Circulation

ORIGINAL RESEARCH ARTICLE

Metabolic Architecture of Acute Exercise Response in Middle-Aged Adults in the Community

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BACKGROUND: Whereas regular exercise is associated with lower risk of cardiovascular disease and mortality, mechanisms of exercise-mediated health benefits remain less clear. We used metabolite profiling before and after acute exercise to delineate the metabolic architecture of exercise response patterns in humans.

METHODS: Cardiopulmonary exercise testing and metabolite profiling was performed on Framingham Heart Study participants (age 53±8 years, 63% women) with blood drawn at rest (n=471) and at peak exercise (n=411).

RESULTS: We observed changes in circulating levels for 502 of 588 measured metabolites from rest to peak exercise (exercise duration 11.9±2.1 minutes) at a 5% false discovery rate. Changes included reductions in metabolites implicated in insulin resistance (glutamate, −29%; $P=1.5\times10^{-55}$; dimethylguanidino valeric acid [DMGV], −18%; $P=5.8\times10^{-18}$) and increases in metabolites associated with lipolysis (1-methylnicotinamide, +33%; $P=6.1\times10^{-67}$), nitric oxide bioavailability (arginine/ornithine + citrulline, +29%; $P=2.8\times10^{-169}$), and adipose browning (12,13-dihydroxy-9Z-octadecenoic acid +26%; $P=7.4\times10^{-38}$), among other pathways relevant to cardiometabolic risk. We assayed 177 metabolites in a separate Framingham Heart Study replication sample (n=783, age 54±8 years, 51% women) and observed concordant changes in 164 metabolites (92.6%) at 5% false discovery rate. Exercise-induced metabolite changes were variably related to the amount of exercise performed (peak workload), sex, and body mass index. There was attenuation of favorable excursions in some metabolites in individuals with higher body mass index and greater excursions in select cardioprotective metabolites in women despite less exercise performed. Distinct preexercise metabolite levels were associated with different physiologic dimensions of fitness (eg, ventilatory efficiency, exercise blood pressure, peak Vo_2). We identified 4 metabolite signatures of exercise response patterns that were then analyzed in a separate cohort (Framingham Offspring Study; n=2045, age 55±10 years, 51% women), 2 of which were associated with overall mortality over median follow-up of 23.1 years ($P\leq0.003$ for both).

CONCLUSIONS: In a large sample of community-dwelling individuals, acute exercise elicits widespread changes in the circulating metabolome. Metabolic changes identify pathways central to cardiometabolic health, cardiovascular disease, and long-term outcome. These findings provide a detailed map of the metabolic response to acute exercise in humans and identify potential mechanisms responsible for the beneficial cardiometabolic effects of exercise for future study.

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Clinical Perspective

What Is New?

- To better understand how acute exercise influences the human metabolome, we measured the levels of 588 circulating metabolites before and immediately after ≈12 minutes of exercise in 411 community-dwelling individuals who underwent cardiopulmonary exercise testing and replicated key findings in a separate sample (n=783).
- Significant changes occurred in >80% of circulating metabolites, including favorable shifts in many metabolites previously linked to cardiometabolic outcomes.
- We observed distinct associations of resting metabolites with physiologic exercise responses captured by cardiopulmonary exercise testing and metabolite signatures of multidimensional exercise responses were associated with the risk of death in a separate sample (n≈2000).

What Are the Clinical Implications?

- We observed favorable exercise-induced changes in circulating levels of metabolites related to insulin resistance, obesity, lipolysis, oxidative stress, inflammation, vascular reactivity, and longevity, with blunting of advantageous changes observed in individuals with higher body mass index and different metabolite excursions in men and women.
- These findings provide a detailed description of the metabolic effects of acute exercise, link specific metabolic pathways with exercise response variables and long-term health outcomes, and spotlight metabolic pathways involved in exercise responses for future study.

Whereas pleiotropic beneficial effects of exercise on metabolic, inflammatory, vascular, cardiac, and other systems are well described,[1–3] the molecular mechanisms underlying these favorable adaptations are incompletely elucidated. Recent investigations in small samples have suggested the role of circulating molecules elaborated during acute exercise exposure (including exosomes, metabolites, proteins, and noncoding RNAs) in potentially mediating exercise-induced cardiometabolic benefits.[4–7] Although these studies demonstrate that excursions in functional biomarkers during acute exercise may be a potent probe of human physiology, most studies remain limited by small sample size, incomplete phenotypic characterization of multidimensional physiologic response patterns during exercise, and lack of linkage to long-term outcome. A necessary first step toward understanding how exercise improves human health is to link heterogeneous physiologic responses to exercise with underlying molecular physiology.

We studied participants of the Framingham Heart Study (FHS) with simultaneous cardiopulmonary exercise testing (CPET) and assessment of the circulating metabolome at rest and at peak exercise. We hypothesized that a short period of maximum incremental exercise would produce a significant shift in the circulating metabolome, with metabolic alterations reflecting important pathways of exercise-mediated cardiometabolic benefit. Moreover, harnessing multidimensional phenotyping of exercise responses during CPET, we evaluated how resting metabolic profiles associated with exercise response patterns that predict future cardiovascular disease (CVD). We also evaluated metabolite signatures of "integrated" responses to exercise (cardiac, systemic and pulmonary vascular, and peripheral) in relation to long-term cardiovascular prognosis. Ultimately, we sought not only to provide a snapshot of metabolic responses to exercise and their relations to multidimensional exercise responses in humans, but also to understand how these patterns may explain the mechanisms underlying the widespread cardiovascular benefits of exercise.

METHODS

Data Sharing

The data supporting the study findings will be made available on reasonable request. FHS data are made 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

The FHS is a prospective cohort study of community-dwelling adults in Framingham, Massachusetts, that began with the original cohort in 1948 (n=5209) and now includes their children and children's spouses (second generation [Gen2], starting in 1971, n=5124)[8] and their grandchildren and grandchildren's spouses (third generation [Gen3], enrolled in 2002–2005, n=4095).[9] Eligible individuals with at least 1 parent enrolled in the Gen2 cohort were enrolled in Gen3 from 2002 to 2005. At the third study visit (2016–2019), CPET was performed as part of the routine examination cycle.[10] For the current study, we included the first 482 individuals to undergo CPET with metabolite profiling available. After excluding 9 individuals for fasting <8 hours before blood sampling and 2 individuals with multiple extreme outlier metabolite values (>10 SD from mean change), a total of 471 individuals had metabolite profiling performed at rest. Blood sampling at peak exercise was not available for 60 individuals owing to participant preference or because the blood draw could not be obtained after 2 attempts, and therefore 411 individuals had metabolite profiling both at rest and at peak exercise. The next consecutive 783 Gen3 participants with metabolite profiling performed on fasting blood samples were used for replication of exercise-induced changes in metabolite levels. Details of the study flow are shown in Figure I in the Data Supplement. Assessment of clinical

covariates and outcomes are described in the Expanded Methods in the Data Supplement.

To evaluate the associations of metabolite signatures of exercise and long-term outcomes, 2066 participants from the Gen2 cohort[8] with metabolite profiling performed previously on stored blood from the fifth examination cycle (1991–1995)[11] were eligible for inclusion. We excluded 21 individuals with inadequate fasting, yielding a sample of 2045 for associating metabolites with death and 1996 individuals without prevalent CVD for associating metabolites with incident CVD. The Boston University Medical Center and Massachusetts General Hospital institutional review boards approved study protocols, and all participants provided written informed consent.

Cardiopulmonary Exercise Testing

Maximal effort CPET was performed on the same cycle ergometer (Lode, the Netherlands) and breath-by-breath gas exchange values were measured by the same metabolic cart (MedGraphics, St Paul, MN) in all participants. Heart rate was monitored continuously and blood pressure was measured every 2 minutes manually using sphygmomanometry. The exercise protocol began with 3 minutes of unloaded (0-watt) exercise, followed by loaded exercise with an incremental ramp protocol. Two ramp protocols were used (15 and 25 watts/min) and participants were assigned to 1 of the 2 protocols based on assessment of estimated peak watts to achieve similar exercise time across participants. Recovery measures were taken during 3 minutes of unloaded cycling and 1 minute of rest. For this study, our primary measure of exercise capacity was peak oxygen uptake (peak Vo_2, scaled to body weight, in mL/kg/min), determined as the highest 30-second average during the final 90 seconds of exercise. The maximum predicted Vo_2 was calculated using the Wasserman equation.[12] All measures were adjudicated by 2 trained exercise physiologists (M. Tanguay and J.B. Blodgett) and reviewed by the study principal investigator (G.D. Lewis) blinded to individual participant metabolite levels.

Metabolite Profiling

Blood was sampled at rest (before exercise) and at peak exercise in potassium ethylenediaminetetraacetic tubes. The median time between peak exercise and blood collection was 56 seconds (first and third quartiles, 44 to 72 seconds). Ethylenediaminetetraacetic blood collection tubes were centrifuged for 22 minutes at 2500 *g*, 4 °C, and then stored at –80 °C without freeze–thaw cycles until assayed. Metabolite profiling was subsequently performed at the Broad Institute of Harvard and Massachusetts Institute of Technology (Cambridge) using 4 complementary liquid chromatography tandem mass spectrometry methods providing broad coverage of the following metabolite classes: (1) amino acids, amino acid metabolites, acylcarnitines, dipeptides, and other cationic polar metabolites (hydrophobic interaction liquid chromatography [HILIC] in the positive ionization mode [HILIC-positive]); (2) sugars, organic acids, purines, pyrimidines, and other anionic polar metabolites (HILIC in the negative ionization mode [HILIC-negative]); (3) lipids (C8-positive); and (4) free fatty acids, lipid-derived mediators (eicosanoids), bile acids, and metabolites of intermediate polarity (C18-negative). Detailed metabolite profiling methods are available in the Expanded Methods in the Data Supplement.

