# Can we assess exercise metabolism from skin? Metabolomic profiles in skin dialysate collected during exercise

Katsuhiko Yajima [a], Gulinu Maimaituxun [b], Taito Murakami [a], Shota Mitsuhashi [a], Koichi Watanabe [b], Takeshi Nishiyasu [b,c], Naoto Fujii [b,c]

[a] Laboratory of Nutritional Physiology, Faculty of Pharmaceutical Sciences, Josai University, Saitama, Japan.
[b] Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan
[c] Advanced Research Initiative for Human High Performance (ARIHHP)

**Short title:** *Metabolomics of skin dialysate*

**Corresponding author:**
Naoto Fujii, Ph.D.
Institute of Health and Sport Sciences
University of Tsukuba
Tsukuba, Ibaraki 305-8574, Japan
Tel & Fax: +81-298-53-2675
E-mail: fujii.naoto.gb@u.tsukuba.ac.jp

**Abstract**

Monitoring exercise intensity is essential for optimizing the health benefits of physical activity. Indirect calorimetry is the gold-standard method for assessing metabolic stress during exercise, though, its reliance on extensive equipment for sampling and analyzing exhaled gases restricts its widespread application. Skin interstitial fluid may represent an ideal biofluid for the continuous monitoring of whole-body metabolism and exercise intensity. However, specific metabolites in skin interstitial fluid that are more closely associated with metabolic variables measured by indirect calorimetry remain unknown. We examined which metabolites in skin interstitial fluid most closely reflect metabolic responses assessed by indirect calorimetry during a continuous graded exercise protocol. Twelve young participants (5 females) exercised at low, moderate, and high-intensity phases (25%, 50%, and 75% $VO_2$peak), each lasting 20 min. Skin dialysate, which reflects the composition of skin interstitial fluid, were collected via intradermal microdialysis during each exercise, and metabolites in the dialysate were measured and analyzed. Medium- and long-chain acylcarnitines in skin dialysate increased during moderate-intensity exercise in line with elevations in whole-body fat oxidation. During high-intensity phase, lactic acid in skin dialysate was elevated along with increases in whole-body carbohydrate oxidation. Many metabolites including those mentioned above in skin dialysate were correlated with whole-body metabolic responses. Our preliminary data suggest that metabolite concentrations in skin interstitial fluid may be associated with variables measured by indirect calorimetry. These findings may inform the future development of wearable devices that could potentially be used for continuous and noninvasive monitoring of exercise intensity.

**Keywords:** skin interstitial fluid, wearable device, exercise metabolism, acylcarnitines, exercise prescription.

## Introduction

Regular exercise improves all aspects of human health and is widely accepted as a preventative and therapeutic approach for various diseases (1,2). To maximize the benefits of exercise, setting appropriate intensity is essential. For instance, higher-intensity exercise training leads to greater (3) and more consistent (4) improvements in maximal oxygen uptake. Subjective indices such as ratings of perceived exertion can be used to assess exercise intensity, but it can be influenced by visual and auditory cues (5), caffeine (6) and heat stress (7). Heart rate, another commonly used variable, increases proportionally with exercise intensity. However, it is affected by caffeine intake (6), heat stress (7), and hydration status (8). Furthermore, it is unsuitable for individuals with arrhythmias, cardiac diseases, or hypertension who use medications like beta-blockers that suppress heart rate (9). Blood lactate concentration is widely utilized in clinical exercise stress tests and athletic performance evaluations (10). However, its invasiveness limits its applicability in large populations. Other body fluids, such as sweat or tears, may be used to measure metabolites like lactate. The accuracy of sweat can be significantly compromised by dilution effects at the measurement site and fluctuations in biomarker concentrations due to changes in sweat volume (11). Sampling tears is complex because only small amounts (less than 10 μL) can be collected, and tear metabolites vary in composition depending on collection methods (e.g., basal, reflex, flush, tears immediately after sleep) (12).

Exercise intensity is strongly correlated with whole-body metabolic responses, and the gold-standard method for evaluating metabolic strain during exercise is indirect calorimetry. Based on the data provided by indirect calorimetry, fat oxidation predominates during low-intensity exercise; however, as exercise intensity increases, fat oxidation decreases, and carbohydrate oxidation becomes the primary contributor to total energy expenditure (13). The respiratory exchange ratio, a key respiratory variable, rises in conjunction with the increase in carbohydrate metabolism. However, the required extensive equipment for sampling and analyzing exhaled gases limits the widespread use of indirect calorimetry. Skin interstitial fluid, primarily present in the lowermost skin layer of the dermis (14), increasingly gains attention as a biofluid marker (15,16). Assessing biomarkers from interstitial fluid in the skin offers several advantages compared to traditional biological fluids: 1) interstitial fluid does not clot; 2) the skin is highly accessible; 3) minimally invasive techniques like microneedles can be employed for interstitial fluid sampling in the skin; 4) specific analytes that may be undetectable in the blood could be detectable in interstitial fluid, as interstitial fluid interacts locally with cells, whereas blood analytes result from integration across multiple organs; and 5) the potential for future wearable devices attached to the skin, enabling continuous biomolecule monitoring. Therefore, skin interstitial fluid is an ideal biofluid for continuously monitoring whole-body metabolism during exercise and exercise intensity. However, specific biomarkers in skin interstitial fluid that more reflect whole-body metabolic response assessed by indirect calorimetry remain unknown.

Forearm skin dialysate is abundant in metabolites, including carbohydrates, lipids, proteins, vitamins, and minerals (16). A previous study demonstrated that lactate concentrations in skin dialysate progressively increase with rising exercise intensity (17); lactate is a marker of anaerobic metabolism and is frequently used for metabolic assessment (18–21). However, it remains unclear whether lactate levels in skin interstitial fluid accurately reflect metabolic responses measured via indirect calorimetry. More importantly, to the best of our knowledge, no study has yet investigated the behavior of other metabolic intermediates in skin interstitial fluid across varying exercise intensities, and identified which intermediates closely align with metabolic responses determined by indirect calorimetry over a broad range of exercise intensities. This is important because identifying multiple candidate metabolites would provide a broader range of options for developing future wearable sensors. This, in turn, allows for the selection of more reliable, reproducible, and cost-effective wearable devices.

In the present study, we examined which metabolites in skin dialysate, indicative of skin interstitial fluid metabolic status, are most closely associated with metabolic responses measured via indirect calorimetry during exercise at different intensities.

## Methods

### Ethical approval

The current study was approved by Human Subjects Committee of the University of Tsukuba (#29-24), adhering to the latest version of Declaration of Helsinki. Verbal and written informed consent were obtained from all participants before commencing the experiment.

### Participants

Twelve healthy, physically active young participants (5 females) were enrolled in the current investigation. Their age, body mass, height, body mass index, and peak oxygen uptake ($VO_{2peak}$), expressed as mean and standard deviation, were 22 ± 2 years, 61 ± 7 kg, 1.66 ± 0.06 m, 22 ± 2 kg/m$^2$, and 51 ± 7 mL/kg/min/, respectively. As no relevant prior data existed, a sample size calculation was not conducted. None of the participants were prescribed medication or smokers. All participants abstained from alcohol and caffeine for over 24 hours, rigorous physical activity for more than 12 hours, and food for more than 2 hours before study participation. To mitigate potential confounding influences of sex hormones, female participants were evaluated during their self-reported early follicular phase (i.e., within 9 days following the onset of menstruation). None of the female participants utilized contraceptives. Some participants were not familiar with certain aspects of the experimental procedures, including microdialysis fiber insertion. Although this may have influenced the data, most participants had previously participated in similar experiments, and thus we believe that any such effect was minimal.

**Peak oxygen uptake test**

All participants underwent an incremental cycling test using a bicycle ergometer (818E; Monark, Sweden) modified for semi-recumbent exercise to evaluate their $VO_{2peak}$ in a controlled environment chamber (Fuji Medical Science Co., Ltd, Chiba, Japan) maintained at a temperature of 20 °C and relative humidity of 50% one to two weeks prior to the experimental trial. Participants commenced with a brief low-intensity warm-up session (0.5 kp, 60 rpm) lasting for 3 minutes, succeeded by an incremental exercise test until volitional fatigue. The workload for male participants initiated at 1.5 kp and for female participants at 1.0 kp, with increments of 0.5 kp and 0.25 kp, respectively, every 1 minute until volitional exhaustion. Participants maintained a pedaling rate of 60 rpm, and volitional fatigue was defined as the inability to sustain a pedaling rate exceeding 55 rpm. Oxygen consumption ($VO_2$) and carbon dioxide output ($VCO_2$) were measured at 30-second intervals using indirect calorimetry (AE310s, Minato Medical Science, Osaka, Japan). $VO_{2peak}$ was determined as the maximal oxygen uptake averaged over the final 30-second period.

