Journal of Breath Research

PURPOSE-LED PUBLISHING™

NOTE • **OPEN ACCESS**

The human breath volatilome responds to exercise and recovery: an untargeted profiling study

To cite this article: Mostafa Hashemi *et al* 2026 *J. Breath Res.* **20** 041001

View the article online for updates and enhancements.

You may also like

- Metabolic insights at the finish line: deciphering physiological changes in ultramarathon runners through breath VOC analysis
Hsuan Chou, Kayleigh Arthur, Elen Shaw et al.
- Factors that influence the volatile organic compound content in human breath
L Blanchet, A Smolinska, A Baranska et al.
- Food and lifestyle impact on breath VOCs using portable mass spectrometer—pilot study across European countries
Milena Aleksi, Andrea Simeon, Djordje Vuji et al.





GAS

BreathSpec®

The combination of GC and IMS enables a physical separation to detect volatiles without pre-concentration directly sampled from human breath.

Our GC-IMS based analyzer allows instant breath sampling and analysis of volatiles in minutes.

The transportable GC-IMS facilitates versatile sampling incl. direct exhalation, syringe based and also gas bags for sampling of breath and static body headspace (oral/nasal/skin).

▶▶▶ click for more details

This content was downloaded from IP address 192.167.210.124 on 03/11/2026 at 15:18

Journal of Breath Research

Check for updates

OPEN ACCESS

RECEIVED
25 March 2026

REVISED
28 August 2026

ACCEPTED FOR PUBLICATION
17 September 2026

PUBLISHED
1 October 2026

Original content from this work may be used under the terms of the Creative Commons Attribution 4.0 licence.

Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

NOTE

The human breath volatilome responds to exercise and recovery: an untargeted profiling study

Mostafa Hashemi[1], **Marek Svoboda**[2], **Flavio A Franchina**[3] and **Jane E Hill**[1,4,*]

[1] Department of Chemical and Biological Engineering, University of British Columbia, Vancouver, BC, Canada
[2] Department of Genetics and Genomic Sciences, Mount Sinai Hospital, New York, NY, United States of America
[3] Department of Chemical, Pharmaceutical, and Agricultural Sciences, University of Ferrara, Ferrara, Italy
[4] School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada
[*] Author to whom any correspondence should be addressed.

E-mail: jane.hill@ubc.ca

Keywords: exhaled breath analysis, volatilomics, exercise metabolism, untargeted metabolomics, physical activity, GC×GC-TOFMS

Supplementary material for this article is available online

Abstract

Exercise induces metabolic and physiological changes across multiple organs. These changes have been studied via several human biofluids, including urine and blood; however, they remain mainly underexplored in exhaled breath. In this exploratory pilot study, we performed untargeted profiling of breath volatile compounds (VCs) to investigate how exercise and recovery influence breath chemical composition. Breath samples were collected from 71 university athletes from three sporting disciplines. Across 143 breath samples, a total of 1204 unique breath VCs were detected. There were distinct volatilomic responses to physical activity and recovery, regardless of the athlete's sport. Comparison of paired samples using volcano analysis collected before and during exercise identified 80 breath VCs that significantly increased and 182 that significantly decreased. Similarly, a comparison of samples collected before and after exercise identified 11 compounds that decreased significantly. These findings demonstrate that exercise induces measurable changes in the breath volatilome. However, due to the lack of standardized exercise intensity measures and physiological monitoring, the results should be considered exploratory and interpreted cautiously within the field of exercise science.

1. Introduction

Exercise triggers widespread changes throughout the body, causing rapid demands on multiple physiological and metabolic systems [1]. During physical activity, whole-body energy use rises sharply, reflected in increased oxygen consumption and glucose utilization, which causes a series of metabolic challenges for the body [2]. For example, inside working muscles, adenosine triphosphate (ATP) is broken down at rates far higher than at rest, requiring ATP-producing pathways to increase to maintain energy balance [3]. Exercise also induces hormonal shifts to support cardiovascular function, mobilize fuels, and regulate blood flow [3]. In addition, both resistance and endurance exercise influence muscle protein damage and also, post-exercise, muscle repair and synthesis [3]. These metabolic shifts can be detected through the analysis of a range of biological samples, including serum [4–10], plasma [3, 11–18], saliva [19, 20], and urine [21–23]. Untargeted metabolomic analyses of these biosamples extensively mapped exercise-induced changes, highlighting shifts in glycolysis, the tricarboxylic acid cycle, lipid oxidation, amino acid turnover, and oxidative stress. These comprehensive profiles link metabolic alterations to physiological adaptation, fatigue, recovery, and individual fitness. However, these biosamples are either invasive to obtain or difficult to collect repeatedly, highlighting the need for more accessible sampling methods.

The analysis of volatile compounds (VCs) in exhaled breath has the potential to be a powerful, non-invasive, and unlimited sample source to monitor processes in the body [24, 25]. With less than a minute required for a whole cycle of blood circulation [26] VCs generated by various body parts, airway

© 2026 The Author(s). Published by IOP Publishing Ltd

and gut microbiota, dietary inputs, and environmental exposures can diffuse into the bloodstream and be exhaled via the breath [27]. There are estimated to be approximately 1500 VCs in the exhaled breath of healthy individuals [28]. Changes in the concentration or presence of breath VCs can offer valuable insights into internal metabolic activity and external influences [29].

Despite many studies using other biofluids, exhaled breath analysis remains underused in exercise research [30–40]. Several studies have targeted specific breath VCs, including acetone, isoprene, dimethyl sulfide, and acetaldehyde in the context of exercise, providing a narrow perspective on the broader breath volatilome [30–37]. Untargeted studies examining broader changes in the breath volatilome are even more limited. Heaney and colleagues used a non-targeted approach to examine changes in exhaled VCs in physically active men undergoing a graded maximal exercise test, identifying isoprene as dynamic in response to exercise [38]. Untargeted approaches have also been applied to prolonged endurance exercise. Chou and colleagues studied ultramarathon runners who completed a 174 km trail running competition, identifying 63 breath molecules that significantly altered between breath samples taken 24–72 h before and 1–4 h after the race [39]. Henderson and colleagues examined breath taken during a 30–50 km walk over three consecutive days of repeated field walking in a cohort including both statin users and non-users, identifying notable shifts in nine VCs and also evaluating statin use alongside exercise [40]. While these studies demonstrate that exercise-associated changes can be detected in the breath volatilome, relatively little is known about responses to shorter-duration training involving activities such as drills, sprints, and plyometrics.

