Science Translational Medicine
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Gregory D. Lewis, *et al.*

Sci Transl Med **2**, 33ra37 (2010);
DOI: 10.1126/scitranslmed.3001006

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EXERCISE AND METABOLISM

Metabolic Signatures of Exercise in Human Plasma

Gregory D. Lewis,[1,2,3,4]* Laurie Farrell,[1] Malissa J. Wood,[1] Maryann Martinovic,[1] Zoltan Arany,[5] Glenn C. Rowe,[5] Amanda Souza,[4] Susan Cheng,[1,6,7] Elizabeth L. McCabe,[6] Elaine Yang,[4] Xu Shi,[4] Rahul Deo,[1,8] Frederick P. Roth,[8] Aarti Asnani,[1,2] Eugene P. Rhee,[4,9] David M. Systrom,[10] Marc J. Semigran,[1] Ramachandran S. Vasan,[6,11,12] Steven A. Carr,[4] Thomas J. Wang,[1,6] Marc S. Sabatine,[3,7] Clary B. Clish,[4] Robert E. Gerszten[1,2,3,4]*

(Published 26 May 2010; Volume 2 Issue 33 33ra37)

Exercise provides numerous salutary effects, but our understanding of how these occur is limited. To gain a clearer picture of exercise-induced metabolic responses, we have developed comprehensive plasma metabolite signatures by using mass spectrometry to measure >200 metabolites before and after exercise. We identified plasma indicators of glycogenolysis (glucose-6-phosphate), tricarboxylic acid cycle span 2 expansion (succinate, malate, and fumarate), and lipolysis (glycerol), as well as modulators of insulin sensitivity (niacinamide) and fatty acid oxidation (pantothenic acid). Metabolites that were highly correlated with fitness parameters were found in subjects undergoing acute exercise testing and marathon running and in 302 subjects from a longitudinal cohort study. Exercise-induced increases in glycerol were strongly related to fitness levels in normal individuals and were attenuated in subjects with myocardial ischemia. A combination of metabolites that increased in plasma in response to exercise (glycerol, niacinamide, glucose-6-phosphate, pantothenate, and succinate) up-regulated the expression of nur77, a transcriptional regulator of glucose utilization and lipid metabolism genes in skeletal muscle in vitro. Plasma metabolic profiles obtained during exercise provide signatures of exercise performance and cardiovascular disease susceptibility, in addition to highlighting molecular pathways that may modulate the salutary effects of exercise.

INTRODUCTION

Exercise can confer cardiovascular protection (*1*, *2*), unmask occult organ dysfunction (*3*), and predict disease prognosis (*4*, *5*), but how and why these effects occur are not entirely clear. An understanding of exercise-induced metabolic changes can begin to elucidate these issues. Samples of blood and more invasive biopsies of skeletal muscle from humans and animals during exercise have revealed changes in several subsets of metabolites (*6*–*10*). High-intensity exercise increases the concentrations of lactate (*6*) and products of adenine nucleotide catabolism (*11*) in skeletal muscle and plasma, reflecting heightened anaerobic metabolism and adenosine triphosphate turnover, respectively. In skeletal muscle, acute exercise causes changes in amino acid concentrations, including a modest uptake of glutamate and release of glutamine and alanine to promote ammonia metabolism (*8*), with resultant changes in plasma concentrations of these metabolites (*7*, *10*).

Less is known about effects of exercise on the relative intramuscular and plasma concentrations of other classes of metabolites. For example, skeletal muscle biopsies have demonstrated rapid expansion of span 2 tricarboxylic acid (TCA) cycle intermediates in response to exercise (*9*, *12*), which augments aerobic energy production in cardiac and skeletal muscle (*7*, *13*, *14*). Similarly, glycogenolysis and lipolysis increase in skeletal muscle and adipose tissue, mobilizing substrates for oxidative phosphorylation (*15*). However, the extent to which these tissue changes in substrate utilization can be detected in plasma in response to exercise and whether they reflect fitness or disease status remain poorly characterized.

Emerging metabolite profiling technologies have enhanced the feasibility of acquiring high-throughput "snapshots" of the metabolic status of a whole organism (*16*–*19*). In addition to serving as potential biomarker signatures of disease states (*20*), circulating metabolites may themselves have unanticipated roles as regulatory signals with hormone-like functions (*21*, *22*). The objective of this study was to obtain a systematic view of the metabolic response to exercise by simultaneously assaying a large and diverse set of known metabolites. We developed a targeted liquid chromatography–mass spectrometry (LC-MS)–based metabolomics platform with high analyte specificity (*23*, *24*) and applied it to serial blood samples obtained before and after exercise so that each individual could serve as his or her own control. We defined the magnitude, kinetics, and interrelatedness of plasma metabolic changes in response to acute exercise and their relation to changes elicited by prolonged (marathon-running) exercise. We then tested whether metabolites altered in response to exercise were correlated with a key fitness-related parameter in a prospective cohort. Finally, we examined whether metabolites that changed during exercise can modulate the expression of transcriptional regulators of metabolism in cell culture and animal models.

[1]Cardiology Division and Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA 02114, USA. [2]Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital, Boston, MA 02114, USA. [3]Donald W. Reynolds Cardiovascular Clinical Research Center on Atherosclerosis at Harvard Medical School, Boston, MA 02115, USA. [4]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA. [5]Cardiovascular Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA. [6]Framingham Heart Study, Framingham, MA 01702, USA. [7]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA 02115, USA. [8]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA. [9]Nephrology Division, Massachusetts General Hospital, Boston, MA 02114, USA. [10]Pulmonary and Critical Care Division, Massachusetts General Hospital, Boston, MA 02114, USA. [11]Sections of Epidemiology and Preventive Medicine, Boston University School of Medicine, Boston, MA 02118, USA. [12]Cardiology Section, Boston University School of Medicine, Boston, MA 02118, USA.
*To whom correspondence should be addressed. E-mail: rgerszten@partners.org (R.E.G.); glewis@partners.org (G.D.L.)

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Table 1. Clinical characteristics. Values shown are means ± SD unless otherwise indicated. Exercise blood pressure measurements were taken at the peak exercise time point (~10 min, at conclusion of exercise). NA, creatinine and hemoglobin concentrations and peak exercise measurements were not available for marathon participants; SBP, systolic blood pressure; DBP, diastolic blood pressure, METs, metabolic equivalents.

	ETT derivation cohort (*n* = 45)	ETT validation cohort (*n* = 25)	CPET cohort (*n* = 8)	Marathon cohort (*n* = 25)
Age (years)	58 ± 13	59 ± 12	48 ± 14	42 ± 9*
Male sex (%)	83	92	72	76
Weight (lbs)	194 ± 33	194 ± 33	185 ± 34	164 ± 20*
BMI (kg/m^2)	28 ± 4	28 ± 4	27 ± 4	24 ± 3*
Creatinine (mg/dl)	1.1 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	NA
Hemoglobin (g/dl)	14.9 ± 1.1	14.8 ± 1.1	14.2 ± 1.0	NA
SBP rest (mmHg)	126 ± 16	128 ± 19	146 ± 24	123 ± 14
DBP rest (mmHg)	75 ± 8	75 ± 10	78 ± 10	69 ± 9
SBP exercise (mmHg)	175 ± 26	184 ± 23	192 ± 29	NA
DBP exercise (mmHg)	72 ± 10	75 ± 10	93 ± 15*	NA
Peak heart rate (% predicted)	96 ± 11	96 ± 12	89 ± 11	NA
Estimated peak VO_2 (METs)	11 ± 3	11 ± 4	8 ± 3*	NA

*$P < 0.05$ versus ETT derivation and validation cohorts.

Table 2. Metabolite changes in plasma at the peak exercise time point. Metabolites with $P < 0.005$ in the derivation cohort are shown. Metabolites with identical retention times and parent-daughter ion pairs (for example, isoleucine and leucine) cannot be distinguished by the platform.

Metabolite	Derivation cohort (*n* = 45) [% change (IQR)]	*P*	Validation cohort (*n* = 25) [% change (IQR)]	*P*
Lactate	394 (263–697)	<0.0001	553 (332–697)	<0.0001
Malate	120 (63–232)	<0.0001	143 (96–203)	<0.0001
Succinate	246 (125–379)	<0.0001	345 (217–492)	<0.0001
Glycerol	59 (31–126)	<0.0001	50.8 (25–117)	0.0001
Fumarate	56 (31–83)	<0.0001	75 (47–133)	<0.0001
Pyruvate	169 (78–308)	<0.0001	169 (96–289)	<0.0001
Niacinamide	79 (42–182)	<0.0001	71 (11–148)	0.0005
Pantothenate	22 (1–64)	<0.0001	29 (9–86)	0.0003
Glucose-6-phosphate	20 (34–46)	<0.0001	25 (11–74)	0.002
Alanine	29 (−1 to 49)	<0.0001	30 (3–41)	0.0008
Inosine	83 (7–181)	<0.0001	68 (15–250)	0.0005
Hypoxanthine	87 (31–201)	<0.0001	132 (68–295)	<0.0001
Citrulline	−8 (−19 to −1)	0.0003	−12 (−19 to −3)	0.021
AMP	37 (−10 to 75)	0.0002	56 (10–125)	0.004
Isoleucine-leucine	3 (−1 to 10)	0.0005	9 (3–17)	0.0005
Serine	−14 (−25 to −5)	0.0006	−11 (−22 to −3)	0.0061
Glutamate	−13 (−27 to 0)	0.001	−10 (−30 to 16)	0.21
Xanthine	18 (−5 to 46)	0.002	14 (7–66)	0.002
Cysteine	−19 (−35 to 8)	0.002	−22 (−36 to −15)	0.0001
Allantoin	−28 (−44 to −7)	0.003	−15 (−28 to 3)	0.02
3-Phosphoglycerate	26 (−5 to 43)	0.0035	34 (−12 to 67)	0.06
Homocysteine	−14 (−21 to 4)	0.0039	−15 (−24 to 3)	0.022
Glutamine	8 (1–17)	0.004	6 (−4 to 14)	0.046

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RESULTS

We first performed metabolic profiling in subjects referred for exercise treadmill testing (ETT) who had normal exercise capacity and no evidence of myocardial ischemia (Table 1). We obtained peripheral blood samples from subjects at baseline, peak exercise (~10 min, at conclusion of exercise), and 60 min after completion of ETT to characterize the alterations associated with acute maximum exercise. Because unintentional overfitting of data is a concern in biomarker discovery studies (*25*), we studied metabolic changes in two independent groups of subjects [that is, a derivation cohort (n = 45) and a validation cohort (n = 25)]. There were no significant clinical differences between subjects in the ETT derivation and validation cohorts (Table 1). Twenty-three metabolites changed significantly (nominal $P < 0.005$) at the peak exercise time point in the derivation cohort (Table 2 and Fig. 1), with an estimated false discovery rate of <5% or ~1 of the 23 metabolites that changed (see Materials and Methods). In the validation cohort, significant changes were noted for 21 of the 23 metabolites. For the overall group of metabolites in Table 2, the magnitude of change in individual metabolites in the two cohorts was highly correlated (r = 0.99; $P <$ 0.0001) (table S1 provides additional data on metabolite changes in the combined cohorts).