The median interassay coefficients of variation for the metabolites included in the derivation effort using pooled-plasma samples was 4.94 (25th percentile 2.35, 75th percentile 8.55) and 92% of metabolites had coefficients of variation <20% (Table I in the Data Supplement).

For the replication sample, the single platform with the highest number of metabolites analyzed (HILIC-positive) was run. Metabolite profiling in Gen2 was performed (2009–2011) in the same laboratory (Broad Institute) using previous versions of the HILIC-positive and HILIC-negative platforms, as described.[11]

Statistical Analysis

Data Transformations and Imputation

For "missing" (below-detection concentrations) metabolite values, we imputed data at 50% of the lowest detected value of that metabolite across participants (separately for resting and peak exercise values).[13] Metabolite data were $\log_2$ transformed and fold changes were calculated ($\log_2$[peak]/$\log_2$[rest]); rest, peak, and fold change values were then separately rank normalized[14] to limit skewness and outlier effects.

Metabolic Responses to Exercise

For each metabolite, we compared the observed mean $\log_2$(exercise/rest) versus no change (ie, $\log_2$[exercise/rest]=0) with 2-tailed *t* tests. To account for multiple testing across metabolites, we used the Benjamini-Hochberg false discovery rate (FDR) method, separately for each metabolite platform and with a 5% FDR significance threshold.

Correlates of Metabolic Response to Exercise

To understand relations of metabolic responses with key demographic and clinical measures, we specified linear models for rank-normalized fold change (outcome) versus age, sex, body mass index (BMI), workload, and normalized resting metabolite level (predictors). As input to regression analyses, we standardized the distributions of age, $\log_2$ BMI, and peak watts. Because of large sex differentials in achieved workload, we subtracted the sex-specific mean peak watts from the peak watts value for each participant, pooled men and women, and then standardized (mean 0, variance 1) for analysis. To account for multiple testing, we applied an FDR correction across *P* values for estimated regression coefficients separately for each predictor (eg, age, sex, BMI). Metabolites in each quantified platform (HILIC, C8, C18) were modeled separately, with separate FDR adjustments applied per platform.

Exercise Performance Relative to Resting Metabolites

To model associations between exercise performance data (outcomes) with individual resting metabolites (predictors), we used multivariable linear regression models adjusting for sex and age in 471 individuals with resting metabolite data. In sensitivity analyses, we additionally adjusted for resting systolic blood pressure, diabetes, hypertension treatment status, smoking, and prevalent CVD. The list of exercise performance

variables was as follows: $\log_2$ peak Vo_2, peak heart rate, aerobic efficiency, peak O_2 pulse, $\log_2$ recovery half-time, square root Vo_2 recovery delay, mean arterial pressure (MAP) at 75 watts, and ventilatory efficiency (V_E/Vco_2) nadir (Table 1). Models relating metabolite levels with peak heart rate and MAP at 75 watts were also adjusted for resting heart rate and MAP, respectively. To account for multiple testing across metabolites, we used a 5% FDR significance threshold.

Canonical Correlation Analysis

We next sought to understand the joint relationships between the assayed metabolites and integrated cardiopulmonary exercise responses by CPET using canonical correlation analysis (CCA).[19,20] CCA estimates the joint associations between 2 sets of multiple variables (in our case, 8 CPET measures and 107 individual circulating metabolites) by creating 2 separate sets of composite "canonical variates" (a set of CPET variates and a set of metabolite variates) that are "loaded on" (correlated with) each individual variable. The CPET canonical variates are multivariable "scores" that are loaded on different CPET measures; analogously, the metabolite canonical variates are multivariable metabolite-based scores loaded on different individual metabolites. The loadings of each CPET or metabolite measure in each canonical variate are calculated to maximize joint association between each CPET variate and a corresponding metabolite variate, while being uncorrelated with all the other canonical variates. We used CCA to construct maximum correlations between high-dimensional CPET data (8 measures) and circulating metabolite levels (107 metabolites in the HILIC dataset measured in both FHS Gen2 and Gen3). We selected metabolites measured in both Gen2 and Gen3 to allow us to identify metabolite patterns related to multidimensional exercise responses in Gen3 and subsequently applied the metabolite weights defined by the CCA in Gen3 to Gen2 data to assess the relationship between CPET-related resting metabolite signatures and long-term outcomes. The CCA was based on partial correlations adjusted for age and sex and the maximum number of variates included in our analysis was selected by an F test ($P<0.05$; PROC CANCORR in SAS).

Survival Analysis

Cox proportional hazards regression analysis was used to relate the top 4 CCA-based metabolite scores (standardized to mean 0 and variance 1) to incident CVD and all-cause mortality in Gen2. Models were stratified by sex owing to nonproportional hazards between men and women. We fitted a separate model for each CCA-based metabolite score. For both CVD and mortality, we adjusted for age and BMI, and then additionally for systolic blood pressure, hypertension

Table 1. Cardiopulmonary Exercise Testing (CPET) Variables

CPET variable	Description and physiologic relevance	Relationship to future outcomes	Measurement methodology	Threshold for abnormal	Required exercise duration
Exercise blood pressure	Integrated measure of arterial compliance,[15] autonomic function[16]	Predicts future systemic hypertension,[17] CVD[18]	Measurement of DBP + 1/3 (SBP–DBP) at a standardized workload of 75 watts (% change from resting values)	Women, >118 mm Hg[18]; men, >128 mm Hg[18]	≤8 min
Peak Vo_2	Aerobic capacity, gold standard indicator of cardiorespiratory fitness; integrates cardiopulmonary, vascular, and peripheral skeletal muscle performance	Predicts future metabolic and CVD outcomes including diabetes, heart failure, and death	Highest 30 s mean Vo_2 occurring in final 90 s of incremental exercise	<80% Predicted	Peak
Ventilatory efficiency	Minute ventilation required to exchange 1 L/min of CO_2; reflects cardiac output and pulmonary ventilation–perfusion matching during exercise, correlates with pulmonary arterial pressure but not peripheral skeletal muscle performance	Predicts hospitalization and death from heart failure and pulmonary hypertension	Lowest ratio of minute ventilation (V_E) to CO_2 elimination (Vco_2) during exercise; occurs during early to midexercise and is independent of volitional effort	>34	<8 min
O_2 pulse	Ratio of peripheral oxygen extraction and stroke volume	Predicts CVD outcomes	Slope of Vo_2 versus heart rate relationship	<80% Predicted	Peak
Peak heart rate	Reflects ability to augment heart rate in response to exercise, indicative of autonomic function, conduction system integrity	Predicts conduction system disease, CVD outcomes	Highest achieved continuous measurement of heart rate by ECG	<220–Age	Peak
Aerobic efficiency	Reflects metabolic cost (in terms of O_2 uptake) of performing external work aerobically	Predicts outcomes in established HF	ΔO_2 consumption/Δ work during incremental exercise	<8.5 mL/W/min	<6 min
Vo_2 half-time	Measures of Vo_2 recovery kinetics that reflect the metabolic consequences of an acute bout of exercise with delays indicative of Vo_2 deficit accrued during exercise	Predicts outcomes in established CVD	Time for Vo_2 to decrease to 50% of peak Vo_2 adjusted for resting Vo_2	<65 s	Peak
Vo_2 recovery delay			Time from the end of loaded exercise until Vo_2 permanently falls below peak Vo_2	<15 s	Peak

CVD indicates cardiovascular disease; DBP, diastolic blood pressure; HF, heart failure; SBP, systolic blood pressure; and VE, ventilatory efficiency.

treatment status, diabetes, current smoking, and total and high-density lipoprotein cholesterol level.

All analyses were performed in SAS 9.4 (SAS Institute, Cary NC) or R (The R Foundation for Statistical Computing, version 4.0.0; http://www.rproject.org).

Pathway Analysis

Pathway analysis was performed to identify biological pathways represented by metabolites with statistically significant (5% FDR) changes with exercise. MetaboAnalystR[21] was used to map metabolites to genes. Gene enrichment analyses were then performed with clusterProfiler,[22] WikiPathways, and RCy3[23] against the WikiPathways database of community-curated pathway models.[24]

RESULTS

Characteristics of the Study Samples

Gen3 study participants in the derivation sample had a mean age of 53±8 years, 63% were women, 19% had hypertension, 19% were on treatment with lipid-lowering medications, and 8% had diabetes (Table 2). Gen3 participants in the replication sample displayed similar characteristics with a slightly lower proportion of women. Participants from Gen2 were of similar age (mean age 55±10 years) with 51% women and comparable risk factor burden to Gen3 participants (Table 2).

The mean exercise duration was 11.9±2.1 minutes with a peak respiratory exchange ratio of 1.20±0.09, consistent with peak volitional effort during exercise. Average exercise capacity was relatively preserved compared with predicted values (peak oxygen uptake [Vo_2] 23.1±7.1 mL/kg/min, 92±22% predicted; Table 2).

Table 2. Characteristics of the Study Samples

Characteristic	Generation 3, derivation (n=471)	Generation 3, replication (n=783)	Generation 2 (n=2045)
Age, y	53±8	54±8	55±10
Women, %	296 (63)	396 (51)	1047 (51)
Body mass index, kg/m^2	27.4±5.5	27.7±5.1	27.6±5.0
Hypertension, %	88 (19)	153 (20)	412 (20)
Current smoking, %	30 (6)	47 (6)	384 (19)
Diabetes, %	36 (8)	42 (5)	135 (7)
Total cholesterol, mg/dL	188±32	192±34	207±37
HDL cholesterol, mg/dL	62±19	62±21	49±15
Exercise duration, min	11.9±2.1	11.9±2.0	—
Peak respiratory exchange ratio	1.2±0.1	1.2±0.1	—
Peak Vo_2, mL/kg/min	23.1±7.1	23.8±7.3	—

Entries are mean±SD for continuous variables or n (%) for categorical variables. HDL indicates high-density lipoprotein.

