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ORIGINAL RESEARCH

Blood-Based Fingerprint of Cardiorespiratory Fitness and Long-Term Health Outcomes in Young Adulthood

Ravi V. Shah, MD*; Patricia Miller, MS; Laura A. Colangelo, MS; Ariel Chernofsky, MS; Nicholas E. Houstis, MD; Rajeev Malhotra, MD; Raghava S. Velagaleti, MD, MPH; David R. Jacobs Jr, PhD; Kelley Pettee Gabriel, PhD; Jared P. Reis, PhD; Donald M. Lloyd-Jones, MD, ScM; Clary B. Clish, PhD; Martin G. Larson, SD; Ramachandran S. Vasan, MD; Venkatesh L. Murthy, MD, PhD*; Gregory D. Lewis, MD*, Matthew Nayor, MD, MPH*

BACKGROUND: Cardiorespiratory fitness is a powerful predictor of health outcomes that is currently underused in primary prevention, especially in young adults. We sought to develop a blood-based biomarker of cardiorespiratory fitness that is easily translatable across populations.

METHODS AND RESULTS: Maximal effort cardiopulmonary exercise testing for quantification of cardiorespiratory fitness (by peak oxygen uptake) and profiling of >200 metabolites at rest were performed in the FHS (Framingham Heart Study; 2016–2019). A metabolomic fitness score was derived/validated in the FHS and was associated with long-term outcomes in the younger CARDIA (Coronary Artery Risk Development in Young Adults) study. In the FHS (derivation, N=451; validation, N=914; age 54±8 years, 53% women, body mass index 27.7±5.3 kg/m^2), we used LASSO (least absolute shrinkage and selection operator) regression to develop a multimetabolite score to predict peak oxygen uptake (correlation with peak oxygen uptake r=0.77 in derivation, 0.61 in validation; both P<0.0001). In a linear model including clinical risk factors, a ≈1-SD higher metabolomic fitness score had equivalent magnitude of association with peak oxygen uptake as a 9.2-year age increment. In the CARDIA study (N=2300, median follow-up 26.9 years, age 32±4 years, 44% women, 44% Black individuals), a 1-SD higher metabolomic fitness score was associated with a 44% lower risk for mortality (hazard ratio [HR], 0.56 [95% CI, 0.47–0.68]; P<0.0001) and 32% lower risk for cardiovascular disease (HR, 0.68 [95% CI, 0.55–0.84]; P=0.0003) in models adjusted for age, sex, and race, which remained robust with adjustment for clinical risk factors.

CONCLUSIONS: A blood-based biomarker of cardiorespiratory fitness largely independent of traditional risk factors is associated with long-term risk of cardiovascular disease and mortality in young adults.

Key Words: body mass index ■ cardiorespiratory fitness ■ exercise test ■ linear models ■ longitudinal studies

Cardiorespiratory fitness (CRF) has a powerful, sustained impact on cardiometabolic health and longevity across the spectrum of underlying cardiovascular risk and different disease states.[1–6] The association of CRF with improved health outcomes is independent of standard cardiovascular disease (CVD) risk factors, reflecting its role as an integrative assessment of multiorgan system function and metabolism.[7] Accordingly, CRF has been proposed as a fifth vital sign to complement routine clinical assessment and to

Correspondence to: Matthew Nayor, MD, MPH, Boston University Department of Medicine, 72 E Concord St, Suite L-516, Boston, MA 02118.
Email: mnayor@bu.edu
*R. V. Shah, V. L. Murthy, G. D. Lewis, and M. Nayor contributed equally.

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CLINICAL IMPLICATIONS

What Is New?

- We developed a resting blood-based biomarker signature of directly measured cardiorespiratory fitness in a mostly middle-aged sample.
- This biomarker signature included ≈70 circulating metabolites and was correlated with cardiorespiratory fitness across age and sex.
- In a separate sample of younger adults, the biomarker signature was associated with long-term morality and cardiovascular disease risk.

What Are the Clinical Implications?

- Cardiorespiratory fitness is an important predictor of adverse health outcomes that is challenging to measure at the population level.
- Our findings demonstrate the feasibility and prognostic relevance of a blood-based signature of fitness that, if further validated, may facilitate broader incorporation of this important metric in primary prevention settings.

Nonstandard Abbreviations and Acronyms

CARDIA	Coronary Artery Risk Development in Young Adults
CPET	cardiopulmonary exercise testing
CRF	cardiorespiratory fitness
FHS	Framingham Heart Study
VO_2	oxygen uptake; LASSO, least absolute shrinkage and selection operator

improve risk prediction.[6] An important strength of CRF as a clinical vital sign is that it is readily modifiable with lifestyle changes and can be helpful in promoting healthy behaviors.[8] As a result, CRF assessment may be especially relevant for risk prediction in younger individuals who are often at low predicted short-term risk because of their young age, regardless of the presence of risk factors.[9]

Despite its broad potential clinical usefulness, fitness assessment in primary prevention has not been widely adopted, primarily because of cost, feasibility, patient burden, and inaccessibility in some settings.[6] The gold-standard assessment of CRF is peak oxygen uptake (VO_2) by maximal cardiopulmonary exercise testing (CPET), which provides a quantitative value that directly measures metabolic response to exercise and can account for differences in body size and volitional effort.[10] However, CPET requires specialized equipment, trained personnel, and interpretation expertise, limiting its widespread implementation. As a result, substantial efforts have been directed at predicting CRF without having to perform an exercise test, using standard risk factors[11–14] or high-dimensional biomarker techniques.[15–17] However, a blood-based biomarker of CRF that is validated against relevant health outcomes in a large sample of younger community-dwelling adults has not yet been identified. There is good reason to think that the blood metabolome may be able to reflect CRF well because it provides a snapshot of diverse biological processes that are relevant to accommodating the increased metabolic demand of acute exercise.

Here, we use the circulating metabolome to develop a blood-based biomarker of directly measured peak VO_2 in a large sample of community-dwelling adults from the FHS (Framingham Heart Study). Given the importance of CRF in young adulthood, when traditional CVD risk prediction is challenging, we evaluated the association of this multimetabolite score with incident CVD and mortality over ≈25 years in young adults in the CARDIA (Coronary Artery Risk Development in Young Adults) study. Our ultimate goal was to evaluate the hypothesis that peak VO_2 could be represented by a blood-based metabolic biomarker translatable across populations, and that this biomarker would contribute to risk assessment in a younger population.

METHODS

Data Sharing

The data supporting the study findings will be made available on reasonable request. FHS and CARDIA data are publicly available and can be accessed through the National Institutes of Health database of genotypes and phenotypes (https://www.ncbi.nlm.nih.gov/gap/).

