Supplemental Materials

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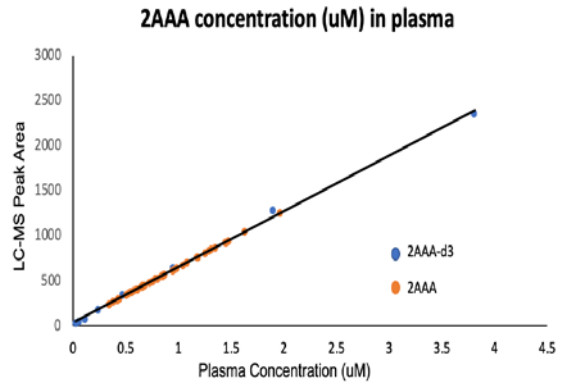
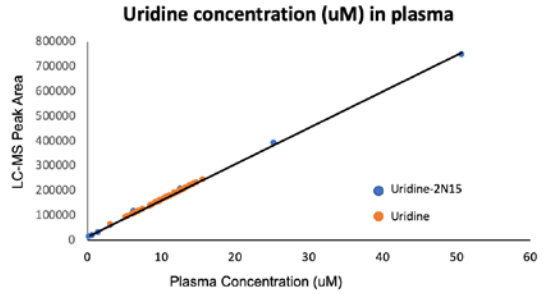
Supplemental Methods
Methods for Absolute Quantification of Representative Metabolites

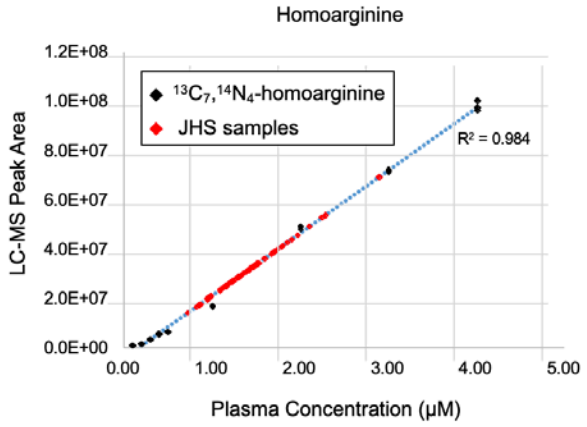
Of the significant metabolites highlighted in the manuscript, we selected four for which we were able to obtain stable isotope labeled reference material and created calibration curves by serially diluting each in pooled reference plasma. Calibration curves were fitted using linear regression and we re-analyzed a subset of 50 JHS plasma samples that were representative the peak area range for each metabolite measured in the full dataset. The squared regression coefficient (R^2) value for each stable isotope labeled reference standard indicates a high correlation between concentration and peak area in the plasma matrix ($^{13}C_7$,$^{14}N_4$-homoarginine 0.984; Choline-d_4 0.981; 3-Methylhistidine-d_3 0.996; ; 2AA-d3 0.998; Uridine 15N2 0.999). These analyses also showed that that range of peak areas measured in the JHS samples fell within the linear response range of the LC-MS analysis. Last, the re-analysis of the 50 representative JHS samples demonstrated excellent reproducibility in comparison to the original dataset. The Pearson correlation coefficients between the re-analyzed and original sample data were: homoarginine 0.912, choline 0.914, 3-methylhistidine 0.866, , 2AA-d3 0.968, Uridine 15N2 0.999.



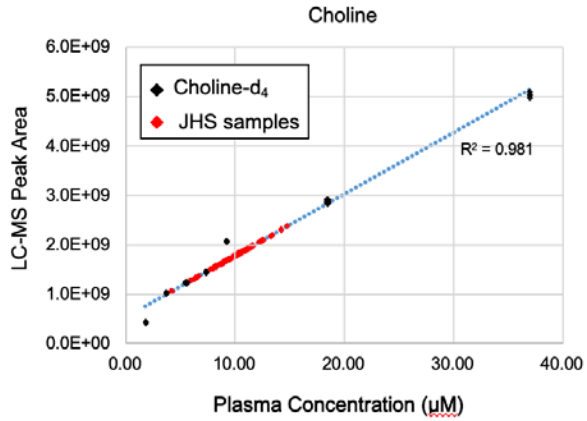
HMDB Info:	
Biospecimen	Value
Blood	0.3-3.4 uM
JHS plasma	***0.84 +/- 0.37 uM***