In this exploratory, pilot study, we aimed to profile and compare overall breath composition before, during, and after short-term exercise (<2 h) in a cohort of university-aged athletes during pre-season training.

2. Materials and methods

2.1. Study participants

Seventy-one university undergraduate students from Dartmouth College in the tier 2 athletic [41] group were recruited for this study. Tier 2 indicates that subjects regularly trained and participated in organized sport. The study was approved by the Dartmouth Committee for the Protection of Human Subjects (STUDY00030484). Subjects consented to study participation. Participants engaged in pre-season training for either American football, ice hockey, or rugby union. The training sessions sampled in this study took place on the sporting field or, for ice hockey, at the rink, rather than in the weight room or during resistance training sessions. The specific activities and exercise intensity varied by sport and individual athlete and were not directly measured or standardized [42]. Baseline fitness level was also not controlled or assessed. Breath samples were collected between September and December 2017. The athletes' fasting status is unknown. The first breath sample was collected from athletes before beginning exercise (aka, 'pre-exercise'). The second sample was collected during the exercise session, following approximately 90 min of physical activity (aka, 'during-exercise'). The third sample was collected approximately two hours after completing the exercise (aka, 'post-exercise'). Paired pre- and post-exercise samples were collected from male subjects training for American football and ice hockey, while paired pre- and during-exercise samples were collected from male and female rugby union athletes and male American football athletes. The age, sex distribution, number of samples per group, and primary sport played by the athlete are summarized in table 1.

2.2. Breath sample collection

Breath collection was performed using a mixed expiratory, fixed-volume sampling method following the guidelines of the European Respiratory Society [43]. Before collecting breath, subjects rinsed their mouth with water [44]. Breath samples were collected using one-liter Tedlar bags (SKC Inc., PA, USA). Prior to use in breath sampling, each Tedlar bag was conditioned by flushing three times with nitrogen gas with incubation periods of 30 min after each nitrogen gas fill. Subjects exhaled normally into the preconditioned bags through a disposable mouthpiece (Intersurgical Ltd., Wokingham, UK) until the bags filled around 80%–90%, without force-inflation, corresponding to when subjects encountered increased resistance. Less than one hour after breath collection, the contents of each Tedlar bag were transferred through a 13 mm, 0.22 μm PTFE filter (Avantor, Inc., PA, USA) onto a pre-conditioned 3-bed thermal desorption tube (TDT; Carbopack™ B and X, and Carboxen™ 1000; Gerstel, Linthicum, MD, USA) using a vacuum pump (SKC Inc., PA, USA) operated at a calibrated flow rate of 150 ml min^{-1} for 6 min. The pump flow rate was verified and calibrated using an external gas flowmeter after every 100 uses. Pre-conditioning of TDTs involved using a tubeconditioner 2 device (Gerstel Inc., Linthicum, Maryland, USA), in which the temperature was increased from 50 °C–350 °C over 30 min, maintained at 350 °C for 120 min, and then cooled to 50 °C before removal. Before sampling, a portion of 20% of the tubes was verified by a two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) system (LECO Corp., MI, USA), ensuring the absence of carryover and proper cleanliness. Immediately after

Table 1. Participant demographics, sport involvement, and breath sample collection summary.

Category	Details
Number of Subjects	71
Median Age (range), years	20 (17–22)
Sex (Female:Male)	22:49
Sport participation (female:male)	Rugby Union (22:10)
	American Football (0:24)
	Ice Hockey (0:15)
Total Samples Collected	143
Pre-exercise	71
During-exercise	56
Post-exercise	16

sample transfer, the TDTs were capped, placed individually within conical tubes, and transported in a Styrofoam cooler with ice packs. The tubes were then stored at 4 °C, with storage temperature monitored using a temperature logger, for an average of 2.5 months until analysis [45, 46].

2.3. Sample analysis

TDTs containing breath samples were moved automatically using a multi-purpose sampler (Gerstel Inc., MD, USA) into the thermal desorption unit (TDU). In the TDU, the temperature was raised to 330 °C at the rate of 5 °C s^{-1} to desorb molecules from TDTs. Desorbed molecules were then concentrated into a cold injection system at −120 °C before being heated to 270 °C at the rate of 12 °C s^{-1} for transfer onto the chromatographic columns. Concentrated samples were analyzed using a GC × GC-TOFMS system. The column combination in GC × GC was an SLB®-5 ms (30 m × 0.25 mm; 0.25 μm film thickness) followed by an SLB®-50 ms (1.27 m × 0.25 mm; 0.25 μm film thickness) (Supelco Inc., PA, USA), using helium as the carrier gas with the flow rate of 1.0 ml min^{-1}. A modulation period of 2.5 s was applied, consisting of a 0.85 s hot pulse and a 0.40 s cold time. TOFMS spectra were collected over an *m/z* range of 40–400 at an acquisition rate of 150 Hz. Instrument performance was monitored daily by analyzing a quality control standard mixture (Programmed Test Mix, Supelco Inc., PA, USA).

2.4. Chromatographic data processing

Chromatographic data were processed and aligned using ChromaTOF Tile Software v1.2.6.0 (LECO Corp., MI, USA). Samples were grouped as pre-, during-, and post-exercise. Peak detection was performed using a 6-modulation by 60-spectra tile size, with no F-ratio threshold applied. A signal-to-noise (S/N) ratio threshold of 50:1 in at least three samples was required for peak detection.

Initial putative peak identification was performed using the National Institute of Standards and Technology 2011 library. Metabolite annotation followed the Metabolomics Standards Initiative (MSI) framework for levels 2–4 identifications [47]. As the first step, peaks were visually verified, and those that appeared primarily as tailing from a main peak and could not be considered distinct were excluded. Visually verified peaks were assigned a putative name (MSI level 2) if both the forward and reverse similarity scores were greater than 850 and the match probability exceeded 70% [47]. For peaks with similarity scores between 700 and 850, a chemical formula was assigned (MSI level 3) if the top four library hits shared the same formula. If scores ranged between 600 and 700, a chemical class was assigned based on consistency across the top four hits. Peaks that did not meet any of these criteria were labeled as unknown (MSI level 4) [47]. Because this was an untargeted profiling study, we did not determine compound-specific limits of detection or quantification, and we used peak areas as relative measures of signal abundance rather than absolute concentrations.