**Experimental protocol**

Figure 1 illustrates the experimental procedure. All participants visited the laboratory at 7:00 a.m., except for one participant who arrived at 11:00 a.m. Upon arrival at the laboratory, body mass was obtained using a digital weighing platform (model ICS429; Mettler Toledo, Schwerzenbach, Switzerland). Participants then underwent a period of rest in an environmental chamber (Fuji Medical Science Co., Ltd) maintained at a room temperature of 25°C and a relative humidity of 50%. Subsequently, four 25-gauge needles were inserted into different skin sites of the unanesthetized forearm dermis using aseptic technique, with entry and exit points separated by approximately 2.5 cm. A microdialysis fiber, composed of a commercially available 10 mm regenerated cellulose membrane with a 50 kDa cutoff (EICOM, Kyoto, Japan), was inserted through the lumen of each needle. Following insertion, the needles were withdrawn, leaving the four membranes beneath the skin. Approximately 5−10 minutes after the placement of the four fibers, lactic acid Ringer's solution (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan) was continuously perfused until the conclusion of the experiment. The perfusion rate at each skin site was maintained at 2.0 μL/min throughout utilizing a micro infusion pump (BASi Bee Hive controller and Baby Bee syringe drive; Bioanalytical Systems, West Lafayette, IN, USA). Although a lower perfusion rate would theoretically increase metabolite concentrations in skin dialysate, it requires a longer sample collection period. A longer exercise duration during high-intensity exercise is not feasible for the population studied; therefore, we selected a perfusion rate of 2.0 μL/min. We have also previously reported that this perfusion rate or a similar rate is sufficient for metabolomic analysis of skin dialysate (16,22). Following an initial continuous infusion of lactic acid Ringer's solution lasting ≥60 minutes to mitigate trauma associated with fiber insertion (23), a 20-minute baseline measurement commenced. Subsequently, participants initiated cycling exercise at 25%, 50%, and 75% of $VO_{2peak}$ (defined as Ex 1, Ex 2, and Ex 3), each sustained for 20 minutes without intermissions. Regardless of exercise intensity, a pedaling rate of 60 rpm was maintained. Skin dialysate was gathered into sample tubes during the final 15 minutes of each stage (i.e., baseline and each exercise intensity). Dialysate collected from the four intradermal microdialysis sites (2.0 μL × 15 minutes × 4 sites) at each stage was consolidated into a microtube (#11150, Sorenson Bioscience Inc, Salt Lake City, USA), resulting in a total volume of approximately ~120 μL per each stage, which was preserved at -80°C until subsequent analysis. The microdialysis-treated site was shielded by a local heater (PeriFlux Heater PF 450, Perimed) maintaining a local skin temperature of 33°C throughout the experiment. To minimize temperature- and blood flow–induced changes in metabolite concentrations in skin dialysate (16), we maintained skin temperature at a thermoneutral level (33 °C), which is known to keep skin blood flow low. Venous blood samples were obtained by a skilled nurse from the median cubital vein of the right upper arm using an 8.5 mL vacutainer pre-filled with EDTA-2Na as an anticoagulant (NP-EN0557, NIPRO, Japan) at end of baseline and each exercise. Collected venous blood samples were allowed to stand at room temperature (~25°C) for ~15 minutes and subsequently centrifuged at 1,500 × g for 10 minutes at 4°C. The plasma was then extracted and pipetted into microcentrifuge tubes (#11150, Sorenson Bioscience Inc, Salt Lake City, USA), which were frozen at -80°C until further analysis.

**Indirect calorimetry**

During the experimental session, respiratory gases were continuously collected using the breath-by-breath indirect calorimetry (AE310s, Minato Medical Science, Osaka, Japan). $VO_2$, $VCO_2$, respiratory exchange ratio (RER = $VCO_2/VO_2$), total energy expenditure (24) and substrate oxidation (25) were calculated from the measured $O_2$ consumption and $CO_2$ production. For the measurement of total energy expenditure, carbohydrate oxidation and fat oxidation, stoichiometric equations were applied according to the methodology described by Peronnet and Massicotte (25).

Energy expenditure (kcal/min) = 3.941 $VO_2$ (L/min) – 1.106 $VCO_2$ (L/min)

Carbohydrate oxidation (g/min) = 4.585 $VCO_2$ (L/min) − 3.226 $VO_2$ (L/min)

Fat oxidation (g/min) = 1.695 $VO_2$ (L/min) − 1.701 $VCO_2$ (L/min)

When the RER was equal to or greater than 1.0, calculated fat oxidation values that became negative were set to zero.

**Assessment of metabolites in venous plasma and skin dialysate**

100 µL of plasma or skin dialysate sample were combined with 300 µL of 0.1% formic acid in acetonitrile and 30 µL of an internal standard solution. This mixture was agitated in a vortex mixer (Lab Companion VM-96A, MITSUWA FRONTECH, Japan) for 3 minutes. The solution was then centrifuged at 16,000 rpm for 5 minutes at 4 ℃, and the supernatant was collected. Prior to sample loading, a solid phase extraction column was conditioned with 1 mL of 0.1% formic acid in acetonitrile. The sample was subsequently loaded into the solid phase extraction column. All dissolved samples were collected in

test tubes, and the eluate was evaporated using nitrogen gas. The residue was re-dissolved in 30 μL of 50% isopropanol. Metabolites from venous plasma and skin dialysate sample were analyzed using a liquid chromatograph interfaced with a Shimadzu LCMS-8050 tandem mass spectrometer featuring an electrospray ionization source. The chromatographic system was equipped with a CTO-40C column oven (maintained at 40°C), LC-40D XS pumps, and an SCL-40 system controller, all from Shimadzu, Kyoto, Japan. Chromatographic conditions were described in the previous study (26) and are included in the supplementary material (Tables S1 and S2). Metabolites were quantified based on the internal standard method and analyzed using Shimadzu LC Solution Software, Version 1.22 SP1. Limits of detection (LOD) and limits of quantification (LOQ) were determined for representative metabolites using a signal-to-noise–based approach. LOD was defined as a signal-to-noise ratio of 3, and LOQ was defined as three times the LOD. LOD and LOQ were determined using authentic standards, including lactic acid (Tokyo Chemical Industry, Tokyo, Japan), succinic acid and valine (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and acylcarnitine standards (NeoSMAAT® AC; Sekisui Medical Co., Ltd., Tokyo, Japan). The LOD and LOQ values are provided in table S3.

**Data analyses**

Delta (Δ) changes in the concentrations of each metabolite from baseline to exercise were calculated for each sample (i.e., skin dialysate fluid and venous plasma). The ratio of the sum of C16 (palmitoylcarnitine) and C18 (stearoylcarnitine) to C0 (free carnitine) [(C16 + C18) / C0] was calculated as a surrogate index of carnitine palmitoyltransferase I (CPT1)-mediated fatty-acid transport, wherein C0 represents a substrate of CPT1, and C16 + C18 shows the sum of acylcarnitines with a 16 or 18 carbon length that were products of CPT1. This calculation is a modified version of the original calculation for the assessment of CPT 1 (27–29). Liquid chromatography quantification provided data on 49 energy metabolism-related metabolites in skin dialysate and venous plasma. The obtained dataset was imported into SIMCA-P multivariate analysis software (version 13.0.3, Umetrics, Umea, Sweden) for statistical analysis and modeling of score and loading plots. PCA models was described by two main principal components, scaled by Pareto mode and Autofit function. Unsupervised separation and score contribution plots of skin dialysate and venous plasma samples up to exercise at low, moderate, and high intensities were analyzed.

## Statistical analyses

All statistical analyses were performed using SPSS (version 23.0; SPSS Japan), SIMCA 13 (Umetrics AB, Umeå, Sweden), and RStudio (2026.01.0 + 392, Posit Software, PBC). α was set at 0.05. Normal distribution of data was checked by Q-Q plots. All values are reported as mean (standard deviation) for normally distributed variables and as median (interquartile range) for non-normally distributed variables. Respiratory variables were analyzed using one-way repeated measures ANOVA with time (baseline and exercise at three different intensities) as a factor, followed by Bonferroni's multiple comparison test. Changes in metabolite concentrations were examined using Friedman test with time (same as above) as a factor, followed by Wilcoxon's signed ranks tests. To account for multiple comparisons in metabolite analyses, p-values were adjusted using false discovery rate (FDR) control based on the Storey method, as implemented in the qvalue package in Rstudio (30). Correlation analysis between exhaled gas and metabolite concentrations in skin dialysate and venous plasma was performed using Spearman's nonparametric correlation coefficient. For correlation analyses involving multiple metabolites, p-values were further adjusted by the aforementioned FDR correction (30). The Wilcoxon's signed ranks tests were employed to compare baseline metabolite concentrations between skin dialysate and venous plasma.

PCA was performed using SIMCA 13, where linear regression was applied to the metabolite data from skin dialysate and venous plasma, and the variables were Pareto-scaled prior to model construction; two principal components were then selected. The choice of principal components was established based on the fitting (R2X, 0.802) and predictive (Q2X, 0.426) values. Associations between venous plasma and skin dialysate metabolite concentrations were assessed using three complementary approaches: (i) Spearman's rank correlation was applied to raw (untransformed) data to evaluate overall cross-sectional associations across all participants and time points. (ii) Agreement analysis was performed using Bland–Altman plots on log-transformed concentrations to account for skewed distributions and proportional bias. (iii) Within-subject relationships were examined using repeated-measures correlation (rmcorr) on raw data to quantify common intra-individual trends across repeated measurements. These analyses described above were conducted in Rstudio using the repeated-measures correlation analysis (rmcorr) (31).

# Results

## Whole-body metabolic responses

Figure 2 illustrates whole-body metabolic responses during the continuous graded exercise assessed by indirect calorimetry. As anticipated, energy expenditure (panel A) and carbohydrate oxidation (panel B) increased with elevations in exercise intensity. Fat oxidation (panel C) was higher during Ex 1 and Ex 2 relative to baseline, while at Ex 3, it was comparable to baseline. RER (panel D) was elevated during Ex 2 and Ex 3 compared to baseline. These metabolic responses were relatively stable after 5 min from the initiation of exercise (Figure S1), during which skin dialysate was collected. Other respiratory variables are detailed in Table S4 and the time course of respiration-related variables is illustrated at 30-second intervals (Figure S1).

**Metabolites in skin dialysate and venous plasma**

Figure 3 illustrates a heat map showing changes in skin dialysate metabolites during the continuous graded exercise protocol relative to baseline for skin dialysate (panel A) and venous plasma (panel B). In skin dialysate, C6-, C8-, C10-, C12-, C14-, C16-, and C18-acylcarnitine showed an increase during the Ex 2 phase of the continuous graded exercise protocol compared to baseline, with only C8-acylcarnitine remaining elevated during Ex 3 (Figure 3A, Figure S2C - I). Glycolytic metabolites including lactic acid, along with succinic acid, a TCA cycle metabolite, were elevated in the skin dialysate during Ex 3 relative to baseline (Figure 3A, Figure S2A and B). Other assessed metabolites in skin dialysate did not exhibit significant increases at any exercise intensity. In venous plasma, lactic acid, C12-, C14-, C16- and C18-acylcarnitines showed increases during Ex 3 compared to baseline (Figure 3B, Figure S3A - E). Other metabolites in venous plasma did not exhibit significant increases at any exercise intensity.