HMDB Info	
Biospecimen	Value
Blood	6.15 +/- 5.33
JHS plasma	***10.6 +/- 2.8***



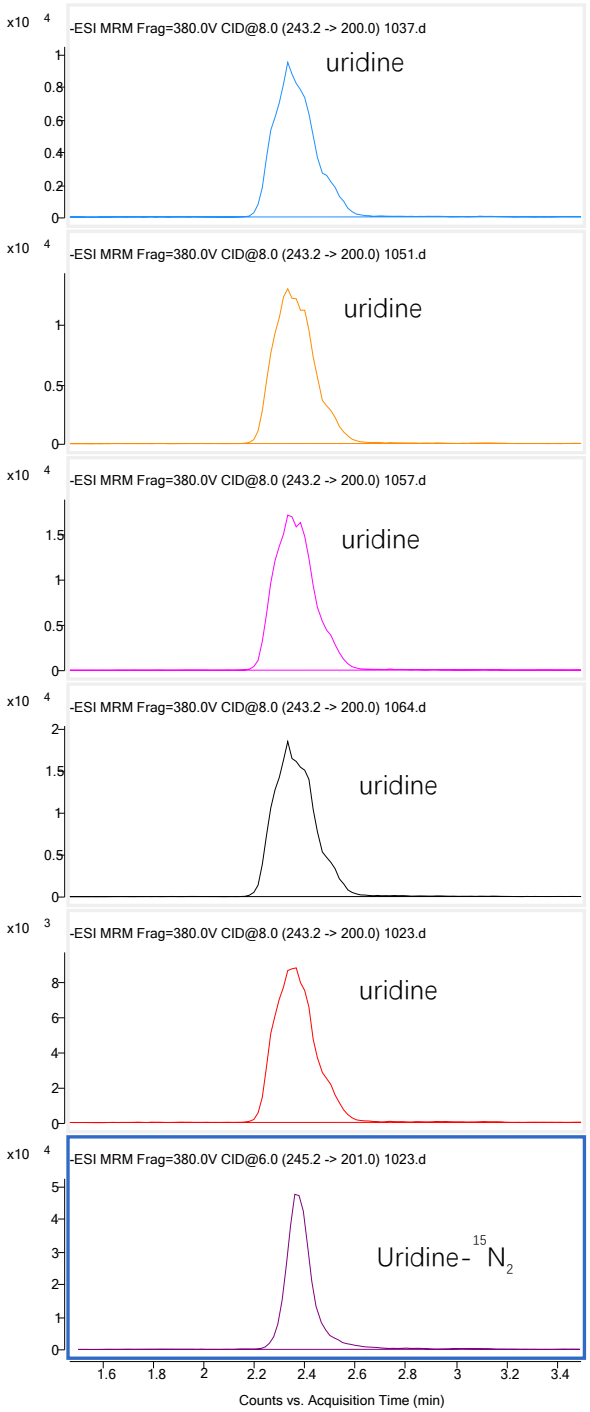
HMDB Info	
Biospecimen	Value
Blood	1.12 +/- 0.60
JHS plasma	***1.41 +/- 0.41***



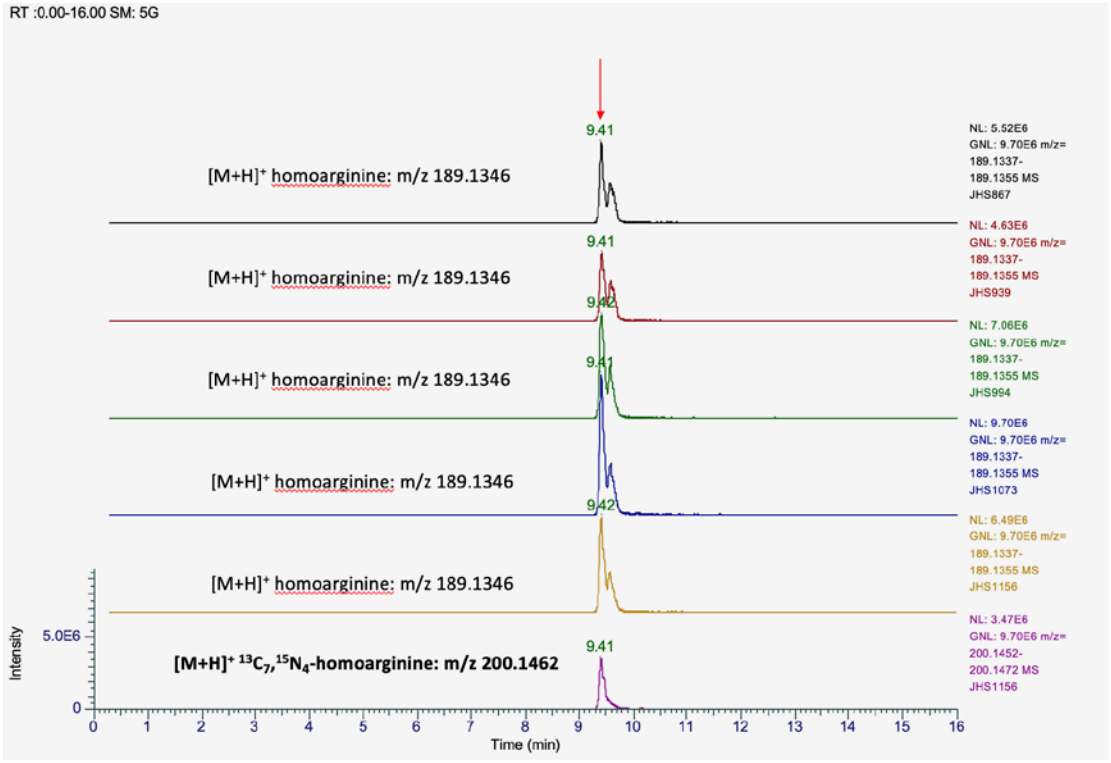
HMDB Info:	
Biospecimen	Value
Blood	10.6 +/- 1.9 uM
JHS plasma	***9.51 +/- 2.22 uM***

Spectral Peaks for Highlighted Metabolites: Random sample spectral peaks overlaying stable isotope peak (bottom)

