Systemic metabolite kinetics mirror skeletal muscle energy metabolism during acute aerobic exercise

Running head: Exercise kinetics of serum metabolites

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Abstract

Acute exercise increases energy demand in skeletal muscle and releases metabolic intermediates into circulation, yet the serum kinetics of exercise-mobilized metabolites remain poorly characterized. By applying high-frequency serial blood sampling and targeted metabolomics in a longitudinal exercise trial with 12 young, healthy adults (6 female, 6 male), we assessed temporal alterations in energy-related metabolites during acute aerobic exercise and after 1 hour of recovery. We provide evidence for 42 exercise-responsive metabolites, including end products of glycolysis, tricarboxylic acid cycle intermediates, ketone bodies and amino acids. Overall, the observed metabolic alterations closely resembled skeletal muscle energy metabolism, thereby refining fundamental principles of exercise biochemistry through detailed serum kinetics, including novel, so far uncharacterized responses in systemic energy homeostasis and inter-organ crosstalk.

New and noteworthy

In our study we provide detailed serum kinetics of energy-related metabolites during acute aerobic exercise and after 1 hour of recovery. Semantic interpretation of our results against the backdrop of fundamental principles of exercise biochemistry indicated that serum metabolites mirror skeletal muscle energy metabolism, thus providing new insights into systemic energy homeostasis and inter-organ crosstalk.

Keywords: exercise, energy metabolism, metabolomics, inter-organ crosstalk, systemic communication

INTRODUCTION

Acute physical exercise is marked by a transient increase in energy demand, which is supported by various metabolic pathways including glycolysis and the tricarboxylic acid (TCA) cycle. Previous studies demonstrated that metabolic intermediates of energy production are not only increased in skeletal muscle (1,2) but also in systemic circulation (3). Once systemically available, these metabolites can serve distinct purposes. On the one hand, they can act as metabolic precursors for energy production in distant organs, thus supporting the exchange of energetic intermediates between metabolically active and inactive tissues in the context of systemic energy homeostasis (4). On the other hand, they can act as signaling molecules, also known as exerkines, for inter-organ crosstalk and tissue adaptation (5,6). A prime example of this is lactate which acts as a metabolic intermediate that is shunted from contracting skeletal muscle to other tissues such as the liver via systemic circulation where it can be converted back to glucose via the Cori cycle (7). Besides gluconeogenesis, lactate can also fuel energy production directly via oxidation to pyruvate or act as a signaling molecule across a wide range of peripheral tissues (7,8). Against this multi-facetted role of lactate, research on exercise metabolism is rapidly discovering similar exercise-secreted metabolites such as succinate and L-β-aminoisobutyric acid (L-BAIBA), both of which are mobilized by exercise and were demonstrated to induce peripheral tissue adaptation (9–11).

Our knowledge on the exercise-induced release of energetic intermediates into systemic circulation is primarily based on studies comparing resting baseline to post-exercise samples, often with a focus on individual or a few metabolites (3). While these studies offer a valuable starting point in our understanding of metabolic communication, follow-up investigations are at least of equal importance to establish physiological details such as the tissues of origin, potential target tissues, or the time-course of systemic release (6). Although acute exercise is known to trigger complex systemic alterations in metabolic homeostasis (12), the temporal kinetics and dynamic mobilization patterns of various intermediates of energy metabolism remain largely unexplored to date.

To address this gap, we performed high-frequency serial blood sampling and employed state-of-the-art targeted metabolomics to comprehensively detect temporal alterations of metabolic intermediates in blood serum during acute aerobic exercise and after 1h of recovery (Fig. 1a). We conducted a longitudinal exercise trial with 12 healthy adults (6 female, 6 male) and drew blood at baseline, immediately after the warm-up, after 5, 10, 15, 30, and 40 minutes of exercise (end of exercise session), and after 1h of recovery. Our targeted metabolomics panel comprised a total of 42 metabolites involved in energy metabolism (for details see Supplemental material). Based on current knowledge, we subdivided these metabolites into six groups: end products of glycolysis, TCA cycle intermediates, metabolites replenishing pyruvate and acetyl-CoA, metabolites replenishing TCA cycle intermediates, ketone bodies, and additional/other related metabolites (Fig. 1b). We hypothesized that the temporal kinetics of serum metabolites would reflect skeletal muscle energy metabolism during exercise and recovery.

