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Plasma Metabolite Profiles in Response to Chronic Exercise

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Abstract

Purpose—High throughput profiling of metabolic status (metabolomics) allows for the assessment of small-molecule metabolites that may participate in exercise-induced biochemical pathways and corresponding cardiometabolic risk modification. We sought to describe the changes in a diverse set of plasma metabolite profiles in patients undergoing chronic exercise training and assess the relationship between metabolites and cardiometabolic response to exercise.

Methods—secondary analysis was performed in 216 middle-aged abdominally obese men and women ([mean (SD)], 52.4 (8.0) years) randomized into one of four groups varying in exercise amount and intensity for 6 months duration: high amount high intensity, high amount low intensity, low amount low intensity, and control. 147 metabolites were profiled by liquid chromatography-tandem mass spectrometry.

Results—No significant differences in metabolite changes between specific exercise groups were observed; therefore, subsequent analyses were collapsed across exercise groups. There were no significant differences in metabolite changes between the exercise and control groups after 24 weeks at a Bonferroni-adjusted statistical significance ($p < 3.0 \times 10^{-4}$). Seven metabolites changed in the exercise group compared to the control group at $p < 0.05$. Changes in several metabolites from distinct metabolic pathways were associated with change in cardiometabolic risk traits, and three baseline metabolite levels predicted changes in cardiometabolic risk traits.

Conclusion—Metabolomic profiling revealed no significant plasma metabolite changes between exercise compared to control after 24-weeks at Bonferroni significance. However, we identified circulating biomarkers that were predictive or reflective of improvements in cardiometabolic traits in the exercise group.

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Conflict of Interest

The authors have no conflicts of interest to disclose. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Keywords

Exercise; metabolomics; risk factors; global assessment

Introduction

Increased physical activity and improved cardiorespiratory fitness (CRF) are associated with improvements in individual cardiometabolic risk factors as well as global cardiovascular disease risk (1-5), however the molecular mechanisms underlying the salutary effects of exercise remain unclear. Substantial heterogeneity exists in the individual response to exercise training (6-8) thus, insight into the biochemical pathways involved in exercise offers promise towards early detection of cardiometabolic disease (9) and tailoring exercise "prescriptions" to the individual level (10).

Metabolites are substrates and by-products of metabolism with a diverse set of biochemical actions that serve as both biomarkers and effectors of disease states (11, 12). The metabolome's interface between gene-protein functional state and phenotype, and its dynamic response to environmental stimuli makes it well suited for the study of physiologic perturbations such as exercise. Advancements in high-throughput platforms have enabled the systematic assessment of large numbers of small-molecule metabolites in tissue and plasma samples that may participate in exercise-induced biochemical pathways (12, 13).

Work by our group and others have characterized changes in plasma metabolites following a single aerobic exercise session using advanced metabolite profiling technologies (13-15). Findings from these studies demonstrate exercise-induced changes in several key metabolites involved in glycolysis, lipolysis, glycogenolysis, the citric acid (TCA) cycle, and amino acid metabolism, and identified differential substrate utilization among more and less fit individuals. By contrast, far less is known about the metabolic response to prolonged exercise training. Huffman et al (16) examined the change in 69 plasma metabolites after six months of chronic exercise training in a small (n=53) cohort of sedentary, middle-aged, overweight or mildly obese men and women, and found significant changes in leptin, monocyte chemoattractant protein (MCP-1) and arachidoyl carnitine (C20) compared to the inactive control group. Notably, this study focused on free fatty acids, acylcarnitines and a subset of amino acids. More recently, the same group employed a larger, targeted metabolomics platform in skeletal muscle biopsies in an expanded sample, and found that a high amount of vigorous intensity aerobic exercise significantly increased concentrations of even-, medium-, and long-chain acylcarnitines, while resistance training and a low-amount of vigorous intensity aerobic exercise preferentially increased short- and medium-chain acylcarnitines (17).

The purpose of this study was to characterize the impact of a prolonged exercise intervention on a diverse set of circulating plasma metabolites. We employed a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) platform in peripheral blood samples before and after participation in a six-month randomized controlled exercise trial among 216 middle-aged, abdominally obese men and women. We tested whether metabolite

changes differed between groups, and if metabolites were associated with changes in cardiometabolic traits.