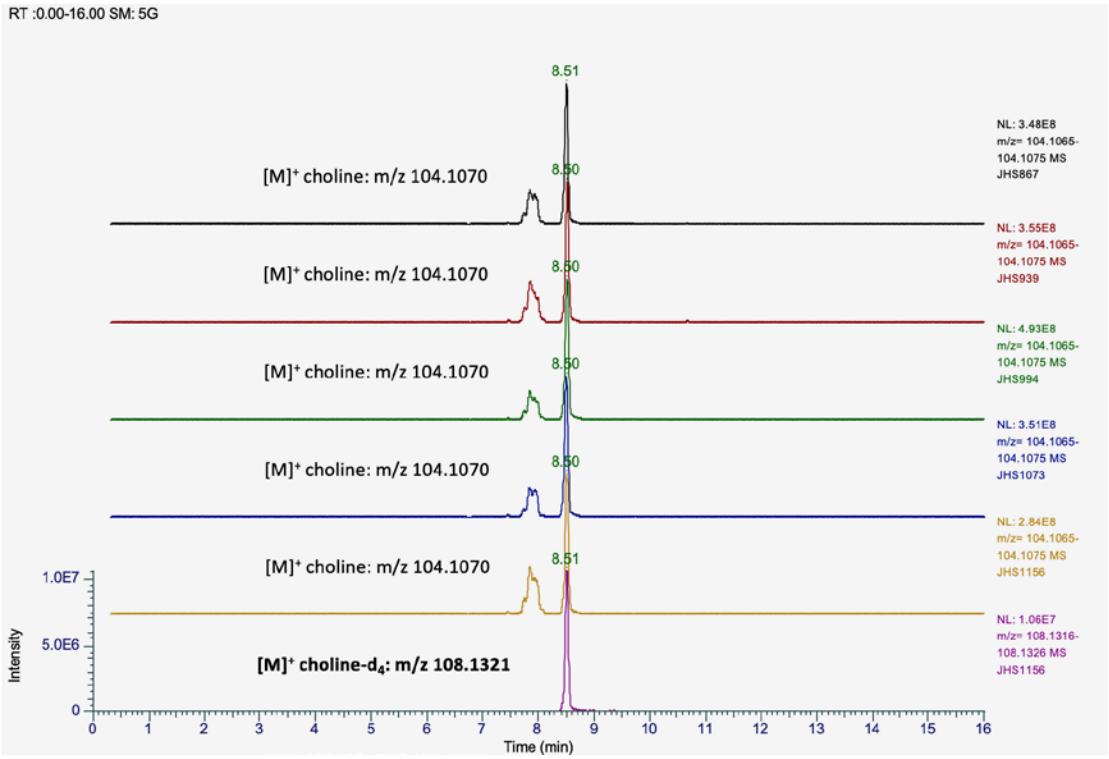
1. **Uridine**



2. Homoarginine



3. Choline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8.51
[M]+ choline: m/z 104.1070
8.50
NL: 3.48E8
m/z= 104.1065-104.1075 MS JHS867
[M]+ choline: m/z 104.1070
8.50
NL: 3.55E8
m/z= 104.1065-104.1075 MS JHS939
[M]+ choline: m/z 104.1070
8.50
NL: 4.93E8
m/z= 104.1065-104.1075 MS JHS994
[M]+ choline: m/z 104.1070
8.50
NL: 3.51E8
m/z= 104.1065-104.1075 MS JHS1073
[M]+ choline: m/z 104.1070
8.51
NL: 2.84E8
m/z= 104.1065-104.1075 MS JHS1156
[M]+ choline-d4: m/z 108.1321
NL: 1.06E7
m/z= 108.1316-108.1326 MS JHS1156
Intensity
1.0E7
5.0E6
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (min)

4. 2-Aminoadipic acid

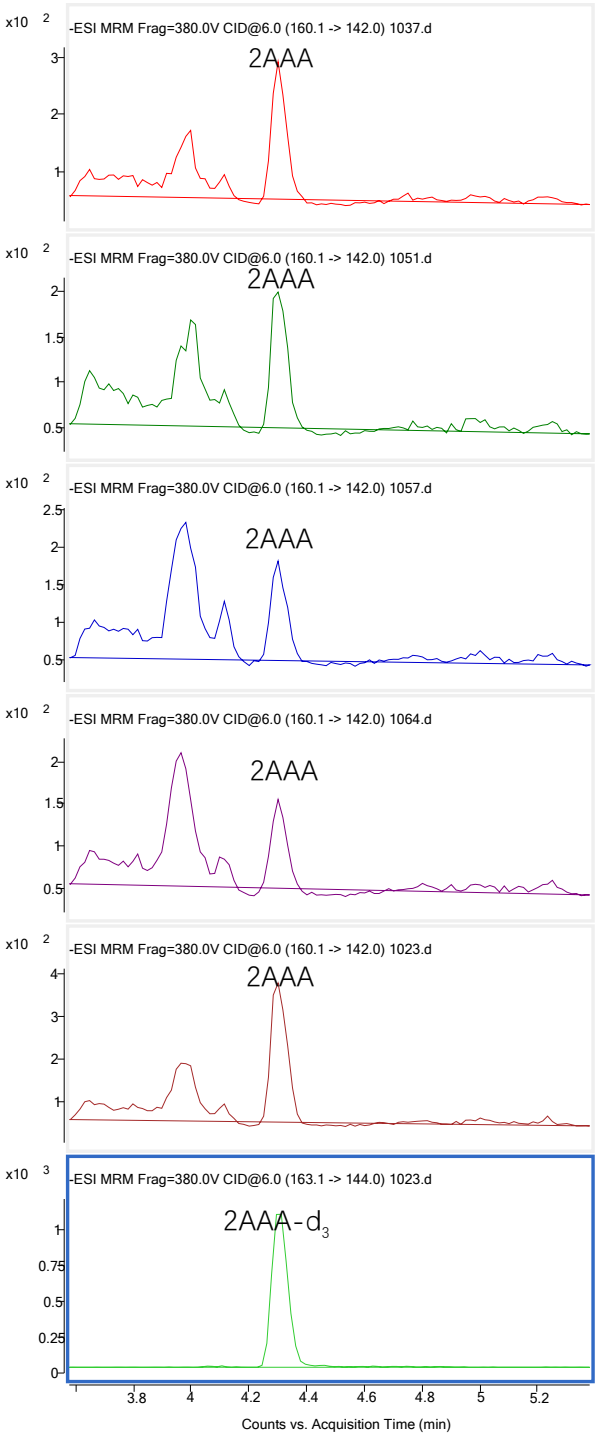

Table I: Clinical characteristics between the entire Jackson Heart Study, available and profiled samples.

