Volatile organic compounds in exhaled breath reflect physiological changes in ultramarathon runners

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Aim

The aim for this study is to dentify potential biomarkers of exhaustive exercise by characterizing VOCs that differ in abundance between samples collected before and after an ultra-marathon. Ultimately these VOCs will be compared to a comprehensive series of physiological measures obtained before and after the race.

1. Background and Objectives

Exhaustive exercise, typified by ultra-marathons, represents an extreme activity that triggers unique physiological responses. It also provides an opportunity to identify and study markers of inflammation or 'injury' resulting from physical stress. In this particular study we focus on the respiratory system.

An interest in the effect of exhaustive exercise on the lungs makes Breath Biopsy an ideal tool for identifying volatile organic compound (VOC) biomarkers that relate to this kind of activity.

Blood biomarkers have started to be investigated, but currently, very few studies have explored the potential of breath VOCs as biomarkers to reflect metabolic changes induced by exhaustive exercise. These studies measured subject breath VOCs through exhaustive exercise with either a standard ramp-like protocol [1] or a graded maximal exercise protocol [2]. To date, no studies have explored breath VOC changes in the context of running an ultramarathon, where can range from 31-200+ miles.

The objective of this study was to evaluate VOCs in exhaled breath that differ in abundance between matched pairs of samples collected before and after the Ultra-Trail du Mont Blanc (UTMB) ultra-marathon (Figure 1). Breath Biopsy® technology (Owlstone Medical), which allows for VOC biomarker discovery, was utilized in this study to identify potential breath biomarkers of exhaustive exercise. The analyses reported here are based on 24 matched sample pairs collected before and after the race. The results will contribute toward a better understanding of the physiological changes that occur during exhaustive exercise.

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

2. Methods

Breath Biopy samples were collected before and after the race using the ReCIVA® Breath Sampler (**Figure 2**), developed by Owlstone Medical, and analysed with thermal desorption gas chromatography mass spectrometry (TD-GC-MS) using the Breath Biopsy Platform including GC-Orbitrap™.

A total of 29 volunteers had samples collected pre-race, two of these did not finish the race and three sample sets were excluded due to sample curation (97% pass rate), leaving 24 samples pairs for analysis.

Relative quantification of VOCs was possible by comparison to eight deuterated internal standard compounds. This allowed instrument variability across this study to be measured at a median relative standard deviation (RSD) of 3.88%.

	Sex	Age	BMI	Halitosis	Last food/drink included		
					Coffee	Citrus drink	Fruit
Pre-race	3F/21M	38.8 ± 8.9	22.2 ± 2.1	0	11	4	8
Post-race	3F/21M	38.8 ± 8.9	21.5 ± 2.1	5	3	9	11

Table 1: Test subject groups and demographic overview. 21/24 sample pairs were provided by males. Ages ranged from 26 to 57 years. The median BMI decrease over the course of the race was 0.79. More subjects had detectable halitosis after the race.

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

After feature extraction and quality control, a total of 811 different features were identified. To reduce noise in the data, features that were present in only a small fraction of the samples were removed, and those that were present in 40% or more of the total samples were kept. Missing values for each feature were imputed at 80% of the minimum value. This resulted in 793 features for further analysis. Among these features, 74 features were assigned with a VOC compound identity based on comparisons to the Breath Biopsy HRAM Reference Library.

Principal component analysis assesses the full dataset to identify sources of variation. PC1 reflects the greatest amount of variation with subsequent components declining in significance. The results indicated that the separation of breath VOCs between pre- and post-race samples was more evident in PC3 and PC4 than PC1 and PC2, suggesting that while race-relevant effects were not the most significant source of variation across all samples, they were a crucial factor in the differences of VOC abundance observed before and after the race (Figure 3). Despite the modest separation in PC1 and PC2, important VOCs that may reflect physiological changes in exhaustive exercise were identified in the dataset with further analysis between pre- and post-race.

After correcting for multiple testing (Benjamini–Hochberg False Discovery Rate, p <0.05), 63 VOCs showed significant differences between pre- and post-race samples (12 decreased and 51 increased) Of these, 18 had identities assigned from the Breath Biopsy Library. (Figure 4)

Upon considering the median levels of VOCs above blanks and the current understanding of their role in biology, seven VOCs that significantly increased in post-running samples are suggested to reflect the different underlying physiological changes in exhaustive exercise (Figure 5). Increased levels of acetone suggest lipolysis while increased isoprene has been proposed to be released from working muscles at the onset of exercise. Increased levels of ketones 2-butanone and 2-heptanone could be a result of utilizing lipids as an alternative energy source during exhaustive exercise. It can also suggest inflammation, which is associated with the oxidation of membrane lipids, resulting in the final production of ketones. Increased levels of acetate, 2,3-butanedione and 2,3-butanediol, which are products of microbial fermentation, suggest the effects of exhaustive exercise on gut microbiome activities.

