REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
09-08-19	Journal Article	

4. TITLE AND SUBTITLE
Metabolomic stability of exercise-induced sweat

5a. CONTRACT NUMBER
FA8650-17-F-6831

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sean W. Harshman[a],*, Rhonda L. Pitsch[b], Nicole M. Schaeublin[a], Zachary K. Smith[a], Kraig E. Strayer[a], Mandy S. Phelps[a], Anthony V. Qualley[a], Daniel W. Cowan[a], Sharon D. Rose[a], Maegan L. O'Connor[c], Jason J. Eckerle[d], Tapas Das[e], Aron K. Barbey[f], Adam J. Strang[g], Jennifer A. Martin[g]

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
H0V3

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
a UES Inc., AFRL, 711th HPW/RHXBC, 2510 Fifth Street, Area B, Bldg 840, Wright-Patterson AFB, OH 45433
b The Henry M. Jackson Foundation for the Advancement of Military Medicine, Air Force Research Laboratory, 711th HPW/RHXB, Wright-Patterson AFB, OH 45433
c Oak Ridge Institute of Science & Education, Air Force Research Laboratory, 711th Human Performance Wing/RHCP, Wright-Patterson AFB, OH 45433
d InfoSciTex Corp., AFRL, 711th HPW/RHCP, Wright-Patterson AFB, OH 4543
e Abbott Nutrition, Columbus, OH 43219
f Beckman Institute, University of Illinois, Urbana, IL 61801

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
G Air Force Materiel Command
Air Force Research Laboratory
711th Human Performance Wing
Airman Systems Directorate
Airman Bioengineering Division
Applied Biotechnology Branch
Wright-Patterson AFB, OH 45433

10. SPONSORING/MONITORING AGENCY ACRONYM(S)
711 HPW/RHBB

11. SPONSORING/MONITORING AGENCY REPORT NUMBER(S)
AFRL-RH-WP-JA-2019-0030

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution A: Approved for public release. 88ABW-2019-0963, cleared 12 March 2019

13. SUPPLEMENTARY NOTES
Journal of Chromatography B https://www.sciencedirect.com/science/article/pii/S1570023219307196

14. ABSTRACT
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15. SUBJECT TERMS
Metabolomics, HILIC, LC-MS, Exercise, Sample preparation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT:	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON (Monitor)
a. REPORT	b. ABSTRACT	c. THIS PAGE			Dr. Jennifer Martin
Unclassified	Unclassified	Unclassified	SAR	9	19b. TELEPHONE NUMBER *(Include Area Code)*

Journal of Chromatography B 1126–1127 (2019) 121763

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Metabolomic stability of exercise-induced sweat

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[a] UES Inc., Air Force Research Laboratory, 711th Human Performance Wing/RHXBC, 2510 Fifth Street, Area B, Building 840, Wright-Patterson AFB, OH 45433, USA
[b] The Henry M. Jackson Foundation for the Advancement of Military Medicine, Air Force Research Laboratory, 711th Human Performance Wing/RHXB, Wright-Patterson AFB, OH 45433, USA
[c] Oak Ridge Institute of Science & Education, Air Force Research Laboratory, 711th Human Performance Wing/RHCP, Wright-Patterson AFB, OH 45433, USA
[d] InfoSciTex Corp., Air Force Research Laboratory, 711th Human Performance Wing/RHCP, Wright-Patterson AFB, OH 45433, USA
[e] Abbott Nutrition, Columbus, OH 43219, USA
[f] Beckman Institute, University of Illinois, Urbana, IL 61801, USA
[g] Air Force Research Laboratory, 711th Human Performance Wing/RH, Wright-Patterson AFB, OH 45433, USA

ARTICLE INFO

Keywords:
Sweat
Metabolomics
HILIC
LC-MS
Exercise
Sample preparation

ABSTRACT

Due to increased interest in the use of excreted sweat for biomarker discovery, data must be generated supporting sample collection and handling methods to allow for controlled, large-scale biomarker discovery studies to be performed. In this manuscript, twelve amino acids were quantitated from exercise-induced excreted sweat held at room temperature or a simulated body temperature of 37 °C for up to 90 min. The data illustrate a large dynamic range exists among amino acids in sweat. Additionally, the amino acid quantities vary across individuals and among the same individual under different storage conditions, with alanine, arginine, and threonine showing a significant statistical difference between sampling events ($p < 0.05$). Furthermore, the results establish amino acids are relatively invariant, at both storage temperatures tested, for up to 90 min illustrated by < 10% (15/156) of the amino acids measurements demonstrating change greater than 10% from the time zero value. An untargeted metabolomics approach was also applied to the data set to evaluate global changes to the metabolome. The results show more than 88% of all data points fall within the established limits, regardless of temperature condition and ionization mode. Collectively, this study demonstrates that sweat is largely invariant at two distinct temperatures for up to 90 min. These results establish sweat collection and sample handling is possible for up to 90 min with minimal changes in metabolite abundances.

1. Introduction

Metabolomic biomarker discovery efforts have been dominated by blood-based sources, plasma and serum, to characterize a host of variables from cancer to performance [1–4]. However, as the general population becomes increasingly interested in real-time continuous personal heath monitoring, blood is not an ideal biosource to fit this need. Therefore, investigations of novel, non-invasive methods must be evaluated for excreted biological materials to accommodate this demand. However, sources of biomaterial that will allow for real-time continuous personal monitoring are few, such as sweat, exhaled breath, exhaled breath condensate, saliva, and urine. Of these biomaterials, sweat is the least problematic for on-demand sampling, analytical capabilities, and ultimately real-time sensing feasibility due to improved availability and continuous collection potential. As a result, sweat has been thrust into the research spotlight as a potential source of real-time personal biomonitoring.