Clinical Trait	Full Cohort (N=5306)	Available Samples (N= 3221)	Samples Profiled (N=2750)
Age, mean (SD)	55.4 (12.8)	55.6 (12.8)	56.4 (12.7)
BMI, mean (SD)	31.8 (7.2)	32 (7.3)	31.7 (7.1)
eGFR, mean (SD)	85.7 (18.5)	85.5 (18.8)	84.8 (19)
TC, mean (SD)	199 (40.1)	199.4 (40.9)	200 (41)
HDL, mean (SD)	51.8 (14.6)	51.6 (14.7)	51.8 (14.7)
sbp, mean (SD)	127 (18.4)	127.5 (16.6)	127 (18.2)
Male, n (%)	1934 (36.5)	1224 (38)	1046 (38)
Current Smoker, n (%)	693 (13.1)	419 (13)	335 (12.2)
HTN, n (%)	3252 (61.3)	1836 (57)	1746 (63.5)
Diabetes, n (%)	1152 (21.7)	741 (23)	659 (24)

BMI: body mass index; eGFR: estimated glomerular filtration rate; TC: total cholesterol, HDL: high density lipid lipoprotein; sbp: systolic blood pressure; HTN: hypertension

Table II: Age, sex and batch adjusted analysis for metabolites and incident HF

Metabolites	Hazard ratio	P value	FDR
pseudouridine	1.68 (1.51-1.87)	5.69E-22	1.75E-19
4-acetamidobutanoate	1.55 (1.4-1.7)	1.01E-18	1.55E-16
D.Gluconic.acid	1.72 (1.51-1.96)	7.93E-16	8.14E-14
2.deoxyuridine	1.69 (1.48-1.92)	1.13E-15	8.67E-14
N2.N2.dimethylguanosine	1.49 (1.34-1.65)	1.39E-13	7.12E-12
DMGV	1.9 (1.6-2.25)	1.36E-13	8.38E-12
creatinine	1.57 (1.38-1.78)	3.03E-12	1.33E-10
1-methyladenosine	1.57 (1.37-1.81)	2.22E-10	8.55E-09
Uridine	0.719 (0.648-0.798)	4.38E-10	1.50E-08
methylhistidine	1.5 (1.31-1.72)	4.23E-09	1.30E-07
N.carbamoyl.beta.alanine	1.31 (1.19-1.43)	8.24E-09	2.12E-07
choline	1.53 (1.32-1.77)	8.11E-09	2.27E-07
N6.acetyllysine	1.42 (1.26-1.61)	1.01E-08	2.40E-07
N4.acetylcytidine	1.47 (1.29-1.68)	1.71E-08	3.75E-07
C4.carnitine	1.43 (1.25-1.62)	5.06E-08	1.04E-06
1-5-AG...1.deoxyglucose	0.739 (0.661-0.826)	9.73E-08	1.87E-06
allantoin	1.52 (1.3-1.78)	1.84E-07	3.34E-06
diacetylspermine	1.45 (1.25-1.69)	9.04E-07	1.46E-05
N1.acetylspermidine	1.4 (1.23-1.61)	8.56E-07	1.47E-05
C3.DC.CH3.carnitine	1.42 (1.24-1.64)	1.10E-06	1.70E-05
N.acetylalanine	1.42 (1.23-1.64)	1.99E-06	2.67E-05
trigonelline	1.44 (1.24-1.67)	1.94E-06	2.72E-05
Quinolinic.acid	1.37 (1.2-1.55)	1.94E-06	2.84E-05
acetylglutamic.acid	1.34 (1.18-1.51)	3.68E-06	4.54E-05
trimethylamine.N.oxide	1.36 (1.19-1.54)	3.55E-06	4.55E-05
N6.N6.dimethyllysine	1.32 (1.17-1.49)	4.10E-06	4.86E-05
S.Adenosyl.L.homocysteine	1.35 (1.19-1.54)	6.06E-06	6.67E-05
N.acetylputrescine	1.38 (1.2-1.59)	5.89E-06	6.72E-05
1-methylnicotinamide	0.733 (0.638-0.842)	1.08E-05	0.000114
phenylacetylglutamine	1.44 (1.22-1.7)	1.28E-05	0.000132
cystine	1.5 (1.25-1.82)	1.99E-05	0.000198
N6.N6.N6.trimethyllysine	1.34 (1.17-1.54)	2.15E-05	0.000207
biliverdin	0.751 (0.655-0.861)	3.98E-05	0.000361
homoarginine	0.748 (0.651-0.859)	3.89E-05	0.000363
ectoine	1.34 (1.16-1.54)	5.06E-05	0.000445
C7.carnitine	1.33 (1.16-1.52)	5.28E-05	0.000452
hydroxyproline	1.3 (1.15-1.49)	5.59E-05	0.000466

