Journal of the American Heart Association

ORIGINAL RESEARCH

Altered Acylcarnitine Metabolism Is Associated With an Increased Risk of Atrial Fibrillation

Einar Smith, MD; Celine Fernandez, PhD; Olle Melander, MD, PhD; Filip Ottosson, PhD

BACKGROUND: Atrial fibrillation (AF) is the most common cardiac arrhythmia, but the pathogenesis is not completely understood. The application of metabolomics could help in discovering new metabolic pathways involved in the development of the disease.

METHODS AND RESULTS: We measured 112 baseline fasting metabolites of 3770 participants in the Malmö Diet and Cancer Study; these participants were free of prevalent AF. Incident cases of AF were ascertained through previously validated registers. The associations between baseline levels of metabolites and incident AF were investigated using Cox proportional hazard models. During 23.1 years of follow-up, 650 cases of AF were identified (incidence rate: 8.6 per 1000 person-years). In Cox regression models adjusted for AF risk factors, 7 medium- and long-chain acylcarnitines were associated with higher risk of incident AF (hazard ratio [HR] ranging from 1.09; 95% CI, 1.00–1.18 to 1.14, 95% CI, 1.05–1.24 per 1 SD increment of acylcarnitines). Furthermore, caffeine and acisoga were also associated with an increased risk (HR, 1.17; 95% CI, 1.06–1.28 and 1.08; 95% CI, 1.00–1.18, respectively), while beta carotene was associated with a lower risk (HR, 0.90; 95% CI, 0.82–0.99).

CONCLUSIONS: For the first time, we show associations between altered acylcarnitine metabolism and incident AF independent of traditional AF risk factors in a general population. These findings highlight metabolic alterations that precede AF diagnosis by many years and could provide insight into the pathogenesis of AF. Future studies are needed to replicate our finding in an external cohort as well as to test whether the relationship between acylcarnitines and AF is causal.

Key Words: acylcarnitines ■ atrial fibrillation ■ metabolomics

Atrial fibrillation (AF) is the most common cardiac arrhythmia, with a worldwide prevalence of ≈33 million as estimated by the 2010 Global Burden of Disease Study.[1] In the European Union, the number of patients with AF is projected to increase from 8.8 million in 2010 to 16.9 million in 2060.[2] AF is associated with increased morbidity in the form of heart failure, stroke, and dementia as well as increased mortality.[1,3] The pathogenesis of AF is a complex, not fully understood multifactorial combination of electrical remodeling, structural remodeling, and inflammation.[4] Recently, the application of metabolomics has been suggested as a tool to improve our understanding of AF pathogenesis.[5]

In regard to new-onset AF, metabolomics has so far been underutilized when compared with the research done in cardiometabolic diseases. To our knowledge, 3 cohort studies utilizing metabolomics to investigate the development of new-onset AF have been published.[6–8] A recent study from the ARIC (Atherosclerosis Risk in Communities) study with a sample size of 3922 and a mean follow-up time of 20 years found that the 4 metabolites glycochenodeoxycholate, acisoga, pseudouridine, and uridine were associated with incidence of AF in adjusted Cox regression models.[6] In a longitudinal analysis of the Framingham Heart Study with 2458 subjects and 10 years of follow-up time, no

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.016737

For Sources of Funding and Disclosures, see page 8.

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

CLINICAL PERSPECTIVE

What Is New?

- For the first time, we show associations between altered acylcarnitine metabolism and incident atrial fibrillation during a median follow-up time of >20 years.
- The circulating levels of medium- and long-chain acylcarnitines are associated with a higher risk of developing atrial fibrillation, independent of traditional atrial fibrillation risk factors.

What Are the Clinical Implications?

- The metabolic disturbances shown to precede atrial fibrillation diagnosis by several years could be future targets for medical or lifestyle interventions.

Nonstandard Abbreviation and Acronym

MDC	Malmö Diet and Cancer Study

plasma metabolites were found to be associated with the risk of new-onset AF after adjustment for multiple comparisons.[7] Lastly, in a metabolomics study done on 2023 patients undergoing coronary angiography from the Measurement to Understand Reclassification of Disease of Cabarrus/Kannapolis (MURDOCK) Horizon 1 CV (Horizon 1 Cardiovascular Disease) Study, several metabolite principal component analysis factors composed of medium- and long-chain acylcarnitines were found to associate with new onset of AF after a median follow-up time of 3.5 years.[8] Together, these studies indicate that metabolic changes can occur several years before AF diagnosis, but that either a large cohort with a long follow-up time (ARIC), or a cohort with high risk for AF (MURDOCK) is needed in order to gain enough statistical power to find the changes that predispose to AF.

In the present study, we measured fasting plasma levels of 112 metabolites from the baseline examination of a Swedish population-based prospective cohort study, the MDC (Malmö Diet and Cancer) Study, comprising 3770 individuals without AF at study entry. The associations of metabolite levels and the development of AF were assessed during a median follow-up time of 23 years. Our aim was to identify metabolites associated with AF risk in order to highlight metabolic changes that predispose to the development of AF, with the opportunity to discover new pathways involved in the complex pathogenesis of AF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

The MDC is a population-based prospective cohort study of individuals who attended baseline examinations between 1991 and 1996 in Malmö, Sweden. The methodology and population have been previously described.[9,10] A random sample of 3833 participants in the cardiovascular cohort (MDC-CC)[11] was included for metabolite measurement. Participants with prevalent AF (n=35) or unknown vital status at follow-up (n=28) were excluded from all analyses. The remaining 3770 participants constituted our study sample in this post hoc analysis of incident AF. All participants provided written informed consent and the study was approved by the Ethics Committee of Lund University, Lund, Sweden (LU 51–90).

Data on covariates were collected at baseline, and have previously been described.[12] The consumption of alcohol was defined by a 4-category variable by combining a 7-day menu book and a food frequency questionnaire as previously described.[13]NT-proBNP (N-terminal pro-B-type natriuretic peptide) was measured using the automated Dimension Vista (R) Intelligent Lab System method (Siemens Healthcare Diagnostics Inc., Deerfield, IL). Because of nonnormality, NT-proBNP underwent logarithmic transformation.[14]

Cases of new-onset AF were ascertained until December 31, 2016 by linkage of Swedish personal identification numbers to the Swedish Hospital Discharge Register and the Swedish Cause of Death Register. Given the similarity of the 2 diseases, AF was defined as persistent or recurring AF or flutter using diagnosis codes 427.92 (*International Classification of Diseases, Eighth Revision* [*ICD-8*]), 427D (*ICD-9*), and I48 (*ICD-10*). This end point has previously been validated.[15,16] Prevalent heart failure was defined as codes 427.00, 427.10, and 428.99 (*ICD-8*), 428 (*ICD-9*), and I50 and I11.0 (*ICD-10*).[16] Prevalent diabetes mellitus was defined as a fasting whole blood glucose ≥6.1 mmol/L (corresponding to a plasma glucose level of ≥7.0 mmol/L) or a history of physician diagnosis of diabetes mellitus or being on antidiabetic medication or having been registered in any of the 6 different national and regional diabetes mellitus registers.[12] Ischemic stroke was coded according to the *ICD-9* code 434 (cerebral infarction) and verified by computed tomography scan or autopsy. To further enrich the end point, only ischemic stroke events that were preceded by or coincided (within 1 month) with a diagnosis of AF were included in order to find plausible cardioembolic stroke cases.