2.5. Statistical data analysis

We performed statistical analysis using R version 4.3.2 (R Core Team, Vienna, Austria). As the first step in statistical analysis, we removed contaminant peaks from the sampling and separation systems. These peaks were identified based on their association with column coatings, laboratory plastics, and common solvents or cleaning agents used in the labs [48]. The remaining peaks were considered part of the pan-volatilome and were grouped into three categories based on how frequently they appeared across samples. Peaks detected in all samples were classified as core. Peaks found in less than 10% of samples were labeled rare. Peaks appearing in more than 10% but not all samples were considered accessory. Missing intensities for features imputed with zero. Peak intensities were normalized using probabilistic quotient normalization to reduce sample-wide multiplicative differences in signal intensity [49], followed by $\log_{10}$ transformation and mean centering [48]. A two-dimensional principal component analysis (PCA) score plot was used to visualize the samples. Significantly different peaks were identified with

a volcano plot approach [50]. Intensities were compared between pre- and during exercise and pre- and post-exercise samples using a two-sided unpaired *t*-test [51]. Log_2 fold change (log_2FC) was calculated as log_2(mean Group 2/mean Group 1). Peaks with a *p*-value < 0.05 and a $|log_2FC| \geqslant 0.6$ ($\sim$1.5-fold [52]) were considered significantly changing.

3. Results and discussion

3.1. Pan-volatilome overview and distribution of identified chemical classes

Across all breath samples, we identified 1204 unique breath features constituting an athlete's pan volatilome. Initial library matching allowed us to assign names to 741 (62%) breath features. Chemical class identification of these breath features revealed that aromatic hydrocarbons, alcohols, and carboxylic acids were the most abundant classes, together accounting for over 70% of all annotated compounds (figure 1(a)). In particular, hydrocarbons account for around 30% of the breath volatilome. The hydrocarbons were dominated by aromatic compounds (26%), with smaller contributions from alkanes (2%) and alkenes (2%). This distribution is consistent with breath volatilome reviews, which also report hydrocarbons as the predominant VC class in healthy human breath [28]. The pan volatilome consisted of 517 (43%) core breath features, 633 (53%) accessory breath features, and 54 (4%) rare breath features. The core breath features (figure S-1) were primarily composed of alcohols (33%) and aromatic hydrocarbons (27%), followed by carboxylic acids (14%). Philips and colleagues reported 27 compounds consistently present across 50 healthy adults [53], as well as 585 VCs detected in more than 80% of samples from 90 healthy participants [54]. Among our 517 core features, 84 features exhibited low variability (variance below the 10th percentile across all groups), minimal fold change ($<$1.1), and non-significant *p*-values ($>$0.05). These 84 breath features were predominantly alcohols (71%), alkenes (15%), and carboxylic acids (7.0%) (figure S-1).

3.2. Exercise-induced shifts in breath volatile composition and chemical class distribution

In a two-dimensional PCA plot capturing 61% of the variance, we observed a clear separation between pre-, during-, and post-exercise breath samples (figure 1(b)). Separate paired PCA analyses (figure S-2), supported by linear mixed-effects models, indicated that the dominant separation was primarily associated with sampling timepoint. Timepoint significantly influenced PC2 in the pre vs during comparison and PC1 in the pre vs post comparison (both $p < 0.0001$), while subject-level variance on these components was near zero. Among the 741 breath features with putatively identified chemical classes (figure 1(c)), levels of aldehydes, ketones, alkanes, ethers, and alcohols remained relatively consistent with less than 10% change between pre- and during-exercise breath samples. Carboxylic acids, aromatic hydrocarbons, alkenes, and esters, however, varied by more than 10% in relative class intensity. Similar findings have been reported in studies using other biosamples including urine [55, 56], plasma [57], and skeletal muscle extracts [58], and across different exercise modalities, where clear differences between pre- and post-exercise metabolic profiles were consistently observed [39, 40].

3.3. Identification of key breath molecules significantly altered during- and post-exercise

Given the differences observed between the breath profile groups, we further examined the breath features that showed significant changes during- and post-exercise compared to the pre-exercise condition.

3.3.1. Significant changes during-exercise (vs pre-exercise)

We analyzed 56 pairs of breath samples collected pre- and during-exercise from subjects training for rugby union or American football. From those, 80 breath features were found to be significantly higher and 182 that significantly lower in the during-exercise compared to the pre-exercise condition (figure 2(a), table S-1). The remaining breath features did not show significant changes. Compounds in the breath at a higher amount during-exercise were predominantly carboxylic acids (74%), followed by alcohols (11%) and ketones (7.0%). In contrast, 49% of decreasing compounds were aromatic hydrocarbons, 22% carboxylic acids, 10% esters, and 8.0% alcohols. Consistent shifts in these chemical classes were observed in normalized intensities, with higher levels of breath features rising by 0.35 ± 0.06 (figure 2(b)) and lower levels of breath features falling by 0.26 ± 0.04 (figure 2(c)).

The abundance of five ketones changed significantly during exercise. Specifically, two increased, including acetone, and three decreased (table S-1). The significantly increased level of breath acetone during exercise is consistent with previous studies in both breath [30, 31, 37, 39] and saliva [20] which showed that acetone levels increased progressively with increasing exercise intensity and duration, irrespective of fasting status, potentially due to enhanced β-oxidation of lipids [59]. The other ketones could only be identified to the level of chemical formula, so we are unable to compare with other studies nor speculate on their origin. However, these findings should be interpreted cautiously.