Table 1 specifies the exercise variables measured, their physiologic and prognostic relevance, and previously established abnormal thresholds, with sex-specific values for these measures indicated in Table 3.

Widespread Changes in the Metabolome With Acute Exercise Exposure

Figure 1 demonstrates the magnitude of change in circulating metabolites with exercise. We observed widespread excursions in the assayed metabolome, with 502 of 588 metabolites (85%) demonstrating statistically significant changes with exercise (Table II in the Data Supplement). In a sensitivity analysis restricted to the 365 individuals with peak respiratory exchange ratio ≥1.1, we observed consistency of our findings (Figure II in the Data Supplement).

Metabolite changes with exercise represented a variety of biological pathways with an approximately equal number of metabolites increasing and decreasing (Table 4). There was an increase in metabolic intermediates reflecting enhanced anaerobic and aerobic respiration, consistent with peak effort exercise: (1) a 246% increase in circulating lactate (P=4.6×10^{-197}, anaerobic

Table 3. Exercise Characteristics of the Generation 3 (Derivation) Study Sample

Exercise variable	Women (n=296)	Men (n=175)
Exercise duration, min	11.5±2.1	12.6±2.0
Peak respiratory exchange ratio	1.2±0.1	1.2±0.1
Peak Vo_2, mL/kg/min	21.3±6.4	26.2±7.2
Peak Vo_2 % predicted	93.4±23.3	89.9±19.5
Peak Vo_2 absolute, mL/min	1454.2±390.9	2340.1±567.3
Aerobic efficiency, mL/W*	9.1±0.9	9.6±0.7
V_E/Vco_2 nadir	27.7±3	26.1±2.7
O_2 pulse peak, mL/beat	9.7±2.2	15.4±3.2
Vo_2 T 1/2 recovery, s*	84.4±37.8	69.1±24.4
Vo_2 recovery delay, s*	12.5±10.6	10.6±17.8
Workload achieved, W	132.6±35.9	214.2±55.3
Resting heart rate, bpm	73.9±11.8	69.5±11.8
Peak heart rate, bpm	152.3±18.4	154.3±20.9
Resting systolic blood pressure, mm Hg	123.9±16.4	131.6±15.5
Systolic blood pressure at 75 W, % change*	28.5±12.7	20.4±10.3
Resting diastolic blood pressure, mm Hg	79.8±8.1	84.7±8.4
Diastolic blood pressure at 75 W, % change*	2.3±8.4	0.7±6.4
Mean arterial pressure at 75 W, % change*	13.6±8	9.2±6

Entries are mean±SD for continuous variables.

*n = *470 for aerobic efficiency, 461 for Vo_2 T 1/2 recovery, 465 for Vo_2 recovery delay*, and 459 for blood pressure at 75 W.

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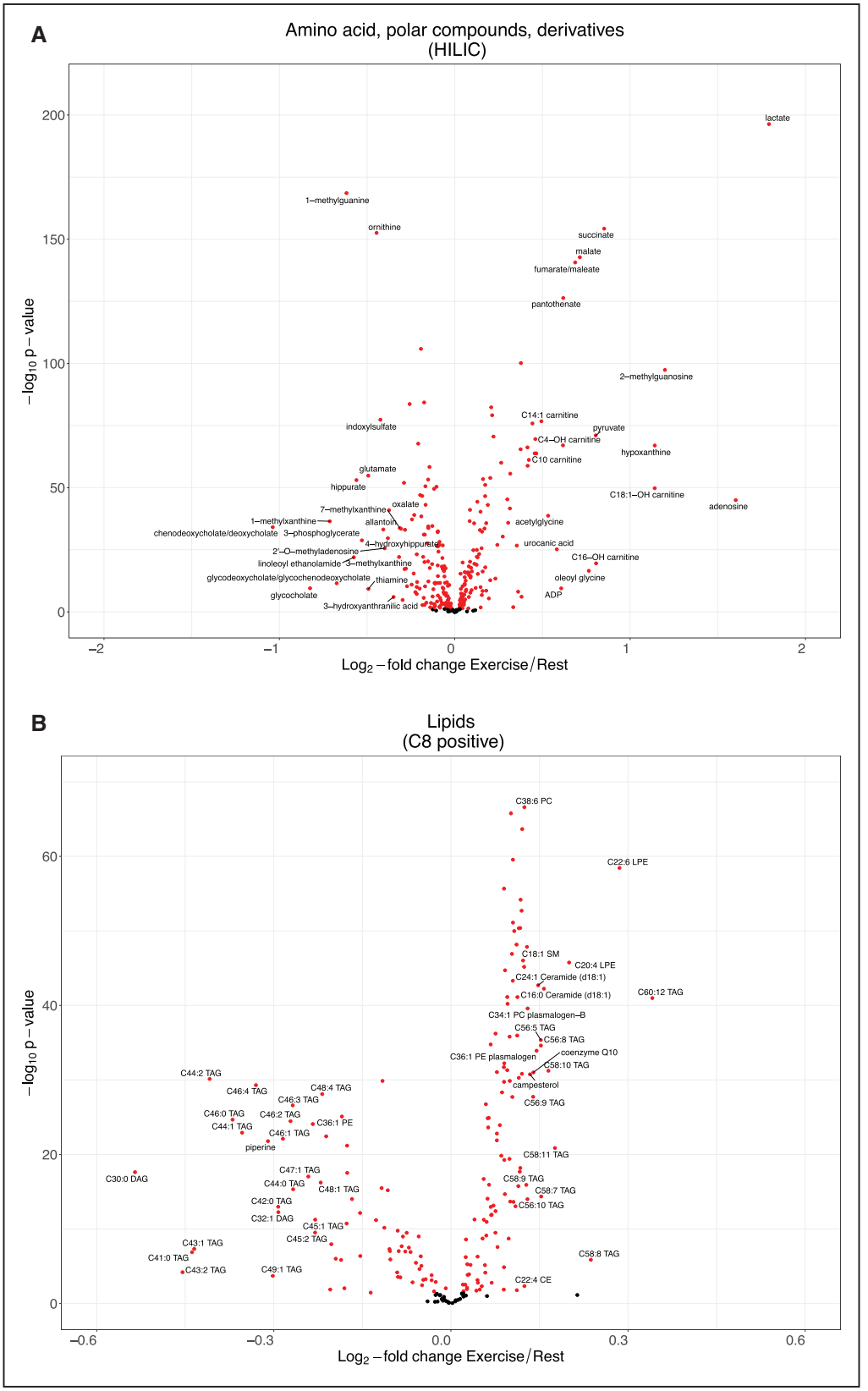

Figure 1. Response of the circulating metabolome to acute exercise.
A–**C**, A series of volcano plots of the change in individual metabolites with exercise for different metabolite platforms. Red indicates metabolite levels that are significant at a 5% false discovery rate (FDR) level. Metabolites are labeled arbitrarily. Inosine is not displayed in the hydrophilic interaction liquid chromatography (HILIC) plot for visualization purposes, because it had an extreme increase with exercise ($\log_2$ fold change = 3.2, raw P=5.70×10^{-111}). The full fold change and significance levels are shown in Table II in the Data Supplement. **D**, Selected ratios indicative of distinct metabolic phenotypes, including allantoin/urate, which is related to oxidative stress; kynurenine/tryptophan, which is related to higher indoleamine 2,3-dioxygenase activity and systemic inflammation; (*Continued*)