Study Samples and Clinical Characterization

Framingham Heart Study

Enrollment of the FHS Generation 3 and Omni Minority Generation 2 cohorts and their participation in maximum effort CPET at a routine study visit (2016–2019) are reported.[17–21] From the 3117 participants who performed CPET, we excluded individuals with nonfasting status (N=61), missing peak VO_2 (N=12), missing covariates (N=6), or with inadequate volitional effort during exercise (peak respiratory exchange ratio <1.05; N=136), yielding 2902 individuals, of whom 1365 had blood metabolite profiling performed (Figure 1). This subsample was divided into derivation (N=451) and validation (N=914) samples for the present investigation (assignment based on quantification batch to minimize batch effects). Blood metabolite levels were quantified using hydrophobic interaction chromatography in

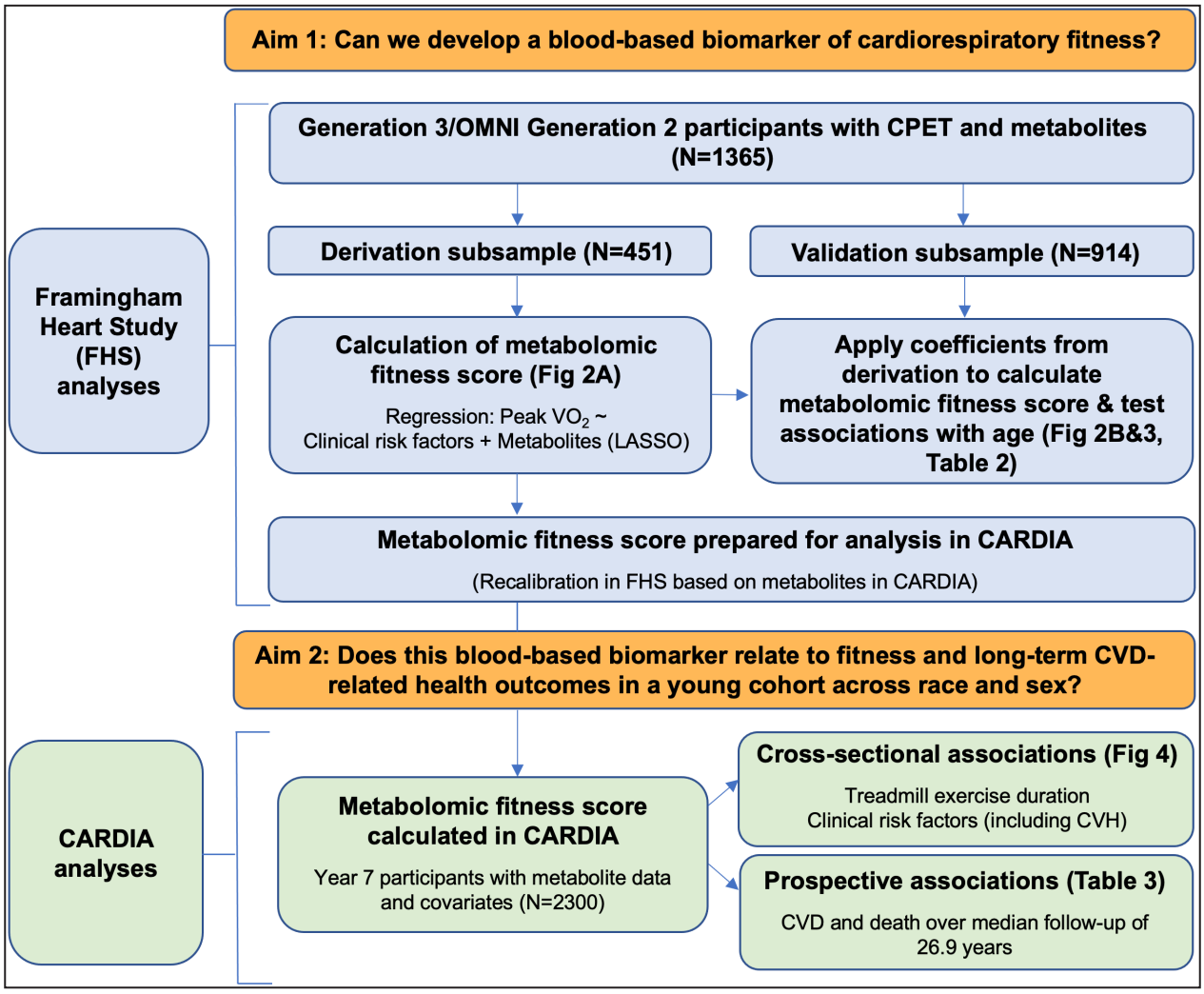

Figure 1. Overview of the study design.
The research questions and study design are displayed. CARDIA indicates Coronary Artery Risk Development in Young Adults; CPET, cardiopulmonary exercise testing; CVD, cardiovascular disease; CVH, cardiovascular health score (Life's Simple 7); FHS, Framingham Heart Study; LASSO, least absolute shrinkage and selection operator; and VO_2, oxygen uptake.

the positive ionization mode, which measures amino acids, lipids, and select polar compounds.[17] Detailed methods for metabolite quantification are available in Data S1. Diabetes was defined as fasting blood glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, or use of glucose lowering medications. Smoking was ascertained by self-report as current or former smoking. Prevalent CVD was defined as a history of myocardial infarction, stroke, peripheral arterial disease, or heart failure.

Coronary Artery Risk Development in Young Adults Study

Methods for the CARDIA study have been previously described.[22–25] Our analytic subsample included 2300 participants from the year 7 study visit in the CARDIA study (1992–1993; baseline for this analysis) with available metabolite measurements and covariates.[26] Metabolites were quantified using the same procedures and in the same laboratory as the FHS samples. Symptom-limited maximal exercise testing at year 7 and year 20 in the CARDIA study were performed as described.[27,28] Tests in which participants reached 85% maximal predicted heart rate (as defined by published formulas) were included,[29] and cohort-approved imputation strategies were used for tests from the Minnesota CARDIA site to address a protocol violation.[30] Standard risk factors and cardiovascular health score (based on the American Heart Association Life's Simple 7) were measured at year 7.[31,32] Diabetes was defined as fasting blood glucose ≥126 mg/mL, use of

glucose lowering medications, or self-report. Physical activity was assessed via questionnaire.[33] Cigarettes smoked per day was assessed by questionnaire, and estimated lifetime pack-years exposure to cigarettes was also determined. CVD included incident fatal/nonfatal myocardial infarction, acute coronary syndrome, transient ischemic attack, stroke, hospitalization for heart failure, or intervention for carotid or peripheral artery disease.

Study protocols were approved by the institutional review boards at Boston University Medical Campus, the Massachusetts General Hospital, and each CARDIA study site. All participants provided written informed consent.