acisoga	1.35 (1.16-1.56)	6.57E-05	0.000532
C4.OH.carnitine	1.32 (1.15-1.51)	7.40E-05	0.000585
ADMA	1.31 (1.15-1.5)	7.65E-05	0.000589
imidazole.propionate	1.3 (1.14-1.49)	9.21E-05	0.000692
4-aminohippuric.acid	1.29 (1.13-1.47)	0.000131	0.00096
3-hydroxyhippurate	1.3 (1.13-1.48)	0.000157	0.00112
1-methylguanine	1.3 (1.13-1.49)	0.000185	0.0013
kynurenic.acid	1.31 (1.14-1.51)	0.000194	0.0013
SDMA	1.3 (1.13-1.49)	0.000192	0.00132
N.acetylserine	1.31 (1.13-1.5)	0.000203	0.00133
N.acetyltryptophan	1.32 (1.14-1.53)	0.000284	0.00182
C5.carnitine	1.28 (1.12-1.47)	0.000396	0.00249
C5.1.carnitine	1.3 (1.12-1.5)	0.000569	0.00351
C3.carnitine	1.29 (1.12-1.49)	0.000591	0.00357
C6.carnitine	1.25 (1.1-1.42)	0.000675	0.00385
N.acetylaspartic.acid	1.27 (1.11-1.46)	0.000657	0.00389
hydroxycotinine_impute	1.24 (1.1-1.41)	0.00067	0.00389
1-methylhistamine	1.26 (1.1-1.44)	0.000827	0.00455
N.Acetyl.L.Glutamic.acid	1.28 (1.11-1.48)	0.00082	0.00459
tryptophan	0.79 (0.688-0.908)	0.00085	0.00459
2-Hydroxyglutaric.acid	1.26 (1.1-1.45)	0.00096	0.0051
urate	1.28 (1.11-1.49)	0.00111	0.00578
Hydroxyphenylpyruvic.acid	1.34 (1.12-1.6)	0.00146	0.00748
Indole.3.lactic.acid	1.26 (1.09-1.45)	0.00157	0.00791
C10.2.carnitine	1.27 (1.09-1.48)	0.00176	0.00873
7-methylguanine	1.25 (1.08-1.44)	0.00202	0.00989
C5.DC.carnitine	1.24 (1.08-1.42)	0.00219	0.0105
Oleoyl.Leucine	0.784 (0.67-0.918)	0.00242	0.0113
C38.4.PE	1.25 (1.08-1.45)	0.00239	0.0113
1-methylguanosine	1.24 (1.08-1.42)	0.0025	0.0115
Aconitic.acid	1.32 (1.1-1.58)	0.00284	0.0129
cotinine	1.21 (1.07-1.38)	0.00348	0.0155
Orotic.acid	1.18 (1.05-1.32)	0.00536	0.0236
C36.4.PE	1.23 (1.06-1.42)	0.00591	0.0257
dimethylglycine	1.17 (1.05-1.31)	0.00614	0.0263
Oxaloacetic.acid	1.2 (1.05-1.37)	0.00629	0.0266
trimethylbenzene	0.822 (0.714-0.946)	0.00645	0.0269
histidine	0.831 (0.724-0.953)	0.00825	0.0339
threonine	0.826 (0.715-0.953)	0.00885	0.0359
lysine	0.84 (0.737-0.958)	0.00932	0.0373

hippurate	1.2 (1.05-1.38)	0.00953	0.0376
methionine	0.832 (0.723-0.959)	0.0108	0.0422
C34.2.PE	1.21 (1.04-1.39)	0.0111	0.0427
C36.2.PE	1.2 (1.04-1.39)	0.0125	0.0475

Table III: Coefficient of variation for metabolites associated with incident heart failure in multivariate model

Metabolite	CV %*
N1-acetylspermidine	2.1
choline	2.1
methylhistidine	2.9
4-acetamidobutanoate	3.1
pseudouridine	3.5
DMGV	3.6
uridine	4.1
ectoine	4.3
homoarginine	5.1
2-deoxyuridine	8.7
N2-N2-dimethylguanosine	9.0
diacetylspermine	15.4
2-aminoadipic acid	16.3
N-acetyl-l-alanine	18.4

*Averaged across batches
CV: Coefficient of Variation

Table IV: Baseline Characteristics for Cases and Controls of Incident Heart Failure in the Framingham Heart Study

	Cases (n=216)	Controls (n=2157)	Cohort (n=2373)
Age, y	63 (9)	54 (10)	55(10)
Female sex n (%)	93 (43)	1164 (54)	1257 (53)
BMI	29.8 (5.5)	27.3 (4.8)	27.5 (4.9)
SBP	137 (21)	125 (18)	126 (19)
Hypertension (%)	137 (63)	686 (32)	823 (35)
Total cholesterol (mg/dl)	207 (41)	206 (36)	206 (37)
HDL (mg/dl)	45 (14)	50 (15)	50 (15)
Smoker n (%)	36 (17)	397 (18)	433 (18)
CHD History n (%)	12 (6)	21 (1)	33 (1)
Diabetes n (%)	43 (20)	107 (5)	150 (6)
eGFR (mL/min/1.73 m^2)	81.2 (19.9)	89.5 (19.0)	88.8 (19.2)

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; HDL: high density lipoprotein; eGFR: estimated glomerular filtration rate; CHD: coronary heart disease; HTN: hypertension

Table V: Association of metabolites with echocardiographic variables in multi-variate model