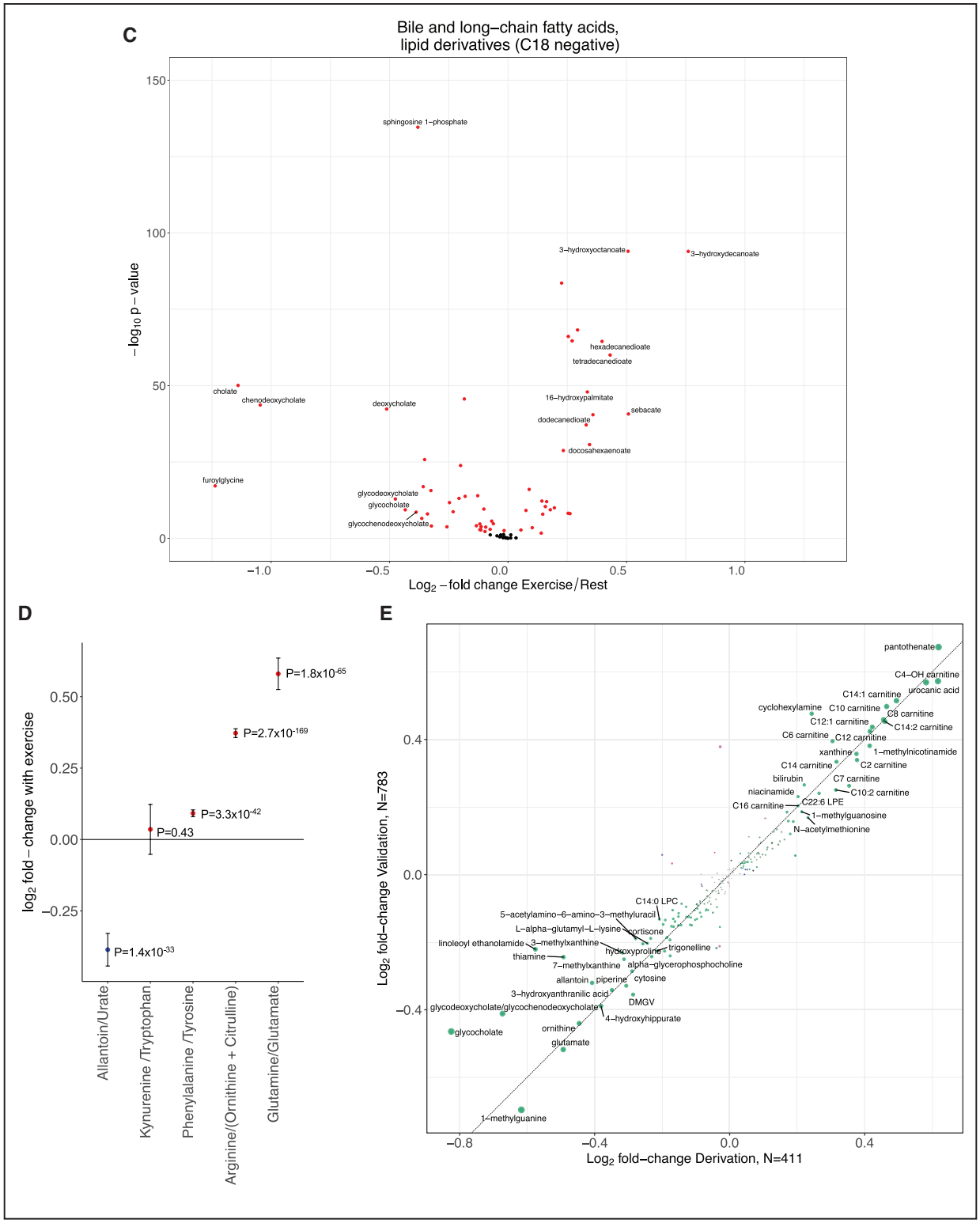

Figure 1 Continued. phenylalanine/tyrosine, which is related to higher inflammation; arginine/(ornithine + citrulline), which is related to greater nitric oxide synthesis, improved endothelial function, and lower metabolic syndrome risk; and glutamine/glutamate, which is related to lower cardiometabolic risk. Bars represent the 95% CI for the $\log_2$ fold change. *P* values are shown in the diagram. Point estimates are shown in red for increasing with exercise and blue for decreasing with exercise. **E**, Plot of the metabolite $\log_2$ fold change with exercise in the replication sample versus the derivation sample (Table III in the Data Supplement). The sizes of the points are proportional to the sum of the $\log_2$ fold change in the derivation and replication samples. The dashed line represents equal fold change in both replication and derivation ($y=x$). Colors indicate the following: green, significant (at 5% FDR) with same directionality in both samples; red, significant (at 5% FDR) with opposite directionality in both samples; blue, significant (at 5% FDR) in the derivation sample only; and magenta, significant (at 5% FDR) in the replication sample only. 2-Methylguanosine was not shown because of a greater proportion of levels below the detection limit in the replication versus derivation sample limiting interpretation. ADP indicates adenosine triphosphate; CE, cholesterol esters; DAG, diacylglycerol; DMGV, dimethylguanidino valeric acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; and TAG, triacylglycerol.

Table 4. Metabolic Response to Exercise

Metabolite class	Metabolites	Δ With exercise	Adverse pattern (human/model)	Clinical correlate and proposed mechanism
Insulin sensitivity/ vascular risk	1-Methylnicotinamide	↑ (33%)	↓	Antithrombotic and anti-inflammatory (via prostacyclin),[25] promotes muscle lipolysis[26]
	DMGV	↓ (–18%)	↑	Associated with diabetes, hepatic steatosis, and reduced with long-term exercise[27–29]
	Glutamate Glutamine	↓ (–29%) ↑ (6%)	↑ ↓	Associated with diabetes and CVD risk,[30,31] visceral fat; glutamine metabolism linked to adipose tissue inflammation[32]
	Branched chain and aromatic amino acids (Ile, Val, Tyr, Phe)	↓ (–9% to –2%)	↑	Greater diabetes and cardiovascular risk[11,33]; proinflammatory effects (aromatic)[34]; modulation of branched chain amino acid catabolic pathway[35]
	Betaine	↑ (3%)	↓	Lower levels associated with diabetes; favorably influenced by lifestyle intervention[36]
	2-Aminoadipate	↓ (–3%)	↑/↓	Higher 2-aminoadipic acid levels associated with higher incident diabetes; administration of 2-aminoadipic acid may increase β-cell insulin secretion[37]
	Dimethylglycine	↓ (–3%)	↑	Higher levels associated with greater risk of diabetes[38]
	Taurine	↑ (4%)	↓	Pleotropic effects on oxidative stress, angiotensin/NO pathway, and blood pressure[39]
	Oleoyl glycine	↑ (70%)	↓	Increases insulin sensitivity in adipocytes in vitro[40]
Nucleotides and derivatives	Adenosine	↑ (204%)	↓	Lower levels of adenosine in individuals with obstructive coronary disease,[41] potentially via modulation of macrophage activation[42]
	Hypoxanthine Inosine	↑ (121%, 817%)	↑/↓	Bioproducts of ischemia–reperfusion in cardiac muscle,[43] and may have anti-inflammatory effects[44]
	Allantoin Urate	↓ (–25%, –1%)	↑	Allantoin is a product of oxidation of uric acid[45] (reactive oxygen stress); urate associated with CVD[46]
NO pathway	Arginine	↑ (4%)	↓	Arginine (NO precursor) deficiency related to endothelial dysfunction[47]; supplementation improves vascular function[48]
	Ornithine Citrulline	↓ (–27%, –8%)	↑/↓	Arginine–citrulline cycle involved in NO generation; ornithine is product of arginase and a precursor to glutamate; higher ornithine/citrulline ratios associated with metabolic syndrome risk[49]
Oxidative stress	Cystine	↓ (–11%)	↑	Higher levels associated with greater mortality in patients with coronary artery disease[50]
	13-HODE	↑ (12%)	↑	See following; marker of increased oxidative stress[51]
	Cys–Gly oxidized	↑ (12%)	↑	Marker of oxidative stress; higher in individuals who develop myocardial infarction[52]
	Methionine sulfoxide	↓ (–11%)	↑	Oxidative product of methionine; protein methionine oxidation may reflect ischemic oxidative stress in stroke (via NF-kB)[53]
Bile acids	Chenodeoxycholate Deoxycholate Glycodeoxycholate Glycoursodeoxycholate Taurodeoxycholate Taurocholate Taurochenodeoxycholate	↓ (–52% to –16%)	↑/↓	Higher plasma levels in obese individuals and type 2 diabetics, inversely related to cognitive restraint of eating. Previously reported to decrease with strenuous exercise[54]; complex regulation may depend on type of exercise[55]
Lipids, lipokines, and lipid precursors	12,13-diHOME	↑ (26%)	↓	Exercise-induced brown adipose tissue-derived lipokine, increases skeletal muscle fatty acid uptake[56]
	13-HODE	↑ (12%)	↑/↓	Classically considered a marker of oxidative stress, but may act to decrease proinflammatory cytokine release (G-CSF, IL-6)[51]
	Arachidonate	↑ (11%)	↑/↓	Eicosanoid precursor (regulation of inflammation, vascular dysfunction, platelet reactivity)
	Eicosapentaenoate/ docosapentaenoate	↑ (7%, 15%)	↑	Implicated in reduced CVD risk, inflammation
	16-Hydroxypalmitate Hexadecanedioate	↑ (26%, 32%)	NA	Palmitate metabolism

(*Continued*)

Table 4. Continued

Metabolite class	Metabolites	Δ With exercise	Adverse pattern (human/model)	Clinical correlate and proposed mechanism
	Sphingosine Sphingosine-1 phosphate	↓ (–13%, –23%)	↑/↓	HDL-bound sphingosine-1 phosphate may be anti-atherogenic[57]; associated with CVD[58,59]
	Select sphingomyelins (C16:1 SM, C22:1 SM, C18:1 SM)	↑ (8% to 12%)	↓	Selected sphingomyelins (lower levels) and TAGs (higher levels) associated with higher risk of diabetes[38]
	Selected triacylglycerols (C48:0 TAG, C50:0 TAG, C46:0 TAG, C42:0 TAG, C44:0 TAG)	↓ (–23% to –12%)	↑	
	Medium- and long-chain acylcarnitines (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0)	↑ (11% to 38%)	↑	Related to presence of CAD and future CAD risk[60] and are higher in obesity and diabetes[61]
Microbial metabolism	Hippurate	↓ (–32%)	↑/↓	Mixed directionality of association for metabolic disease and CVD[62,63]; higher levels may be related to higher CVD mortality
	Trimethylamine-N-oxide	↓ (–11%)	↑	Related to increased CVD risk[64]
Tryptophan metabolites	Indoxylsulfate	↓ (–25%)	↑	Metabolite of tryptophan; higher levels related to CVD in chronic kidney disease[65]
	3-Hydroxyanthranilic acid	↓ (–21%)	↑	Higher level related to greater vascular risk and aneurysm formation, induced by angiotensin II[66]
	Quinolinate	↓ (–14%)	↑	Associated with increased carotid vascular disease[67] and pulmonary vascular dysfunction[68]
	Tryptophan Kynurenine	↓ (–10%, –8%)	↑	Reflects IDO-tryptophan metabolism; characteristic of proinflammatory states
Collagen/fibrosis metabolites	Hydroxyproline	↓ (16%)	↑	Tissue expression of hydroxyproline may reflect increased fibrotic potential[69]
Steroids	Pregnenolone sulfate	↑ (10%)	↑	Involved in long-term potentiation in the hippocampus[70]
Glycolysis/ anerobic metabolism	Lactate Pyruvate 3-phosphoglycerate	↑ (246%, 75%) ↓ (–31%)	NA	Glycolytic metabolism
Krebs cycle intermediates and precursors	Succinate Fumarate Aconitate α-Ketoglutarate	↑ (9% to 81%)	NA	Aerobic metabolism; enhanced redox availability to the electron transport chain for aerobic respiration
CoA biosynthesis	Pantothenate	↑ (54%)	NA	Central vitamin cofactor (B5) for fatty acid metabolism and Krebs cycle

