Online Methods

SUPPLEMENTARY METHODS

The KORA S4 and MONICA/KORA S2 studies

Primary discovery took place in the Multinational Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) / Cooperative Health Research in the Region of Augsburg (KORA) surveys, [1 2] specifically the KORA S4 and MONICA/KORA S2 surveys.

The baseline KORA survey 4 (S4) consists of 4,261 individuals (aged 25-74 years) examined between 1999 and 2001.[3] Metabolite profiling was performed on 1,610 individuals (1,545 without prior MI) aged 55 to 74 years who provided serum samples at the baseline examination. For the discovery cohort 203 individuals were removed due to their non-fasting status. This left 1,342 participants for the study of which 67 had an incident MI with seven years of average follow-up time (follow up conducted through 2009).

MONICA/KORA S2 is a survey of 4,940 participants who were all enrolled from 1989-1990.[4] A case-cohort study was formed from them by taking all incident MI cases identified before 2003 (N = 112) and combining it with a sex stratified cohort of randomly selected participants free of prevalent MI at baseline (N = 549). Only incident cases that occurred before the age of 75 were included.

Two definitions of MI were used over the time period spanning the MONICA/KORA S2 and KORA S4 cohort. These MI definitions are complimentary and represent the best practices at the time of diagnosis. Until December 2000, the diagnosis of a major non-fatal MI event was based on the MONICA algorithm taking into account symptoms, cardiac enzymes and ECG changes. Since January 1, 2001, MIs are diagnosed by the European

Society of Cardiology and American College of Cardiology criteria. Deaths from MI were validated by autopsy reports, death certificates, chart reviews, and information from the last treating physician.[5]

In MONICA/KORA S2, hsCRP concentrations were measured using a high-sensitivity immunoradiometric assay (IRMA) (men aged 45–74 years) [6] or a high-sensitivity latex enhanced nephelometric assay on a BN II analyzer (men aged 35–44 years and all women) (Dade- Behring, Marburg, Germany). [7] Both methods gave similar results when the same samples were analyzed. [8] In KORA S4, hsCRP concentrations were assessed by a high sensitivity latex enhanced nephelometric assay on a BN II analyser (Dade Behring, Marburg, Germany).[7] The intra- and interassay coefficients of variation of quality control test sera for CRP were as follows: 1) CRP-IRMA, 4.0% and 12.0%; 2) CRP nephelometric assay, <5% and <10%, respectively.

The AGES and REFINE Reykjavik studies

For biomarker association refinement in an independent cohort we utilized the Age, Gene/Environment Susceptibility (AGES) Reykjavik and the Risk Evaluation For INfarct Estimates (REFINE) studies, two population-based cohort studies conducted in Iceland in the past decade.[9] The AGES Reykjavik (aged 66-96 years, mean age 75±6, 58% females) is a study of all survivors of the 40-year-long prospective Reykjavik study (n=30,000), an epidemiologic study focusing on four biologic systems: vascular, neurocognitive (including sensory), musculoskeletal, and body composition/metabolism.[10] The REFINE Reykjavik study (20-73, mean age 49±12, 56% females) was designed with the aim of increasing the predictability of risk factors for the development of CAD on an individual basis in relatively young people, using comprehensive evaluation of the arterial condition. AGES and REFINE

subjects have participated in follow-up studies (4-5 years from baseline). For the present study we selected controls that had no history of CHD including MI, PCI, CABG (from hospital records or self report) at entry, and incident MI cases that were diagnosed within 6 years after enetering the study. Further, we selected an age and sex matched case-control cohort of 37 incident MI cases and 74 healthy MI-free controls at mean age of 57 (range 36-72 years) from REFINE, and 50 incident MI cases and 100 MI-free controls at mean age of 71 (range 68-74 years) from the AGES study.

For both AGES and REFINE, hsCRP was measured on a Hitachi 912, using reagents from Roche Diagnostics (Mannheim, Germany). The coefficient of variation of the method was 1.3% for intra-assay and 3.4% for inter-assay through the period of data collection.

Metabolite measurements

For the KORA S4 and MONICA S2 surveys, 188 metabolites have been simultaneously quantified out of 10 µL serum using the Absolute*IDQ*^{TM} P180 kit (BIOCRATES Life Science AG, Innsbruck, Austria). Liquid handling of serum samples was performed with Hamilton star robot (Hamilton Bonaduz AG, Bonaduz, Switzerland). Samples were measured by LC-MS/MS and FIA-MS/MS (liquid chromatography-/flow injection analysis-tandem mass spectrometry). Details of the measurement methods and explanations for abbreviations were described in previous publications.[11-14] The complete analytical process was monitored by quality control steps, reference samples and the Met*IDQ*^{TM} software package, which is an integral part of the Absolute *IDQ*^{TM} kit.

For the AGES and REFINE Reykjavik studies metabolites were quantified using the Biocrates Absolute*IDQ*^{TM} P150 kit which assayed 163 metabolites.[15-17] The quality control procedure was the same as that used for the KORA S4 and S2 surveys.[11]

Statistical Methods

Cox regression models were used to model the association between metabolites and MI for KORA S4 and KORA S2. For the KORA S2 cohort a sex-stratified weighting was used to account for over-sampling of cases in the case-cohort design. This weighting follows Breslow's method,[5] and is also suggested to be proper for a stratified case-cohort design, which is used in the KORA S2 study. For the AGES and REFINE Reykjavik studies conditional logistic regression models were used to analyze the case-control samples.

The 140 metabolites from the KORA S4 cohort went through several filtering steps to arrive at a final list of potential biomarkers (Online Figure S2). The first step was to associate each metabolite with MI via Cox regression in KORA S4 to determine those metabolites showing nominal ($P < 0.05$) associations. Two Cox regression models were used to evaluate the independence of the metabolites from clinical factors associated with MI

1. Basic model adjusting for age and sex,
2. Multivariable model adjusting for body mass index (BMI), smoking status, alcohol consumption, diabetes, systolic blood pressure, high density lipoprotein cholesterol (HDL-C), and total cholesterol

In the next step in the procedure, metabolites with non-zero regression estimates after L1-regularized (lasso) regression on all metabolites in KORA S4 were retained. Those metabolites that remained after the regression were filtered via backward stepwise regression.

The backward stepwise selection was guided by the Akaike Information Criterion. The metabolites remaining after this step were referred to as the potential biomarkers.

We used a case-cohort study sampled from the KORA S2 cohort and a nested case-control study within the AGES and REFINE Reykjavik studies to replicate and validate any improved prediction seen from the potential biomarkers and thus be able to label them as metabolite biomarkers in the event that their predictive value replicated. In the KORA S2 case-cohort we used a sex-stratified weighting in our Cox regression models to account for the over-sampling of cases in the case-cohort design.[5] Conditional logistic regression models were used to analyze the matched case-control samples from the AGES and REFINE Reykjavik studies. Sensitivity analyses were performed to evaluate the influence of potential confounders on significant results.

The metabolite biomarkers were those potential biomarkers whose improved MI risk prediction relative to the Framingham risk score (FRS) replicated in the independent cohorts. Receiver operating characteristic (ROC) curves were used to compare the MI risk. Added predictive values of our biomarkers for incident MI were indicated by the increase in the area under the ROC curves (AUC), net reclassification improvement (NRI) and integrated discrimination improvement (IDI) statistics, which were assessed in all models and compared with the FRS.[18] All estimates of the incremental predictive values were based on leave-one-out cross-validation. We use the term novel biomarkers to describe those candidate biomarkers that showed improvements in prediction performance with respect to incident MI.