Fitness Assessment

In the FHS, maximal effort CPET was performed on a cycle ergometer (Lode, Groningen, the Netherlands).[21] The exercise protocol consisted of 3 minutes of unloaded exercise followed by incremental ramp exercise (15–25 W/min incremental protocol). We obtained breath-by-breath gas exchange measures (MedGraphics, St. Paul, MN), and peak VO_2 was defined as the highest 30-second median value during the final minute of loaded exercise.[17,20] In the CARDIA study, maximal symptom-limited treadmill exercise testing was performed using a modified Balke graded protocol.[30]

Statistical Analysis

Development of a Metabolomic Fitness Score in the FHS

Metabolites in the FHS were processed with imputation, log-transformation, and rank normalization as described,[17] and mean-centered and standardized across combined derivation and validation sets for analysis. We used penalized regression (least absolute shrinkage and selection operator [LASSO]) to create a multimetabolite score of peak VO_2 (metabolomic fitness score) in the FHS derivation subsample (N=451). LASSO regression was selected to avoid overfitting, address collinearity across metabolites, and provide a parsimonious multimetabolite model for peak VO_2. LASSO regression has the benefit of selecting a subset of metabolites to optimize model fit. Our aim was to develop a metabolite score independent of standard risk factors, so we forced (unpenalized) adjustment for age, sex, body mass index (BMI), systolic blood pressure, hypertension treatment status, total cholesterol, high-density lipoprotein cholesterol, prevalent CVD, diabetes, and smoking status in the LASSO model. Continuous covariates and peak VO_2 were log-transformed; forced adjustments in LASSO were not standardized. Given that we sought to generate a metabolite-only score (conditioned on the clinical covariates), our final score only included metabolites: the LASSO coefficient for each metabolite was multiplied by the corresponding metabolite level, which was summed across all metabolites to generate the metabolomic fitness score. This score was standardized across the FHS derivation and validation sample. We tested the correlation of this score with measured peak VO_2 in the derivation and validation subsamples (Pearson correlation). To visualize the relations among age, metabolomic fitness score, and measured peak VO_2, we estimated marginal means for peak VO_2 (log_2-transformed for modeling and re-exponentiated back for visualization) in a linear model adjusted for the clinical covariates forced into the initial LASSO model. We evaluated for effect modification by sex and age on the association of the metabolomic fitness score and peak VO_2 using multiplicative interaction terms.

Defining the Metabolomic Fitness Score in the CARDIA Study

Of the 74 metabolites selected in the LASSO model in the FHS, 61 were also quantified in the CARDIA study. We used linear regression in the FHS with the full metabolomic fitness score as the dependent variable and the 61 overlapping metabolites (with the CARDIA study) as independent variables. The coefficients for these 61 metabolites were used to generate the metabolomic fitness score in the CARDIA study. Of note, correlation of the full score (based on 74 metabolites) and the 61 metabolites' score in the FHS was high (r=0.97). The coefficients from the reduced metabolomic fitness score were applied to log-transformed, mean-centered, and standardized metabolite levels in the CARDIA study to generate metabolomic fitness scores for each participant. The score was calculated in all individuals with metabolites and standardized (N=2358). The analytic cohort was those with complete clinical covariates (N=2300).

Cross-Sectional and Prospective Associations of the Metabolomic Fitness Score in the CARDIA Study

We related the metabolomic fitness score with year 7 (ie, contemporaneous with metabolite profiling) treadmill exercise duration, cardiovascular health score, and standard cardiometabolic risk factors using Spearman correlations. To evaluate the association of the metabolomic fitness score measured at year 7 with future fitness (year 20 treadmill exercise duration), we constructed a linear model, adjusted for age, sex, race, year 7 BMI, and year 7 treadmill time. Using Cox regression, we measured the association of the metabolomic fitness score with long-term mortality and CVD in the CARDIA study. We performed 3 sets of models: (1)

adjusted for age, sex, and race; (2) additionally adjusted for cardiovascular health score (Life's Simple 7); and (3) adjusted for age, sex, race, and standard clinical risk factors (lifetime pack-years smoking status, cigarettes per day, physical activity, total and high-density lipoprotein cholesterol, diabetes, BMI, systolic blood pressure). For comparison, we constructed models for mortality and CVD as a function of year 7 treadmill time (standardized). C statistics were computed via the method of Harrell.[34]

All analyses were performed in R (www.rproject.org) or SAS (SAS Institute, Cary, NC). A P value <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics

The characteristics of the FHS derivation and validation samples are shown in Table 1. These groups were well matched, with a higher proportion of women in the derivation sample. Overall, FHS participants had a mean age of 54±8 years, BMI of 27.7±5.3 kg/m^2, peak VO_2 of 23.6±7.2 mL/kg per min, and were generally representative of the larger FHS cohort including individuals without metabolite quantification (Table S1). By comparison, CARDIA study participants were younger (age 32±4 years at CARDIA year 7), with 44% women and 44% Black individuals (Table 1). Accordingly, overall cardiometabolic risk was lower in CARDIA study participants, with a lower BMI and lower prevalence of diabetes compared with the FHS sample.

Development and Characterization of a Metabolomic Fitness Score in the FHS

We constructed a multimetabolite model for the prediction of peak VO_2 with LASSO regression from 201 assayed metabolites. Selected metabolites and their regression coefficients are shown in Figure 2A and Table S2. The metabolite coefficients from this LASSO regression were used to construct a metabolomic fitness score, which demonstrated good correlation with directly measured peak VO_2 (FHS derivation r=0.77, FHS validation r=0.61; both P<0.0001; Figure 2B).

Several metabolites previously implicated in cardiometabolic disease emerged in the final metabolomic fitness score in directions consistent with prior disease associations, including higher betaine (choline metabolite inversely associated with diabetes risk[35]), pantothenate (vitamin B5 cofactor for fatty acid metabolism[36]), 1-methylnicotinamide (implicated in muscle lipolytic capacity and inflammation[37,38]), arginine (NO biosynthetic precursor, implicated in endothelial health[39]), and lower trimethylamine N-oxide (a dietary choline metabolite produced from gut microbiota and related to CVD risk[40]) and dimethylguanidino valeric acid (linked to liver steatosis and responsive to exercise[41–43]), as well as a series of carnitines (involved in fatty acid oxidation capacity and mitochondrial function[16]).

In our sample, peak VO_2 was lower at older ages, but there was variability in the peak VO_2 values observed for a given age (Figure 3A). We therefore explored the joint relations of the metabolomic fitness score and age with peak VO_2. In a multivariable linear model including age, clinical risk factors, and the metabolomic

Table 1. Characteristics of the Study Samples

Characteristic	FHS		CARDIA, N=2300
	Derivation, N=451	Validation, N=914	
Age, y	53±8	54±8	32±4
Women, n (%)	282 (63)	444 (49)	1021 (44)
White race, n (%)	444 (98)	904 (99)	1277 (56)
Body mass index, kg/m^2	27.3±5.5	27.9±5.2	25.7±5.0
Systolic blood pressure, mm Hg	118±14	119±14	108±11
Total cholesterol, mg/dL	188±33	192±34	176±33
HDL cholesterol, mg/dL	62±19	61±20	54±14
Prevalent CVD, n (%)	15 (3)	38 (4)	0 (0)
Diabetes, n (%)	27 (6)	59 (7)	46 (2)
Smoking, n (%)*	166 (37)	336 (37)	903 (39)
Peak VO_2, mL/kg per min	23.4±6.9	23.7±7.3	…
Treadmill duration, min*	…	…	9.9±2.6
Cardiovascular health score*	…	…	10.2±1.8
CARDIA physical activity score, exercise units	…	…	375±282

Displayed values are mean±SD for continuous variables and n (%) for categorical variables. CARDIA variables are at year 7. CARDIA indicates Coronary Artery Risk Development in Young Adults; CVD, cardiovascular disease; FHS, Framingham Heart Study; HDL, high-density lipoprotein; and VO_2, oxygen uptake.

