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ORIGINAL ARTICLE

# Novel Correlations Between Lung Function and Gut Microbial-Produced VOCs in the Exhaled Breath of Ultramarathon Runners: Insights from the 2019 Ultra-Trail du Mont Blanc

**Hsuan Chou[1] · Amy Craster[1] · Kayleigh Arthur[1] · Billy Boyle[1] · Max Allsworth[1] · Eli F. Kelley[2] · Glenn M. Stewart[2,3] · Courtney M. Wheatley-Guy[4] · Jesse Schwartz[2] · Caitlin C. Fermoyle[4,5] · Briana L. Ziegler[2] · Kay A. Johnson[2] · Paul Robach[6] · Patrick Basset[7] · Bruce D. Johnson[2]**

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## Abstract

**Purpose** Volatile organic compounds (VOCs) in exhaled breath change significantly after ultramarathons and could help monitor athletes' physiological status to optimize training. In this study, we investigated how breath VOCs are linked to clinical variables that reflect the cardiovascular and respiratory system.

**Methods** Correlation analysis was performed between blood and respiratory data collected in pre- and post-race samples from 24 elite runners who participated in the 2019 Ultra-Trail du Mont Blanc (UTMB®) ultramarathon. Correlation analysis was then performed between these clinical data and previously published breath VOC data collected from the same individuals.

**Results** Post-race clinical data showed decreased lung function compared to pre-race. Notably, respiratory parameters, vital capacity (VC) and forced expiratory volume (FEV1), showed positive moderate correlation with VOC 2,3-butanediol ($r=0.53$, $r=0.63$), a compound produced by bacterial metabolism. We hypothesize that the increase in 2,3-butanediol in post-race breath results from exercise-induced changes in gut microbiome activity, potentially protecting against lung injury. Additionally, correlations between lung function and respiratory muscle function strengthened post-race (VC/FEV1, $r=0.67$ to $r=0.84$; forced vital capacity (FVC)/maximal expiratory pressure (MEP), $r=0.57$ to $r=0.75$; FEV1 and MEP, $r<0.5$ to $r=0.73$). This suggests that exercise-induced changes in gut microbiome activity may indirectly influence these functions.

**Conclusion** Our findings support the notion of an intricate relationship between exhaustive exercise, altered gut microbiome activity, and lung function, and together they can influence the physiological status and performance of athletes.

**Keywords** Breath · Volatile Organic Compounds · Exhaustive Exercise · Gut Microbiome · Respiratory

## Introduction

Exhaustive exercise, including ultramarathon running, can induce physiological changes in the lungs and other parts of the body [22, 24, 25, 26, 29]. Blood biomarkers have been shown to reflect metabolic changes in ultramarathon runners [13, 16], however, the process of drawing blood multiple times for longitudinal analysis can be daunting and impractical for participants. Alternatively, breath sampling, a non-invasive approach for understanding physiological changes, has been on the rise for biomarker development in the last few decades [5]. The volatile organic compounds (VOCs) detectable in breath can be derived from metabolic processes, and literature has reported VOCs abundance changes in breath that are associated with inflammation (including alkanes and aldehydes), or

✉ Bruce D. Johnson
johnson.bruce@mayo.edu

1 Owlstone Medical, Cambridge, UK

2 Department of Cardiovascular Diseases, Mayo Clinic, Rochester, USA

3 Perkins Centre and Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Charles Camperdown, Australia

4 Department of Cardiovascular Diseases, Mayo Clinic, Scottsdale, USA

5 Sydney Medical School, University of Sydney, Sydney, Australia

6 Ecole Nationale des Sports de Montagne, Chamonix, France

7 Ultra Sports Science Foundation, Grenoble, France

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altered gut microbiome activity (including short-chain fatty acids) [15, 20]. Several studies have demonstrated the changes of exhaled breath VOCs resulting from exercise [2, 9, 12], and the first study comparing exhaled breath VOC changes in ultramarathon runners before and after the race was recently published [4]. The findings suggested the involvement of fatty acid oxidation, inflammation, and possible altered gut microbiome activity [4]. However, it remains unclear if these VOC changes, especially those produced by bacterial metabolism, are related to the effects of running an ultramarathon on the respiratory system, respiratory muscle fatigue, or the cardiovascular system.

The potential impact of lung function on ultramarathon performance has been reported [23], with negative associations found between performance and forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and peak expiratory flow (PEF). The decreases in lung function post-race are unlikely to be a clinical concern as most runners remain above the lower limits of normal, although those with preexisting respiratory disorders (e.g., asthma) may be affected [26]. On the other hand, abundance change in specific gut microbes post-marathon and the impact on metabolic changes has been reported [25], and cross-talk between the gut microbiota and lung diseases has been proposed [31]. Although decreased lung function in post-race differs from the more complexed pathophysiological changes in lung diseases, studying the physiological changes of ultramarathon runners provides an opportunity to look at the cross-talk between gut microbiota changes and lung function. The understanding of these complex relationships may provide a more holistic view on the impacts of ultramarathon running. Additionally, the non-invasive and side-effect-free sampling and analysis of breath biomarkers may potentially be used for optimizing athlete's training for better performance.