MATERIALS AND METHODS

Study design

Twelve healthy participants (6 women, 6 men) attended two separate laboratory sessions in an overnight-fasted state without any dietary control the day prior. Participants were asked to refrain from exercise, alcohol consumption and caffeine intake in the 48h prior to each visit. Inclusion criteria comprised age between 20 and 35, an BMI below 30, and fluent knowledge of German (due to questionnaires used). Exclusion criteria comprised any type of acute or chronic disease as well as ongoing or past medication in the last six months, pregnancy or breast-feeding, use of oral contraceptives or nutritional supplements, current smoking, or more than 3 resistance exercise sessions or 300 minutes of endurance exercise per week in the last 6 months. All tests were performed in an exercise physiology laboratory under standardized environmental conditions (25.3 ± 2.4 °C, 60.9 ± 6 % humidity, controlled interior ventilation) between 08:00 and 10:00 AM to account for potential environmental and circadian effects on physiological and biological outcomes. The study was approved by the institutional ethics committee of the Leibniz Research Centre for Working Environment and Human Factors (Dortmund, Germany). Details on the study procedures were previously published (13). In brief, all participants signed written informed consent before inclusion into the study. During the first visit, anthropometric measurements including bioimpedance analysis (SECA mBCA 525) were taken. In addition, a cardiopulmonary exercise test on a cycle ergometer was performed to determine $\dot{V}O_{2peak}$ (METAMAX® 3B, Cortex, Germany). At least 72 hours later, participants performed a 40-minute aerobic exercise session on the same cycle ergometer at an intensity corresponding to 60 % of the individual $\dot{V}O_{2peak}$. To ensure that participants cycled at a constant exercise intensity during the 40-minute exercise session, internal load was assessed by spirometry and power output was dynamically adjusted. Blood draws were performed on an intravenous cannula that was placed on the anterior portion of the forearm. The blood samples were processed immediately after collection. Serum was isolated by centrifugation and stored at -80°C until further analysis.

Targeted metabolomics

The analyses of metabolites and amino acids (platform B) were carried out by BEVITAL AS (www.bevital.no), using gas chromatography-tandem mass chromatography (GC-MS/MS) after derivatization with methylchloroformate, using a slight modification of a method published previously (14). Metabolites (e.g. amino acid catabolites and TCA metabolites) not initially included in the original validated method were added individually along with their isotope labelled internal standard and the same validation procedures were performed (including linearity testing, accuracy, precision, and recovery), followed by cross-validation of the original assay ensuring quantitation and chromatographic performance were unaffected. The sample volume requirement was 50 µL. Highest analytical accuracy and reproducibility was ensured by calibrated measurement procedures and participation in external quality control programs. Within- and between-day coefficients of variation for the measured metabolites ranged from 1-6% and 1-7%, respectively.

Statistical analysis

All statistical analyses were performed using R (version 4.4.1). The final data set consisted of 12 individuals (IDs), 8 time points and 42 quantified metabolites. Metabolite values were expressed as absolute concentrations and CVs were calculated according to the following formula: CV = (standard deviation / mean) ×

100. The median CV of each measurement time point was used to calculate an overall mean.

Metabolite values were $\log_2$-transformed, and z-score normalized. A t-SNE analysis was performed using the `Rtsne` package with parameters dims = 2, perplexity = 8, max_iter = 1000, and theta = 0.05, chosen based on the moderate sample size and to ensure stable convergence of the algorithm. PCA was performed using the packages `FactoMineR` and `factoextra`. After checking normality of the residuals via Shapiko-Wilk test and Q-Q plots, linear mixed-effects models (LMMs) were fitted with the `nlme` package to identify time effects. Each metabolite was modelled individually using time and sex as fixed effects and ID as a random intercept. We also examined $\dot{V}O_{2peak}$ as a fixed effect to account for interindividual differences in fitness. However, after correcting for multiple tests, $\dot{V}O_{2peak}$ was not found to be significantly associated with any metabolite and was therefore not included in the final models. Each model included 77 degrees of freedom and 96 observations (see Supplemental material for full results), and the residuals were approximately normally distributed based on Shapiro-Wilk tests and Q-Q plots. P-values for time effects were adjusted for multiple testing using the Benjamini-Hochberg (BH) procedure. Metabolites were considered significantly different from baseline if at least one time point had a BH-adjusted p value < 0.05.

The $\log_2$ fold changes relative to baseline were calculated and visualized using a heatmap. Hierarchical clustering was applied to the rows of the heatmap (metabolites) to highlight patterns of co-regulation. For each time point, the mean and standard error of the mean (SEM) of the $\log_2$ fold changes across participants were calculated. The SEM was calculated as the standard deviation divided by the square root of the sample size (SD/√n). Additionally, the area under the curve (AUC) of the absolute metabolite levels during exercise was calculated using the trapezoidal rule. All figures were created using the R packages `ggplot2` or `ggpubr`. Schematic figures were created using www.biorender.com.

RESULTS

Participant characteristics

Participants exhibited a mean age of 25.5 ± 2.71 (standard deviation), a body mass index of 22.81 ± 3.14, and a relative fat mass of 20.7 ± 5.65. Peak oxygen uptake ($\dot{V}O_{2peak}$) and peak power output (PPO) during a cardiopulmonary exercise test on a cycle ergometer was 45.55 ± 9.72 mL/min/kg and 3.81 ± 0.88 W/kg, respectively. During the acute aerobic exercise session, participants cycled at an intensity of 65.37 ± 6.57 % $\dot{V}O_{2peak}$ or 50.92 ± 2.23 % PPO (Fig. 1c). Detailed participant characteristics are provided in Table 1.