*Cardiovascular health score is available in 2205 individuals. Smoking is reported as current/former smoking. Treadmill duration reported at year 7 in CARDIA in participants achieving 85% maximal predicted heart rate (N=1985/2102 with available tests and metabolites).

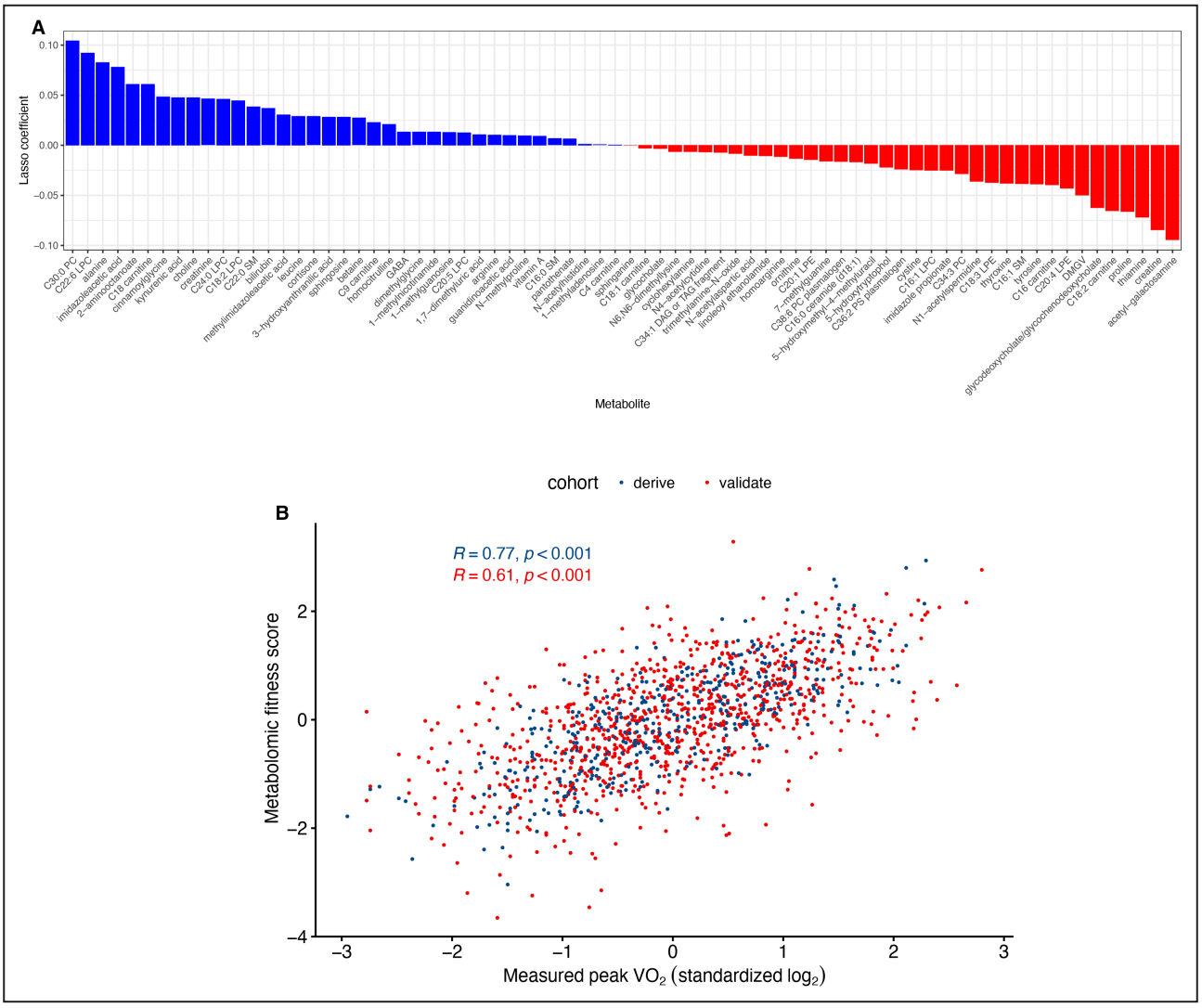

Figure 2. Developing the metabolomic fitness score in the FHS (Framingham Heart Study).
A, Coefficients of metabolites from least absolute shrinkage and selection operator (LASSO) regressions that are used in calculation of the metabolomic fitness score. Abbreviations for metabolite names are expanded in Table S2. **B**, Correlation between the metabolomic fitness score and peak oxygen uptake (VO_2). Peak VO_2 is log_2-transformed and standardized. The blue dots represent the FHS derivation group, and the red dots represent the FHS validation group. The noted correlation coefficients (*R*) and corresponding *P* values refer to Pearson correlations.

fitness score, a ≈1-SD higher metabolomic fitness score had the equivalent magnitude of association with log-transformed peak VO_2 of a 9.2-year age increment (Table 2). As shown in Figure 3B, at any given age, individuals with a 1-SD higher metabolomic fitness score displayed a peak VO_2 value equivalent to people nearly 10 years younger. We did not observe evidence of effect modification by age or sex on the relation between the metabolomic fitness score and peak VO_2.

Relations of the Metabolomic Fitness Score With Future CRF and Long-Term Mortality in the CARDIA Study

The metabolomic fitness score was refitted for use in the CARDIA study using metabolites measured in both cohorts (FHS and CARDIA), resulting in a reduced 61-metabolite score that was highly correlated with the original 74-metabolite score (r=0.97; Table S2). The metabolomic fitness score was associated with treadmill exercise duration at year 7 in the CARDIA study (ρ=0.56, P<0.0001; Figure 4A), but was not strongly associated with standard cardiometabolic risk factors and composite measures, such as the cardiovascular health score (Figure 4B). Among individuals with treadmill exercise duration at year 7 and year 20 who achieved a target 85% maximally predicted heart rate, we observed a mean decline in exercise duration between year 7 and year 20 of 2.1 minutes, with a higher metabolomic fitness score at year 7 associated with greater exercise capacity at year 20 (β=0.283 minutes