Profiling of plasma metabolites was performed using liquid chromatography–mass spectrometry, and has been previously described in detail.[17] Measured metabolites are listed in an in-house metabolite library and categorized according to normalization method (Table S1). Thirty-three of the metabolites were adjusted with an internal standard and 79 were normalized with standard curves calculated from the quality control samples as previously described.[12] The mass spectrometry method was initially created to measure 35 polar metabolites and amino acids.[18] A subsequent study expanded the method to investigate the relationship between metabolites and development of cardiovascular disease and type 2 diabetes mellitus[12] and managed to identify 77 additional metabolites, thus measuring 112 metabolites with suspected relationship to cardiometabolic disease.

R (V.3.6.0) was used for all statistical analysis. Because of nonnormality, metabolite data were log transformed and scaled to multiples of 1 SD and centered on zero before statistical analyses. Outliers that differed >4 SD from the mean after normalization were excluded from the analysis. Percentages of removed samples are reported in the in-house metabolite library (Table S1).

To assess the associations between baseline fasting levels of metabolite levels and incident AF, Cox proportional hazard models were used. First, hypothesis-generating analyses were performed with models adjusted for sex and age, and corrected for multiple comparison using false discovery rate. False discovery rate was used instead of a more stringent multiple testing method such as Bonferroni because these methods assume that the statistical tests being performed are independent. In this case, the tests are not independent because the levels of acylcarnitines are closely correlated and using Bonferroni correction would increase the risk of false negatives. Metabolites with significant associations were further analyzed in Cox proportional hazard models adjusted for sex, age, body mass index, baseline smoking status, systolic blood pressure, alcohol intake, use of antihypertensive medicine, NT-proBNP, prevalent diabetes mellitus, prevalent heart failure, and prevalent ischemic heart disease. Schoenfeld residuals test was used to check the proportional hazard assumptions.

Because of the high amount of samples with miniscule caffeine levels (16%) (Table S1), additional Cox regression models were made using quintiles of caffeine levels including all samples. The lowest quintile was set as reference quintile.

The relationships between metabolites were investigated using Spearman correlations, displayed in a heat map with metabolites ordered by their first component.

To test the associations between metabolite levels and risk factors, partial Spearman correlations adjusted for sex and age were used. The associations between metabolite levels and sex were only adjusted for age, and the associations between metabolite levels and age were only adjusted for sex.

The associations between AF-associated metabolites and likely cardioembolic stroke were tested using Cox proportional hazard models adjusted for sex and age. Before analyses, patients with prevalent stroke, incident subarachnoid hemorrhage, or incident intracerebral hemorrhage were excluded. Associations were further tested in Cox proportional hazard models adjusted for sex, age, body mass index, baseline smoking status, systolic blood pressure, alcohol intake, use of antihypertensive medicine, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, prevalent diabetes mellitus, prevalent heart failure, and prevalent ischemic heart disease. Additional adjustments were made with prevalent chronic obstructive pulmonary disease, prevalent cancer, and estimated glomerular filtration rate.

RESULTS

The average baseline age was 57.7 years, and 59% were female. General characteristics of the study population can be found in Table. Among the 3770 participants free from prevalent AF, we identified 650 incident cases of AF during a median follow-up time of 23.1 years (incidence rate: 8.6 per 1000 person-years).

Using sex- and age-adjusted Cox proportional hazard models, out of 112 metabolites measured, 15 were associated with an increase or decrease in risk for developing AF (false discovery rate <0.05). In fully adjusted models, 11 metabolites remained significantly associated with AF incidence, most of which were acylcarnitines (Figure 1 and Table S2). Caffeine was associated with the highest increase in risk for AF (hazard ratio [HR] per 1 SD increment of caffeine, HR, 1.17; 95% CI, 1.06–1.28, P=0.001) in the fully adjusted model followed by acylcarnitine 16:1 (HR per 1 SD increment of acylcarnitine 16:1, HR, 1.13; 95% CI, 1.04–1.23, P=0.004). The proportional hazard assumptions were met for all statistically significant models shown in Figure 1. Further adjustments for prevalent chronic obstructive pulmonary disease, prevalent cancer, and estimated glomerular filtration rate did not change the results (Table S3).

Since there were many samples with caffeine levels below limit of detection (16%) (Table S1), additional analysis of caffeine split into quintiles was made including all samples. In these Cox regression models with quintile 1 as reference, quintile 5 had a significantly

Table. General Characteristics of Study Participants

	Total No.=3770 Mean (SD) or % (No.)	Non-Incident No.=3120 Mean (SD) or %	Incident AF No.=650 Mean (SD) or %	*P* Value
N	3770	3120 (83%)	650 (17%)	
Age, y	58 (6.0)	57 (6.0)	60 (5.4)	<0.001
Sex (% female)	59% (2224)	61% (1914)	48% (310)	<0.001
BMI, kg/m^2	25.7 (3.9)	25.5 (3.8)	26.5 (4.3)	<0.001
LDL-C, mmol/L	4.16 (1.0)	4.17 (1.0)	4.13 (0.9)	0.3
HDL-C, mmol/L	1.40 (0.4)	1.40 (0.4)	1.39 (0.4)	0.4
Glucose, mmol/L	5.20 (1.4)	5.15 (1.3)	5.41 (1.6)	<0.001
NT-proBNP, ng/L	96 (151)	89.1 (139)	129.1 (152)	<0.001
eGFR, mL/min per 1.73 m^2	75.7	76.2	73.4	<0.001
Systolic blood pressure, mm Hg	142 (19)	141 (19)	148 (19)	<0.001
Diastolic blood pressure, mm Hg	87 (9.5)	86.5 (9.5)	88.7 (9.2)	<0.001
Antihypertensive treatment	15.9% (599)	14.1% (439)	24.6% (160)	<0.001
Smoking status (3% missing)	27% (995)	28% (840)	25% (155)	0.11
Alcohol intake, g/d (3% missing)	10.4 (12)	10.0 (12)	11.8 (14)	0.002
Prevalent coronary artery disease	2.1% (79)	1.4% (45)	5.2% (34)	<0.001
Prevalent diabetes mellitus	9.8% (368)	9.3% (289)	12.2% (79)	0.03
Prevalent heart failure	0.1% (5)	0.1% (3)	0.31% (2)	0.4
Prevalent COPD	0.6% (24)	0.6% (20)	0.6% (4)	1
Prevalent cancer	5.8% (219)	5.6% (172)	7.2% (47)	0.1

Values are displayed as mean (SD) or percentages. BMI indicates body mass index; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

higher risk for developing AF in the sex- and age-adjusted model (Figure 2). The fully adjusted models showed no significant associations between quintiles of caffeine and AF risk.