In this study, we aim to determine whether changes in exhaled breath VOCs during ultramarathon running, particularly those produced by bacterial metabolism, are linked to alterations in the respiratory and the cardiovascular systems. While logistically challenging, we have collected and analyzed clinical variables, including blood data, lung function data, forced oscillation technique measurements, and other data from a small group of elite runners who participated in the 2019 Ultra-Trail du Mont Blanc (UTMB®) ultramarathon. Through establishing the relationship between these clinical variables and the previously published exhaled breath VOCs that showed significant changes in the same participants [4], we present a novel connection between lung function FEV1, vital capacity (VC), and the gut microbial-produced 2,3-butanediol in post-race samples, allowing a comprehensive view of the physiological changes occurring in ultramarathon runners.

## Methods

### Study Population and Design

The protocol was approved by both the Mayo Clinic Institutional Review Board and the Comite de Protection des Personnes (CPP) Sud-Ouest Et Outre-Mer II. All aspects of the study conformed to the Declaration of Helsinki and Health Insurance Portability and Accountability Act (HIPAA) guidelines, and all participants provided written informed consent. Thirty-two healthy participants in the 2019 UTMB ultramarathon volunteered for the study. The UTMB (171 km, ~ 10,000 m ascent, Fig. 1) commenced in Chamonix, France, between August 26th to September 1st, and the course undulates through alpine regions that remain predominantly above 1000 m with intermittent bouts of altitude exposure over 2500 m.

Participants were asked to visit a dedicated laboratory space for physiological measurements 24–72 h before (pre) and 1–4 h (post) after participating in the UTMB. The varied collection times are limitations associated with collecting breath samples in a real-world setting, though no clear separation was found in PCA in the breath VOC profiles by collection time [4]. During each visit, participants completed a series of physiological assessments, including blood sampling, pulmonary function testing, breath VOC collection, and a submaximal cycling exercise (intensity at 20-, 30- & 40-W with each stage lasting for 3 min) to assess simultaneous lung diffusion (Fig. 1). Data for cycling was collected at rest (averaged with two duplicates) and at the last 30 s of exercise; the design allows for increased cardiac output and ventilation while remaining manageable for participants in the post-race setting.

### Breath Collection and Analyses

Breath samples for VOC analysis were collected using the ReCIVA® Breath Sampler (Owlstone Medical), a detailed method for sample collection, analysis, and data processing can be found in the previous publication [4]. Briefly, collected samples were analyzed by thermal desorption-gas chromatography mass spectroscopy (TD-GC-MS) using the Breath Biopsy Platform including GC-Orbitrap™. The analysis was followed by feature extraction using Compound Discoverer (v 3.2) (deconvolution of features) and a list of features was identified via the in-house Breath Biopsy high resolution accurate mass (HRAM) library of chemicals. The relative abundance of identified VOCs was quantified through comparison to eight deuterated internal standard compounds. The final analysis consisted of 48 breath samples from 24 subjects,

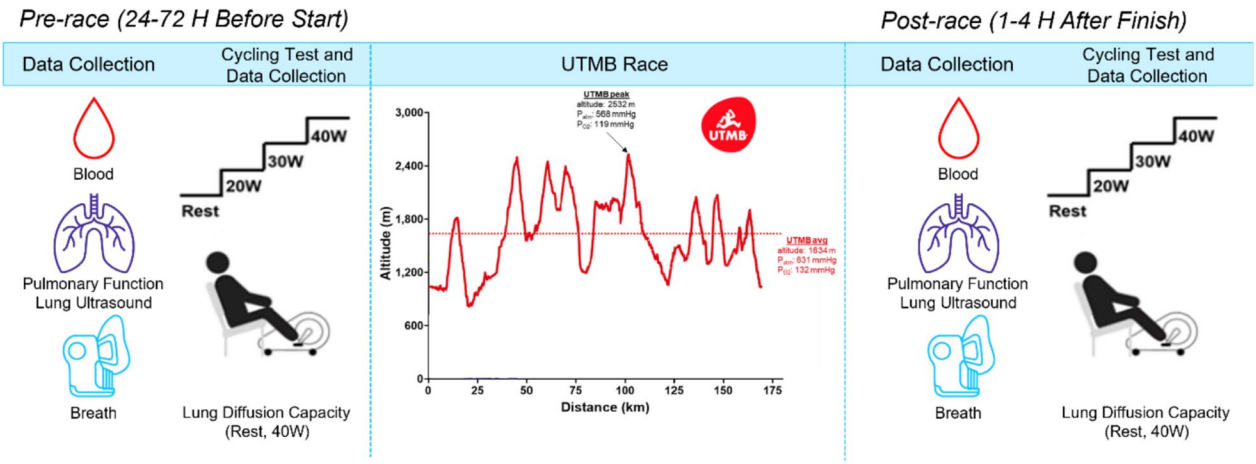


**Fig. 1** Schematic of the Study design. Experimental data collection is shown in chronological order. The chart illustrates the altitude variations and running distance during the 2019 UTMB ultramarathon

with each subject sampled pre- and post-race. Wilcoxon signed rank test was used to determine whether the peak area of each VOC differed significantly between pre- and postrace breath samples. The Benjamini–Hochberg False Discovery Rate was used to adjust *P*-values for multiple testing. Due to the difficulty of ultra-trail events, subjects were not requested to fast prior to giving breath samples.