Metabolites		Beta-coefficient	P value
Pseudouridine	IVS	0.097 (0.066-0.128)	**0.00175**
	LVEDD	0.0177 (-0.014-0.0495)	0.577
	PWT	0.122 (0.0914-0.153)	**7.49E-05**
	LAESD	0.0566 (0.0256-0.0876)	**0.0681**
	MV E/A	0.00749 (-0.0214-0.0364)	0.796
	RWT	0.141 (0.101-0.181)	**0.000447**
2.Deoxyuridine	IVS	0.0513 (0.0254-0.0772)	**0.0476**
	LVEDD	0.0212 (-0.00531-0.0477)	0.424
	PWT	0.0723 (0.0465-0.098)	**0.00506**
	LAESD	0.0145 (-0.0114-0.0404)	0.576
	MV E/A	0.0277 (0.00351-0.0518)	0.252
	RWT	0.0842 (0.051-0.117)	**0.0113**
4.Acetamidobutanoate	IVS	0.0579 (0.0275-0.0884)	0.0572
	LVEDD	-0.0292 (-0.0604-0.00196)	0.349
	PWT	0.0522 (0.0219-0.0825)	0.0852
	LAESD	0.0209 (-0.00959-0.0514)	0.493
	MV E/A	-0.00272 (-0.031-0.0256)	0.923
	RWT	0.115 (0.0747-0.156)	**0.00449**
Methylhistidine	IVS	-0.00246 (-0.0274-0.0225)	0.922
	LVEDD	0.0559 (0.0304-0.0814)	**0.0286**
	PWT	-0.0101 (-0.0349-0.0147)	0.685
	LAESD	0.0418 (0.0169-0.0668)	0.0939
	MV E/A	0.0092 (-0.0141-0.0325)	0.692
	RWT	0.0258 (-0.00642-0.0581)	0.423
N1.Acetylspermidine	IVS	0.0355 (0.0134-0.0577)	0.109
	LVEDD	-0.0161 (-0.0388-0.00662)	0.479
	PWT	0.0305 (0.00839-0.0525)	0.168
	LAESD	0.0681(0.0459-0.0902)	**0.00215**
	MV E/A	0.0219 (0.00131-0.0425)	0.288
	RWT	0.0438 (0.0153-0.0722)	0.124
DMGV	IVS	0.0486 (0.0248-0.0724)	**0.0412**
	LVEDD	-0.000574 (-0.0249-0.0238)	0.981
	PWT	0.0611 (0.0374-0.0848)	**0.0099**
	LAESD	0.0193 (-0.00459-0.0431)	0.42
	MV E/A	-0.000787 (-0.0228-0.0212)	0.971
	RWT	0.0639 (0.0342-0.0937)	**0.032**

Diacetylspermine	IVS	0.000786 (-0.0194-0.0209)	0.969
	LVEDD	-0.0332 (-0.0538--0.0126)	0.107
	PWT	0.0202 (0.000141-0.0403)	0.314
	LAESD	0.0105 (-0.00966-0.0307)	0.602
	MV E/A	0.0147 (-0.00369-0.0332)	0.424
	RWT	0.0436 (0.0175-0.0698)	0.0959
Uridine	IVS	0.000828 (-0.0221-0.0237)	0.971
	LVEDD	-0.0486 (-0.0721--0.0251)	**0.0385**
	PWT	-0.0016 (-0.0244-0.0212)	0.944
	LAESD	-0.0155 (-0.0384-0.0074)	0.499
	MV E/A	-0.0151 (-0.0363-0.00611)	0.477
	RWT	0.047 (0.0156-0.0785)	0.135
Choline	IVS	0.0429 (0.02-0.0659)	0.0615
	LVEDD	0.0154 (-0.00809-0.0389)	0.512
	PWT	0.052 (0.0292-0.0749)	0.0228
	LAESD	0.0223 (-0.000651-0.0453)	0.331
	MV E/A	0.0636 (0.0425-0.0848)	**0.00269**
	RWT	0.0718 (0.0428-0.101)	**0.0135**
N.Acetyl.L.Alanine	IVS	0.0587 (0.0288-0.0887)	**0.0499**
	LVEDD	-0.0109 (-0.0409-0.0191)	0.717
	PWT	0.108 (0.0781-0.138)	**0.000314**
	LAESD	0.000639 (-0.0288-0.0301)	0.983
	MV E/A	0.00528 (-0.0225-0.033)	0.849
	RWT	0.0857 (0.0469-0.124)	**0.0274**
N2.N2.dimethylguanosine	IVS	0.0666 (0.0409-0.0924)	**0.00967**
	LVEDD	-0.0203 (-0.0467-0.00607)	0.441
	PWT	0.089 (0.0634-0.115)	**0.000517**
	LAESD	0.0383 (0.0125-0.0642)	0.138
	MV E/A	0.0295 (0.00554-0.0534)	0.218
	RWT	0.0901 (0.0567-0.124)	**0.00712**
Ectoine	IVS	-0.0218 (-0.0432--0.000523)	0.306
	LVEDD	0.0251 (0.00325-0.0469)	0.251
	PWT	-0.0292 (-0.0504--0.00798)	0.169
	LAESD	0.0504 (0.0291-0.0718)	**0.0183**
	MV E/A	-0.00494 (-0.0248-0.0149)	0.804
	RWT	0.0185 (-0.00932-0.0463)	0.506
2.Aminoadipic.acid	IVS	0.0404 (0.0194-0.0614)	0.0548
	LVEDD	-0.00413 (-0.0257-0.0174)	0.848

	PWT	0.0482 (0.0273-0.0692)	0.0212
	LAESD	-0.00596 (-0.027-0.0151)	0.777
	MV E/A	-0.0428 (-0.0622--0.0234)	**0.0273**
	RWT	0.0422 (0.0161-0.0683)	0.106
Homoarginine	IVS	0.023 (0.00137-0.0447)	0.288
	LVEDD	0.0144 (-0.00778-0.0366)	0.516
	PWT	0.0388 (0.0172-0.0604)	0.072
	LAESD	-0.0284 (-0.0501--0.00673)	0.19
	MV E/A	-0.000364 (-0.0205-0.0197)	0.986
	RWT	0.0589 (0.0321-0.0858)	**0.0283**

IVS: intraventricular septum; LVEDD: left ventricular end diastolic diameter (mm); PWT: posterior wall thickness (mm); LAESD: left atrial end systolic diameter (mm); RWT: relative wall thickness (mm)

Table VI: Competing risk model for metabolites associated with incident heart failure, for incident HFpEF vs. HFrEF