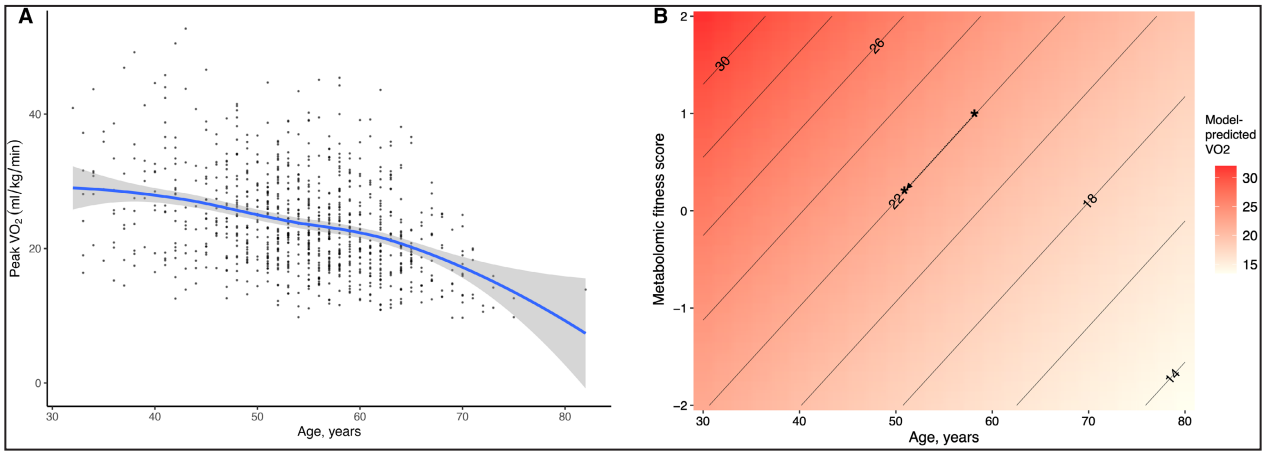

Figure 3. Age, metabolomic fitness score, and peak oxygen uptake (VO_2).
A, Relationship between peak VO_2 and age in the Framingham Heart Study (in the validation subsample). The blue line with overlaying gray confidence intervals represents a Loess spline across the data. **B**, Joint effects plot depicting the relation between age, the metabolomic fitness score, and peak VO_2. We estimated marginal means for peak VO_2 across age and metabolomic fitness score using a fully adjusted linear model as described (see Methods). The contour lines and heat colors represent model-estimated peak VO_2. The metabolic VO_2 score is displayed as the mean (0), and each number represents a standard deviation higher or lower than the mean. An illustrative example is shown with an asterisk (*) on the plot; a 60-year-old with a metabolomic fitness score ≈1 SD above the mean (ie, 1) has the same peak VO_2 as someone ≈51 years old with a metabolomic fitness score at the mean (ie, 0).

per standard deviation, $P<0.0001$), after adjustment for age, sex, race, year 7 BMI, and year 7 exercise duration.

Finally, we examined whether the metabolomic fitness score was associated with long-term mortality and incident CVD in the CARDIA study (Table 3). We observed 153 deaths and 116 CVD events in 2300 individuals during a median follow-up of 26.9 years. A 1-SD higher metabolomic fitness score was associated with a 44% lower risk for mortality and 32% lower risk for CVD in models adjusted for age, sex, and race. These associations were robust to additional adjustment for clinical risk factors or cardiovascular health score (Table 3). Inclusion of the metabolomic fitness score improved discrimination for mortality, but not for CVD, in models fully adjusted for clinical risk factors. By comparison, year 7 treadmill time was associated with a similar reduced hazard of death (hazard ratio [HR], 0.56 per SD [95% CI, 0.45–0.70]; $P<0.0001$) and a lower hazard of CVD (HR, 0.53 per SD [95% CI, 0.41–0.68]; $P<0.0001$) in age, sex, and race-adjusted models.

Table 2. Association of Metabolomic Fitness Score and Clinical Risk Factors With Peak VO_2

Variable	β coefficient±SE	*P* value
Age	−0.033±0.003	<0.0001
Female sex	−0.831±0.055	<0.0001
Cardiovascular disease	−0.084±0.106	0.43
Diabetes	−0.356±0.091	0.0001
Hypertension treatment	−0.099±0.058	0.09
Smoking	−0.193±0.043	<0.0001
Log body mass index	−1.144±0.100	<0.0001
Log systolic blood pressure	0.210±0.136	0.12
Log total cholesterol	−0.209±0.087	0.02
Log HDL cholesterol	0.210±0.059	0.0004
Metabolomic fitness score	0.303±0.025	<0.0001

This linear model was built in the Framingham Heart Study validation cohort (N=914 individuals). Logarithms are base 2. The dependent variable in this model is peak oxygen uptake (VO_2) (expressed as milliliters per kilogram per minute, log-transformed, and standardized). The ratio of the estimated regression coefficient for the metabolomic fitness score (0.303 per ≈1 SD in the score) was divided by the regression coefficient for age (−0.033 per year) to yield the relative association effect size of 1 SD of the score relative to years of age (≈9.2 years lower age has an equivalent association with log-peak VO_2 as ≈1 SD higher in the metabolomic fitness score).

DISCUSSION

We developed and validated a circulating multimetabolite biomarker of peak VO_2 in 2 complementary samples with representation across the adult life course. The metabolomic fitness score explained a significant amount of the variation in exercise capacity in both samples. In the FHS, a 1-SD higher metabolomic fitness score was associated with the same increment in peak VO_2 as nearly 10 years younger age, reflecting the importance of metabolic health in age-related CRF. In the CARDIA study, a higher metabolomic fitness score was not only associated with a greater exercise treadmill time measured at the same exam cycle, but also with an attenuated decline in fitness over the ensuing decade. In addition, during ≈25 years of follow-up, the