**A surrogate index of CPT1 activity in skin dialysate and venous plasma**

Figure 4 illustrates the surrogate index of CPT1 activity in skin dialysate (panel A) and venous plasma (panel B). The surrogate index of CPT1 activity in skin dialysate was higher during Ex 1 and Ex 2 compared to baseline, while during Ex 3, it was comparable to baseline. The surrogate index of CPT1 activity in venous plasma was higher during Ex3 compared to baseline.

**Comparison between whole-body metabolism and metabolite concentration in skin dialysate and venous plasma**

Figure 5A illustrates the correlation relationships between respiratory variables and metabolic concentrations in skin dialysate using a heat map. Several metabolites showed significant correlations during exercise, particularly during Ex 2 and Ex 3. Several acylcarnitines, free fatty acids, and vitamins displayed significant negative correlations with RER or carbohydrate oxidation during Ex 2 and Ex 3, whereas C8-AC and C10-AC demonstrated significant positive correlations with fat oxidation during Ex 3. Conversely, no significant correlations were observed between respiratory variables and metabolite concentrations in venous plasma after FDR correction (Figure 5B).–

**Comparison between skin dialysate and venous plasma**

During the baseline, the concentrations of intermediates of the glycolytic pathway, amino acids, short-chain acylcarnitines and long-chain fatty acids were higher in skin dialysate than in venous plasma (Tables S5 and S6). Concentrations of medium- and long-chain acylcarnitines and polyunsaturated fatty acids in skin dialysate concentrations were lower than in venous plasma (Tables S5 and S6). For PCA models, the chosen PC1 and PC2 explained 23.5 % and 22.2 % of the variance, respectively, for a total explained variance of 45.7 % (Figure 6A). TCA circuit intermediates, amino acids, and short-chain acylcarnitine features showed the highest contributions to skin dialysate, whereas medium- and long-chain acylcarnitines and long-chain free fatty acid features showed high positive contributions to plasma (Figure S4). Skin dialysate and venous plasma showed positive correlations for many metabolites involved in glycolysis, the TCA cycle, amino acids, and fatty acids at baseline and during Ex 1 (Figure 6B). In the agreement analysis, the vast majority of pairs fell within the ± 1.96 standard deviation limits across exercise intensities (Figure S5A-I). In contrast, within-subject repeated-measures correlations revealed a divergent pattern among acylcarnitines (Figure S6A - H).

## Discussion

Key findings of our preliminary study are as follows. First, during a continuous graded exercise protocol, concentrations of medium- and long-chain acylcarnitines in skin dialysate increased during moderate-intensity exercise and subsequently decreased during high-intensity exercise compared with resting conditions. These changes closely resembled whole-body fat oxidation assessed by indirect calorimetry. Second, concentrations of several metabolites in skin dialysate, including medium- and long-chain acylcarnitines, were correlated with respiratory variables, such as the respiratory exchange ratio, as well as with carbohydrate and fat oxidation across low- to high-intensity exercise. Our preliminary data suggest that metabolite concentrations in skin interstitial fluid, specifically medium- and long-chain acylcarnitines, are associated with respiratory metrics of indirect calorimetry during graded exercise.

*Fat metabolism*

Whole-body fat oxidation assessed by respiratory gas during Ex1 was elevated relative to baseline and further increased during Ex 2 (Figure 2C), then decreased during Ex 3. Parallel to whole-body fat oxidation, acylcarnitines in the skin dialysate increased during Ex2 relative to baseline but decreased during Ex 3, returning to near baseline levels, except for C8-acylcarnitine (Figs 3A and S2). These findings suggest that acylcarnitines in skin interstitial fluid may track changes in whole-body fat metabolism associated with increasing exercise intensities during graded exercise.

Parallel changes in whole-body fat metabolism evaluated by respiratory gas and acylcarnitines in the skin dialysate could be attributed to the fact that acylcarnitines readily migrate from skeletal muscle to other organs via the bloodstream. Acylcarnitines increase in cells and blood when the production of acetyl-CoA, the end product of mitochondrial β-oxidation, exceeds the capacity of the TCA cycle (32,33). The accumulation of acylcarnitines in muscle, particularly medium- and long-chain acylcarnitines, can induce reductive and/or oxidative stress due to mitochondrial overload, potentially leading to various dysfunctions in the body, including insulin resistance, if left unchecked (34). To avoid these detrimental effects, medium- and long-chain acylcarnitines produced within skeletal muscle may be rapidly distributed via the bloodstream to the brain, kidneys, and other tissues (35). Since acylcarnitines are highly membrane-permeable, they rapidly diffuse into the skin interstitium (36) , and are perhaps relatively stable, which may underlie the parallel changes observed in whole-body metabolism and acylcarnitine levels in the skin dialysate. We do not know, however, the time required for acylcarnitines produced in active skeletal muscle to reach the skin interstitial fluid. In this context, acylcarnitine levels in the gastrocnemius medialis increase within 15 minutes of the onset of moderate-intensity exercise (37,38) . Thus, at least 15 minutes may be required to observe elevations in acylcarnitines in skin interstitial fluid after the initiation of exercise, although this requires direct investigation in future studies.

Alternatively, acylcarnitines may be locally produced by increased lipid metabolism in skin tissue during exercise. CPT1, which promotes lipid metabolism by binding carnitine to fatty acids in muscle tissue, is present in human skin (39) and may be activated by exercise (40). The surrogate index of CPT1 activity in skin dialysate increased predominantly during Ex1 and Ex2 compared to baseline (Figure 4A), which partially explains the increase in long-chain productions. However, future studies are warranted to directly assess CPT1 activity in the skin during exercise and validate this possibility. In contrast to medium- and long-chain acylcarnitines, short-chain acylcarnitines (i.e., C3- and C5-acylcarnitines) did not increase during exercise, regardless of exercise intensity. This is possible because most C3- and C5-acylcarnitines are formed by transferring the acyl groups produced by the catabolism of branched-chain amino acids to carnitine (41), a process that is not active during exercise.

*Carbohydrate metabolism*

Carbohydrate oxidation and RER increased with elevations in exercise intensity, consistent with previous findings (42). In line with this, lactic acid in venous plasma and skin dialysate increased during Ex 3 relative to resting states (Figs 3 A, B, S2 and S3), which is consistent with previous findings (43,44). Increases in lactic acid concentrations in skin dialysate are plausible, considering that lactate can easily move from active skeletal muscle to several organs (45). Alternatively, the elevated lactic acid concentrations in skin dialysate may be attributed to increased glycolytic activity in the skin, as glycolytic activity is high in the epidermis (46), which requires future scrutiny.

*Correlation analysis*

Whole-body fat oxidation was positively correlated with medium- and long-chain acylcarnitines in the skin dialysate during Ex 3 (Figure 5A). Conversely, a negative correlation was observed between whole-body carbohydrate oxidation and medium- and long-chain acylcarnitines in the skin dialysate during Ex 2 or Ex 3, which may reflect the inhibition of lipolysis associated with pyruvic acid and lactate production (47). Based on the aforementioned exercise-intensity-dependent data as discussed above and correlation outcomes, several metabolites specifically medium- and long-chain acylcarnitines, and lactic acid in the skin dialysate are candidates for predicting metabolic rate during exercise at different intensities.

By contrast, most of the acylcarnitines in the plasma were not correlated with whole-body fat oxidation (Figure 5B). This can be explained by the fact that acylcarnitines in the blood would rapidly diffuse into the skin interstitium as noted above. Alternatively, since acylcarnitines are continuously metabolized in major organs such as heart, liver, and skeletal muscles and released into the bloodstream (48), concentrations of acylcarnitines in the blood may not precisely reflect whole-body metabolic rate.

*Metabolite profiles and correlations in skin dialysate and venous plasma*

Succinic acid and C8-acylcarnitine increased exclusively in skin dialysate during Ex 3, whereas long-chain acylcarnitines (C12–18) increased only in venous plasma (Figs 3A and B). PCA clearly separated the skin dialysate and venous plasma (Figure 6A).

Moreover, although significant correlations between skin dialysate and venous plasma were observed for multiple metabolites under resting and low-intensity exercise condition, these correlations disappeared as exercise intensity increased (Figure 6B). Repeated correlation analysis further showed no significant correlations for most major metabolites (Figure S6). Collectively, these findings indicate that plasma and skin dialysate do not reflect identical metabolic profiles; rather, they capture distinct yet complementary aspects of metabolic regulation during exercise. Furthermore, correlations between respiratory variables and metabolite concentrations differed markedly between skin dialysate and venous plasma, with a greater number of significant correlations observed in skin dialysate (Figs 5A and B). Therefore, assessing skin interstitial fluid may be more suitable for estimating whole-body metabolic state during exercise compared to blood analysis, encouraging the development of wearable devices for continuous monitoring of metabolites in skin interstitial fluid. However, the lower concentrations of metabolites in skin dialysate (Tables S5 and S6) may affect precision and reproducibility. Notably, several metabolites show higher concentrations in skin dialysate compared to venous plasma (Table S5), making them potential candidates for estimating whole-body metabolism. It is important to note that acylcarnitines, which are candidates for assessing whole-body fat metabolism, exhibit lower concentrations in skin dialysate vs. venous plasma as the carbon chain length increases (Table S6). Therefore, short- and medium-chain acylcarnitines may be more suitable for detection in skin interstitial fluid. Our findings offer valuable insights into the development of wearable devices that access human skin interstitial fluid to assess whole-body metabolic state during exercise.

*Implication*

Metabolites in skin interstitial fluid potentially track whole-body metabolic status assessed by indirect calorimetry during low-to-high-intensity exercise as discussed above. If a wearable device is developed to continuously monitor the concentrations of these metabolites in skin interstitial fluid, similar to continuous glucose monitoring, it may enable continuous, non-invasive monitoring of whole-body metabolic stress during exercise without expensive equipment. This may support exercise prescription, training intensity optimization, and evaluation of training effects. Wearable-based monitoring of skin interstitial fluid may also facilitate the collection of large-scale metabolic data during exercise, thereby contributing to advances in exercise physiology and metabolic research. It should be noted that our data are preliminary; therefore, further research is needed to rigorously characterize the metabolic profile of skin interstitial fluid during exercise including repeatability for the future development of wearable devices.