Since many of the metabolites significantly associated with AF were in the same class of metabolites, namely, acylcarnitines, the correlations between AF-associated metabolites levels were examined. The

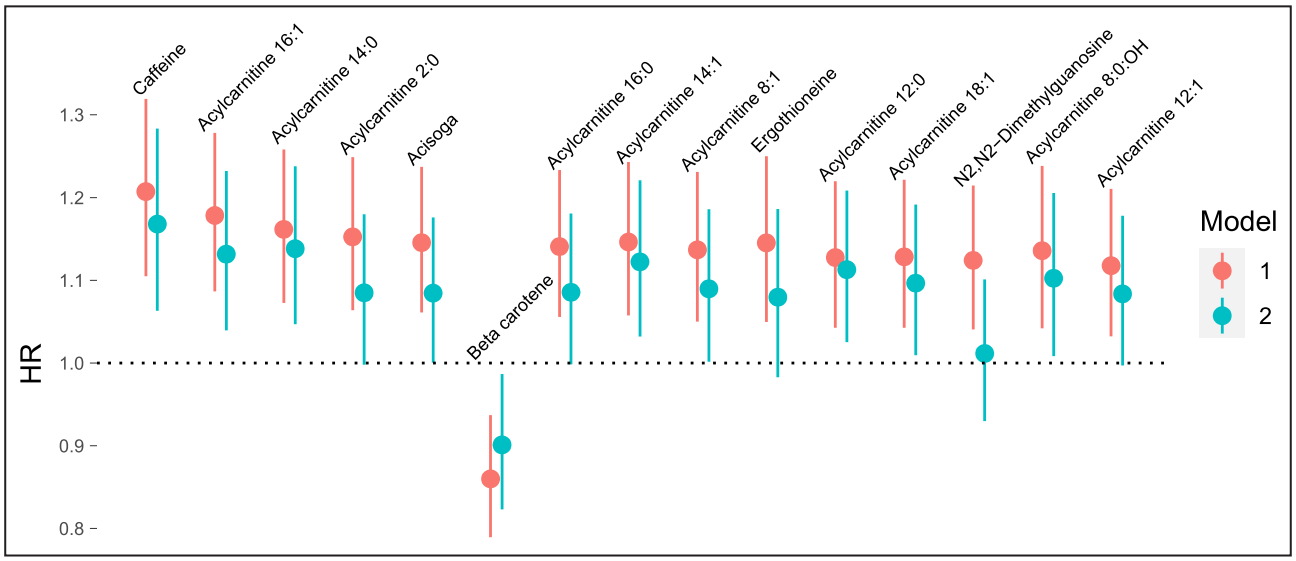

Figure 1. Cox proportional hazard models comparing circulating metabolite levels with risk for atrial fibrillation during the median follow-up time of 23.1 years.
Model 1 was adjusted for sex and age. Model 2 was adjusted for sex, age, smoking, body mass index, systolic blood pressure, alcohol intake, use of hypertensive medicine, N-terminal pro-B-type natriuretic peptide, and prevalent diabetes mellitus, heart failure, and coronary artery disease. The HR is calculated as the increase or decrease in risk per 1 SD increment of metabolite levels with 95% CI. HR indicates hazard ratio.

heat map with Spearman correlations show that acylcarnitines correlate strongly with each other, except for acylcarnitine 8:1, which had a weaker correlation with other acylcarnitines (Figure 3).

In partial Spearman correlations comparing metabolite levels to risk factors, acylcarnitines were associated with higher age (Figure 4). The strongest correlation was between ergothioneine and alcohol intake. The correlations with the other risk factors, systolic blood pressure, use of antihypertensive treatment, smoking status, body mass index, and NT-proBNP were small overall.

In a median follow-up time of 23.2 years, 329 cases of ischemic stroke were identified after exclusion of patients with prevalent stroke (n=29), incident subarachnoid hemorrhage (n=7), incident intracerebral hemorrhage (n=48), or unspecified incident stroke subtype (n=13) (n after exclusion 3681). Out of these 329 cases, 83 were preceded by or coincided (within a month) with AF diagnosis and were therefore deemed likely to be cardioembolic in nature.

Associations between likely cardioembolic stroke and AF-associated metabolites had similar effect sizes as risk for incident AF, but after multiple test corrections, no associations were significant (Table S4).

DISCUSSION

In this metabolomics study of 3770 individuals in a prospective cohort, we found 10 metabolites to associate significantly with new-onset AF in models adjusted for AF risk factors. Most of the metabolites associated with incident AF were acylcarnitines, suggesting a dysfunction of carnitine metabolism that precedes AF diagnosis by many years and which also might contribute to the pathogenesis of AF.

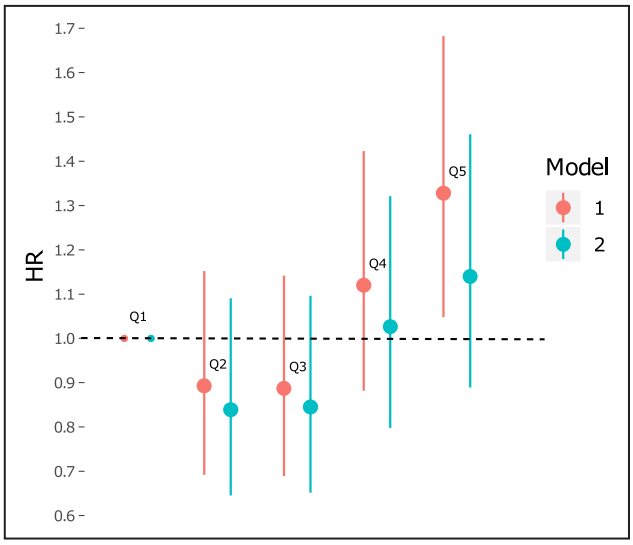

Figure 2. Cox proportional hazard models comparing caffeine levels with risk for atrial fibrillation.
Model 1 was adjusted for sex and age. Model 2 was adjusted for sex, age, smoking, body mass index, systolic blood pressure, alcohol intake, use of hypertensive medicine, N-terminal pro-B-type natriuretic peptide, and prevalent diabetes mellitus, heart failure, and coronary artery disease. The HR is calculated as the increase or decrease in risk per 1 SD increment of metabolite levels with 95% CI. HR indicates hazard ratio.

In a metabolomics study done on 2023 patients from the MURDOCK Study, several metabolite factors composed of medium- and long-chain acylcarnitines were found to associate with new onset of AF.[8] The cohort consisted of patients subjected to coronary angiography with a high risk of new-onset AF, and 12.3% of study participants developed AF during the 2.8-year follow-up. We extend these findings displaying associations between several acylcarnitines and risk for AF in a general population without increased risk for AF. Furthermore, we show the associations between AF and levels of specific acylcarnitines to be independent of several risk factors for AF, including NT-proBNP. In the ARIC study mentioned earlier, some acylcarnitines were associated with an increased risk of AF, but the associations were not significant after multiple test correction.

Acylcarnitines are mostly derived from mitochondrial fatty acid oxidation, but can be formed from almost any coenzyme A ester.[19] Changed levels of acylcarnitines in circulation have been suggested to provide indirect evidence of altered mitochondrial metabolism, and accumulation of acylcarnitines could be seen as a sign of poor metabolic status.[20] Stressed myocardial cells can change from fatty acid oxidation in the mitochondria to glycolysis,[21] and the subsequent accumulation of long-chain acylcarnitines in the cytoplasm could contribute to membrane instability by inhibiting the exchange of sodium and calcium ions in the sarcolemma and thus lead to the development of arrhythmia.[22] In a study of patients undergoing coronary artery bypass grafting surgery, adenosine-diphosphate-stimulated mitochondrial respiration supported by acylcarnitine 16:0 was significantly lower in patients who developed postoperative AF.[23] Levels of short-, medium-, and long-chain acylcarnitines have all been associated with an increased risk of cardiovascular death and acute myocardial infarction.[24]

Furthermore, circulating levels of long-chain acylcarnitines have been associated with maladaptive left ventricular remodeling.[25] The increased levels of acylcarnitines could therefore be associated with either electrical remodeling or structural remodeling, which together with inflammation form the 3 dominant theories on AF pathogenesis.[4] The structural remodeling seems to be independent of the heart-failure-associated remodeling, given that the associations displayed in the fully adjusted models were independent of NT-proBNP levels. Additional studies are needed to replicate our finding and to further investigate the potentially