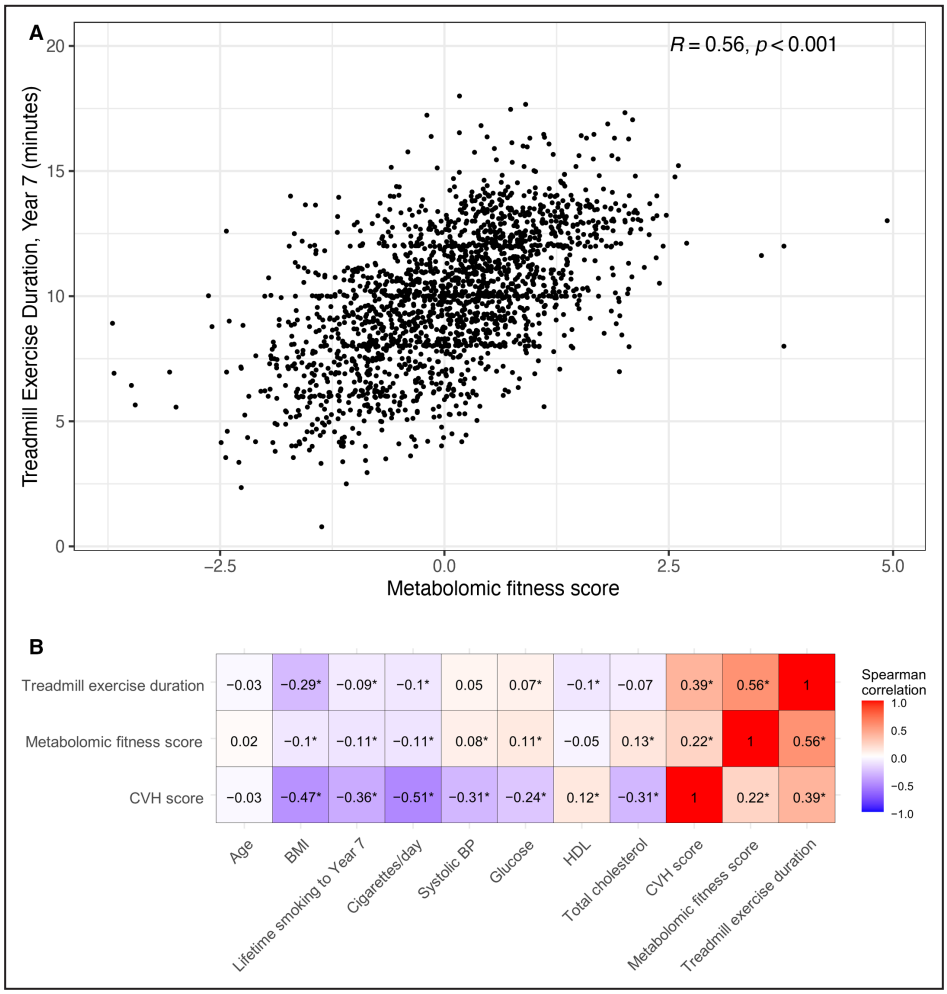

Figure 4. Correlation of the metabolomic fitness score with treadmill time and cardiovascular risk factors in the CARDIA (Coronary Artery Risk Development in Young Adults) study.

A, Scatter plot of the year 7 treadmill exercise duration in the CARDIA study vs the metabolomic fitness score. This plot included 1985 individuals at year 7 in the CARDIA study. *R* represents Spearman correlation. **B**, Spearman correlation matrix of cardiovascular risk factors and key metrics of fitness at year 7 in the CARDIA study. Each cell shows a Spearman correlation coefficient between each risk factor and treadmill exercise duration at year 7, the metabolomic fitness score, or cardiovascular health (CVH) score. Asterisks represent statistical significance at 0.05 level with Bonferroni type 1 error correction ($P<0.0016$). BMI indicates body mass index; BP, blood pressure; and HDL, high-density lipoprotein.

metabolomic fitness score was significantly associated with incident CVD and all-cause mortality after adjusting for standard CVD risk factors. For mortality, the metabolomic fitness score provided significant though modest improvement in model discrimination beyond standard clinical risk factors and cardiovascular health score. Of note, several metabolites within the final score had been previously implicated in fitness and training effects. Collectively, these findings support not only the importance of metabolic health to CRF, but they also demonstrate that a blood-based fitness biomarker can augment CRF prediction beyond clinical risk factors, provide prognostic information at an early age, and potentially enable widespread application of blood-based metabolic fitness assessment for CVD risk prevention.

Despite decades of study establishing CRF itself as a powerful prognostic indicator, it has not reached widespread clinical use because of cost, added patient burden, and relative inaccessibility.[4,6,44,45] Importantly, fitness testing may be more likely to occur in higher resource settings, potentially serving to exacerbate health care disparities.[6] A biomarker of CRF that can be assessed from a single blood sample at rest would directly address these challenges, with substantial potential as a tool in the prevention armamentarium.

Table 3. Association of Metabolomic Fitness Score With Outcomes in CARDIA

Outcome	No. of events/no. at risk	Standardized HR (95% CI)	*P* value	C index, base model	C index, base model+metabolomic fitness score	Δ C index (95% CI)
Mortality						
Model 1: age-, sex-, race-adjusted	153/2300	0.56 (0.47 to 0.68)	<0.0001	0.641	0.706	0.064 (0.031 to 0.097)
Model 2: age-, sex-, race-, CVH-adjusted	144/2205	0.60 (0.50 to 0.73)	<0.0001	0.698	0.727	0.030 (0.006 to 0.053)
Model 3: age-, sex-, race-, risk factor-adjusted	153/2300	0.64 (0.52 to 0.77)	<0.0001	0.728	0.746	0.018 (0.001 to 0.036)
Cardiovascular disease						
Model 1: age-, sex-, race-adjusted	116/2300	0.68 (0.55 to 0.84)	0.0003	0.675	0.698	0.024 (0.000 to 0.048)
Model 2: age-, sex-, race-, CVH-adjusted	107/2205	0.75 (0.59 to 0.95)	0.016	0.739	0.746	0.008 (–0.008 to 0.023)
Model 3: age-, sex-, race-, risk factor-adjusted	116/2300	0.77 (0.61 to 0.98)	0.034	0.764	0.772	0.009 (–0.004 to 0.021)

Clinical risk factors for model adjustment included: diabetes, body mass index, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, cigarettes per day at year 7, lifetime pack-years smoking history up to year 7, CARDIA physical activity at year 7. CARDIA indicates Coronary Artery Risk Development in Young Adults study; CVH, cardiovascular health score; and HR, hazard ratio.

Because CRF can be improved with increased physical activity,[8] a biomarker of CRF may also prove to be an important tool for evaluating the response to behavioral modifications. Accordingly, we observed effect sizes for the metabolomic fitness score that were comparable to directly measured treadmill duration in the CARDIA study. The improved discrimination (C index) observed for mortality only is intriguing and hypothesis generating, especially given the lack of strong association between CVD risk factors and the metabolomic fitness score (Figure 4B). Although this is likely partially because of low CVD event rates in the CARDIA study (a younger sample), the metabolomic fitness score may also capture elements of metabolism relevant to a broader array of outcomes linked to CRF (eg, cancer[46]), which is an intriguing possibility that requires study in larger cohorts. More broadly, the validation of the metabolomic score in a younger group (CARDIA) not at high short-term CVD risk[9,47] implicates serial, early assessment of fitness via a blood-based biomarker as a novel method to improve prognostic risk assessment to promote health at a preclinical stage in disease.

The study of 2 large cohorts across a broad age range enables unique insights into the role of CRF and metabolism in cardiovascular aging. Given its reliance on the function of multiple organ systems[48,49] and established prognostic importance,[1,2] CRF is considered an integrative candidate measure of cardiovascular aging.[50] Recent work observed that metabolism (as captured by daily energy expenditure) is stable from young adulthood to older age, and then begins to decline, suggesting transitions in metabolism occurring at distinct points in life.[51] We observed that individuals with higher metabolomic fitness scores in the FHS exhibited a similar expected peak VO_2 as younger individuals. Furthermore, a higher metabolomic fitness score at the index visit was associated with an attenuation of age-related decline in CRF in the CARDIA study, implicating metabolic health (by circulating metabolite profiling) in explaining some of the variation in age-related declines in CRF. Although the average energy expenditure may remain constant over much of the adult age range, the ability of the entire system to augment oxygen consumption declines with age (Figure 3), and the variation in that decline may be reflected in heterogeneity in metabolism at the individual level.

