SUPPLEMENTAL MATERIAL

Supplemental Methods

Study participants

This study was based on a nested case-control study of CHD within the SCCS[49]. Briefly, the SCCS enrolled 84,735 primarily low-income, uninsured/underinsured Black and White Americans aged 40-79 years from 12 southeastern US states between 2002-2009, with >50% having household income <$15,000/y and ~86% were uninsured or underinsured[49]. Participants were surveyed for a wide range of information at baseline and followed up regularly for morbidity and mortality outcomes. Venous blood samples were collected at the same time as the baseline survey, and plasma samples were aliquoted and stored at -80°C for long-term use. A nested case-control study of CHD within SWMHS was used as the validation cohort. The SWMHS enrolled 74,940 women aged 40-70 years and 61,480 men aged 40-74 years from Shanghai, China, between 1996-2000 and 2002-2006[50,51]. These cohort studies were approved by the Institutional Review Boards of the Vanderbilt University Medical Center, Meharry Medical College, and/or Shanghai Cancer Institute. Informed consent was obtained from all enrolled participants.

For the nested case-control studies, participant inclusion criteria were 1) no history of CHD, stroke, heart failure, cancer, or end-stage renal disease at baseline; 2) available baseline plasma samples and data on fasting time and in SCCS, the time between sample collection and lab processing; 3) no use of antibiotics nor cold/flu in last 7 days before blood collection to minimize the potential influence of acute disease and medications on gut microbiota-

related metabolites; 4) in SCCS, participants were eligible for Centers for Medicare & Medicaid Services (CMS) and had ≥2 claims after cohort enrollment; in SWMHS, participants' medical records were accessible for our study. In SCCS, nonfatal CHD cases were identified through CMS data, including acute myocardial infarction, coronary revascularization, and other CHD, and CHD deaths were identified through the National Death Index. In SWMHS, CHD cases were first identified by self-reported diagnoses during follow-up visits and then confirmed by medical records. After applying inclusion criteria as of August 2020, there were 322 Black women, 365 Black men, 304 White women, 266 White men, 305 Chinese women, and 395 Chinese men identified as incident CHD cases in the SCCS and SWMHS. In each race (Black, White, or Chinese) and sex (male or female), 150 incident CHD cases were randomly selected and 1:1 matched to controls who had no CHD, heart failure, stroke, or cancer at the time of case diagnosis, by enrollment age (±2 years), fasting time (±2 hours), and time between sample collection and lab processing (±4 hours, for SCCS samples; all SWMHS samples were processed within 6 hours after collection). After excluding eight plasma samples that failed the metabolomics quality control, a total of 1792 participants, including 597 CHD case-control pairs in SCCS (299 pairs of Black Americans and 298 pairs of White Americans) and 299 pairs of Chinese adults in SWMHS were included in the present study (**Fig. I** in the Data Supplement).

Life's Essential 8

We constructed each LE8 component metric and total LE8 score according to AHA guidelines[3,52], with some modifications based on the characteristics of our data, as shown in

Table I in the Data Supplement. Specifically, dietary quality was assessed by the Dietary Approaches to Stop Hypertension (DASH) score[53-55], which was calculated based on validated food frequency questionnaires (FFQ). The SCCS FFQ included 89 food/beverage items capturing the main sources of energy and nutrient intakes for adults living in the southeastern US[56,57], which was validated through 24-hour dietary recalls and demonstrated a high level of agreement (kappa was 0.82 to 0.96 for macronutrients and 0.73 to 0.95 for micronutrients)[56]. The SWMHS FFQs included 77 to 81 food items commonly consumed in Shanghai, China, which were validated through 24-hour dietary recalls with correlation coefficients being 0.38 to 0.66 for macronutrients and 0.33 to 0.59 for micronutrients[58,59]. Physical activity component was assessed by total minutes of leisure-time moderate and vigorous physical activity per week, with each minute of vigorous physical activity counted as 2 minutes toward the total minutes. The reliability and validity of the physical activity questionnaires used in SCCS and SWMHS were evaluated against physical activity monitors or logs in random samples of cohort participants and published previously[60-62]. Nicotine exposure was assessed by active tobacco smoking and secondhand smoking exposure. Sleep health was measured by the weighted average of self-reported sleep hours per day during weekdays and weekends. BMI was calculated as weight (kg)/height $(m)^2$ using self-reported weight and height at the baseline survey. Blood lipids component was assessed by non-HDL cholesterol (mg/dL) (plasma total cholesterol minus HDL cholesterol), which were measured by nuclear magnetic resonance (NMR) using the Bruker In Vitro Diagnostic Research (IVDr) Platform at the Vanderbilt NMR Facility Core. Because concentrations of fasting glucose and HbA1c were not measured in our study, blood glucose component was scored based on

diabetes diagnosis, use of anti-diabetic medications, and relative level of plasma glucose measured simultaneously with other metabolites (see following "metabolite profiling"). Blood pressure was unavailable in SCCS, thus blood pressure component was scored based on hypertension status and use of anti-hypertensive medications. Total LE8 score was obtained by calculating the arithmetic mean of 8 individual component scores, ranging from 0 to 100[3]. We also calculated scores reflecting alignment with health behaviors (ie, diet, physical activity, smoking, and sleep) and health factors (ie, BMI, lipids, glucose, and blood pressure), respectively based on their component scores.