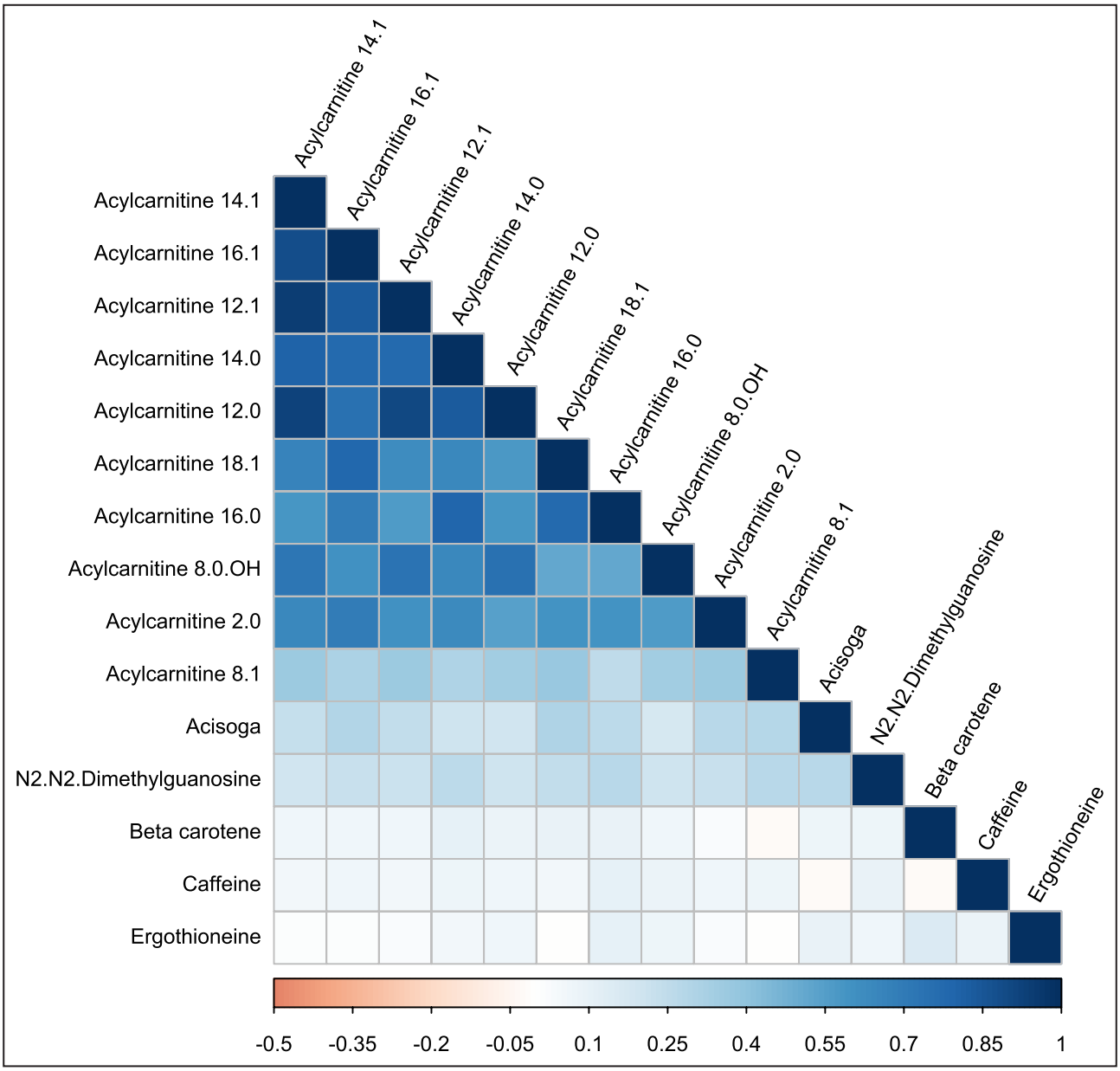

Figure 3. A heat map of Spearman correlation coefficients between metabolites that were associated with incident atrial fibrillation. Metabolites are ordered by their first component.

causal associations between increased acylcarnitines levels and AF.

The finding of caffeine being associated with new-onset AF was surprising, because caffeine exposure has not been shown to increase the risk of AF in a systematic review of >100 000 individuals.[26] The results from this large meta-analysis even suggest that low-dose caffeine may have a protective effect. Given that many of our participants had caffeine levels below the limit of detection, quintile analyses were made with all samples included. This analysis showed that the potential association between plasma caffeine levels and AF is not linear. Caffeine quintile 5 displayed a significantly higher risk for AF compared with quintile 1, but other associations were not significant.

To our knowledge, our study is the first to study caffeine levels in plasma in relation to AF instead of studying caffeine exposure, based on reported intake, in relation to AF risk. Participants of the study were overnight fasting, and data on caffeine exposure preceding days before the analyses are not available. There are numerous factors influencing caffeine intake, absorption, metabolism, and physiologic and functional effects, which all could affect caffeine plasma levels, such as age, sex, hormonal status, diet, smoking, exposure to drugs, and genetic background.[27] Adjustments for genetic polymorphism of CYP1A2 (cytochrome P450