To understand the impact of the metabolite biomarkers on inflammation we used linear regression to associate the metabolites with hsCRP in KORA and the AGES and REFINE Reykjavik studies. The β estimations were calculated as the change in log-transformed hsCRP level for each standard deviation (SD) increase of the log-transformed

metabolite concentration after adjustment for MI risk factors. Associations were defined as significant at the $P < 0.05$ cutoff. All statistical analyses were performed in R (version 3.0.1, http://cran.r-project.org/), using the packages penalized,[19] pROC,[20] and PredictABEL.[21]

Sensitivity analysis

As KORA S2 was non-fasting we tested the influence of fasting status on the metabolite biomarkers by including 203 non-fasting samples (22 MI cases and 181 non-cases) in the Cox regression analyses for the KORA S4 cohort. In addition, we tested whether the association of the replicated biomarkers with incident MI was confounded by statins or modified within subgroups of the populations assessed.

References

1. Wichmann HE, Gieger C, Illig T, Group MKS. KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. Gesundheitswesen 2005;**67 Suppl 1**:S26-30 doi: 10.1055/s-2005-858226[published Online First: Epub Date]|.
2. Holle R, Happich M, Lowel H, Wichmann HE, Group MKS. KORA--a research platform for population based health research. Gesundheitswesen 2005;**67 Suppl 1**:S19-25 doi: 10.1055/s-2005-858235[published Online First: Epub Date]|.
3. Keil U, Liese AD, Hense HW, et al. Classical risk factors and their impact on incident non-fatal and fatal myocardial infarction and all-cause mortality in southern Germany. Results from the MONICA Augsburg cohort study 1984-1992. Monitoring Trends and Determinants in Cardiovascular Diseases. European heart journal 1998;**19**(8):1197-207
4. Koenig W, Khuseyinova N, Baumert J, Meisinger C. Prospective study of high-sensitivity C-reactive protein as a determinant of mortality: results from the MONICA/KORA Augsburg Cohort Study, 1984-1998. Clinical chemistry 2008;**54**(2):335-42 doi: 10.1373/clinchem.2007.100271[published Online First: Epub Date]|.
5. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case-cohort designs. Journal of clinical epidemiology 1999;**52**(12):1165-72
6. Hutchinson WL, Koenig W, Frohlich M, Sund M, Lowe GD, Pepys MB. Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. Clinical chemistry 2000;**46**(7):934-8

7. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clinical chemistry 1999;**45**(12):2136-41
8. Khuseyinova N, Imhof A, Trischler G, et al. Determination of C-reactive protein: comparison of three high-sensitivity immunoassays. Clinical chemistry 2003;**49**(10):1691-5
9. Harris TB, Launer LJ, Eiriksdottir G, et al. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. American journal of epidemiology 2007;**165**(9):1076-87 doi: 10.1093/aje/kwk115[published Online First: Epub Date]|.
10. Harris TB, Launer LJ, Eiriksdottir G, et al. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol. 2007;**165**(9):1076-87.
11. Wang-Sattler R, Yu Z, Herder C, et al. Novel biomarkers for pre-diabetes identified by metabolomics. Molecular systems biology 2012;**8**:615 doi: 10.1038/msb.2012.43[published Online First: Epub Date]|.
12. Xu T, Holzapfel C, Dong X, et al. Effects of smoking and smoking cessation on human serum metabolite profile: results from the KORA cohort study. BMC medicine 2013;**11**:60 doi: 10.1186/1741-7015-11-60[published Online First: Epub Date]|.
13. Goek ON, Prehn C, Sekula P, et al. Metabolites associate with kidney function decline and incident chronic kidney disease in the general population. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2013;**28**(8):2131-8 doi: 10.1093/ndt/gft217[published Online First: Epub Date]|.
14. Zukunft S, Sorgenfrei M, Prehn C, Möller G, Adamski J. Targeted Metabolomics of Dried Blood Spot Extracts. Chromatographia 2013;**10**
15. Römisch-Margl W, Prehn C, Bogumil R, Roehring C, Suhre K, J. A. Procedure for tissue sample preparation and metabolite extraction for high-throughput targeted metabolomics. Metabolomics 2012
16. Illig T, Gieger C, Zhai G, et al. A genome-wide perspective of genetic variation in human metabolism. Nat Genet 2010;**42**(2):137-41 doi: ng.507 [pii] 10.1038/ng.507[published Online First: Epub Date]|.
17. Yu Z, Zhai G, Singmann P, et al. Human serum metabolic profiles are age dependent. Aging cell 2012;**11**(6):960-7 doi: 10.1111/j.1474-9726.2012.00865.x[published Online First: Epub Date]|.
18. D'Agostino RB, Sr., Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;**117**(6):743-53 doi: 10.1161/CIRCULATIONAHA.107.699579[published Online First: Epub Date]|.
19. Goeman JJ. L1 penalized estimation in the Cox proportional hazards model. Biometrical journal. Biometrische Zeitschrift 2010;**52**(1):70-84 doi: 10.1002/bimj.200900028[published Online First: Epub Date]|.
20. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC bioinformatics 2011;**12**:77 doi: 10.1186/1471-2105-12-77[published Online First: Epub Date]|.
21. Kundu S, Aulchenko YS, van Duijn CM, Janssens AC. PredictABEL: an R package for the assessment of risk prediction models. European journal of epidemiology 2011;**26**(4):261-4 doi: 10.1007/s10654-011-9567-4[published Online First: Epub Date]|.