	HFrEF		HFpEF		Difference
Metabolites	**Hazard ratio**	**P value**	**Hazard ratio**	**P value**	**P value**
Pseudouridine	1.82 (1.53-2.16)	0.00046	1.35 (1.16-1.57)	0.049	0.311
2.Deoxyuridine	1.53 (1.31-1.79)	0.0057	1.43 (1.26-1.63)	0.0063	0.793
4.Acetamidobutanoate	1.45 (1.25-1.67)	0.0095	1.22 (1.06-1.41)	0.16	0.511
Methylhistidine	1.29 (1.11-1.51)	0.1	1.26 (1.12-1.42)	0.046	0.952
N1.Acetylspermidine	1.32 (1.17-1.49)	0.023	1.24 (1.13-1.37)	0.027	0.75
DMGV	1.52 (1.28-1.8)	0.014	1.21 (1.05-1.4)	0.17	0.287
Diacetylspermine	1.32 (1.17-1.48)	0.016	1.29 (1.14-1.45)	0.042	0.903
Uridine	0.848 (0.76-0.947)	0.13	0.838 (0.757-0.927)	0.081	0.983
Choline	1.19 (1.04-1.36)	0.19	1.33 (1.15-1.53)	0.042	0.59
N.Acetyl.L.Alanine	1.58 (1.4-1.79)	0.00016	1.08 (0.879-1.33)	0.71	0.156
N2.N2.dimethylguanosine	1.32 (1.17-1.49)	0.025	1.17 (1.01-1.36)	0.3	0.619
Ectoine	1.11 (0.982-1.26)	0.39	1.33 (1.19-1.48)	0.01	0.366
2.Aminoadipic.acid	0.872 (0.767-0.991)	0.28	0.808 (0.698-0.937)	0.15	0.699
Homoarginine	0.747 (0.662-0.843)	0.016	0.845 (0.759-0.941)	0.12	0.481

Table VII: Interaction analysis between metabolites, diabetic status and incident heart failure

Metabolite	Interaction HR*	Low 95% CI	High 95% CI	Interaction P value*
DMGV	**1.48**	**1.04**	**2.12**	**0.0302**
Choline	1.28	0.936	1.75	0.122
2.Deoxyuridine	1.21	0.919	1.58	0.176
N1.Acetylspermidine	0.853	0.651	1.12	0.248
N2.N2.dimethylguanosine	1.15	0.899	1.47	0.266
Diacetylspermine	0.864	0.627	1.19	0.372
Pseudouridine	1.11	0.88	1.4	0.378
Uridine	0.899	0.705	1.15	0.394
N.acetylalanine	0.888	0.675	1.17	0.399
Homoarginine	0.888	0.66	1.2	0.434
Ectoine	1.12	0.825	1.52	0.467
4.Acetamidobutanoate	1.08	0.868	1.35	0.479
2.Aminoadipic.acid	0.917	0.675	1.25	0.58
Methylhistidine	1.04	0.779	1.39	0.79

*Hazard ratio and p value is of the interaction term between metabolite and diabetes status

Table VIII: Metabolites Associated with Incident Heart Failure and Left Ventricular Mass Index

Metabolites	β-coefficient	P value
pseudouridine	0.0954 (0.0706-0.12)	0.0001
2-deoxyuridine	0.0743 (0.05080.0979)	0.0016
choline	0.0785 (0.0558-0.101)	0.0005
***N2*-*N2*-dimethylguanosine**	0.0724 (0.04920.0956)	0.0018
***N*-Acetyl-L-Alanine**	0.0701 (0.04550.0946)	0.0044
4-acetamidobutanoate	0.0799 (0.0546-0.105)	0.00164
uridine	-0.065 (-0.088--0.042)	0.0047
DMGV	0.046 (0.024-0.0681)	0.0366

Model adjusted for age, sex and batch

Figure I: Pearson correlation matrix between metabolites associated with incident heart failure