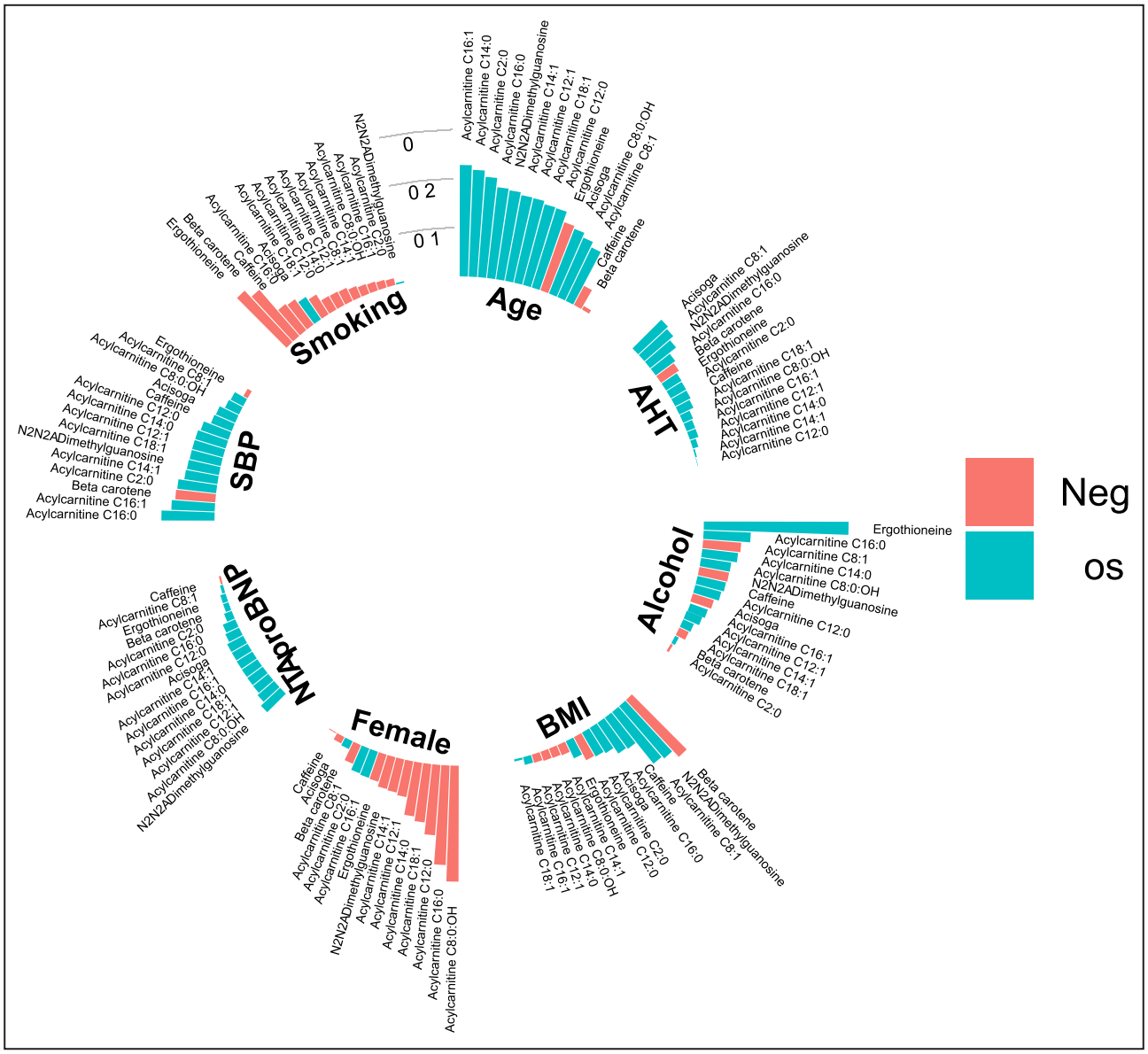

Figure 4. Partial Spearman correlation coefficients adjusted for sex and age between risk factors and metabolite levels. The directions of the associations are color coded. AHT indicates antihypertensive treatment; BMI, body mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and SBP, systolic blood pressure.

1A2), the main metabolizer of caffeine, has not shown any improvement in AF risk prediction, but the addition of caffeine concentration measurements to such studies could bring more insight into the caffeine–AF interaction.[28]

An article published last year studying incident AF in 3922 individuals from the ARIC study found that the 4 metabolites acisoga, glycochenodeoxycholate, pseudouridine, and uridine were associated with AF.[6] The polyamine acisoga is a breakdown product of spermidine, and its precise role is unknown.[29] In the present study, acisoga was found to associate with AF in a sex- and age-adjusted Cox regression model, but the relationship was strongly attenuated after adjustment. The liquid chromatography–mass spectrometry method used in our study did not measure the other 3 metabolites that were significantly associated with AF in the ARIC study.

Ergothioneine and N2N2-dimethylguanosine were both associated with an increased risk for AF in sex- and age-adjusted models, but the associations were not significant after full adjustment. We have previously found that ergothioneine was associated with a decreased risk of cardiovascular disease, cardiovascular mortality, and all-cause mortality[12] and N2N2-dimethylguanosine with incident type 2 diabetes mellitus.[18] The attenuated association between ergothioneine and AF could further be explained by the

strong association between ergothioneine and alcohol intake, a known risk factor for AF.[30]

Beta carotene was associated with a decreased risk of AF in sex- and age-adjusted models, an association that was attenuated after full adjustment. Beta carotene has previously been associated with higher risk for AF, which contradicts our findings.[31] The association found in the present study between beta carotene and AF could be explained by an association with heart failure, a disease with closely aligned risk factors and pathogenesis with AF.[32,33]

When testing the association between metabolite levels and likely cardioembolic stroke, associations showed the same directionality and effect size as risk for incident AF. However, the results were nonsignificant, which could partly have been explained by a lack of statistical power because of the low incidence rate and because we did not have sufficient information about stroke subtypes to validate the end point cardioembolic stroke.

In a short perspective, our results must be validated before they are integrated into clinical care. If the results are generalizable, test for acylcarnitines might be a part of AF risk stratification as we move towards personalized medicine. If further research shows a causative link between altered acylcarnitine metabolism and AF, acylcarnitine metabolism might become a target for drug development in order to lower the risk of AF.

The main strengths of our study are the large cohort with follow-up data of good quality combined with a large number of AF cases found by a previously validated method. Since AF cases were diagnosed via registers and not through ECGs at follow-up visits, there is the potential that undiagnosed cases are excluded. There were no data available on electrophysiological parameters or cardiac imaging, which might have provided additional information about what parameters the acylcarnitines affect. Moreover, with the prospective but observational study design of the MDC study, the causal link between metabolites and AF cannot be tested.

CONCLUSIONS

For the first time, we show associations between altered acylcarnitine metabolism and incident AF independent of traditional risk factors in a general population. These findings highlight metabolic alterations that precede AF diagnosis by many years. Future studies are needed to replicate our finding in an external cohort as well as to test whether the relationship between acylcarnitines and AF is causal.

ARTICLE INFORMATION

Received March 26, 2020; accepted September 17, 2020.

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Acknowledgments

We thank all the participants and staff in the Malmö Diet and Cancer—Cardiovascular Cohort.

Sources of Funding

Smith was supported by the Hulda and Conrad Mossfelt Foundation. Melander was supported by research grants from the Knut and Alice Wallenberg Foundation, Göran Gustafsson Foundation, the Swedish Heart- and Lung Foundation, the Swedish Research Council, the Novo Nordisk Foundation, Region Skåne, Skåne University Hospital and Lund University. Fernandez was supported by the Albert Påhlsson Research Foundation, the Crafoord Research Foundation, the Ernhold Lundström Research Foundation, the Royal Physiographic Society of Lund, and the Åke Wiberg Foundation. Ottosson was supported by Ernhold Lundströms Research Foundation.

Disclosures

None.

Supplementary Material

Tables S1–S4

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SUPPLEMENTAL MATERIAL

Table S1. In house metabolite library.