Supplementary Figures

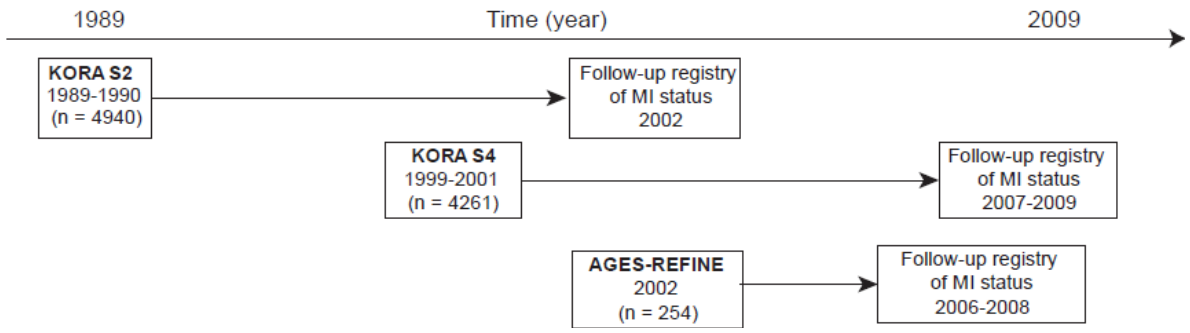

Figure S1. The timeline of the three cohorts

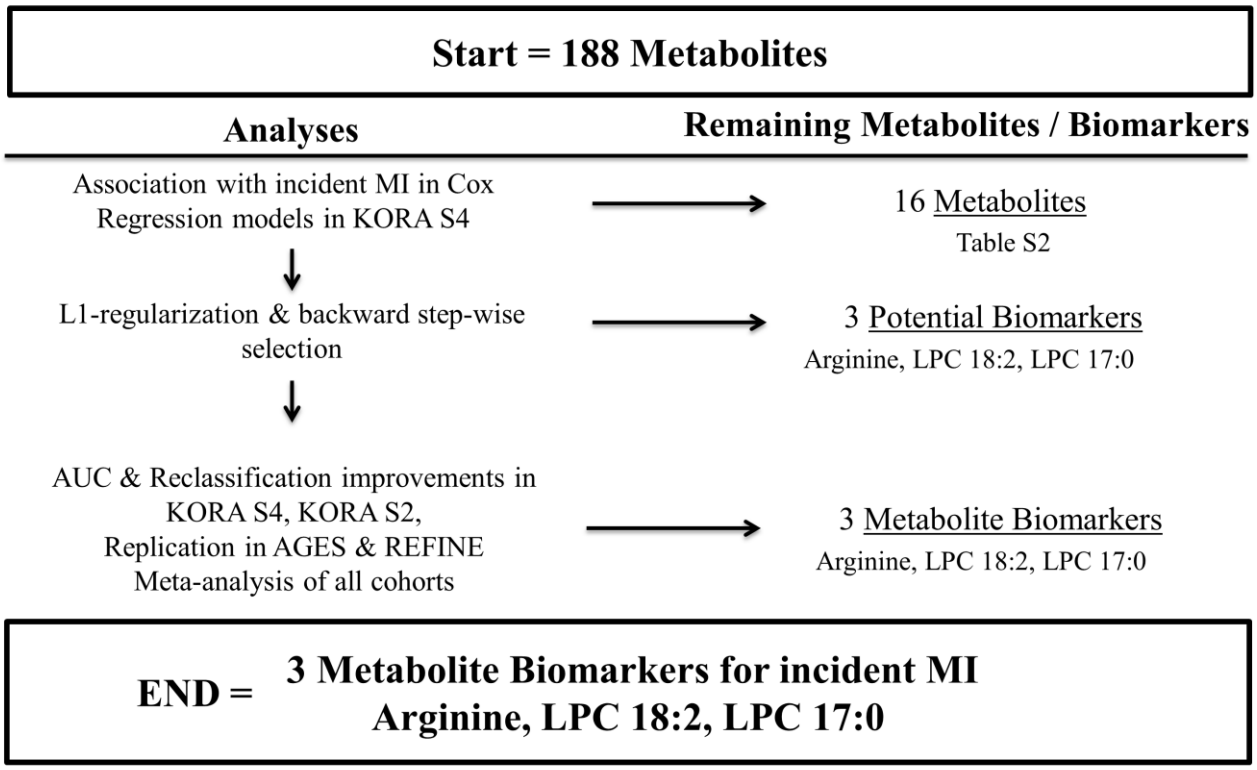

Figure S2. Procedures for the selection and validation of the candidate biomarkers

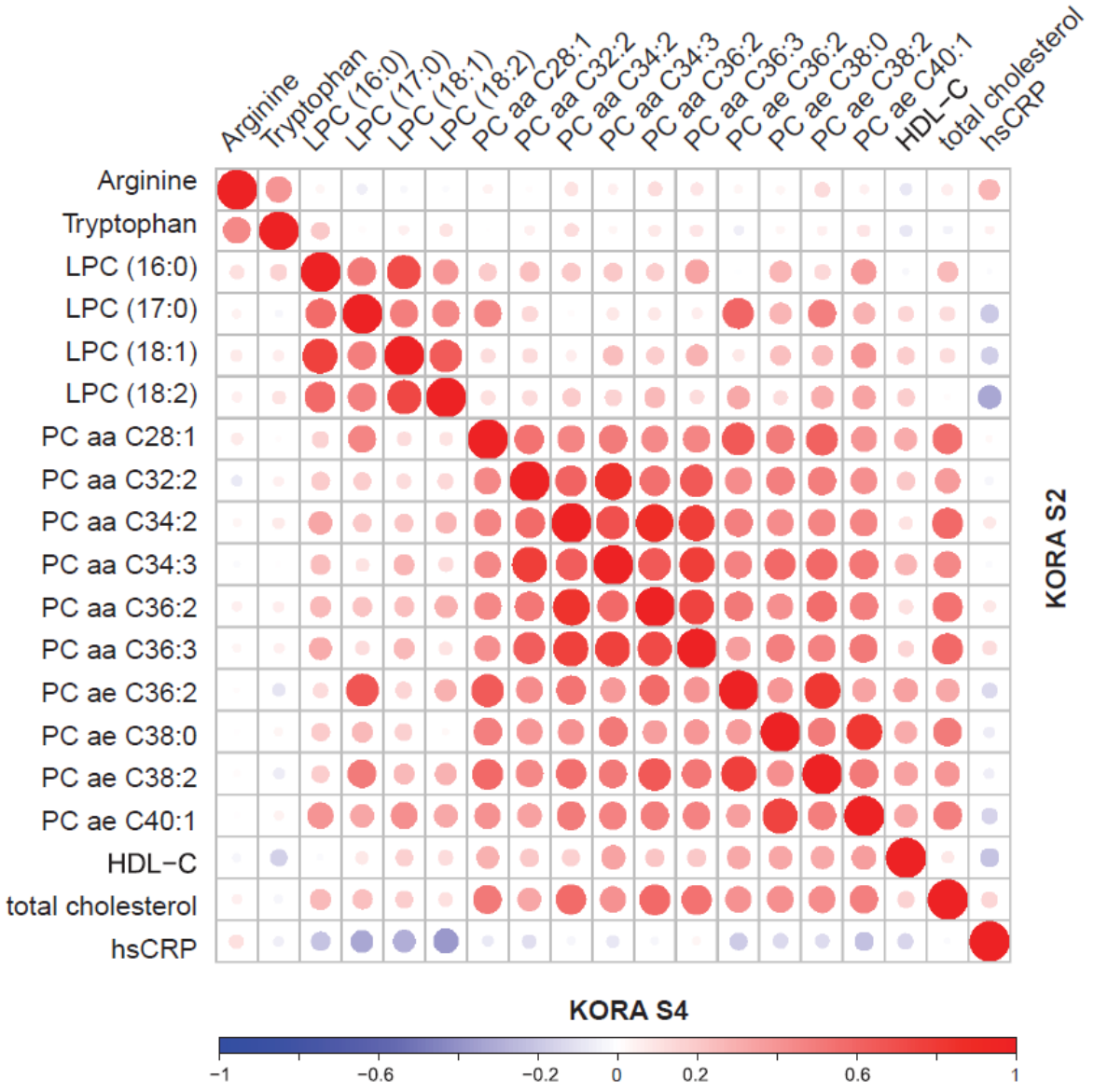

Figure S3. Correlations between metabolites and CVD risk factors in KORA S4 and KORA S2

The Spearman correlation coefficients for each pair of metabolites and CVD risk factors were calculated in KORA S4 (lower triangle) and KORA S2(upper triangle). The strength of correlations is indicated by colour as shown in the figure legend.