*Limitations*

This study has several limitations. First, metabolite concentrations in skin dialysate are generally lower than those in skin interstitial fluid, suggesting that the actual concentrations in skin interstitial fluid would be higher (49) . We did not perform recovery rate experiments; therefore, we could not estimate the absolute concentration of each metabolite. It is worth highlighting that some metabolites exhibit much higher concentrations in skin dialysate compared to venous plasma, indicating that high-sensitivity sensors may not be necessary to detect these metabolites in skin interstitial fluid. Second, the interpretations are confined to a limited number of metabolites. Extracting more sample material and/or utilizing additional analytical platforms would provide a more comprehensive metabolomics profile, including very low-abundance metabolites across multiple chemical classes. Third, our participants were healthy young men and women. It remains unknown whether age, training status, sex differences, disease states, or the use of topical medications influence concentrations of metabolites in skin interstitial fluid. Moreover, although female participants were tested during the self-reported early follicular phase, circulating female hormone levels were not measured and may have varied among participants, potentially affecting the results. Fourth, the influence of dietary intake on our data remains unclear. Although a standardized meal was provided during the experiments to minimize variability in diet-derived metabolites, the timing of food intake was not strictly controlled. Additionally, certain metabolites originating from dietary sources may remain in our body for extended periods. Fifth, individual variability in metabolic responses during Ex 3 was greater than that during Ex 1 or Ex 2, which might underlie the larger number of significant correlations observed at this intensity. Sixth, we cannot exclude the possibility that time/ carry-over effects influenced our data, given we employed a graded exercise protocol. Nevertheless, we believe that this effect is relatively small, as the metabolic data were relatively stable during the last 5 minutes of each exercise stage (Fig S1), during which we collected skin dialysate. Seventh, it was not possible to precisely match the collection time of the venous plasma samples with that of the dialysate, which might have affected the correlation analysis between venous plasma and skin dialysate.

*Conclusion*

Medium- and long-chain acylcarnitine levels increased during moderate intensity exercise compared to resting conditions, along with elevations in whole-body fat oxidation measured indirect calorimetry. Lactic acid increased during high-intensity exercise relative to resting levels, which aligns with elevations in whole-body carbohydrate oxidation. Concentrations of several metabolites, including medium- and long-chain acylcarnitines in skin dialysate, were correlated with respiratory variables during graded exercise. Our preliminary findings suggest that metabolite concentrations in skin interstitial fluid may be associated with respiratory variables measured by indirect calorimetry during graded exercise. These results may inform the future development of wearable devices for estimating whole-body metabolic states during exercise.

## Author's contributions

N.F, K.Y, and G.M, conceived and designed experiments. G.M, contributed to data collection. N.F, K.Y, and G.M, contributed to data analysis. N.F, K.Y, G.M, T.M, S.M, K.W, and T.N, interpreted the experimental results. N.F, and K.Y, drafted the manuscript. All authors edited and revised the manuscript and approved the final version of the manuscript. All experiments took place at the Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan.


## Acknowledgements

We thank all participants who joined the present study.


## Disclosures

The authors declare that they have no conflicts of interest regarding the publication of this article. There are no financial conflicts of interest to disclose. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.


## Grants

None.


## Data Availability Statement

The datasets and supplemental file associated with the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.20176685

## References


[1] Lear SA, Hu W, Rangarajan S, Gasevic D, Leong D, Iqbal R, et al. The effect of physical activity on mortality and cardiovascular disease in 130 000 people from 17 high-income, middle-income, and low-income countries: the PURE study. The Lancet 2017;390:2643–54.

[2] Qiu Y, Fernández-García B, Lehmann HI, Li G, Kroemer G, López-Otín C, et al. Exercise sustains the hallmarks of health. J Sport Health Sci 2023;12:8–35.

[3] Franklin BA, Eijsvogels T, Pandey A, Quindry J, Toth P. Physical activity, cardiorespiratory fitness, and cardiovascular health: A clinical practice statement of the American Society for Preventive Cardiology Part II: Physical activity, cardiorespiratory fitness, minimum and goal intensities for exercise training, prescriptive methods, and special patient populations. Am J Prev Cardiol 2022;12:100425.

[4] Bacon AP, Carter RE, Ogle EA, Joyner MJ. $VO_2$max trainability and high intensity interval training in humans: A meta-analysis. PLoS One 2013;8.

[5] Olsson KSE, Ceci R, Wahlgren L, Rosdahl H, Schantz P. Perceived exertion can be lower when exercising in field versus indoors. PLoS One 2024;19.

[6] Fujii N, Fujimoto T, Yinhang C, Dobashi K, Matsutake R, Amano T, et al. Caffeine Exacerbates Hyperventilation and Reductions in Cerebral Blood Flow in Physically Fit Men Exercising in the Heat. Med Sci Sports Exerc 2021;53:845–52.

[7] Nybo L, Nielsen B. Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. J Appl Physiol (1985). 2001;91:2017-23.

[8] Castro-Sepúlveda M, Cerda-Kohler H, Pérez-Luco C, Monsalves M, Andrade DC, Zbinden-Foncea H, et al. Hydration status after exercise affect resting metabolic rate and heart rate variability. Nutr Hosp 2015;31:1273–7.

[9] Kraal JJ, Sartor F, Papini G, Stut W, Peek N, Kemps HMC, et al. Energy expenditure estimation in beta-blocker-medicated cardiac patients by combining heart rate and body movement data. Eur J Prev Cardiol 2016;23:1734–42.

[10] Goodwin ML, James E Harris, Andrés Hernández, et al. Blood lactate measurements and analysis during exercise: a guide for clinicians. J Diabetes Sci Technol. 2007;1:558-69.

[11] Harshman SW, Strayer KE, Davidson CN, et al. Rate normalization for sweat metabolomics biomarker discovery. Talanta. 2021;223.

[12] Khanna RK, Catanese S, Emond P, Corcia P, Blasco H, Pisella PJ. Metabolomics and lipidomics approaches in human tears: A systematic review. Surv Ophthalmol 2022;67:1229–43.

[13] Muscella A, Stefàno E, Lunetti P, Capobianco L, Marsigliante S. The regulation of fat metabolism during aerobic exercise. Biomolecules 2020;10:1–29.

[14] Pierre G, Agache PAPH. Measuring The Skin. Philipp M, Ed., (Springer-Verlag Berlin Heidelberg New York, 2004) (Springer Science & Business Media, 2004).

[15] Samant PP, Niedzwiecki MM, Raviele N, Tran V, Mena-Lapaix J, Walker DI, et al. Sampling interstitial fluid from human skin using a microneedle patch. Sci Transl Med. 2020;12.

[16] Oharazawa A, Maimaituxun G, Watanabe K, Nishiyasu T, Fujii N. Metabolome analyses of skin dialysate: Insights into skin interstitial fluid biomarkers. J Dermatol Sci 2024;114:141–7.

[17] De Boer J, Plijter-Groendijk H, Visser KR, et al. Continuous monitoring of lactate during exercise in humans using subcutaneous and transcutaneous microdialysis. Eur J Appl Physiol Occup Physiol. 1994;69:281-6.

[18] Peake JM, Tan SJ, Markworth JF, Broadbent JA, Skinner TL, Cameron-Smith D. Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. Am J Physiol Endocrinol Metab. 2014. 307; E539–52.

[19] San-Millán I, Brooks GA. Assessment of metabolic flexibility by means of measuring blood

lactate, fat, and carbohydrate oxidation responses to exercise in professional endurance athletes and less-fit individuals. Sports Med 2018, 48; 467-79.

[20] San-Millán I, Stefanoni D, Martinez JL, Hansen KC, D'Alessandro A, Nemkov T. Metabolomics of endurance capacity in world tour professional cyclists. Front Physiol 2020; 11: 578.

[21] Nemkov T, Cendali F, Stefanoni D, Martinez JL, Hansen KC, San-Millán I, D'Alessandro A. Metabolic signatures of performance in elite world tour professional male cyclists. Sports Med 2023; 53;1651-65.

[22] Fujii N, Omori S, Kataoka Y, Maimaituxun G, Bailey SJ, Lloyd AB, Arnold JT, Amano T, Tanabe Y, Omi N, Watanabe K, Nishiyasu T. Dietary nitrate supplementation increases nitrate and nitrite concentrations in human skin interstitial fluid. Nitric Oxide 2023;10-16.

[23] Anderson C, Andersson T, Wardell K. Changes in skin circulation after insertion of a microdialysis probe visualized by laser Doppler perfusion imaging. J Invest Dermatol. 1994;102:807-11.

[24] De JB, Weir V. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949;109:1-9.

[25] F Péronnet DM. Table of nonprotein respiratory quotient: an update. Can J Sport Sci 1991;16:23–9.

[26] Park I, Yoshitake R, Kioka K, Ishihara A, Yajima K, Kawana F, et al. Orexin receptor antagonist increases fat oxidation and suppresses protein catabolism during sleep in humans. iScience 2024;27;110212.

[27] Mavroudakis L, Lanekoff I. Ischemic Stroke Causes Disruptions in the Carnitine Shuttle System. Metabolites 2023;13;278

[28] Wu T, Zheng X, Yang M, Zhao A, Li M, Chen T, et al. Serum lipid alterations identified in chronic hepatitis B, hepatitis B virus-associated cirrhosis and carcinoma patients. Sci Rep 2017;7;42710.

[29] Li S, Gao D, Jiang Y. Function, detection and alteration of acylcarnitine metabolism in hepatocellular carcinoma. Metabolites 2019;9;36.

[30] Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003; 100; 9440-5.

[31] Bakdash JZ, Marusich LR. Repeated measures correlation. Front Psychol 2017; 8:456.

[32] Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 2008;7:45–56.

[33] Horst Schulz. Beta oxidation of fatty acids. Biochim Biophys Acta 1991;1081:109–20.