The observed changes in plasma metabolites immediately after cessation of exercise, which occurred ~10 min after baseline sampling, reflect rapid up-regulation of several metabolic pathways responsible for skeletal muscle substrate utilization (Fig. 2, top panel). The metabolite profiling platform captured previously reported increases in plasma concentrations of glycolysis products (lactate and pyruvate), lipolysis products (glycerol), and amino acids (alanine and glutamine). Plasma concentrations of the ketone body acetoacetate fell in response to acute exercise, as described (*26*).

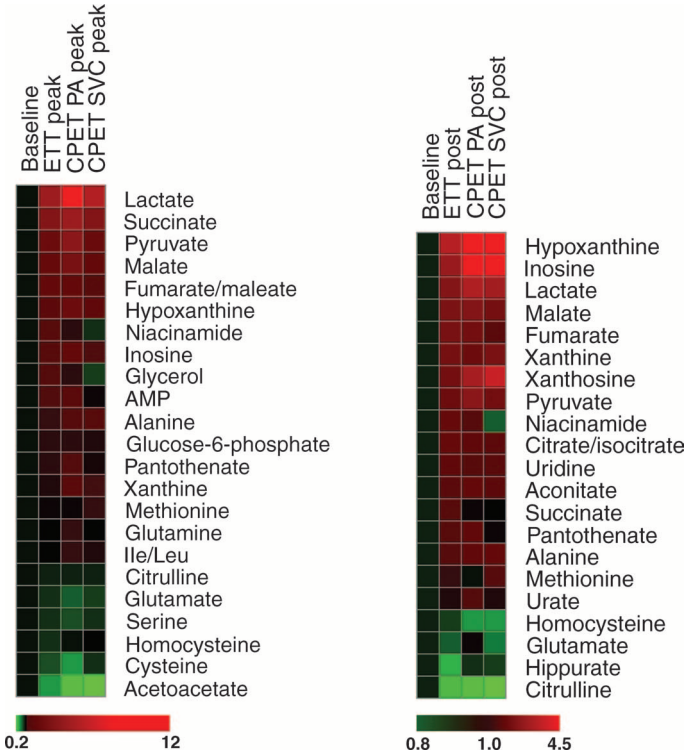

Fig. 1. Relative changes in metabolites in response to exercise. Heat maps show changes in metabolites compared to baseline at the peak exercise time point (left) and at 60 min after exercise (right). Shades of red and green represent fold increase and fold decrease of a metabolite, respectively, relative to baseline metabolite concentrations (see color scale). Three distinct plasma samples are represented: peripheral plasma from the ETT cohort, PA plasma from subjects undergoing CPET, and SVC plasma from subjects undergoing CPET.

Our broad metabolomics approach enabled detection of coordinate changes in multiple components of various metabolic pathways. We documented increases in sequential products of adenine nucleotide catabolism [adenosine monophosphate (AMP), inosine, hypoxanthine, and xanthine], including phosphorylated metabolites that are usually confined to intracellular compartments (Fig. 3A). We observed particularly prominent changes in sequential span 2 TCA cycle intermediates (Table 2 and Fig. 3B). Notably, the changes in individual TCA cycle intermediates in plasma closely mirrored previously reported changes in skeletal muscle obtained from invasive intramuscular biopsies taken during exercise testing (*9*).

We also detected plasma metabolic changes in pathways not previously associated with exercise. For example, niacinamide, which enhances insulin release and improves glycemic control (*27*, *28*), increased by 79% [interquartile range (IQR), 42 to 182%]. We observed heterogeneity in niacinamide elevation in response to exercise, in that leaner individuals [subjects with body mass index (BMI) less than the median value of 28] had an increase in niacinamide in response to exercise that was more than double the increase seen in individuals with BMI above the median [102% (IQR, −1 to 117%) versus 41% (IQR −7 to 80%), respectively; P = 0.04]. Concentrations of allantoin, a product of uric acid oxidation that has been implicated as an indicator of oxidative stress (*29*), fell after acute exercise. Finally, plasma concentrations of glycogenolysis intermediates (3-phosphoglycerate and glucose-6-phosphate) increased with ETT. Hierarchical clustering of metabolites to determine the interrelatedness of metabolic changes with exercise is displayed in fig. S2.

Although previous studies have documented that heart rate and hemodynamic parameters rapidly return to baseline after acute exercise (*30*, *31*), we observed metabolic changes that persisted 60 min after the cessation of exercise (Table 3). Metabolites that were changed only at the 60-min time point included TCA cycle intermediates (citrate-isocitrate and aconitate), a homocysteine metabolism pathway intermediate (methionine), and two adenine nucleotide degradation products (xanthosine and uric acid). Other metabolites that were changed only at the 60-min time point included uridine, a pyrimidine nucleoside, and the amino acid ornithine. Adenine nucleotides concordantly increased incrementally at 60 min compared to the peak exercise time point (Fig. 3A), whereas span 2 TCA intermediates decreased in a concerted fashion (Fig. 3B).

Localization of metabolic changes through multisite blood sampling

We performed metabolic profiling on eight subjects who underwent comprehensive cardiopulmonary exercise testing (CPET), which included bicycle ergometry with invasive hemodynamic monitoring and multisite blood sampling, to assess the cause of shortness of breath upon exertion. The CPET subjects showed normal exercise responses, although estimated peak oxygen uptake (VO_2) values were lower in the CPET cohort than in the ETT cohort (Table 1), commensurate with the smaller metabolic demand imposed by bicycle

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ergometry CPET than by treadmill ETT (*32*). We compared samples from the superior vena cava (SVC), which contains blood from the nonexercising upper body, with samples from the pulmonary artery (PA), which reflects the venous effluent from the exercising lower extremity skeletal muscle as well as cardiac muscle. Previous studies suggest that there is a 50% contribution of inferior vena cava (IVC) blood at rest while upright on a bicycle and a 73% contribution during bicycle ergometry exercise (*33*). Thus, we could assess instantaneous metabolite gradients in distinct vascular beds and so localize the site of metabolic changes. Analysis of bicycle ergometry also allowed us to test whether our findings from treadmill ETT could be generalized to other exercise modalities.

Cycle ergometry exercise yielded very similar metabolic changes to those observed with treadmill ergometry (Fig. 1, heat map, and Fig. 3). At peak exercise, most metabolites showed significantly larger changes in PA plasma than in SVC plasma, implicating a subdiaphragmatic skeletal muscle or cardiac source of these metabolic changes, likely from the exercising lower limbs. As expected, steep instantaneous PA-to-SVC gradients were evident for the purine degradation products (ΔPA/ΔSVC ratio range, 2.2 to 9.0; Fig. 3A) and span 2 TCA cycle intermediates (ΔPA/ΔSVC ratio range, 1.7 to 2.3; Fig. 3B). By contrast, a subset of metabolites that were significantly changed in both the ETT and the CPET cohorts, including several amino acids, acetoacetate, and glucose-6-phosphate, was similarly changed in the SVC and PA samples. This may be due to endocrine-like signaling effects from the exercising muscle (*34*). At 60 min after exercise, significant instantaneous PA-to-SVC gradients were no longer present for any of the metabolite classes.

Relation between metabolic changes and fitness in ETT

We next examined whether exercise-induced excursions of metabolites that were confirmed in both the ETT derivation and the validation cohorts, either at peak exercise or 60 min (n = 28 metabolites total), were correlated with exercise performance. For these analyses, we divided the ETT cohort of individuals with overall normal exercise capacity into two groups on the basis of median percent predicted peak oxygen uptake (%pVO_2). The more and less fit subgroups did not differ with regard to age (58 ± 11 versus 59 ± 14 years), sex (94% versus 86% male), weight (193 ± 35 versus 194 ± 31 lbs), or the product of heart rate and systolic blood pressure achieved (27,500 ± 4770 versus 26,750 ± 6900 mmHg/min), respectively ($P > 0.05$ for all comparisons). At peak exercise, glycerol increased to a greater extent in the group of individuals who achieved higher %pVO_2 (98%; IQR, 38 to 143%) compared to individuals who achieved lower %pVO_2 (48%; IQR, 24 to 69%; $P < 0.005$; Fig. 4), suggesting that more fit individuals have a greater capacity for lipolysis in response to acute exercise. The magnitude of the changes in glycerol was most closely related to %pVO_2 ($r = 0.54$; $P < 0.001$). We further investigated glycerol excursions in a distinct cohort of subjects with and without exercise-induced myocardial ischemia, with no differences in exercise exposure or cardiac risk factors, including age, sex, BMI, or diabetes status (see table S2). Exercise-induced increases in plasma glycerol concentrations were significantly smaller in subjects