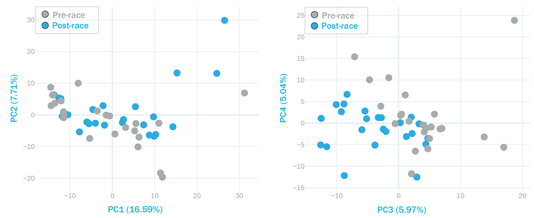

Figure 3: Principal component analysis of breath samples coloured by stage of collection. Pre- and post-race samples are not separated by PC1 and PC2 (left) but are separated by PC3 and PC4 (right) suggesting that the effects of the race provide a notable but not primary source of variation within the dataset.

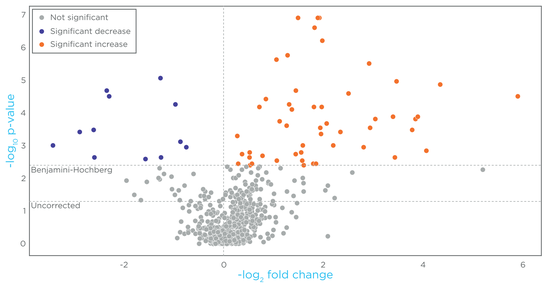

Figure 4: Volcano plot of fold change and p-value between pre- and post-race samples for detected features. 161 show significant difference with 63 persisting after correction for multiple testing. 12 of these decrease post-race and 51 increase.

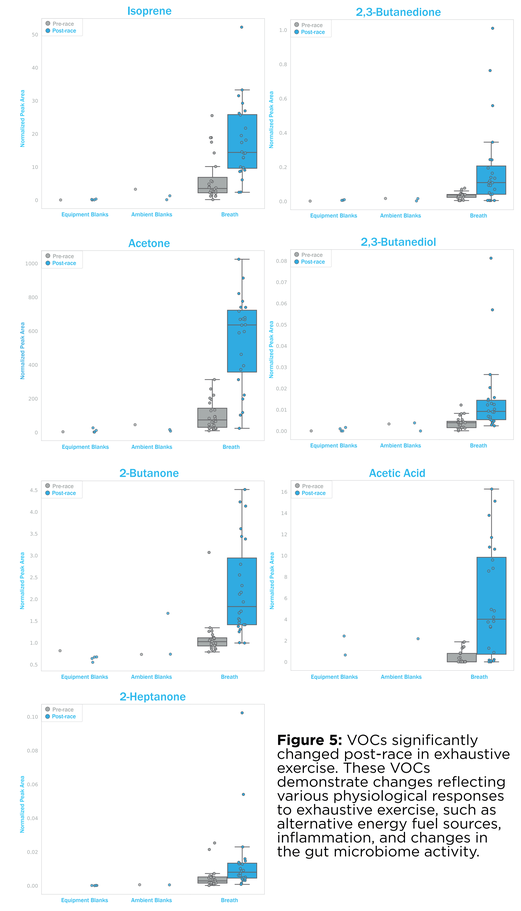

Figure 5: VOCs significantly changed post-race in exhaustive exercise. These VOCs demonstrate changes reflecting various physiological responses to exhaustive exercise, such as alternative energy fuel sources, inflammation, and changes in the gut microbiome activity.

4. Conclusions

This study demonstrates that 161 features showed significant differences between pre- and post-race samples, with 63 retained after correction for multiple testing. Of these the majority (51) increased post-race, several (18/63) were assigned molecular identities on the basis of comparison to the Breath Biopsy HRAM Library of VOCs. The differences in exhaled breath VOCs between pre-race and post-race ultramarathon runners potentially reflect various physiological responses to exhaustive exercise, such as alternative energy fuel sources, inflammation, and changes in the gut microbiome activity. Moving forward, the findings from this pilot study should be validated in a larger and more diverse cohort with a similar study design. Moreover, additional research on VOC changes, along with other physiological measurements, could provide further insight into the biological associations between VOCs and exhaustive exercise.

References

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