Metabolite profiling

Baseline plasma samples of selected CHD case-control pairs were retrieved and placed adjacently and randomly in the same assay batch. Laboratory persons were blinded to the case-control status of samples. Pooled plasma samples were inserted after every ~30 study samples as quality control (QC) samples in addition to metabolite profiling QC samples. Untargeted metabolite profiling was performed using ultra-high-performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry (MS) by Metabolon Inc. (Morrisville, NC, USA) following a standard assay protocol[63]. Briefly, plasma samples were extracted with methanol and split into four aliquots for analysis by UHPLC-MS/MS in both positive and negative ion modes using a combination of reverse phase and HILIC chromatography methods. Metabolites were identified by automated comparison of mass spectra features to a reference library of >4,000 authenticated standard compounds followed by visual inspection for quality control. Peaks were quantified using area-under-the-curve. A

total of 1502 metabolites were detected in our samples. The majority of metabolites (>80%) were annotated based on internal standards. Metabolites that were annotated only by a match to a known MS spectrum or chemical formula were marked by '*' and '**', respectively. The median coefficient of variance for all metabolites among our QC samples was 10.3% (interquartile range: 5.4%-19.1%). We excluded metabolites detected in <10% of participants, resulting in 1322 metabolites. Metabolites with missing values were imputed by half of the minimal value in the non-missing samples. The values of all metabolites were log-transformed and standardized to mean 0 and unit variance.

Statistical analysis

The characteristics of the study participants were presented as mean (standard deviation [SD]) for continuous variables and frequency (percentage) for categorical variables. *Spearman* correlations between LE8 score and individual component scores were assessed. Elastic net regression was used to identify metabolites associated with LE8[12,14,64], with hyperparameters determined via a ten-repeated tenfold cross-validation framework, using R package caret (version: 6.0-88)[65] and glmnet (version: 4.1-3)[64,66]. The MetaSig was calculated through a leave-one-out cross-validation approach in the SCCS dataset without using CHD outcome information. In addition, we externally validated the identified signature in SWMHS using weights (ie, elastic net regression coefficients) of the selected metabolites obtained from SCCS. We calculated *Spearman* correlations between LE8 score and MetaSig among all participants and by race, sex, age (≥60y/<60y), incident CHD status, diabetes status, hypertension status, dyslipidemia status, and fasting status.

We then examined the associations of LE8 and its MetaSig with risk of CHD using conditional logistic regression, adjusting for age, education, income, alcohol intake, and family history of CHD. We also included LE8 score and MetaSig in the same model to assess their independent associations with CHD and potential mediating effect of MetaSig on LE8-CHD association. The potential multicollinearity was assessed by variance inflation factor (VIF) using R package car (version: 3.1-0) with VIF >10 indicating multicollinearity among variables. The ranges of VIF for LE8 score and its MetaSig were 1.08-1.37, confirming no multicollinearity. The causal mediation analysis was performed using R package mediation (version: 4.5.0)[67], with assumptions of a linear dependency among LE8 score→MetaSig→ CHD and sequential ignorability[68], adjusting for age, sex, race, education, income, alcohol intake, family history of CHD, and fasting status. Subgroup analyses to evaluate the associations of LE8 and MetaSig with incident CHD were performed by race, age group, sex, education, household income, diabetes status, hypertension status, and dyslipidemia status, with *P* value for interaction obtained from the corresponding interaction term in the model.

The same methods were used to identify MetaSigs for health behaviors and health factors included in LE8 and evaluate their associations with incident CHD, potential mediation effects, and their external validity. Mutual adjustments of health behaviors and health factors scores were performed in corresponding statistical models. All analyses were performed using R (version 4.1.1). Two-sided $P < 0.05$ was considered statistically significant. An overview of our study design is presented in **Fig. I** in the Data Supplement.

Supplemental Tables

Table I. Measurement of alignment with Life's Essential 8 components

LE8 components	Measurement	Scoring			
		SCCS		SWMHS	
Diet score	DASH score	Points	Quantile	Points	Quantile
		100	≥95th percentile	100	≥95th percentile
		80	75th-94th percentile	80	75th-94th percentile
		50	50th-74th percentile	50	50th-74th percentile
		25	25th-49th percentile	25	25th-49th percentile
		0	≤24th percentile	0	≤24th percentile
Physical activity score	Self-reported total minutes of leisure-time moderate and vigorous physical activity per week	Points	Minutes	Points	Minutes
		100	≥150	100	≥150
		90	120-149	90	120-149
		80	90-119	80	90-119
		60	60-89	60	60-89
		40	30-59	40	30-59
		20	1-29	20	1-29
		0	0	0	0
Smoking score	Tobacco smoking and secondhand smoke	Points	Status	Points	Status
		100	Never smoker	100	Never smoker
		75	Former smoker, quit ≥5y	75	Former smoker, quit ≥5y
		50	Former smoker, quit 1-<5y	50	Former smoker, quit 1-<5y