12,13-diHOME indicates 12,13-dihydroxy-9Z-octadecenoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; CVD, cardiovascular disease; Cys-Gly, cysteinyl-glycine; DMGV, dimethylguanidino valeric acid; G-CSF, granulocyte colony-stimulating factor; NF, nuclear factor; HDL, high-density lipoprotein; IL, interleukin; NO, nitric oxide; SBP, systolic blood pressure; SM, sphingomyelin; and TAG, triacylglycerol.

respiration); (2) an increase in Krebs cycle span 2 intermediates (succinate, 81% increase, $P=5.6\times10^{-155}$; fumarate, 61% increase, $P=2.2\times10^{-141}$, aerobic respiration); (3) changes in metabolite levels reflecting glycolytic flux (pyruvate, 75% increase, $P=9.4\times10^{-72}$; 3-phosphoglycerate, 31% reduction, $P=1.7\times10^{-29}$); (4) a generalized increase in medium-chain acylcarnitines (mitochondrial fatty acid oxidation); and (5) a 54% increase in pantothenate ($P=4.7\times10^{-127}$), reflecting increased coenzyme A bioavailability and Krebs cycle activity (Table II in the Data Supplement). A discrete bout of exercise also led to directionally favorable changes in circulating levels of metabolites for which resting levels have been previously implicated in cardiometabolic disease (Table 4). We observed favorable changes with exercise for metabolites associated with insulin resistance and excess adiposity, such as a 29% decrease in glutamate ($P=1.5\times10^{-55}$), for which higher levels are associated with visceral adiposity, diabetes, and hypertension[30,31]; 2% to 9% reductions in branched chain amino acids, which are associated with diabetes and CVD[11,60]; 18% decrease in DMGV ($P=5.8\times10^{-18}$), implicated in hepatic steatosis and diabetes[27]; reductions in triacylglycerols (TAGs) with low carbon and double bond numbers, implicated in risk for diabetes[36,71]; and decreases in bile acids, implicated in adipose tissue metabolism and appetite stimulation.[55] Increases in metabolites with inverse associations with cardiometabolic risk were also observed, with a 29% increase in arginine/ornithine + citrulline, representing higher levels of nitric oxide bioavailability[49] ($P=2.8\times10^{-169}$); 33% increase in 1-methylnicotinamide ($P=6.1\times10^{-67}$), which is associated with lipolysis[26]; and a 26% increase in 12,13-diHOME (12,13-dihydroxy-9Z-octadecenoic acid; $P=7.4\times10^{-38}$),

a brown adipose tissue–derived lipokine implicated in skeletal muscle fatty acid metabolism.[56]

As demonstrated in previous studies, metabolites representing pathways related to oxidative stress and inflammation demonstrated varying directions of activity in response to the physiologic stress of acute exercise. We observed reductions in several metabolites correlated with inflammation, with decreases in cystine (oxidized cysteine[50]), allantoin, and their ratio, and lower kynurenine, which is associated with proinflammatory states. Conversely, levels of 13-HODE (13-hydroxyoctadecadienoic acid)[51] and arachidonate (both implicated in oxidative stress, inflammation, and its counterregulation) were increased with exercise.

Of the 177 metabolites significant at a 5% FDR level from the HILIC-positive platform in our derivation cohort and measured in the replication cohort, we observed consistent signals in the direction of exercise-induced metabolite change in 164 (92.6%, at 5% FDR; Figure 1E and Table III in the Data Supplement).

In Silico Pathway Analysis

Filtering for metabolites that changed significantly with exercise (at a 5% FDR) and with a specified Human Metabolome Database identifier produced sets of 251 metabolites in the HILIC platforms, 173 metabolites in the C8 platform, and 58 metabolites in the C18 platform. Using MetaboAnalystR[21] to perform compound gene mapping, 106 of 251 HILIC metabolites were mapped to 2141 genes, 4 of 173 C8 metabolites mapped to 7 genes, and 16 of 58 C18 metabolites mapped to 291 genes. The genes from these mappings were then used to perform enrichment analysis. The top pathway analysis results are displayed as dot plots (Figure III in the Data Supplement). We observed enrichment of pathways related to amino acid metabolism, peroxisome proliferator–activated receptor signaling, eicosanoid and prostaglandin metabolism, insulin signaling, angiopoietin-like protein 8 regulation, and brain-derived neurotrophic factor signaling, all of which have been implicated in cardiovascular and metabolic diseases.

Determinants of Metabolite Changes With Exercise

To evaluate the relations of metabolite changes with exercise to clinical variables (age, sex, and BMI) and to the amount of exercise performed (workload achieved), we specified multivariable-adjusted linear regression models (Figure IV and Tables IV–VI in the Data Supplement). As expected, we observed increases in metabolites involved in glycolysis and anaerobic metabolism (eg, lactate, pyruvate, fumarate) to be related to higher amounts of exercise (Figure V in the Data Supplement). In contrast, exercise-induced changes in many metabolites related to increased insulin sensitivity, greater nitric oxide bioavailability, and favorable shifts in the circulating lipidome were not associated with the amount of exercise performed.

We observed significant heterogeneity in exercise-induced change in several metabolites by BMI and sex (Figure 2 and Figure IV and Tables IV–VI in the Data Supplement). For example, DMGV (with higher levels associated with hepatic steatosis) decreased with exercise, with a magnitude of decrease associated with BMI, such that individuals with higher BMI had a less marked reduction in DMGV with exercise (Figure 2A). These findings suggest that there may be less metabolic plasticity with exercise in individuals who have a higher BMI for certain pathways important to cardiometabolic health. We also observed distinct metabolic responses to exercise by sex, with higher excursions in metabolites linked to anaerobic metabolism (eg, pyruvate, lactate) with greater muscle mass in men, yet greater favorable changes in select metabolites involved in cardiometabolic health (eg, reductions in DMGV and proinflammatory tryptophan-kynurenine metabolites and increases in hexadecanedioate and oleoyl glycine) in women (Figure 2B). After accounting for the other variables in a multivariable model, excursions of only a small number of metabolites (n=3: sorbitol, C10:2 acylcarnitine, C14:2 acylcarnitine) were significantly related to age (Figure IV in the Data Supplement).

Exercise Responses in Relation to the Resting Metabolome

In age- and sex-adjusted models, resting blood metabolite levels were distinctly associated with 1 or more exercise response patterns measured by CPET (Figure 3 and Tables VII–IX in the Data Supplement). These findings were largely consistent in models additionally adjusted for clinical factors including resting systolic blood pressure, diabetes, hypertension treatment status, smoking, and prevalent CVD (Figure VI and Tables X–XII in the Data Supplement). We focused on patterns of unique metabolite associations with 3 complementary exercise measures: global aerobic capacity (peak Vo_2), systemic vascular function (mean arterial pressure at 75 watts), and right heart–pulmonary vascular performance during exercise (V_E/Vco_2), as illustrated in Figure 3D. As expected, given its dependence on both central cardiac and peripheral factors, peak Vo_2 was associated with the largest number of metabolites (Figure 3A–3C). We observed associations between lower peak Vo_2 and higher levels of metabolites reflecting impaired peripheral muscle and fat metabolism (DMGV, 1-methylnicotinomide, 2-aminoadipate), insulin resistance (branched chain amino

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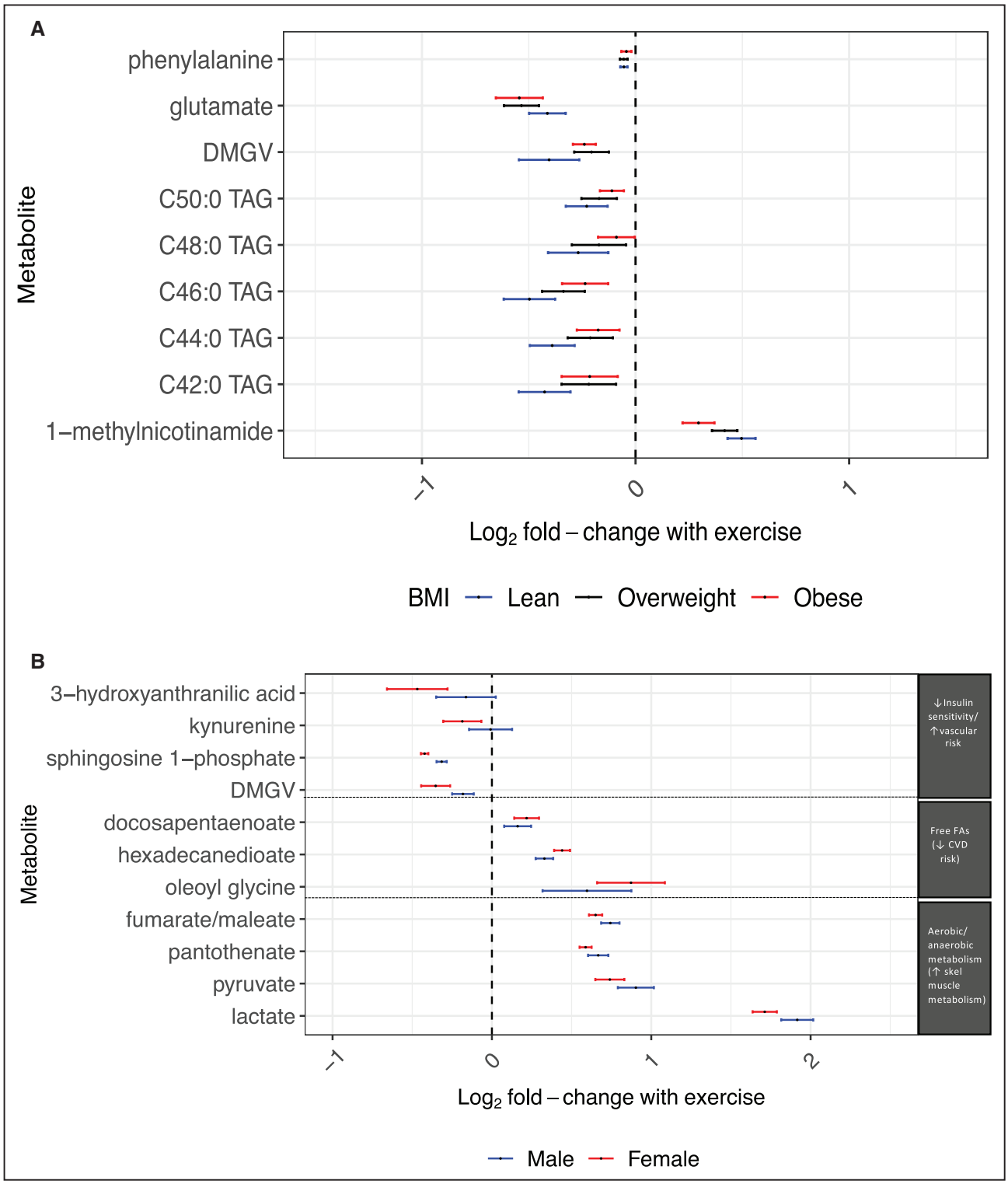