[34] Dambrova M, Makrecka-Kuka M, Kuka J, Vilskersts R, Nordberg D, Attwood MM, et al. Acylcarnitines: Nomenclature, biomarkers, therapeutic potential, drug targets, and clinical trials.

pharmacol Rev 2022;74:506–51.
[35] Jones LL, McDonald DA, Borum PR. Acylcarnitines: Role in brain. Prog Lipid Res 2010;49:61–75.
[36] Klepochová R, Niess F, Meyerspeer M, Slukova D, Just I, Trattnig S, et al. Correlation between skeletal muscle acetylcarnitine and phosphocreatine metabolism during submaximal exercise and recovery: interleaved 1H/31P MRS 7 T study. Sci Rep 2024;14: 3254.
[37] Friedel M, Thompson IAP, Kasting G, Polsky R, Cunningham D, Soh HT, et al. Opportunities and challenges in the diagnostic utility of dermal interstitial fluid. Nat Biomed Eng 2023;7:1541–55.
[38] Ibrahim R, Nitsche JM, Kasting GB. Dermal clearance model for epidermal bioavailability calculations. J Pharm Sci 2012;101:2094–108.
[39] Setoyama D, Fujimura Y, Miura D. Metabolomics reveals that carnitine palmitoyltransferase-1 is a novel target for oxidative inactivation in human cells. Genes to Cells 2013;18:1107–19.
[40] Maunder E, Rothschild JA, Fritzen AM, Jordy AB, Kiens B, Brick MJ, et al. Skeletal muscle proteins involved in fatty acid transport influence fatty acid oxidation rates observed during exercise. Pflugers Arch 2023;475:1061–72.
[41] Rousseau M, Guénard F, Garneau V, Allam-Ndoul B, Lemieux S, Pérusse L, et al. Associations between dietary protein sources, plasma BCAA and short-chain acylcarnitine levels in adults. Nutrients 2019;11.
[42] Capostagno B, Bosch A. Higher fat oxidation in running than cycling at the same exercise intensities. Int J Sport Nutr Exerc Metab 2010;20:44–55.
[43] Ming DK, Jangam S, Gowers SA, Wilson R, Freeman DM, Boutelle MG, et al. Real-time continuous measurement of lactate through a minimally invasive microneedle patch: a phase I clinical study. BMJ Innov 2022; 8: 87-94.
[44] MacRae HS, Dennis SC, Bosch AN, Noakes TD, S-h H, Cdennis S. Effects of training on lactate production and removal during progressive exercise in humans. J Appl Physiol (1985). 1992;72:1649-56.
[45] Louzada RA, Bouviere J, Matta LP, Werneck-De-Castro JP, Dupuy C, Carvalho DP, et al. Redox signaling in widespread health benefits of exercise. antioxid redox signal 2020;33:745–60.
[46] Li F, Li S, Xie H. The effects of physical activity on skin health: A narrative review. Clin Cosmet Investig Dermatol 2025; 18: 2189-200.
[47] Huang S, Shangguan R, Chen S, Lai X, Han H, Sun J. Mechanism of fatty acid metabolism and regulation by lactate during exercise in white adipose and skeletal muscle tissue: A review. Sports Med Open 2025; 11: 76.
[48] Dambrova M, Cecatto C, Vilskersts R, Liepinsh E. Mitochondrial metabolites acylcarnitines: therapeutic potential and drug targets. Bioenerg Commun 2022; 15:1-10.

[49] Benfeldt E, Serup J, Menné T. Microdialysis vs. suction blister technique for in vivo sampling of pharmacokinetics in the human dermis. Acta Derm Venereol 1999; 79: 338-42.

## Figure legend

**Fig 1.**

A schematic of the experimental protocol. Following baseline measurements, participants exercised at low, moderate, and high intensities, corresponding to 25%, 50%, and 75% of $VO_{2peak}$ (designated as Ex 1, Ex 2, and Ex 3, respectively). Skin dialysate samples were collected during the final 15 minutes of each stage, while blood samples were obtained 12.5 minutes into each stage.

**Fig 2**. Changes in respiratory variables from baseline (BL) to exercise at low, moderate, and high intensities, corresponding to 25%, 50%, and 75% of $VO_{2peak}$ (defined as Ex1, Ex2, and Ex 3, respectively). Data includes energy expenditure (panel A), carbohydrate oxidation (panel B), fat oxidation (panel C) and respiratory exchange ratio (RER, panel D) (n=12 for panel A-D). All data are averaged over the final 15 minutes of each stage and presented as mean (standard deviation). Data were analyzed by one-way repeated measure ANOVA, followed by Bonferroni. A main effect of exercise intensity was significant for all variables ($P < 0.01$). Statistical significance at levels of $P < 0.05$, $P < 0.01$, and $P < 0.001$ is denoted by *, **, and ***, respectively.

**Fig 3**. Heat map illustrating changes in metabolite concentrations in skin dialysate (panel A) and venous plasma (panel B) from baseline (BL) to exercise at low, moderate, and high intensities, corresponding to 25%, 50%, and 75% of $VO_{2peak}$ (defined as Ex1, Ex2, and Ex 3, respectively). Data are normalized to baseline values (% change from baseline; BL = 100%) and presented as medians (n = 12). The colour scale represents percent change from baseline, with increases shown in red (up to 400%) and decreases shown in blue (down to 0%). All data in panel A are medians of concentrations in skin dialysate collected at 2 µL/min during the final 15 minutes of each stage. All data in panel B are medians of concentrations in venous plasma collected at the end of baseline and each exercise. Metabolites exhibiting a significant increase relative to baseline are annotated with *. Statistical significance at the $P < 0.05$ levels is indicated by *. Data were analyzed using the Friedman one-way repeated measures analysis of variance, followed by Wilcoxon's signed ranks tests, with FDR correction applied using Storey's method ($q < 0.05$). G6P, Glucose 6-phosphate; F6P, Fructose 6-phosphate; Gr3P, Glycerol 3-phosphate; G1P, Glucose 1-phosphate; GA3P, Glyceraldehyde 3-phosphate; TCA, tricarboxylic acid; 3MHis, 3-methyl histidine; AC, acylcarnitine; FFA, free fatty acid;

ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

**Fig 4**. Changes in the (C16 + C18) / C0 ratio in skin dialysate (panel A) and venous plasma (panel B). The ratio of the sum of long-chain acylcarnitines (C16 + C18) to free carnitine (C0) was computed as a surrogate index of CPT1 activity, where free carnitine serves as the substrate for CPT1, whereas the long-chain acylcarnitines are products of CPT1. Data are presented as median (interquartile range) at baseline (BL), and during exercise at low, moderate, and high intensity, corresponding to 25%, 50%, and 75% of $VO_{2peak}$ (defined as Ex 1, Ex 2, and Ex 3, respectively). (C16 + C18) / C0 ratio exhibiting a significant increase relative to baseline are annotated with *. Statistical significance at the $P < 0.05$ and $P < 0.01$ levels is indicated by * and **, respectively. Data were analyzed by The Friedman one-way repeated measures analysis of variance, followed by Wilcoxon's signed ranks tests. Readers should be cautious because this index does not necessarily reflect CPT1 activity.

**Fig 5**. Heat map illustrating correlations between metabolite concentrations in skin dialysate (panel A) or venous plasma (panel B) and metabolic variables assessed by respiratory gases. Data are presented at baseline (BL), and during exercise at low, moderate, and high intensity, corresponding to 25%, 50%, and 75% of $VO_{2peak}$ (defined as Ex 1, Ex 2, and Ex 3, respectively). Spearman's non-parametric test was employed for correlation analyses. The colour scale represents Spearman's correlation coefficients (q), ranging from −1 to +1, with positive correlations shown in red and negative correlations shown in blue. Statistical significance was assessed using FDR correction based on Storey's method, with $q < 0.05$ indicated by *. RER, respiratory exchange ratio. G6P, Glucose 6-phosphate; F6P, Fructose 6-phosphate; Gr3P, Glycerol 3-phosphate; G1P, Glucose 1-phosphate; GA3P, Glyceraldehyde 3-phosphate; TCA, tricarboxylic acid; 3MHis, 3-methyl histidine; AC, acylcarnitine; FFA, free fatty acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

**Fig 6**. Unsupervised separation (panel A) of skin dialysate and venous plasma samples from baseline (BL) to exercise at low, moderate, and high intensities corresponding to 25%, 50%, and 75% of $VO_2$peak (defined as Ex 1, Ex 2, and Ex 3, respectively). The numbers in panel A indicate subject ID (Subjects 1–12) and exercise condition (BL, Ex1, Ex2, and Ex 3 labeled 1–4, respectively). After log-transformation of energy metabolism–related metabolite concentrations, principal component analysis (PCA) revealed distinct metabolomic profiles between skin dialysate (blue) and venous plasma (red). The model included 49 metabolites, with principal components selected based on goodness of fit ($R2X = 0.802$) and predictive ability ($Q2 = 0.426$); the oval indicates Hotelling's $T^2$ (95% confidence limits). Panel B shows a heat map illustrating correlations between skin dialysate and venous plasma metabolite concentrations at BL and during Ex 1–Ex 3. Spearman's non-parametric correlation analysis was performed. The colour scale represents Spearman's correlation coefficient (q), ranging from −1 to 1, with positive correlations shown in red and negative correlations in blue. Statistical significance was assessed using FDR correction based on Storey's method, with $q <$ 0.05, 0.01, and 0.001 indicated by *, **, and ***. G6P, Glucose 6-phosphate; F6P, Fructose 6-phosphate; Gr3P, Glycerol 3-phosphate; G1P, Glucose 1-phosphate; GA3P, Glyceraldehyde 3-phosphate; TCA, tricarboxylic acid; 3MHis, 3-methyl histidine; AC, acylcarnitine; FFA, free fatty acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

