Exhaustive Exercise Alters Exhaled Breath Volatile Organic Compounds: Insights from Elite Ultra-Marathon Runners

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Aim

The aim for this study is to identify breath VOC biomarkers of exhaustive exercise, with better understanding of their potential roles through correlation analysis of clinical metadata collected before and after an ultra-marathon.

1. Background and Objectives

- Exhaustive exercise can induce unique physiological responses in the lungs (i.e. lung injury, due to the increased susceptibility of the respiratory tract to infections and inflammation) and other parts of the human body.
- The volatile organic compounds (VOCs) in exhaled breath are ideal for studying the effects of exhaustive exercise on the lungs due to the proximity of the breath matrix to the respiratory tract. As breath VOCs can originate from the bloodstream, changes in abundance should also indicate broader physiological effects of exhaustive exercise on the body.
- In partnership with Mayo Clinic, this is the first study that explored breath VOC changes in the context of running an ultramarathon, which can range from 31-200+ miles.
- The objective of this study was to evaluate VOCs in exhaled breath collected before and after the Ultra-Trail du Mont Blanc (UTMB) ultra-marathon. Through correlation with clinical metadata, it will help better understand the potential roles of significantly changed VOCs.

Figure 1: An overview of the 2019 Ultra-Trail du Mont Blanc (UTMB) ultra-marathon.

2. Methods

- Breath Biopsy samples from 24 elite runners participated in the 2019 UTMB ultra-marathon were collected using the ReCIVA® Breath Sampler (Figure 2), developed by Owlstone Medical.
- Each participant provided two breath samples, one before and one after the race. All samples were analyzed using the Breath Biopsy Platform with GC-Orbitrap™ via thermal desorption gas chromatography-mass spectrometry (TD-GC-MS).
- A list of features was identified via the in-house high resolution accurate mass (HRAM) library, a database of authenticated chemical standards.
- The relative abundance of identified VOCs was quantified through comparison to eight deuterated internal standard compounds.
- The Wilcoxon signed-rank test was used to determine whether VOC abundances differed between pre- and post-race breath samples (adjusted $p < 0.05$).
- The Mann-Whitney U test was used to determine whether the clinical variables differed between pre- and post-race.
- Spearman's correlation analysis was used 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.

Total Participants	N = 24	
Gender (Male)	N = 21 (87.5%)	
Median Age	38.8 (±8.9)	
	Pre-race	Post-race
Median BMI	22.2 (±2.1)	21.5 (±2.1)
Halitosis Detection	N = 0	N = 5 (20.8%)
Coffee/Citrus Drinks	N = 15 (62.5%)	N = 12 (50%)
Fruit Consumption	N = 8 (33%)	N = 11 (45.8%)

Table 1: Demographics of the 24 subjects that participated in the 2019 UTMB

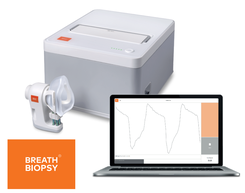


Figure 2: The Breath Biopsy® Collection Station, consisting of ReCIVA® Breath Sampler (left), CASPER™ Portable Air Supply (top) and Breath Biopsy Collect Software (lower right).

3. Results

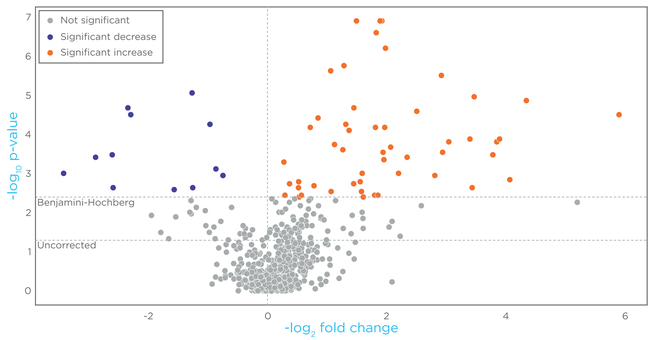


Figure 3. Volcano plot of fold change and p-value between pre- and post-race samples for detected features. Correction for multiple testing shows that 63 VOCs are significant different between pre- and post-race samples (12 decreased, 51 increased).