Supplementary Tables

Table S1. Metabolite panels of KORA S4 and KORA S2 case-cohort

Biochemical name	KORA S4				KORA S2			
	CV (%)	Above LOD (%)	Concentration	Application	CV (%)	Above LOD (%)	Concentration	Application
Carnitine	5.8	99.63	41.25 ± 8.99	Used	9.41	100.00	43.74±9.66	Used
Acetylcarnitine	6.3	99.63	8.64 ± 2.91	Used	5.83	100.00	7.36±2.81	Used
Propionylcarnitine	10	99.63	0.48 ± 0.15	Used	7.49	100.00	0.51±0.2	Used
Propenoylcarnitine	32.8	3.72	0.01 ± 0	Excluded	24.63	6.20	0.02±0	Excluded
Hydroxypropionylcarnitine	44.7	2.85	0.12 ± 0.1	Excluded	63.57	0.00	0.08±0.05	Excluded
Butyrylcarnitine	9.7	99.63	0.23 ± 0.13	Used	8.11	100.00	0.21±0.09	Used
Butenylcarnitine	22.2	46.25	0.02 ± 0.01	Excluded	17.88	9.43	0.03±0.01	Excluded
Hydroxybutyrylcarnitine	21.1	18.95	0.06 ± 0.03	Excluded	18.82	50.65	0.05±0.03	Used
Valerylcarnitine	10.8	98.7	0.17 ± 0.11	Used	8.51	99.87	0.17±0.06	Used
Tiglylcarnitine	22.9	1.8	0.05 ± 0.01	Excluded	15.64	0.13	0.05±0.01	Excluded
Glutaconylcarnitine	40	24.83	0.01 ± 0.01	Excluded	23.07	13.57	0.02±0	Excluded
Glutarylcarnitine	29.4	61.36	0.02 ± 0.01	Excluded	21.23	47.29	0.02±0.01	Excluded
Methylglutarylcarnitine	28	2.48	0.03 ± 0.01	Excluded	14.42	4.52	0.05±0.05	Excluded
Hydroxyvalerylcarnitine	26.9	19.69	0.03 ± 0.01	Excluded	25.08	18.48	0.04±0.01	Excluded
Hexanoylcarnitine	21.8	65.33	0.09 ± 0.08	Used	21.65	43.28	0.07±0.04	Excluded
Hexenoylcarnitine	30.7	5.2	0.02 ± 0.01	Excluded	23.52	5.81	0.02±0.01	Excluded
Pimelylcarnitine	18.4	70.53	0.05 ± 0.02	Used	13.73	63.70	0.03±0.01	Used
Octanoylcarnitine	13.2	60.62	0.27 ± 0.24	Used	9.06	46.90	0.14±0.09	Excluded
Nonaylcarnitine	23.6	97.28	0.05 ± 0.02	Used	21.26	94.70	0.05±0.02	Excluded
Decanoylcarnitine	11.7	99.07	0.39 ± 0.3	Used	8.52	89.15	0.23±0.13	Used
Decenoylcarnitine	11.2	74.8	0.18 ± 0.08	Used	7.91	46.12	0.13±0.05	Excluded
Decadienylcarnitine	16	94.86	0.04 ± 0.01	Used	10.32	88.37	0.04±0.02	Used
Dodecanoylcarnitine	12.2	96.41	0.16 ± 0.07	Used	9.85	58.14	0.1±0.04	Used
Dodecenoylcarnitine	15.2	26.75	0.17 ± 0.06	Excluded	10.08	38.24	0.09±0.04	Excluded
Dodecanedioylcarnitine	12.3	0	0.07 ± 0.01	Excluded	10.25	0.00	0.07±0.01	Excluded
Tetradecanoylcarnitine	15.8	96.66	0.06 ± 0.02	Used	12.59	85.40	0.05±0.01	Used
Tetradecenoylcarnitine	11.4	99.63	0.15 ± 0.05	Used	10.95	100.00	0.09±0.03	Used

Hydroxytetradecenoylcarnitine	28.9	74.92	0.02 ± 0.01	Excluded	20.01	60.08	0.01±0	Excluded
Tetradecadienylcarnitine	18.3	98.33	0.04 ± 0.02	Used	12.29	95.87	0.02±0.01	Used
Hydroxytetradecadienylcarnitine	35.1	47	0.01 ± 0	Excluded	16.17	35.01	0.01±0.01	Excluded
Hexadecanylcarnitine	11.3	99.63	0.14 ± 0.03	Used	10.48	100.00	0.13±0.03	Used
Hexadecenoylcarnitine	18.1	77.83	0.04 ± 0.01	Used	13.83	61.37	0.03±0.01	Used
Hydroxyhexadecenoylcarnitine	26.5	26.01	0.01 ± 0	Excluded	24.69	45.09	0.01±0	Excluded
Hexadecadienylcarnitine	34	87.49	0.01 ± 0	Excluded	16.35	72.35	0.01±0	Used
Hydroxyhexadecadienylcarnitine	30.1	5.76	0.01 ± 0	Excluded	21.38	0.13	0.01±0	Excluded
Hydroxyhexadecanoylcarnitine	33	16.28	0.01 ± 0	Excluded	22.03	51.03	0.01±0	Excluded
Octadecanoylcarnitine	15.7	99.63	0.06 ± 0.01	Used	15.04	100.00	0.06±0.02	Used
Octadecenoylcarnitine	9.7	99.57	0.15 ± 0.04	Used	8.76	100.00	0.17±0.05	Used
Hydroxyoctadecenoylcarnitine	44.6	7.37	0.01 ± 0	Excluded	24.11	2.07	0.01±0	Excluded
Octadecadienylcarnitine	10.5	99.57	0.05 ± 0.02	Used	8.32	100.00	0.07±0.02	Used
Alanine	13.7	99.5	419.93 ± 100.25	Used	5.97	100.00	474.5±97.72	Used
Arginine	13.2	99.26	127.38 ± 27.01	Used	12.26	100.00	135.03±30.41	Used
Asparagine	11.1	99.57	46.08 ± 8.47	Used	22.48	100.00	56.13±12.52	Excluded
Aspartate	12.2	99.44	30.05 ± 9.93	Used	18.39	100.00	51.14±12.92	Used
Citrulline	12.7	99.44	35.11 ± 9.94	Used	11.20	100.00	36.92±11.83	Used
Glutamine	12.8	99.57	579.79 ± 118.55	Used	15.13	100.00	612.01±115.41	Used
Glutamate	15.8	99.57	81.53 ± 35.4	Used	10.53	100.00	170.97±75.34	Used
Glycine	13.2	99.5	263.02 ± 74.35	Used	16.76	100.00	347.87±81.28	Used
Histidine	12.9	99.38	82.39 ± 15.13	Used	12.47	100.00	98.59±17.24	Used
Isoleucine	13.9	99.63	72.65 ± 19.67	Used	12.03	100.00	98.52±32.16	Used
Leucine	12.9	98.58	160.26 ± 43.19	Used	10.41	100.00	204.78±54.9	Used
Lysine	15.5	99.69	166.36 ± 37.05	Used	14.05	100.00	176.72±34	Used
Methionine	13.5	99.69	23.94 ± 6.85	Used	13.62	100.00	26.33±7.76	Used
Ornithine	14.9	99.63	59.34 ± 15.12	Used	15.48	100.00	94.12±21.6	Used
Phenylalanine	12.2	99.57	77.51 ± 17.62	Used	12.11	100.00	109.69±18.79	Used
Proline	11.8	99.63	195.17 ± 60.16	Used	9.23	99.87	250.07±63.9	Used
Serine	13.6	99.44	126.75 ± 28.92	Used	13.60	100.00	161.02±29.55	Used
Threonine	18.3	99.13	120.31 ± 31.71	Used	5.55	100.00	123.6±28.35	Used
Tryptophan	12.9	99.63	60.23 ± 12.64	Used	11.50	100.00	68.65±14.31	Used