*** Insert Table 1 here ***

Analytic quality of the dataset and descriptive results

Before conducting formal statistical analysis, we evaluated the analytic quality of our dataset. Inter-individual variability, assessed as coefficients of variation (CV), displayed a mean CV of 21.84 ± 1.13 % across the employed measurement time points (Fig. 1d). This suggests considerable variability between study participants but low variance in CVs across time points. To further dissect the variability in our data introduced by study participants, we applied t-distributed stochastic neighbour embedding (t-SNE) and observed high metabolic individuality, as indicated by separate clusters for each participant and an overall separation of our samples by

sex (Fig. 1e). Principal component analysis (PCA) additionally revealed a clear separation of the post-exercise and post-recovery samples from baseline samples, confirming as expected, metabolic alterations induced by exercise and recovery (Fig. 1f). Principal component (PC) 1 explained 34.2 % of the variance in our data while PC 2 accounted for 15.4 %. Further dissection of the PCA results suggested that metabolites involved in glycolysis and the TCA cycle were correlated with PC1 and 2 in a similar manner as post-exercise samples, while ketone bodies were better resembled by post-recovery samples (Fig. 1f, g). This suggests that acute exercise alters metabolites involved in glycolysis and the TCA cycle, while the response of ketone bodies is more confined to the recovery phase. Confirming these results, we observed a similar pattern in the ranked contributions of metabolites to PC1 and 2 (Fig. 1h). Overall, this suggests that acute exercise and recovery are marked by distinct metabolic responses in blood serum.

*** Insert Figure 1 here ***

Acute aerobic exercise triggers alterations in serum metabolites

We next performed formal statistical analysis by applying linear mixed models to our metabolomics dataset and found significant fluctuations from baseline for all 42 metabolites as well as sex differences for sarcosine, leucine, tyrosine, phenylalanine, methionine, and β-alanine. Considering the small sample size of six participants per sex, we did not address sex differences in more detail (see Supplemental material for full results including effect sizes and 95% confidence intervals). Interestingly, the acute exercise bout elicited a significant increase in 33 metabolites while none of the metabolites decreased below baseline levels. In contrast, after 1h of recovery more metabolites were decreased compared to baseline (Fig. 1i). Proportional changes from time point to time point revealed similar results and additionally suggested complex temporal kinetics during and after exercise (Fig. 1j). In summary, this confirms that acute exercise and recovery are marked by distinct metabolic responses in blood serum and that these responses depend on different metabolic pathways involved in energy supply.

Serum metabolite kinetics mirror skeletal muscle energy metabolism

We next performed hierarchical clustering to identify similar exercise kinetics across all metabolites. Hierarchical clustering yielded two main clusters, which mirrored the literature-based categorization of our metabolomics panel (Fig. 2a). Cluster 1 contained canonical end products of glycolysis and TCA cycle intermediates, including pyruvate, lactate, succinate, fumarate, and malate, all of which demonstrated a pronounced increase in response to exercise. While serum levels of pyruvate and lactate increased rapidly after 15 min of exercise, respectively, a more pronounced elevation until the end of the exercise session was found for succinate, fumarate, and malate (Fig. 2b, c).

These kinetics align with the release of metabolic intermediates from contracting skeletal muscle via monocarboxylate transporters (MCTs), which are ubiquitously expressed across different tissues and enable transport across plasma membranes dependent upon the local proton motive force and the concentration gradient of the substrate monocarboxylate (8). In accordance with fundamental principles of exercise biochemistry, the rapid increase in serum levels of lactate and pyruvate (Fig. 2b) might be attributed to the physiological decrease in skeletal muscle pH during exercise (i.e., an increase in proton motive force) as well as a cytosolic accumulation of pyruvate and lactate due to increased glycolytic flux. Both effects would create a

driving force for transport of these metabolites across skeletal muscle plasma membranes via MCTs, thus resulting in elevated serum levels.

While these principles explain the early mobilization of lactate and pyruvate into systemic circulation, they fall short in explaining the more pronounced increase in central TCA metabolites like succinate, fumarate, or malate, which are present as dicarboxylates and thus unavailable for MCT-mediated transport. In a landmark paper (11), Reddy and colleagues demonstrated that the physiological acidification of skeletal muscle during exercise protonates succinate, thus rendering the monocarboxylic form a transport substrate for MCT1. In detail, it was shown that the higher monocarboxylic pKa of succinate (pKa = 5.69) compared to malate (pKa = 5.13) and fumarate (pKa = 4.22) explains its preferential protonation and subsequent release from contracting skeletal muscle (11). This is mirrored by our obtained kinetics, which demonstrate an earlier and steeper increase of serum succinate levels compared to malate and fumarate (Fig. 2c). However, although malate and fumarate do not follow a pH-gated release via MCT1 – a result attributable to their different physicochemical properties (11) – we also observed a pronounced increase in serum levels of these metabolites. The fact that previous studies have demonstrated an initial increase in intracellular malate, fumarate, and other TCA cycle intermediates, followed by a decrease towards baseline levels during acute exercise (15,16), suggests rising concentration gradients across skeletal muscle plasma membranes and a potential release into circulation by so far unknown transporters. Of note, we observed a similar but less pronounced kinetic for α-ketoglutarate, another dicarboxylate, and slightly different kinetics for the tricarboxylates citrate and isocitrate (Fig. 2c). Taken together, our data suggest that acute exercise-induced increases in skeletal muscle energy metabolism are mirrored by systemic metabolite levels during aerobic exercise.