Seven aldehydes changed significantly during exercise. Specifically, four decreased, including acetaldehyde, and three increased (table S-1). Our observation for decreased level of acetaldehyde due to an increase in exercise intensity is consistent with

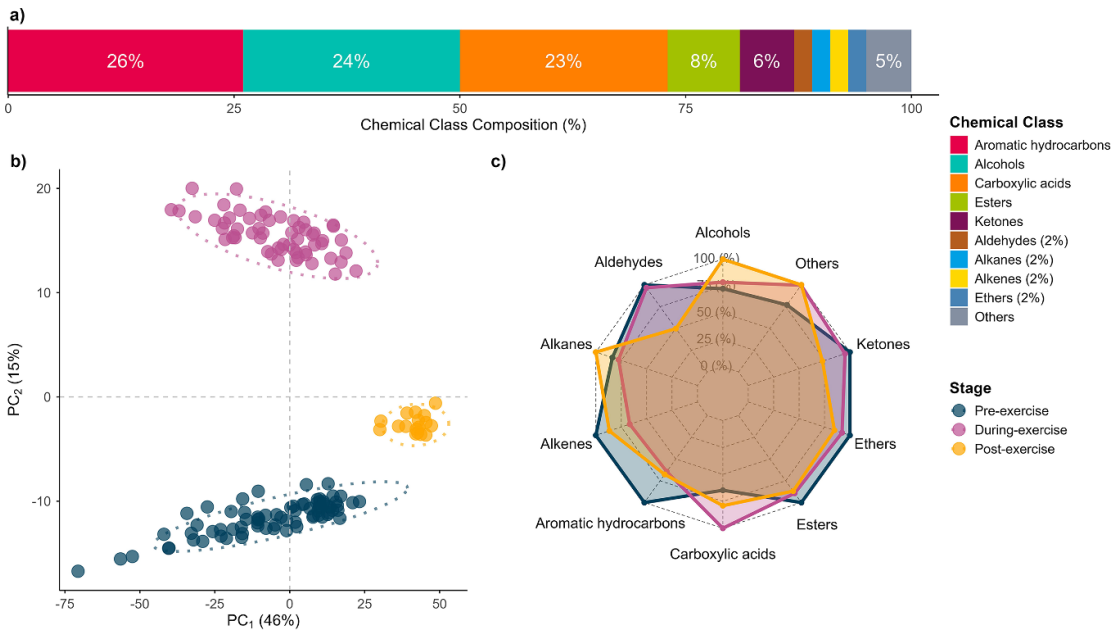


Figure 1. (a) Stacked bar chart illustrating the cumulative chemical class composition of 1204 breath features identified across 143 samples, based on the percentage distribution of breath feature intensities. (b) Two-dimensional PCA plot illustrating the overall breath volatilome profile across exercise stages. Each point represents a sample, colored by stage (pre-exercise in dark blue, during-exercise in purple, and post-exercise in yellow). The first two principal components explain 46% and 15% of the total variance, respectively. Dotted ellipses denote the 95% confidence intervals for each group; (c) spider plot comparing the breath feature intensities of chemical classes of 1204 breath features between pre- (dark blue), during- (purple), and post-exercise (yellow) samples. Each axis represents a chemical class, and the radial distance indicates the relative percentage contribution of each class to the total breath feature intensity within each.

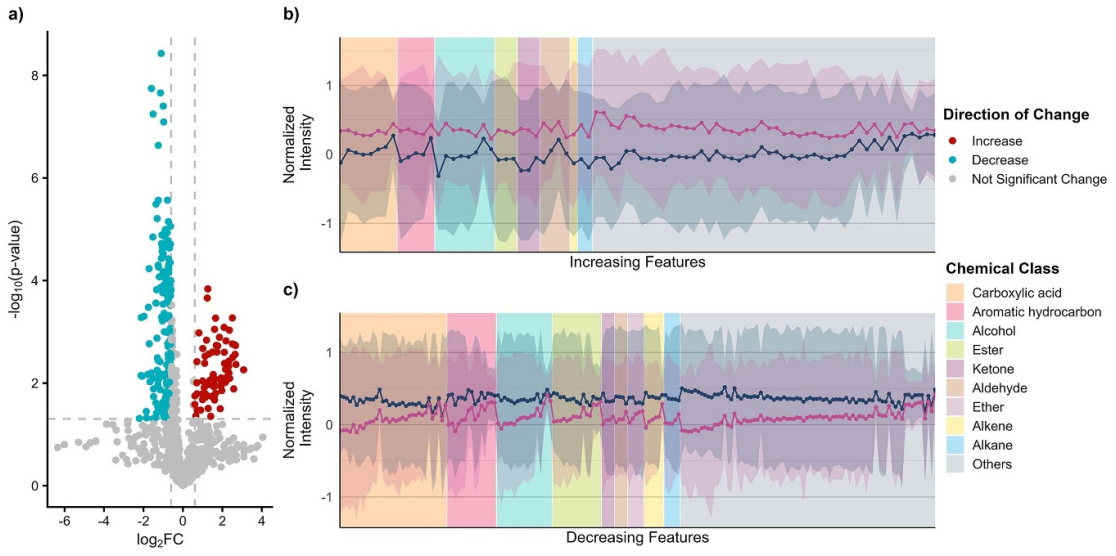


Figure 2. (a) Volcano plot illustrating changes in 1204 breath features during-exercise compared to pre-exercise. Each point represents a breath feature, with red and blue indicating significant increases (n = 80) and decreases (n = 182), respectively; gray points denote non-significant changes. The horizontal dashed line represents the *p*-value threshold of 0.05, while the vertical dashed lines indicate $\log_2$ fold-change cutoffs of ±0.6. (b) and (c) ribbon plots showing normalized intensities of significantly changing breath features between pre-exercise and during-exercise samples, sorted by chemical classes and the differences in average normalized intensities. Panel (b) presents features that increased during exercise, and panel (c) shows those that decreased. Each line represents the mean normalized feature intensity for pre-exercise (dark blue) and during-exercise (purple), with shaded areas indicating the standard deviation.

published research [30, 33]. This decrease can be linked to lipid peroxidation [60]. For other aldehydes, less information is available. Aldehydes are generally produced through reactive oxygen species (ROS)-induced lipid peroxidation and then rapidly oxidized by aldehyde dehydrogenase (ALDH) [61]. However, ALDH activity is localized. Increased ROS production may exceed local detoxification capacity, leading to variable aldehyde accumulation and thus resulting in different aldehyde levels in breath during

exercise [62]. Nevertheless, the proposed biochemical explanations should be interpreted cautiously.

Eleven alcohols significantly decreased during exercise (table S-1). To-date, changes in alcohol levels in the breath of athletes has not been reported. We suspect that alcohol levels are likely influenced by aldehyde metabolism, as the lower availability of some aldehydes due to ALDH activity may limit alcohol formation, given that aldehydes serve as precursors for producing alcohols [63]. Additionally, increased mitochondrial respiration during exercise shifts the cellular redox balance from NADH toward NAD^+, further limiting alcohol production [64]. However, these metabolic links are inferred and remain speculative.