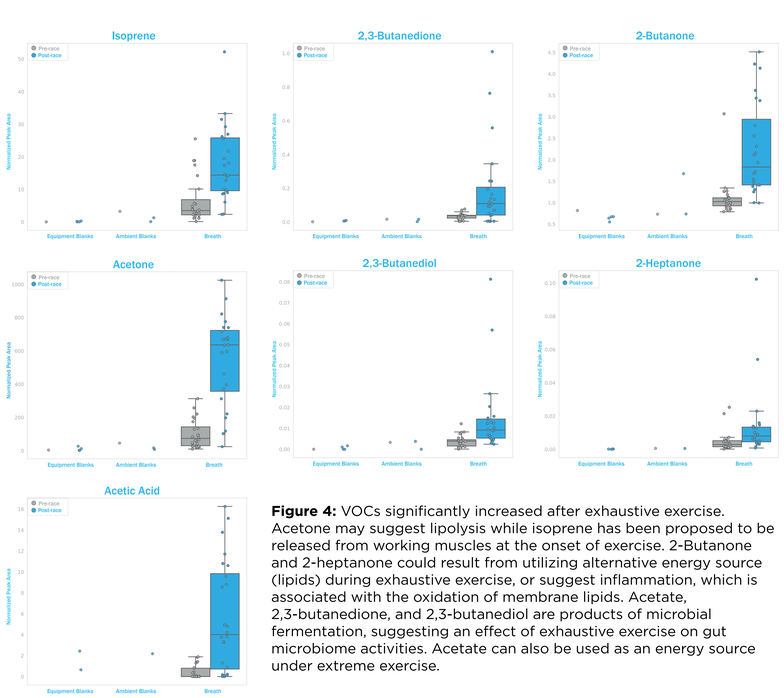
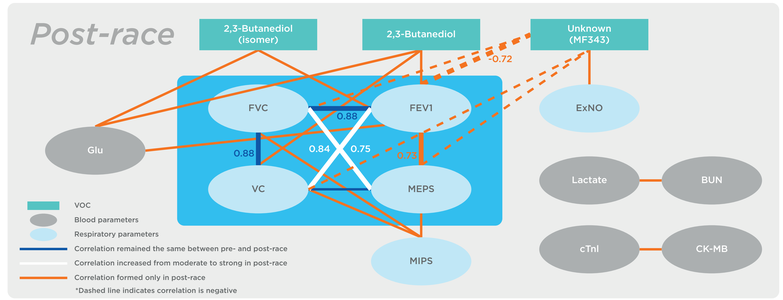


Figure 4: VOCs significantly increased after exhaustive exercise. Acetone may suggest lipolysis while isoprene has been proposed to be released from working muscles at the onset of exercise. 2-Butanone and 2-heptanone could result from utilizing alternative energy source (lipids) during exhaustive exercise, or suggest inflammation, which is associated with the oxidation of membrane lipids. Acetate, 2,3-butanedione, and 2,3-butanediol are products of microbial fermentation, suggesting an effect of exhaustive exercise on gut microbiome activities. Acetate can also be used as an energy source under extreme exercise.



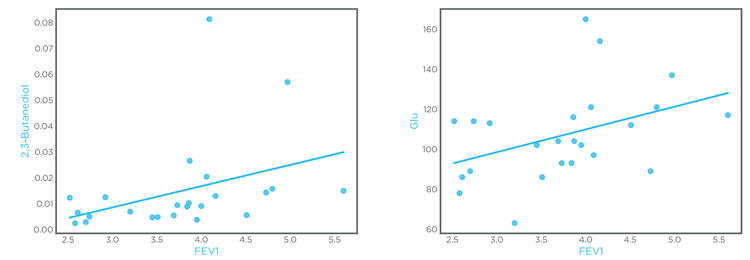


Figure 5. Correlation between selected clinical metadata and VOCs in post-race samples. Clinical metadata and VOCs presented in this graph only include those that reached statistical significance between pre- and post-race comparison. Metadata within the turquoise box suggests clustering. Unless indicated with correlation coefficient, all correlation presented are moderate (0.5-0.7). Correlation between 2,3-butanediol, lung function data (in particular FEV1) and blood glucose was observed in post-race samples, potentially being influenced by the strengthened relationship of FVC/ MEPS and FEV1/ VC.

2,3-Butanediol is a downstream product of glucose and is produced by the gut microbiota. Crosstalk between the gut microbiota and lung diseases has been proposed1, and community shifts in gut microbiota were found associated with lung function decline in COPD subjects2. A longitudinal study showed diabetic subjects with higher blood glucose level had more severe lung function decline3. The findings suggest there is an intricate relationship between exhaustive exercise, altered gut microbiome activity, and lung function.

4. Conclusions

- This first study in exploring breath VOC changes in ultramarathon runners suggests significant differences between pre-race and post-race samples. These VOCs potentially reflect various physiological responses to exhaustive exercise, including fatty acid oxidation, inflammation and
- altered activity in the gut microbiome.
- Correlation analysis between clinical metadata and exhaled breath VOCs suggest an intricate relationship between exhaustive exercise, altered gut microbiome activity, and lung function.

5. References

- Zhang, D., et al., The Cross-Talk Between Gut Microbiota and Lungs in Common Lung Diseases. Front Microbiol, 2020. 11:301
- Chiu, Y., et al., 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, 10
- Li, W., et al., Association of lung function and blood glucose level: a 10-year study in China. BMC Pulm Med , 2022. 22, 444