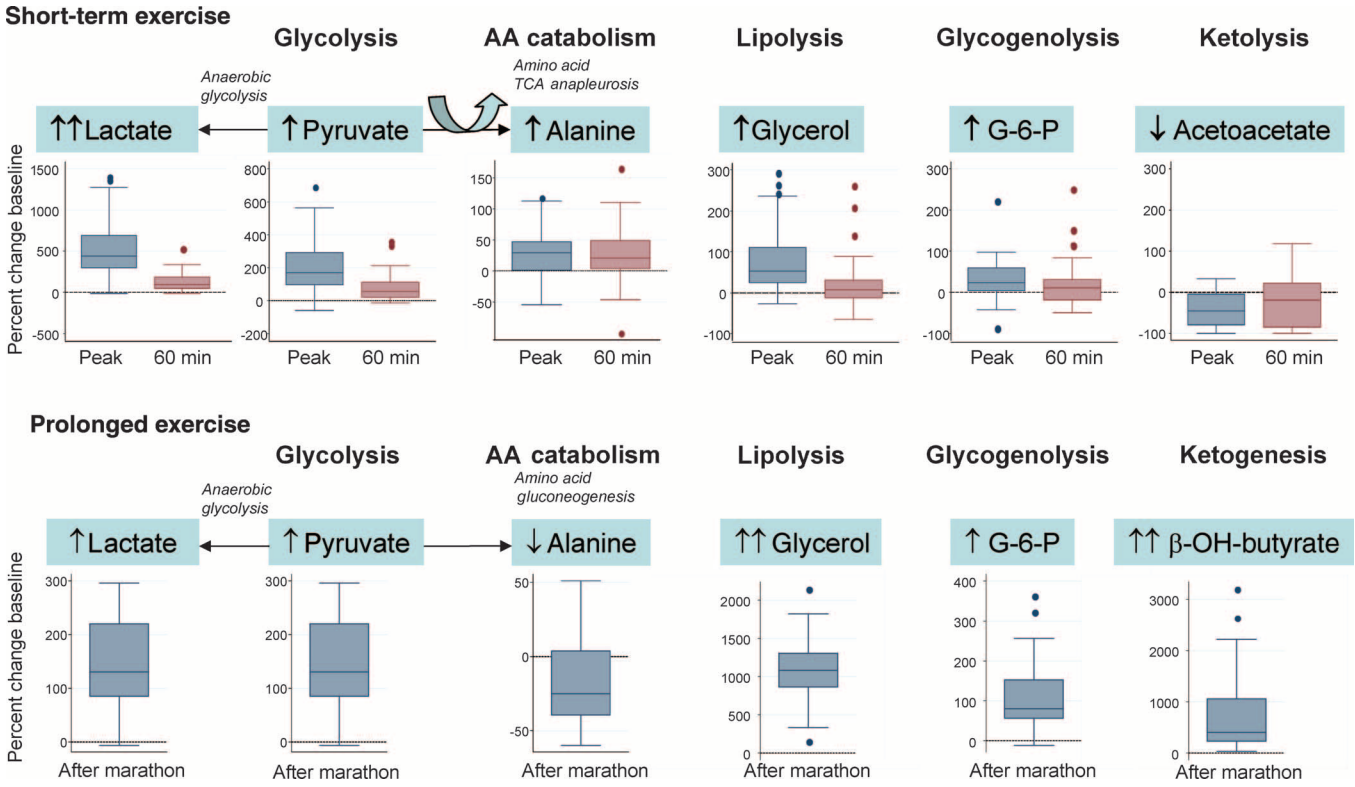

Fig. 2. Fuel substrate mobilization during exercise. Top: box-and-whisker plots indicating changes in metabolites at the peak exercise time point in ETT. The lines in the boxes indicate the median percent change in the metabolite concentrations. The lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the 5th and 95th percentiles. Bottom: box-and-whisker plots indicating changes in metabolites in response to prolonged exercise in the form of marathon running. AA, amino acid; G-6-P, glucose-6-phosphate; β-OH-butyrate, β-hydroxybutyrate.

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with inducible ischemia than in controls [18% (IQR, −10 to 79%) versus 66% (IQR, 24 to 109%) in controls; $P = 0.0015$]. The evidence for reduced exercise-induced lipolysis in the ischemic and less fit cohorts, as indicated by the smaller elevations in glycerol, may be indicative of maladaptive responses to exercise in which lipid utilization is impaired.

Pantothenate also increased to a greater extent in more fit individuals, whereas methionine excursions were greater in the less fit subgroup (Fig. 4). Changes in glutamine concentrations were also greater in the less fit group, likely reflecting greater skeletal muscle release of ammonia during exercise. The metabolic changes in each

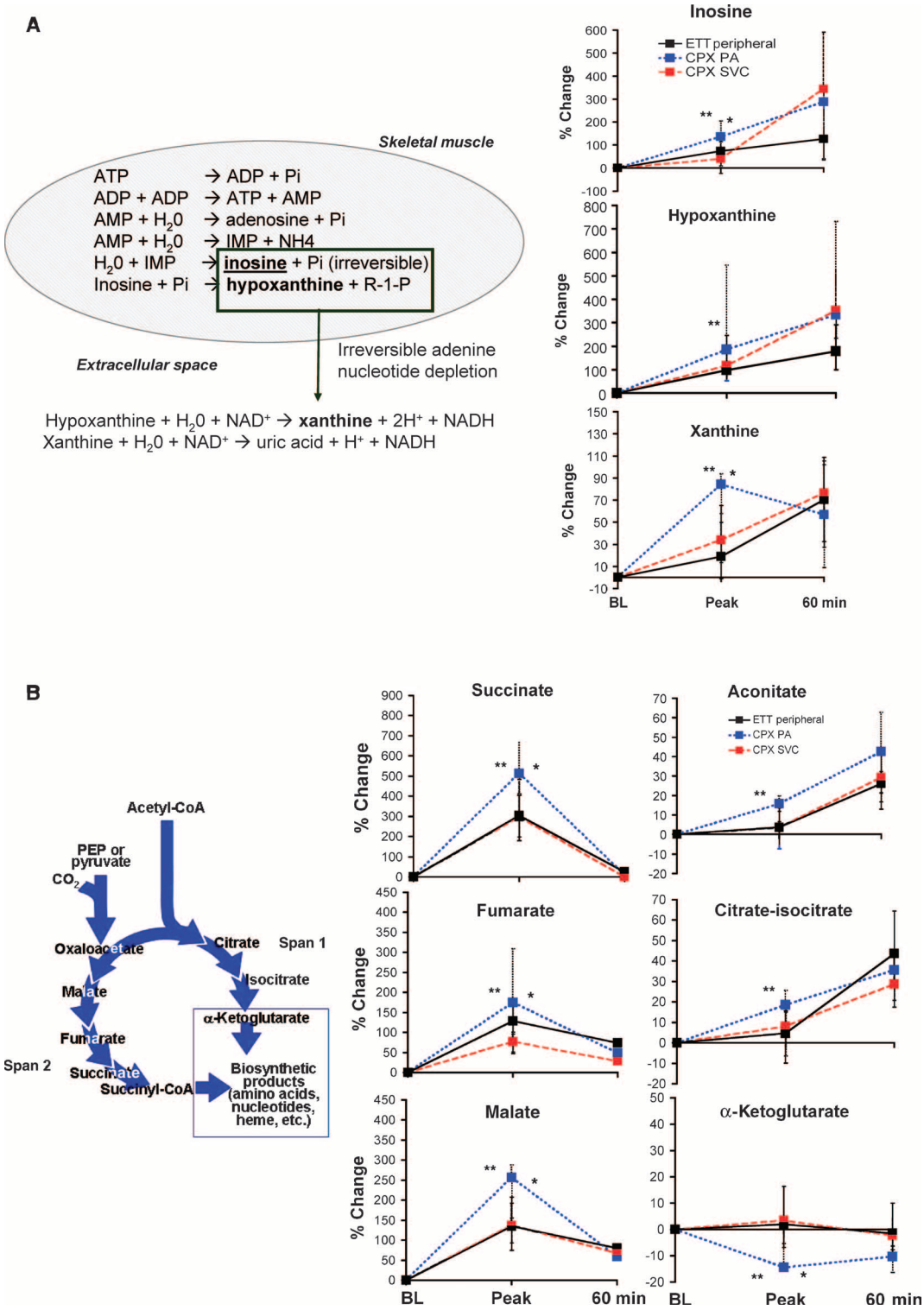

Fig. 3. (**A**) Enrichment of adenine nucleotide catabolites in PA blood during exercise. Left: intramuscular and extracellular metabolic reactions in the adenine nucleotide catabolism pathway. Right: patterns of change of individual metabolites in peripheral plasma from subjects undergoing ETT as well as PA and SVC plasma from subjects undergoing CPET. *$P < 0.05$ for between-group differences in metabolite levels in PA plasma versus SVC plasma; **$P < 0.05$ for comparison of PA plasma metabolite levels versus baseline (BL). (**B**). Enrichment of TCA cycle intermediates in PA blood during exercise. Right: patterns of change in individual TCA cycle intermediates in peripheral plasma from subjects undergoing ETT as well as in PA and SVC plasma from subjects undergoing CPET. *$P < 0.05$ for between-group differences in metabolite levels in PA plasma versus SVC plasma; **$P < 0.05$ for comparison of PA plasma metabolite levels versus baseline.

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of these metabolites persisted 60 min after cessation of exercise (Fig. 4). By contrast, lactate concentrations did not differ between more and less fit individuals at either peak exercise or 60 min after exercise. Notably, the differential changes in glycerol, pantothenate, glutamine, and methionine between more and less fit subjects persisted after we adjusted for the amount of exercise performed (table S3).

Relation between metabolic changes and prolonged exercise

We acquired metabolic profiles of 25 subjects who ran the 26.2-mile Boston Marathon, with an average time of 247 ± 46 min. As expected, extensive changes in plasma metabolite concentrations were evident at the end of the race as compared to prerace concentrations (Table 4 and Fig. 2). We documented marked elevations in glycerol (1128%; IQR, 897 to 1315%; $P < 0.0001$) and β-hydroxybutyrate (401%; IQR, 224 to 1060%; $P < 0.0001$; Fig. 2), consistent with extensive lipolysis and ketone body production, respectively, in subjects who completed the marathon. In contrast to our findings after acute exercise, we saw a reduction in gluconeogenic amino acids (alanine, threonine, serine, proline, valine, histidine, glutamine, and asparagine; Table 4) and unexpected increases in tryptophan metabolites (kynurenate, quinolinate, and anthranilate).

To investigate whether the metabolic changes that correlated with fitness after acute exercise also correlated with fitness after prolonged exercise, we stratified marathon runners into two groups on the basis of their finish time above and below the median (240 min). The groups did not differ in age (faster group, 41 ± 11 versus 42 ± 7 years), sex (76% versus 69% male), or BMI (25 ± 4 versus 24 ± 2 kg/m^2; all $P > 0.05$). Fumarate changes, which correlated with fitness during acute exercise performance, were also related to marathon speed [faster group: +200% (IQR, 110 to 281%) versus +108% (IQR, 16 to 137%); $P < 0.05$]. Additional span 2 TCA intermediary metabolites with greater excursions in the faster marathoners included succinate [+227% (IQR, 194 to 411%) versus +66% (IQR, 30 to 94%)] and malate [+227% (IQR, 133 to 269%) versus 87% (IQR, 49 to 109%); all $P < 0.05$]. An equally weighted sum of TCA cycle intermediate concentrations after marathon completion differentiated the faster from the slower runners (Fig. 5). There were nonsignificant trends toward greater increases in both pantothenate [+40% (IQR, 6 to 88%) versus +22% (IQR, −15 to 40%)] and glycerol [+1172% (IQR, 993 to 1469%) versus 986% (IQR, 827 to 1295%)] in the faster marathon group, in the same direction as was observed in acute exercise.

In the marathon runners, other metabolites that changed during acute exercise differed in the faster and slower runners. Faster run-

Table 3. Metabolite changes in plasma at 60 min after completion of exercise testing. Metabolites with $P < 0.005$ in the derivation cohort are shown.