Methods

The study was approved by the Queen's University Health Sciences Research Ethics Board and all participants provided written informed consent. Details of the study design and methods have been published previously (18). Briefly, the 24-week exercise intervention randomized 300 inactive, abdominally obese (waist circumference (WC) >102 cm for men, 88 cm for women) participants into one of four exercise groups: control (no exercise), low amount low intensity (LALI; 180 and 300 kcal/session for women and men, respectively, at 50% VO_2peak), high amount low intensity (HALI; 360 and 600 kcal/session, respectively, at 50% VO_2peak), and high amount high intensity (HAHI; 360 and 600 kcal/session, respectively, at 75% VO_2peak). Cardiorespiratory fitness was assessed using standard open-circuit spirometry during a maximal graded exercise test. Waist circumference (WC) and blood pressure were measured at baseline, 16 and 24 weeks. Fasting insulin (pmol/L), homeostatic model assessment insulin resistance (HOMA-IR), 2-hour glucose (mmol/L) and insulin area under the curve (AUC) were derived from a 2-hour, 75-g oral glucose tolerance test (OGTT) at baseline, 16 and 24 weeks.

In the current analysis, participants from the original trial were excluded if they did not complete the 24-week intervention (n=84), which resulted in a study sample of 216 participants (Control, n=53; HAHI, n=47; HALI, n=60; LALI, n=56).

Plasma Sampling and Metabolite Profiling

Fasting plasma samples were acquired via the antecubital vein at baseline prior to the OGTT and again 48 hours after the last exercise session at 16 and 24 weeks. The plasma was immediately separated by centrifugation (10 minutes at 4250 rpm) and stored at -80°C.

We used two distinct, LC-MS/MS based methods to profile 147 analytes including amino acids, organic acids, bile acids, indoles, nucleotides and sugars. These platforms have been previously used to characterize a diverse set of biochemical pathways implicated in metabolic status (19, 20). Briefly, fasting plasma samples (EDTA, 10μL and 30μL for positive and negative ion modes, respectively) were deproteinized using extraction solvent containing stable isotope labeled internal standards. Samples were vortexed and centrifuged, and aliquots were transferred to 2mL autosampler vials with glass inserts for LC-MS analysis. In positive mode, normal phase hydrophilic interaction chromatography (HILIC) using a 2.1×150mm 3μm Atlantis column (Waters) was coupled to a 4000 QTrap triple quadrupole mass spectrometer (Applied Biosystems/Sciex) equipped with an electrospray ionization source for targeted detection of 78 metabolites using a dynamic multiple reaction monitoring (dMRM) mechanism. In negative mode, HILIC chromatography using a 2.1×100mm 3.5 μm Xbridge Amide column (Waters) was coupled to an Agilent 6490 triple quadrupole mass spectrometer equipped with an electrospray ionization source for targeted detection of 69 metabolites using dMRM. Metabolite peak areas were integrated using Sciex MultiQuant software (positive mode) or Agilent Masshunter Quantitative software (negative mode). All metabolite peaks were manually reviewed for peak quality in a blinded manner.

In addition, pooled plasma samples were interspersed within each analytic run at standardized intervals, enabling the monitoring and correction for temporal drift in MS performance. The median coefficient of variation (CV) in metabolites was 5.88% in positive mode and 5.23% in negative mode.

Statistical Analysis

All metabolites were log-transformed to approximate a normal distribution and analyses were adjusted for age and sex. Repeated measures ANOVA was used to compare metabolite changes within and between the control, LALI, HALI, and HAHI groups. In secondary analyses, principal component analysis (PCA) was used to reduce dimensionality of the metabolite dataset at baseline. Thirty-seven main principal factors were derived after orthogonal Varimax rotation (Table 1). Factors were retained if they had an eigenvalue >1 and individual metabolites with a factor load >|0.4| for a given PCA-derived factor were included. Scores were calculated for each participant based on standardized scoring coefficients. Cross-sectional associations between PCA factor scores and cardiometabolic risk traits (body weight, BMI, WC, 2-hour glucose, fasting insulin, insulin AUC, HOMA-IR, systolic blood pressure (SBP), diastolic blood pressure (DBP)) were assessed with multiple linear regression adjusted for age and sex. Additionally, multiple linear regression was used to determine cross-sectional associations between individual metabolites and cardiometabolic risk traits, baseline and change in metabolite concentrations, and change in cardiometabolic traits. All analyses were performed using SPSS Statistics (Version 23) and statistical significance was determined at the Bonferroni-corrected p-value $< 3.0\times10^{-4}$ (0.05/147). Nominal significance was set at $p < 0.05$.