Further evaluation of our hierarchical clustering suggested that cluster 2 was subdivided into two subclusters. Cluster 2a contained many metabolites that were barely altered by exercise but exhibited a decline below baseline levels after 1h of recovery (Fig. 2a). Semantic interpretation of these metabolites revealed that such kinetics were mainly displayed by amino acids that replenish pyruvate and acetyl-CoA (Fig. 2d) or by amino acids that replenish TCA cycle intermediates (Fig. 2e). This is in line with the notion that acute exercise, which depicts a transient state of high metabolic turnover in skeletal muscle, is followed by a post-exercise recovery period that is marked by uptake of metabolic precursors for regeneration and tissue repair (17). In contrast, cluster 2b was more heterogeneous since it contained metabolites that increased during exercise (albeit less pronounced than in cluster 1) and then either returned to baseline or remained elevated until 1h after (Fig. 2a). These kinetics are in line with three further fundamental principles of exercise biochemistry.

First, the increase in α-ketoglutarate (Fig. 2c) and α-hydroxyglutaric acid (Fig. 2e) suggests that the accumulation of TCA cycle intermediates in skeletal muscle (i.e., α-ketoglutarate) can have immediate effects on other metabolites that are in close metabolic proximity (i.e., α-hydroxyglutaric acid). Although α-hydroxyglutaric acid is not directly involved in energy metabolism itself, it can be formed from α-ketoglutarate under conditions of hypoxia and acidic/decreasing pH via lactate dehydrogenase A (18). High glycolytic flux and elevated NADH levels in skeletal muscle during acute exercise, would render the reaction from α-ketoglutarate to α-hydroxyglutaric acid a reductive mechanism that regenerates NAD^+, thus ensuring undisrupted glycolytic flux and maintenance of cellular redox balance. As for α-

ketoglutarate, the elevated serum levels of α-hydroxyglutaric acid are thus likely attributable to accumulation in skeletal muscle and a subsequent release into circulation.

Second, the increase in serum alanine levels (Fig. 2d) depicts a prime example of alanine's function as a shuttle system for amino groups between skeletal muscle and the liver. This process, which is known as glucose-alanine cycle (19), binds nitrogen groups arising from increased branched-chain amino acid (BCAA) catabolism during acute exercise, to pyruvate, thus forming alanine. Alanine is then shuttled to the liver where it can serve as metabolic precursor for glucose production after transamination (20). The increased alanine levels observed in our data might thus be interpreted as a systemic resemblance of the elevated clearance of amino groups from skeletal muscle via the glucose-alanine cycle. This mechanism maintains systemic glucose homeostasis and protects skeletal muscle from accumulation of toxic ammonia derived from increased BCAA catabolism during exercise (20).

Third, cluster 2b also contained the ketone bodies β-hydroxybutyrate and acetoacetate, which displayed a progressive increase in response to acute exercise until 1h after exercise cessation (Fig. 2f). Since ketone bodies are produced by the liver as alternative fuel source during fasting, exercise of longer duration, and in the post-exercise recovery period (21), our obtained kinetics suggest that this is also true for shorter exercise bouts, especially following overnight-fasted conditions as employed by us. Alternatively, the elevated serum levels could reflect reduced tissue uptake during acute exercise, or a combination of both. Regarding the metabolic fate of systemically available ketone bodies, quantification of nutrient fluxes in mice has revealed that β-hydroxybutyrate is taken up by a wide range of peripheral tissues in response to acute exercise (4). However, the sustained increase of ketone bodies into the post-exercise recovery phase observed in our data suggests that ketone body production exceeds tissue uptake.

To validate our findings of increased mobilization of metabolic intermediates during acute exercise and an increased uptake of replenishing amino acids from blood serum in the subsequent recovery period, we calculated areas under the curve (AUC) for all metabolites, ranked these according to magnitude, and compared them to ranked fold changes from baseline after 1h of recovery. Interestingly, we observed that end products of glycolysis, TCA cycle intermediates, and ketone bodies displayed the highest AUC, thus suggesting that these metabolites are preferentially mobilized into systemic circulation during acute exercise (Fig. 2g). Conversely, amino acids demonstrated the highest negative fold changes 1h after exercise, indicating uptake from circulation, most likely into skeletal muscle, for regeneration and tissue repair (Fig. 2h).