Historically, sweat represents a minimally explored medium, relative to other biosources such as serum, for metabolomic biomarker discovery. Previous sweat metabolomic studies have evaluated the sweat metabolome by both NMR and mass spectrometry-based methodologies which established amino acids as the most commonly identified compounds in this medium [5–15]. Beyond identification alone, several sweat metabolomic biomarker discovery studies have also been

* Corresponding author at: Air Force Research Laboratory, 711th Human Performance Wing, Human Biosignatures Branch, 2510 Fifth Street, Area B, Bldg. 840, Wright-Patterson Air Force Base, OH 45433, USA.
E-mail address: sean.harshman.ctr@us.af.mil (S.W. Harshman).

https://doi.org/10.1016/j.jchromb.2019.121763
Received 7 May 2019; Received in revised form 12 July 2019; Accepted 8 August 2019
Available online 09 August 2019

performed across a wide array of diseases and conditions [7,8,11,16]. For instance, Macedon et al. showed differences in the untargeted sweat metabolome between infants affected by cystic fibrosis and those unaffected [7]. Furthermore, del Mar Delgado-Povedano et al. discerned specific sweat metabolite abundances between lung cancer patients and risk factor controls [8]. While these studies have provided the foundation for future sweat biomarker discovery, true biomarker discovery can only be accomplished if the factors influencing sample collection, sample handling, and data normalization are rigorously investigated.

Although blood analytes, such as lactate, have been linked to performance metrics, linking sweat analytes to human performance is in its infancy [1,2]. Due to the relative novelty, researchers have utilized a large heterogeneous number of sampling approaches and collection durations. For example, literature suggests sampling of sweat from individuals for 10 min to 211 min. However, it is unclear if metabolomic reactivity occurs over the duration of the collection time. To address this gap, sweat metabolomic stability was evaluated at simulated body temperature (37 °C) and room temperature (24.7 °C) using both targeted and untargeted metabolomic approaches. The results establish sweat is invariant for up to 90 min at either temperature for greater than 88% of the data points measured. This manuscript provides data in support of extended sampling protocols without impact to the sweat metabolome.

2. Experimental

2.1. Human participant information

All participants were male active duty military volunteers ($n = 4$) stationed at Wright-Patterson Air Force Base. Prior to initiation of the study, written permission was obtained from the Wright-Patterson AFB Institutional Review Board (IRB# FWR20150032H). Additionally, all participants were informed of the protocol and provided written informed consent.

2.2. Sweat induction protocol

Participants taking part in an unrelated exercise and nutrition intervention experiment were recruited for this study. In that experiment, a series of metabolic resistance circuits and cardiovascular exercise routines were prescribed (Monday through Friday) over a 12-week training intervention. For this study, researchers sampled sweat during two cardiovascular training routines within that larger study that had identical heart rate zone prescriptions. This allowed for the dual focus of examining room temperature (RT) stability (Training Session #1) and 37 °C stability (Training Session #2).

For these routines, subjects freely selected an exercise modality (i.e., stationary bike, treadmill, or elliptical) and then performed a bout of continuous cardiovascular exercise with intensity modulated by prescribed changes in percent (%) max heart rate (HR). Maximum HR was determined prior to the training intervention using a VO2 max running test using a Parvo Medics TrueOne 2400 metabolic cart (Polar Electro, Kempele, Finland, Parvo Medics, Sandy, UT, USA). Heart rate zones were warm up/cool down: 60–69% of max HR, easy: 70–79% of max HR, moderate: 80–89% of max HR, and hard: 90 + % of max HR. A summary of the 20-minute exercise protocol, heart rate zones, and each participant's VO2 max test results are provided in Supplemental Data 1.

2.3. Sweat collection & sample preparation

Prior to initiation of the exercise protocol, participant's forearms were prepared for sweat collection by wiping both inner forearms with sealed isopropyl alcohol wipes (one per arm, BD Biosciences, San Jose, CA, USA) followed by thoroughly rinsing 5–10 s with tap water. A modified version of a sweat patch initially described by Brisson et al., was placed on the center (wrist to elbow) of the each participant's air-dried forearms [17]. Briefly, the patch consisted of four pieces of heat-fused 100 μm nylon mesh (1.75″ × 2.5″, 4.45 cm × 6.35 cm) covered by virgin polyethylene film (2″ × 2.75″, 5.08 cm × 6.99 cm) (nylon mesh, Amazon, Seattle, WA, USA, polyethylene film, Fantapak, Livonia, MI, USA). The nylon mesh was placed on each participant's arm, creating a pouch under the polyethylene film, with a 4″ × 4.25″ (10.16 cm × 10.80 cm) piece of Opsite adhesive tape (Smith and Nephew, London, UK). A diagram and photo of the collection patch are provided in Supplemental Data 2.

Following exercise, free sweat within the patch was removed by aspiration via 5 mL syringe affixed with a blunt tipped needle (Hamilton, Reno, NV, USA). The aspirated sweat from each arm was placed in the top of separate Ultrafree-CL 0.1 μm PVDF membrane centrifugal filters (Millipore, Burlington, MA, USA). The nylon mesh within the patch was removed with forceps and added to the top of the membrane filters. One filter was used per arm sampled. Filters were centrifuged individually at 3000 × *g* for 10 min at 4 °C. The filtrates from each arm per subject were combined, briefly vortexed, and aliquoted into five 250 μL aliquots (except for Subject #5 of Training Session #2), where only two aliquots were obtained. These aliquots were assigned times 0 and 90.