Results

Participant characteristics

Participant characteristics pre- and post-treatment are summarized in Table 2. Briefly, participants were abdominally obese, inactive, middle-aged adults with a relatively healthy metabolic profile. Significant reductions in body weight, WC, fasting insulin, HOMA-IR and insulin AUC, and increases in CRF were observed in exercise groups compared to control ($p < 0.05$).

Baseline associations between metabolites and cardiometabolic traits

PCA was used to reduce the large number of circulating metabolites into fewer factors containing highly correlated metabolites that are biologically related (Table 1). We detected strong associations between several principal factors, cardiometabolic risk factors and CRF prior to the initiation of exercise (Table 3). Both PCA factor and individual metabolite associations revealed several well-established relationships between metabolic pathways and clinical traits, including body mass (tryptophan derivatives, aromatic amino acids, glutamate, uric acid and adenine catabolism products) and glucose and insulin homeostasis (branched chain amino acids, aromatic amino acids, tryptophan derivatives, and glutamate) (21-23). The hexosamine end-product, UDP-N-acetylglucosamine (UDP-GlcNac), was also strongly and positively related to baseline levels of fasting and 2-hour insulin, insulin AUC, and HOMA-IR. Additionally, we identified novel inverse relationships between the aromatic

amino acid, tyrosine ($p = 2.9\times10^{-4}$); tryptophan precursor, anthranilic acid ($p = 1.5\times10^{-4}$); and the adenine nucleotide catabolism product, inosine ($p = 2.1\times10^{-4}$); and directly measured cardiorespiratory fitness.

Change in metabolites at 24 weeks

There were no significant metabolite changes between exercise and control groups after 24 weeks at the Bonferroni-adjusted threshold ($p < 3.0 \times 10^{-4}$). When exercise arms were collapsed to create a single exercise group (N = 163), we found 7 metabolites that changed at nominal significance ($p < 0.05$) compared to the control group. These included tryptophan metabolites [(indoxylsulfate (increased) and indole-3-lactic acid (increased)]; metabolites derived from energy metabolism pathways [(aconitic acid (decreased), pyruvic acid (decreased), ATP (increased), malonic acid (increased)]; and the purine degradation product xanthine (decreased). Changes in indole-3-lactic acid, indoxylsulfate, pyruvic acid and xanthine appear to be driven by the high amount exercise groups upon examination of within-group changes. We further identified several metabolites that changed from baseline levels within exercise groups at the Bonferroni-adjusted level of significance Products of adenine nucleotide metabolism (cyclic AMP, inosine, xanthine, and xanthosine); lipolysis (glycerol); the nucleotide sugar, UDP-GlcNac; and non-polar amino acids and intermediates (alanine and homogentisic acid) decreased, whereas serine and acetoacetic acid, a fatty-acid intermediate, both increased in HALI. Xanthine and indole-3-lactic acid also decreased and increased in LALI and HAHI, respectively.

Metabolite changes associated with changes in cardiometabolic traits

We detected strong associations between analyte changes and changes in cardiometabolic traits ($p < 3 \times 10^{-4}$, Table 4). Changes in the branched chain amino acids leucine and isoleucine, and UDP-GlcNAc were inversely associated with changes in CRF (ml/kg/min), whereas change in the TCA cycle intermediate, isocitric acid, was positively correlated. These changes stayed significant even after adjustment for known relationships (BMI, fasting glucose). Changes in gluconeogenic amino acids (alanine, tyrosine, and proline) and UDP-GlcNAc were positively associated with change in BMI; UDP-GlcNAc was similarly associated with WC and insulin metabolism (insulin and insulin AUC). Xanthurenic acid was also associated with change in insulin AUC. These relationships remained significant after further adjusting for change in weight.

