Chobanian & Avedisian School of Medicine, Boston, MA (L.X., M.G.F., J.L.F., D.M.G.); Section of Cardiovascular Medicine, Department of Medicine, Boston University Chobanian & Avedisian School of Medicine/Boston Medical Center, Boston, MA (N.A., C.A., D.K., E.J.B., R.S.V., D.M.G.); Evans Department of Medicine, Section of Cardiovascular Medicine and Department of Epidemiology, Boston University, Boston, MA (E.J.B., R.S.V.); Population Sciences Branch, Division of Intramural Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD (D.L.); Section of Preventive Medicine and Epidemiology, Department of Medicine, Boston University Chobanian & Avedisian School of Medicine, Boston, MA (R.S.V., V.X.); Department of Biostatistics, Boston University School of Public Health, Boston, MA (M.G.L., J.R., V.X., C.L.); and Framingham Heart Study, Framingham, MA (E.J.B., D.L., R.S.V., M.G.L., V.X., C.L.).

Sources of Funding

This research was supported by K01 HL143142 (J.L.F), American Heart Association #17FTF33670369 (D.M.G.), Boston University Career Development Award (D.M.G), Boston University CTSI 1UL1TR001430 (D.M.G.), Grant 2021261 from the Doris Duke Charitable Foundation through the COVID-19 Fund to Retain Clinical Scientists collaborative grant program, which was made possible through the support of Grant 62288 from the John Templeton Foundation (D.M.G.). E.J.B. reports support from the National Heart, Lung, and Blood Institute R01HL092577 and American Heart Association AF AHA_18SFRN34110082. R.S. Vasan is partly supported by the Evans Medical Foundation and Jay and Louis Coffman Endowment from the Department of Medicine at Boston University Chobanian & Avedisian School of Medicine.

Disclosures

None.

Supplemental Material

Tables S1–S6
Figures S1–S7

REFERENCES

1. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, et al. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation*. 2010;121:586–613. doi: 10.1161/CIRCULATIONAHA.109.192703
2. Strasser T. Reflections on cardiovascular diseases. *Interdiscip Sci Rev*. 1978;3:225–230. doi: 10.1179/030801878791925921
3. Folsom AR, Yatsuya H, Nettleton JA, Lutsey PL, Cushman M, Rosamond WD; ARIC Study Investigators. Community prevalence of ideal cardiovascular health, by the American Heart Association definition, and relationship with cardiovascular disease incidence. *J Am Coll Cardiol*. 2011;57:1690–1696. doi: 10.1016/j.jacc.2010.11.041
4. Lachman S, Peters RJ, Lentjes MA, Mulligan AA, Luben RN, Wareham NJ, Khaw K-T, Boekholdt SM. Ideal cardiovascular health and risk of cardiovascular events in the EPIC-Norfolk prospective population study. *Eur J Prev Cardiol*. 2016;23:986–994. doi: 10.1177/2047487315602015
5. Ommerborn MJ, Blackshear CT, Hickson DA, Griswold ME, Kwatra J, Djoussé L, Clark CR. Ideal cardiovascular health and incident cardiovascular events. *Am J Prev Med*. 2016;51:502–506. doi: 10.1016/j.amepre.2016.07.003
6. Xanthakis V, Enserro DM, Murabito JM, Polak JF, Wollert KC, Januzzi JL, Wang TJ, Tofler G, Vasan RS. Ideal cardiovascular health: associations with biomarkers and subclinical disease and impact on incidence of cardiovascular disease in the Framingham Offspring Study. *Circulation*. 2014;130:1676–1683. doi: 10.1161/CIRCULATIONAHA.114.009273
7. Ford ES, Greenlund KJ, Hong Y. Ideal cardiovascular health and mortality from all causes and diseases of the circulatory system among adults in the United States. *Circulation*. 2012;125:987–995. doi: 10.1161/CIRCULATIONAHA.111.049122
8. Folsom AR, Shah AM, Lutsey PL, Roetker NS, Alonso A, Avery CL, Miedema MD, Konety S, Chang PP, Solomon SD. American Heart Association's Life's Simple 7: avoiding heart failure and preserving cardiac structure and function. *Am J Med*. 2015;128:970–976.e2. doi: 10.1016/j.amjmed.2015.03.027
9. Nayor M, Enserro DM, Vasan RS, Xanthakis V. Cardiovascular health status and incidence of heart failure in the Framingham Offspring Study. *Circulation: Heart Failure*. 2016;9:e002416. doi: 10.1161/CIRCHEARTFAILURE.115.002416
10. Ogunmoroti O, Oni E, Michos ED, Spatz ES, Allen NB, Rana JS, Virani SS, Blankstein R, Aronis KN, Blumenthal RS, et al. Life's Simple 7 and incident heart failure: the Multi-Ethnic Study of Atherosclerosis. *J Am Heart Assoc*. 2017;6:6. doi: 10.1161/JAHA.116.005180
11. Garg PK, O'Neal WT, Ogunsua A, Thacker EL, Howard G, Soliman EZ, Cushman M. Usefulness of the American Heart Association's Life Simple 7 to predict the risk of atrial fibrillation (from the REasons for Geographic And Racial Differences in Stroke [REGARDS] study). *Am J Cardiol*. 2018;121:199–204. doi: 10.1016/j.amjcard.2017.09.033
12. Nishikawa T, Tanaka Y, Tada H, Tsuda T, Kato T, Usui S, Sakata K, Hayashi K, Kawashiri M-A, Hashiba A, et al. Association between cardiovascular health and incident atrial fibrillation in the general Japanese population aged ≥40 years. *Nutrients*. 2021;13:3201. doi: 10.3390/nu13093201
13. Ogunmoroti O, Michos ED, Aronis KN, Salami JA, Blankstein R, Virani SS, Spatz ES, Allen NB, Rana JS, Blumenthal RS, et al. Life's Simple 7 and the risk of atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis*. 2018;275:174–181. doi: 10.1016/j.atherosclerosis.2018.05.050
14. Cheng S, Shah SH, Corwin EJ, Fiehn O, Fitzgerald RL, Gerszten RE, Illig T, Rhee EP, Srinivas PR, Wang TJ, et al. Potential impact and study considerations of metabolomics in cardiovascular health and disease: a scientific statement from the American Heart Association. *Circulation: Cardiovasc Genet*. 2017;10:e000032. doi: 10.1161/HCG.0000000000000032
15. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham offspring study. *Am J Epidemiol*. 1979;110:281–290. doi: 10.1093/oxfordjournals.aje.a112813
16. Desai CS, Ning H, Liu K, Reis JP, Gidding SS, Armstrong A, Lima JA, Lloyd-Jones DM. Cardiovascular health in young adulthood and association with left ventricular structure and function later in life: the Coronary Artery Risk Development in Young Adults study. *J Am Soc Echocardiogr*. 2015;28:1452–1461. doi: 10.1016/j.echo.2015.07.026
17. McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. *N Engl J Med*. 1971;285:1441–1446. doi: 10.1056/NEJM197112232852601
18. Ho JE, Larson MG, Ghorbani A, Cheng S, Chen M-H, Keyes M, Rhee EP, Clish CB, Vasan RS, Gerszten RE, et al. Metabolomic profiles of body mass index in the Framingham Heart Study reveal distinct cardiometabolic phenotypes. *PLoS One*. 2016;11:e0148361. doi: 10.1371/journal.pone.0148361
19. Rhee EP, Cheng S, Larson MG, Walford GA, Lewis GD, McCabe E, Yang E, Farrell L, Fox CS, O'Donnell CJ, et al. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. *J Clin Investig*. 2011;121:1402–1411. doi: 10.1172/JCI44442
20. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, et al. Metabolite profiles and the risk of developing diabetes. *Nat Med*. 2011;17:448–453. doi: 10.1038/nm.2307
21. Wang TJ, Ngo D, Psychogios N, Dejam A, Larson MG, Vasan RS, Ghorbani A, O'Sullivan J, Cheng S, Rhee EP, et al. 2-aminoadipic acid is a biomarker for diabetes risk. *J Clin Investig*. 2013;123:4309–4317. doi: 10.1172/JCI64801
22. Garg PK, O'Neal WT, Chen LY, Loehr LR, Sotoodehnia N, Soliman EZ, Alonso A. American Heart Association's Life Simple 7 and risk of atrial fibrillation in a population without known cardiovascular disease: the ARIC (Atherosclerosis Risk in Communities) study. *J Am Heart Assoc*. 2018;7. doi: 10.1161/JAHA.117.008424
23. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc: Series B (Methodological)*. 1995;57:289–300.
24. Pang Z, Zhou G, Ewald J, Chang L, Hacariz O, Basu N, Xia J. Using MetaboAnalyst 5.0 for LC–HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat Protoc*. 2022;17:1735–1761. doi: 10.1038/s41596-022-00710-w
25. Rhee EP, Clish CB, Ghorbani A, Larson MG, Elmariah S, McCabe E, Yang Q, Cheng S, Pierce K, Deik A, et al. A combined epidemiologic and metabolomic approach improves CKD prediction. *J Am Soc Nephrol*. 2013;24:1330–1338. doi: 10.1681/ASN.2012101006
26. Lee H, Herbert RD, McAuley JH. Mediation analysis. *JAMA*. 2019;321:697. doi: 10.1001/jama.2018.21973
27. Vanderweele T, Vansteelandt S. Mediation analysis with multiple mediators. *Epidemiol Methods*. 2014;2:2. doi: 10.1515/em-2012-0010
28. VanderWeele TJ. Causal mediation analysis with survival data. *Epidemiology*. 2011;22:582–585. doi: 10.1097/EDE.0b013e31821db37e
29. Weng L-C, Preis SR, Hulme OL, Larson MG, Choi SH, Wang B, Trinquart L, McManus DD, Staerk L, Lin H, et al. Genetic predisposition, clinical risk factor burden, and lifetime risk of atrial fibrillation. *Circulation*. 2018;137:1027–1038. doi: 10.1161/CIRCULATIONAHA.117.031431
30. Staerk L, Wang B, Preis SR, Larson MG, Lubitz SA, Ellinor PT, McManus DD, Ko D, Weng L-C, Lunetta KL, et al. Lifetime risk of atrial fibrillation