# Can we assess exercise metabolism from skin?
# Metabolomic profiles in skin dialysate collected during exercise.

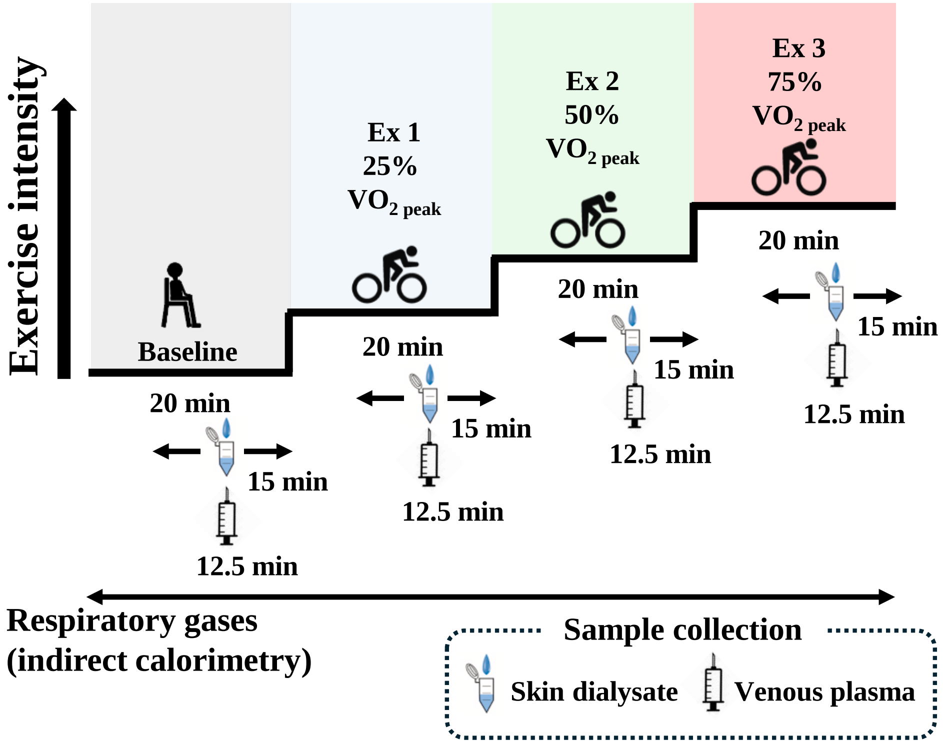
**Participants:**
12 young participants
(7 males and 5 females)

**Purpose:** To identify skin interstitial metabolites associated with metabolic responses measured by indirect calorimetry during graded exercise.

Metabolites in skin dialysate, reflecting the metabolic state of the skin interstitial fluid, were analyzed during graded exercise.

## Experimental protocol:

Respiratory gases, skin dialysate, and venous plasma were collected at baseline and during each stage of a graded exercise protocol consisting of three exercise stages (Ex 1–Ex 3).

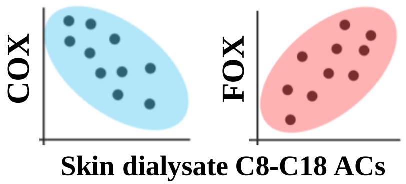



## Results:

⇧ indicates a significant increase vs. baseline

| | Baseline | Ex 1 (Low) | Ex 2 (Moderate) | Ex 3 (High) |
|---|---|---|---|---|
| Respiratory gases ($CO_2$, $O_2$) | | COX ⇧<br>FOX ⇧ | COX ⇧<br>FOX ⇧ | COX ⇧ |
| Skin dialysate | | | C6-C18 ACs ⇧ | Lactic acid ⇧<br>Succinic acid ⇧<br>C8 AC ⇧ |
| Venous plasma | | | | Lactic acid ⇧<br>C12-C18 ACs ⇧ |

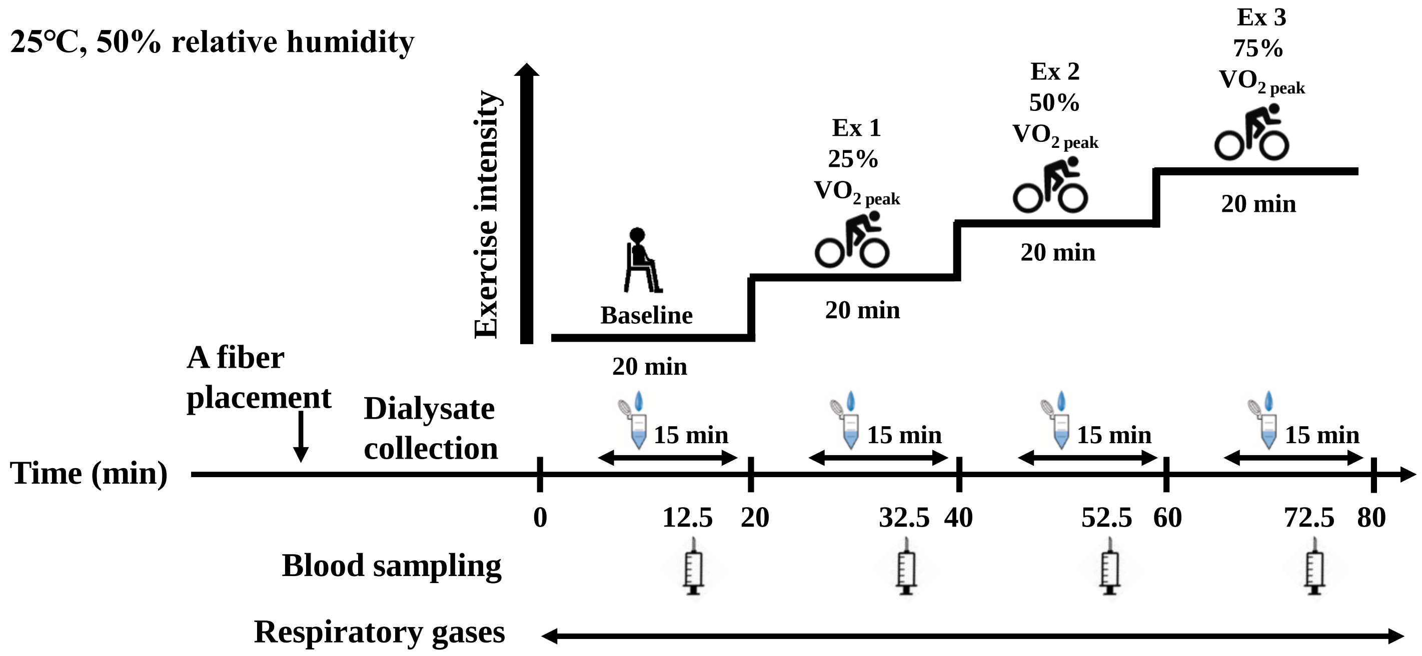
Correlation analysis




**Many metabolites including acylcarnitines in skin dialysate were correlated with whole-body metabolism**

COX, carbohydrate oxidation; FOX, fat oxidation; AC, acylcarnitine.

**Conclusion:** Skin interstitial metabolite profiles may be associated with respiratory variables measured by indirect calorimetry during graded exercise.



25°C, 50% relative humidity
Exercise intensity
Baseline
20 min
Ex 1
25%
VO2 peak
20 min
Ex 2
50%
VO2 peak
20 min
Ex 3
75%
VO2 peak
20 min
A fiber placement
Dialysate collection
15 min
15 min
15 min
15 min
Time (min)
0
12.5
20
32.5
40
52.5
60
72.5
80
Blood sampling
Respiratory gases


Figure 1.

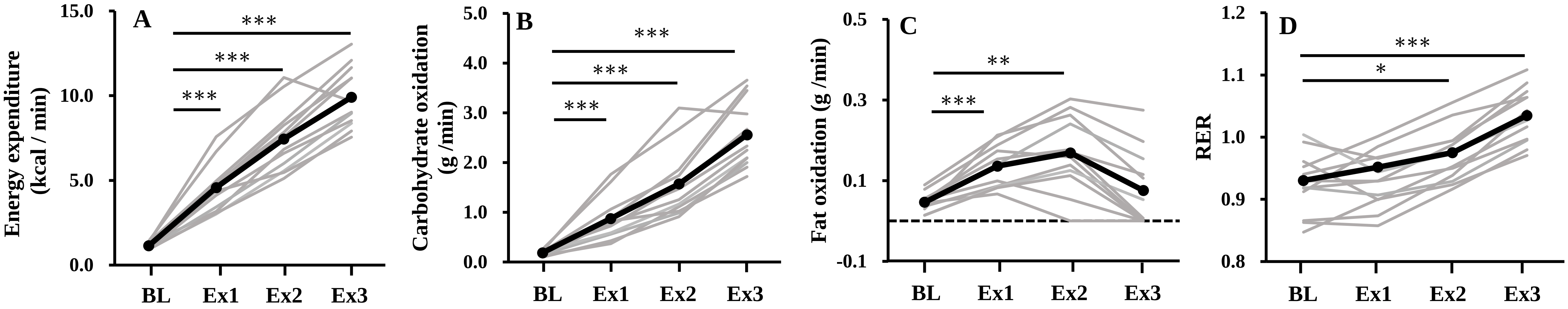

A
***
***
***
Energy expenditure (kcal / min)
15.0
10.0
5.0
0.0
BL
Ex1
Ex2
Ex3
B
***
***
***
Carbohydrate oxidation (g /min)
5.0
4.0
3.0
2.0
1.0
0.0
BL
Ex1
Ex2
Ex3
C
**
***
Fat oxidation (g /min)
0.5
0.3
0.1
-0.1
BL
Ex1
Ex2
Ex3
D
***
*
RER
1.2
1.1
1.0
0.9
0.8
BL
Ex1
Ex2
Ex3

Figure 2.