	exposure	25 Former smoker, quit <1y 0 Current smoker Subtract 20 points (unless score is 0) for exposing to secondhand smoking	25 Former smoker, quit <1y 0 Current smoker Subtract 20 points (unless score is 0) for exposing to secondhand smoking
Sleep score	Average self-reported sleep hours per day	Points Level 100 7-<9 90 9-<10 70 6-<7 40 5-<6 or ≥10 20 4-<5 0 <4	Points Levels 100 7-<9 90 9-<10 70 6-<7 40 5-<6 or ≥10 20 4-<5 0 <4
BMI score	Weight (kg)/height $(m)^2$	Points Level 100 <25 70 25.0-29.9 30 30.0-34.9 15 35.0-39.9 0 ≥40.0	Points Level 100 <23 75 23.0-24.9 50 25.0-29.9 25 30.0-34.9 0 ≥35.0
Lipids score	Non-HDL cholesterol (mg/dL)	Points Level 100 <130 60 130-159 40 160-189 20 190-219	Points Level 100 <130 60 130-159 40 160-189 20 190-219

		0 ≥220 If drug-treated level, subtract 20 points (unless score is 0)	0 ≥220 If drug-treated level, subtract 20 points (unless score is 0)
Glucose score	T2D status and glucose abundance measured by untargeted metabolomic profiling	Points Level Without diabetes 100 Glucose <50th percentile 60 Glucose ≥50th percentile With diabetes 40 Glucose <20th percentile 30 Glucose 20th-<40th percentile 20 Glucose 40th-<60th percentile 10 Glucose 60th-<80th percentile 0 Glucose ≥80th percentile	Points Level Without diabetes 100 Glucose <50th percentile 60 Glucose ≥50th percentile With diabetes 40 Glucose <20th percentile 30 Glucose 20th-<40th percentile 20 Glucose 40th-<60th percentile 10 Glucose 60th-<80th percentile 0 Glucose ≥80th percentile
Blood pressure score	SCCS: hypertension status and number of medications; SWMHS:	Points Level 100 Without hypertension With hypertension 50 Without taking medications	Points Level 100 SBP<120 and DBP<80 75 SBP 120-<130 and DBP<80 50 SBP 130-<140 or DBP 80-<90

	SBP (mmHg) and DBP (mmHg)	25	Taking only 1 medication	25	SBP 140-<160 or DBP 90-<100
		0	Taking >1 medication	0	SBP≥160 or DBP≥100
				Subtract 20 points if participant receive anti-hypertension treatment (minimal score is 0)	

Table II. Life's Essential 8 scores between incident CHD and control groups among male and female participants in the Southern Community Cohort Study

	Male participants		Female participants	
	CHD (*N*=297)	Control (*N*=297)	CHD (*N*=300)	Control (*N*=300)
Life's Essential 8 score	46.6 (13.5)	51.9 (14.0)	44.3 (11.9)	51.9 (12.7)
Life's Essential 8 score category, *n* (%)				
High (80-100)	3 (1.0)	10 (3.4)	0 (0.0)	7 (2.3)
Moderate (50-79)	119 (40.1)	160 (53.9)	95 (31.7)	162 (54.0)
Low (0-49)	175 (58.9)	127 (42.8)	205 (68.3)	131 (43.7)
Health behaviors score	41.0 (19.2)	41.7 (21.2)	44.7 (18.3)	52.0 (19.0)
Health factors score	51.9 (21.5)	61.5 (20.4)	43.4 (18.5)	51.5 (19.7)
Diet score	31.8 (28.5)	31.5 (27.6)	45.0 (32.6)	50.6 (32.1)
Physical activity score	29.3 (44.0)	26.7 (42.2)	13.5 (32.2)	18.8 (36.9)
Smoking score	29.9 (37.1)	33.2 (40.3)	53.0 (41.9)	60.8 (39.5)
Sleep score	73.6 (28.8)	76.1 (28.0)	69.9 (30.0)	79.1 (25.0)
Body mass index score	58.1 (35.2)	67.2 (32.0)	42.4 (31.5)	47.5 (34.7)
Blood lipids score	34.9 (33.5)	43.0 (31.9)	27.7 (28.6)	29.6 (28.4)
Blood glucose score	59.8 (33.6)	70.6 (27.3)	55.4 (34.8)	72.4 (29.5)
Blood pressure score	54.7 (36.8)	65.3 (37.8)	47.9 (36.7)	56.4 (38.7)

Table III. Characteristics of study participants in the Shanghai Women's and Men's Health Studies