Metabolites	% excluded before analysis	mass (m/z)	retention time (min)	HMDB ID	Normalization method
Urea	0.2%	60.032	1.98	HMDB00294	LOESS
Trimethylamine-N-oxide	3.2%	75.068	4.28	HMDB00925	LOESS
Alanine	0.2%	89.048	7.5	HMDB00161	Internal Standard
Sarcosine	11.0%	89.048	7.27	HMDB00271	LOESS
Dimethylglycine	1.0%	103.063	6.59	HMDB00092	Internal Standard
2-Aminoisobutyric acid	0.5%	103.064	6.9	HMDB01906	LOESS
Choline	0.2%	104.108	4.01	HMDB00097	Internal Standard
Serine	0.3%	105.043	8.56	HMDB00187	Internal Standard
Creatinine	0.2%	113.059	4.55	HMDB00562	LOESS
Proline	0.4%	115.063	6.64	HMDB00162	Internal Standard
Guanidineacetate	0.0%	117.054	7.56	HMDB00128	LOESS
Betaine	0.4%	118.087	6.14	HMDB00043	Internal Standard
Valine	0.0%	118.087	6.57	HMDB00883	Internal Standard
Threonine	0.6%	119.058	7.85	HMDB00167	Internal Standard
Nicotinamide	0.6%	122.048	1.36	HMDB01406	Internal Standard
Taurine	0.2%	125.015	6.62	HMDB00251	Internal Standard
Pyroglutamate	0.7%	129.043	2.56	HMDB00267	LOESS
N.Methylproline	6.3%	129.079	6.25	HMDB94696	LOESS
Pipecolate	1.8%	129.079	6.84	HMDB00716	LOESS
Creatine	0.1%	131.07	7.19	HMDB00064	LOESS
Isoleucine	0.3%	131.095	6.09	HMDB00172	Internal Standard
Leucine	0.1%	131.095	5.92	HMDB00687	Internal Standard
Asparagine	0.2%	132.053	8.7	HMDB00168	Internal Standard
Ornithine	0.3%	132.09	11.58	HMDB00214	Internal Standard
Hypoxanthine	0.2%	136.039	3.8	HMDB00157	LOESS
Trigonelline	5.9%	137.048	6.38	HMDB00875	LOESS
Methylnicotinamide	0.8%	137.071	4.96	HMDB03152	Internal Standard
Urocanate	4.7%	138.042	2.38	HMDB00301	LOESS
Proline betaine	0.8%	143.095	6.21	HMDB04827	LOESS
Glutamine	0.1%	146.069	8.48	HMDB00641	Internal Standard
Lysine	0.2%	146.106	11.4	HMDB00182	Internal Standard
4-Trimethylammoniobutanoic acid	0.6%	146.118	4.58	HMDB01161	LOESS
Glutamate	0.2%	147.053	8.14	HMDB00148	Internal Standard
Methionine	0.2%	149.051	6.32	HMDB00696	Internal Standard
N-Methyl-4-pyridone-3-carboxamide	1.1%	152.058	1.92	HMDB04194	LOESS
N-Methyl-2-pyridone-5-carboxamide	1.00%	152.0586	1.72	NA	LOESS
Homostachydrine	2.3%	157.11	6.02	HMDB33433	LOESS
Methyllysine	0.0%	160.121	10.66	HMDB02038	LOESS

Carnitine	1.4%	162.113	6.19	HMDB00062	Internal Standard
Methionine.S.oxide	6.0%	165.046	8.23	HMDB02005	LOESS
7-Methylguanine	0.3%	165.065	4.77	HMDB00897	LOESS
Phenylalanine	0.4%	165.079	5.88	HMDB00159	Internal Standard
1-Methylhistidine	0.4%	169.085	9.72	HMDB00001	LOESS
3-Methylhistidine	2.3%	169.085	10.82	HMDB00479	LOESS
Acetylornithine	0.7%	174.102	7.86	HMDB03357	LOESS
Arginine	0.2%	174.112	10.92	HMDB00517	Internal Standard
Dimethyllysine	0.10%	174.137	9.95	NA	LOESS
Citrulline	0.3%	175.095	9.04	HMDB00904	Internal Standard
Cotinine	71.10%	176.095	1.33	NA	LOESS
Hippurate	2.4%	179.058	1.14	HMDB00714	LOESS
Paraxanthine	2.1%	180.065	1.33	HMDB01860	LOESS
Tyrosine	6.3%	181.074	6.64	HMDB00158	Internal Standard
Acisoga	0.4%	184.121	1.63	HMDB61384	LOESS
Histidine	0.3%	188.127	10.56	HMDB00177	Internal Standard
Homoarginine	0.3%	188.127	10.59	HMDB00670	LOESS
NMMA	0.2%	188.127	10.2	HMDB29416	LOESS
Kynurenate	0.8%	189.043	5.06	HMDB00715	LOESS
Homocitrulline	3.3%	189.111	8.86	HMDB00679	LOESS
Trimethyllysine	1.0%	189.16	10.14	HMDB01325	LOESS
Hydroxycotinine	96.80%	192.09	1.75	NA	LOESS
Caffeine	16.2%	194.08	1	HMDB01847	LOESS
5-Acetylamino-6-amino-3-methyluracil	13.8%	198.075	4.61	HMDB04400	LOESS
DMGV	1.7%	202.119	6.01	HMDB0240212	LOESS
Asymmetric dimethylarginine	1.3%	202.143	9.52	HMDB01539	Internal Standard
Symmetric dimethylarginine	2.0%	202.143	9.51	HMDB03334	LOESS
Tryptophan	0.3%	204.09	5.93	HMDB00929	Internal Standard
Acylcarnitine C2:0	0.3%	204.124	4.45	HMDB00201	Internal Standard
3-Hydroxytrimethyllysine	0.7%	205.155	10.8	HMDB01422	LOESS
Kynurenine	0.3%	208.085	6	HMDB00684	LOESS
Acetylarginine	0.3%	216.122	7.08	HMDB04620	LOESS
Acylcarnitine C3:0	0.4%	218.139	4.06	HMDB00824	Internal Standard
Pantothenate	1.2%	219.111	1.63	HMDB00210	LOESS
Ergothioneine	1.1%	229.088	7.35	HMDB03045	LOESS
Acylcarnitine C4:0	1.2%	232.154	3.63	HMDB02013	Internal Standard
Cystine	0.1%	240.024	13.02	HMDB00192	LOESS
Tiglylcarnitine	2.9%	243.147	3.46	HMDB02366	LOESS
Acylcarnitine C5:0	0.6%	245.163	3.23	HMDB00688	LOESS
Acylcarnitine C4:0-OH	23.90%	247.142	4.7	NA	LOESS
Glycerophosphocholine	0.5%	258.11	8.71	HMDB00086	LOESS
Acylcarnitine C6:0	0.7%	260.186	2.88	HMDB00756	LOESS
Phenylacetylglutamine	0.6%	264.11	1.98	HMDB06344	LOESS
Acetylcarnosine	0.4%	268.117	8.04	HMDB12881	LOESS

1-Methyladenosine	0.2%	281.113	6.46	HMDB03331	LOESS
Piperine	1.50%	285.137	0.85	NA	LOESS
Acylcarnitine C8:1	0.6%	286.202	2.73	HMDB13324	LOESS
Acylcarnitine C8:0	0.9%	288.217	2.49	HMDB00791	Internal Standard
5-Methylthioadenosine	4.1%	297.09	2.08	HMDB01173	LOESS
Acylcarnitine C9:0	0.7%	302.233	2.23	HMDB13288	LOESS
Acylcarnitine C8:0-OH	0.5%	304.212	4.06		LOESS
Acylcarnitine C10:3	0.8%	310.202	2.42		LOESS
N2.N2.Dimethylguanosin e	0.5%	311.123	4.74	HMDB04824	LOESS
Acylcarnitine C10:2	0.6%	312.217	2.44		LOESS
Acylcarnitine C10:1	1.0%	314.232	2.34	HMDB13205	LOESS
Acylcarnitine C10:0	0.7%	316.249	2.18	HMDB00651	LOESS
Acylcarnitine C11:1	0.3%	328.248	2.28		LOESS
Acylcarnitine C11:0	0.6%	330.263	2.14	HMDB13321	LOESS
Acylcarnitine C10:0-OH	1.0%	332.244	3.52		LOESS
Acylcarnitine C12:2	1.0%	340.25	2.23		LOESS
Acylcarnitine C12:1	0.5%	342.263	2.16	HMDB13326	LOESS
Acylcarnitine C12:0	0.4%	344.279	2.13	HMDB02250	LOESS
Acylcarnitine C13:1	0.8%	356.279	2.1		LOESS
Acylcarnitine C13:0	0.8%	358.295	2.03		LOESS
Acylcarnitine C14:2	0.5%	368.28	2.08	HMDB13331	LOESS
Acylcarnitine C14:1	0.4%	370.294	2.03	HMDB0240588	LOESS
Acylcarnitine C14:0	0.4%	372.311	1.94	HMDB05066	Internal Standard
Acylcarnitine C16:1	0.4%	398.326	1.93	HMDB13207	LOESS
25-Hydroxyvitamin D3	0.7%	400.334	0.81	HMDB03550	LOESS
Acylcarnitine C16:0	0.3%	400.343	1.85	HMDB00222	Internal Standard
Acylcarnitine C18:2	0.4%	424.346	1.87		LOESS
Acylcarnitine C18:1	0.3%	426.357	1.86		LOESS
Acylcarnitine C18:0	0.4%	428.372	1.84	HMDB00848	LOESS
Beta-carotene	0.6%	536.438	0.83	HMDB00561	LOESS

In-house metabolite library. Samples with differed more 4SD from the mean after normalization were excluded from analyses.