Metabolite	Derivation cohort (n = 45) [% change (IQR)]	P	Validation cohort (n = 25) [% change (IQR)]	P
Malate	69 (36–121)	<0.0001	85 (51–118)	<0.0001
Citrate-isocitrate	44 (18–62)	<0.0001	39 (13–65)	<0.0001
Uridine	36 (21–53)	<0.0001	32 (2–49)	<0.0001
Fumarate	76 (39–138)	<0.0001	74 (19–117)	0.0001
Aconitic acid	25 (11–38)	<0.0001	27 (13–49)	<0.0001
Lactate	81 (42–170)	<0.0001	141 (78–196)	<0.0001
Pyruvate	47 (21–96)	<0.0001	81 (18–134)	0.0001
Hypoxanthine	166 (86–269)	<0.0001	256 (104–403)	<0.0001
Xanthine	71 (30–109)	<0.0001	68 (36–113)	0.0002
Methionine	10 (2–18)	<0.0001	13 (−5 to 27)	0.019
Alanine	21 (7–49)	<0.0001	20 (1–49)	0.009
Succinate	20 (8–44)	<0.0001	31 (4–43)	0.0003
Uridine triphosphate	42 (7–100)	<0.0001	6 (−25 to 52)	0.35
Niacinamide	53 (7–11)	<0.0001	8 (−13 to 73)	0.042
Inosine	106 (37–333)	<0.0001	154 (44–368)	0.0001
Xanthosine	43 (5–120)	<0.0001	93 (31–211)	0.0001
Pantothenate	18 (−4 to 46)	0.0002	25 (15–60)	0.0035
Cystathione	−38 (−52 to −20)	0.0006	−30 (−38 to −12)	0.068
Glutamine	12 (6–17)	0.0017	5 (−1 to 16)	0.09
Hippurate	−12 (−31 to 4)	0.0018	−17 (−30 to 1)	0.008
Uric acid	8 (−3 to 16)	0.001	6 (−7 to 15)	0.10
Ornithine	−19 (−28 to −8)	0.002	−8 (−11 to −1)	0.01
Allantoin	−33 (−40 to −15)	0.003	−15 (−28 to 3)	0.02

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ners had more modest increases in ketone bodies (β-hydroxybutyrate, +362% versus +855%) and in allantoin (+9% versus +28%), a marker of oxidative stress, than did slower runners. Citrulline, which modulates arginine bioavailability for nitric oxide synthesis, showed attenuated reduction in faster runners (−14% versus −41%), whereas argininosuccinate concentrations (+79% versus +25%) and niacinamide (+253% versus +53%) were higher in faster marathon runners ($P < 0.05$ for all).

Metabolic predictors of fitness in a large prospective cohort

We next determined whether baseline plasma concentrations of metabolites that were altered in response to exercise (Table 2) were associated with cardiovascular fitness in an independent cohort of subjects from the Framingham Heart Study ($n = 302$; see table S3). Heart rate is a known independent predictor of exercise capacity (*35*) that has been directly related to cardiovascular outcomes in the Framingham Heart Study (*36*) and was measured at the time of blood sampling in this cohort.

We specifically evaluated whether the metabolites that were modulated by acute exercise were correlated with resting heart rate in this cohort. Of the acute exercise metabolites, 12 were also measured in a subset of subjects in the Framingham Heart Study cohort (table S5). Glycerol concentrations were significantly correlated with resting heart rate ($r = 0.22$; $P = 0.0002$), whereas glutamine concentrations were inversely related to heart rate ($r = -0.21$; $P = 0.0002$). Both of these differences remained significant after adjustment for age, BMI, and gender (table S5). Subjects in the fourth heart rate quartile had glycerol concentrations that were higher by a factor of 1.44 than those in the first quartile ($P = 0.001$). Glutamate concentrations in the fourth heart rate quartile tended to be higher by a factor of 1.21 ($P = 0.08$), whereas glutamine concentrations were lower by a factor of 0.87 ($P = 0.03$). These data are notable given the greater increase in glycerol and attenuated decrease in glutamine in response to exercise in more fit subjects in the ETT cohort.

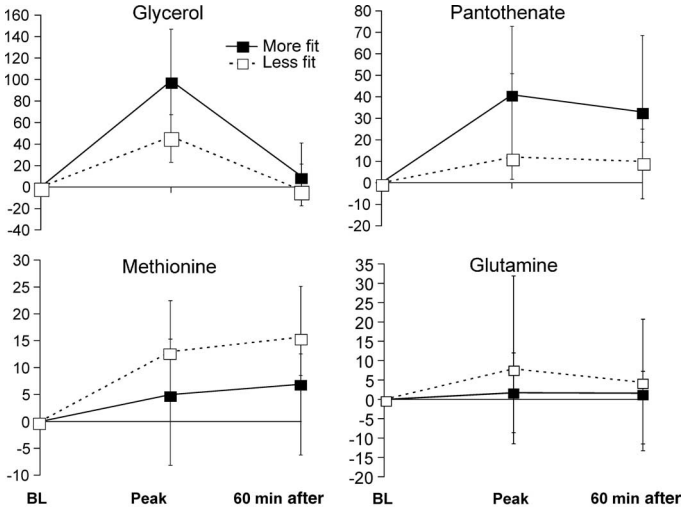

Fig. 4. Fitness levels and differential metabolic changes during ETT. Patterns of metabolite changes in subjects who achieved higher (more fit) (solid line) and lower (less fit) (dashed line) percent predicted peak VO_2 in response to exercise.

Activation of the nur77 pathway by exercise-induced metabolites

We further hypothesized that a subset of metabolites that increased in plasma after exercise could also modulate pathways relevant to cellular respiration and substrate utilization. We performed experiments with six exercise-induced metabolites of biological interest, specifically assaying their effects on 11 transcriptional regulators of metabolism in cultured myotubes. We found that a mixture of physiologically relevant concentrations of glycerol, niacinamide, glucose-6-phosphate, pantothenate, and succinate rapidly up-regulates the expression of nr4a1c (or nur77) (Fig. 6A), a recently described transcriptional regulator of glucose utilization and lipid metabolism genes in skeletal muscle (*37*, *38*). No individual metabolite triggered a similar response. Consistent with these findings, nur77 expression was induced by a factor of 5 in mouse quadriceps after 30 min of exercise (Fig. 6B).

DISCUSSION

Metabolomics technologies can be used to systematically define phenotypic patterns of small molecules in blood and urine (*23*, *24*, *39*). Here, we applied an LC-MS–based platform to characterize the metabolic response to exercise. As expected, we identified changes in metabolites reflecting heightened utilization of fuel substrates in several metabolic pathways, including increased glycolysis (*6*), lipolysis (*15*, *26*), adenine nucleotide catabolism (*11*), and amino acid catabolism (*8*). These data corroborate previous studies examining the metabolic response to exercise, which have typically focused on one metabolite or a discrete set of identified small molecules (*40*, *41*). By contrast, the breadth of our approach also enabled the identification of previously undescribed metabolic changes in response to exercise, including increases in indicators of glycogenolysis (glucose-6-phosphate and 3-phosphoglycerate) and small molecules that reflect oxidative stress (allantoin) and that modulate insulin sensitivity (niacinamide). The sensitivity of the platform also allowed us to monitor metabolic changes that had been previously documented only by invasive skeletal muscle sampling during exercise. Rigorous clinical phenotyping further enabled us to link distinct metabolic excursions with key clinical parameters, including exercise performance, myocardial ischemia, and resting heart rate. Finally, we found that a mixture of exercise-induced metabolites rapidly triggered expression of the transcription factor nur77, which controls glucose and lipid metabolism in skeletal muscle.

Plasma metabolic profiles reflect intracellular metabolism

Our method detected metabolites in plasma typically thought to be confined to intracellular compartments because of their phosphorylation state or site of bioactivity (*9*, *12*). Although intracellular TCA cycle and adenine nucleotide changes in muscle during exercise are well characterized (*9*, *11*, *13*), our high-sensitivity LC-MS platform allowed us to examine whether these changes occurred in plasma in a fitness-dependent way. Indeed, the exercise-induced changes in plasma concentrations of TCA cycle intermediates that we measured were similar to those reported in skeletal muscle after a similar amount of exercise (*9*). Specifically, there were increases in individual TCA cycle span 2 constituents (factor of 2 to 4 increases for fumarate, malate, and succinate), with more modest increases in citrate-isocitrate, and no change in α-ketoglutarate (*9*, *12*). Similarly, we found plasma signatures of sequential products of purine degradation and intra-

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muscular glycogenolysis, which had previously been documented to change within muscle (*11*).

Furthermore, selective catheterization during exercise allowed us to localize metabolic changes. The PA-to-SVC gradients at peak exercise of both purines and TCA intermediaries are consistent with marked release of these small molecules from exercising lower extremity skeletal muscle or cardiac muscle. By contrast, excursions in amino acids, acetoacetate, and glucose-6-phosphate were all induced by exercise but similarly so in the SVC and PA samples. This result suggests that these metabolites reflect coordinated metabolic changes in both exercising and nonexercising tissues, potentially triggered by circulating hormone-like molecules (*34*). However, the data from selective catheterization of the SVC and PA cannot rule out rapid modulation of metabolite concentrations by organs such as the liver that would attenuate differences between PA and SVC levels of metabolites.

Performance correlates of metabolic changes

In our studies, the most fit individuals in both the ETT and the marathon cohorts showed greater augmentation of pantothenate and fumarate concentrations than less fit individuals. Pantothenate, which has not been previously associated with acute exercise performance, plays an active role in fatty acid metabolism and facilitates acetyl–coenzyme A (CoA) entry into the TCA cycle. Pantothenate deficiency is rare, but when present, it is associated with postural hypotension, increased heart rate, and impaired cardiac pyruvate utilization (*42*, *43*), which may reflect a reduced capacity to augment cardiac stroke volume for a given workload.

The span 2 TCA intermediary fumarate also increased to a greater extent in the more fit people, that is, those achieving a higher percent predicted peak VO_2 during acute exercise. Furthermore, increases in fumarate and other span 2 TCA intermediates were associated with faster marathon times. It has been suggested that an increase in the total concentration of intramuscular TCA intermediates is necessary to augment and maintain TCA cycle flux during exercise (*9*, *12*). In our studies, the size of the plasma TCA intermediate pool may serve as a barometer of adequate muscle TCA pool size to maintain oxidative metabolism during prolonged exercise.

We identified previously undescribed exercise-induced increases in plasma concentrations of allantoin and niacinamide. Allantoin is a marker of oxidative stress generated in humans by nonenzymatic oxidation of uric acid by reactive oxygen species (*44*, *45*). Allantoin concentrations increased to a greater extent in less fit individuals compared to more fit individuals during prolonged exercise, indicating attenuated oxidative stress associated with fitness. Niacinamide is an amide of niacin that participates in intracellular respiration to oxidize fuel substrates. Niacinamide regulates insulin sensitivity, and studies in small cohorts suggest that niacinamide may promote glycemic control in early-stage diabetes (*27*, *28*). Thus, the persistent increases in niacinamide after brief exercise, which were more marked in lean individuals, and the greater increase in niacinamide among faster marathon runners, provide a possible link between acute and chronic insulin-sensitizing effects of exercise.