Changes in metabolites that were associated with changes in cardiometabolic traits at nominal significance are reported (see Table, Supplemental Digital Content 1, Associations between change in metabolites and change in cardiometabolic traits, http://links.lww.com/MSS/B221).

Baseline metabolites associated with change in cardiometabolic traits

We detected baseline metabolite levels measured prior to the initiation of exercise that predicted a favorable cardiometabolic trait response after 24 weeks of chronic exercise, even after adjusting for the baseline clinical trait (Table 5). Baseline taurine and asymmetric dimethylarginine (ADMA)/symmetric dimethylarginine (SDMA) concentrations were inversely associated with changes in WC and 2-hour glucose, respectively, while glutamate

was positively associated with change in DBP ($p < 3 \times 10^{-4}$). Additional associations between baseline metabolites and change in cardiometabolic risk factors and CRF at nominal significance are reported (see Table, Supplemental Digital Content 2, Associations between baseline metabolites and change in cardiometabolic risk factors, http://links.lww.com/MSS/B222).

Discussion

Advancements in metabolomics technologies have enabled efforts to systematically profile blood-based metabolites in order to characterize changes predictive (13, 24) or reflective (9) of physiologic states. Here, we employed a targeted LC-MS/MS platform to describe the changes in a large and varied set of plasma metabolites in a cohort of 216 abdominally obese subjects randomized to 24 weeks of inactivity or one of three aerobic exercise interventions. We found no significant differences between metabolites in the control and exercise arms at our *a priori* level of statistical significance. Products of lipolysis, adenine nucleotide metabolism, and both polar- and non-polar amino acids changed within individual exercise groups, albeit not differently from control. We confirmed cross-sectional relationships between metabolites and cardiometabolic risk traits. Additionally, we demonstrated associations between changes in metabolite level and changes in these traits.

Prior metabolomics investigations into the effects of chronic exercise training were either limited by a small sample size and less expansive metabolite platform (16), or characterized changes in skeletal muscle rather than blood (17). Huffman et al. randomized participants into exercise groups varying in amount and intensity and a control group and observed only three nominally significant metabolite changes between groups (16). The same group also studied changes in skeletal muscle metabolite concentrations after exercise training and found nominally ($p < 0.05$) significant increases in even-chain acylcarnitines and TCA cycle intermediates, whereas skeletal muscle concentrations of amino acids, glycolytic metabolites (lactate and pyruvate) and all other TCA cycle intermediates did not change (17).

Similarly, our group did not find significant differences in metabolites between exercise and control groups at the Bonferroni-adjusted level of significance ($p < 4 \times 10^{-4}$) but did detect significant metabolite changes within groups. Several potential explanations exist for these findings. First, the use of a strict statistical threshold may have masked biologically relevant associations, particularly when several of the metabolites under scrutiny cluster together and participate in known biologic pathways (11). Second, the presence of significant inter-individual variability may have blunted group comparisons. Within group analysis using each individual as his/her own biologic control revealed significant changes within the exercise intervention arms, an experimental design that has been previously employed in small metabolomics (9, 13, 16) and proteomic (25) pertubational studies. While we cannot attribute exercise effects to the changes seen within individual exercise arms, our findings underscore the importance of performing much larger trials that are better powered to characterize the molecular response to physical activity (26).

The cross-sectional associations between baseline metabolites and clinical risk factors demonstrated here extend previous findings. Elevations in aromatic (tyrosine) and branched

chain amino acids, and tryptophan breakdown products (anthranilic acid, kynurenines) have been strongly correlated with elevated body mass and insulin resistance phenotypes (23, 27, 28). In addition, we observed novel inverse associations between tyrosine, anthranilic acid, and inosine, and peak oxygen consumption (VO_2peak) which is an established predictor of morbidity and mortality.