Tryosine	14.7	99.57	72.51 ± 20.2	Used	11.62	100.00	82.62±21.04	Used
Valine	13.5	99.63	228.02 ± 51.89	Used	12.00	100.00	243.31±50.16	Used
Acetylornithine	20.8	79.07	0.75 ± 0.46	Used	37.51	96.38	1.35±1.08	Excluded
Asymmetric dimethylarginine	17.4	66.5	0.57 ± 0.38	Used	26.42	100.00	0.49±0.13	Excluded
Symmetric dimethylarginine	32.4	97.34	0.77 ± 0.26	Excluded	41.52	99.22	0.56±0.28	Excluded
Total dimethylarginine	20.3	99.2	1.22 ± 0.25	Used	25.49	89.92	0.71±0.34	Excluded
alpha-Aminoadipic acid	32	97.34	0.67 ± 0.29	Excluded	26.89	95.87	1.03±0.67	Excluded
Carnosine	89.8	4.02	0.13 ± 0.06	Excluded	NA	0.78	0.07±0.1	Excluded
Creatinine	14.7	99.38	76.07 ± 24.21	Used	5.79	100.00	80.44±44.11	Used
Histamine	43.5	89.97	0.39 ± 0.17	Excluded	77.23	53.62	0.15±0.16	Excluded
Kynurenine	11.3	97.28	2.93 ± 0.76	Used	21.59	99.87	2.67±0.91	Excluded
Methioninesulfoxide	20.9	96.66	0.76 ± 0.27	Used	20.55	99.22	1.22±0.6	Excluded
Nitrotyrosine	58.4	7.55	0.66 ± 0.32	Excluded	NA	0.39	0.35±0.37	Excluded
Hydroxyproline	NA	2.11	5.34 ± 5.15	Excluded	165.29	2.07	1.67±6.16	Excluded
Phenylethylamine	NA	0.56	0.09 ± 0.01	Excluded	20.14	0.52	0.02±0.04	Excluded
Putrescine	53.2	93.75	0.15 ± 0.05	Excluded	56.69	69.38	0.06±0.26	Excluded
Sarcosine	28.7	4.4	93.9 ± 223.85	Excluded	83.02	99.61	11.52±14.13	Excluded
Serotonin	38	99.32	0.69 ± 0.36	Excluded	131.40	99.22	0.57±0.29	Excluded
Spermidine	24.1	98.51	0.27 ± 0.07	Used	19.46	74.03	0.23±0.27	Used
Spermine	8.5	9.29	0.28 ± 0.13	Excluded	19.74	63.82	0.28±0.3	Used
Taurine	13.7	96.9	93.65 ± 24.37	Used	11.42	100.00	141.64±22.76	Used
DOPA	19.5	44.58	0.49 ± 0.58	Excluded	47.12	20.67	0.2±0.11	Excluded
Dopamine	NA	0.06	0.61 ± NA	Excluded	77.04	1.42	0.11±2.12	Excluded
lysoPhosphatidylcholine acyl C14:0	6.8	0	6.28 ± 0.8	Excluded	5.45	42.38	5.82±1.53	Excluded
lysoPhosphatidylcholine acyl C16:0	6.9	99.81	122.71 ± 26.13	Used	6.75	100.00	233.73±55.43	Used
lysoPhosphatidylcholine acyl C16:1	7	99.69	3.71 ± 1.25	Used	7.59	100.00	6.73±2.16	Used
lysoPhosphatidylcholine acyl C17:0	7.3	99.63	2.08 ± 0.67	Used	7.54	100.00	4.58±1.67	Used
lysoPhosphatidylcholine acyl	7.2	99.81	32.73 ± 8.11	Used	8.01	100.00	64.98±18.47	Used

C18:0 lysoPhosphatidylcholine acyl	6.8	99.75	21.5 ± 6.28	Used	7.65	100.00	35.79±9.82	Used
C18:1 lysoPhosphatidylcholine acyl	6.9	99.75	28.09 ± 8.99	Used	7.18	100.00	46.06±15.11	Used
C18:2 lysoPhosphatidylcholine acyl	8.8	99.63	2.29 ± 0.68	Used	7.41	100.00	3.63±1.11	Used
C20:3 lysoPhosphatidylcholine acyl	7.3	99.69	6.2 ± 1.88	Used	7.32	100.00	10.38±3.03	Used
C20:4 lysoPhosphatidylcholine acyl	32	23.22	0.21 ± 0.07	Excluded	23.98	64.47	0.32±0.11	Excluded
C24:0 lysoPhosphatidylcholine acyl	44.4	43.72	0.3 ± 0.16	Excluded	45.37	98.06	0.55±0.31	Excluded
C26:0 lysoPhosphatidylcholine acyl	9.5	0	1.68 ± 0.17	Excluded	39.74	99.87	0.25±0.12	Excluded
C26:1 lysoPhosphatidylcholine acyl	37	23.47	0.33 ± 0.12	Excluded	34.67	95.35	0.55±0.23	Excluded
C28:0 lysoPhosphatidylcholine acyl	35.5	98.64	0.47 ± 0.16	Excluded	29.45	100.00	0.69±0.23	Excluded
C28:1 Phosphatidylcholine diacyl C24:0	45.9	69.35	0.09 ± 0.05	Excluded	39.09	96.51	0.16±0.08	Excluded
Phosphatidylcholine diacyl C26:0	27.2	5.63	0.74 ± 0.26	Excluded	27.32	29.46	1.16±0.41	Excluded
Phosphatidylcholine diacyl C28:1	9.5	99.63	3.58 ± 0.84	Used	7.65	100.00	3.22±0.82	Used
Phosphatidylcholine diacyl C30:0	9.4	99.63	5.85 ± 1.71	Used	7.28	100.00	4.59±1.43	Used
Phosphatidylcholine diacyl C30:2	89.9	31.33	0.03 ± 0.07	Excluded	51.75	89.28	0.12±0.09	Excluded
Phosphatidylcholine diacyl C32:0	8.4	99.81	14.86 ± 2.85	Used	5.91	100.00	14.16±3.08	Used
Phosphatidylcholine diacyl C32:1	9.2	99.81	20.81 ± 10.63	Used	7.95	100.00	15.37±10.01	Used
Phosphatidylcholine diacyl C32:2	12.3	99.81	4.31 ± 1.66	Used	8.64	100.00	4.42±1.65	Used
Phosphatidylcholine diacyl C32:3	9.2	99.75	0.54 ± 0.14	Used	9.25	100.00	0.6±0.18	Used
Phosphatidylcholine diacyl C34:1	7.1	99.88	222.04 ± 46.61	Used	5.78	100.00	205.3±53.16	Used
Phosphatidylcholine diacyl C34:2	7	99.88	364.87 ± 50.66	Used	5.86	100.00	351.39±63.18	Used
Phosphatidylcholine diacyl C34:3	6.3	99.88	18.18 ± 5.25	Used	6.51	100.00	16.73±4.97	Used
Phosphatidylcholine diacyl C34:4	6.8	99.81	2.18 ± 0.77	Used	7.43	100.00	2.24±0.76	Used
Phosphatidylcholine diacyl C36:0	11.6	99.63	2.98 ± 0.75	Used	9.15	100.00	2.63±0.72	Used
Phosphatidylcholine diacyl C36:1	6.9	99.88	53.54 ± 14.16	Used	5.58	100.00	55.79±14.67	Used