## Clinical Data Collection

Venous blood samples were extracted into 4 mL lithium heparin and EDTA vacutainers via venipuncture for subsequent analysis. Cardiac troponin I (cTnI), brain natriuretic peptide (BNP), creatine kinase-MB (CK-MB), glucose (Glu), lactate, urea nitrogen (BUN), creatinine (Crea), base excess (BE), Partial pressure of Carbon Dioxide ($PCO_2$) and acidity level (pH) were assessed from whole fresh venous blood using a commercially available portable blood analyzer and cartridges (i-STAT Corporation, New Jersey, USA).

Pulmonary function testing was performed following the guidelines prescribed by the American Thoracic Society (ATS) and European Respiratory Society (ERS) [6, 8]. Spirometry measures, including forced vital capacities (FVC), forced expiratory volume in 1-s (FEV1), vital capacities (VC), forced expiratory flow between 25% and 75% of FVC (FEF25-75), and expiratory reserve volume (ERV) were assessed using a portable spirometer and software (Breeze Suite 8.5 and CPFS/D USB™, Medgraphics Corporation, Minnesota, USA). Respiratory muscle testing, including Maximal Inspiratory Pressure (MIP) and Maximal Expiratory Pressure (MEP) was performed using a Micro RPM system (Vyaire™, Mettawa, IL) following the ATS/ERS guidelines [1]. Respiratory mechanics, including inspiratory/expiratory total airway resistance and reactance were measured via a Forced Oscillation Technique (FOT) with a Resmon Pro® (MCG Diagnostics, Saint Paul, MN) following the ERS guidelines [11]. Fractional exhaled nitric oxide (FeNO) was measured using a handheld device (FeNObreath, Bedfont, Rochester, UK).

Transthoracic ultrasound (CX50 and S5-1 transducer, Philips Healthcare, Netherlands) was performed following the American Society of Echocardiography and European Association of Cardiovascular Imaging guidelines [19]. As a measure of extravascular lung fluid, the number of B-lines of 'comet tails' present during lung ultrasound was determined via transthoracic sonography, as previously described [21, 28].

Simultaneous measurements of lung diffusing capacity for carbon monoxide (DLco) and nitric oxide (DLno), carbon monoxide membrane conductance (Dmco), and capillary blood volume (Vc) were assessed using a single-breath technique in a semi-recumbent position at rest and during three stages of cycling exercise (Fig. 1). The assessment of lung diffusion during exertion offers a more sensitive assessment of pulmonary alveolar-capillary function rather than only at rest [7]. Details of the single-breath DLco/DLno technique and the calculation of Dmco and Vc have been previously published [7, 18, 30].

## Statistical Analyses

All data were analyzed using the Python programming language (Python Software foundation, Python Language Reference, version 3.10.12 https://www.python.org/). To determine whether the clinical variables differed between pre- and post-race, the Wilcoxon Signed-Rank test was

performed and variables with $P$-value < 0.05 were considered statistically significant. Spearman's correlation analysis was also performed to determine the correlation between clinical variables pre- and post-race, as well as the correlation between clinical variables and VOCs pre- and post-race. Correlation coefficients between 0.5 and 0.7 were considered moderate whereas > 0.7 were considered a strong correlation. Analysis on VOCs abundance change between pre- and post-race breath samples was performed with the Wilcoxon signed-rank test and previously published [4]. VOCs with an adjusted $P$-value < 0.05 were considered to have strong evidence of an association with exhaustive exercise. The Benjamini–Hochberg False Discovery Rate was used to adjust $P$-values for multiple testing.

## Results

### Demographics, Clinical Variables, and VOCs

Participant demographics, clinical variables (blood and respiratory data) in pre-race and post-race samples, as well as breath VOCs with a significant fold change in post-race samples that are above background levels (breath VOC data are from previous published work [4]), are presented in Table 1. Blood data reflects the cardiovascular system, and all variables except for pH reached statistical significance ($P$ value < 0.05) between pre-race and post-race samples. Lung function data reflects the respiratory system, and a significant decrease was found in variables FEV1 ($P = 0.01$), FVC ($P = 0.0002$), and VC ($P = 0.0001$) in post-race samples. Forced oscillation technique and additional data reflect respiratory mechanics and respiratory muscle fatigue, respectively. Significant changes were found in variables expiratory reactance (X5exsp, $P = 0.001$), FeNO ($P < 0.001$), comet tails ($P < 0.001$), MIP ($P < 0.001$), and MEP ($P < 0.002$) between pre-race and post-race samples. Lung diffusion capacity (DLco, DLno, and Vc) was assessed simultaneously during a submaximal cycling exercise before and after the race (Fig. 1). The cycling exercise was designed at a low intensity to increase cardiac output while minimizing the risk of non-completion of the exercise stages in the post-race period. The results showed lower lung diffusion capacity in post-race than pre-race samples regardless of whether at rest or at the different stages of the cycling test. However, the delta (stage 3 versus at rest) lung diffusion capacity data showed no significant differences between pre-race and post-race samples. This suggests that despite lowered lung diffusion capacity upon running an ultramarathon, it did not have an effect on the submaximal cycling exercise.

