Breath Biopsy® of Elite Runners Engaging in Exhaustive Exercise

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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 there is no published data on breath[1,2]. Given that forms of lung injury may occur at or near the alveolar-capillary membrane, there is clear reason to expect that VOCs in breath could prove to be potent biomarkers.

Samples were collected from volunteers who ran in the 2019 Ultra-Trail du Mont-Blanc (UTMB) ultra-marathon (**Figure 1**). The analyses reported here are based on 24 matched sample pairs collected before and after the race. This study was an enhanced repeat of a similar study performed at UTMB 2018, with improved study design and analytical pipeline.

Aim: Identify 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.

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%.

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).

	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.

3. Results

Across all samples, a total of 811 different VOC signals were identified, a significant improvement over 203 in the previous version of this study. Of these, 793 were detected with suitable frequency (40% or above) to enable their use for further analysis. The Breath Biopsy HRAM Library of verified VOCs allowed 74 of the 811 features to be assigned verified compound identities.

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. A plot of PC1 and PC2 coloured by time demonstrates a trend in post race sample composition (**Figure 3B**). Similarly PC3 and PC4 separate pre- and post-race samples somewhat (**Figure 4B**). As expected, this indicates effects related to the race are not the most significant source of variation across all samples, but they are a key factor.

After correction for multiple testing, 63 features showed significant differences between pre- and post-race samples (12 decreased, 51 increased) (**Figure 5**). Of these, 10 had identities assigned from the Breath Biopsy Library.

Of particular interest was a feature that limited analysis in the preceding study by causing detector saturation. Qualitative analysis in previous study suggested that this compound generally occured in higher abundance in post-race samples. The higher dynamic range of the GC-Orbirap allowed this to be measured and prospectively identified as acetic acid, which showed a 59-fold increase post-race (**Figure 6**). Further examples of other features of interest are included in **Figure 7**.

Figure 5: 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 3: Principal component analysis by time of sampling

Post-race samples appear to separate along the PC1 axis in accordance with approximate time of sample collection (or possibly ambient pressure). This suggests that the timing of sampling was a factor in variation between samples, which may impact results.