Phosphatidylcholine diacyl C36:2	6.6	99.88	255.06 ± 43.56	Used	5.25	100.00	241.28±44.22	Used
Phosphatidylcholine diacyl C36:3	6.5	99.88	151.97 ± 29.94	Used	5.97	100.00	155.15±31.97	Used
Phosphatidylcholine diacyl C36:4	6.3	99.94	208.67 ± 44.62	Used	5.86	100.00	201.24±44.34	Used
Phosphatidylcholine diacyl C36:5	6.7	99.81	30.78 ± 15.23	Used	6.11	100.00	29.18±13.97	Used
Phosphatidylcholine diacyl C36:6	9.5	99.75	1.11 ± 0.43	Used	7.54	100.00	1.33±0.51	Used
Phosphatidylcholine diacyl C38:0	8.8	99.63	3.35 ± 0.86	Used	6.57	100.00	3.84±0.92	Used
Phosphatidylcholine diacyl C38:1	27	99.75	1.38 ± 0.43	Excluded	17.47	100.00	1.58±0.42	Used
Phosphatidylcholine diacyl C38:3	6.9	99.88	58.03 ± 14.11	Used	5.02	100.00	54.98±13.85	Used
Phosphatidylcholine diacyl C38:4	5.7	99.88	119.01 ± 29.37	Used	5.26	100.00	111.23±26.31	Used
Phosphatidylcholine diacyl C38:5	5.6	99.88	62.89 ± 15.21	Used	5.54	100.00	56.45±13.65	Used
Phosphatidylcholine diacyl C38:6	6.9	100	89.9 ± 26.07	Used	5.85	100.00	92.7±24.75	Used
Phosphatidylcholine diacyl C40:1	11.7	14.24	0.42 ± 0.09	Excluded	9.39	11.24	0.5±0.11	Excluded
Phosphatidylcholine diacyl C40:2	14.9	99.63	0.37 ± 0.11	Used	10.52	100.00	0.48±0.14	Used
Phosphatidylcholine diacyl C40:3	13.9	99.75	0.69 ± 0.16	Used	7.60	100.00	0.82±0.21	Used
Phosphatidylcholine diacyl C40:4	6.8	99.81	4.15 ± 1.22	Used	5.58	100.00	4.2±1.2	Used
Phosphatidylcholine diacyl C40:5	6.5	99.75	12.75 ± 3.6	Used	5.23	100.00	13.43±4.04	Used
Phosphatidylcholine diacyl C40:6	6.1	99.63	32.47 ± 10.04	Used	5.48	100.00	34.36±10.66	Used
Phosphatidylcholine diacyl C42:0	9.2	99.88	0.55 ± 0.15	Used	8.30	100.00	0.52±0.15	Used
Phosphatidylcholine diacyl C42:1	12	99.69	0.27 ± 0.07	Used	9.76	100.00	0.26±0.07	Used
Phosphatidylcholine diacyl C42:2	13.5	99.69	0.2 ± 0.05	Used	9.13	100.00	0.24±0.06	Used
Phosphatidylcholine diacyl C42:4	11	99.81	0.21 ± 0.04	Used	10.46	100.00	0.17±0.04	Used
Phosphatidylcholine diacyl C42:5	11.3	99.69	0.44 ± 0.13	Used	8.32	100.00	0.51±0.15	Used
Phosphatidylcholine diacyl C42:6	10.7	95.42	0.6 ± 0.14	Used	8.39	69.51	0.72±0.17	Used
Phosphatidylcholine acyl-alkyl C30:0	19.7	99.57	0.45 ± 0.13	Used	14.01	97.93	0.41±0.12	Used
Phosphatidylcholine acyl-alkyl C30:1	77.9	82.35	0.13 ± 0.11	Excluded	53.44	99.74	0.23±0.11	Excluded
Phosphatidylcholine acyl-alkyl C30:2	25.2	99.57	0.13 ± 0.04	Excluded	16.46	95.87	0.17±0.04	Used
Phosphatidylcholine acyl-alkyl C32:1	9.3	99.81	2.79 ± 0.56	Used	6.82	100.00	2.7±0.57	Used
Phosphatidylcholine acyl-alkyl C32:2	12.2	99.63	0.71 ± 0.16	Used	9.66	100.00	0.78±0.18	Used
Phosphatidylcholine acyl-alkyl	9.6	99.81	1.67 ± 0.43	Used	6.84	100.00	1.85±0.49	Used

C34:0								
Phosphatidylcholine acyl-alkyl C34:1	7.4	99.81	10.4 ± 2.19	Used	6.29	100.00	9±2.07	Used
Phosphatidylcholine acyl-alkyl C34:2	7.2	99.88	11.96 ± 2.96	Used	6.33	100.00	11.07±2.69	Used
Phosphatidylcholine acyl-alkyl C34:3	6.9	99.88	7.51 ± 2.12	Used	6.22	100.00	8.01±2.32	Used
Phosphatidylcholine acyl-alkyl C36:0	22.7	99.63	0.93 ± 0.28	Used	12.83	100.00	1.08±0.27	Used
Phosphatidylcholine acyl-alkyl C36:1	7.9	99.75	8.79 ± 2.05	Used	6.25	100.00	8.2±1.99	Used
Phosphatidylcholine acyl-alkyl C36:2	7	99.88	15.06 ± 3.84	Used	5.84	100.00	15.84±4.33	Used
Phosphatidylcholine acyl-alkyl C36:3	7.1	99.88	7.91 ± 1.85	Used	6.37	100.00	8.54±1.84	Used
Phosphatidylcholine acyl-alkyl C36:4	6.3	99.88	19.99 ± 4.78	Used	6.33	100.00	17.87±4.09	Used
Phosphatidylcholine acyl-alkyl C36:5	6.1	99.81	12.96 ± 3.22	Used	6.28	100.00	13.01±3.29	Used
Phosphatidylcholine acyl-alkyl C38:0	8.1	99.63	2.22 ± 0.66	Used	6.18	100.00	2.76±0.78	Used
Phosphatidylcholine acyl-alkyl C38:1	14.7	99.5	0.62 ± 0.26	Used	11.96	34.75	0.09±0.17	Excluded
Phosphatidylcholine acyl-alkyl C38:2	11.7	99.75	2.1 ± 0.48	Used	7.05	100.00	2.48±0.57	Used
Phosphatidylcholine acyl-alkyl C38:3	7	99.94	4.25 ± 0.98	Used	6.04	100.00	5.1±1.2	Used
Phosphatidylcholine acyl-alkyl C38:4	6.1	100	15.36 ± 3.11	Used	5.73	100.00	14.36±2.77	Used
Phosphatidylcholine acyl-alkyl C38:5	5.9	100	19.25 ± 3.86	Used	5.76	100.00	19.64±3.74	Used
Phosphatidylcholine acyl-alkyl C38:6	6.5	99.88	8.7 ± 2.05	Used	5.93	100.00	8.57±1.91	Used
Phosphatidylcholine acyl-alkyl	11.1	99.63	1.58 ± 0.37	Used	9.42	100.00	1.83±0.41	Used

C40:1								
Phosphatidylcholine acyl-alkyl C40:2	8.3	99.88	2.12 ± 0.49	Used	6.20	100.00	2.17±0.53	Used
Phosphatidylcholine acyl-alkyl C40:3	9	99.94	1.18 ± 0.24	Used	6.29	100.00	1.18±0.32	Used
Phosphatidylcholine acyl-alkyl C40:4	8.7	99.63	2.68 ± 0.51	Used	6.57	100.00	2.83±0.56	Used
Phosphatidylcholine acyl-alkyl C40:5	6.5	99.88	3.66 ± 0.69	Used	5.72	100.00	4.4±0.88	Used
Phosphatidylcholine acyl-alkyl C40:6	6.9	99.94	5.42 ± 1.3	Used	5.62	100.00	5.7±1.35	Used
Phosphatidylcholine acyl-alkyl C42:0	13.8	36.35	0.52 ± 0.1	Excluded	8.46	15.89	0.69±0.12	Excluded
Phosphatidylcholine acyl-alkyl C42:1	16	99.57	0.38 ± 0.09	Used	13.43	100.00	0.48±0.11	Used
Phosphatidylcholine acyl-alkyl C42:2	11.5	99.69	0.63 ± 0.14	Used	8.13	100.00	0.72±0.16	Used
Phosphatidylcholine acyl-alkyl C42:3	9.8	99.88	0.84 ± 0.19	Used	8.46	100.00	0.86±0.18	Used
Phosphatidylcholine acyl-alkyl C42:4	7.8	99.63	0.95 ± 0.22	Used	6.21	100.00	0.96±0.22	Used
Phosphatidylcholine acyl-alkyl C42:5	7.4	99.57	2.19 ± 0.46	Used	5.55	100.00	2.26±0.49	Used
Phosphatidylcholine acyl-alkyl C44:3	24.3	99.69	0.13 ± 0.04	Used	14.93	100.00	0.14±0.03	Used
Phosphatidylcholine acyl-alkyl C44:4	12.1	99.69	0.38 ± 0.1	Used	9.21	100.00	0.36±0.08	Used
Phosphatidylcholine acyl-alkyl C44:5	7.4	99.69	1.73 ± 0.46	Used	6.18	100.00	1.46±0.38	Used
Phosphatidylcholine acyl-alkyl C44:6	7.8	99.63	1.25 ± 0.33	Used	6.16	100.00	1.02±0.28	Used
Hydroxysphingomyeline C14:1	11	99.63	9.43 ± 2.53	Used	7.18	100.00	9.88±2.94	Used
Hydroxysphingomyeline C16:1	11	100	5.15 ± 1.35	Used	8.16	100.00	4.85±1.39	Used
Hydroxysphingomyeline C22:1	11.2	99.88	20.18 ± 4.56	Used	8.63	100.00	18.97±4.74	Used