### Correlation Between Different Clinical Data in Pre- and Post-race

To investigate whether there is a relationship between the cardiovascular system, the respiratory system, respiratory mechanics, and respiratory muscle fatigue, and how ultramarathon running affects these relationships, we performed a Spearman correlation analysis on significantly altered clinical variables resulting from exhaustive exercise (Table 1). Interestingly, only respiratory related variables formed at least a moderate correlation ($r > 0.5$) in pre- and post-race samples (Table 2). Moreover, the results showed that the relationship between VC and FEV1 was strengthened after ultramarathon running ($r = 0.67$ in pre-race, $r = 0.84$ in post-race), as well as FVC and MEP ($r = 0.57$ in pre-race, $r = 0.75$ in post-race), indicating the impact of exhaustive exercise on lung function and respiratory muscle fatigue. We also observed a few blood and respiratory variables forming a moderate or strong correlation only in the post-race samples (Table 2). This suggests that these variables are independent under normal physiological conditions but may be indirectly affected by exhaustive exercise. Additionally, we performed correlation between race time and the clinical variables presented in Table 1 but observed no relationship.

### Correlation Between VOC and Clinical Data in Pre- and Post-race

We performed Spearman correlation analysis to understand whether the altered breath VOCs observed in the previous publication (Table 1) [4] are connected to the effects of running an ultramarathon on the cardiovascular system, the respiratory system, respiratory mechanics, and respiratory muscle fatigue. We observed VOCs 2,3-butanediol, 2,3-butanediol isomer, and an unknown compound are correlated with specific clinical variables, particularly respiratory data and blood glucose (Table 3). However, these correlations are only found in post-race samples. Data integration and visualization shows that the correlation between these three VOCs and respiratory data are connected to the strengthened correlation between VC and FEV1, as well as FVC and MEP. This indicates that there may be an interactive relationship between these VOCs and lung/respiratory muscle function as a result of exhaustive exercise (Supplementary Fig. 1).

## Discussion

The significant changes in breath VOCs reported in a previous publication suggested inflammation and altered gut microbiome activity in ultramarathon runners [4]. In this study, we explored how ultramarathon running affects the

**Table 1** Demographics and clinical data presented with median and median absolute deviation

| Total Participants: $n=21$ (Male), $n=3$ (Female) | | | |
|---|---|---|---|
| Median Age: 38.8 ( ± 8.9) years | | | |
| Variable | Pre-race | Post-race | *P*- value |
| **Blood Data** | | | |
| pH | 7.4 (0.0) | 7.4 (0.0) | 0.83 |
| BUN (mg/dL) | 17.5 (3.5) | 32.0 (5.0) | < 0.001 |
| BNP (ng/L) | 16.0 (2.0) | 93.0 (39.0) | < 0.001 |
| P $CO_2$ (mmHg) | 44.45 (3.85) | 37.1 (4.5) | 0.001 |
| cTnI (µg/L) | 0.0 (0.0) | 0.02 (0.02) | 0.002 |
| CK-MB (µg/L) | 5.05 (1.95) | 77.5 (36.6) | < 0.001 |
| Glu (mg/dL) | 90.0 (5.5) | 104.0 (13.0) | 0.01 |
| Lactate (mmol/L) | 1.33 (0.295) | 1.75 (0.36) | 0.001 |
| BE (mmol/L) | 2.0 (1.5) | – 2.0 (2.0) | < 0.001 |
| Crea (mg/dL) | 0.9 (0.1) | 1.1 (0.1) | < 0.001 |
| **Pulmonary Function Data** | | | |
| FEV1 (L/s) | 4.055 (0.64) | 3.85 (0.53) | 0.01 |
| FVC (L) | 5.12 (0.81) | 4.89 (0.52) | 0.0002 |
| VC (L) | 5.65 (0.1) | 5.01 (0.55) | 0.0001 |
| FEF25-75 (L/s) | 3.47 (0.64) | 3.34 (0.58) | 0.58 |
| ERV (L) | 1.58 (0.31) | 1.77 (0.31) | 0.49 |
| **Forced Oscillation Technique Data** | | | |
| R5insp ($cmH_2O$/(L/s)) | 2.48 (0.68) | 1.99 (0.38) | 0.44 |
| R5exsp ($cmH_2O$/(L/s)) | 2.42 (0.54) | 2.25 (0.56) | 0.94 |
| X5insp ($cmH_2O$/(L/s)) | – 0.54 (0.23) | – 0.59 (0.20) | 0.51 |
| X5exsp ($cmH_2O$/(L/s)) | – 0.5 (0.19) | – 0.66 (0.16) | 0.001 |
| Additional Data | | | |
| FeNO (ppb) | 18.3 (6.0) | 11.5 (5.0) | < 0.001 |
| Comet Tails (n) | 0.0 (0.0) | 3.5 (3.0) | < 0.001 |
| MIP ($cmH_2O$) | 116.0 (19.5) | 104.5 (20.5) | < 0.001 |
| MEP ($cmH_2O$) | 163.5 (24.0) | 129.0 (31.0) | 0.002 |
| **Cycling Exercise Data** | | | |
| Δ DLCO | 5.49 (1.55) | 4.94 (1.43) | 0.55 |
| Δ DLNO | 20.39 (8.17) | 17.45 (8.07) | 0.34 |
| Δ DmCO | 14.97 (11.07) | – 1.92 (34.48) | 0.12 |
| Δ Vc | 15.04 (5.64) | 13.66 (3.92) | 0.68 |