	CHD cases (*N*=299)	Controls (*N*=299)
Age, years	61.5 (8.3)	61.4 (8.3)
Male, *n* (%)	149 (49.8)	149 (49.8)
Education, *n* (%)		
Less than high school	181 (60.5)	184 (61.5)
Completed high school	67 (22.4)	61 (20.4)
Vocational school or some college	29 (9.7)	22 (7.4)
College or graduate school	22 (7.4)	32 (10.7)
Income, *n* (%)*		
Low	59 (19.7)	54 (18.1)
Middle	224 (74.9)	231 (77.3)
High	16 (5.4)	14 (4.7)
Alcohol intake, *n* (%)†		
None	258 (86.3)	255 (85.3)
Moderate	22 (7.4)	31 (10.4)
Heavy	19 (6.4)	13 (4.3)
Family history of CHD, *n* (%)	43 (14.4)	33 (11.0)
History of diabetes, *n* (%)	58 (19.4)	25 (8.4)
History of dyslipidemia, *n* (%)	34 (11.4)	22 (7.4)
History of hypertension, *n* (%)	151 (50.5)	101 (33.8)

Life's Essential 8 score	50.7 (12.0)	57.2 (12.8)
Life's Essential 8 score category, *n* (%)‡		
High (80-100)	1 (0.3)	7 (2.3)
Moderate (50-79)	160 (53.5)	207 (69.2)
Low (0-49)	138 (46.2)	85 (28.4)
Health behaviors score	53.6 (21.8)	58.0 (22.3)
Health factors score	47.8 (15.6)	56.2 (15.7)
Diet score	35.5 (30.4)	44.0 (31.8)
Physical activity score	44.6 (49.0)	46.4 (48.9)
Smoking score	61.4 (40.4)	67.7 (38.2)
Sleep score	79.9 (24.6)	79.9 (25.0)
Body mass index score	68.4 (25.8)	73.2 (22.9)
Blood lipids score	38.0 (31.9)	48.3 (32.5)
Blood glucose score	66.9 (30.2)	76.4 (25.5)
Blood pressure score	15.0 (27.2)	25.6 (35.8)

Data were mean (standard deviation) or *n* (%) as indicated.
*Annual income per capita < ¥ 6,000, ¥ 6,000 to < ¥ 10,000, and ≥ ¥ 10,000 for low, middle, and high levels of income, respectively in Chinese men and < ¥ 4000, ¥ 4,000 to < ¥ 8,000, and ≥ ¥ 8,000 for low, middle, and high levels of income, respectively in Chinese women.
†Alcohol intake was grouped as none, moderate (>0 to ≤2 drinks per day in men or >0 to ≤1 drink per day in women; 1 drink = 14 g ethanol), and heavy drinking (>2 drinks per day in men or >1 drink per day in women).
‡The cutoffs were provided by the American Heart Association[3].

Table IV. Full list of metabolites related to LE8 in elastic net regression model. (see separate excel file)

Table V. MetaSig-LE8 score correlation in SCCS (see separate excel file)

Table VI. Excluded metabolite (see separate excel file)

Supplemental Figures

Fig. I. Overview of the current study design. This study involved two nested case-control studies within the Southern Community Cohort Study (SCCS, primary cohort) and Shanghai Women's and Men's Health Studies (SWMHS, validation cohort). In the follow-up visits, participants with incident coronary heart disease (CHD) were identified and matched with controls by age, sex, race, fasting time, and time between sample collection and lab processing. After excluding eight samples that did not pass metabolomics quality control, 1194 SCCS participants and 598 SWMHS participants were included. Metabolite signature (MetSig) of Life's Essential 8 (LE8) was identified using elastic net regression with leave-one-out cross-validation in SCCS. The associations of LE8 score and its metabolite signature with risk of CHD were evaluated by conditional logistic regression adjusted for confounders. Mediation analysis was performed to assess the potential mediating role of metabolite signature on the LE8-CHD association. External validity of the metabolite signature related to LE8 score and CHD risk was investigated in SWMHS.