Three carboxylic acids showed significant changes during exercise. Specifically, we report an increase in acetic acid and decreases in two others (table S-1). Previous studies reported an increase in acetic acid in both breath [40] and urine [65] immediately after prolonged exercise. These studies suggest that two main pathways can lead to increased acid levels. One is increased activity of the gut microbiota [40, 66], which increases the production of short-chain fatty acids. The other is linked to increased fatty acid metabolism, where faster β-oxidation creates more acetyl-CoA than the Krebs cycle capacity [65], leading the excess to be converted into acetic acid. Green and colleagues also found that circulating short-chain fatty acid changes were not reflected in exhaled breath condensate, suggesting that local respiratory production, including microbial metabolism, may also contribute to breath acetic acid, which could be particularly relevant during exercise, when ventilation increases [67]. Carboxylic acid levels may also be influenced by shifts in aldehyde metabolism, since aldehydes are commonly oxidized into carboxylic acids, which could help explain the observed decrease in the other two carboxylic acids with limited aldehyde availability due to enzyme detoxification [59]. However, these interpretations remain tentative.

Nine esters significantly changed during-exercise. Specifically, six decreased and three increased (table S-1). Esters can be produced through reactions between fatty acids and alcohols [68] or via lactic acid dehydration [69]. The observed decrease in six esters might be attributed to reduced alcohol availability and increased fatty acid oxidation during exercise [68]. Moreover, lactate accumulation might contribute to increased lactic acid dehydration, explaining the rise in two other esters [69]. However, this link is quite speculative.

The evidence for changes in the other chemical classes and molecules in relation to exercise is even more limited, so we cannot speculate further. In addition, exercise intensity and physiological responses were not standardized or directly monitored in this study; therefore, we cannot attribute the observed breath VC changes specifically to exercise intensity. We recognize that there are many factors that could be contributing to the changes in breath profiles between rest and exercise and that these are further confounded by gross physiological changes, such as increased cardiac output and alveolar ventilation during exercise, which elevate the ventilation-perfusion ratio, diluting numerous breath compounds [31, 37, 70]. These factors likely also contribute to at least some of the breath feature changes we observed.

3.3.2. Significant changes post-exercise (vs pre-exercise)

We compared 16 paired pre-exercise and post-exercise samples collected from subjects training for American football or ice hockey. We identified 11 breath features significantly decreasing post-exercise without any increasing features (figure 3(a), table S-1). The chemical class of decreasing breath features were mainly carboxylic acids, alkanes, and esters. Consistent shifts in these chemical classes were observed in normalized intensities, with decreasing breath features falling by 1.06 ± 0.43 (figure 3(b)).

We observed only a few significantly altered molecules post-exercise compared to pre-exercise, suggesting that participants had largely returned to their baseline breath profile at the sampling point. This observation potentially reflects partial recovery from exertion and the moderate intensity of the exercise protocol. This interpretation is consistent with our study design, where breath was collected after a defined recovery period rather than immediately post-exercise. The limited number of persistent VC changes suggests that major metabolic perturbations detectable in breath had already diminished by this stage [71, 72]. This is in line with the moderate exercise intensity used in our protocol, which is expected to induce smaller metabolic shifts compared to high-intensity or prolonged exercise. However, as exercise intensity and physiological responses were not standardized or directly monitored, the underlying mechanisms and recovery-related changes cannot be confirmed from our data alone. Future studies would benefit from sampling breath at additional post-exercise time points alongside direct physiological monitoring.

4. Conclusion and future steps

This study demonstrated that exercise induces measurable changes in the human breath volatilome, likely reflecting key metabolic processes such as fatty acid oxidation, ketogenesis, and oxidative stress. These findings support the potential of exhaled breath analysis as a non-invasive tool to monitor exercise-induced metabolic dynamics. However, this is very much a pilot study, and the lack of standardized exercise prescription data and direct physiological monitoring substantially limits the interpretation

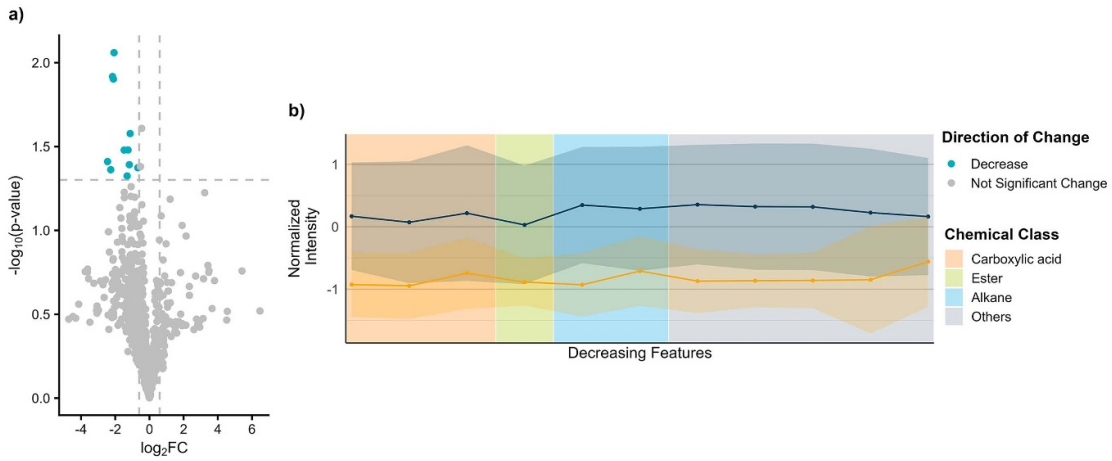


Figure 3. (a) Volcano plot illustrating changes in 1204 breath features post-exercise compared to pre-exercise. Each point represents a breath feature, with blue indicating significant decreases (n = 11; $p < 0.05$, $\log_2$FC <-0.6), and gray denoting non-significant changes. The horizontal dashed line marks the p-value threshold of 0.05, and vertical dashed lines indicate the $\log_2$ fold change cutoffs of ±0.6; (b) Ribbon plot, showing normalized breath feature areas of significantly decreasing breath features post-exercise compared to pre-exercise, sorted by chemical classes and the differences in average normalized intensities. Each line represents the mean normalized breath feature intensities for pre-exercise (dark blue) and post-exercise (yellow) samples, with shaded bands indicating standard deviation.