Hydroxysphingomyeline C22:2	11.2	99.88	16.33 ± 4.09	Used	8.94	100.00	14.95±4.16	Used
Hydroxysphingomyeline C24:1	15.1	99.75	1.99 ± 0.49	Used	10.86	100.00	1.76±0.48	Used
Sphingomyeline C16:0	10.6	99.88	150.28 ± 24.31	Used	7.28	100.00	146.17±24.55	Used
Sphingomyeline C16:1	9.9	99.88	23.81 ± 4.67	Used	7.33	100.00	23.75±5.19	Used
Sphingomyeline C18:0	9.8	99.81	33.18 ± 7.02	Used	7.76	100.00	31.52±7.17	Used
Sphingomyeline C18:1	9.4	99.88	16.7 ± 4.09	Used	7.30	100.00	15.29±4.12	Used
Sphingomyeline C20:2	16.2	99.81	0.66 ± 0.23	Used	9.59	100.00	1.21±0.62	Used
Sphingomyeline C22:3	NA	0.37	0 ± 0.01	Excluded	41.07	78.94	0.39±0.41	Excluded
Sphingomyeline C24:0	11.9	99.75	30.26 ± 5.82	Used	8.81	100.00	28.77±5.78	Used
Sphingomyeline C24:1	12.1	99.88	76.6 ± 14.93	Used	8.79	100.00	66.12±13.01	Used
Sphingomyeline C26:0	31.8	99.81	0.3 ± 0.09	Excluded	32.47	100.00	0.21±0.07	Excluded
Sphingomyeline C26:1	21.2	99.75	0.65 ± 0.2	Used	17.62	100.00	0.57±0.17	Used
Hexose	5.2	99.81	5368.96 ± 1010.34	Used	5.53	100.00	5255.8±1967.3 3	Used

Table S2. Metabolites associated with MI in KORA S4

	Basic model (MI: 67, non-MI: 1275)		Multivariable model (MI: 67, non-MI: 1263)		Multivariable model + hsCRP (MI: 67, non-MI: 1250)	
Metabolites	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Arginine	1.40(1.10,1.78)	0.006	1.35(1.06,1.72)	0.016	1.32(1.04,1.69)	0.024
Tryptophan	0.79(0.62,1.00)	0.050	0.72(0.57,0.92)	0.01	0.77(0.60,0.99)	0.044
LPC 16:0	0.65(0.51,0.83)	0.001	0.66(0.51,0.86)	0.002	0.69(0.53,0.91)	0.008
LPC 17:0	0.65(0.52,0.82)	2.5×10^{-4}	0.69(0.53,0.89)	0.005	0.71(0.55,0.93)	0.01
LPC 18:1	0.65(0.52,0.82)	0.001	0.71(0.54,0.94)	0.017	0.75(0.56,1.00)	0.05
LPC 18:2	0.71(0.56,0.91)	5.3×10^{-5}	0.65(0.49,0.87)	0.004	0.70(0.52,0.94)	0.017
PC aa C28:1	0.66(0.51,0.84)	0.012	0.66(0.49,0.91)	0.01	0.66(0.48,0.90)	0.01
PC aa C32:2	0.60(0.46,0.77)	2.1×10^{-4}	0.64(0.50,0.81)	2.2×10^{-4}	0.67(0.52,0.86)	0.002
PC aa C34:2	0.72(0.56,0.93)	0.036	0.62(0.47,0.83)	0.001	0.64(0.48,0.85)	0.002
PC aa C34:3	0.66(0.53,0.82)	0.007	0.69(0.52,0.91)	0.009	0.72(0.54,0.96)	0.026
PC aa C36:2	0.72(0.57,0.92)	0.028	0.7(0.52,0.94)	0.017	0.73(0.54,0.97)	0.033
PC aa C36:3	0.72(0.56,0.92)	0.045	0.68(0.51,0.92)	0.011	0.69(0.51,0.94)	0.016
PC ae C36:2	0.79(0.62,1.00)	0.030	0.67(0.49,0.91)	0.011	0.69(0.51,0.94)	0.019
PC ae C38:0	0.78(0.61,0.99)	0.002	0.7(0.52,0.95)	0.022	0.73(0.54,0.99)	0.041
PC ae C38:2	0.75(0.58,0.97)	0.009	0.74(0.55,0.98)	0.038	0.74(0.56,0.99)	0.043
PC ae C40:1	0.67(0.52,0.87)	0.007	0.67(0.50,0.91)	0.009	0.71(0.52,0.97)	0.029

The basic model was adjusted for age and sex. The multivariable model was additionally adjusted for BMI, diabetes, systolic blood pressure, smoking status, (current smoker, former smoker and never smoker), alcohol consumption (≥20 g/day for women; ≥40 g/day for men), total cholesterol, and HDL-C. The final column shows the multivariable model with an additional adjustment for hsCRP. Values are provided as estimated HR (95% CI) for each SD increase of the log-transformed metabolite concentration. Abbreviations: HR, hazard ratio; CI: confidence interval.

Table S3. Sensitivity analysis of the three metabolite biomarkers association with MI

	Arginine	LPC 17:0	LPC 18:2
KORA S4			
Adjust for statin use (67 incident MI / 1275 non-MI)			
HR (95% CI)	1.32(1.04,1.69)	0.71 (0.55, 0.93)	0.70 (0.52, 0.94)
P value	0.02	0.01	0.02
Include non-fasting samples (86 incident MI / 1417 non-MI)			
HR (95% CI)	1.31(1.06,1.62)	0.79 (0.63, 0.99)	0.75 (0.60, 0.94)
P value	0.014	0.04	0.011
Exclude participants with diabetes (54 incident MI / 1171 non-MI)			
HR (95% CI)	1.31 (1.02, 1.67)	0.69 (0.53, 0.90)	0.67 (0.49, 0.90)
P value	0.03	0.006	0.008
Exclude statin users (64 incident MI / 1161 non-MI)			
HR (95% CI)	1.30 (1.01, 1.70)	0.69 (0.52, 0.91)	0.70 (0.50,0.97)
P value	0.04	9.5×10^{-3}	0.03
KORA S2			
Adjust for statin use (112 incident MI / 549 non-MI)			
HR(95% CI)	1.17(0.97,1.42)	0.75 (0.60, 0.95)	0.84 (0.68, 1.03)
P value	0.10	0.01	0.08
Exclude participants with diabetes (92 incident MI / 532 non-MI)			
HR (95% CI)	1.13 (0.93,1.38)	0.71 (0.56,0.90)	0.72 (0.57, 0.90)
P value	0.22	0.005	0.005
Exclude statin users (112 incident MI / 546 non-MI)			
HR(95% CI)	1.17 (0.98, 1.42)	0.75 (0.60, 0.95)	0.83 (0.68, 1.03)
P value	0.08	0.015	0.09

Sensitivity analyses were conducted by including non-fasting samples in KORA S4 and S2, and excluding participants with diabetes and those taking statins. Associations between our novel biomarkers and incident MI were assessed in age, sex and smoking subgroups using Cox regression models with adjustment for age, sex, BMI, alcohol consumption, systolic blood pressure, diabetes status, total cholesterol, HDL cholesterol, and hsCRP. Values in the table are provided as estimated hazard ratio and associated 95% confidence interval for each standard deviation increase of the log-transformed metabolite concentrations. Abbreviations: HR, hazard ratio; hsCRP, high-sensitivity CRP

Table S4. Spearman correlation amongst metabolite biomarkers and hsCRP for KORA S4 (lower triangle) and KORA S2 (upper triangle)

		KORA S2			
		Arginine	LPC 17:0	LPC 18:2	hsCRP
KORA S4	Arginine		-0.06 (0.13)	-0.01 (0.71)	0.27 ($1.8x10^{-14}$)
	LPC 17:0	0.04 (0.12)		0.46 ($1.5x10^{-40}$)	-0.20 ($4.5x10^{-08}$)
	LPC 18:2	0.06 (0.03)	0.49 ($3.1x10^{-80}$)		-0.32 ($6.0x10^{-20}$)
	hsCRP	0.11 ($2.8x10^{-05}$)	-0.32 ($3.3x10^{-33}$)	-0.37 ($7.4x10^{-45}$)	
		KORA S4			KORA S2

The Spearman correlation and associated P value (in parentheses) for arginine, LPC 17:0, LPC 18:2, and high sensitivity C-reactive protein (hsCRP) for KORA S4 (lower triangle) and KORA S2 (upper triangle).