A single aliquot was placed into liquid nitrogen (time zero), while the remaining aliquots were allowed to stand in closed LoBind Eppendorf tubes at room temperature (RT, 24.7 °C, Training Session #1) to mimic degradation attributed to sample handling, i.e. a sweat sample left on a lab bench, or 37 °C (Training Session #2) to mimic skin temperature found during sample collection, for 30, 60, or 90 min prior to liquid nitrogen submersion to determine if storage time had an effect on metabolite stability. The 30, 60, and 90-minute time points were selected to simulate short, mid-range and long exercise protocols. A diagram of the experimental design and a plot of the room temperature for Training Session #1 are provided in Supplemental Data 2. All aliquots were removed from liquid nitrogen, lyophilized (Labconco, Kansas City, MO, USA) overnight to dryness to allow for buffer exchange, and stored at −80 °C until analysis.

Lyophilized sweat samples ($n = 16$ for RT, $n = 14$ for 37 °C) were reconstituted in 250 μL of sample buffer which consisted of 50% acetonitrile aq. supplemented with 25 μmol isotopically-labeled Metabolomics Amino Acid Mix Standard (Optima® MS Grade, Fisher Scientific, Waltham, MA, USA, C^{13}/N^{15}, Cambridge Isotope Laboratories, Tewksbury, MA, USA). A pooled sample was generated by mixing 25 μL of each sample in a LoBind Eppendorf tube. Samples were put into vials and immediately run, in a randomized order, with blanks between each sample injection. Additionally, a pooled sample was run prior to and after sample injections with additional injections every 10 sample injections throughout the run.

Two collection blank samples were created by pipetting 1 mL of distilled water onto both the nylon mesh with the polyethylene film and Ultrafree-CL PVDF membrane centrifugal filter. All collection blank samples were left at room temperature for 20 min and centrifuged at 3000 × *g* for 10 min at 4 °C as described previously for sweat collection. 250 μL of the collection blank filtrates were placed into LoBind Eppendorf tubes, frozen in liquid nitrogen, and lyophilized to dryness. Collection blank samples were reconstituted in 250 μL of sample buffer, 50% acetonitrile (aq), and immediately run. The chromatograms of the blank sample injections are found in Supplemental Data 3.

2.4. Metabolomic hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS) analysis

The polar metabolites were separated on a Thermo Scientific Vanquish UPLC at 500 μL min^{-1} using 0.01 M ammonium formate in 4.5% acetonitrile (aq) at pH 3.0 for mobile phase A and 0.01 M ammonium formate in 95% acetonitrile (aq) at pH 3.0 as mobile phase B (≥ 99.0%, Sigma-Aldrich, St. Louis, MO, USA). A gradient of 97% B for 0–3 min, 65% B from 3 to 9 min, 50% B from 9 to 9.5 min, hold at 50%

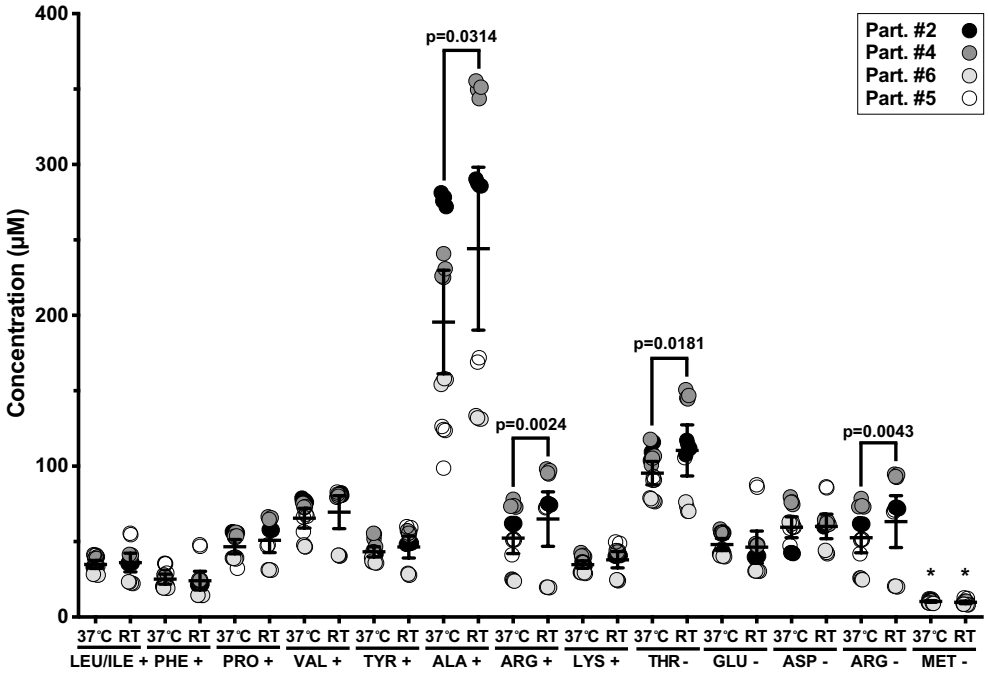

Fig. 1. A scatter plot of the 12 quantified amino acid values from all samples stored at 37 °C and room temperature (24.7 °C). The data show a large dynamic range among amino acid concentrations from excreted sweat, independent of storage temperature. Additionally, large variability among individual participants and within participants was also observed. Bars indicate the mean and 95% confidence interval. A paired Student's *t*-test was used to determine significance across storage temperatures. * indicates values are estimated due to data falling below the lower calibration point.