and application of these findings within sport and exercise science. Several methodological limitations related to breath sampling should also be considered. Mixed-expiratory sampling may introduce variability from dead-space, upper airway, and ambient air contributions. Breath transfer to TDTs and refrigerated storage may also cause compound-specific contamination, analyte loss, or analyte transformation. Although consistent handling procedures minimized these effects, they cannot be fully excluded, and future studies should directly measure and standardize exhaled breath volume. To strengthen and expand upon our exploratory insights, future studies would benefit from employing controlled exercise intensities that can be monitored via baseline fitness assessments (e.g. VO_2max) and monitored using both real-time exercise metrics (e.g. heart rate, lactate) and post-exercise physiological recovery markers (e.g. cortisol, IL-6) at earlier post-exercise time points. In addition, the analytical pipeline should be expanded to include additional, well-studied VCs, such as isoprene [30–33, 35, 37, 39, 73]. Also, collecting longitudinal samples at more frequent time points during exercise and throughout recovery, or using online/real-time breath measurements where feasible, for example, over a 24 h recovery window, would also provide a more complete picture of VCs and metabolic trajectories. Implementing standardized and reproducible exercise protocols would also improve the correlation between exercise parameters and metabolic responses. These steps will help establish breath analysis as a reliable tool for exercise physiology and personalized performance monitoring.

Acknowledgments

We would like to sincerely thank all study participants for their time and commitment, without whom this research would not have been possible. We are also grateful to the students working under JEH's and MS's supervision for their assistance with participant recruitment and sample collection. MS contributed to study design, coordination, Institutional Review Board (IRB) approval, subject recruitment, and sample collection. FAF was responsible for running the samples and supporting the analytical workflow. MH performed data alignment, result generation, and manuscript writing. JEH contributed to study funding, study design, IRB approval, co-author supervision, manuscript drafting and writing, and overall project oversight. This work was partially supported by private, anonymous funding to JEH when at Dartmouth College. Additional support was provided for MH: University of British Columbia Four-Year Doctoral Fellowship (4YF); JEH: Canada Research Chair in Breath Science and Technologies (CRC-2020-00151). We also acknowledge valuable feedback from our colleagues and collaborators, including Jonathan Little and Seth McCarthy, whose input helped shape the interpretation of this study.

Data availability statement

The data cannot be made publicly available upon publication because they contain sensitive personal information. The data that support the findings of this study are available upon reasonable request from the authors.

Supplementary Figures & Table available at: https://doi.org/10.1088/1752-7163/aea926/data1.

ORCID iDs

Mostafa Hashemi 0009-0003-0433-7778
Marek Svoboda 0000-0002-5166-673X
Flavio A Franchina 0000-0001-7236-4266
Jane E Hill 0000-0003-3534-5765