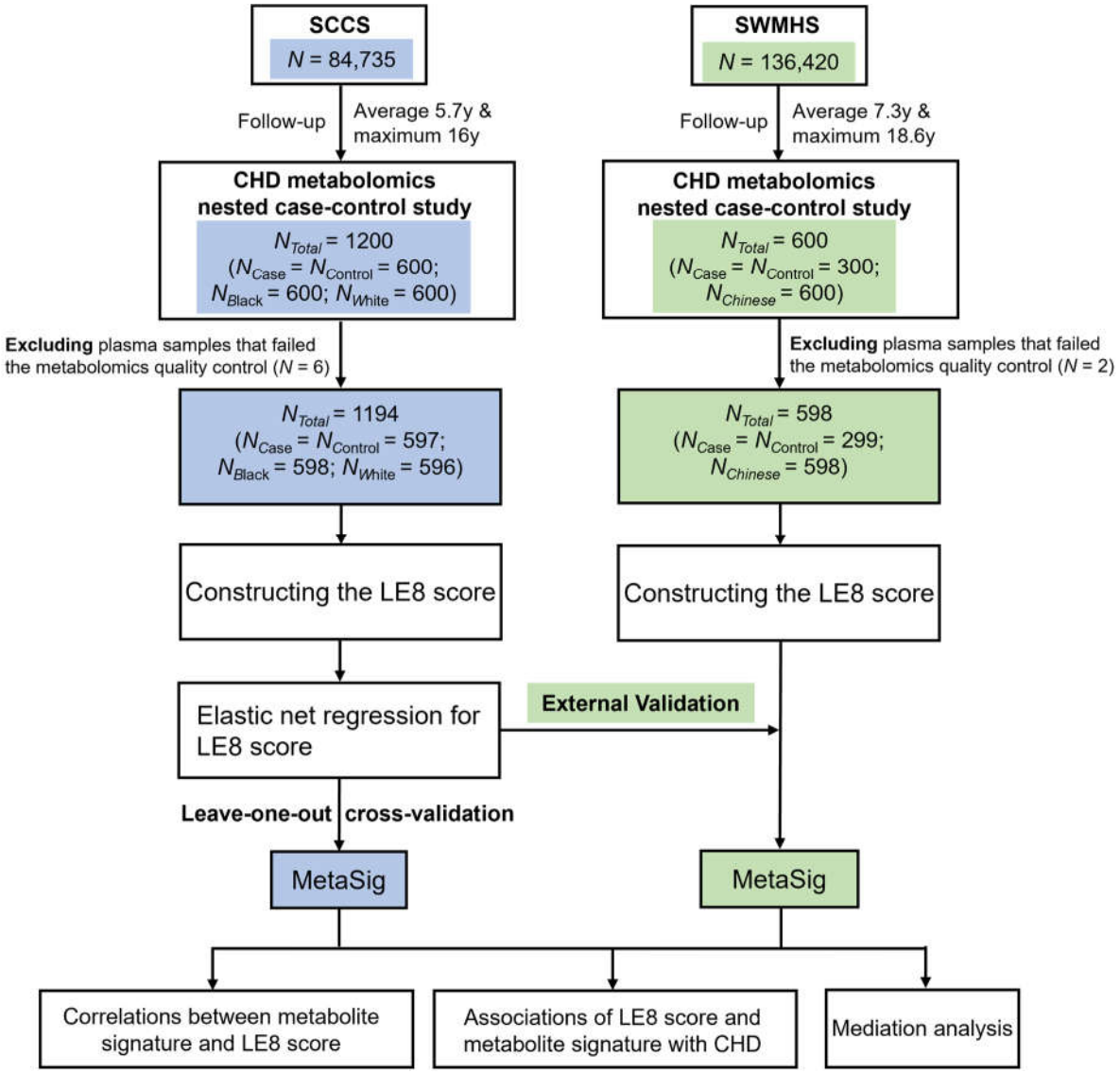

SCCS
N = 84,735
Follow-up
Average 5.7y & maximum 16y
CHD metabolomics nested case-control study
N_Total = 1200
(N_Case = N_Control = 600;
N_Black = 600; N_White = 600)
Excluding plasma samples that failed the metabolomics quality control (N = 6)
N_Total = 1194
(N_Case = N_Control = 597;
N_Black = 598; N_White = 596)
Constructing the LE8 score
Elastic net regression for LE8 score
Leave-one-out cross-validation
MetaSig
SWMHS
N = 136,420
Follow-up
Average 7.3y & maximum 18.6y
CHD metabolomics nested case-control study
N_Total = 600
(N_Case = N_Control = 300;
N_Chinese = 600)
Excluding plasma samples that failed the metabolomics quality control (N = 2)
N_Total = 598
(N_Case = N_Control = 299;
N_Chinese = 598)
Constructing the LE8 score
External Validation
MetaSig
Correlations between metabolite signature and LE8 score
Associations of LE8 score and metabolite signature with CHD
Mediation analysis

Fig. II. Correlations between Life's Essential 8 score and its individual component scores in the SCCS (A) and SWMHS (B). Values in the figures are *Spearman* correlation coefficients. Colors represent the extent of correlations. LE8, life's Essential 8; BMI, body mass index. SCCS, Southern Community Cohort Study; SWMHS, Shanghai Women's and Men's Health Studies.