according to optimal, borderline, or elevated levels of risk factors: cohort study based on longitudinal data from the Framingham Heart Study. *BMJ*. 2018;361:k1453. doi: 10.1136/bmj.k1453
31. Cheng S, Larson MG, McCabe EL, Murabito JM, Rhee EP, Ho JE, Jacques PF, Ghorbani A, Magnusson M, Souza AL, et al. Distinct metabolomic signatures are associated with longevity in humans. *Nat Commun*. 2015;6:6791. doi: 10.1038/ncomms7791
32. Ooi GJ, Meikle PJ, Huynh K, Earnest A, Roberts SK, Kemp W, Parker BL, Brown W, Burton P, Watt MJ. Hepatic lipidomic remodeling in severe obesity manifests with steatosis and does not evolve with non-alcoholic steatohepatitis. *J Hepatol*. 2021;75:524–535. doi: 10.1016/j.jhep.2021.04.013
33. Tonks KT, Coster AC, Christopher MJ, Chaudhuri R, Xu A, Gagnon-Bartsch J, Chisholm DJ, James DE, Meikle PJ, Greenfield JR, et al. Skeletal muscle and plasma lipidomic signatures of insulin resistance and overweight/obesity in humans. *Obesity*. 2016;24:908–916. doi: 10.1002/oby.21448
34. Petersen MC, Madiraju AK, Gassaway BM, Marcel M, Nasiri AR, Butrico G, Marcucci MJ, Zhang D, Abulizi A, Zhang XM, et al. Insulin receptor Thr1160 phosphorylation mediates lipid-induced hepatic insulin resistance. *J Clin Invest*. 2016;126:4361–4371. doi: 10.1172/JCI86013
35. Takai Y. Reprint of "unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system". *Biochem Biophys Res Commun*. 2012;425:571–577. doi: 10.1016/j.bbrc.2012.08.026
36. Hameed A, Mojsak P, Buczynska A, Suleria HAR, Kretowski A, Ciborowski M. Altered metabolome of lipids and amino acids species: a source of early signature biomarkers of T2DM. *J Clin Med*. 2020;9:2257. doi: 10.3390/jcm9072257
37. van Diepen JA, Robben JH, Hooiveld GJ, Carmone C, Alsady M, Boutens L, Bekkenkamp-Grovenstein M, Hijmans A, Engelke UFH, Wevers RA, et al. SUCNR1-mediated chemotaxis of macrophages aggravates obesity-induced inflammation and diabetes. *Diabetologia*. 2017;60:1304–1313. doi: 10.1007/s00125-017-4261-z
38. Alonso A, Yu B, Qureshi WT, Grams ME, Selvin E, Soliman EZ, Loehr LR, Chen LY, Agarwal SK, Alexander D, et al. Metabolomics and incidence of atrial fibrillation in African Americans: the Atherosclerosis Risk in Communities (ARIC) study. *PLoS One*. 2015;10:e0142610. doi: 10.1371/journal.pone.0142610
39. Alonso A, Yu B, Sun YV, Chen LY, Loehr LR, O'Neal WT, Soliman EZ, Boerwinkle E. Serum metabolomics and incidence of atrial fibrillation (from the Atherosclerosis Risk in Communities study). *Am J Cardiol*. 2019;123:1955–1961. doi: 10.1016/j.amjcard.2019.03.017
40. Emmert DB, Vukovic V, Dordevic N, Weichenberger CX, Losi C, D'Elia Y, Volpato C, Hernandes VV, Gögele M, Foco L, et al. Genetic and metabolic determinants of atrial fibrillation in a general population sample: the CHRIS study. *Biomolecules*. 2021;11:1663. doi: 10.3390/biom11111663
41. Jung Y, Cho Y, Kim N, Oh I-Y, Kang SW, Choi E-K, Hwang G-S. Lipidomic profiling reveals free fatty acid alterations in plasma from patients with atrial fibrillation. *PLoS One*. 2018;13:e0196709. doi: 10.1371/journal.pone.0196709
42. Lai S, Hua X, Gao R, Zeng L, Song J, Liu J, Zhang J. Combinational biomarkers for atrial fibrillation derived from atrial appendage and plasma metabolomics analysis. *Sci Rep*. 2018;8:8. doi: 10.1038/s41598-018-34930-6
43. Lind L, Salihovic S, Sundström J, Broeckling CD, Magnusson PK, Prenni J, Fall T, Ärnlöv J. Multicohort metabolomics analysis discloses 9-decenoylcarnitine to be associated with incident atrial fibrillation. *J Am Heart Assoc*. 2021;10:10. doi: 10.1161/JAHA.120.017579
44. Ko D, Riles EM, Marcos EG, Magnani JW, Lubitz SA, Lin H, Long MT, Schnabel RB, McManus DD, et al. Metabolomic profiling in relation to new-onset atrial fibrillation (from the Framingham Heart Study). *Am J Cardiol*. 2016;118:1493–1496. doi: 10.1016/j.amjcard.2016.08.010
45. Hensley CT, Wasti AT, Deberardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. *J Clin Investig*. 2013;123:3678–3684. doi: 10.1172/JCI69600
46. Lu V, Dahan P, Ahsan FM, Patananan AN, Roy IJ, Torres A, Nguyen RMT, Huang D, Braas D, Teitell MA. Mitochondrial metabolism and glutamine are essential for mesoderm differentiation of human pluripotent stem cells. *Cell Res*. 2019;29:596–598. doi: 10.1038/s41422-019-0191-2
47. Salabei JK, Lorkiewicz PK, Holden CR, Li Q, Hong KU, Bolli R, Bhatnagar A, Hill BG. Glutamine regulates cardiac progenitor cell metabolism and proliferation. *Stem Cells*. 2015;33:2613–2627. doi: 10.1002/stem.2047