References

[1] Sakaguchi C A, Nieman D C, Signini E F, Abreu R M and Catai A M 2019 Metabolomics-based studies assessing exercise-induced alterations of the human metabolome: a systematic review *Metabolites* **9** 164
[2] Schranner D, Kastenmüller G, Schönfelder M, Römisch-margl W and Wackerhage H 2020 Metabolite concentration changes in humans after a bout of exercise: a systematic review of exercise metabolomics studies *Sports Med. Open* **6** 11
[3] Koay Y C, Stanton K, Kienzle V, Li M, Yang J, Celermajer D S and O'Sullivan J F 2021 Effect of chronic exercise in healthy young male adults: a metabolomic analysis *Cardiovasc. Res.* **117** 613–22
[4] Al-Khelaifi F, Diboun I, Donati F, Botrè F, Alsayrafi M, Georgakopoulos C, Suhre K, Yousri N A and Elrayess M A 2018 A pilot study comparing the metabolic profiles of elite-level athletes from different sporting disciplines *Sports Med. Open* **4** 2
[5] Shi R *et al* 2020 Runners' metabolomic changes following marathon *Nutr. Metab.* **17** 19
[6] Davison G, Vinaixa M, McGovern R, Beltran A, Novials A, Correig X and McClean C 2018 Metabolomic response to acute hypoxic exercise and recovery in adult males *Front. Physiol.* **9** 1682
[7] Stander Z, Luies L, Mienie L J, Keane K M, Howatson G, Clifford T, Stevenson E J and Loots D T 2018 The altered human serum metabolome induced by a marathon *Metabolomics* **14** 150
[8] Pechlivanis A, Kostidis S, Saraslanidis P, Petridou A, Tsalis G, Veselkov K, Mikros E, Mougios V and Theodoridis G A 2013 1H NMR study on the short-and long-term impact of two training programs of sprint running on the metabolic fingerprint of human serum *J. Proteome Res.* **12** 470–80
[9] Hintikka J E, Ahtiainen J P, Permi P, Jalkanen S, Lehtonen M and Pekkala S 2023 Aerobic exercise training and gut microbiome-associated metabolic shifts in women with overweight: a multi-omic study *Sci. Rep.* **13** 11228
[10] Nieman D C, Shanely R A, Gillitt N D, Pappan K L and Lila M A 2013 Serum metabolic signatures induced by a three-day intensified exercise period persist after 14 h of recovery in runners *J. Proteome Res.* **12** 4577–84
[11] Karl J P, Margolis L M, Murphy N E, Carrigan C T, Castellani J W, Madslien E H, Teien H-K, Martini S, Montain S J and Pasiakos S M 2017 Military training elicits marked increases in plasma metabolomic signatures of energy metabolism, lipolysis, fatty acid oxidation, and ketogenesis *Physiol. Rep.* **5** e13407
[12] Danaher J, Gerber T, Wellard R M, Stathis C G and Cooke M B 2016 The use of metabolomics to monitor simultaneous changes in metabolic variables following supramaximal low volume high intensity exercise *Metabolomics* **12** 7
[13] Howe C C F, Alshehri A, Muggeridge D, Mullen A, Boyd M, Spendiff O, Moir H and Watson D 2018 Untargeted metabolomics profiling of an 80.5 km simulated treadmill ultramarathon *Metabolites* **8** 14
[14] Manaf F A, Lawler N G, Peiffer J J, Maker G L, Boyce M C, Fairchild T J and Broadhurst D 2018 Characterizing the plasma metabolome during and following a maximal exercise cycling test *J. Appl. Physiol.* **125** 1193–203
[15] Lewis G D *et al* 2010 Metabolic signatures of exercise in human plasma *Sci. Transl. Med.* **2** 33ra37
[16] Lehmann R *et al* 2010 Medium chain acylcarnitines dominate the metabolite pattern in humans under moderate intensity exercise and support lipid oxidation *PLoS One* **5** e11519
[17] Nieman D C, Sha W and Pappan K L 2017 IL-6 linkage to exercise-induced shifts in lipid-related metabolites: a metabolomics-based analysis *J. Proteome Res.* **16** 970–7
[18] Schader J F, Haid M, Cecil A, Schoenfeld J, Halle M, Pfeufer A, Prehn C, Adamski J, Nieman D C and Scherr J 2020 Metabolite shifts induced by marathon race competition differ between athletes based on level of fitness and performance: a substudy of the enzy-magIC study *Metabolites* **10** 87
[19] Ra S G, Maeda S, Higashino R, Imai T and Miyakawa S 2014 Metabolomics of salivary fatigue markers in soccer players after consecutive games *Appl. Physiol. Nutr. Metab.* **39** 1120–6
[20] Khoramipour K, Gaeini A A, Shirzad E, Gilany K, Chashniam S and Sandbakk Ø 2021 Metabolic load comparison between the quarters of a game in elite male basketball players using sport metabolomics *Eur. J. Sport Sci.* **21** 1022–34
[21] Cao B, Liu S, Yang L and Chi A 2020 Changes of differential urinary metabolites after high-intensive training in teenage football players *Biomed Res. Int.* **2020** 2073803
[22] Zhao J, Wang Y, Zhao D, Zhang L, Chen P and Xu X 2020 Integration of metabolomics and proteomics to reveal the metabolic characteristics of high-intensity interval training *Analyst* **145** 6500–10
[23] Quintas G, Reche X, Sanjuan-Herráez J D, Martínez H, Herrero M, Valle X, Masa M and Rodas G 2020 Urine metabolomic analysis for monitoring internal load in professional football players *Metabolomics* **16** 45
[24] Heng W, Yin S, Chen Y and Gao W 2025 Exhaled breath analysis: from laboratory test to wearable sensing *IEEE Rev. Biomed. Eng.* **18** 50–73
[25] Das S and Pal M 2020 Review—non-invasive monitoring of human health by exhaled breath analysis: a comprehensive review *J. Electrochem. Soc.* **167** 037562
[26] Guyton A C and E H J 2020 *Guyton and Hall Textbook of Medical Physiology* (Elsevier)
[27] Belizário J E, Faintuch J and Malpartida M G 2021 Breath biopsy and discovery of exclusive volatile organic compounds for diagnosis of infectious diseases *Front. Cell. Infect. Microbiol.* **10** 564194
[28] Drabińska N, Flynn C, Ratcliffe N, Belluomo I, Myridakis A, Gould O, Fois M, Smart A, Devine T and Costello B D L 2021 A literature survey of all volatiles from healthy human breath and bodily fluids: the human volatilome *J. Breath Res.* **15** 034001
[29] Chou H, Godbeer L, Allsworth M, Boyle B and Ball M L 2024 Progress and challenges of developing volatile metabolites from exhaled breath as a biomarker platform *Metabolomics* **20** 72
[30] Pugliese G, Trefz P, Weippert M, Pollex J, Bruhn S, Schubert J K, Miekisch W and Sukul P 2022 Real-time metabolic monitoring under exhaustive exercise and evaluation of ventilatory threshold by breathomics: independent validation of evidence and advances *Front. Physiol.* **13** 946401
[31] King J, Mochalski P, Kupferthaler A, Unterkofler K, Koc H, Filipiak W, Teschl S, Hinterhuber H and Amann A 2010 Dynamic profiles of volatile organic compounds in exhaled breath as determined by a coupled PTR-MS/GC-MS study *Physiol. Meas.* **31** 1169–84
[32] Schubert R, Schwoebel H, Mau-Moeller A, Behrens M, Fuchs P, Sklorz M, Schubert J K, Bruhn S and Miekisch W 2012 Metabolic monitoring and assessment of anaerobic threshold by means of breath biomarkers *Metabolomics* **8** 1069–80