*** Insert Figure 2 here ***

DISCUSSION

Our data confirms the increased systemic availability of metabolic intermediates which fulfill a dual function in response to acute exercise. On one side, they foster energy transfer between metabolically active and inactive tissues in the context of systemic energy homeostasis (4), as suggested by our kinetics for lactate and pyruvate as well as TCA cycle intermediates and amino acids. Regarding this, we observed a fine-tuned release of energetic intermediates into systemic circulation during exercise, followed by a decrease of serum amino acid levels in the

subsequent recovery phase (Fig. 2i). On the other side, some of the metabolites are also known to have endocrine function as signaling molecules which communicate metabolic state between organs and help regulate physiological processes to maintain homeostasis and coordinate the response to different (internal and external) stimuli (22). Succinate, a prime example, has been shown to act on brown adipose tissue in the context of cold exposure-induced shivering (23) and skeletal muscle in the context of exercise (11,24). However, the physiological relevance of other increased metabolites like fumarate, malate, α-ketoglutarate, or α-hydroxyglutaric acid are less well investigated in an exercise context and remain topics for future research.

Although the high temporal resolution during acute exercise applied by us offers profound insights into the systemic response of metabolic intermediates to acute exercise, there are several limitations that should be addressed in future research to generate more conclusive evidence. First, without a passive control group, it is hard to distinguish between exercise-induced alterations and potential other triggers such as prolonged fasting or circadian fluctuations. Additionally, different macronutrient composition due to the lack of standardized nutrition the day prior, might depict a confounding factor in the changes observed in this study. Addressing these limitations in a randomized cross-over study, preferably with a larger sample size, would not only pave the way to more conclusive insights into metabolic regulation during exercise, but also reduce inter-individual variability and allow for an adequately powered analysis of sex-specific differences.

Second, from our study setup, it remains unclear whether elevated serum metabolites are attributable to an increased release or a decreased uptake by peripheral tissues (or a combination of both). Additionally, whether these metabolites originate from skeletal muscle or other tissues, remains topic of future research. While we generally believe that contracting skeletal muscle – which is subject to high energetic demand during an acute exercise session as applied by us – is likely responsible for most of the metabolic alterations observed in blood serum, quantitative flux analyses in study setups employing arteriovenous blood sampling are needed to answer these questions conclusively (25). In this context, technological advances in exercise metabolism research now allow for systems-level integration of metabolomics data, which offers profound insights into the complex interactions of different systems during exercise and improves our understanding of the molecular underpinnings of exercise-mediated health benefits (12,26).

In summary, we provide detailed serum kinetics for 42 exercise-responsive metabolites during acute aerobic exercise and after 1h of recovery. Our data suggests a fine-tuned orchestration of release and uptake of energetic intermediates during and after exercise. Building upon fundamental principles of exercise biochemistry, we expand existing knowledge by detailed serum kinetics and uncover novel, so far undescribed systemic responses. With our analysis, we aim to inform ongoing research efforts investigating the metabolic foundation of exercise and its beneficial impact in health and disease.

SUPPLEMENTAL MATERIAL

Supplemental file: 10.6084/m9.figshare.30533117

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Data availability

Raw concentrations of all quantified metabolites (in µmol per liter) separated by participant, sex, and measurement time point as well as full statistical results are displayed in the supplementary file hosted on https://figshare.com under the following digital object identifier (DOI): 10.6084/m9.figshare.30533117. Additional data and analysis scripts are available from the corresponding author upon reasonable request.

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Author contributions

N.J., D.W. and P.Z. conceptualized the study. T.W., S.T. and N.J. designed and performed the experiments. A.M. and P.M.U. designed and conducted outcome measurement. C.W., D.W. analyzed and interpretated data. D.W., C.W., L.H., J.S. drafted the manuscript. N.J., P.Z. edited the manuscript and provided important intellectual input. All authors revised and approved the final version of the paper. Figures were created using BioRender.