B for 1 min, return to 97% B over 1 min and held at 97% B for 10 min was used to separate the samples on a Phenomenex Luna® HILIC 3 μm, 200 Å, 100 × 3 mm column held at 40 °C (Torrance, CA, USA). 2 μL injections were introduced into a Thermo Scientific Q Exactive HF mass spectrometer affixed with a heated electrospray ionization source setup for polarity switching. For both positive and negative mode, the source was operated at 4.0 kV, sheath gas 25, aux gas 10, and a capillary temperature of 300 °C. Scans were made at 15,000 resolution across 75–300 *m/z* in positive mode and 113–300 *m/z* in negative mode.

2.5. Amino acid calibration curve and quantitation

Duplicate calibration curves were generated for each training session analysis utilizing the Thermo Fisher Amino Acid Standard H mix containing 18 amino acids. Briefly, 0 μM, 10 μM, 20 μM, 40 μM, 100 μM, 200 μM, and 500 μM of each PHE, LEU/ILE, PRO, MET, VAL, TYR, THR, ALA, GLU, ASP, ARG, & LYS from Amino Acid Standard H were diluted in sample buffer. Each amino acid calibration curve was analyzed prior to and following sample acquisition. Additionally, a 40 μM amino acid standard was analyzed following each pooled sample as described above.

The determination of MS^1 ion peak areas for the amino acid calibration and quantitation were performed in the Tracefinder EFS software suite (v. 3.2, Thermo Scientific). Calibration curves were generated for 12 amino acids by plotting the C^{13}/N^{15} normalized amino acid peak areas (y-axis) by the theoretical concentration (10 μM–500 μM, x-axis). The retention times, quantitative ions, calibration curve results summary, and a pooled/40 μM standard sample results summary are provided in Supplemental Data 4 & 5. Unknown amino acid concentrations from sweat samples were determined by calculating the C^{13}/N^{15} normalized amino acid peak areas from sweat samples and comparing them to the individual amino acid calibration curves. All statistical analyses on quantitated amino acid results were performed in the Prism GraphPad software suite (v.8.0.0, LaJolla, CA, USA).

2.6. Untargeted metabolomic data processing and statistical analysis

Individual positive and negative mzXML files were extracted from the original raw data files using the MSConvert command line tool as part of the ProteoWizard software package (v. 2.1.x). Positive and negative mzXML files were uploaded as separate batches to the XCMS Online software suite (v. 2.3.0) and processed for retention time alignment and feature detection [18–25]. The MSConvert and XCMS settings are provided in Supplemental Data 6. The XCMS software produced 2001 (RT) and 830 (37 °C) features from positive mode data and 1908 (RT) and 1784 (37 °C) features from negative mode data. The data were filtered to retain only the features that met the following criteria: retention time (RT): ≤10.0 min; $m/z \leq 206$; and mean abundance of each individual feature for each pooled sample greater than or equal to the mean of the overall pooled feature abundance. Data was further reduced by manually filtering the features list by removing features corresponding to noise and those pertaining to C^{13}/N^{15} labeled amino acids added as part of the sample buffer. The final data set was a result of retaining features with a ≤10.0% RSD of the repeated pooled sample injections ($n = 4$). The filter parameters yielded 52 positive and 28 negative features from the room temperature data set and 38 positive and 32 negative features from the 37 °C data set. The filter criteria were selected and applied to remove features corresponding to background noise, added standards, and those with large instrumental drift over the analysis. Resulting feature abundances were normalized to the time zero sample abundance for each participant and feature and then $\log_2$ transformed. Time zero normalization was performed to determine the $\log_2$ fold change in metabolite abundance from time zero which was considered the best-case collection scenario. Transformed data was plotted and analyzed using the Prism GraphPad software (LaJolla, CA, USA).

The features removed from the data set corresponding to the C^{13}/N^{15} labeled amino acids were plotted separately to determine their variances (Supplemental Data 7). As these compounds were internal standards spiked into each sample, the abundances of the C^{13}/N^{15} labeled amino acids should be invariant across each sample and represent the variability in each injection. The two and three standard deviations of the C^{13}/N^{15} labeled amino acids were calculated and applied to the overall features list to establish limits for stability over time.