Figure 2. Metabolic architecture of acute exercise.
A, Mean (dot) and 95% CI (bar) for $\log_2$ fold changes across 3 different body mass index (BMI) strata: lean (BMI <25 kg/m^2), overweight (BMI 25 to <30 kg/m^2), and obese (BMI ≥30 kg/m^2). These are crude (unadjusted) fold changes for each metabolite across BMI strata. Metabolites were selected for display based on significance for fold change with exercise (Table II in the Data Supplement), significance for BMI in fully adjusted regressions (Tables IV–VI in the Data Supplement), and curation into pathways of cardiometabolic health (Table 4). **B**, Mean (dot) and 95% CI (bar) for $\log_2$ fold changes for men and women. With exercise, women displayed greater reductions in metabolites associated with impaired insulin sensitivity[28] and increased vascular risk[58] and greater increases in cardioprotective free fatty acids. By contrast, men demonstrated higher excursions in metabolites involved in cellular metabolism, likely attributable to greater muscle mass. CVD indicates cardiovascular disease; DMGV, dimethylguanidino valeric acid; FA, fatty acid; skel, skeletal; and TAG, triacylglycerol.

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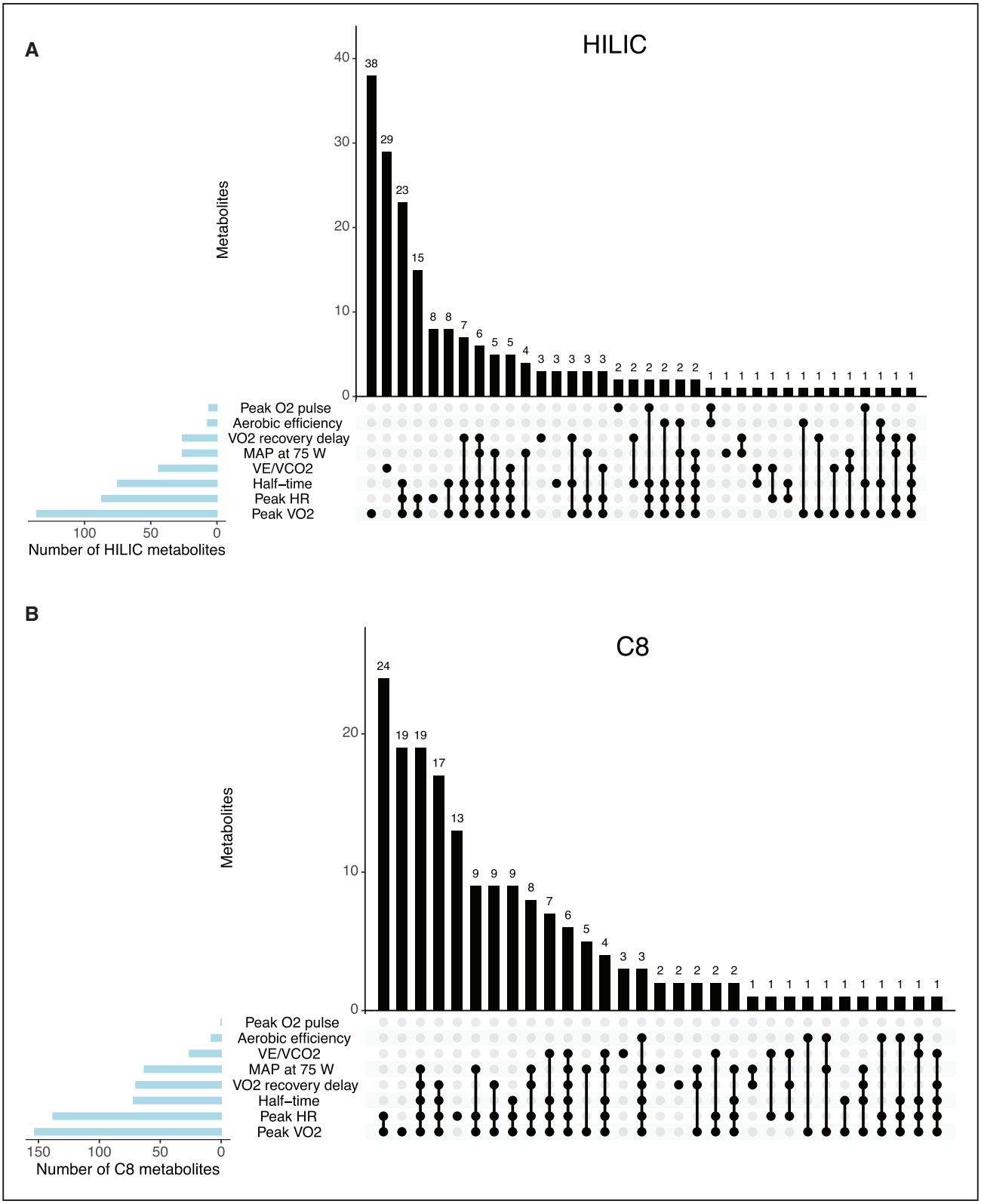


Figure 3. Resting metabolite profiles are differentially associated with multidimensional physiologic measurements during exercise.
A–C, Number of resting metabolite levels (per platform, in columns) that are significantly associated (at a 5% false discovery rate [FDR]) with combinations of cardiopulmonary exercise testing (CPET) measures (in rows) in age- and sex-adjusted linear regression models. **D**, Estimated regression coefficients for select metabolites in regressions for 3 key physiologic exercise responses: peak Vo_2, V_E/Vco_2 nadir, and mean arterial pressure (MAP) at 75 watts. Each CPET variable demonstrates a pattern of associations with metabolites representing distinct physiologic processes as noted. Details of metabolite functions and associations with cardiometabolic traits are shown in Table 4. Asterisks are used to denote statistical significance as follows: *FDR >0.01 to ≤0.05; **FDR >0.001 to ≤0.01; ***FDR ≤0.001. Raw data for these plots are shown in Tables VII–IX in the Data Supplement. 12,13-diHOME indicates (*Continued*)