| VOC | Median Fold Change (Post- relative to Pre-race) | Multiple Testing Corrected *P*-value* |
|---|---|---|
| **Breath VOC Data** [2] | | |
| Acetate | 59.3 | 0.001 |
| Acetone | 7.5 | 0.00003 |
| Isoprene | 4.7 | 0.02 |
| 2,3-Butanedione | 3.9 | 0.0002 |
| Isopropyl Alcohol | 3.8 | 0.0007 |
| 2,3-Butanediol | 2.8 | 0.00003 |
| 4-Heptanone | 2.7 | 0.03 |
| Methyl Vinyl Ketone | 2.4 | 0.0006 |
| 2-Butanone | 1.8 | 0.0002 |
| 2-Pentanone | 1.6 | 0.0002 |
| Methyl Formate | 2.08 | 0.0003 |

**Table 1** (continued)

| VOC | Median Fold Change (Post- relative to Pre-race) | Multiple Testing Corrected *P*-value* |
|---|---|---|
| Methyl Acetate | 3.94 | 0.0001 |
| 2,3-Butanediol (isomer) | 3.70 | 0.00003 |
| 1,4-Dimethylimidazole | 16.67 | 0.02 |
| 1,5-Dimethylimidazole | 7.64 | 0.01 |
| MF343 | 0.094 | 0.02 |

VOCs are presented with median fold change

*BUN* Urea Nitrogen, *BNP* Beta-type Natriuretic Peptide, $PCO_2$ Partial pressure of Carbon Dioxide, *cTnl* Troponin, *CK-MB* Creatinine Kinase MB, *Glu* Glucose, *BE* base excess, *Create* Creatinine, *FEV1* Forced Expired Volume in 1 s, *FVC* Forced Vital Capacity, *VC* Vital Capacity, *FEF25-75* Forced Expired Flow at 25%–75% of expiration, *ERV* Expiratory Reserve Volume, *R5insp* 5 htz median inspiratory resistance, *R5exsp* 5 htz median expiratory resistance, *X5insp* 5 htz median inspiratory reactance, *X5exsp* 5 htz median expiratory reactance, *FeNO* Exhaled nitric oxide, *MIP* Maximum Inspiratory Pressure, *MEP* Maximum Expiratory Pressure, *Dlco* Lung diffusing capacity for carbon monoxide, *Dlno* Lung diffusing capacity for nitric oxide, *Dmco* membrane diffusing capacity for carbon monoxide, *Vc* Vital capacity

*The Benjamini–Hochberg False Discovery Rate was used to adjust *P*-values for multiple testing

**Table 2** Correlation analysis between clinical variables in pre- and post-race samples

| Variables | Variables | Coefficient (*r*), pre-race | Coefficient (*r*), post-race |
|---|---|---|---|
| Variables correlate ($r > 0.5$) but no change between pre- and post-race | | | |
| FVC | VC | **0.78** | **0.88** |
| FVC | FEV1 | **0.95** | **0.87** |
| MIP | FVC | 0.53 | 0.63 |
| MIP | VC | 0.65 | 0.52 |
| Variables correlate ($r > 0.5$) and strengthened ($r > 0.7$) in post-race | | | |
| VC | FEV1 | 0.67 | **0.84** |
| FVC | MEP | 0.57 | **0.75** |
| Variables correlate ($r > 0.5$) only in post-race | | | |
| BUN | Lactate | | 0.51 |
| Glu | FEV1 | | 0.52 |
| cTnI | CK-MB | | 0.62 |
| FEV1 | MEP | | **0.73** |

All variables here are at least moderately correlated ($r > 0.5$) with each other. Variables strongly correlated ($r > 0.7$) are in bold. Only variables with significant difference between pre- and post-race (see Table 1) are presented

**Table 3** Correlation analysis between post-race significantly changed breath VOCs and Clinical variables

| VOCs | Variables | Coefficient (*r*), pre-race | Coefficient (*r*), post – race |
|---|---|---|---|
| 2,3-Butanediol | VC | – | 0.53 |
| | FEV1 | – | 0.63 |
| | Glu | – | 0.51 |
| 2,3-Butanediol (isomer) | FEV1 | – | 0.55 |
| | Glu | – | 0.62 |
| Unknown VOC (MF343) | FEV1 | – | **– 0.72** |
| | FVC | – | – 0.55 |
| | VC | – | – 0.52 |
| | FeNO | – | 0.54 |
| | MEP | – | – 0.56 |

All variables here are at least moderately correlated ($r > 0.5$ or $r < -0.5$) with each other. Variables strongly correlated ($r > 0.7$ or $r < -0.7$) are in bold

relationships between various clinical variables and how exhaled breath VOCs are connected to these alterations. Our findings demonstrated: (i) strengthened correlation between VC and FEV1, as well as FVC and MEP from pre- to post-race samples implying the impact of exhaustive exercise on lung function and respiratory muscle fatigue; (ii) strong correlation between MEP and FEV1 in post-race samples, implying an indirect impact by exhaustive exercise; and (iii) moderately positive correlation between FEV1 and microbial-produced VOC 2,3-butanediol despite implications of decreased lung function post-race, suggesting a possible protection mechanism to ameliorate lung injury. Overall, there is a complex relationship between gut microbiome activity and respiratory function in the context of exhaustive exercise.