48. Shen Y, Zhang Y, Li W, Chen K, Xiang M, Ma H. Glutamine metabolism: from proliferating cells to cardiomyocytes. *Metabolism*. 2021;121:154778. doi: 10.1016/j.metabol.2021.154778
49. Chambers KT, Leone TC, Sambandam N, Kovacs A, Wagg CS, Lopaschuk GD, Finck BN, Kelly DP. Chronic inhibition of pyruvate dehydrogenase in heart triggers an adaptive metabolic response. *J Biol Chem*. 2011;286:11155–11162. doi: 10.1074/jbc.M110.217349
50. Cluntun AA, Badolia R, Lettlova S, Parnell KM, Shankar TS, Diakos NA, Olson KA, Taleb I, Tatum SM, Berg JA, et al. The pyruvate-lactate axis modulates cardiac hypertrophy and heart failure. *Cell Metab*. 2021;33:629–648.e10. doi: 10.1016/j.cmet.2020.12.003
51. Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, Han X, Gross RW, Kozak R, Lopaschuk GD, et al. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. *J Clin Invest*. 2002;109:121–130. doi: 10.1172/JCI0214080
52. Neglia D, De Caterina A, Marraccini P, Natali A, Ciardetti M, Vecoli C, Gastaldelli A, Ciociaro D, Pellegrini P, Testa R, et al. Impaired myocardial metabolic reserve and substrate selection flexibility during stress in patients with idiopathic dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2007;293:H3270–H3278. doi: 10.1152/ajpheart.00887.2007
53. Stride N, Larsen S, Hey-Mogensen M, Sander K, Lund JT, Gustafsson F, Køber L, Dela F. Decreased mitochondrial oxidative phosphorylation capacity in the human heart with left ventricular systolic dysfunction. *Eur J Heart Fail*. 2013;15:150–157. doi: 10.1093/eurjhf/hfs172
54. McCommis KS, Kovacs A, Weinheimer CJ, Shew TM, Koves TR, Ilkayeva OR, Kamm DR, Pyles KD, King MT, Veech RL, et al. Nutritional modulation of heart failure in mitochondrial pyruvate carrier–deficient mice. *Nat Metab*. 2020;2:1232–1247. doi: 10.1038/s42255-020-00296-1
55. Schwartz B, Gjini P, Gopal DM, Fetterman JL. Inefficient batteries in heart failure: metabolic bottlenecks disrupting the mitochondrial ecosystem. *JACC Basic Transl Sci*. 2022;7:1161–1179. doi: 10.1016/j.jacbts.2022.03.017
56. Murashige D, Jang C, Neinast M, Edwards JJ, Cowan A, Hyman MC, Rabinowitz JD, Frankel DS, Arany Z. Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science*. 2020;370:364–368. doi: 10.1126/science.abc8861
57. Garland PB, Randle PJ, Newsholme EA. Citrate as an intermediary in the inhibition of phosphofructokinase in rat heart muscle by fatty acids, ketone bodies, pyruvate, diabetes and starvation. *Nature*. 1963;200:169–170. doi: 10.1038/200169a0
58. Ritterhoff J, Tian R. Metabolism in cardiomyopathy: every substrate matters. *Cardiovasc Res*. 2017;113:411–421. doi: 10.1093/cvr/cvx017
59. Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, et al. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation*. 2014;129:1821–1831. doi: 10.1161/CIRCULATIONAHA.113.002500
60. Wittenbecher C, Eichelmann F, Toledo E, Guasch-Ferré M, Ruiz-Canela M, Li J, Arós F, Lee C-H, Liang L, Salas-Salvadó J, et al. Lipid profiles and heart failure risk. *Circ Res*. 2021;128:309–320. doi: 10.1161/CIRCRESAHA.120.317883
61. Potter EL, Hopper I, Sen J, Salim A, Marwick TH. Impact of socioeconomic status on incident heart failure and left ventricular dysfunction: systematic review and meta-analysis. *Eur Heart J Qual Care Clin Outcomes*. 2019;5:169–179. doi: 10.1093/ehjqcco/qcy047
62. Rao VN, Mentz RJ, Coniglio AC, Kelsey MD, Fudim M, Fonarow GC, Matsouaka RA, DeVore AD, Caughey MC. Neighborhood socioeconomic disadvantage and hospitalized heart failure outcomes in the American Heart Association Get With The Guidelines-Heart Failure registry. *Circ Heart Fail*. 2022;15:e009353. doi: 10.1161/CIRCHEARTFAILURE.121.009353
63. Benjamin EJ, Thomas KL, Go AS, Desvigne-Nickens P, Albert CM, Alonso A, Chamberlain AM, Essien UR, Hernandez I, Hills MT, et al. Transforming atrial fibrillation research to integrate social determinants of health: a National Heart, Lung, and Blood Institute workshop report. *JAMA Cardiol*. 2023;8:182–191. doi: 10.1001/jamacardio.2022.4091
64. Suffee N, Baptista E, Piquereau J, Ponnaiah M, Doisne N, Ichou F, Lhomme M, Pichard C, Galand V, Mougenot N, et al. Impacts of a high-fat diet on the metabolic profile and the phenotype of atrial myocardium in mice. *Cardiovasc Res*. 2022;118:3126–3139. doi: 10.1093/cvr/cvab367