Despite its established role as a marker of general health and CVD risk, the underlying mechanisms linking greater CRF with protection from CVD are incompletely understood. With increasing availability of high-throughput biomarker assays (eg, metabolomics, proteomics), our group and others have recently demonstrated molecular correlates of CRF in circulating blood.[15–17] These studies have implicated pathways involved in regulation of energy balance, metabolic health, immune response, skeletal muscle integrity, and adiposity as potential mediators of CRF.[15–17] A consistent finding across these studies is that greater CRF is closely linked to favorable systemic metabolic health. The current investigation builds on these prior reports by demonstrating that a score comprising circulating markers of diverse metabolic processes can be developed to partially capture the links between metabolism, fitness, and cardiovascular health outcomes. Individual metabolites comprising the metabolomic fitness score include several previously implicated in

cardiometabolic disease (eg, dimethylguanidino valeric acid,[41–43] betaine,[35] trimethylamine N-oxide,[40] arginine[39]), broad metabolic pathways related to energy use (eg, pantothenate,[36] 1-methylnicotinamide,[37,38] carnitine species), and several novel metabolites with less well-defined roles in metabolic health and CRF. Future studies with dedicated study designs are warranted to evaluate whether modulation of any of these metabolic pathways leads to improvements in CRF.

Overall, the current study adds to the increasing data linking the molecular basis for exercise responses to clinical outcome.[15–17,43,52] The unique advantages of the present investigation include the use of a large sample of individuals with careful CRF phenotyping with extension to a geographically and temporally separate population with a younger age and racial diversity for validation. Nevertheless, the study has several limitations that merit mention. A major barrier to clinical translation of small molecule-based methods is the need for absolute quantification to determine appropriate clinical thresholds.[53] In addition, the metabolomic fitness score was standardized in our samples, and its distribution is likely to differ in other populations; larger, confirmatory studies are necessary to establish population-level distributions and for further validation and refinement of the metabolomic fitness score. Although we cannot specifically identify all sources of confounding for metabolic biomarkers of fitness, the methods used here (forced adjustment in LASSO models for risk factors) allowed us to derive a score conditioned on established CVD risk factors. We cannot comment on whether the metabolomic fitness score is mutable over time with intervention (eg, fitness, diet, or weight change); ongoing work in large National Institutes of Health consortia[52] and other cohort studies will address this premise. The techniques and modalities for exercise testing differed in the FHS and CARDIA study. Although this might influence the relation of the metabolomic fitness score derived in the FHS with exercise duration in the CARDIA study, the fact that the correlation is comparable in the 2 samples serves to improve the generalizability of our findings. We did not compare the metabolomic fitness score with other methods to estimate CRF and its relative strengths and weaknesses compared with other methods (eg, submaximal fitness tests or additional resting assessments), and therefore that cannot be directly addressed. Finally, we did not assess changes in the metabolomic fitness score over time. Therefore, how the metabolomic fitness score changes with aging and whether this might differ across different trajectories of age-related fitness declines remains an open question.

In conclusion, we developed a blood-based multimetabolite score of peak VO_2, demonstrating association with age-related differences in CRF and long-term CVD and mortality in young adults. Higher scores in older adults were associated with a similar CRF as younger individuals with lower scores, implicating maintenance of healthy metabolism over the life course in CRF and cardiovascular aging broadly (given association with CVD and mortality). These efforts support the notion that blood-based biomarkers of CRF are feasible and underpin future efforts focused on translation of biomarkers of CRF as modifiable markers of lifestyle interventions and CVD risk.

ARTICLE INFORMATION

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Affiliations

Vanderbilt Translational and Clinical Research Center, Cardiology Division, Vanderbilt University Medical Center, Nashville, TN (R.V.S.); Department of Biostatistics, Boston University School of Public Health, Boston, MA (P.M., A.C., M.G.L.); Department of Preventive Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL (L.A.C., D.M.L.); Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA (N.E.H., R.M., G.D.L.); Cardiology Section, Department of Medicine, Boston VA Healthcare System, West Roxbury, MA (R.S.V.); Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN (D.R.J.); Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL (K.P.G.); Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, Bethesda, MD (J.P.R.); Division of Cardiology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL (D.M.L.); Broad Institute of MIT and Harvard, Cambridge, MA (C.B.C.); Boston University's and National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA (M.G.L., R.S.V.); Sections of Cardiovascular Medicine and Preventive Medicine and Epidemiology, Department of Medicine, Boston University School of Medicine, Boston, MA (R.S.V., M.N.); Department of Epidemiology, Boston University School of Public Health, and the Center for Computing and Data Sciences, Boston University, Boston, MA (R.S.V., V.L.M.); Division of Cardiovascular Medicine, Department of Medicine, and Frankel Cardiovascular Center University of Michigan, Ann Arbor, MI (V.L.M.); and Pulmonary Critical Care Unit, Massachusetts General Hospital, Boston, MA (G.D.L.).

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Shah has served as a consultant for Myokardia, Best Doctors, Amgen, and Cytokinetics. Dr Shah is a coinventor on a patent for ex-RNAs signatures of cardiac remodeling. Dr Shah's spouse works for UpToDate (Wolters Kluwer). Dr Murthy owns stock in Amgen, General Electric, and Cardinal Health. He has received speaking honoraria from, serves as a scientific advisor for and owns stock options in Ionetix. He has received research funding and speaking honoraria from Siemens Medical Imaging. He has received nonfinancial research support from INVIA Medical Imaging Solutions. Dr Malhotra receives research funding from the National Institutes of Health, the American Heart Association, Bayer, and Amgen; serves as a consultant for Myokardia/BMS and Third Pole; and is a cofounder of Patch, Inc. Dr Malhotra is a coinventor for a patent on pharmacologic bone morphogenic protein inhibitors (along with Mass General Brigham) for which he receives royalties from Keros Therapeutics. Dr Malhotra also receives royalties from UpToDate for scientific content authorship. Dr Lewis has research funding from the National Institutes of Health and the American Heart Association as well as Amgen, Cytokinetics, Applied Therapeutics, AstraZeneca, and Sonivie in relation to projects and clinical trials investigating exercise capacity that are distinct from this work. He has served as a scientific advisor for Pfizer, Merck, Boehringer-Ingelheim, Novartis, American Regent, Relypsa, Cyclerion, Cytokinetics, and Amgen and receives royalties from UpToDate for scientific content authorship related to exercise physiology. Dr Nayor has received speaking honoraria from Cytokinetics. The remaining authors have nothing to disclose. The views expressed in this article are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute, the National Institutes of Health, or the U.S. Department of Health and Human Services.