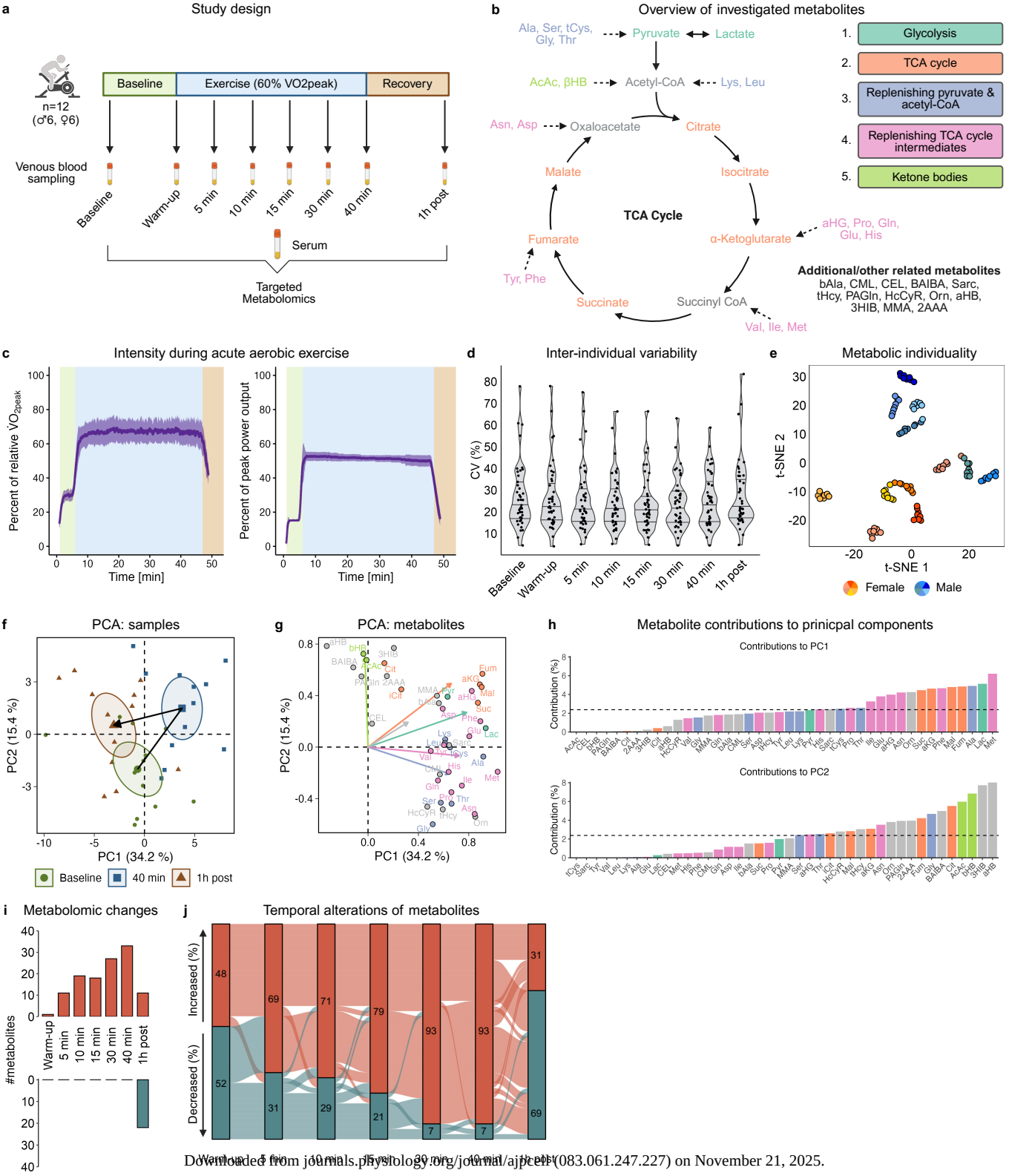
Disclosures

The authors declare no competing interests.

FIGURE LEGENDS

Figure 1. Study design, overview of investigated metabolites, and descriptive results. a, Study design. **b,** Overview of the metabolite panel. **c,** Exercise intensity (mean ± SD, 60-point moving average). **d,** Inter-individual variability expressed as coefficients of variation (CV) separated by time point. **e,** Metabolic individuality identified by t-distributed stochastic neighbour embedding (t-SNE). **f,** Principal component analysis (PCA) of the samples obtained at baseline, post-exercise, and post-recovery (means and 95 % confidence intervals for principal component (PC) 1 and 2). **g,** PCA of metabolites separated by metabolic process. Arrows indicate the mean correlation with PC1 and 2 of each metabolic process. **h,** Ranked metabolite contributions to PC1 and 2. Dashed lines indicate the hypothetical average contribution if all 42 metabolites were equally weighted (2.38 %). **i,** Number of significantly altered metabolites relative to baseline (red=increase, blue=decrease). **j,** Kinetics of all metabolites relative to baseline. 2AAA, 2-Aminoadipic acid; aHB, 2-Hydroxybutyrate; bHB, 3-Hydroxybutyrate; 3HIB, 3-Hydroxyisobutyrate; AcAc, Acetoacetate; aHG, α-Hydroxyglutaric acid; Ala, Alanine; Asn, Asparagine; Asp, Aspartic acid; bAla, β-Alanine; BAIBA, L-β-aminoisobutyric acid; CEL, Carboxyethyllysine; CML, Carboxymethyllysine; Glu, Glutamic acid; Gln, Glutamine; Gly, Glycine; His, Histidine; Ile, Isoleucine; Leu, Leucine; Lys, Lysine; Met, Methionine; MMA, Methylmalonic acid; Orn, Ornithine; PAGln, Phenylacetylglutamine; Phe, Phenylalanine; Pro, Proline; Sarc, Sarcosine; Ser, Serine; Thr, Threonine; tCys, Total cysteine; tHcy, Total homocysteine; HcCyR, homocysteine to cysteine ratio; Tyr, Tyrosine; Val, Valine