Supplemental Material

Table S1. Ideal Cardiovascular Health Components and Scoring System

CVH Metric	Poor (0 point)	Intermediate (1 point)	Ideal (2 points)
Smoking	Current smoker	Former smoker (≤ 12 months)	Non-smoker or not smoking > 12 months
Body mass index	BMI ≥ 30 kg/m^2	25 kg/m^2 ≤ BMI ≤ 29 kg/m^2	BMI < 25 kg/m^2
Blood pressure	Systolic BP ≥ 140 mmHg OR Diastolic BP ≥ 90 mmHg	120 mmHg ≤ Systolic BP ≤ 139 mmHg OR 80 mmHg ≤ Diastolic BP ≤ 89 mmHg OR On medication and treated to goal	Systolic BP ≤ 120 mmHg Diastolic BP ≤ 80 mmHg AND Not on medication
Total cholesterol	TC ≥ 240 mg/dL	TC between 200-239 mg/dL	TC < 200 mg/dL AND Not on cholesterol medication
Physical activity index	Lowest 20th percentile on PAI	21-60th percentile on PAI	Top 40th percentile on PAI
Diet	0-1 components	2-3 components	4-5 components
Fasting blood glucose	FBG ≥ 126 mg/dL	FBG between 100-125 mg/dL OR On medication and treated to goal	FBG < 100 mg/dL AND Not on medication

CVH, cardiovascular health; body mass index, BMI; BP, blood pressure; TC, total cholesterol; PAI, physical activity index, FBG, fasting blood glucose

Supplemental Table 2. FHS Participant Characteristics with CVH and Metabolomics Data

Characteristic	Women (n=1099)	Men (n=960)
Age, years	54 (10)	55 (10)
Body mass index, kg/m^2	26.7 (5.4)	28.2 (4.1)
Systolic blood pressure, mmHg	124 (20)	129 (17)
Diastolic blood pressure, mmHg	73 (10)	77 (10)
Antihypertensive medications, %	181 (17)	195 (20)
Total cholesterol, mg/dL	209 (38)	203 (35)
HDL, mg/dL	56 (15)	43 (11)
Fasting glucose, mg/dL	97 (25)	104 (28)
Diabetes medication, %	21 (2)	36 (4)
eGFR, mL/min/1.73m^2	68 (15)	74 (16)
Smoking, %	200 (18)	163 (17)
CVH score metrics		
CVH score	9 (2)	8 (2)
CVH category frequency, %		
Smoke		
Poor	200 (18)	163 (17)
Intermediate	0 (0)	0 (0)
Ideal	899 (82)	797 (83)
Body mass index		
Poor	239 (22)	256 (27)
Intermediate	359 (33)	510 (53)
Ideal	501 (46)	194 (20)
Blood pressure		
Poor	230 (21)	261 (27)
Intermediate	418 (38)	447 (47)
Ideal	451 (41)	252 (26)
Total cholesterol		
Poor	209 (19)	128 (13)
Intermediate	447 (40)	401 (42)
Ideal	443 (40)	431 (45)
Fasting blood glucose		
Poor	49 (5)	70 (7)
Intermediate	229 (21)	340 (35)
Ideal	821 (75)	550 (57)
Physical activity		
Poor	247 (23)	154 (16)
Intermediate	511 (46)	347 (36)
Ideal	341 (31)	459 (48)
Diet		
Poor	374 (34)	446 (47)
Intermediate	676 (62)	476 (50)
Ideal	49 (5)	38 (4)

Continuous variables represent mean (standard deviation); categorical variables represent number (percentage); HDL, high-density lipoprotein; CVH, cardiovascular health

Table S3. Age- and Sex-Adjusted Association between CVH Score and Components with Incident AF and HF

CVH component	HR (95% CI)	*P* value
Incident AF		
CVH score (per 1-unit increase)	0.93 (0.89-0.97)	<0.001
Individual components of CVH score		
Body mass index	0.79 (0.71-0.89)	<0.001
Blood pressure	0.79 (0.70-0.90)	<0.001
Fasting blood glucose	0.83 (0.73-0.95)	0.007
Total cholesterol	0.96 (0.85-1.08)	0.51
Smoking	1.02 (0.90-1.16)	0.74
Physical activity	1.05 (0.94-1.19)	0.38
Diet	0.95 (0.81-1.10)	0.48
Incident HF		
CVH score (per 1-unit increase)	0.79 (0.73-0.84)	<0.001
Individual components of CVH score		
Body mass index	0.63 (0.51-0.76)	<0.001
Blood pressure	0.67 (0.54-0.83)	<0.001
Fasting blood glucose	0.54 (0.43-0.66)	<0.001
Total cholesterol	1.08 (0.88-1.34)	0.45
Smoking	0.73 (0.60-0.89)	0.001
Physical activity	0.77 (0.63-0.93)	0.008
Diet	1.04 (0.80-1.36)	0.77

Values represent hazard ratios (HR) with 95% confidence intervals (CI) per 1-unit increase in CVH score; individual component scores were modelled as continuous variables with HR representing per 1-unit increase in component scale

CVH, cardiovascular health score; AF, atrial fibrillation, HF, heart failure

Table S4. Association of Metabolites with CVH Score and Components

CVH Metric	Number of metabolites	Raw *P value*
Total CVH score	144	0.021
Total cholesterol	135	0.018
BMI	116	0.017
Fasting blood glucose	111	0.019
Blood pressure	94	0.021
Diet	60	0.020
Smoking status	58	0.020
Physical activity	6	0.018

*Total metabolites in the targeted metabolomics platform (n=212); CVH, cardiovascular health; BMI, body mass index; all raw *P* values shown correspond with false-discovery rate-adjusted P value < 0.05.