As respiratory muscle function and lung function are intricately connected and mutually dependent, it is not surprising to observe a correlation between respiratory muscle strength and lung function parameters in both pre- and post-race samples. However, it is interesting to note that the correlation between MEP and FVC strengthened ($r = 0.57$ to $r = 0.75$) in post-race samples, despite both values decreasing (Tables 1 and 2). This finding suggests that ultramarathon running has an impact on expiratory

effort. Similarly, a strong correlation between MEP and FEV1 only emerged in post-race samples ($r < 0.5$ to $r = 0.73$) (Table 2). It is anticipated that these connections may weaken or disappear upon recovery from ultramarathon running. Further research is needed to determine whether the strengthened relationship between respiratory muscle function and lung function is associated with the duration and intensity of exercise or the fitness and training of athletes.

The physiological implications and potential origins of exhaled breath VOCs found significantly changed in post-race runners (Table 1) have been discussed in great lengths previously [4]. In this study, we focused on the relationship between these VOCs and clinical metadata. We found VOC 2,3-butanediol correlated with respiratory parameters FEV1 and VC, and an unknown compound (MF343) correlated with FEV1, FVC, VC, and MEP (Table 3). However, the correlations were only observed in post-race samples, not pre-race samples, suggesting that these relationships are formed as a result of ultramarathon running. Through data integration and visualization, we found these correlations are linked to the altered relationships between VC and FEV1 in pre- and post-race suggest that ultramarathon running-induced changes in 2,3-butanediol may have an indirect impact on physiology (Supplementary Fig. 1).

2,3-Butanediol is a downstream product of glucose and is produced by the gut microbiota [17]. Cross-talk between the gut microbiota and lung diseases has been proposed [31]. While this study did not involve a disease cohort, the lung injury to which these runners are susceptible may share a similar underlying inflammation mechanism with lung diseases. Studies in cell culture and rats suggest that 2,3-butanediol has an effect on activating innate immunity cells, ameliorating inflammation [10, 14]. Additionally, community shifts in gut microbiota have been found to be associated with lung function changes in COPD subjects [3]. Here, we hypothesize that the increased levels of 2,3-butanediol observed resulted from exercise-induced alterations in gut microbiome activity, which may then ameliorate lung inflammation through an unknown protective mechanism (Fig. 2). A longitudinal analysis of VOCs and lung function from post-race to race-recovery would provide further insight. Moreover, studies aimed at investigating the involvement of gut microbial products and their protective effects should consider administering antibiotics to deplete the gut microbiome in athletes undergoing exhaustive exercise. Alternatively, the increased 2,3-butanediol may act through flavin-containing monooxygenases (FMOs) to promote lipid metabolism as an alternative energy source when glucose is depleted [27]. The previously observed increase in lipid metabolism products aligns with this hypothesis, though whether FMOs are more expressed in response to physical activity has yet to be elucidated.

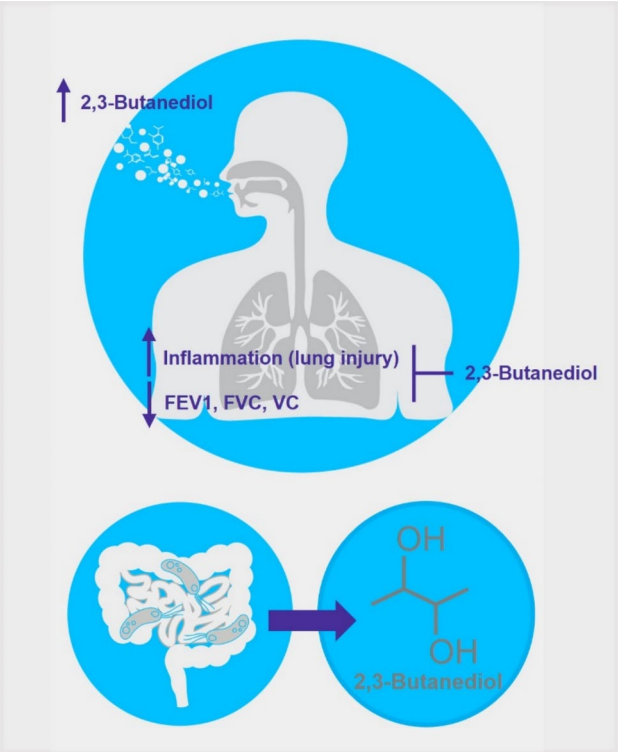


**Fig. 2** Illustration of exhaustive exercise-induced alteration of the gut microbiome activity. We propose that increased levels of microbial product 2,3-butanediol in exhaled breath may ameliorate the reduced lung function caused by inflammation upon recovery from exhaustive exercise

We also attempted to identify MF343, which was matched to tetrahydro-2-methylthiophene in the NIST library, but with poor peak shape. A reference standard confirmed it was not the suggested compound, therefore the identity of MF343 remains unknown. While the NIST library suggested other candidates, these compounds presented low scores and belonged to the thiophene chemical class. Thiophenes are Maillard reactions products, and therefore the likelihood of this compound being relevant to exercise physiology is low. Regardless, the findings of 2,3-butanediol in this study demonstrate the intricate relationship between exhaustive exercise, altered gut microbiome activity, and lung function.