Table S5. Linear association between the metabolite biomarkers and hsCRP

	KORA S4		KORA S2		AGES-REFINE		Meta-analysis	
Metabolite	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**	**β (95% CI)**	**P Value**
Arginine	0.07 (0.01, 0.12)	0.02	0.24 (0.16,0.32)	$2.8x10^{-9}$	0.27 (0.08, 0.46)	0.006	0.13 (0.09, 0.18)	$5.3x10^{-9}$
LPC 17:0	-0.27 (-0.33, -0.21)	$8.8x10^{-19}$	-0.19 (-0.28, -0.11)	$2.2x10^{-5}$	-0.30 (-0.51, -0.09)	0.005	-0.25(-0.30, -0.20)	$1.4x10^{-24}$
LPC 18:2	-0.31 (-0.37, -0.25)	$1.5x10^{-24}$	-0.28 (-0.37, -0.20)	$2.1x10^{-11}$	-0.20 (-0.40, 0.00)	0.05	-0.30 (-0.35,-0.25)	$3.3x10^{-36}$

The β estimates (95% CI) for the linear regression of metabolites on hsCRP. HsCRP, high sensitivity C-reactive protein, CI, confidence interval.

Table S6. Change in the association between hsCRP and incident MI after adding the three metabolite biomarkers

	KORA S4		KORA S2		AGES-REFINE		Meta-analysis	
	HR (95% CI)	**P value**	**HR(95% CI)**	**P value**	**HR(95% CI)**	**P value**	**HR(95% CI)**	**P value**
Without metabolites								
Age	1.27(0.98,1.65)	0.08	2.64(1.99,3.5)	$1.23x10^{-11}$	1.55(0.39,6.11)	0.53	1.79(1.01,3.17)	0.05
Sex*	0.28(0.14,0.55)	$2.61x10^{-4}$	0.33(0.19,0.56)	$3.82x10^{-5}$	0.91(0.47,1.79)	0.79	0.43(0.21,0.88)	0.02
BMI	1.23(0.95,1.59)	0.12	0.88(0.7,1.12)	0.31	0.80(0.55,1.16)	0.24	0.97(0.75,1.25)	0.81
Diabetes	1.8(0.92,3.54)	0.09	3.52(2.02,6.13)	$9.21x10^{-6}$	1.35(0.58,3.16)	0.49	2.18(1.23,3.87)	0.008
Systolic BP	1.25(0.97,1.6)	0.08	1.37(1.12,1.66)	$1.69x10^{-3}$	1.02(0.44,2.40)	0.96	1.31(1.13,1.52)	4.57×10^{-4}
Current smoker †	0.87(0.48,1.59)	0.66	1.59(0.95,2.66)	0.08	1.44(0.73,2.86)	0.29	1.27(0.87,1.86)	0.21
Former smoker †	1.54(0.73,3.24)	0.25	2.27(1.33,3.9)	$2.81x10^{-3}$	1.14(0.84,1.53)	0.4	1.52(0.96,2.38)	0.07
Alcohol intake ‡	0.61(0.3,1.22)	0.16	0.79(0.5,1.27)	0.33	–	–	0.73(0.50,1.08)	0.11
Total cholesterol	1.01(0.77,1.31)	0.97	1.52(1.27,1.82)	$4.71x10^{-6}$	1.41(1.06,1.88)	0.02	1.31(1.02,1.68)	0.04
HDL-C	1.01(0.74,1.38)	0.95	0.73(0.57,0.93)	0.01	0.79(0.55,1.14)	0.21	0.82(0.67,1.02)	0.07
HsCRP	1.19(1.04,1.35)	0.01	1.16(1.02,1.32)	0.02	1.41(1.06,1.89)	0.02	1.19(1.09,1.30)	6.58×10^{-5}
With metabolites								
Arginine	1.69(1.26,2.27)	$4.40x10^{-4}$	1.08(0.87,1.32)	0.49	1.10(0.75,1.63)	0.63	1.26(0.94,1.69)	0.13
LPC 17:0	0.66(0.50,0.87)	$3.20x10^{-3}$	0.71(0.54,0.94)	0.02	1.03(0.68,1.55)	0.89	0.78(0.65,0.94)	0.008
LPC 18:2	0.92(0.65,1.29)	0.62	0.88(0.68,1.13)	0.3	0.69(0.45,1.06)	0.09	0.85(0.71,1.02)	0.08
Age	1.24(0.94,1.63)	0.13	2.67(2.00,3.57)	$3.00x10^{-11}$	1.66(0.38,7.34)	0.51	1.79(0.98,3.28)	0.06
Sex*	0.26(0.12,0.54)	$3.70x10^{-4}$	0.36(0.21,0.64)	$4.70x10^{-4}$	0.89(0.42,1.88)	0.76	0.43(0.22,0.86)	0.02
BMI	1.19(0.90,1.58)	0.22	0.79(0.62,1.02)	0.07	0.76(0.50,1.14)	0.18	0.91(0.68,1.21)	0.5
Diabetes	1.85(0.93,3.66)	0.08	4.91(2.74,8.79)	$8.40x10^{-8}$	1.28(0.51,3.22)	0.6	2.39(1.07,5.32)	0.03
Systolic BP	1.19(0.92,1.54)	0.18	1.39(1.14,1.70)	$9.40x10^{-4}$	1.17(0.47,2.92)	0.74	1.31(1.12,1.53)	5.57×10^{-4}
Current smoker †	0.80(0.44,1.46)	0.47	1.70(0.99,2.91)	0.05	1.52(0.76,3.04)	0.24	1.28(0.79,2.06)	0.31
Former smoker †	1.22(0.57,2.62)	0.61	2.18(1.23,3.84)	0.01	1.15(0.84,1.56)	0.4	1.41(0.93,2.14)	0.11
Alcohol intake ‡	0.60(0.29,1.24)	0.17	0.55(0.33,0.92)	0.02	–	–	0.57(0.37,0.87)	0.01
Total cholesterol	1.12(0.85,1.47)	0.42	1.54(1.27,1.86)	$5.40x10^{-6}$	1.48(1.07,2.05)	0.02	1.38(1.13,1.69)	0.002
HDL-C	0.97(0.71,1.34)	0.87	0.79(0.61,1.02)	0.07	0.79(0.54,1.15)	0.22	0.84(0.71,1.01)	0.06
HsCRP	1.05(0.80,1.38)	0.74	1.10(0.94,1.27)	0.23	1.27(0.91,1.76)	0.16	1.11(0.98,1.25)	0.1

Estimates of hsCRP and the confounders from the multivariable Cox regression model. * women compared with men; † compared with never smokers; ‡ ≥20 g/day for women; ≥40 g/day for men. Abbreviations: HR, hazard ratio; CI, confidence interval.