We further explored whether improvements in cardiometabolic traits were related to changes in circulating metabolite levels. For instance, decreased uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) concentrations were associated with improvements in anthropometric and insulin resistance traits, whereas increased levels were associated with worse CRF. UDP-GlcNAc, a nucleotide sugar, is the end-product of the hexosamine biosynthetic pathway (HBP) and acts as a substrate for the O-linked N-acetylglucosamine transferase enzymes (OGTs). Chronically elevated glucose concentrations are associated with increased UDP-GlcNAc concentrations via flux through the HBP, augmenting O-GlcNAcylation of numerous proteins across the body and altering their function (29). Aberrant OGT activity has been implicated in insulin resistance, dyslipidemia, and diabetic cardiomyopathy (30-32), and increases in graded fashion with increased UDP-GlcNAc concentrations. In addition, increased O-GlcNAc mitochondrial protein expression within myocardium was seen among rats selected for low capacity running in comparison to high capacity runners, suggesting a role for OGT activity in aerobic capacity (33). Investigation into the effects of chronic exercise on OGT activity has been limited to myocardial tissue in different mouse models undergoing heterogeneous exercise interventions, and its role is not well understood (29). Our findings validate UDP-GlcNAc's relationship to metabolic health traits, and further suggest its potential role as either a marker or effector of cardiorespiratory status.

Lastly, we explored whether baseline metabolite levels could predict exercise-induced response in a given cardiometabolic trait and identified three significant associations. Baseline glutamate levels were positively associated with change in DBP. This extends previous cross-sectional findings, wherein glutamate was positively associated with a range of cardiometabolic risk factors (insulin, HOMA-IR, SBP, and DBP) (23). ADMA/SDMA levels were inversely related to changes in 2-hour glucose, a finding previously supported (34). In addition, baseline taurine (a sulfated organic compound) was negatively associated with change in WC. These findings are consistent with previous observations investigating the relationship between taurine and obesity. In animal studies, a decrease in blood taurine concentration was associated with obesity (35) and taurine treatment reduced inflammatory processes in adipose tissue via reduction in macrophage infiltration. Our observations extend prior literature by suggesting that higher plasma taurine concentrations may predict a more favourable exercise response for abdominal obesity.

A limitation of our study is that the sample was relatively homogeneous which may have attenuated the strength of observed relationships. However, given that nearly 40% of US and Canadian adults are abdominally obese (36, 37), our findings are relevant to this population. Furthermore, while we measured a diverse panel of ~150 metabolites in plasma, our analysis was targeted and not comprehensive of the entire plasma metabolome.

In summary, our study provides additional details on the metabolic changes induced by chronic exercise training, in addition to potential predictors of changes in metabolic traits. These data motivate additional studies in larger, heterogeneous cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Principal component analysis factors.