There are several considerations concerning the interpretation of the data in this study. First, we did not separate the clinical variable analysis by gender, as there were too few female subjects participating in the study to perform subgroup analysis with adequate statistical power. Second, although it is recommended to fast in moderation prior to breath VOC sampling, we recorded dietary and beverage intake but did not request subjects to fast in this study as it was not appropriate for the participants [4]. Therefore, increased glucose levels observed in post-race samples may have been proportionally influenced by dietary or beverage

intake. Other limitations of the study that were difficult to control include the participants' age, body composition, race times, recovery times, hydration levels, training levels and cultural backgrounds [4]. Finally, while studies have suggested an association between gut bacteria and exercise-induced metabolic changes [25], further research is needed to determine what specific bacteria are associated with the changes in 2,3-butanediol observed in this study.

To our knowledge, this is the first report correlating breath VOCs with clinical variables in the context of ultra-marathon running. Despite the logistical challenges of data collection, the analysis provided a more comprehensive view, linking breath VOCs with clinical variables that reflect the cardiovascular and respiratory system.

## Conclusion

Our findings suggest that there is a complex relationship between exhaustive exercise, altered gut microbiome activity, and lung function. We hypothesize that the production of 2,3-butanediol results from the impact of exhaustive exercise on the gut microbiome, potentially providing a protective effect to ameliorate lung injury. As exhaled breath VOCs can reflect physiological changes in the human body, there is potential for non-invasive, side-effect-free breath biomarker applications to monitor athletes' health and performance.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s42978-025-00331-1.

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**Author Contributions** EFK, GMS, BDJ, and CMWG conceived and designed the study. HC, AC, KA, EFK, GLM, JS, CCF, BLZ, and KAJ contributed to data collection and analysis. HC wrote the manuscript. All authors reviewed and approved the final manuscript.

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**Data Availability** The data will be made available on reasonable request.

## Declarations

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Ethical Approval** All aspects of the study conformed to the Declaration of Helsinki and Health Insurance Portability and Accountability Act (HIPAA) guidelines.

**Consent to Participate** All participants provided written informed consent.

## References

1. American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. Am J Respir Crit Care Med. 2002;166(4):518–624.
2. Bell LR, Wallen MP, Talpey SW, Myers MA, O'Brien BJ. Can exhaled volatile organic compounds differentiate high and low responders to resistance exercise? Med Hypotheses. 2022;162:110837.
3. Chiu YC, Lee SW, Liu CW, Lan TY, Wu LS. Relationship between gut microbiota and lung function decline in patients with chronic obstructive pulmonary disease: a 1-year follow-up study. Respir Res. 2022;23(1):10.
4. Chou H, Arthur K, Shaw E, Schaber C, Boyle B, Allsworth M, Kelley EF, Stewart GM, Wheatley CM, Schwartz J, Fermoyle CC. Metabolic insights at the finish line: deciphering physiological changes in ultramarathon runners through breath VOC analysis. J Breath Res. 2024;18(2): 026008.
5. Chou H, Godbeer L, Allsworth M, Boyle B, Ball ML. Progress and challenges of developing volatile metabolites from exhaled breath as a biomarker platform. Metabolomics. 2024;20(4):72.
6. Dweik RA. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602–15.
7. Fermoyle CC, Stewart GM, Borlaug BA, Johnson BD. Simultaneous measurement of lung diffusing capacity and pulmonary hemodynamics reveals exertional alveolar-capillary dysfunction in heart failure with preserved ejection fraction. J Am Heart Assoc. 2021;10(16): e019950.
8. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, McCarthy K, McCormack MC, Oropez CE. Standardization of spirometry 2019 update. An official american thoracic society and european respiratory society technical statement. Am J Respir Crit Care Med. 2019;200(8):e70–88.
9. Heaney LM, Lindley MR. Translation of exhaled breath volatile analyses to sport and exercise applications. Metabolomics. 2017;13:1–19.
10. Hsieh SC, Lu CC, Horng YT, Soo PC, Chang YL, Tsai YH, Lin CS, Lai HC. The bacterial metabolite 2,3-butanediol ameliorates endotoxin-induced acute lung injury in rats. Microbes Infect. 2007;9(12–13):1402–9.
11. King GG, Bates J, Berger KI, Calverley P, de Melo PL, Dellacà RL, Farré R, Hall GL, Ioan I, Irvin CG, Kaczka DW. Technical standards for respiratory oscillometry. Eur Respir J. 2020;55(2):1900753.
12. King J, Kupferthaler A, Unterkofler K, Koc H, Teschl S, Teschl G, Miekisch W, Schubert J, Hinterhuber H, Amann A. Isoprene and acetone concentration profiles during exercise on an ergometer. J Breath Res. 2009;3(2): 027006.
13. Kłapcińska B, Waśkiewicz Z, Chrapusta SJ, Sadowska-Krępa E, Czuba M, Langfort J. Metabolic responses to a 48-h ultramarathon run in middle-aged male amateur runners. Eur J Appl Physiol. 2013;113(11):2781–93.
14. Lai HC, Chang CJ, Yang CH, Hsu YJ, Chen CC, Lin CS, Tsai YH, Huang TT, Ojcius DM, Tsai YH, Lu CC. Activation of NK cell cytotoxicity by the natural compound 2,3-butanediol. J Leukoc Biol. 2012;92(4):807–14.
15. Lee JH, Zhu J. Analyses of short-chain fatty acids and exhaled breath volatiles in dietary intervention trials for metabolic diseases. Exp Biol Med (Maywood). 2021;246(7):778–89.
16. Lewis GD, Farrell L, Wood MJ, Martinovic M, Arany Z, Rowe GC, Souza A, Cheng S, McCabe EL, Yang E, Shi X. Metabolic signatures of exercise in human plasma. Sci Transl Med. 2010;2(33):33ra37.