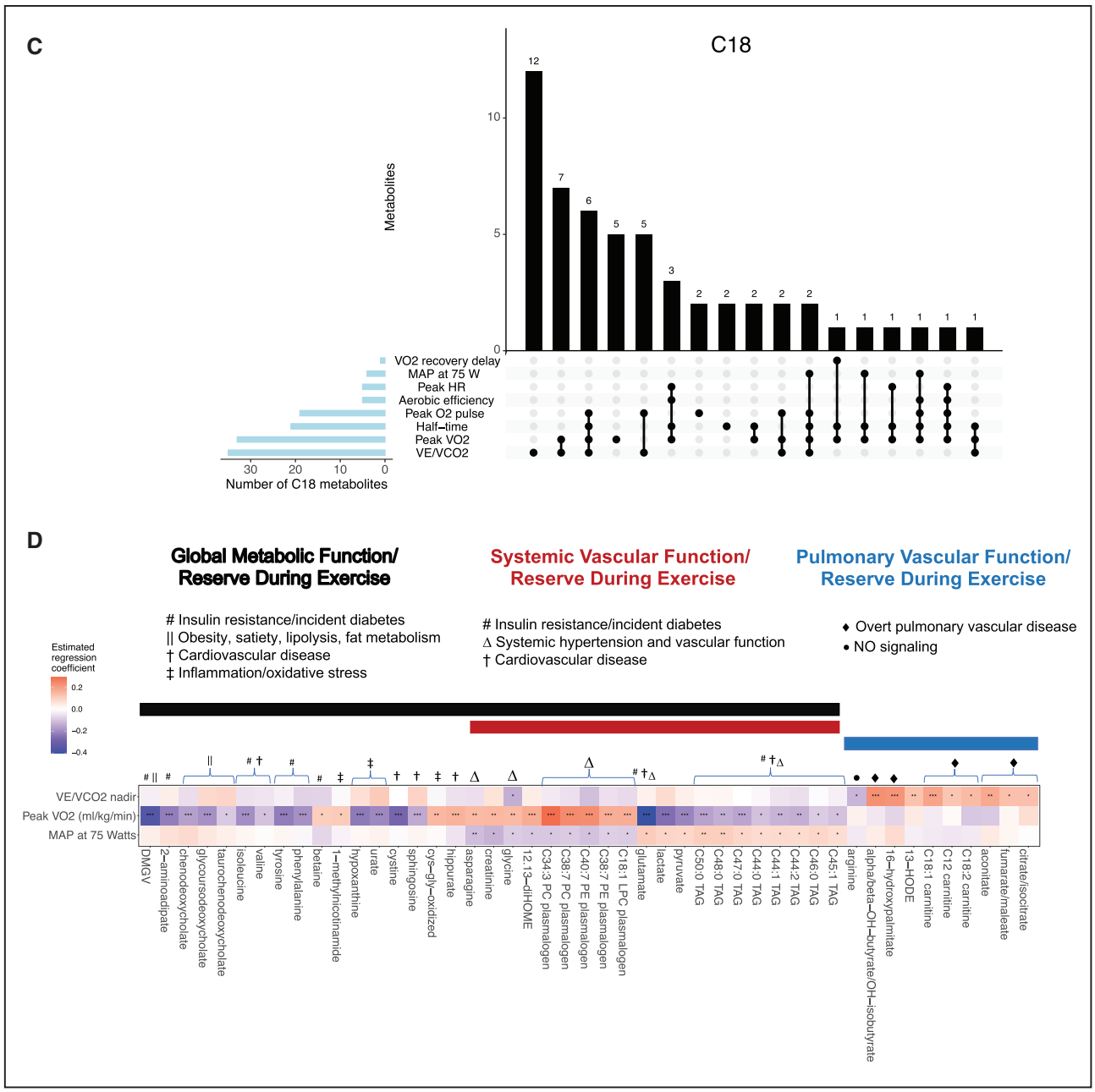


Figure 3 Continued. 12,13-dihydroxy-9Z-octadecenoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; DMGV, dimethylguanidino valeric acid; HILIC, hydrophilic interaction liquid chromatography; HR, heart rate; LPC, lysophosphatidylcholine; NO, nitric oxide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAG, triacylglycerol; and VE/VCO2, ventilatory efficiency.

acids), oxidative stress–related purine degradation (hypoxanthine, urate), and greater reliance on anaerobic metabolism in a state of rest (lactate).

Submaximum exercise blood pressure response is associated with future development of overt resting hypertension[17] and CVD,[18] but the metabolic pathways underlying these associations have not been established. Plasma levels of several plasmalogens were inversely related to exercise blood pressure in models adjusted for resting blood pressure (Figure 3D). Plasmalogens, which serve as endogenous antioxidants and generate vasoactive mediators,[72] are lower in overt hypertension and vascular disease[73] but have not been previously related to exercise blood pressure responses. We also observed associations of MAP at 75 watts with several amino acids and amino acid derivatives previously associated with resting blood pressure (glutamate, associated with higher exercise MAP; and asparagine and glycine, associated with lower MAP).[74] A large number of TAGs were associated with exercise MAP with direct associations noted for TAGs with lower carbon number and double bond content and inverse associations noted with TAGs with higher carbon number and double bond content.

Ventilatory efficiency (V_E/VCO_2) reflects pulmonary ventilation–perfusion matching during exercise, is elevated proportionate to pulmonary vascular resistance, and decreases in response to pulmonary vasodilatory therapy in patients with overt pulmonary hypertension.[10,75] V_E/VCO_2 was uniquely associated with long-chain acylcarnitines, which are elevated in pulmonary arterial hypertension,[68,76] as well as 16-hydroxypalmitate, which is known to become elevated in the setting of impaired fatty acid β-oxidation characteristic of pulmonary hypertension.[77] β-hydroxybutyrate was also related to V_E/VCO_2 and has recently been shown to be elevated in overt pulmonary arterial hypertension.[78] Other free fatty acids (eg, dodecanoate, eicosatrienoate, hexadecanedioate) related to V_E/VCO_2 in this study have not been previously related to pulmonary vascular function.

Metabolite Signatures of Integrated Exercise Responses Are Associated With Long-Term Survival

We next sought to derive resting metabolite signatures of integrated exercise responses, leveraging data across the 8 CPET measures described in Table 1. CCA was used to create metabolite and CPET scores that can be used to define metabolite signatures of multidimensional exercise data (Tables XIII and XIV in the Data Supplement). Figure 4A shows correlations of the first 4 metabolite variates with each CPET variable. Metabolite variate 1 was most strongly correlated with lower peak VO_2, higher submaximal exercise blood pressure, and delayed O_2 recovery kinetics after exercise (Table XV in the Data Supplement). Metabolite variate 2 was correlated with favorable O_2 uptake kinetics (higher peak VO_2, aerobic efficiency, O_2 pulse) and lower 75-watt MAP. Metabolite variate 3 was correlated with lower V_E/VCO_2 and metabolite variate 4 was correlated with higher peak heart rate. The metabolites most highly correlated with each canonical variate are shown in Figure 4B and Table XVI in the Data Supplement.

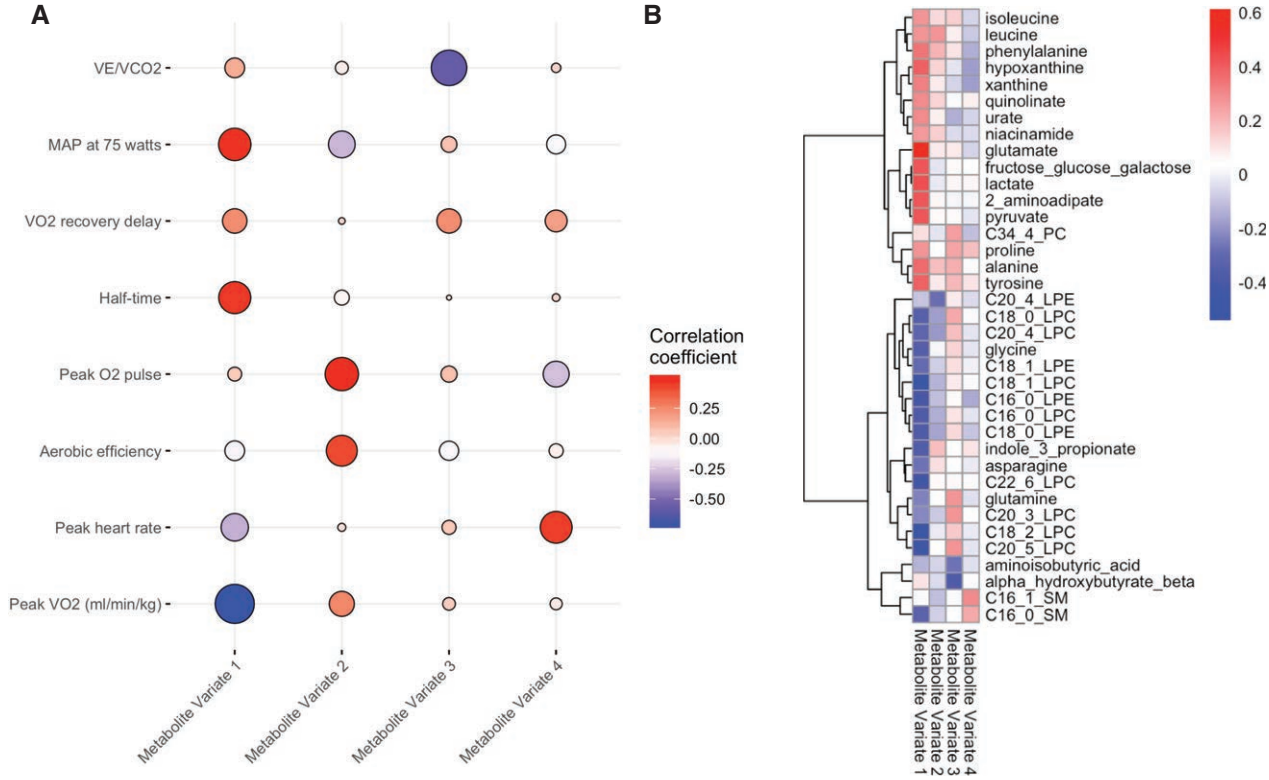
We then evaluated the associations of metabolite variate scores with long-term outcomes (Figure 4C). During a median follow-up of 23.1 years (limits, 0.1–27.3), 702 deaths occurred in 2045 individuals. In age-, sex-, and BMI-adjusted analyses, metabolite variates 1, 2, and 4 were associated with mortality; variates 2 and 4 remained statistically significant after further adjustment for CVD risk factors (Figure 4C). In 1996 individuals without CVD at baseline, 489 CVD events occurred during a median follow-up of 22.7 years (limits, 0.1–27.3). Variate 1 was associated with higher CVD risk in age-, sex-, and BMI-adjusted models, with attenuation of the association after further multivariable adjustment.

DISCUSSION

Exercise is associated with broad benefits on cardiovascular, metabolic, and general health, although precise mechanisms by which these ends are met are unclear. We provide a comprehensive, quantitative assessment of global human metabolism before and after an acute bout of exercise in a large sample of well-phenotyped, community-dwelling individuals. We observed a dramatic shift in >80% of annotated metabolites in the circulating metabolome in response to ≈12 minutes of incremental exercise, with beneficial alterations in levels of metabolites representing key metabolic pathways central to obesity, insulin resistance, oxidative stress, inflammation, vascular reactivity, and longevity, including a variety of novel metabolic mediators and pathways not extensively described. Dynamic changes in metabolite levels varied by BMI and sex but were not associated with age. We observed distinct resting metabolite signatures of abnormal systemic and pulmonary vascular responses to exercise as well as global fitness as captured by peak VO_2. We linked metabolite signatures of 8 CPET measures to mortality and CVD in nearly 2000 individuals with ≈23 years' follow-up. Collectively, these findings underscore the complex, heterogeneous metabolic response to acute exercise and its relation to physiologic mechanisms and long-term prognosis.