Analysis was performed on all data together but is shown on two plots to visually clarify the observed trend in post-races samples.
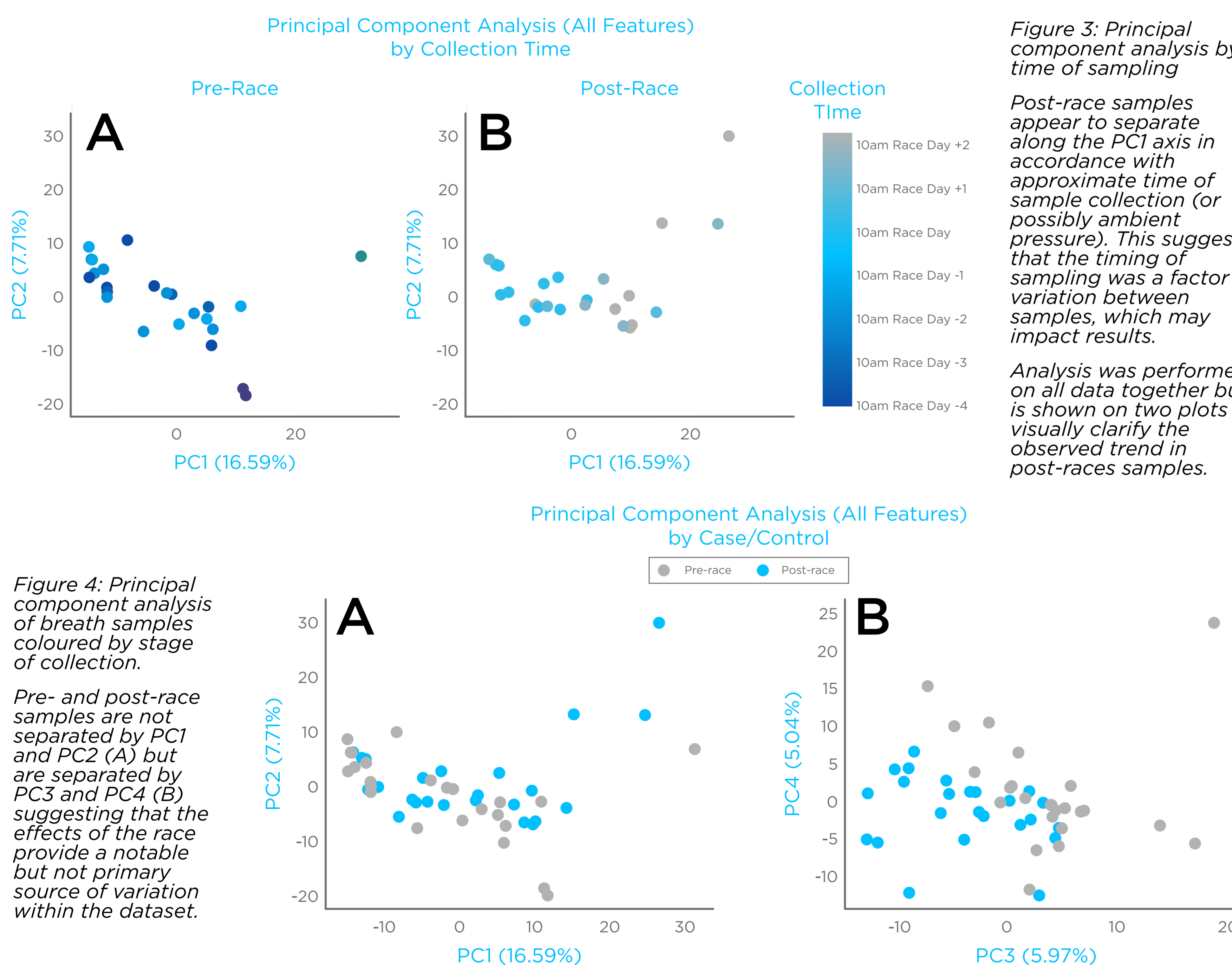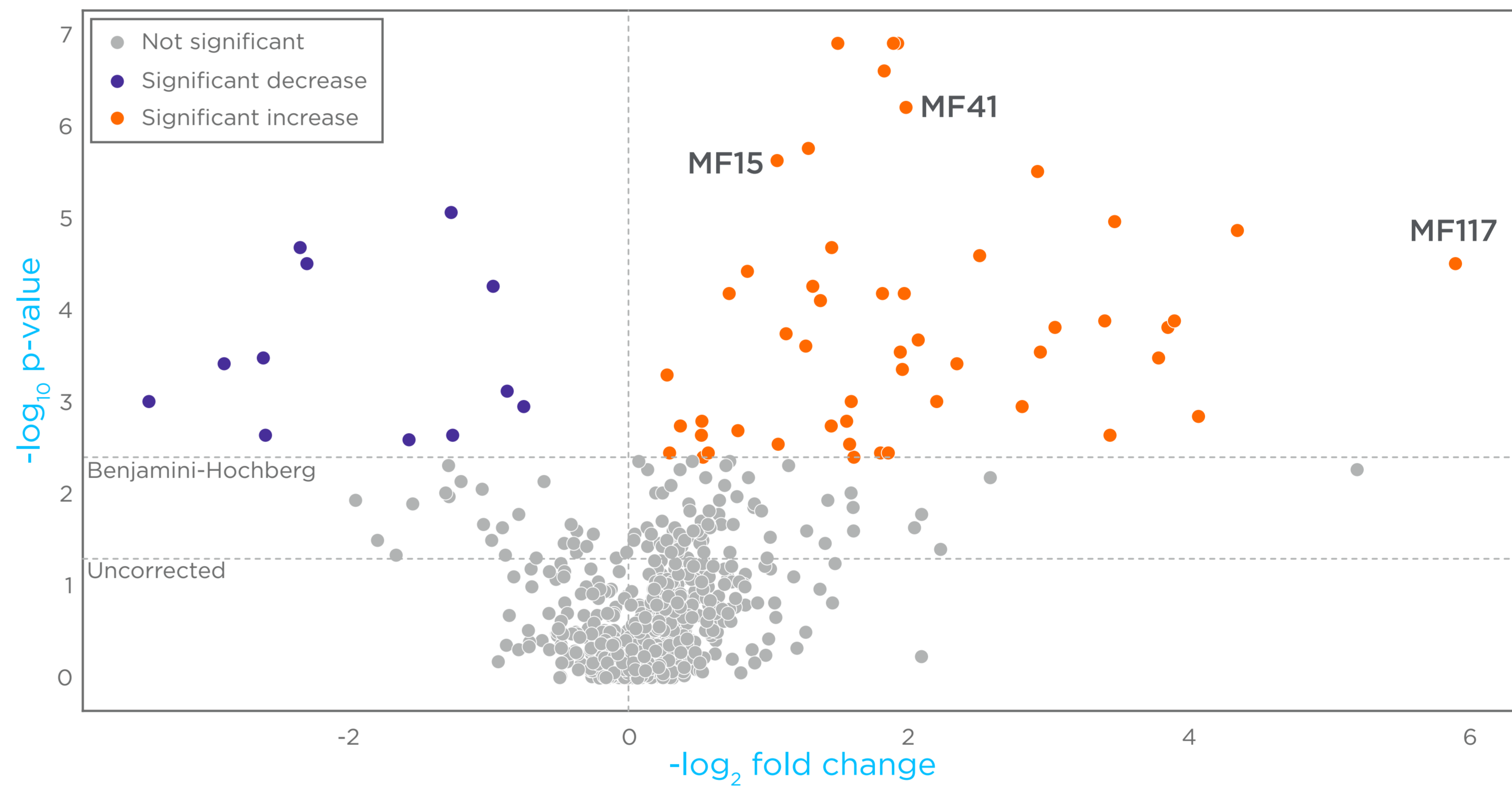

Figure 4: Principal component analysis of breath samples coloured by stage of collection.