Table S2. Cox proportional hazard models associating metabolite levels with risk for incident atrial fibrillation.

Metabolite	P model 1	p_fdr model 1	HR model 1	95% lowint	95% highint	p model 2	HR model 2	95% lowint	95% highint
Caffeine	3E-05	0.003	1.21	1.10	1.32	0.0012	1.17	1.06	1.28
Acylcarnitine C16:1	7E-05	0.004	1.18	1.09	1.28	0.0044	1.13	1.04	1.23
Acylcarnitine C14:0	0.0002	0.009	1.16	1.07	1.26	0.0024	1.14	1.05	1.24
Acylcarnitine C2:0	0.0005	0.01	1.15	1.06	1.25	0.06	1.09	1.00	1.18
Acisoga	0.0005	0.01	1.15	1.06	1.24	0.05	1.08	1.00	1.18
Beta-carotene	0.0006	0.01	0.86	0.79	0.94	0.02	0.90	0.82	0.99
Acylcarnitine C16:0	0.0009	0.01	1.14	1.06	1.23	0.05	1.09	1.00	1.18
Acylcarnitine C14:1	0.0009	0.01	1.15	1.06	1.24	0.007	1.12	1.03	1.22
Acylcarnitine C8:1	0.002	0.02	1.14	1.05	1.23	0.05	1.09	1.00	1.19
Ergothioneine	0.002	0.03	1.15	1.05	1.25	0.1	1.08	0.98	1.19
Acylcarnitine C12:0	0.003	0.03	1.13	1.04	1.22	0.01	1.11	1.03	1.21
Acylcarnitine C18:1	0.003	0.03	1.13	1.04	1.22	0.03	1.10	1.01	1.19
N2.N2.Dimethylguanosine	0.003	0.03	1.12	1.04	1.21	0.8	1.01	0.93	1.10
Acylcarnitine C8:0-OH	0.004	0.03	1.14	1.04	1.24	0.03	1.10	1.01	1.21
Acylcarnitine C12:1	0.006	0.05	1.12	1.03	1.21	0.06	1.08	1.00	1.18
Isoleucine	0.007	0.05	1.12	1.03	1.22				
Trimethylamine-N-oxide	0.009	0.06	1.15	1.04	1.28				
Acylcarnitine C13:0	0.01	0.08	1.11	1.02	1.20				
Acylcarnitine C11:1	0.01	0.09	1.10	1.02	1.20				
Acetylcarnosine	0.02	0.09	0.88	0.79	0.98				
Acylcarnitine C10:0-OH	0.02	0.1	1.10	1.02	1.19				
1-Methyladenosine	0.02	0.1	1.10	1.02	1.19				
Trimethyllysine	0.02	0.1	1.14	1.02	1.27				
Acylcarnitine C13:1	0.02	0.1	1.10	1.02	1.20				
Acylcarnitine C10:3	0.02	0.1	1.11	1.01	1.21				
Acylcarnitine C6:0	0.02	0.1	1.11	1.01	1.21				
Paraxanthine	0.03	0.1	1.10	1.01	1.19				
7-Methylguanine	0.03	0.1	1.09	1.01	1.18				
Glutamate	0.03	0.1	1.09	1.01	1.18				
Acylcarnitine C18:2	0.03	0.1	1.09	1.01	1.18				
1-Methylhistidine	0.03	0.1	1.09	1.01	1.19				
Taurine	0.04	0.1	1.09	1.01	1.18				
Acylcarnitine C8:0	0.04	0.1	1.10	1.01	1.20				
Acylcarnitine C10:2	0.04	0.1	1.10	1.00	1.20				
4-Trimethylammoniobutanoic acid	0.04	0.1	0.91	0.84	1.00				

Kynurenine	0.04	0.1	1.09	1.00	1.18				
25-Hydroxyvitamin D3	0.05	0.1	1.09	1.00	1.19				
Hypoxanthine	0.06	0.2	1.08	1.00	1.17				
Acylcarnitine C10:0	0.07	0.2	1.08	0.99	1.18				
5-Acetylamino-6-amino-3-methyluracil	0.07	0.2	1.09	0.99	1.20				
Guanidineacetate	0.09	0.3	0.93	0.85	1.01				
DMGV	0.09	0.3	1.07	0.99	1.17				
Glutamine	0.1	0.3	0.94	0.87	1.01				
Glycerophosphocholine	0.1	0.3	0.93	0.86	1.02				
Cystine	0.1	0.3	1.06	0.99	1.15				
Methionine.S.oxide	0.1	0.3	1.13	0.97	1.32				
Acylcarnitine C14:2	0.1	0.3	1.07	0.98	1.16				
Acylcarnitine C12:2	0.1	0.3	1.07	0.98	1.15				
Dimethyllysine	0.1	0.3	1.06	0.98	1.15				
2-Aminoisobutyric acid	0.1	0.3	1.07	0.98	1.17				
5-Methylthioadenosine	0.2	0.3	0.94	0.87	1.02				
Acylcarnitine C5:0	0.2	0.3	1.07	0.98	1.16				
Methyllysine	0.2	0.3	1.05	0.98	1.14				
Creatine	0.2	0.3	1.06	0.97	1.16				
Leucine	0.2	0.3	1.06	0.97	1.16				
Choline	0.2	0.3	1.06	0.98	1.15				
Hydroxycotinine	0.2	0.3	0.73	0.46	1.15				
Acetylornithine	0.2	0.3	0.94	0.86	1.03				
Kynurenate	0.2	0.4	1.06	0.97	1.16				
Tyrosine	0.2	0.4	1.06	0.96	1.17				
Homocitrulline	0.2	0.4	1.05	0.97	1.15				
Phenylacetylglutamine	0.2	0.4	0.95	0.87	1.04				
Trigonelline	0.2	0.4	1.06	0.96	1.16				
NMMA	0.3	0.4	1.05	0.97	1.14				
Piperine	0.3	0.4	1.06	0.96	1.16				
Methylnicotinamide	0.3	0.5	0.95	0.86	1.04				
Pipecolate	0.3	0.5	1.07	0.95	1.21				
Proline betaine	0.3	0.5	0.95	0.87	1.04				
N.Methylproline	0.3	0.5	0.95	0.87	1.05				
Acylcarnitine C4:0	0.3	0.5	1.05	0.95	1.15				
Ornithine	0.4	0.6	1.04	0.96	1.13				
Tiglylcarnitine	0.4	0.6	1.04	0.95	1.14				
Asymmetric dimethylarginine	0.4	0.6	1.04	0.96	1.13				
Asparagine	0.4	0.6	0.96	0.89	1.05				