Fatty acids and lipids are preferred substrates for exercising muscle. Fatty acids can undergo reesterification after lipolysis within adipocytes (*46*). By contrast, glycerol is irreversibly liberated by lipolysis and is thus a better reporter of lipolysis in plasma (*47*). Plasma glycerol has been previously reported to increase in response to resistance

Table 4. Metabolite changes after completion of a 26.2-mile marathon. Metabolites with $P < 0.005$ are shown.

Metabolite	Median % change (IQR)	*P*
Serine	−42 (−49 to −29)	<0.0001
Proline	−30 (−40 to −24)	<0.0001
Ornithine	−42 (−49 to −36)	<0.0001
Lysine	−41 (−47 to −32)	<0.0001
Lactate	130 (85–220)	<0.0001
Threonine	−38 (−47 to −23)	<0.0001
Betaine	−25 (−33 to −18)	<0.0001
Asparagine	−45 (−55 to −23)	<0.0001
Glycerol	1129 (897–1316)	<0.0001
Xanthosine	321 (200–414)	<0.0001
Creatinine	53 (28–85)	<0.0001
Dimethylglycine	−29 (−43 to −23)	<0.0001
Glutamine	−25 (−33 to −10)	<0.0001
Glucose-6-phosphate	80 (56–153)	<0.0001
Hypoxanthine	537 (419–1070)	<0.0001
Succinate	187 (66–263)	<0.0001
Kynurenate	189 (65–294)	<0.0001
Aconitate	88 (47–123)	<0.0001
Fumarate	134 (77–214)	<0.0001
β-Hydroxybutyrate	401 (224–1060)	<0.0001
AMP	222 (155–458)	<0.0001
Malate	109 (62–240)	<0.0001
Citrate-isocitrate	46 (23–59)	0.0001
α-Ketoglutarate	42 (19–79)	0.0001
Citrulline	−30 (−45 to −14)	0.0002
Valine	−17 (−28 to −12)	0.0002
Inosine monophosphate	183 (83–300)	0.0002
Creatinine	93 (15–159)	0.0002
Hydroxyphenylpyruvate	21 (3–32)	0.0002
Quinolinate	22 (8–49)	0.0002
Niacinamide	175 (36–492)	0.0002
Glycerol-3-phosphate	96 (62–164)	0.0002
Isoleucine-leucine	−22 (−30 to −12)	0.0003
Arginine	−18 (−38 to −10)	0.0003
Anthranilate	36 (12–124)	0.0004
Homovanilate	237 (116–320)	0.0005
Histidine	−13 (−24 to −10)	0.0006
Argininosuccinate	37 (6–98)	0.0011
Tryptophan	−23 (−38 to −4)	0.0014
Allantoin	21 (−11 to 36)	0.0016
Xanthine	191 (100–349)	0.0018
Pyruvate	90 (49–126)	0.002
Pantothenate	29 (−16 to 59)	0.0059

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exercise (*15*, *26*), and our data now show a greater increase in glycerol during exercise in more fit individuals than in those who are less fit. The lipolysis pathway is a specific indicator of fitness; alterations in glycolysis and purine metabolites did not differ significantly between more and less fit individuals. In addition, glycerol increased less in subjects with exercise-induced myocardial ischemia when compared to healthy subjects matched for age, sex, and exercise exposure. This result suggests that more fit individuals have a greater capacity to activate lipolysis during physical activity than those who are less fit, and that subjects with ischemic myocardium may shift away from fatty acids as a preferred substrate (*48*).

Relation between metabolite concentrations and fitness in a large prospective cohort

Resting heart rate is a fitness metric that predicts coronary heart disease outcomes in the Framingham Heart Study and other cohorts (*36*, *49*). Plasma glycerol concentrations directly correlated with heart rate in the Framingham Heart Study, indicating that higher circulating glycerol concentrations at rest were associated with lower fitness levels. Circulating plasma glycerol originates from lipolysis in adipose tissue (*50*, *51*). Increased adipose mass and resistance to the antilipolytic effects of insulin may contribute to the basal concentrations of glycerol in less fit individuals, although correction for BMI did not attenuate the relation between heart rate and glycerol. In addition, interindividual variability in β-adrenergic signaling, which modulates lipolysis (*52*), may contribute to the association between glycerol and heart rate in that heightened basal β-adrenergic signaling may also be associated with increased heart rate.

Plasma glycerol measured at rest and after physical activity could potentially serve as a biomarker of fitness. Individuals who can activate lipolysis in response to various forms of exercise may be more likely to remain lean, whereas high glycerol and limited activity-induced lipolysis may be a maladaptive phenotype. This biomarker would be analogous to heart rate itself in that fit individuals demonstrate greater heart rate augmentation with exercise but lower baseline heart rates. Whether plasma concentrations of glycerol or glutamine (which were also associated with heart rate in the Framingham cohort) add predictive cardiovascular outcomes to existing biomarkers requires further study.

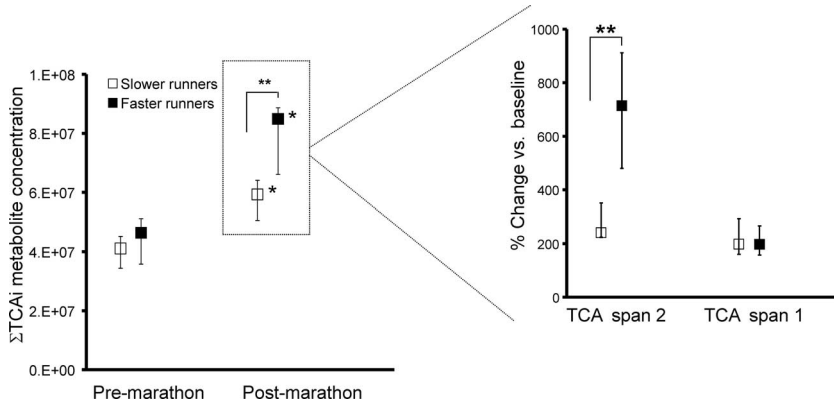

Fig. 5. TCA intermediate changes with marathon running. Left: equally weighted sums of absolute concentrations of TCA cycle intermediates (ΣTCAi, in MS arbitrary units) at baseline and on completion of the marathon in groups of faster and slower runners (medians ± IQR). Span 1 TCA cycle intermediates include citrate-isocitrate, aconitic acid, and α-ketoglutarate. Span 2 TCA cycle intermediates include succinate, malate, and fumarate. *P = 0.0001 versus baseline; **P = 0.004 for between-group comparison. Right: relative percent changes (medians ± IQR) in metabolites from spans 1 and 2 that account for observed differences between the two groups of runners. **P = 0.005 for between-group comparison.

Unanticipated role of metabolites in modulating nur77 transcription

To begin to understand whether circulating metabolites may mediate salutary effects of exercise, we hypothesized that metabolites altered by exercise in our study would induce expression of genes involved in regulating skeletal muscle metabolism. Addition of physiologically relevant concentrations of metabolites to differentiated myotubes in cell culture resulted in a rapid factor of 3 induction in the transcription factor nur77, although no single metabolite augmented gene expression. nur77, primarily studied for its role in apoptosis, is also an important metabolic regulator of glucose (*37*) and lipid metabolism (*38*) in skeletal muscle. nur77 has also been suggested as a potential therapeutic target for the metabolic syndrome (*53*). The expression of nur77 in skeletal muscle is reduced in several models of obesity and type 2 diabetes (ob/ob, db/db, and Zucker Diabetic Fatty rats) and is increased in response to insulin-sensitizing treatments (*54*).

Limitations

The sample size of our patient cohort with multisite sampling was limited by the small number of patients who undergo invasive CPET who also have normal exercise capacity and hemodynamics. Nonetheless, the direction and magnitude of the changes accompanying cycle ergometry were highly consistent with those seen in subjects undergoing ETT, thus validating our observed findings across exercise modalities. In addition, our population was relatively homogeneous because we required normal exercise tolerance for inclusion in the study, likely attenuating correlations between metabolite concentrations and fitness levels.

Conclusions

Metabolic profiling in this study provides a comprehensive snapshot of human metabolism at rest and during exercise and provides small-molecule reporters of fitness. During brief exposure to exercise (~10 min, peak exercise), circulating metabolite concentrations indicate rapid activation of a catabolic program consisting of heightened lipolysis, glycolysis, and glycogenolysis, as well as amino acid and purine catabolism that largely persists for at least 60 min after completion of exercise. This metabolic response is in contrast to the postprandial anabolic state in which insulin suppresses lipolysis and promotes uptake of circulating amino acid and purine metabolites (*24*). Overactivation of this anabolic pathway contributes to obesity and insulin resistance (*55*). The entire spectrum of exercise-induced metabolic changes may therefore provide a favorable counterbalance to a frequently maladaptive net anabolic physiologic state.

The exercise-induced metabolic changes that differentiate more and less fit individuals, in particular, represent attractive candidates as mediators of the salutary effects of exercise. By serially measuring individual metabolites, we found that during exercise more fit individuals activate lipolysis (glycerol), facilitate entry of fatty acids into the TCA cycle (pantothenate), and expand the TCA cycle intermediate pool (fumarate,

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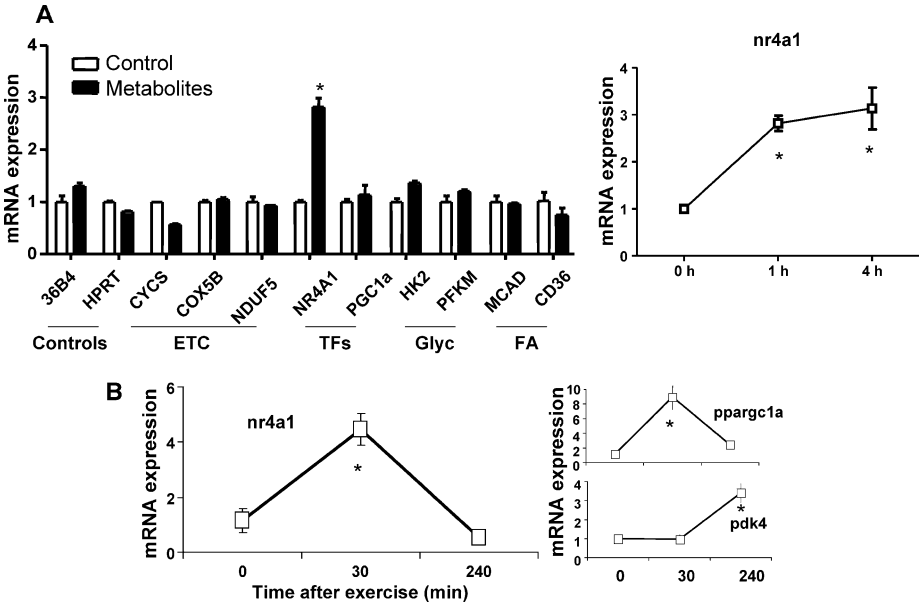