Pre- and post-race samples are not separated by PC1 and PC2 (A) but are separated by PC3 and PC4 (B) suggesting that the effects of the race provide a notable but not primary source of variation within the dataset.
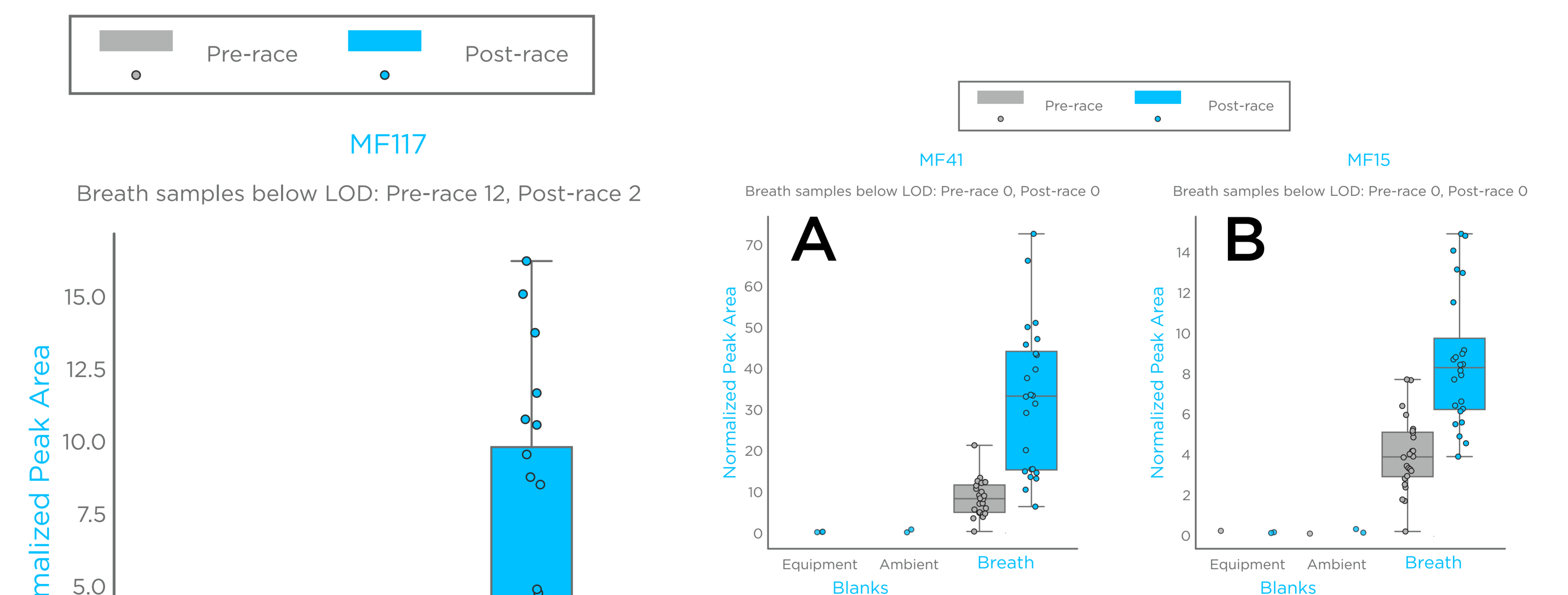

Figure 6: Boxplots of MF117 abundance.

A super abundant peak that previously caused issues with analysis was prospectively identified as acetic acid and shows a 59-fold change from pre- to post-race (p = 0.001). Tentatively identified as acetic acid.

This feature was absent/below the limit of detection in 12 pre-race samples and most blank measurements. In breath samples, where the signal was below the limit of detection it was imputed at 80% of the minimum peak area. For blanks, imputation was not possible and missing values were excluded. Equipment blanks: 1 pre-race, 4 post-race, Ambient blanks: 1 pre-race, 2 post-race

Figure 7: Boxplots of MF15 and MF41 abundance.

Additional examples of features that show significant increase between pre- and post-race samples using the same imputation approach as **Figure 5**.

4. Conclusions

Collected matched pairs of pre- and post-race samples from 24 elite athletes during the 2019 UTMB. An enhanced study design and analysis method provided higher sensitivity and dynamic range resulting in extraction of 811 features and making it possible to measure a super abundant peak that previously confounded analysis.

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 (10/63) were assigned molecular identities on the basis of comparison to the Breath Biopsy HRAM Library of VOCs. Additionally, the super abundant peak was prospectively identified as acetic acid.

The next steps for this work will be to compare VOCs to other markers of physiological stress and inflammation, specific to the respiratory system. The VOCs identified can also be investigated further to assess their origins and interpret their significance in a wider biological context.

Further Resources

Breath Biopsy: The Complete Guide (3rd Edition)
owlstonemedical.com/breath-biopsy-guide

Breath Biopsy Products & Services
owlstonemedical.com/products

Lipid peroxidation as a source of VOCs on breath
owlstonemedical.com/lipid-peroxidation

5. References

1. Al-Khelafi F, et al. "A pilot study comparing the metabolic profiles of elite-level athletes from different sporting disciplines". Sports Medicine. 2018, 4:2.
2. Stander Z, et al. "The altered human serum metabolome induced by a marathon". Metabolomics. 2018, 14:150