In addition to workload-related increases in metabolites reflecting aerobic and anaerobic cellular metabolism (eg, lactate, Krebs cycle intermediates,[4] acylcarnitines,[7,68,79] and purine catabolites indicating heightened ATP turnover[80,81]), we detected favorable shifts in a number of metabolites for which resting levels were previously shown to be associated with cardiometabolic disease. These include reductions in several metabolites associated with future diabetes, including branched chain amino acids,[11,60] glutamate,[30,31] DMGV (associated with hepatic fat[27–29]), as well as select TAGs (lower carbon number and double bond content[71]), and proatherogenic ceramide derivatives (sphingosine and shingosine-1-phosphate[58,59]). We also observed favorable increases in metabolites associated with lower cardiometabolic risk, such as 1-methylnicotinamide (promotes lipolysis[26]), glutamine, and 12,13-diHOME (promotes skeletal muscle fatty acid uptake[56]). Shifts in many of these metabolites were not related to workload achieved, suggesting that a salutary switch may be turned on by brief exercise that results in favorable changes in circulating levels of metabolites, reflecting a healthier metabolic state.

Favorable metabolite shifts may be blunted in those with a higher BMI, even after adjustment for workload, suggesting obesity or its related metabolic perturbations (eg, insulin resistance) may confer resistance to the benefits of exercise. We also observed distinct metabolic responses to exercise between men and women



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Associations of metabolite variates with outcomes in Gen 2

Metabolite variate	Death HR (95% CI)	Death P-value	CVD HR (95% CI)	CVD P-value
Metabolite variate 1				
Age-, sex-, BMI-adj	1.17 (1.07, 1.27)	0.0004	1.11 (1.01, 1.22)	0.03
Age-, sex-, BMI-, and clinical risk factor-adj	1.08 (0.98, 1.18)	0.11	0.99 (0.89, 1.09)	0.82
Metabolite variate 2				
Age-, sex-, BMI-adj	0.88 (0.81, 0.95)	0.0007	1.00 (0.94, 1.06)	0.97
Age-, sex-, BMI-, and clinical risk factor-adj	0.89 (0.82, 0.96)	0.003	0.99 (0.93, 1.05)	0.69
Metabolite variate 3				
Age-, sex-, BMI-adj	0.99 (0.94, 1.04)	0.57	1.00 (0.94, 1.07)	0.93
Age-, sex-, BMI-, and clinical risk factor-adj	0.96 (0.91, 1.01)	0.14	0.99 (0.93, 1.05)	0.71
Metabolite variate 4				
Age-, sex-, BMI-adj	0.93 (0.89, 0.97)	0.002	0.98 (0.93, 1.03)	0.38
Age-, sex-, BMI-, and clinical risk factor-adj	0.93 (0.89, 0.97)	0.001	0.97 (0.92, 1.02)	0.21

Clinical risk factors include: systolic blood pressure, hypertension treatment status, diabetes, current smoking, total/HDL cholesterol
Hazard ratios are expressed for a 1-SD higher value of the variate score

Figure 4. Correlations of metabolites with integrated exercise responses.
A, Age- and sex-adjusted partial correlations of each exercise measure with metabolite variates determined by canonical correlation analysis. The size of each circle is proportional to the absolute value of the correlation coefficient, and its color represents the directionality of correlation. **B**, Heatmap of the age- and sex-adjusted partial correlations of metabolites with metabolite variates. The metabolites shown here have a correlation coefficient ≥0.25 with any of the metabolite variates. **C**, Multivariable-adjusted associations of the 4 metabolite variates with death and incident CVD. MAP indicates mean arterial pressure; and VE/VCO2, ventilatory efficiency.

consistent with known sex heterogeneity in cardiorespiratory fitness. Men appeared to experience greater excursions in metabolites linked to cellular metabolism, while changes in several metabolites with putative beneficial implications were more prominent in women. Whether these are the result of sexual dimorphism in body composition (eg, greater lean muscle mass in men), underlying mechanisms of exercise response, or residual confounding remains a source of future study in large samples specifically designed to address this hypothesis.[82] We observed consistency of metabolite changes with exercise across a broad age spectrum, which corresponds to previous findings of similar clinical benefits of exercise among different age groups.[83]

By combining detailed multidimensional exercise measures obtained using CPET with metabolite profiling,

we were able to evaluate metabolite signatures of discrete physiologic exercise responses. We observed distinct metabolite signatures for peak V_{O_2} (reflecting whole-body metabolic responses), submaximum blood pressure response (reflecting systemic vascular function), and V_E/V_{CO_2} (reflecting pulmonary vascular dysfunction and impaired cardiac performance). Peak V_{O_2} was associated with resting levels of a large number of metabolites, including those related to fat metabolism, insulin resistance, oxidative stress, and anaerobic metabolism, reflecting a combination of central cardiovascular function and peripheral oxygen utilization. Adverse submaximum blood pressure responses were directly associated with higher levels of TAG species related to CVD risk,[71] higher levels of amino acid derivates linked to resting systemic hypertension,[74] and lower levels of vasoprotective plasmalogens.[72,73] V_E/V_{CO_2}, which reflects ventilation–perfusion matching in the pulmonary circulation and is elevated in proportion to pulmonary vascular resistance,[10,75] demonstrated associations with various metabolites related to overt resting pulmonary arterial hypertension including acylcarnitines,[68,76] 16-hydroxypalmitate,[77] and β-hydroxybutyrate.[78] These findings offer mechanistic insight into the metabolic pathways distinctly involved in organ-specific physiologic responses to exercise that lie upstream of overt forms of disease states evident at rest (ie, pulmonary arterial hypertension). Composite metabolite signatures of integrated exercise responses were associated with mortality after adjustment for traditional CVD risk factors, consistent with strong associations between cardiorespiratory fitness and both cardiovascular[84] and noncardiovascular mortality.[85]

Our findings are highly consistent with and extend those from a recent study by Contrepois et al,[7] which reported multi-omic changes with acute exercise in 36 individuals. Similar to this published report, we observed increases in metabolites involved in anaerobic and aerobic respiration, complex lipids such as sphingomyelins, nucleotide derivatives, and reductions in branched chain amino acids, among others.[7] Whereas these consistencies suggest the external validity of our findings, our report fundamentally extends the published literature in this space in several important ways. First, the larger sample size in the present study allowed detection of exercise changes in metabolites relevant to cardiometabolic disease not previously reported, likely attributable to enhanced statistical power. These include exercise-induced decreases in bile acids and modest changes in glutamine[74] (linked with longevity and CVD), furthering the understanding of biological pathways related to exercise responses. In our study, exercise-induced changes in metabolites were replicated in a separate sample, providing strong support of the validity of our key findings. The greater sample size across a broader, heterogeneous population also affords insights into potential modification of observed exercise responses by obesity, age, and sex. Moreover, the comprehensive CPET testing here allowed us to link resting metabolite levels to specific exercise phenotypes reflecting distinct physiologies. These CPET phenotypes may offer insight into metabolic dysfunction related to the early, subclinical stages of CVD development: for example, association of acylcarnitines with V_E/V_{CO_2} slope in the FHS (a population without overt clinical disease) was similar to that in animal models and patients with symptomatic pulmonary arterial hypertension.[76] Similarly, we related metabolite classes such as plasmalogens to exercise blood pressure with adjustment for resting blood pressure, thereby creating signatures that are specific to an exercise response pattern that antedates future hypertension.[17] Lastly, by exporting a metabolomic score representing multidimensional exercise responses to a separate cohort, we demonstrated prognostically significant associations of metabolic exercise signatures with long-term health outcomes. These contributions highlight the importance of broad phenotyping across a large sample to clarify potential disease mechanisms for perturbation, mechanistic evaluation, and targeting.

Our study has several important limitations. Although our sample size was more than an order of magnitude higher than previously published data in this area, we recruited primarily White, middle-aged adults and focused on acute exercise on a cycle. This limits generalizability by age, race, disability, and chronicity and type of exercise, although metabolite excursions observed here are largely consistent with signals from previous studies.[4,7,29] Cycle exercise results in lower maximal CPET measures and is less influenced by body mass compared with treadmill exercise; differences in the activity of metabolic pathways based on exercise modality should be examined in future studies. Whereas we observed sex- and BMI-related heterogeneity in the metabolic response to exercise, true evidence of modification of effect by sex, BMI, and other important clinical features will require larger studies with more comprehensive phenotyping of other confounders known to display differences by sex (eg, muscle and fat distribution) and perturbational studies (ie, weight loss). We observed an approximately equal proportion of metabolites that increased with exercise (that may be affected by exercise-associated hemoconcentration) as metabolites that decreased with exercise (the effect of which would be attenuated by exercise-related hemoconcentration), suggesting that hemoconcentration during this brief period of exercise may not substantially confound the observed changes. The duration of acute, favorable exercise-induced changes in metabolite levels was also not captured because of limitations in feasibility of serial postexercise sampling in a population study. Lastly, we observed metabolite signatures of exercise responses to be more closely associated with future mortality than with incident CVD,

highlighting that exercise-related metabolite pathways associate with prognosis outside of their role in CVD.

Acute exercise exerts specific, widespread changes on the circulating metabolome, many of which are related to favorable health mechanisms. Metabolic responses to exercise are not necessarily related only to the work performed, but may be modulated by key clinical features, including sex and obesity. These results not only provide a detailed resource regarding the metabolic architecture of acute exercise in humans, but also pinpoint specific pathways of prognostically relevant, exercise-mediated metabolic adaptation, suggesting novel avenues for future mechanistic investigation and intervention.

ARTICLE INFORMATION

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Supplemental Materials

Expanded Methods
Data Supplement Figures I–VI
Data Supplement Tables I–XVI
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