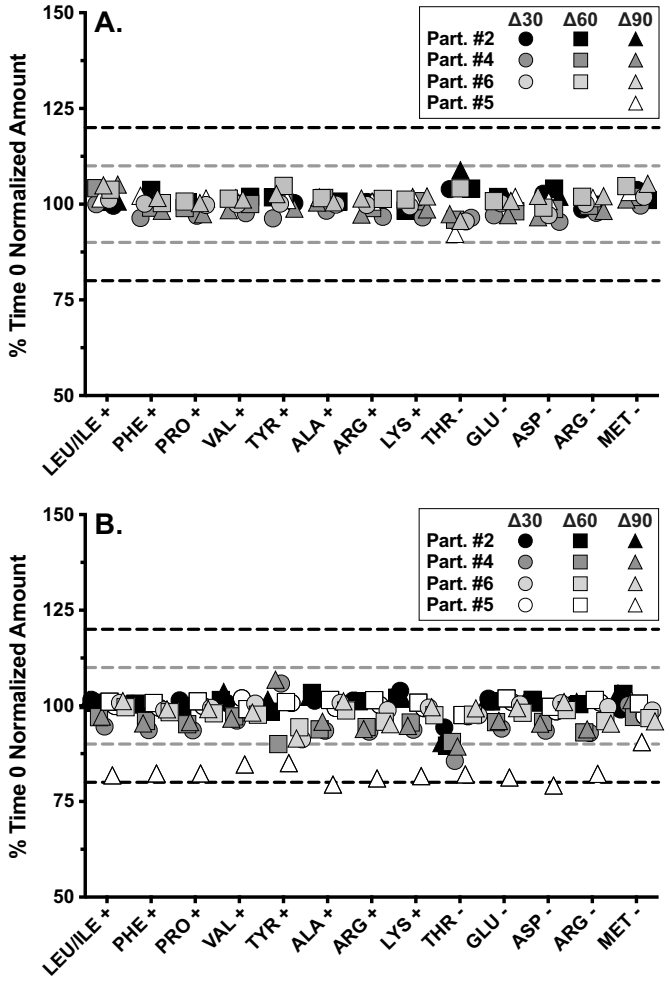

Fig. 2. A plot of the quantitated 13 amino acids normalized to time 0 among stored at A) 37 °C and B) room temperature. The data illustrate little change in amino acid quantity relative to time zero for up to 90 min at either 37 °C or room temperature (24.7 °C). Gray dashed lines indicate 10% deviation and the black dashed lines indicate 20% deviation from 100% of time 0.

3. Results

3.1. Sweat stability: amino acid quantitation

As previous untargeted metabolomic studies have established amino acids as the primary metabolites identified in excreted sweat, these compounds represent a significant group of compounds for potential biomarker discovery efforts from this medium [5–15]. As such, accurately determining the concentration of amino acids and changes resulting from sample collection length (simulated body temperature of 37 °C) and sample handling (room temperature 24.7 °C) is of utmost importance. To address this gap, multiple amino acids (12) were quantified from excreted sweat allowed to stand at 37 °C or room temperature for up to 90 min to establish the stability of this routinely found group of compounds. A scatter plot of all of the quantified values illustrates a large dynamic range in concentration of amino acids is observed in excreted sweat independent of storage condition (Fig. 1). For example, the amino acid alanine is the most abundant amino acid with overall values ranging from 99 μM to 355 μM while a majority of the remaining amino acids are below 100 μM concentration at either storage condition. Additionally, these data show a substantial difference among participants' amino acid concentrations (Fig. 1). For instance, participant #4 had increased threonine (145 μM to 151 μM) at 37 °C storage while participant #6 produced lower concentrations of threonine (76 μM to 79 μM) at the same storage temperature. The data show a significant difference in sweat alanine, arginine, and threonine amounts, based on storage condition, within specific individuals (paired *t*-test, $p < 0.05$, Fig. 1). Collectively, the results illustrate the differences in concentrations attributed to not only the analyte in excreted sweat but also the individual participant from whom the sweat was collected.

As exercise-induced sweat collection requires lengthy periods of time, often from 15 to 90 min, sweat has the potential to react at the sampling location (37 °C) or during sample handling (RT, 24.7 °C). These environmental conditions were simulated on the bench following each training session, to determine the changes of amino acid concentrations due to temperature for up to 90 min. As immediate harvesting and flash freezing of sweat was considered the best-case scenario for minimal reactivity, all quantified amino acid values were normalized to the time zero concentration. Fig. 2A & B show the amino acids in sweat are extremely stable with 90.4% (15/156) of the time 0 normalized values within 10% of the time zero amount at either storage condition. Furthermore, 9 of the 15 measurements that did not fall within 10% of the time zero amount can be attributed to Participant #5's Δ90 minute samples. It is hypothesized that this sample may have had a slight sample handling error or a small injection volume error. Regardless of this potential error, these data illustrate the measured amino acids are stable under simulated collection and lengthy sample handling conditions for up to 90 min. While the experiment was performed on a benchtop and skin was not a factor, the results support sampling strategies that extend up to 90 min with minimal impact to the amino acid content.

3.2. Sweat stability: untargeted metabolomics

Fig. 2A & B suggest amino acids are stable in excreted sweat at 37 °C and RT (24.7 °C) for up to 90 min. However, amino acids are only a portion of the metabolites identified from sweat potentially affected by the simulated conditions [5–15]. To identify global metabolite changes of excreted sweat under the simulated conditions, the dataset was further processed for an untargeted metabolomics approach in both positive and negative ionization modes. Fig. 3 shows the time zero $\log_2$ fold change of filtered features in positive mode (Fig. 3A) and negative mode (Fig. 3B) for samples placed at elevated temperature (37 °C). Due to sample volume constraints with Participant #5, $n = 3$ was obtained for the 30 and 60 min time points and $n = 4$ for the 90 min time point. The data illustrate a small number of features have a time zero $\log_2$ fold change outside 3 standard deviations of C^{13}/N^{15} labeled standards when stored at 37 °C for up to 90 min. For example, of the 380 total data points in positive mode, 97.9% (372/380) of the points fall within three standard deviations of the features corresponding to the C^{13}/N^{15} labeled standard and 97.6% (371/380) fall within two standard deviations (Fig. 3A). As the C^{13}/N^{15} labeled standard was spiked into each sample for the amino acid quantitation, the variability of these features should be representative of the analysis and were used to establish limits for the unknown features (Supplemental Data 7). Similar to the positive mode data, 98.4% (315/320) of the data points are within three standard deviations and 97.2% (311/320) are within two standard deviations of the C^{13}/N^{15} labeled standards when acquired in negative ionization mode (Fig. 3B). Overall, the data points in either ionization mode that do not meet two or three standard deviations of C^{13}/N^{15} labeled standard criterion are representative of seven individual features, suggesting 90.0% (63/70) of features are stable over the entire 90-minute experiment at 37 °C. These results illustrate sweat metabolite stability for up to 90 min at simulated body temperatures, further supporting sweat sampling strategies that require lengthy collection durations, up to 90 min.