Pyroglutamate	0.4	0.6	0.96	0.89	1.05				
Urocanate	0.4	0.6	0.96	0.87	1.06				
Acylcarnitine C3:0	0.4	0.6	0.97	0.88	1.05				
Threonine	0.5	0.7	0.97	0.88	1.06				
3-Hydroxytrimethyllysine	0.5	0.7	1.03	0.95	1.13				
Nicotinamide	0.5	0.7	1.03	0.94	1.13				
Valine	0.5	0.7	1.03	0.95	1.11				
Tryptophan	0.5	0.7	1.03	0.95	1.11				
Homoarginine	0.5	0.7	0.97	0.89	1.06				
N-Methyl-4-pyridone-3-carboxamide	0.5	0.7	1.03	0.93	1.15				
Phenylalanine	0.6	0.7	1.02	0.94	1.11				
Creatinine	0.6	0.7	0.97	0.89	1.07				
Carnitine	0.6	0.7	0.96	0.84	1.10				
Histidine	0.6	0.7	0.98	0.90	1.06				
Arginine	0.6	0.7	0.98	0.90	1.06				
3-Methylhistidine	0.6	0.8	1.02	0.94	1.12				
Citrulline	0.6	0.8	0.98	0.90	1.07				
Methionine	0.7	0.8	0.98	0.91	1.07				
Homostachydrine	0.7	0.8	1.02	0.93	1.13				
N-Methyl-2-pyridone-5-carboxamide	0.8	0.9	1.02	0.91	1.14				
Alanine	0.8	0.9	1.01	0.93	1.10				
Dimethylglycine	0.8	0.9	1.01	0.92	1.11				
Hippurate	0.8	0.9	0.99	0.91	1.08				
Acylcarnitine C18:0	0.8	0.9	1.01	0.92	1.10				
Symmetric dimethylarginine	0.8	0.9	1.01	0.92	1.11				
Acylcarnitine C10:1	0.9	0.9	1.01	0.92	1.10				
Betaine	0.9	0.9	1.01	0.92	1.10				
Serine	0.9	0.9	1.01	0.93	1.09				
Acylcarnitine C9:0	0.9	0.9	0.99	0.91	1.08				
Proline	0.9	0.9	1.01	0.92	1.10				
Pantothenate	0.9	1.0	1.01	0.91	1.12				
Lysine	0.9	1.0	1.00	0.92	1.08				
Cotinine	0.9	1.0	1.01	0.87	1.16				
Acetylarginine	0.9	1.0	1.00	0.93	1.09				
Urea	0.9	1.0	1.00	0.92	1.09				
Acylcarnitine C11:0	1.0	1.0	1.00	0.92	1.08				
Acylcarnitine C4:0-OH	1.0	1.0	1.00	0.90	1.11				
Sarcosine	1.0	1.0	1.00	0.91	1.10				

Atrial Fibrillation incidence, n = 650

Model 1: Adjusted for sex and age

Model 2: adjusted for sex, age, body mass index (BMI), baseline smoking status, systolic blood pressure, alcohol intake, usage of anti-hypertensive medicine, N-terminal pro b-type natriuretic peptide, prevalent diabetes mellitus, prevalent heart failure and prevalent ischemic heart disease

HR = Hazard Ratio

95% lowint = 95% confidence interval, lower

95% highint = 95% confidence interval, higher

Table S3. Cox proportional hazard models further adjusted.

Metabolites	p	HR	95% lowint	95% highint
Caffeine	0.0014	1.16	1.06	1.28
Acylcarnitine.16:1	0.0021	1.14	1.05	1.24
Acylcarnitine.14:0	0.0018	1.14	1.05	1.24
Acylcarnitine.2:0	0.03	1.10	1.01	1.19
Acisoga	0.07	1.08	0.99	1.17
Beta.carotene	0.02	0.90	0.82	0.98
Acylcarnitine.16:0	0.04	1.09	1.00	1.18
Acylcarnitine.14:1	0.004	1.13	1.04	1.23
Acylcarnitine.8:1	0.06	1.08	1.00	1.18
Ergothioneine	0.05	1.10	1.00	1.20
Acylcarnitine.12:0	0.01	1.12	1.03	1.21
Acylcarnitine.18:1	0.02	1.11	1.02	1.20
N2,N2-Dimethylguanosine	0.54	1.03	0.94	1.12
Acylcarnitine.8:0:OH	0.02	1.11	1.02	1.22
Acylcarnitine.12:1	0.05	1.09	1.00	1.18

Cox proportional hazard models adjusted for sex, age, body mass index (BMI), baseline smoking status, systolic blood pressure, alcohol intake, usage of anti-hypertensive medicine, N-terminal pro b-type natriuretic peptide, prevalent diabetes mellitus, prevalent heart failure, prevalent ischemic heart disease, prevalent cancer, prevalent chronic obstructive pulmonary disease and estimated GFR.

HR = Hazard ratio

95 % lowint = 95% confidence interval, lower

95% highint = 95% confidence interval, higher

Table S4. Cox proportional hazard models for cardioembolic stroke.

Metabolites	p	p_fdr	HR	95% lowint	95% highint	P model 2	HR model 2	95% lowint model 2	95% highint model 2
Caffeine	0.03	0.47	1.30	1.02	1.65	0.05	1.29	1.00	1.67
Beta carotene	0.20	0.47	0.85	0.67	1.08	0.15	0.82	0.62	1.07
Acisoga	0.20	0.47	1.15	0.93	1.42	0.91	1.01	0.80	1.28
Acylcarnitine 8:0:OH	0.20	0.47	1.17	0.92	1.48	0.16	1.19	0.93	1.53
Acylcarnitine 16:1	0.21	0.47	1.15	0.92	1.45	0.27	1.15	0.90	1.47
Acylcarnitine 14:1	0.23	0.47	1.15	0.91	1.44	0.26	1.15	0.90	1.47
Acylcarnitine 12:0	0.30	0.47	1.12	0.90	1.40	0.26	1.15	0.90	1.45
N2,N2-Dimethylguanosine	0.33	0.47	1.11	0.90	1.38	0.73	1.04	0.82	1.32
Acylcarnitine 18:1	0.33	0.47	1.12	0.89	1.39	0.22	1.16	0.91	1.47
Acylcarnitine 2:0	0.33	0.47	1.12	0.89	1.40	0.53	1.08	0.84	1.39
Acylcarnitine 12:1	0.34	0.47	1.12	0.89	1.40	0.43	1.10	0.86	1.41
Acylcarnitine 14:0	0.42	0.50	1.10	0.87	1.38	0.52	1.08	0.85	1.39
Acylcarnitine 8:1	0.43	0.50	1.09	0.87	1.37	0.51	1.08	0.85	1.39
Acylcarnitine 16:0	0.66	0.70	1.05	0.84	1.31	0.90	1.02	0.79	1.31
Ergothioneine	0.82	0.82	1.03	0.80	1.34	0.65	0.93	0.70	1.25

Cardioembolic stroke incidence, n = 83

Model 1: Adjusted for sex and age

Model 2: adjusted for sex, age, body mass index (BMI), baseline smoking status, systolic blood pressure, alcohol intake, usage of anti-hypertensive medicine, LDL cholesterol, HDL cholesterol, prevalent diabetes mellitus, prevalent hear failure and prevalent ischemic heart disease.

HR = Hazard ratio

95% lowint = 95% confidence interval, lower

95% highint = 95% confidence interval, higher