Factors	Metabolites	Eigenvalue	Variance, %
1	Glutamine, lysine, arginine, histidine, asparagine, NMMA, citrulline, ornithine, methionine, cysteine, threonine, serine, aspartate, ADMA/SDMA, proline, alanine, tyrosine, glycine	19.047	13.046
2	C10-carnitine, C8-carnitine, C12-carnitine, C6-carnitine, C14-carnitine, C7-carnitine, C5-glutarylcarnitine, C16-carnitine, C9-carnitine, C18-carnitine, C18:1-carnitine, C2-carnitine	10.154	6.955
3	2-aminoadipic acid, 2-hydroxyglutaric acid, phosphocreatine, malic acid, malonic acid, D-gluconic acid, Quinolinic acid, oxalic acid, citric acid isocitric acid	8.143	5.578
4	Lactic acid, pyruvic acid, hypoxanthine, alpha-ketoglutaric acid, alanine, fumaric acid, aconitic acid, hydroxybutyric acid, inosine, ketoisocaproic acid, 2-ketoisovaleric acid	7.101	4.863
5	Methionine, tryptophan, xanthurenate, leucine, isoleucine, indole-3-lactic acid, valine, tyrosine	5.756	3.943
6	C3-carnitine, C4-butyryl-carnitine, carnitine, C5-valeryl carnitine, C4-methylmalonyl carnitine, C2-carnitine	5.108	3.499
7	Quinolinic acid, kynurenine, anthranilic acid, indole-3-carboxylic acid	4.716	3.230
8	Glycocholic acid, taurodeoxycholic acid, taurocholic acid, glycochenodeoxycholic acid,	4.210	2.884
9	C16-carnitine, C26-carnitine, C18-carniitne, C18:1-carnitine, C18:2-carnitine,	3.677	2.519
10	Xanthosine, inosine, xanthine	3.384	2.318
11	3-hydroxybutyric acid, acetoacetic acid, C3-malonyl-carnitine, C2-carnitine	3.115	2.133
12	ADP, AMP, ATP, UDP-glucose-galactose	2.879	1.972
13	Uridine, uracil	2.754	1.886
14	Glucose-fructose-galactose, 2-deoxyuridine, ketoisocaproic acid, 2-ketoisovaleric acid, uric acid	2.639	1.808
15	Indole-3-lactic acid, xanthurenic acid, kynurenic acid	2.417	1.655
16	Pantothenic acid, thiamine, cystamine, niacinamide, glutathione reduced	2.157	1.477
17	Choline, dimethylglycine	2.094	1.434
18	Betaine, allantoin	2.029	1.390
19	3-aminoisobutyric acid, aminoisobutyric acid	1.955	1.339
20	Trimethylamine-N-oxide, cysteamine	1.859	1.273
21	UDP-GlcNAc, homogentisic acid, UDP-glucose-galactose	1.738	1.190
22	Succinic acid, cyclic AMP	1.642	1.125
23	Glyceric acid, pyroglutamic acid	1.629	1.116
24	Glycine, 5-adenosylhomocysteine,serine	1.557	1.066
25	2-deoxycytidine, cytidine	1.480	1.013
26	Psuedouridine, S-adenosyl-L-homocysteine	1.375	0.942
27	Phosphocholine, phenylalanine, N-acetyl-L-glutamic acid	1.322	0.906
28	Arachidonoylglycerol	1.278	0.875
29	Thyroxine, glycerol, creatine	1.254	0.859
30	Indoxylsulfate, oxaloacetic acid	1.204	0.825
31	Indole-3-carboxylic acid, hydroxyphenylpyruvic acid	1.173	0.804
32	N-acetyl-L-glutamic acid, cis/transhydroxproline	1.153	0.790
33	Thymidine, n-acetyl-L-methionine	1.118	0.766
34	5-MTHF	1.096	0.751

Factors	Metabolites	Eigenvalue	Variance, %
35	Homocysteine, indole-3-propanoic acid	1.067	0.731
36	1,5-AG-1-deoxyglucose	1.042	0.713
37	Maleic acid	1.039	0.711

PCA, principal component analysis. Metabolites retained within each factor have factor load ≥ |0.4|.

Table 2

Participant characteristics at baseline and 24 weeks.

	All Exercise (n=163)		Control (n=53)	
Characteristic	Baseline	Change	Baseline	Change
Age, y	52.4 ± 7.8		52.3 ± 8.4	
Sex (% male, % female)	36, 64		34, 66	
Weight (kg)	94.7 ± 15.9	-5.4 ± 4.7*	93.6 ± 16.9	-0.7 ± 4.4
WC (cm)	110.5 ± 11.3	-5.4 ± 4.7*	108.8 ± 10.6	-0.9 ± 4.2
Fasting insulin (pmol/L)	65.6 ± 35.7	-14.2 ± 23.3*	70.7 ± 34.9	-6.6 ± 27.1
HOMA-IR	2.3 ± 1.4	-0.5 ± 0.9*	2.5 ± 1.3	-0.3 ± 1.0
2-hr glucose (mmol/L)	7.2 ± 1.6	-0.2 ± 1.7	7.6 ± 1.7	0.06 ± 1.6
Insulin AUC (pmol/L)	2080.6 ± 1281.9	-458.2 ± 817.8*	2042.4 ± 1021.4	-112.4 ± 661.3
SBP (mmHg)	121.7 ± 12.9	-3.1 ± 10.2	122.1 ± 13.1	-0.4 ± 13.3
DBP (mmHg)	80.2 ± 8.2	-2.9 ± 7.4	79.7 ± 8.1	-0.8 ± 7.8
CRF (VO_2peak)				
L/min	2.7 ± 0.7	0.4 ± 0.3*	2.7 ± 0.8	-0.02 ± 0.2
mL/kg/min	28.4 ± 5.1	6.3 ± 4.1*	29.2 ± 6.0	-0.09 ± 3.0

Mean ± SD. WC, waist circumference; AUC, area under the curve; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRF, cardiorespiratory fitness.