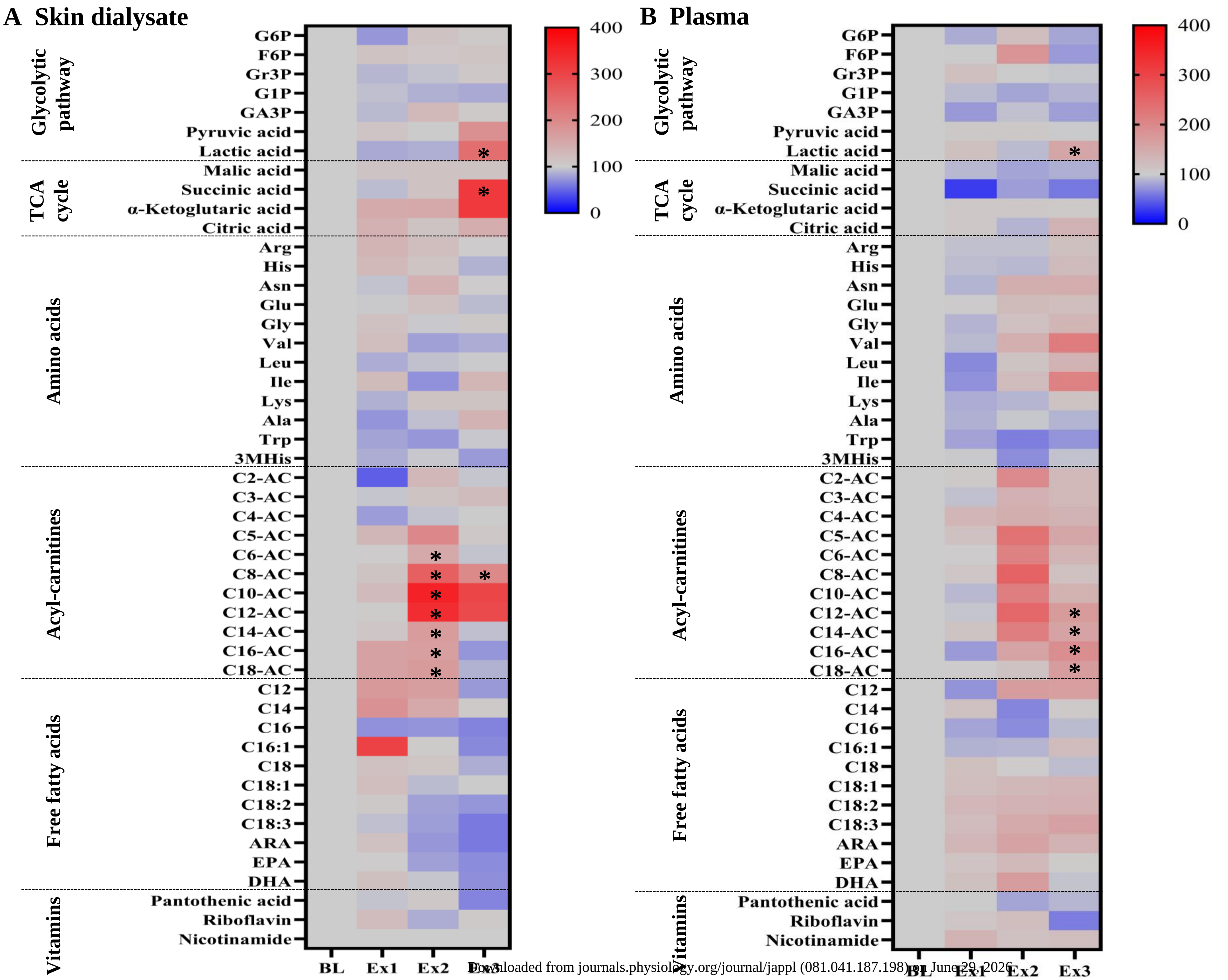

A Skin dialysate
B Plasma
Glycolytic pathway
TCA cycle
Amino acids
Acyl-carnitines
Free fatty acids
Vitamins
G6P
F6P
Gr3P
G1P
GA3P
Pyruvic acid
Lactic acid
Malic acid
Succinic acid
α-Ketoglutaric acid
Citric acid
Arg
His
Asn
Glu
Gly
Val
Leu
Ile
Lys
Ala
Trp
3MHis
C2-AC
C3-AC
C4-AC
C5-AC
C6-AC
C8-AC
C10-AC
C12-AC
C14-AC
C16-AC
C18-AC
C12
C14
C16
C16:1
C18
C18:1
C18:2
C18:3
ARA
EPA
DHA
Pantothenic acid
Riboflavin
Nicotinamide
BL
Ex1
Ex2
Ex3
400
300
200
100
0

Figure 3.

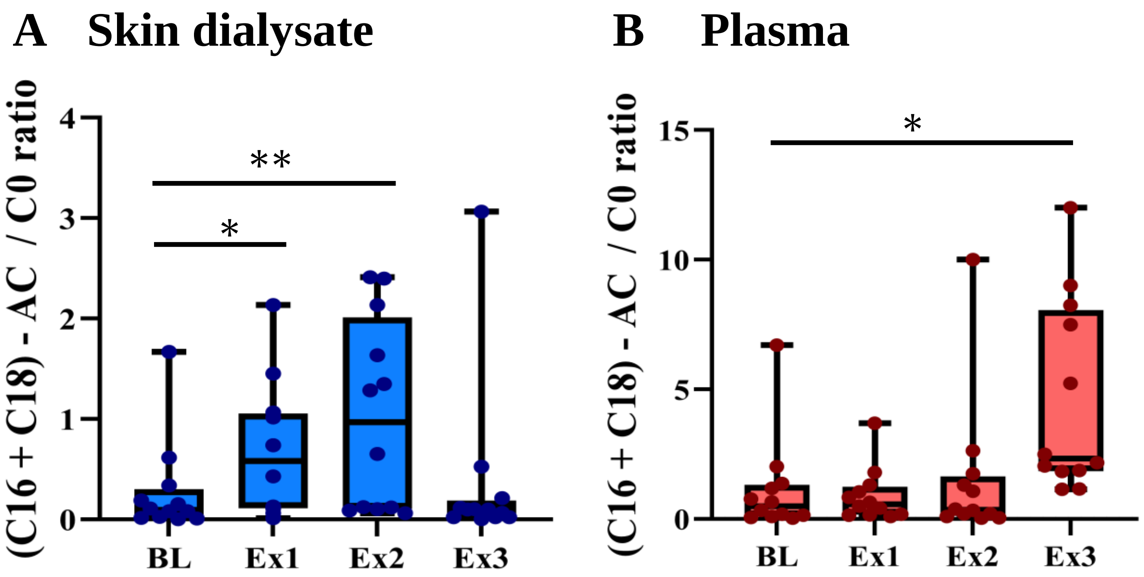

A Skin dialysate
B Plasma
(C16 + C18) - AC / C0 ratio
4
3
2
1
0
**
*
BL
Ex1
Ex2
Ex3
15
10
5
0
*

Figure 4.

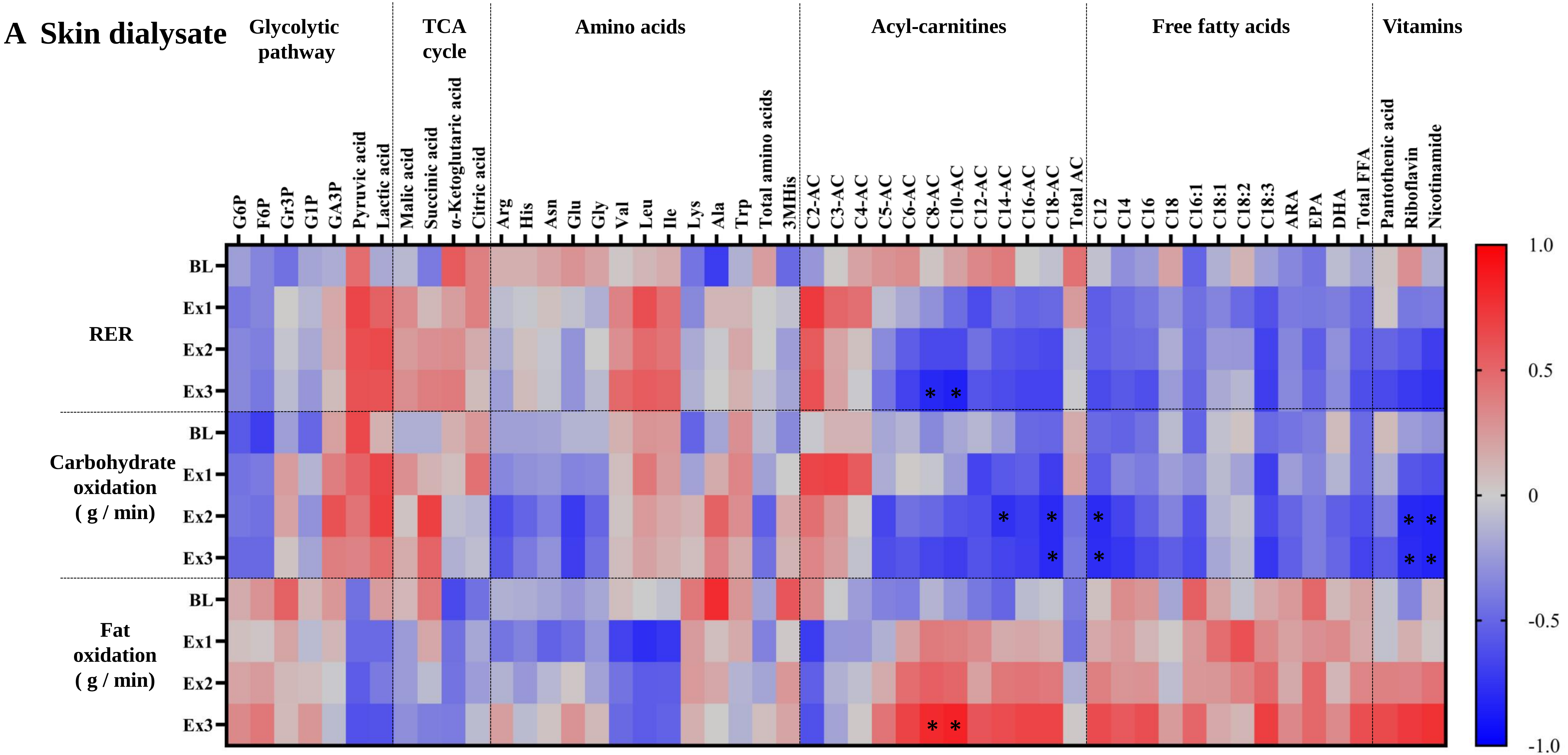
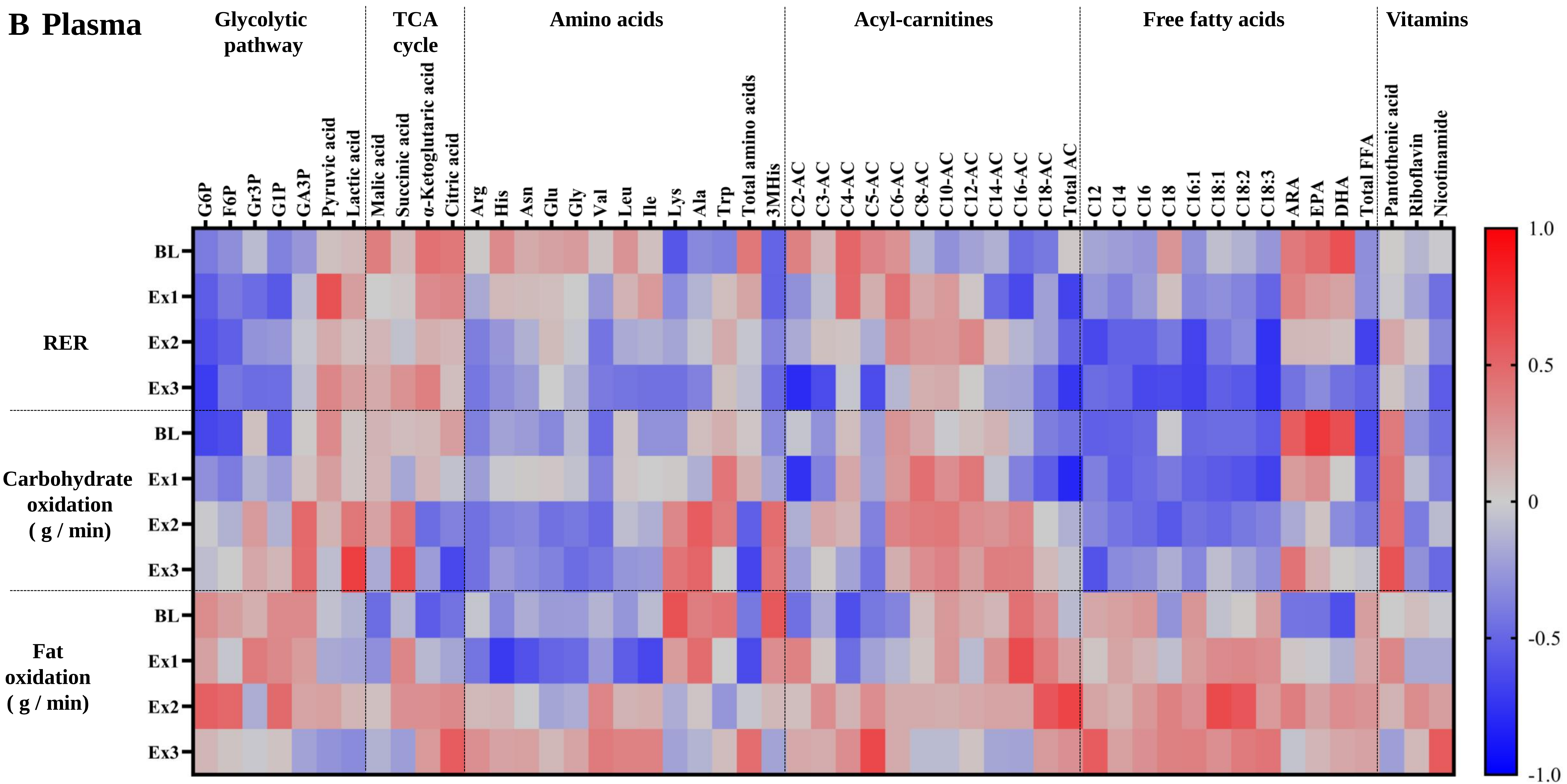