Supplemental Material

Data S1
Tables S1–S2

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SUPPLEMENTAL MATERIAL

Data S1. Supplemental Methods

Metabolite Profiling

The metabolite profiling techniques have been previously published (Nayor *et* al, Circulation, 2020;142:1905-1924) and are reproduced exactly here for fidelity of scientific replication. The hydrophobic interaction chromatography in the positive ionization mode (HILIC-positive) platform consisted of a liquid chromatography mass spectrometry (LC-MS) system composed of a Shimadzu Nexera X2 U-HPLC (Shimadzu Corp) coupled to a Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific). Plasma samples (10 µL) were prepared via protein precipitation with the addition of 9 volumes of 74.9:24.9:0.2 v/v/v acetonitrile/ methanol/formic acid containing stable isotope-labeled internal standards (valine-d8, Sigma-Aldrich; and phenylalanine- d8, Cambridge Isotope Laboratories). Samples were first centrifuged (10 minutes, 9000g, 4°C), and then the supernatants were injected directly onto a 150x2 mm, 3-µm Atlantis HILIC column (Waters). Each column was eluted isocratically with a flow rate of 250 µL/min with 5% mobile phase A (10 mmol/L ammonium formate and 0.1% formic acid in water) for 0.5 minutes followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 minutes. MS analyses were performed by using electrospray ionization in the positive ion mode. The ion spray voltage was 3.5 kV, with a capillary temperature of 350°C and maximum ion time of 250 ms. Raw data from Q Exactive/Exactive Plus instruments were processed using TraceFinder software (Thermo Fisher Scientific) and Progenesis QI (Nonlinear Dynamics), whereas MultiQuant (SCIEX) was used to process 5500 QTRAP data. For each method, metabolite identities were confirmed using authentic reference standards or reference samples. We excluded from each analytical platform: (1) standards and xenobiotics (drugs), and (2) metabolites not detected in >25% participants. Framingham Heart Study samples were quantified in two separate batches.

Table S1. Characteristics of the FHS analytic sample and comparison with the larger FHS Generation 3 and Omni Generation 2 cohorts.

Characteristic	Overall FHS sample with clinical study exclusions (N=2902)	Study subsample (N=1365)
Age, years	54 (9)	54 (8)
Women (%)	1533 (53)	726 (53)
Body mass index, kg/m^2	28.2 (5.4)	27.7 (5.3)
Prevalent CVD (%)	118 (4)	53 (4)
Systolic blood pressure, mmHg	119 (14)	119 (14)
Hypertension treatment	614 (21)	263 (19)
Diabetes (%)	218 (8)	86 (6)
Current or former smoking (%)	1093 (38)	502 (37)
Total cholesterol, mg/dL	191 (36)	191 (34)
HDL cholesterol, mg/dL	60 (19)	61 (20)
Peak VO_2, ml/kg/min	23.0 (6.9)	23.6 (7.2)

Displayed values are the mean (standard deviation) for continuous variables and the N (%) for categorical variables.

Table S2. Framingham Heart Study, LASSO coefficients (74 metabolites) and the coefficients for the 61 metabolites overlapping with CARDIA ("reduced score").

Metabolite	Beta est.	Beta est. for reduced score
1_7_dimethyluric_acid	0.0107	0.0062
1_methyladenosine	0.0007	-0.0165
1_methylguanosine	0.0130	0.0406
1_methylnicotinamide	0.0132	0.0442
2_aminooctanoate	0.0611	0.1398
3_hydroxyanthranilic_acid	0.0283	NA
5_hydroxymethyl_4_methyluracil	-0.0184	NA
5_hydroxytryptophol	-0.0221	NA
7_methylguanine	-0.0163	-0.0205
acetyl_galactosamine	-0.0947	-0.2502
alanine	0.0827	0.1822
arginine	0.0102	0.0126
betaine	0.0274	0.0600
bilirubin	0.0368	NA
C16_0_ceramide__d18_1_	-0.0170	-0.0641
C16_0_SM	0.0067	0.0936
C16_1_LPC	-0.0251	-0.0815
C16_1_SM	-0.0386	-0.1929
C16_carnitine	-0.0398	-0.0986
C18_1_carnitine	-0.0032	0.0210
C18_2_carnitine	-0.0656	-0.1752
C18_2_LPC	0.0447	0.1384
C18_3_LPE	-0.0374	-0.1137
C18_carnitine	0.0610	0.1768
C20_1_LPE	-0.0148	-0.0491
C20_4_LPE	-0.0433	-0.1086
C20_5_LPC	0.0128	-0.0096
C22_0_SM	0.0387	0.0883
C22_6_LPC	0.0924	0.2430
C24_0_LPC	0.0461	0.1361
C30_0_PC	0.1045	0.2742
C34_1_DAG_or_TAG_fragment	-0.0073	NA
C34_3_PC	-0.0289	NA
C36_2_PS_plasmalogen	-0.0243	-0.0742
C38_6_PC_plasmalogen	-0.0166	NA
C4_carnitine	0.0004	-0.0035
C9_carnitine	0.0229	0.0659
choline	0.0477	0.1434
cinnamoylglycine	0.0485	0.1186
cortisone	0.0289	0.0810
creatine	-0.0845	-0.2307
creatinine	0.0464	0.1499
cyclohexylamine	-0.0068	-0.0209
cystine	-0.0250	NA

dimethylglycine	0.0133	0.0198
dimethylguanidino valeric acid (DMGV)	-0.0502	-0.1249
gamma-aminobutyric acid (GABA)	0.0134	0.0398
glycocholate	-0.0035	0.0092
glycodeoxycholate_glycochenodeoxycholate	-0.0626	-0.1734
guanidinoacetic_acid	0.0101	0.0561
homoarginine	-0.0118	-0.0263
homocitrulline	0.0211	0.0466
imidazole_propionate	-0.0254	NA
imidazoleacetic_acid	0.0781	NA
kynurenic_acid	0.0478	0.1669
leucine	0.0290	0.1076
linoleoyl_ethanolamide	-0.0109	-0.0351
methylimidazoleacetic_acid	0.0306	0.0925
N_acetylaspartic_acid	-0.0105	-0.0460
N_acetylhistidine	0.0012	NA
N_methylproline	0.0096	0.0281
N1_acetylspermidine	-0.0363	-0.0957
N4_acetylcytidine	-0.0070	-0.0605
N6_N6_dimethyllysine	-0.0067	-0.0043
ornithine	-0.0133	-0.0514
pantothenate	0.0066	0.0449
proline	-0.0663	-0.1826
sphinganine	-0.0001	0.0261
sphingosine	0.0282	NA
thiamine	-0.0720	-0.1697
thyroxine	-0.0382	-0.0942
trimethylamine_N_oxide	-0.0087	-0.0434
tyrosine	-0.0391	-0.1002
vitamin_A	0.0092	NA

NA signifies the metabolite was not used in the reduced score fit for application to CARDIA

Abbreviations: SM, sphingomyelin; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; DAG, diacylglycerol; TAG, triacylglycerol