Table S5. Metabolites Associated with Incident AF and HF

Metabolites	HR (95% CI)	Raw *P* value	FDR *P* value
Incident AF			
Non-lipid analytes			
Glycerol	1.29 (1.11-1.49)	0.00078	0.037
Lipid analytes			
CE 16:1	1.26 (1.12-1.42)	0.00016	0.022
PC 32:1	1.24 (1.10-1.39)	0.00059	0.037
Incident HF			
Non-lipid analytes			
Glycerol	1.69 (1.32-2.16)	0.00003	0.002
Isocitrate	1.55 (1.24-1.93)	0.00010	0.005
Asparagine	0.70 (0.59-0.85)	0.00018	0.006
Glutamine	0.73 (0.60-0.88)	0.00136	0.033
α-hydroxybutyrate	1.39 (1.12-1.72)	0.00235	0.043
Indolepropionic acid	0.74 (0.61-0.90)	0.00238	0.043
Lipid analytes			
PC-A 36:4	0.58 (0.47-0.72)	<0.00001	<0.001
LPC 18:2	0.74 (0.61-0.89)	0.00138	0.033

HR are per-1 SD increase in log-transformed metabolite level

AF, atrial fibrillation; HF, heart failure; CE, cholesterol ester; PC, phosphatidylcholine; LPC, lysophosphatidylcholine

Lipid nomenclature: the first number indicates the carbon length and the subsequent number after the colon indicates the number of double bonds.

Table S6. Metabolites Associated with Incident HFpEF and HFrEF

Metabolites	HR (95% CI)	Raw *P* value	FDR-adjusted *P* value
Incident HFpEF			
Non-lipid analytes			
Glycerol	2.03 (1.39-2.97)	0.00024	0.027
Glutamine	0.64 (0.50-0.83)	0.00056	0.027
Lipid analytes			
PC-A 36:4	0.57 (0.41-0.78)	0.00042	0.027
Incident HFrEF			
Non-lipid analytes			
Isocitrate	2.05 (1.47-2.86)	0.00002	0.002
Aminoadipate	2.00 (1.41-2.85)	0.00011	0.004
Dimethylglycine	1.74 (1.31-2.31)	0.00014	0.004
Carbamoylalanine	1.59 (1.23-2.06)	0.00047	0.011
Quinolinate	1.71 (1.25-2.34)	0.00072	0.015
Glycolysis and PPP intermediates‡	1.72 (1.24-2.37)	0.00110	0.020
Adenosine monophosphate	1.66 (1.20-2.30)	0.00198	0.031
Phosphoglycerate	1.58 (1.17-2.14)	0.00280	0.038
Ornithine	1.59 (1.17-2.16)	0.00313	0.038
Xanthosine	1.51 (1.14-2.00)	0.00420	0.047
Lipid analytes			
PC-A 36:4	0.51 (0.37-0.70)	0.00003	0.002
LPC 18:2	0.51 (0.37-0.70)	0.00004	0.002
LPC 22:6	0.59 (0.42-0.82)	0.01030	0.031

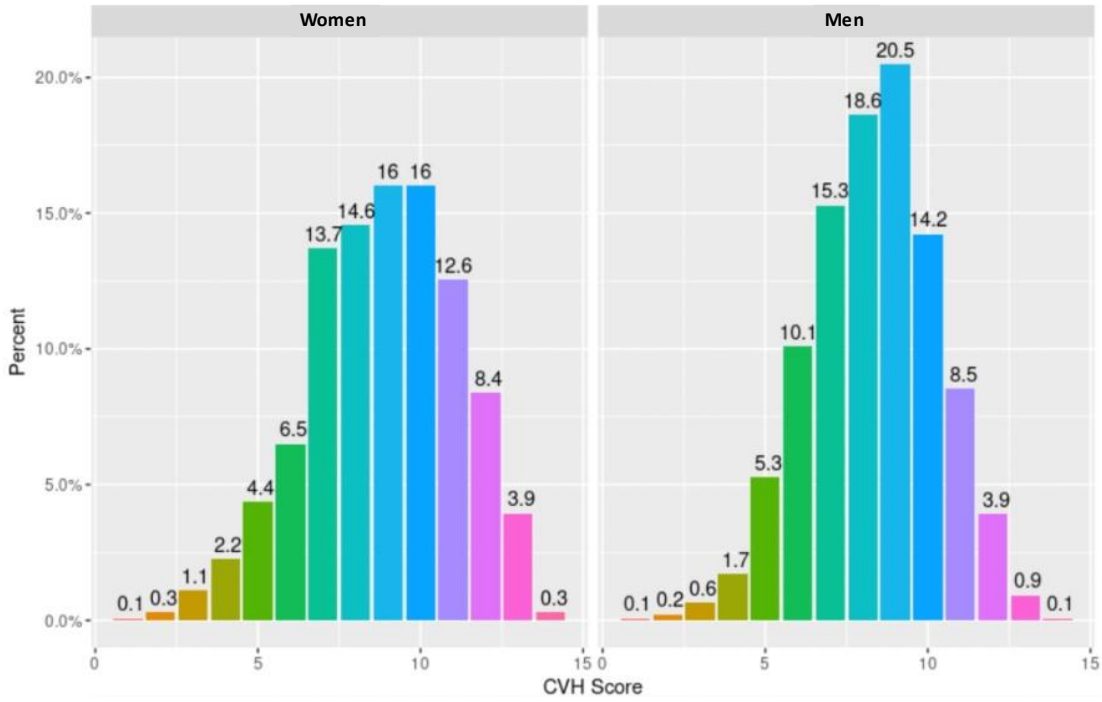
HR are per-1 SD increase in log-transformed metabolite level.

‡Glycolysis and pentose phosphate pathway (PPP) intermediates include fructose-1-phosphate, fructose-6-phosphate, glucose-1-phosphate, and glucose-6-phosphate.

HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; PC, phosphatidylcholine; LPC, lysophosphatidylcholine