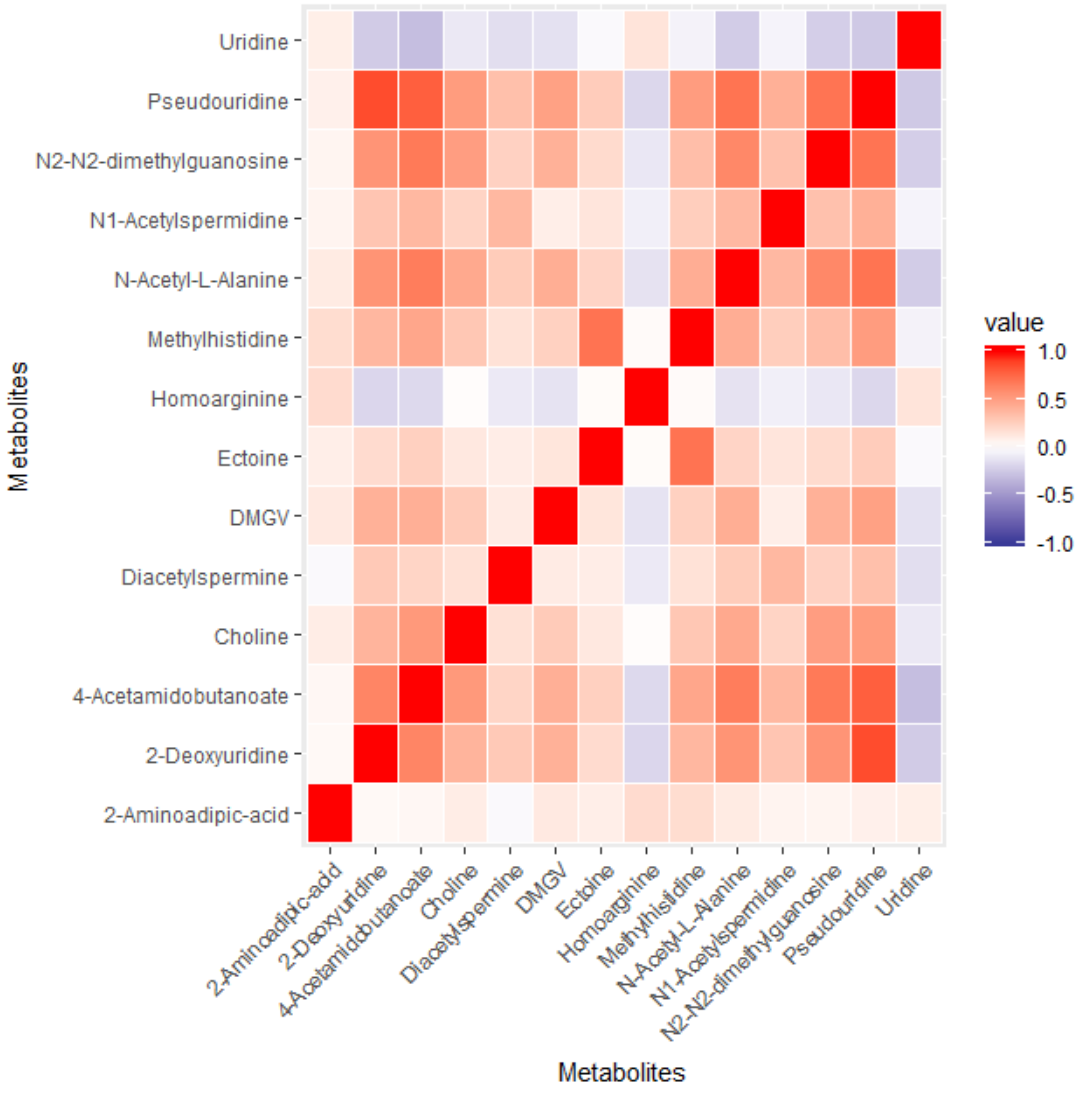

Figure II: Pearson Correlation matrix between metabolites associated with incident heart failure and clinical risk factors

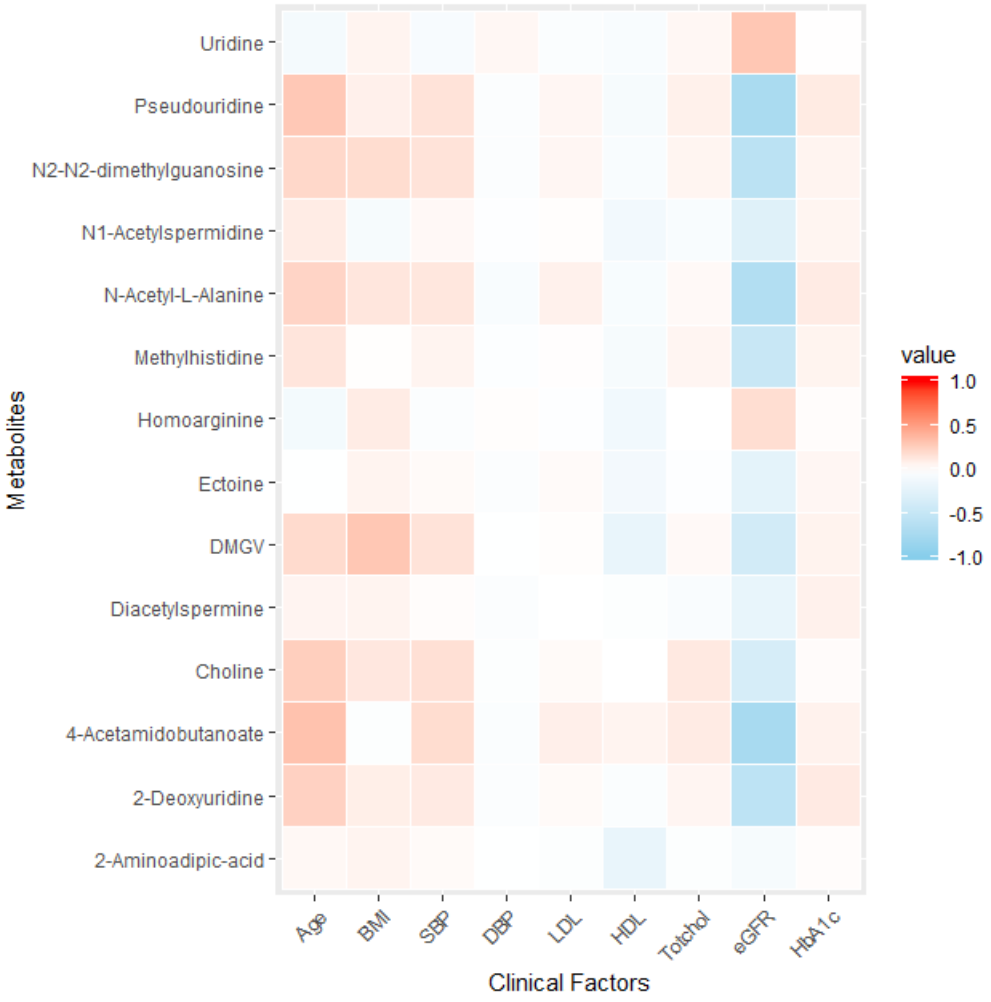

Figure III: Pathway analysis for age and sex adjusted significant metabolites associated with incident heart failure

The pathways enriched for incident heart failure (p value <0.05) are aminoacyl-tRNA synthesis, pyrimidine metabolism and lysine degradation.