Fig. 6. (**A**) Modulation of gene expression by metabolites. Left: mRNA expression of indicated genes [36B4, Rplp0 ribosomal protein, large, P0; HPRT, hypoxanthine guanine phosphoribosyl transferase; CYCS, cytochrome c, somatic; COX5B, cytochrome c oxidase subunit Vb; NDUF5, NADH dehydrogenase (ubiquinone) 1β subcomplex, 5; NR4A1 (or nur77), nuclear receptor subfamily 4, group A, member 1; PGC1a, peroxisome proliferator–activated receptor γ, coactivator 1α; HK2, hexokinase 2; PFKM, phosphofructokinase, muscle; MCAD, acyl-CoA dehydrogenase, medium chain; CD36, fatty acid translocase] in C2C12 cells differentiated into myotubes 60 min after treating with the metabolite cocktail (black bars) versus control (white bars). The pooled metabolites consisted of glycerol, succinate, glucose-6-phosphate, pantothenate, and niacinamide. Right: nr4a1 in cells 0, 60, and 240 min after treating with cocktail. ETC, electron transport chain; TFs, transcription factors; Glyc, glycolysis; FA, fatty acid transport. *$P < 0.05$ versus baseline values. (**B**) Modulation of gene expression by exercise. Left: mRNA expression of nr4a1 in quadriceps 0, 30, and 240 min after running to maximum capacity. Right: mRNA expression of PGC-1α (ppargc1a) and pyruvate dehydrogenase kinase, isoenzyme 4 (pdk4) under the same conditions. *$P < 0.05$ versus baseline values.

malate, and succinate) more robustly than less fit individuals while incurring less oxidative stress (allantoin), attenuated release of a homocysteine precursor (methionine), and greater increases in the insulin-sensitizing small-molecule niacinamide. Moreover, resting basal concentrations of a subset of metabolites that are altered in a fitness-dependent manner in response to exercise (that is, glutamine and glycerol) are also related to fitness in a large prospective cohort.

The capacity of circulating metabolites altered by exercise to activate a transcription factor that regulates glucose and lipid metabolism offers further mechanistic insight into potential salutary metabolic effects of exercise in humans. Exercise-induced metabolic signals may overcome the suppression of nur77 that has been observed in animal models of obesity and diabetes (*53*, *54*). Ultimately, a better understanding of exercise-induced metabolic changes might help to identify the salutary effects of exercise in individuals with and without cardiovascular disease and point to targets for therapeutic modulation.

MATERIALS AND METHODS

Study cohorts and study protocol

All blood sampling was performed as part of human studies protocols approved by the Institutional Review Board Subcommittee on Human Studies of the Massachusetts General Hospital (MGH). Written informed consent was obtained from all subjects.

For exercise testing protocols, we recruited outpatients referred to the MGH Exercise Laboratory for either diagnostic treadmill ETT (n = 70) or bicycle ergometry CPET (n = 8). To define the normal metabolic response to maximum exercise, we selected subjects who met the following inclusion criteria: (i) normal exercise tolerance as defined by estimated peak VO_2 greater than 70% predicted (*56*, *57*), (ii) evident maximum effort on the basis of heart rate response greater than 85% predicted in the absence of β-blockade, and (iii) pre-exercise fasting for at least 4 hours. Exclusion criteria included cessation of exercise by the test supervisor, reversible perfusion defects or electrocardiographic evidence of exercise-induced ischemia, mechanical limitation to exercise, diabetes, or left ventricular ejection fraction <50%.

The myocardial ischemia cohort consisted of subjects with exercise-induced myocardial ischemia (n = 65), as determined by ischemic ST-segment response to exercise (>1-mm horizontal or downsloping ST-segment depression versus none) (*58*), and moderate-to-severe reversible myocardial perfusion defects by ^{99m}Tc-sestamibi imaging. Control subjects (n = 65) were matched on the basis of age, sex, and exercise duration.

The Standard Bruce Protocol was used for treadmill ETT (*59*). CPET was performed by coupling 10 to 25 W/min incremental ramp cycle ergometry with measurement of respiratory gas exchange and continuous hemodynamic monitoring as previously described (*60*). Rest and peak heart rate and blood pressure, duration of the stress test, and either estimated (*57*) or directly measured peak VO_2 were recorded.

For plasma sampling before and after completion of the Boston Marathon, amateur runners scheduled to participate in the Boston Marathon were recruited by open e-mail invitation sent to local running clubs. The initial 25 responders with no history of cardiovascular disease were enrolled in the study. Sample size was limited to enable prompt acquisition of blood samples at the finish line and to avoid subject discomfort related to prolonged delay after a marathon.

Finally, subjects from the Framingham Offspring Study who were initially enrolled in a study of metabolic risk prediction (n = 382), in whom a subset of the metabolites was assayed, were examined. Subjects who were taking nodal agents (that is, β blockers, nondihydropyridine calcium channel blockers, and digoxin) that influence resting heart rate were excluded.

Metabolic profiling analysis

Samples from ETT were obtained from a peripheral venous catheter immediately before initiation of exercise, at peak exercise, and 60 min after completion of exercise. CPET samples were obtained at the same intervals from catheters placed in the SVC and proximal PA. Samples

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were collected in 5-ml K_2EDTA-treated tubes (Becton-Dickinson). All blood samples were immediately centrifuged at 1200*g* for 10 min to pellet cellular elements. The supernatant plasma was then aliquoted and frozen at −80°C to minimize freeze-thaw degradation. For subjects completing the Boston Marathon, peripheral venous plasma samples were obtained within 10 min of marathon completion, immediately centrifuged, placed on ice, and then transferred to −80°C within 120 min.

For metabolite profiling, we incorporated metabolites that were potentially relevant to cardiovascular and metabolic disease and amenable to measurement by LC-MS as previously described (*23*). The detailed description of our LC-MS methodology, which was applied in this study, has been previously described (*23*) and is summarized in the Supplemental Material.

Gene expression profiling experiments

Cell culture. C2C12 cells were differentiated into myotubes for 7 days. Cells were then placed in Hanks' balanced salt solution for 24 hours and treated with the pooled metabolites [succinate (9 μg/ml), pantothenate (6 μg/ml), niacinamide (0.3 μg/ml), glucose-6-phosphate (45 μg/ml), and glycerol (30 μg/ml), to mimic relative plasma concentrations] (*61*) for 60 min. Cells were washed once in phosphate-buffered saline, and messenger RNA (mRNA) was isolated using a TurboCapture method (Qiagen). RNA was reverse-transcribed, and quantitative polymerase chain reaction (qPCR) was performed, using an ABI 7700HT qPCR machine. Gene expression was normalized to expression of TATA box–binding protein (TBP).

Exercise in mice. Mice were tested by forced running on motor-driven treadmills (Columbus Instruments). Mice were first acclimated for 5 min a day for 3 days at a low rate of 14 m/min and 0% incline. On the test day (day 4), the treadmill was set to a constant 10% incline and started at 10 m/min. Every 2 min, the speed was then increased by 2 m/min, and the mice were forced to run to exhaustion. Exhaustion was determined by the unwillingness of mice to keep running on the treadmill despite stimulus by a small electric shock on the stationary platform of the treadmill. Once determined to be exhausted, mice were returned to their cage and, after the indicated times, killed. Quadriceps was removed, and total RNA was isolated using the Trizol method. RNA was reverse-transcribed, and qPCR was performed, using an ABI 7700HT qPCR machine. Expression is normalized to expression of TBP.

Statistical analysis

For clinical characteristics of subjects undergoing exercise in each cohort, continuous variables were compared using Student's *t* test, and categorical variables were compared with Fisher's exact test. From the 70 subjects from whom peripheral samples were collected in the ETT study, 45 subjects were randomly selected for analysis as a derivation set. The significance of change in metabolite concentrations from pretest to posttest values was assessed by either paired Student's *t* test or Wilcoxon signed rank test, as appropriate. We used a significance threshold of $P < 0.005$ in the derivation cohort because this threshold would be expected to yield about one false-positive discovery from 210 analyzed metabolites, assuming independent hypotheses. Conservatively high estimates of false discovery rate were obtained via the Benjamini-Hochberg procedure (*62*). The procedure was applied to the set of 210 measured metabolites for the comparisons of peak and post-exercise with baseline, as well as post-marathon with baseline.

Metabolites that changed significantly at peak exercise or at the 60-min time points in the ETT derivation cohort were selected as candidate exercise biomarkers for testing in the ETT validation cohort (25 patients) and the CPET validation cohort (8 patients). Criteria for validation were $P < 0.05$ by Wilcoxon signed rank test, with the direction of change concordant with that observed in the derivation cohort. The conjoint probability of $0.005 \times 0.05 = 2.5 \times 10^{-4}$ approximates a Bonferroni correction ($0.05/210 = 2.4 \times 10^{-4}$). The relation between changes in metabolites in the derivation and validation cohorts was assessed with a Spearman rank correlation coefficient. The marathon cohort was used only as a derivation group ($n = 25$). For this group, we applied the same significance threshold that was used for the ETT derivation group ($P < 0.005$).

Further analysis was carried out in the subgroup of eight patients undergoing CPET with simultaneous plasma sampling from the SVC and PA at baseline, peak exercise, and 60 min after exercise to localize metabolite changes. During cycle ergometry lower extremity exercise, there is a disproportionately large contribution of IVC blood (reflecting skeletal muscle venous effluent) compared to SVC blood that constitutes mixed venous blood in the PA. Previously reported relative contributions of IVC and SVC blood flow to overall venous return are 73% and 27%, respectively, during cycle ergometry (*33*). Therefore, to identify twofold IVC versus SVC enrichment patterns for metabolic changes, we used criteria consisting of a PA-to-SVC ratio of greater than 1.73:1 ($2 \times 0.73 + 1 \times 0.27$) and $P < 0.05$ by Wilcoxon signed rank test for relative changes in metabolites in the SVC and PA.

Metabolic excursions in subsets of patients stratified by median percent predicted peak VO_2 or by finish time were compared by Wilcoxon signed rank tests. Similarity between metabolites in terms of similar profiles of change across many subjects was assessed by hierarchical clustering. Pearson or Spearman correlation coefficients are reported depending on whether data were normally distributed.