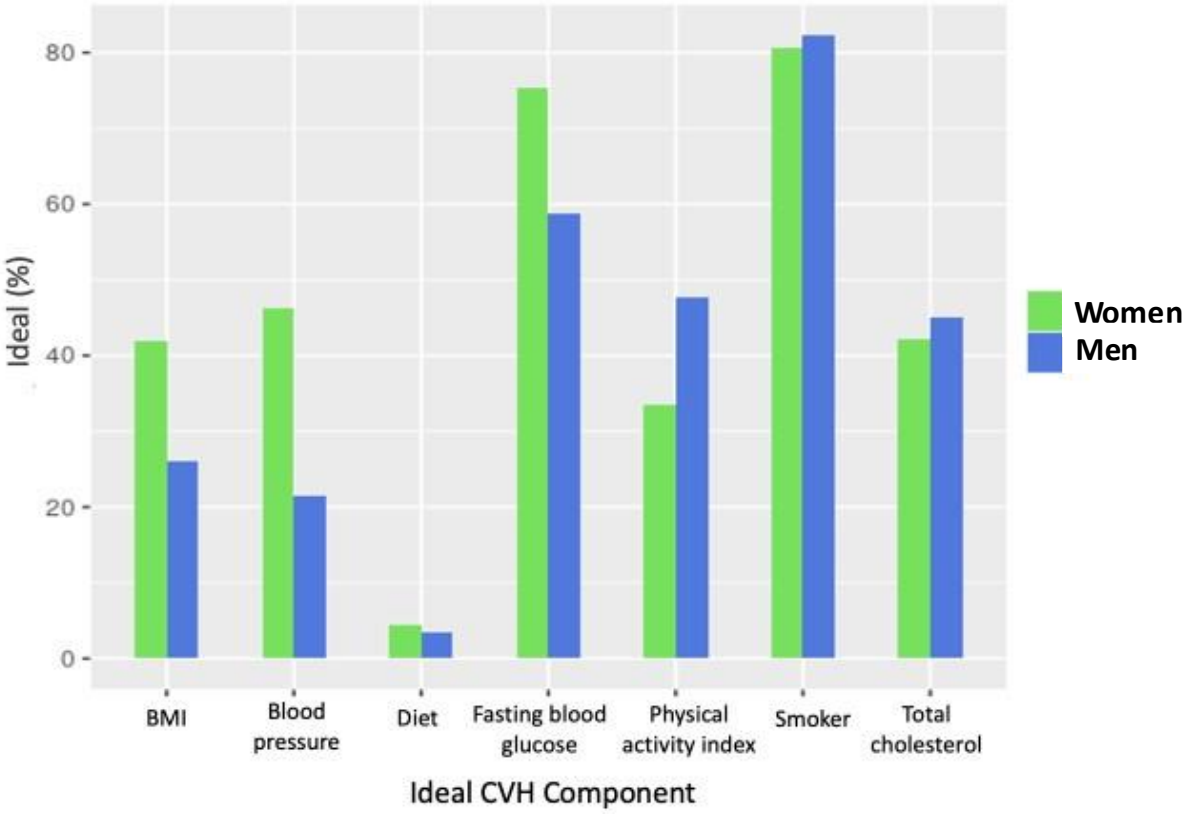
Lipid nomenclature: the first number indicates the carbon length and the subsequent number after the colon indicates the number of double bonds.

Figure S1. Distribution of CVH Scores by Sex.



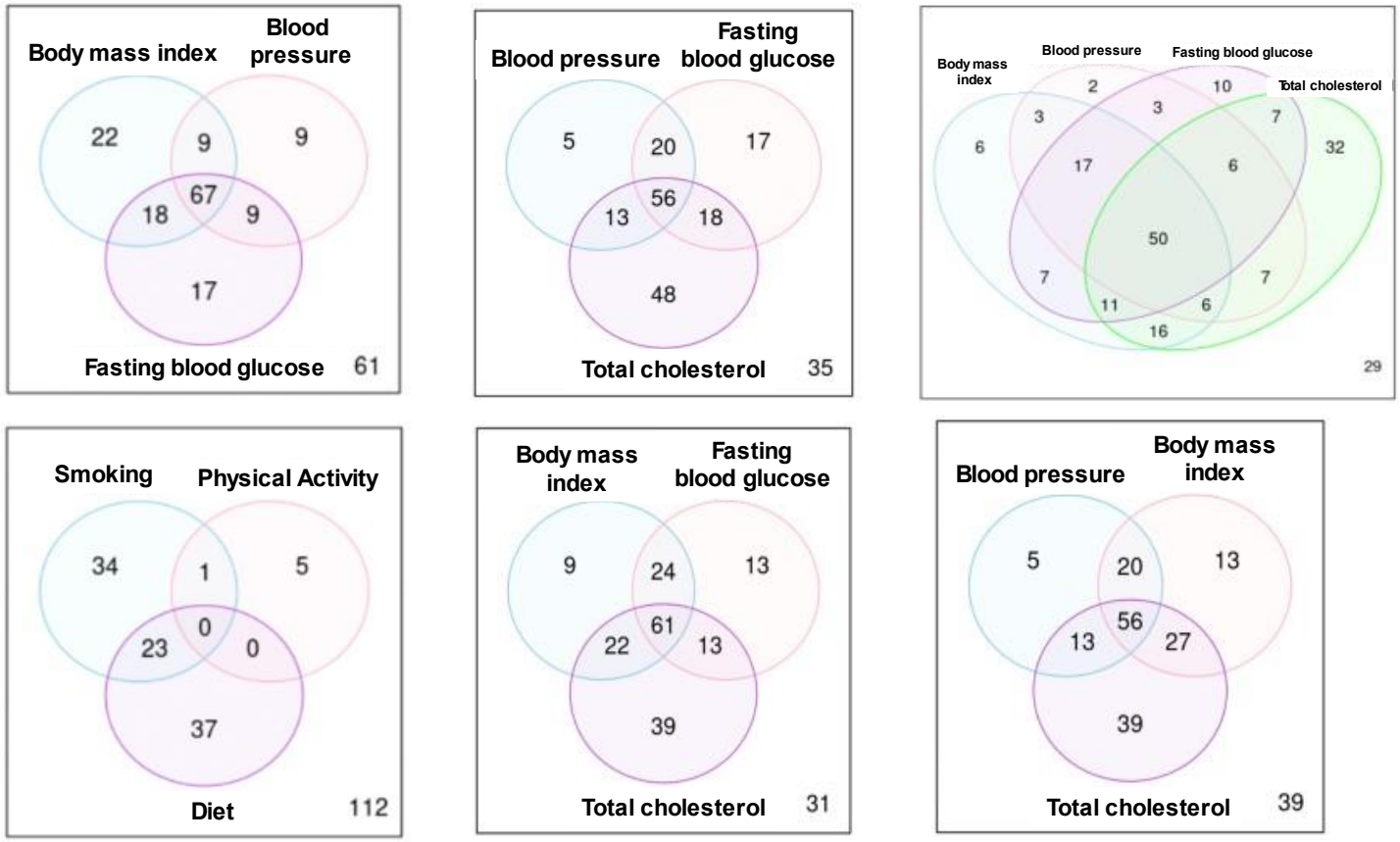
In Sample 1 (n=3056), CVH scores were normally distributed for both women and men. Most participants had intermediate CVH scores with women on average having higher scores compared to men (mean score 8.8 ± 2.2 vs. 8.3 ± 2.0, respectively). A lower percentage of men compared to women classified to the highest CVH scores. CVH, cardiovascular health.

Figure S2. Distribution of Ideal CVH Components at Exam 5 by Sex.