Figure 2. Temporal regulation of metabolic intermediates during acute exercise. a, Exercise-induced fold changes in metabolite levels relative to baseline. Main clusters identified by hierarchical clustering are shown. **b – f,** Kinetics of individual metabolites categorized by metabolic process. **g,** Area under the curve (AUC) during exercise color-coded according to metabolic process. **h,** Fold change 1h after exercise color-coded according to metabolic process. **i**, Schematic of the proposed mode of action regarding the systemic regulation of metabolic intermediates. Data are expressed as mean ± standard error. 2AAA, 2-Aminoadipic acid; aHB, 2-Hydroxybutyrate; bHB, 3-Hydroxybutyrate; 3HIB, 3-Hydroxyisobutyrate; AcAc, Acetoacetate; aHG, α-Hydroxyglutaric acid; aKG, α-Ketoglutarate; Ala, Alanine; Asn, Asparagine; Asp, Aspartic acid; bAla, β-Alanine; BAIBA, L-β-aminoisobutyric acid; CEL, Carboxyethyllysine; CML, Carboxymethyllysine; Cit, Citrate; Fum, Fumarate; Glu, Glutamic acid; Gln, Glutamine; Gly, Glycine; His, Histidine; iCit, Isocitrate; Ile, Isoleucine; Lac, Lactate; Leu, Leucine; Lys, Lysine; Mal, Malate; Met, Methionine; MMA, Methylmalonic acid; Orn, Ornithine; PAGln, Phenylacetylglutamine; Phe, Phenylalanine; Pro, Proline; Pyr, Pyruvate; Sarc, Sarcosine; Ser, Serine; Suc, Succinate; Thr, Threonine; tCys, Total cysteine; tHcy, Total homocysteine; HcCyR, homocysteine to cysteine ratio; Tyr, Tyrosine; Val, Valine