To estimate the impact of sample handling, a global metabolomics approach was applied to the dataset stored at room temperature (24.7 °C). Fig. 4 shows a plot of the filtered time zero $\log_2$ fold change of

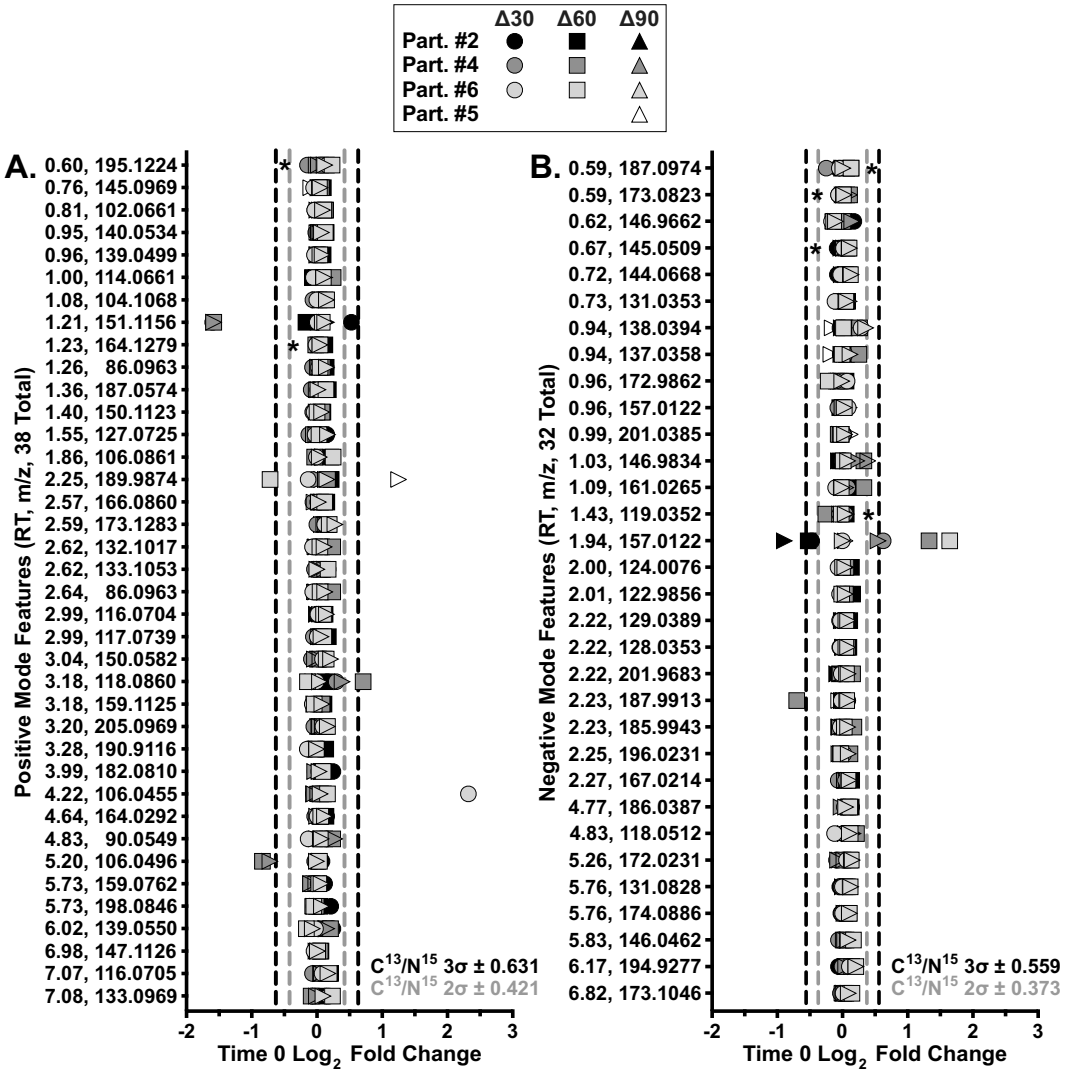

Fig. 3. A plot of the individual metabolite filtered feature abundances (time zero normalized log_2 transformed) from samples placed at 37 °C for 30, 60, and 90 min in A) positive ionization mode and B) negative ionization mode. Lines represent two (gray) and three (black) standard deviations of features corresponding to the C^{13}/N^{15} labeled amino acid standards. The data demonstrate a low change in feature abundance, relative to time zero; 90% of all feature measurements are within the three standard deviation limits for times up to 90 min at 37 °C. * represents features attributed to blank injections.