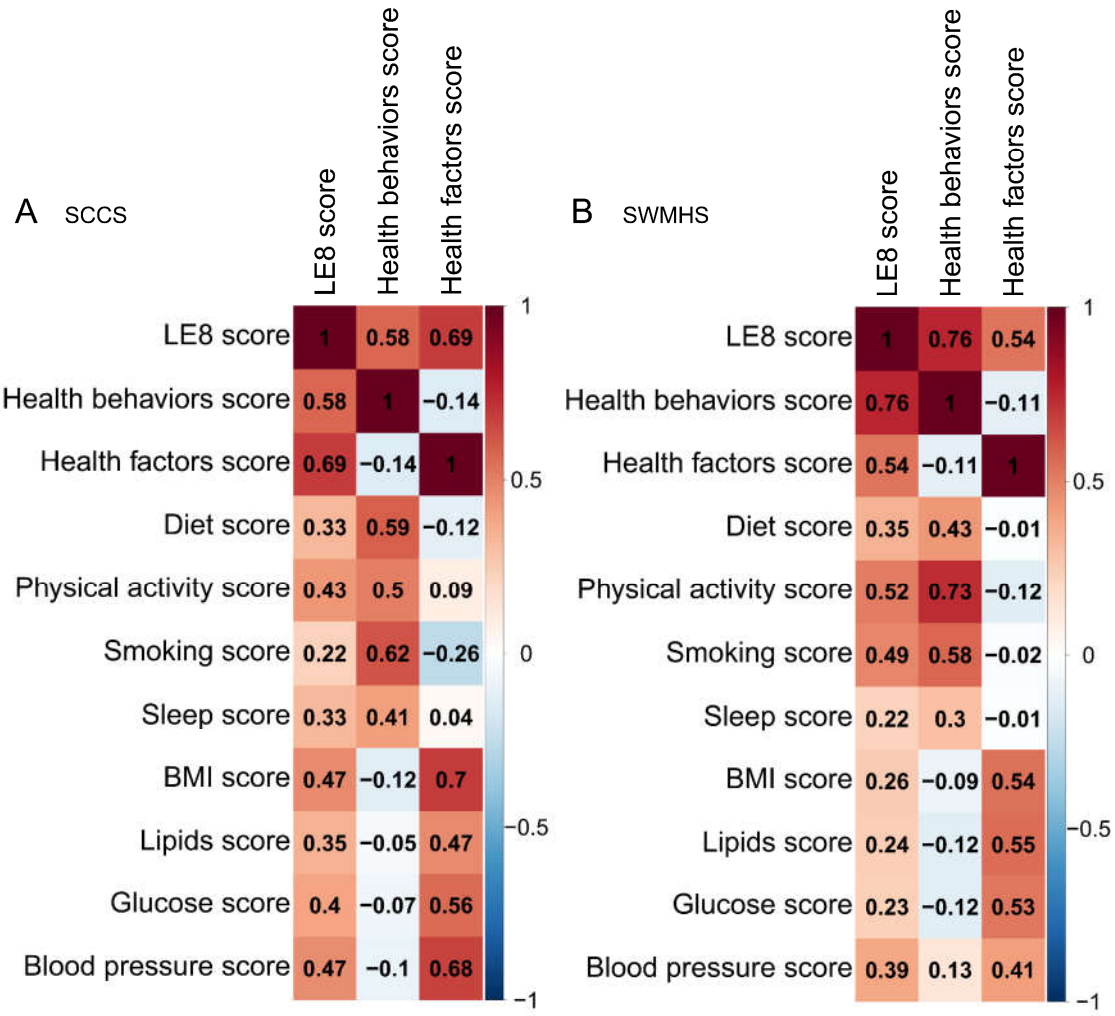


Fig. III. The metabolite signature of health behaviors and its association with risk of CHD. (A) Top 30 metabolites selected by elastic net regression in SCCS. Metabolites were ranked by the absolute value of regression coefficients. **(B)** *Spearman* correlation between MetaSig and health behaviors score in SCCS. **(C)** *Spearman* correlation between MetaSig and health behaviors score in SWMHS. **(D)** The mediation effect of MetaSig on the association between health behaviors score and risk of CHD in SCCS. **(E)** The mediation effect of MetaSig on the association between health behaviors score and risk of CHD in SWMHS. SCCS, Southern Community Cohort Study; SWMHS, Shanghai Women's and Men's Health Studies; MetaSig, metabolite signature; ACME, average causal mediation effects; ADE, average direct effects; CHD, coronary heart disease.