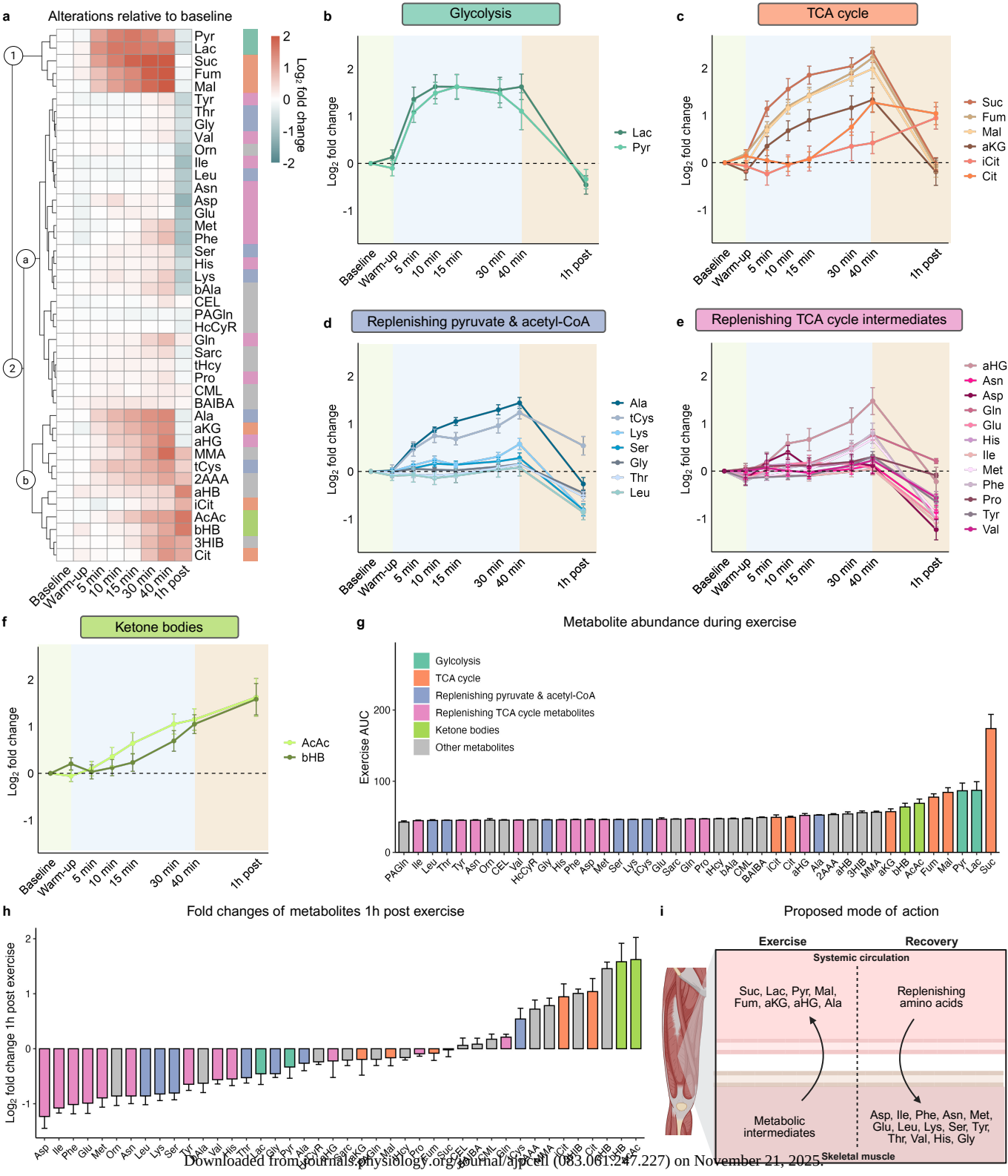


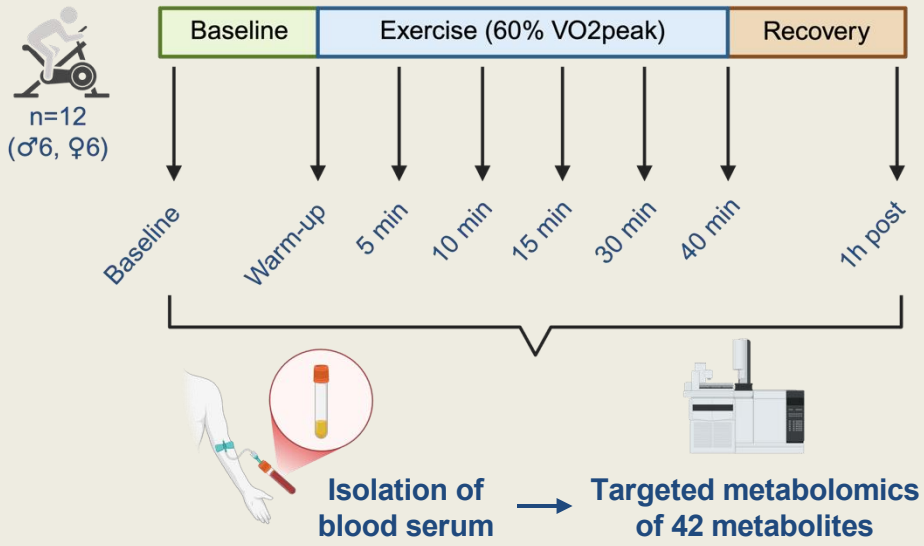
Table 1 Participant characteristics.

	Mean ± standard deviation [min; max]
Demographic and anthropometric characteristics	
Sex, m/f	6/6
Age, yr	25.50 ± 2.71 [21; 30]
Height, cm	173.81 ± 9.43 [161.0; 189.2]
Weight, kg	69.59 ± 14.85 [45.1; 96.3]
BMI, kg/m^2	22.81 ± 3.14 [17.10; 27.06]
Relative fat mass, %	20.70 ± 5.65 [12.73; 31.02]
Fat-free mass, %	79.30 ± 5.65 [68.98; 87.27]
Skeletal muscle mass, %	38.00 ± 3.80 [32.23; 43.45]
Cardiopulmonary exercise test	
$\dot{V}O_{2peak,}$ mL/min/kg	45.55 ± 9.72 [31.08; 56.96]
Maximal heart rate, bpm	185.67 ± 6.06 [173; 192]
Absolute PPO, W	271.67 ± 96.19 [106; 406]
Relative PPO, W/kg	3.81 ± 0.88 [2.35; 4.97]
RER at $\dot{V}O_{2peak}$, AU	1.14 ± 0.07 [0.95; 1.21]
TTE, min:s	10:04.3 ± 02:04.6 [05:45.2; 12:36.4]
Exercise session	
Percent $\dot{V}O_{2peak,}$ %	65.37 ± 6.57 [56.62; 82.78]
Percent PPO, %	50.92 ± 2.23 [47.86; 55.26]
Mean $\dot{V}O_2$, mL/min/kg	29.49 ± 5.84 [19.84; 36.26]
Mean power output, W	136.49 ± 49.78 [51.41; 222.13]
Mean heart rate, bpm	159.85 ± 8.98 [145.78; 169.40]
Mean RER, AU	0.93 ± 0.03 [0.88; 0.97]

AU, arbitrary unit; BMI, body mass index; PPO, peak power output; RER, respiratory exchange ratio; TTE, time to exhaustion; $\dot{V}O_{2peak}$, peak oxygen uptake. Due to technical reasons heart rate data was only recorded from 9 participants.

Detailed serum kinetics of metabolic intermediates during acute exercise

STUDY DESIGN

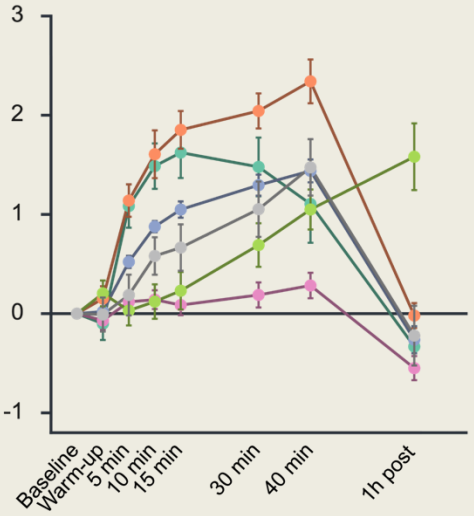


RESULTS

Subdivision into 6 groups

1. Glycolysis
2. TCA cycle
3. Replenishing pyruvate & acetyl-CoA
4. Replenishing TCA cycle intermediates
5. Ketone bodies
5. Other related metabolites

Detailed serum kinetics



Acute exercise triggers alterations in systemic metabolites involved in energy homeostasis and inter-organ crosstalk