features acquired in positive (Fig. 4A) and negative ionization modes (Fig. 4B) from room temperature stored sweat. The data show, in positive mode, 96.8% of data points (604/624) fall within the more stringent two standard deviations of the C^{13}/N^{15} labeled standard. These results represent 13 individual features suggesting 75% of features (39/52) do not largely deviate when stored up to 90 min at room temperature in positive ionization mode (Fig. 4A). Of these data, 8 data points that do not fall within two standard deviations of the C^{13}/N^{15} labeled standard and 6 data points that do not fall within three standard deviations of the C^{13}/N^{15} labeled standard criteria can be attributed to our sample preparation technique, suggesting sweat feature stability may ultimately be greater than 96.8% of the data points (Fig. 4A). While positive mode illustrates many features well within the established limits, data acquired in negative ionization mode from the same room temperature experiment show more data points outside the limits (Fig. 4B). For example, of the 336 data points 37 (11.0%) do not meet the three standard deviations of the C^{13}/N^{15} labeled standard limits in this ionization mode. Of these data points, only 7 features have all the corresponding data points within the 3 standard deviation limits (Fig. 4B). The greater number of features outside the established limits in negative ionization mode seems to arise from more stringent limits. For instance, the standard deviation limits in positive mode for the room temperature experiment are more than twice the limits of negative mode for the same experiment (± 0.673 in positive, ± 0.293 in negative). Furthermore, although the negative mode RT data has values outside the limits, only four data points have a greater than two-fold change, i.e., log_2 fold change greater than one or less than negative one. These results suggest excreted sweat is stable when stored at room temperature for up to 90 min although more divergence is observed in the negative mode data. Collectively, these data illustrate sweat has minimal reactivity and changes to the global metabolome when stored at elevated or room temperatures for up to 90 min.

4. Discussion

As the medical, athletic, and civilian communities push toward more real-time, on-demand, personalized performance monitoring, novel non-invasive biological materials must be explored to fit this need. Additionally, from these novel sources, new markers of physiological, biological, and cognitive states must be evaluated. While sweat provides a unique and non-invasive source to meet these goals, many basic experiments must be performed prior to large scale discovery efforts using excreted sweat. For example, the literature has reports of sweat collection times ranging from 10 min to 211 min [6,9,26].