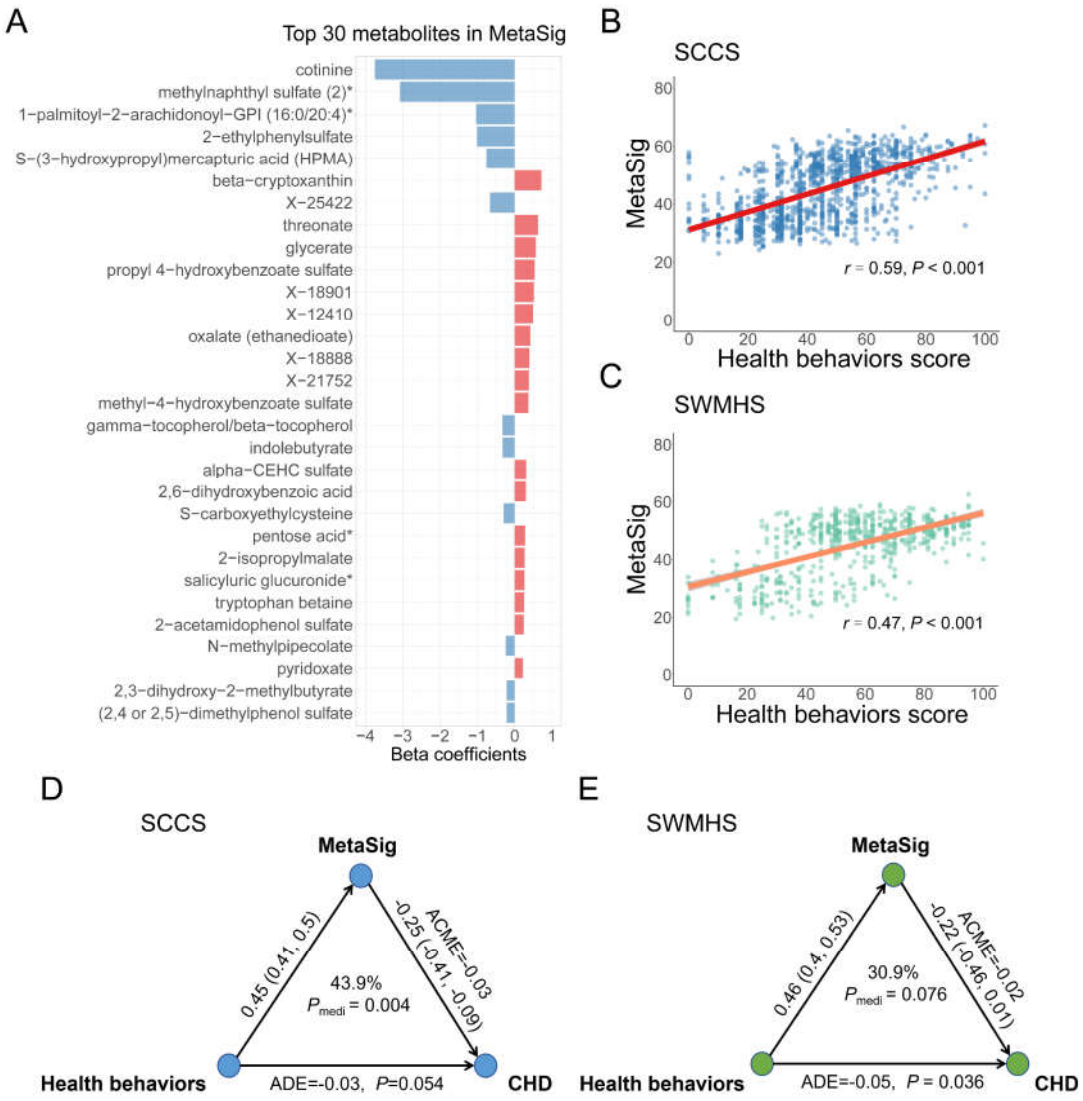
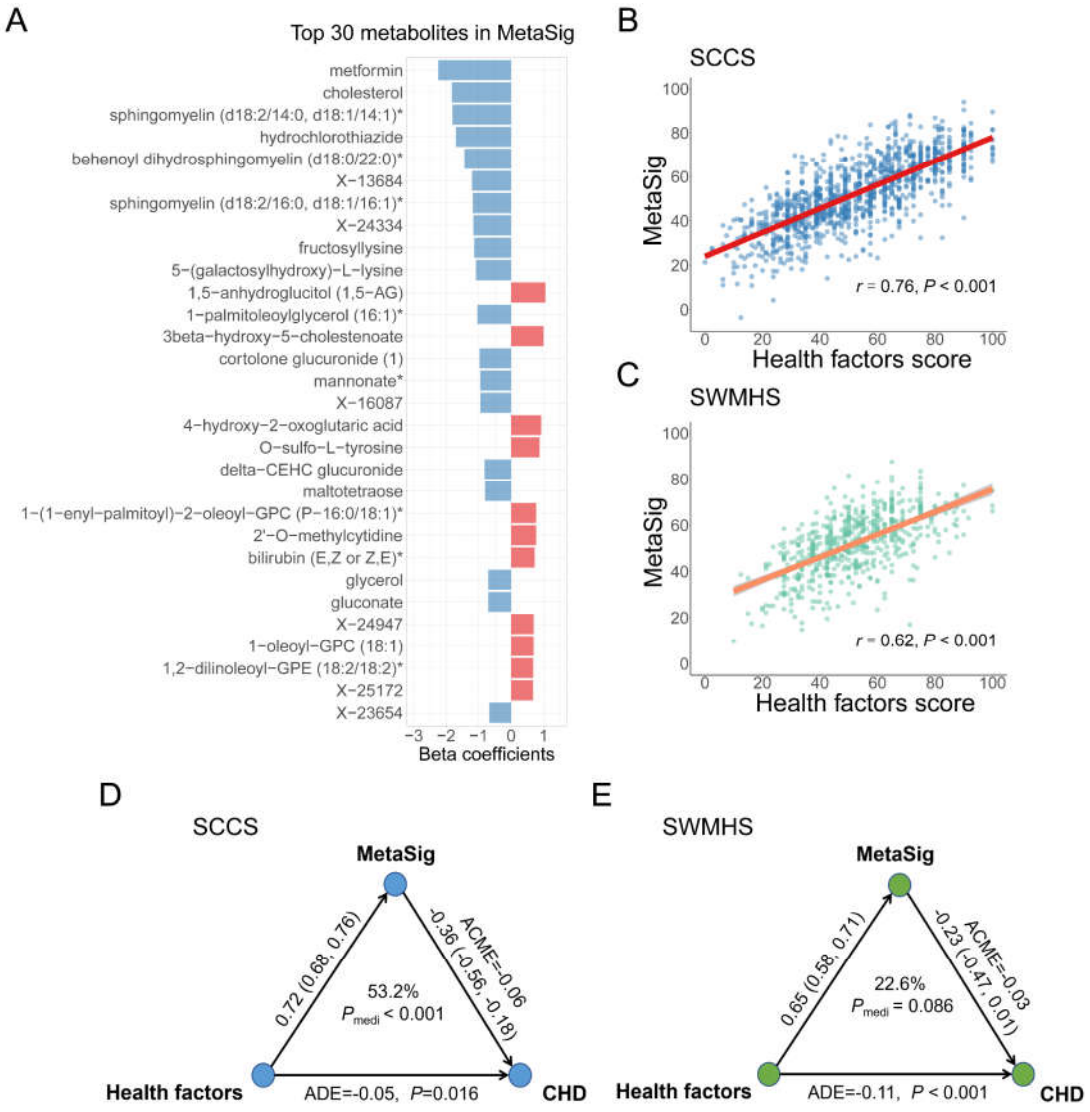