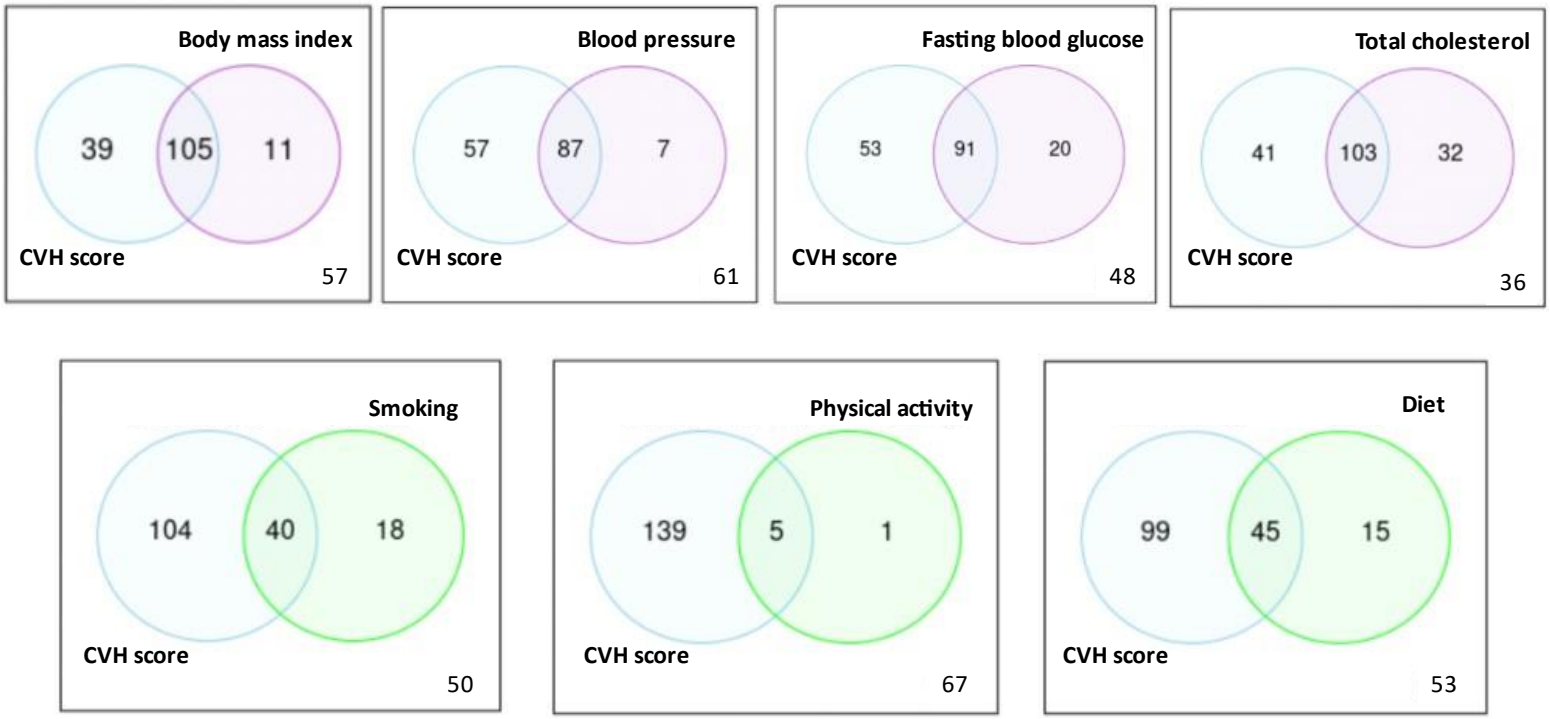
Among participants with complete CVH data, women had a higher percentage of ideal CVH components, including the cardiometabolic phenotype (BMI, blood pressure, and fasting blood glucose), compared to men, except for the physical activity index domain. CVH, cardiovascular health; BMI, body mass index.

Figure S3. **Venn Diagrams of the Number of Metabolites Shared with Various Combinations of CVH Score Components**.



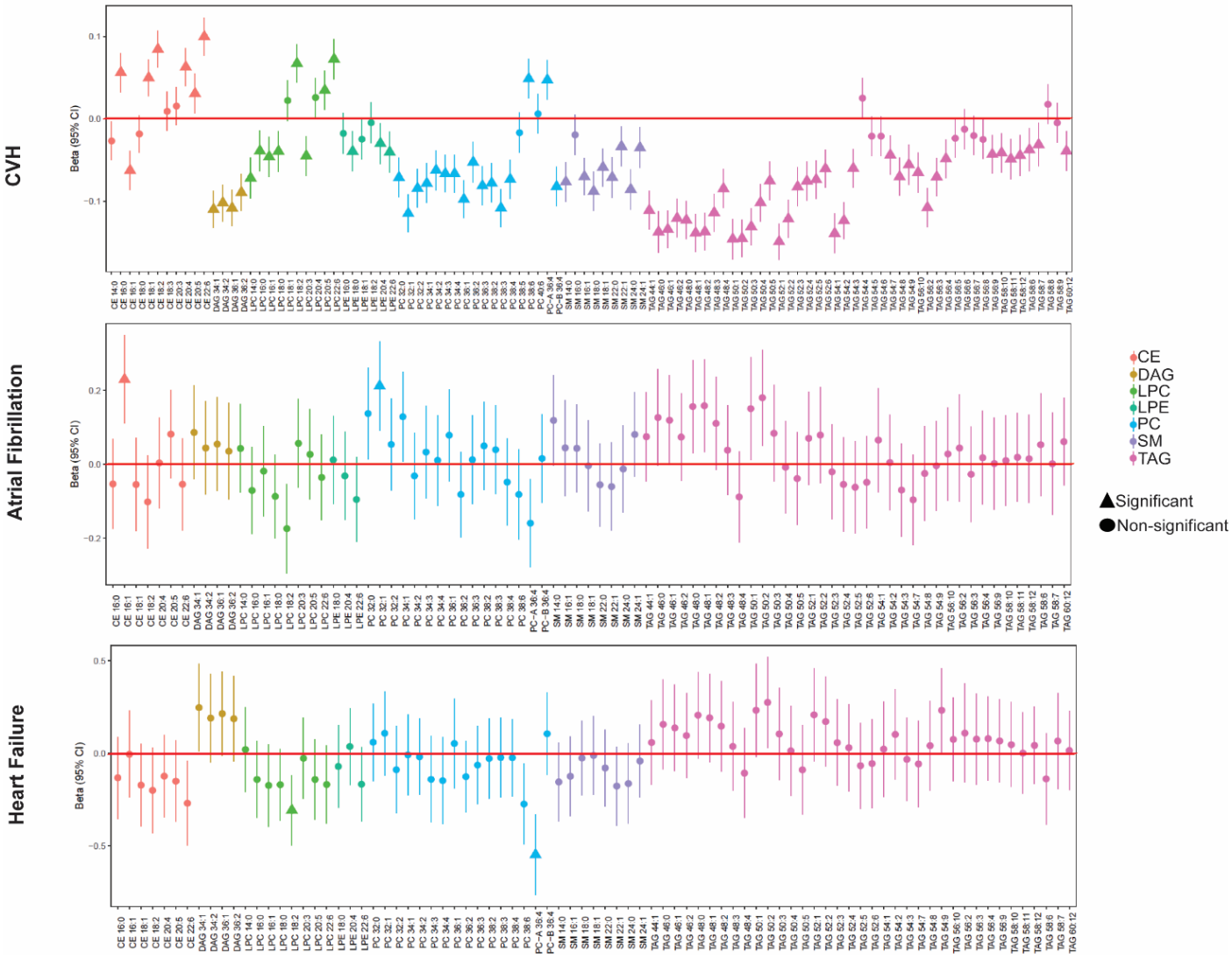
Ideal BMI, ideal blood pressure, and ideal fasting blood glucose shared the greatest number of metabolites collectively. Ideal smoking, ideal diet, and ideal physical activity had no metabolites in common.