17. Li NN, Li W, Feng JX, Du B, Zhang R, Du SH, Liu SY, Xue GH, Yan C, Cui JH, Zhao HQ. High alcohol-producing *Klebsiella pneumoniae* causes fatty liver disease through 2,3-butanediol fermentation pathway in vivo. Gut Microbes. 2021;13(1):1979883.
18. Macintyre N, Crapo RO, Viegi G, Johnson DC, Van Der Grinten CP, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, Gustafsson P. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J. 2005;26(4):720–35.
19. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, Horton K, Ogunyankin KO, Palma RA, Velazquez EJ. Guidelines for performing a comprehensive transthoracic echocardiographic examination in adults: recommendations from the American society of echocardiography. J Am Soc Echocardiogr. 2019;32(1):1–64.
20. Peltrini R, Cordell RL, Wilde M, Abuhelal S, Quek E, Zounemat-Kermani N, Ibrahim W, Richardson M, Brinkman P, Schleich F, Stefanuto PH. Discovery and validation of a volatile signature of eosinophilic airway inflammation in asthma. Am J Respir Crit Care Med. 2024;210:1101–12.
21. Picano E, Frassi F, Agricola E, Gligorova S, Gargani L, Mottola G. Ultrasound lung comets: a clinically useful sign of extravascular lung water. J Am Soc Echocardiogr. 2006;19(3):356–63.
22. Robson-Ansley P, Howatson G, Tallent J, Mitcheson K, Walshe I, Toms C, Toit GD, Smith M, Ansley L. Prevalence of allergy and upper respiratory tract symptoms in runners of the London marathon. Med Sci Sports Exerc. 2012;44(6):999–1004.
23. Salinero JJ, Soriano ML, Ruiz-Vicente D, Gonzalez-Millan C, Areces F, Gallo-Salazar C, Abian-Vicen J, Lara B, Del Coso J. Respiratory function is associated to marathon race time. J Sports Med Phys Fitness. 2016;56(12):1433–8.
24. Sato M, Suzuki Y. Alterations in intestinal microbiota in ultramarathon runners. Sci Rep. 2022;12(1):6984.
25. Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, Wibowo MC, Wurth RC, Punthambaker S, Tierney BT, Yang Z. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nat Med. 2019;25(7):1104–9.
26. Tiller NB, Stewart GM, Illidi CR, Levine BD. Exercise is medicine? The cardiorespiratory implications of ultra-marathon. Curr Sports Med Rep. 2020;19(8):290–7.
27. Veeravalli S, Varshavi D, Scott FH, Varshavi D, Pullen FS, Veselkov K, Phillips IR, Everett JR, Shephard EA. Treatment of wild-type mice with 2,3-butanediol, a urinary biomarker of Fmo5 (-/-) mice, decreases plasma cholesterol and epididymal fat deposition. Front Physiol. 2022;13: 859681.
28. Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, Kirkpatrick AW, Melniker L, Gargani L, Noble VE, Via G, Dean A. International evidence-based recommendations for point-of-care lung ultrasound. Intensive Care Med. 2012;38(4):577–91.
29. Webner D, DuPrey KM, Drezner JA, Cronholm P, Roberts WO. Sudden cardiac arrest and death in United States marathons. Med Sci Sports Exerc. 2012;44(10):1843–5.
30. Zavorsky GS, Hsia CC, Hughes JM, Borland CD, Guénard H, Van Der Lee I, Steenbruggen I, Naeije R, Cao J, Dinh-Xuan AT. Standardisation and application of the single-breath determination of nitric oxide uptake in the lung. Eur Respir J. 2017;49(2):1600962.
31. Zhang D, Li S, Wang N, Tan HY, Zhang Z, Feng Y. The cross-talk between gut microbiota and lungs in common lung diseases. Front Microbiol. 2020;11:301.

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