[33] Schwoebel H, Schubert R, Sklorz M, Kischkel S, Zimmermann R, Schubert J K and Miekisch W 2011 Phase-resolved real-time breath analysis during exercise by means of smart processing of PTR-MS data *Anal. Bioanal. Chem.* **401** 2079–91
[34] Bikov A, Paschalaki K, Logan-Sinclair R, Horváth I, Kharitonov S A, Barnes P J, Usmani O S and Paredi P 2013 Standardised exhaled breath collection for the measurement of exhaled volatile organic compounds by proton transfer reaction mass spectrometry *BMC Pulm. Med.* **13** 43
[35] Décombaz J, Grathwohl D, Pollien P, Schmitt J A J, Borrani F and Lecoultre V 2013 Effect of short-duration lipid supplementation on fat oxidation during exercise and cycling performance *Appl. Physiol. Nutr. Metab.* **38** 766–72
[36] Szabó A, Ruzsanyi V, Unterkofler K, Mohácsi Á, Tuboly E, Boros M, Szabó G, Hinterhuber H and Amann A 2015 Exhaled methane concentration profiles during exercise on an ergometer *J. Breath Res.* **9** 016009
[37] King J, Kupferthaler A, Unterkofler K, Koc H, Teschl S, Teschl G, Miekisch W, Schubert J, Hinterhuber H and Amann A 2009 Isoprene and acetone concentration profiles during exercise on an ergometer *J. Breath Res.* **3** 027006
[38] Heaney L M, Kang S, Turner M A, Lindley M R and Paul Thomas C L 2022 The impact of a graded maximal exercise protocol on exhaled volatile organic compounds: a pilot study *Molecules* **27** 370
[39] Chou H *et al* 2024 Metabolic insights at the finish line: deciphering physiological changes in ultramarathon runners through breath VOC analysis *J. Breath Res.* **18** 026008
[40] Henderson B *et al* 2021 Exhaled breath reflects prolonged exercise and statin use during a field campaign *Metabs.* **11** 192
[41] McKay A K A, Stellingwerff T, Smith E S, Martin D T, Mujika I, Goosey-Tolfrey V L, Sheppard J and Burke L M 2022 Defining training and performance caliber: a participant classification framework *Int. J. Sports Physiol. Perform.* **17** 317–31
[42] Argus C K, Gill N, Keogh J, Hopkins W G and Beaven C M 2010 Effects of a short-term pre-season training programme on the body composition and anaerobic performance of professional rugby union players *J. Sports Sci.* **28** 679–86
[43] Horváth I *et al* 2017 A European Respiratory Society technical standard: exhaled biomarkers in lung disease *Eur. Respir. J.* **49** 1600965
[44] Caddy G R, Sobell M B and Sobell L C 1978 Alcohol breath tests: criterion times for avoiding contamination by 'mouth alcohol' *Behav. Res. Methods Instrum.* **10** 814-8
[45] Libardoni M 2006 Analysis of human breath samples with a multi-bed sorption trap and comprehensive two-dimensional gas chromatography (GC×GC) *J. Chromatogr. B* **842** 13–21
[46] Marsh R L *et al* 2025 Assessing the feasibility of breath collection from conscious young children to support volatilome analysis: insights into age limitations and breath sampling requirements *J. Breath Res.* **19** 026009
[47] Sumner L W *et al* 2007 Proposed minimum reporting standards for chemical analysis: chemical analysis working group (CAWG) metabolomics standards initiative (MSI) *Metabolomics* **3** 211–21
[48] Bobak C A *et al* 2021 Breath can discriminate tuberculosis from other lower respiratory illness in children *Sci. Rep.* **11** 2704
[49] Dieterle F, Ross A, Schlotterbeck G and Senn H 2006 Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in1H NMR metabonomics *Anal. Chem.* **78** 4281–90
[50] Jin W, Riley R M, Wolfinger R D, White K P, Passador-Gurgel G and Gibson G 2001 The contributions of sex, genotype and age to transcriptional variance in Drosophila melanogaster *Nat. Genet.* **29** 389–95
[51] Ruxton G D 2006 The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test *Behav. Ecol.* **17** 688–90
[52] Gómez I, López M C, Egui A, Palacios G, Carrilero B, Benítez C, Simón M, Segovia M, Carmelo E and Thomas M C 2023 Differential expression profile of genes involved in the immune response associated to progression of chronic Chagas disease *PLoS Negl. Trop. Dis.* **17** e0011474
[53] Phillips M *et al* 1999 Variation in volatile organic compounds in the breath of normal humans *J. Chromatogr. B* **729** 75-88
[54] Arulvasan W *et al* 2024 High-quality identification of volatile organic compounds (VOCs) originating from breath *Metabolomics* **20** 102
[55] Enea C, Seguin F, Petitpas-Mulliez J, Boildieu N, Boisseau N, Delpech N, Diaz V, Eugène M and Dugué B 2010 1H NMR-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise *Anal. Bioanal. Chem.* **396** 1167–76
[56] Ali A M *et al* 2016 Metabolomic profiling of submaximal exercise at a standardised relative intensity in healthy adults *Metabolites* **6** 9
[57] Dünnwald T, Paglia G, Weiss G, Denti V, Faulhaber M, Schobersberger W and Wackerhage H 2022 High intensity concentric-eccentric exercise under hypoxia changes the blood metabolome of trained athletes *Front. Physiol.* **13** 904618
[58] Zagatto A M, Bishop D J, Antunes B M, Beck W R, Malta E S, de Poli R A B, Cavaglieri C R, Chacon-Mikahil M P T and Castro A 2022 Impacts of high-intensity exercise on the metabolomics profile of human skeletal muscle tissue *Scand. J. Med. Sci. Sports* **32** 402–13
[59] Evans M, Cogan K E and Egan B 2017 Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation *J. Physiol.* **595** 2857–71
[60] Zhang Q, Zheng J, Qiu J, Wu X, Xu Y, Shen W and Sun M 2017 ALDH2 restores exhaustive exercise-induced mitochondrial dysfunction in skeletal muscle *Biochem. Biophys. Res. Commun.* **485** 753–60
[61] Powers S K and Jackson M J 2008 Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production *Physiol. Rev.* **88** 1243–76
[62] Liu J, Yeo H C, Övervik-Douki E, Hagen T, Doniger S J, Chu D W, Brooks G A and Ames B N 2000 Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants (available at: www.jap.org)
[63] O'Brien P, Siraki A and Shangari N 2005 Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health *Crit. Rev. Toxicol.* **35** 609–62
[64] Cederbaum A I 2012 Alcohol metabolism *Clin. Liver Dis.* **16** 667–85
[65] Samudrala D, Geurts B, Brown P A, Szymańska E, Mandon J, Jansen J, Buydens L, Harren F J M and Cristescu S M 2015 Changes in urine headspace composition as an effect of strenuous walking *Metabolomics* **11** 1656–66
[66] Allen J M, Mailing L J, Niemiro G M, Moore R, Cook M D, White B A, Holscher H D and Woods J A 2018 Exercise alters gut microbiota composition and function in lean and obese humans *Med. Sci. Sports Exerc.* **50** 747–57
[67] Green C G, Bempong J, Ong M L Y, Shah A, Mallia P, Johnston S L, Singanayagam A, Reynolds J C and Heaney L M 2025 Evaluating short-chain fatty acids in breath condensate as surrogate measurements for systemic levels and investigation into alternative respiratory sample matrices *Clin. Sci.* **139** 1287–300
[68] Bondioli P 2004 The preparation of fatty acid esters by means of catalytic reactions *Top. Catal.* **27** 77–82
[69] Cunha B L C *et al* 2022 Lactide: production routes, properties, and applications *Bioengineering* **9** 164
[70] Farhi L E and Yokoyama T 1967 Effects of ventilation-perfusion inequality on elimination of inert gases *Respir. Physiol.* **3** 12–20

[71] Laforgia J, Withers R T and Gore C J 2006 Effects of exercise intensity and duration on the excess post-exercise oxygen consumption *J. Sports Sci.* **24** 1247–64
[72] Gaesser G A and Brooks G A 1984 Metabolic bases of excess post-exercise oxygen consumption *Med. Sci. Sports Exerc.* **16** 29–43
[73] van den Broek J, Mochalski P, Königstein K, Ting W C, Unterkofler K, Schmidt-Trucksäss A, Mayhew C A, Güntner A T and Pratsinis S E 2022 Selective monitoring of breath isoprene by a portable detector during exercise and at rest *Sens. Actuators B* **357** 131444