Figure S4. Venn Diagrams Depicting the Overlap between Individual Components of CVH Score and Total CVH Score.



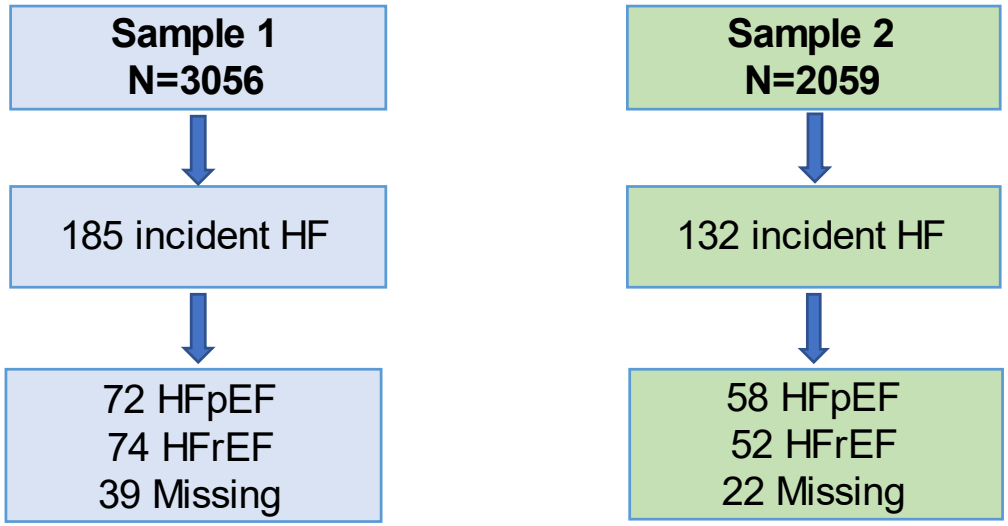
Ideal body mass index, ideal blood pressure, ideal fasting blood glucose, and ideal total cholesterol shared the greatest overlap with CVH score; physical activity shared the least of all seven components with CVH score.

Figure S5. Association of CVH Score, Incident AF, and Incident HF with Lipidomic Length and Saturation.



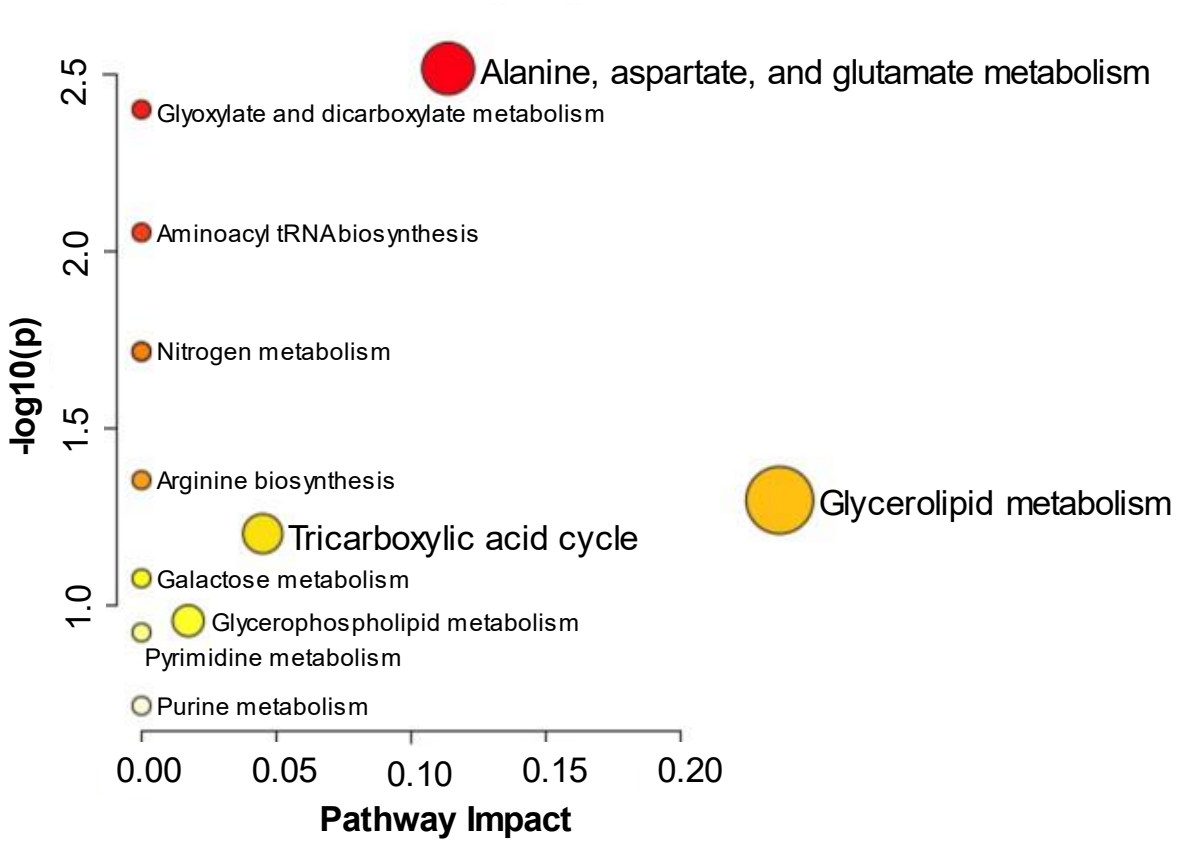
Longer carbon length lipids with greater unsaturation with hydrogen (more double bonds) was positively associated with higher CVH scores in some lipid species. A similar trend was noted for incident AF and HF, but most associations were not statistically significant.

Figure S6. Heart Failure Subgroups in Samples 1 and 2 of Incident Heart Failure.



Flow diagram denoting heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction numbers in both Sample 1 and Sample 2 with missing data numbers.

Figure S7. Pathway Analysis of Significant Metabolites that Mediate the Association of CVH Score and Incident Heart Failure



Pathway analysis of significant metabolites that mediated the association of CVH score and incident heart failure highlights three main pathways: (1) alanine, glutamine, and glutamate metabolism; (2) citric acid cycle metabolism; and (3) glycerolipid metabolism.