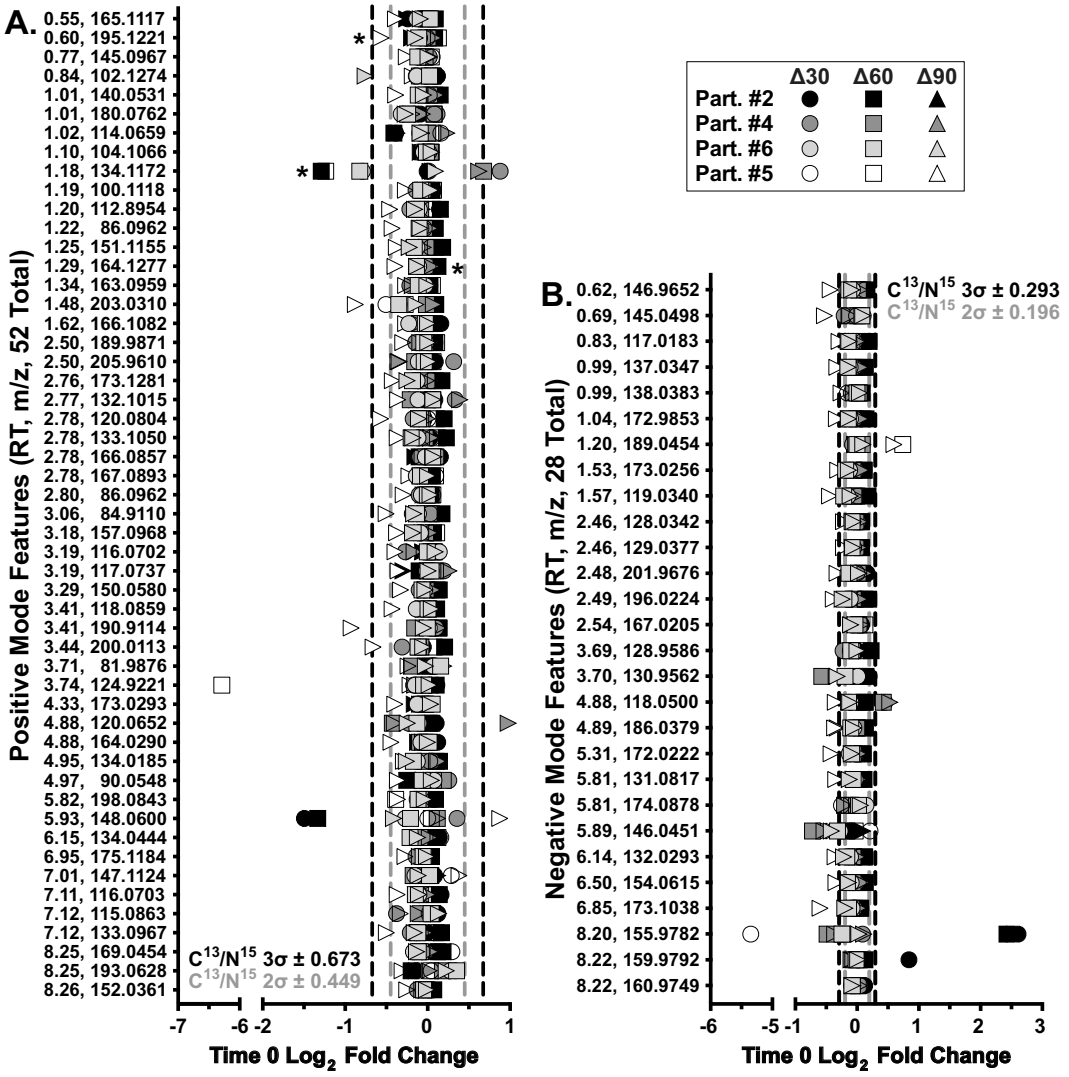

Fig. 4. A plot of the individual metabolite filtered feature abundances (time zero normalized $\log_2$ transformed) from samples placed at room temperature (24.7 °C) for 30, 60, and 90 min detected in A) positive ionization mode and B) negative ionization mode. Lines represent two (gray) and three (black) standard deviations of features corresponding to the C^{13}/N^{15} labeled amino acid standards. The data illustrate a low change in feature abundance, relative to time zero; 75% of all feature measurements in positive mode meeting two standard deviations of the C^{13}/N^{15} labeled standard limits for lengths up to 90 min at room temperature. However in negative ionization mode, only seven features have all measurements within three standard deviations of the C^{13}/N^{15} labeled standard. * represents features attributed to blank injections.

However, it is unclear if there is metabolomic reactivity during the collection period. To address this gap, data was provided to establish the stability of sweat at elevated temperature to simulate reactivity during collection, and at room temperature to simulate sample handling. Overall, the results illustrate sweat is unreactive at both storage conditions for up to 90 min, suggesting extended collection times can be used without degradation of the sample. Lengthy collection times will allow for many interesting studies such as generating bulk sweat for multiple analyses from a single sample or time- course samples over the duration of a long exercise, pushing discovery within this medium to new levels.

While this study has addressed a gap in sample collection and handling, many gaps still persist. First, this study utilizes a small group of participants to demonstrate sweat stability. Ideally a greater number of diverse participants would benefit the study. However due to limitations outside our control, participants were limited both in number and to male active duty military members. Therefore, children, the elderly, and female participants were not included in this study. While the literature suggests potential differences in sweat analyte abundance among these groups, it is not anticipated that sweat would illustrate a high rate of metabolomic degradation, but these experiments still need to be completed [27]. Next, the data presented in Fig. 1 show variability among individuals and also within an individual with different storage conditions. While stored at different temperatures, it is hypothesized that the differences among individuals observed between the two sampling events shown in Fig. 1, is a result of the variable physiological state of the individual on any given day i.e. sweat rate, hydration status, etc., rather than due to storage condition. However, additional experimentation is required to accurately evaluate this hypothesis. Although methods exist to accurately estimate sweat rate, such as the whole body wash down method, these methods evaluate global sweat rates of an individual [28]. As sweat rates at specific places on the body are not uniform, localized sweat rate at the site of collection needs to be accurately measured [29]. However, this determination has proven difficult [9]. Recent advances in wearable technologies may hold the key for this long sought-after measurement [30,31]. Finally, beyond localized sweat rate, other considerations must be explored. For example, while it was recently shown that the sweat metabolome is similar when collected from stimulation with pilocarpine, heat, or exercise, it remains unclear if metabolomic stability is affected by these

other stimulation methods [14]. Additionally, it is unknown what impact hygiene and bacteria on the skin surface have on sweat analyte content. Furthermore, questions still persist regarding impact of diet on sweat metabolomic content. These experiments must be addressed for sweat to become a viable means of monitoring human performance.

Finally, as other fields have realized, field-wide standardized collection procedures are critical for comparison of data across labs and, ultimately, true biomarker discovery. For example, elevated exhaled nitric oxide (NO) has been well characterized in asthmatics resulting in the American Thoracic Society establishing standardized sampling procedures for NO [32]. Standardized sampling has allowed for utilization of this marker of treatment response within this clinical group [33]. Without defined sample collection parameters, it would be nearly impossible for widespread use of NO as a non-invasive biomarker in asthma. A similar approach must be adopted to use sweat for biomarker discovery. For instance, the literature supports more than seven different sweat collection methods, from skin pouches to catching drips, and at least four different approaches, mostly centered around gravimetric methods, to estimate sweat rate [5–12,14,16,26–28,34–49]. This large diversity can only confound results among labs. As sweat continues to progress for biomarker discovery, such issues must be experimentally evaluated and standardized across all facets of sample collection.

5. Conclusions

While correlations of blood metabolites to human performance have been shown, unpacking a similar link between sweat analytes and performance has proven difficult. Correlations between sweat analytes and human performance can only be accomplished if the factors influencing sample collection and handling are investigated in a rigorous manner. In this manuscript, sample handling at two distanced temperatures was investigated by LC-MS. The data demonstrates, by targeted analysis, a large dynamic range exists among amino acids from excreted sweat. Furthermore, the results show the variability among individuals and within an individual, supporting the need for accurate, localized measurement of sweat rate. An untargeted metabolomics approach demonstrates minimal metabolomic reactivity of excreted sweat at two distinct temperatures. Overall these data give support for longer sample collection protocols and room temperature sample handling without detrimental effects to samples, expanding the possibilities of biomarker discovery from this biological medium.

Declaration of competing interest

The authors have no competing interests. Opinions, interpretations, conclusions and recommendations are those of the authors and not necessarily endorsed by the United States Government.

Acknowledgements

The authors would like to thank Ms. Christina Davidson, Mr. Gregory Sudberry, Ms. Tenika Dearmond, Ms. Heather Lyons, and Ms. Stephanie Ambler for their help and scholarly input for this manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2019.121763.

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