STATA (version 10.0) and SAS version 9.1.3 (SAS Institute) software were used to perform statistical analyses. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

SUPPLEMENTARY MATERIAL

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Materials and Methods

Fig. S1. Relation between plasma lactate concentrations measured by LC-MS and the MGH clinical chemistry laboratory.

Fig. S2. Dendrogram illustrating hierarchical clustering of metabolites that changed with exercise.

Table S1. Metabolites that change within plasma in response to exercise in the entire exercise tolerance test cohort ($n = 70$; $P < 0.05$).

Table S2. Clinical characteristics of subjects with exercise-induced myocardial ischemia and control subjects without inducible ischemia.

Table S3. Differential changes in metabolite concentrations per MET achieved during exercise tolerance testing among more and less fit individuals.

Table S4. Clinical characteristics of Framingham Heart Study participants in whom metabolites were measured.

Table S5. Relation between plasma metabolite concentrations and resting heart rate in Framingham Heart Study participants.

References

REFERENCES AND NOTES

1. P. D. Thompson, D. Buchner, I. L. Pina, G. J. Balady, M. A. Williams, B. H. Marcus, K. Berra, S. N. Blair, F. Costa, B. Franklin, G. F. Fletcher, N. F. Gordon, R. R. Pate, B. L. Rodriguez, A. K. Yancey, N. K. Wenger; American Heart Association Council on Clinical Cardiology Subcommittee on Exercise, Rehabilitation, and Prevention; American Heart Association

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Council on Nutrition, Physical Activity, and Metabolism Subcommittee on Physical Activity, Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: A statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* **107**, 3109–3116 (2003).

2. R. J. Gibbons, G. J. Balady, J. T. Bricker, B. R. Chaitman, G. F. Fletcher, V. F. Froelicher, D. B. Mark, B. D. McCallister, A. N. Mooss, M. G. O'Reilly, W. L. Winters Jr., R. J. Gibbons, E. M. Antman, J. S. Alpert, D. P. Faxon, V. Fuster, G. Gregoratos, L. F. Hiratzka, A. K. Jacobs, R. O. Russell, S. C. Smith Jr.; American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1997 Exercise Testing Guidelines), ACC/AHA 2002 guideline update for exercise testing: Summary article: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1997 Exercise Testing Guidelines). *Circulation* **106**, 1883–1892 (2002).
3. J. F. McNeer, J. R. Margolis, K. L. Lee, J. A. Kisslo, R. H. Peter, Y. Kong, V. S. Behar, A. G. Wallace, C. B. McCants, R. A. Rosati, The role of the exercise test in the evaluation of patients for ischemic heart disease. *Circulation* **57**, 64–70 (1978).
4. J. Myers, M. Prakash, V. Froelicher, D. Do, S. Partington, J. E. Atwood, Exercise capacity and mortality among men referred for exercise testing. *N. Engl. J. Med.* **346**, 793–801 (2002).
5. C. E. Snader, T. H. Marwick, F. J. Pashkow, S. A. Harvey, J. D. Thomas, M. S. Lauer, Importance of estimated functional capacity as a predictor of all-cause mortality among patients referred for exercise thallium single-photon emission computed tomography: Report of 3,400 patients from a single center. *J. Am. Coll. Cardiol.* **30**, 641–648 (1997).
6. G. Van Hall, M. Jensen-Urstad, H. Rosdahl, H. C. Holmberg, B. Saltin, J. A. Calbet, Leg and arm lactate and substrate kinetics during exercise. *Am. J. Physiol. Endocrinol. Metab.* **284**, E193–E205 (2003).
7. K. Sahlin, A. Katz, S. Broberg, Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *Am. J. Physiol.* **259**, C834–C841 (1990).
8. J. Henriksson, Effect of exercise on amino acid concentrations in skeletal muscle and plasma. *J. Exp. Biol.* **160**, 149–165 (1991).
9. M. J. Gibala, D. A. MacLean, T. E. Graham, B. Saltin, Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *Am. J. Physiol.* **275**, E235–E242 (1998).
10. L. S. Eriksson, S. Broberg, O. Björkman, J. Wahren, Ammonia metabolism during exercise in man. *Clin. Physiol.* **5**, 325–336 (1985).
11. K. Sahlin, J. Gorski, L. Edström, Influence of ATP turnover and metabolite changes on IMP formation and glycolysis in rat skeletal muscle. *Am. J. Physiol.* **259**, C409–C412 (1990).
12. M. J. Gibala, D. A. MacLean, T. E. Graham, B. Saltin, Anaplerotic processes in human skeletal muscle during brief dynamic exercise. *J. Physiol.* **502**, 703–713 (1997).
13. J. K. Hiltunen, E. J. Davis, The disposition of citric acid cycle intermediates by isolated rat heart mitochondria. *Biochim. Biophys. Acta* **678**, 115–121 (1981).
14. J. J. Aragón, J. M. Lowenstein, The purine-nucleotide cycle. Comparison of the levels of citric acid cycle intermediates with the operation of the purine nucleotide cycle in rat skeletal muscle during exercise and recovery from exercise. *Eur. J. Biochem.* **110**, 371–377 (1980).
15. M. J. Ormsbee, J. P. Thyfault, E. A. Johnson, R. M. Kraus, M. D. Choi, R. C. Hickner, Fat metabolism and acute resistance exercise in trained men. *J. Appl. Physiol.* **102**, 1767–1772 (2007).
16. J. K. Nicholson, I. D. Wilson, Understanding 'global' systems biology: Metabonomics and the continuum of metabolism. *Nat. Rev. Drug Discov.* **2**, 668–676 (2003).
17. L. M. Raamsdonk, B. Teusink, D. Broadhurst, N. Zhang, A. Hayes, M. C. Walsh, J. A. Berden, K. M. Brindle, D. B. Kell, J. J. Rowland, H. V. Westerhoff, K. van Dam, S. G. Oliver, A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat. Biotechnol.* **19**, 45–50 (2001).
18. J. Allen, H. M. Davey, D. Broadhurst, J. K. Heald, J. J. Rowland, S. G. Oliver, D. B. Kell, High-throughput classification of yeast mutants for functional genomics using metabolic footprinting. *Nat. Biotechnol.* **21**, 692–696 (2003).
19. J. An, D. M. Muoio, M. Shiota, Y. Fujimoto, G. W. Cline, G. I. Shulman, T. R. Koves, R. Stevens, D. Millington, C. B. Newgard, Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. *Nat. Med.* **10**, 268–274 (2004).
20. M. S. Sabatine, E. Liu, D. A. Morrow, E. Heller, R. McCarroll, R. Wiegand, G. F. Berriz, F. P. Roth, R. E. Gerszten, Metabolomic identification of novel biomarkers of myocardial ischemia. *Circulation* **112**, 3868–3875 (2005).
21. P. Sapieha, M. Sirinyan, D. Hamel, K. Zaniolo, J. S. Joyal, J. H. Cho, J. C. Honoré, E. Kermorvant-Duchemin, D. R. Varma, S. Tremblay, M. Leduc, L. Rihakova, P. Hardy, W. H. Klein, X. Mu, O. Mamer, P. Lachapelle, A. Di Polo, C. Beauséjour, G. Andelfinger, G. Mitchell, F. Sennlaub, S. Chemtob, The succinate receptor GPR91 in neurons has a major role in retinal angiogenesis. *Nat. Med.* **14**, 1067–1076 (2008).
22. W. He, F. J. Miao, D. C. Lin, R. T. Schwandner, Z. Wang, J. Gao, J. L. Chen, H. Tian, L. Ling, Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* **429**, 188–193 (2004).
23. G. D. Lewis, R. Wei, E. Liu, E. Yang, X. Shi, M. Martinovic, L. Farrell, A. Asnani, M. Cyrille, A. Ramanathan, O. Shaham, G. Berriz, P. A. Lowry, I. F. Palacios, M. Taşan, F. P. Roth, J. Min, C. Baumgartner, H. Keshishian, T. Addona, V. K. Mootha, A. Rosenzweig, S. A. Carr, M. A. Fifer, M. S. Sabatine, R. E. Gerszten, Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. *J. Clin. Invest.* **118**, 3503–3512 (2008).
24. O. Shaham, R. Wei, T. J. Wang, C. Ricciardi, G. D. Lewis, R. S. Vasan, S. A. Carr, R. Thadhani, R. E. Gerszten, V. K. Mootha, Metabolic profiling of the human response to a glucose challenge reveals distinct axes of insulin sensitivity. *Mol. Syst. Biol.* **4**, 214 (2008).
25. D. F. Ransohoff, Rules of evidence for cancer molecular-marker discovery and validation. *Nat. Rev. Cancer* **4**, 309–314 (2004).
26. K. Goto, N. Ishii, S. Sugihara, T. Yoshioka, K. Takamatsu, Effects of resistance exercise on lipolysis during subsequent submaximal exercise. *Med. Sci. Sports Exerc.* **39**, 308–315 (2007).
27. A. Crinò, R. Schiaffini, S. Manfrini, C. Mesturino, N. Visalli, G. Beretta Anguissola, C. Suraci, D. Pitocco, S. Spera, S. Corbi, M. C. Matteoli, I. P. Patera, M. L. Manca Bitti, C. Bizzarri, P. Pozzilli; IMDIAB group, A randomized trial of nicotinamide and vitamin E in children with recent onset type 1 diabetes (IMDIAB IX). *Eur. J. Endocrinol.* **150**, 719–724 (2004).
28. F. Pociot, J. I. Reimers, H. U. Andersen, Nicotinamide—biological actions and therapeutic potential in diabetes prevention. IDIG Workshop, Copenhagen, Denmark, 4–5 December 1992. *Diabetologia* **36**, 574–576 (1993).
29. M. Grootveld, B. Halliwell, Measurement of allantoin and uric acid in human body fluids. A potential index of free-radical reactions in vivo? *Biochem. J.* **243**, 803–808 (1987).
30. K. Ellis, C. E. Pothier, E. H. Blackstone, M. S. Lauer, Is systolic blood pressure recovery after exercise a predictor of mortality? *Am. Heart J.* **147**, 287–292 (2004).
31. M. R. Carnethon, D. R. Jacobs Jr., S. Sidney, B. Sternfeld, S. S. Gidding, C. Shoushtari, K. Liu, A longitudinal study of physical activity and heart rate recovery: CARDIA, 1987-1993. *Med. Sci. Sports Exerc.* **37**, 606–612 (2005).
32. *Principles of Exercise Testing and Interpretation*, K. Wasserman, J. Hansen, D. Sue, W. Stringer, B. Whipp, Eds. (Lippincott Williams and Wilkins, Philadelphia, ed. 4, 2005), p. 217.
33. C. P. Cheng, R. J. Herfkens, C. A. Taylor, Inferior vena caval hemodynamics quantified in vivo at rest and during cycling exercise using magnetic resonance imaging. *Am. J. Physiol. Heart Circ. Physiol.* **284**, H1161–H1167 (2003).
34. B. K. Pedersen, Edward F. Adolph distinguished lecture: Muscle as an endocrine organ: IL-6 and other myokines. *J. Appl. Physiol.* **107**, 1006–1014 (2009).
35. J. A. Laukkanen, D. Laaksonen, T. A. Lakka, K. Savonen, R. Rauramaa, T. Mäkikallio, S. Kurl, Determinants of cardiorespiratory fitness in men aged 42 to 60 years with and without cardiovascular disease. *Am. J. Cardiol.* **103**, 1598–1604 (2009).
36. W. B. Kannel, C. Kannel, R. S. Paffenbarger Jr., L. A. Cupples, Heart rate and cardiovascular mortality: The Framingham Study. *Am. Heart J.* **113**, 1489–1494 (1987).
37. L. C. Chao, Z. Zhang, L. Pei, T. Saito, P. Tontonoz, P. F. Pilch, Nur77 coordinately regulates expression of genes linked to glucose metabolism in skeletal muscle. *Mol. Endocrinol.* **21**, 2152–2163 (2007).
38. M. A. Maxwell, M. E. Cleasby, A. Harding, A. Stark, G. J. Cooney, G. E. Muscat, Nur77 regulates lipolysis in skeletal muscle cells. Evidence for cross-talk between the β-adrenergic and an orphan nuclear hormone receptor pathway. *J. Biol. Chem.* **280**, 12573–12584 (2005).
39. A. Sreekumar, L. M. Poisson, T. M. Rajendiran, A. P. Khan, Q. Cao, J. Yu, B. Laxman, R. Mehra, R. J. Lonigro, Y. Li, M. K. Nyati, A. Ahsan, S. Kalyana-Sundaram, B. Han, X. Cao, J. Byun, G. S. Omenn, D. Ghosh, S. Pennathur, D. C. Alexander, A. Berger, J. R. Shuster, J. T. Wei, S. Varambally, C. Beecher, A. M. Chinnaiyan, Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* **457**, 910–914 (2009).
40. E. Chorell, T. Moritz, S. Branth, H. Antti, M. B. Svensson, Predictive metabolomics evaluation of nutrition-modulated metabolic stress responses in human blood serum during the early recovery phase of strenuous physical exercise. *J. Proteome Res.* **8**, 2966–2977 (2009).
41. E. Pohjanen, E. Thysell, P. Jonsson, C. Eklund, A. Silfver, I. B. Carlsson, K. Lundgren, T. Moritz, M. B. Svensson, H. Antti, A multivariate screening strategy for investigating metabolic effects of strenuous physical exercise in human serum. *J. Proteome Res.* **6**, 2113–2120 (2007).
42. R. E. Olson, F. J. Stare, The metabolism in vitro of cardiac muscle in pantothenic acid deficiency. *J. Biol. Chem.* **190**, 149–164 (1951).
43. Paper presented at the Joint Food and Agriculture Organization/World Health Organization expert consultation, Bangkok, Thailand, 1998.
44. L. S. Tam, E. K. Li, V. Y. Leung, J. F. Griffith, I. F. Benzie, P. L. Lim, B. Whitney, V. W. Lee, K. K. Lee, G. N. Thomas, B. Tomlinson, Effects of vitamins C and E on oxidative stress markers and endothelial function in patients with systemic lupus erythematosus: A double blind, placebo controlled pilot study. *J. Rheumatol.* **32**, 275–282 (2005).
45. J. Lagendijk, J. B. Ubbink, W. J. Vermaak, The determination of allantoin, a possible indicator of oxidant status, in human plasma. *J. Chromatogr. Sci.* **33**, 186–193 (1995).
46. B. Shapiro, I. Chowers, G. Rose, Fatty acid uptake esterification in adipose tissue. *Biochim. Biophys. Acta* **23**, 115–120 (1957).