A
Top 30 metabolites in MetaSig
cotinine
methylnaphthyl sulfate (2)*
1−palmitoyl−2−arachidonoyl−GPI (16:0/20:4)*
2−ethylphenylsulfate
S−(3−hydroxypropyl)mercapturic acid (HPMA)
beta−cryptoxanthin
X−25422
threonate
glycerate
propyl 4−hydroxybenzoate sulfate
X−18901
X−12410
oxalate (ethanedioate)
X−18888
X−21752
methyl−4−hydroxybenzoate sulfate
gamma−tocopherol/beta−tocopherol
indolebutyrate
alpha−CEHC sulfate
2,6−dihydroxybenzoic acid
S−carboxyethylcysteine
pentose acid*
2−isopropylmalate
salicyluric glucuronide*
tryptophan betaine
2−acetamidophenol sulfate
N−methylpipecolate
pyridoxate
2,3−dihydroxy−2−methylbutyrate
(2,4 or 2,5)−dimethylphenol sulfate
−4 −3 −2 −1 0 1
Beta coefficients
B
SCCS
MetaSig
0 20 40 60 80
r = 0.59, P < 0.001
0 20 40 60 80 100
Health behaviors score
C
SWMHS
MetaSig
0 20 40 60 80
r = 0.47, P < 0.001
0 20 40 60 80 100
Health behaviors score
D
SCCS
MetaSig
0.45 (0.41, 0.5)
-0.25 (-0.41, -0.09)
ACME=-0.03
43.9%
P_medi = 0.004
Health behaviors
ADE=-0.03, P=0.054
CHD
E
SWMHS
MetaSig
0.46 (0.4, 0.53)
-0.22 (-0.46, 0.01)
ACME=-0.02
30.9%
P_medi = 0.076
Health behaviors
ADE=-0.05, P = 0.036
CHD

Fig. IV. The metabolite signature of health factors and its association with risk of CHD. **(A)** Top 30 metabolites selected by elastic net regression in SCCS. Metabolites were ranked by the absolute value of regression coefficients. **(B)** *Spearman* correlation between MetaSig and health factors score in SCCS. **(C)** *Spearman* correlation between MetaSig and health factors score in SWMHS. **(D)** The mediation effect of MetaSig on the association between health factors score and risk of CHD in SCCS. **(E)** The mediation effect of MetaSig on the association between health factors score and risk of CHD in SWMHS. SCCS, Southern Community Cohort Study; SWMHS, Shanghai Women's and Men's Health Studies; MetaSig, metabolite signature; ACME, average causal mediation effects; ADE, average direct effects; CHD, coronary heart disease.


A
Top 30 metabolites in MetaSig
metformin
cholesterol
sphingomyelin (d18:2/14:0, d18:1/14:1)*
hydrochlorothiazide
behenoyl dihydrosphingomyelin (d18:0/22:0)*
X−13684
sphingomyelin (d18:2/16:0, d18:1/16:1)*
X−24334
fructosyllysine
5−(galactosylhydroxy)−L−lysine
1,5−anhydroglucitol (1,5−AG)
1−palmitoleoylglycerol (16:1)*
3beta−hydroxy−5−cholestenoate
cortolone glucuronide (1)
mannonate*
X−16087
4−hydroxy−2−oxoglutaric acid
O−sulfo−L−tyrosine
delta−CEHC glucuronide
maltotetraose
1−(1−enyl−palmitoyl)−2−oleoyl−GPC (P−16:0/18:1)*
2'−O−methylcytidine
bilirubin (E,Z or Z,E)*
glycerol
gluconate
X−24947
1−oleoyl−GPC (18:1)
1,2−dilinoleoyl−GPE (18:2/18:2)*
X−25172
X−23654
−3 −2 −1 0 1
Beta coefficients
B
SCCS
MetaSig
Health factors score
r = 0.76, P < 0.001
C
SWMHS
MetaSig
Health factors score
r = 0.62, P < 0.001
D
SCCS
MetaSig
0.72 (0.68, 0.76)
-0.36 (-0.56, -0.18)
ACME=-0.06
53.2%
P_medi < 0.001
Health factors
ADE=-0.05, P=0.016
CHD
E
SWMHS
MetaSig
0.65 (0.58, 0.71)
-0.23 (-0.47, 0.01)
ACME=-0.03
22.6%
P_medi = 0.086
Health factors
ADE=-0.11, P < 0.001
CHD