A Skin dialysate
Glycolytic pathway
TCA cycle
Amino acids
Acyl-carnitines
Free fatty acids
Vitamins
G6P
F6P
Gr3P
G1P
GA3P
Pyruvic acid
Lactic acid
Malic acid
Succinic acid
α-Ketoglutaric acid
Citric acid
Arg
His
Asn
Glu
Gly
Val
Leu
Ile
Lys
Ala
Trp
Total amino acids
3MHis
C2-AC
C3-AC
C4-AC
C5-AC
C6-AC
C8-AC
C10-AC
C12-AC
C14-AC
C16-AC
C18-AC
Total AC
C12
C14
C16
C18
C16:1
C18:1
C18:2
C18:3
ARA
EPA
DHA
Total FFA
Pantothenic acid
Riboflavin
Nicotinamide
RER
Carbohydrate oxidation ( g / min)
Fat oxidation ( g / min)
BL
Ex1
Ex2
Ex3
1.0
0.5
0
-0.5
-1.0

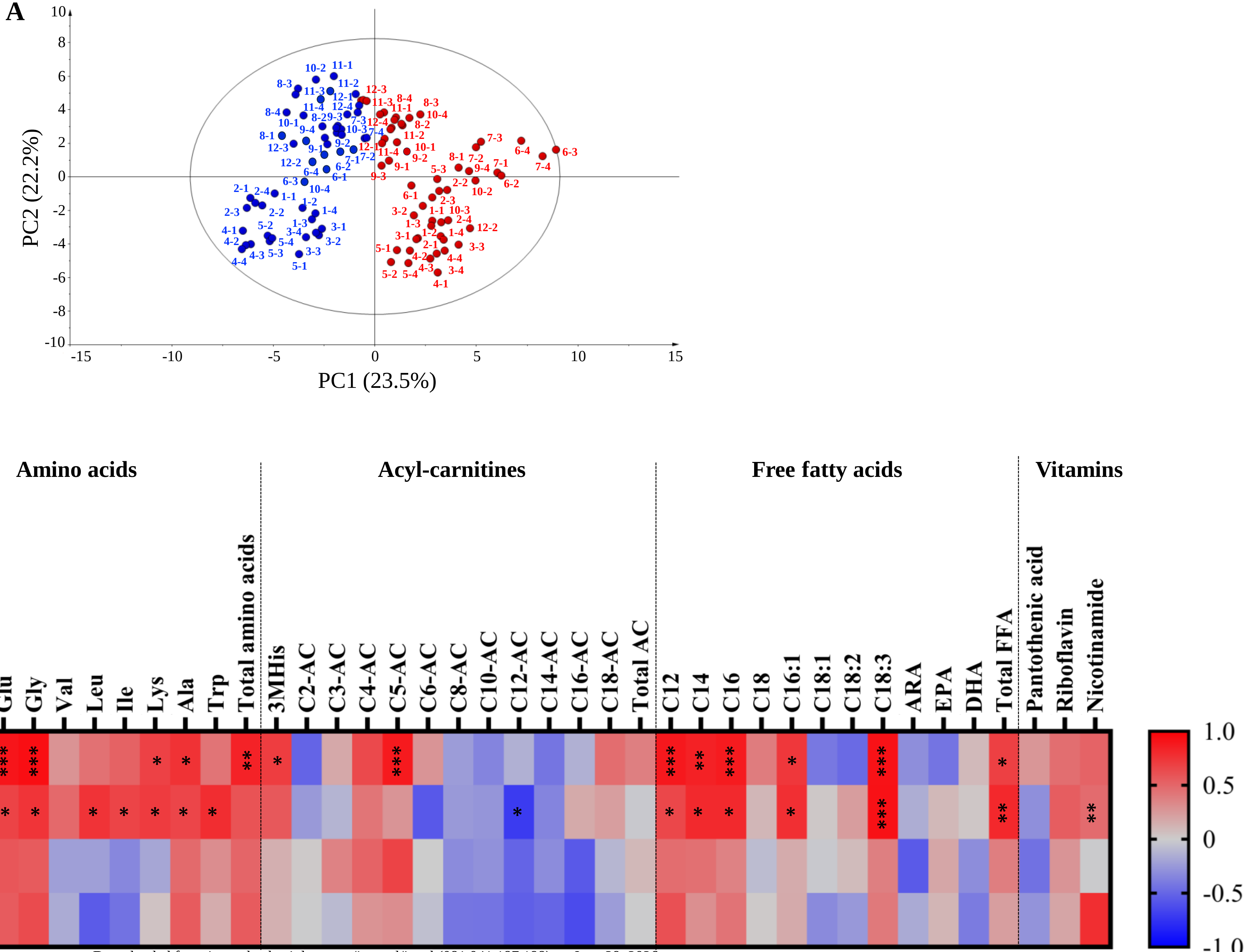
Figure 5.



B Plasma
Glycolytic pathway
TCA cycle
Amino acids
Acyl-carnitines
Free fatty acids
Vitamins
G6P
F6P
Gr3P
G1P
GA3P
Pyruvic acid
Lactic acid
Malic acid
Succinic acid
α-Ketoglutaric acid
Citric acid
Arg
His
Asn
Glu
Gly
Val
Leu
Ile
Lys
Ala
Trp
Total amino acids
3MHis
C2-AC
C3-AC
C4-AC
C5-AC
C6-AC
C8-AC
C10-AC
C12-AC
C14-AC
C16-AC
C18-AC
Total AC
C12
C14
C16
C18
C16:1
C18:1
C18:2
C18:3
ARA
EPA
DHA
Total FFA
Pantothenic acid
Riboflavin
Nicotinamide
RER
Carbohydrate oxidation ( g / min)
Fat oxidation ( g / min)
BL
Ex1
Ex2
Ex3
1.0
0.5
0
-0.5
-1.0


Figure 5.

Figure 6.


A
PC1 (23.5%)
PC2 (22.2%)
-15
-10
-5
0
5
10
15
10
8
6
4
2
0
-2
-4
-6
-8
-10
B
Glycolytic pathway
TCA cycle
Amino acids
Acyl-carnitines
Free fatty acids
Vitamins
G6P
F6P
Gr3P
G1P
GA3P
Pyruvic acid
Lactic acid
Malic acid
Succinic acid
α-Ketoglutaric acid
Citric acid
Arg
His
Asn
Glu
Gly
Val
Leu
Ile
Lys
Ala
Trp
Total amino acids
3MHis
C2-AC
C3-AC
C4-AC
C5-AC
C6-AC
C8-AC
C10-AC
C12-AC
C14-AC
C16-AC
C18-AC
Total AC
C12
C14
C16
C18
C16:1
C18:1
C18:2
C18:3
ARA
EPA
DHA
Total FFA
Pantothenic acid
Riboflavin
Nicotinamide
BL
Ex1
Ex2
Ex3
1.0
0.5
0
-0.5
-1.0