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47. M. Vaughan, The production and release of glycerol by adipose tissue incubated in vitro. *J. Biol. Chem.* **237**, 3354–3358 (1962).
48. H. Taegtmeyer, Energy metabolism of the heart: From basic concepts to clinical applications. *Curr. Probl. Cardiol.* **19**, 59–113 (1994).
49. J. Nauman, T. I. Nilsen, U. Wisløff, L. J. Vatten, Combined effect of resting heart rate and physical activity on ischaemic heart disease: Mortality follow-up in a population study (the HUNT study, Norway). *J. Epidemiol. Community Health* **64**, 175–181 (2010).
50. N. Nurjhan, F. Kennedy, A. Consoli, C. Martin, J. Miles, J. Gerich, Quantification of the glycolytic origin of plasma glycerol: Implications for the use of the rate of appearance of plasma glycerol as an index of lipolysis in vivo. *Metabolism* **37**, 386–389 (1988).
51. N. Nurjhan, A. Consoli, J. Gerich, Increased lipolysis and its consequences on gluconeogenesis in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* **89**, 169–175 (1992).
52. J. W. Jocken, E. E. Blaak, Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. *Physiol. Behav.* **94**, 219–230 (2008).
53. A. G. Smith, G. E. Muscat, Orphan nuclear receptors: Therapeutic opportunities in skeletal muscle. *Am. J. Physiol. Cell Physiol.* **291**, C203–C217 (2006).
54. S. J. Lessard, D. A. Rivas, Z. P. Chen, B. J. van Denderen, M. J. Watt, L. G. Koch, S. L. Britton, B. E. Kemp, J. A. Hawley, Impaired skeletal muscle β-adrenergic activation and lipolysis are associated with whole-body insulin resistance in rats bred for low intrinsic exercise capacity. *Endocrinology* **150**, 4883–4891 (2009).
55. M. Cnop, Fatty acids and glucolipotoxicity in the pathogenesis of type 2 diabetes. *Biochem. Soc. Trans.* **36**, 348–352 (2008).
56. J. E. Hansen, D. Y. Sue, K. Wasserman, Predicted values for clinical exercise testing. *Am. Rev. Respir. Dis.* **129**, S49–S55 (1984).
57. R. A. Bruce, F. Kusumi, D. Hosmer, Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am. Heart J.* **85**, 546–562 (1973).
58. D. B. Mark, M. A. Hlatky, F. E. Harrell Jr., K. L. Lee, R. M. Califf, D. B. Pryor, Exercise treadmill score for predicting prognosis in coronary artery disease. *Ann. Intern. Med.* **106**, 793–800 (1987).
59. G. F. Fletcher, G. J. Balady, E. A. Amsterdam, B. Chaitman, R. Eckel, J. Fleg, V. F. Froelicher, A. S. Leon, I. L. Piña, R. Rodney, D. A. Simons-Morton, M. A. Williams, T. Bazzarre, Exercise standards for testing and training: A statement for healthcare professionals from the American Heart Association. *Circulation* **104**, 1694–1740 (2001).
60. G. D. Lewis, J. Lachmann, J. Camuso, J. J. Lepore, J. Shin, M. E. Martinovic, D. M. Systrom, K. D. Bloch, M. J. Semigran, Sildenafil improves exercise hemodynamics and oxygen uptake in patients with systolic heart failure. *Circulation* **115**, 59–66 (2007).
61. D. S. Wishart, D. Tzur, C. Knox, R. Eisner, A. C. Guo, N. Young, D. Cheng, K. Jewell, D. Arndt, S. Sawhney, C. Fung, L. Nikolai, M. Lewis, M. A. Coutouly, I. Forsythe, P. Tang, S. Shrivastava, K. Jeroncic, P. Stothard, G. Amegbey, D. Block, D. D. Hau, J. Wagner, J. Miniaci, M. Clements, M. Gebremedhin, N. Guo, Y. Zhang, G. E. Duggan, G. D. Macinnis, A. M. Weljie, R. Dowlatabadi, F. Bamforth, D. Clive, R. Greiner, L. Li, T. Marrie, B. D. Sykes, H. J. Vogel, L. Querengesser, HMDB: The Human Metabolome Database. *Nucleic Acids Res.* **35**, D521–D526 (2007).
62. Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Statist. Soc. B* **57**, 289–300 (1995).

63. **Acknowledgments:** We thank the staff in the MGH Exercise Testing Laboratories and the individuals who assisted with orchestrating plasma sampling in the Boston Marathon study. **Funding:** NIH grants K23HL091106 (G.D.L.), R01 HL072872 (R.E.G. and M.S.S.), U01HL083141 (R.E.G., M.S.S., and F.P.R.), and R01DK081572 (R.E.G.); Donald W. Reynolds Foundation (R.E.G. and M.S.S.); Fondation Leducq (R.E.G.); American Heart Association Fellow-to-Faculty Award (G.D.L.); and Established Investigator Award (R.E.G.). This work was also supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195). A.A. was supported by a predoctoral award from the Sarnoff Cardiovascular Research Foundation. F.P.R. was also supported in part by NIH–National Human Genome Research Institute grants HG003224, HG0017115, NS054052, and HG004233 and by the Keck Foundation. **Author contributions:** G.D.L. conceived the study, designed the experiments, performed the primary data analysis, and wrote the manuscript. M.J.W. led the effort to recruit and phenotype marathon subjects. L.F. and M.M. recruited subjects, processed samples, and assisted with experimental design. Z.A. and G.C.R. designed and performed the gene expression profiling experiments. A.S., E.Y., X.S., A.A., S.A.C., and C.B.C. developed the metabolic profiling platform, performed MS experiments, and analyzed the data. S.C., E.L.M., T.J.W., and R.S.V. designed the experiments and analyzed the data from the Framingham Heart Study cohort. R.D. and F.P.R. assisted with statistical analysis and constructed the metabolite interrelatedness dendrogram. E.P.R. contributed to MS data analysis and helped to write the manuscript. D.M.S. and M.J.S. contributed to the CPET metabolic profiling experiment. M.S.S. helped to conceive and design the ETT studies and assisted in data interpretation and in writing the manuscript. R.E.G. conceived the study, designed the experiments, analyzed the data, and wrote the manuscript. **Competing interests:** The authors declare that they have no competing interests.

Submitted 23 February 2010
Accepted 5 May 2010
Published 26 May 2010
10.1126/scitranslmed.3001006

Citation: G. D. Lewis, L. Farrell, M. J. Wood, M. Martinovic, Z. Arany, G. C. Rowe, A. Souza, S. Cheng, E. L. McCabe, E. Yang, X. Shi, R. Deo, F. P. Roth, A. Asnani, E. P. Rhee, D. M. Systrom, M. J. Semigran, R. S. Vasan, S. A. Carr, T. J. Wang, M. S. Sabatine, C. B. Clish, R. E. Gerszten, Metabolic signatures of exercise in human plasma. *Sci. Transl. Med.* **2**, 33ra37 (2010).

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