*Exercise differs from control ($p<0.05$).

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Table 3

Cross-sectional associations between PCA factors and cardiometabolic risk factors.

Risk Factor	PCA Factors										
	2	3	4	5	7	9	10	11	12	13	14
CRF	—	-0.16*	—	—	-0.15*	—	-0.14*	—	—	0.12*	—
Weight	—	—	—	—	0.23†	—	—	—	—	—	—
WC	—	—	0.14*	—	0.27†	—	—	—	—	—	—
Fasting Insulin	—	—	0.20*	0.20*	0.30†	-0.17*	0.18*	—	-0.19*	—	—
HOMA IR	—	0.14*	0.20*	0.19*	0.30†	-0.19*	0.16*	—	-0.21*	—	—
Insulin AUC		—	0.21*	0.19*	0.29†	-0.18*	—	—	-0.15*	—	—
2-hr Glucose	0.15*	—	0.16*	—	—	—	—	0.14*	—	—	—
SBP	—	—	—	—	—	—	0.14*	—	—	-0.17*	-0.15*
DBP	—	0.15*	—	—	—	—	0.18*	—	-0.14*	-0.13*	—
	PCA Factors (cont'd)										
	16	19	21	23	24	26	28	30	32	36	
CRF	—	—	—	—	—	—	—	—	-0.20†	-0.13*	
Weight	—	—	—	—	—	—	—	—	0.15*	0.18†	
WC	—	—	—	-0.12*	—	—	—	—	0.19*	0.19*	
Fasting Insulin	—	-0.24†	0.27†	-0.15*	—	—	—	0.16*	0.15*	—	
HOMA IR	—	-0.24†	0.27†	-0.14*	—	—	—	0.16*	0.14*	—	
Insulin AUC	—	-0.25†	0.22*	-0.16*	-0.16*	—	—	0.17*	—	—	
2-hr Glucose	—	-0.14*	0.20*	—	—	—	—	0.23†	—	—	
SBP	-0.13*	—	0.19*	—	—	—	-0.13*	0.14*	—	—	
DBP	—	-0.19*	0.24†	—	—	0.15*	—	—	—	—	

Table displays factors for which a significant association as observed with at least one variable.

*P≤.05,

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$^{\dagger}P \leq .001$ (Bonferroni-corrected; 0.05/37 PCA factors).

Table 4

Associations between change in metabolites and change in cardiometabolic traits ($p < 3.0 \times 10^{-4}$).

Δ Risk Factor	Δ Metabolite	Beta Coefficient	p-Value
CRF (mL/kg/min)	UDP-GlcNAc	-0.47	1.94×10^{-8}
	Isoleucine	-0.34	9.56×10^{-5}
	Citric Acid/Isocitric Acid	0.33	1.30×10^{-4}
	Leucine	-0.33	1.60×10^{-4}
WC	UDP-GlcNAc	0.51	7.55×10^{-11}
BMI	UDP-GlcNAc	0.49	3.99×10^{-10}
	Alanine	0.42	9.82×10^{-8}
	Tyrosine	0.33	3.99×10^{-5}
	Proline	0.30	2.59×10^{-4}
Fasting Insulin	UDP-GlcNAc	0.36	2.02×10^{-4}
Insulin AUC	UDP-GlcNAc	0.37	3.10×10^{-4}
	Xanthurenic Acid	0.36	3.56×10^{-4}

WC, waist circumference; AUC, area under the curve; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRF, cardiorespiratory fitness. Multiple regression adjusted for age, sex and baseline risk factor.

Table 5

Associations between baseline metabolites and change in cardiometabolic traits ($p < 3.0 \times 10^{-4}$).

Δ Risk Factor	Δ Metabolite	Beta Coefficient	p-Value
WC	Taurine	-0.28	3.0×10^{-4}
2-hour Glucose	ADMA/SDMA	-0.25	2.5×10^{-4}
DBP	Glutamate	0.29	2.5×10^{-4}

WC, waist circumference; DBP, diastolic blood pressure; ADMA/SDMA, asymmetric dimethylarginine/symmetricdimethylarginine.