

Aims

- Identify breath VOCs associated with the early and late phase airway response to allergen exposure in asthma patients.
- Correlate the levels of the breath VOCs identified to be associated with allergen exposure response with levels of sputum/blood markers.

1. Background and Objectives

Lung inflammation is a factor in several illnesses and as a response to pollution and other irritants. Inflammation is reported to release volatile organic compounds (VOCs) in breath that could be non-invasively detected and monitored to assess inflammation. Currently, breath (via Breath Biopsy) is increasingly used as a tool to discover and monitor biomarkers of exposure, metabolism, and disease. The allergen challenge model is considered the gold standard for investigating airway inflammation. It is used by pharmaceutical companies to test the ability of new drugs to reduce inflammation. The challenge model determines patient-specific allergens in steroid naïve asthma patients and then exposes them to their specific allergen or a

placebo exposure. The allergen causes rapid bronchoconstriction (the early asthma response) followed by T-cell mediated eosinophilic inflammation (the late asthma response).

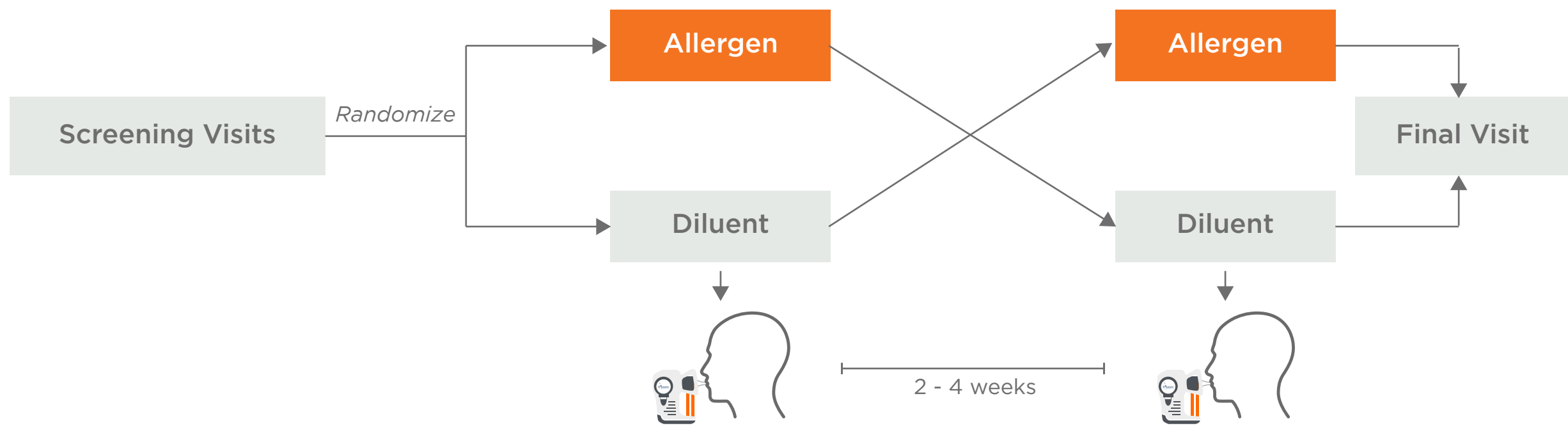
We investigated VOCs in exhaled breath, using Breath Biopsy, associated with lung inflammation induced by the allergen challenge in asthmatic individuals. Our hypothesis was that exposure to a mild allergen induces inflammation of epithelial lung tissue, which will result in the production of specific VOCs in exhaled breath. The primary objective of the study is to investigate the potential links between breath VOCs and acute inflammation in asthmatic population.

2. Methods

Experimental Design

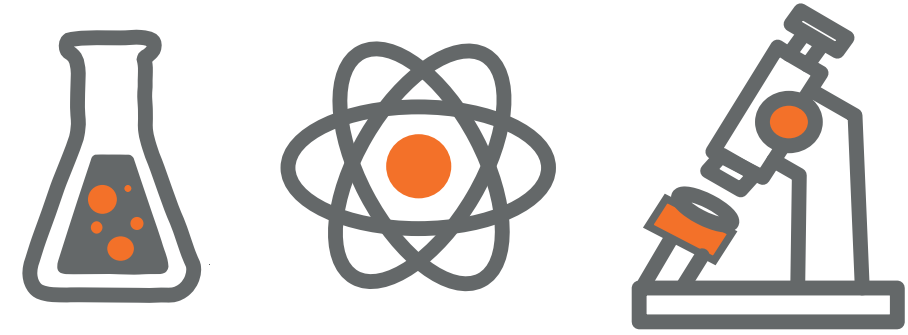
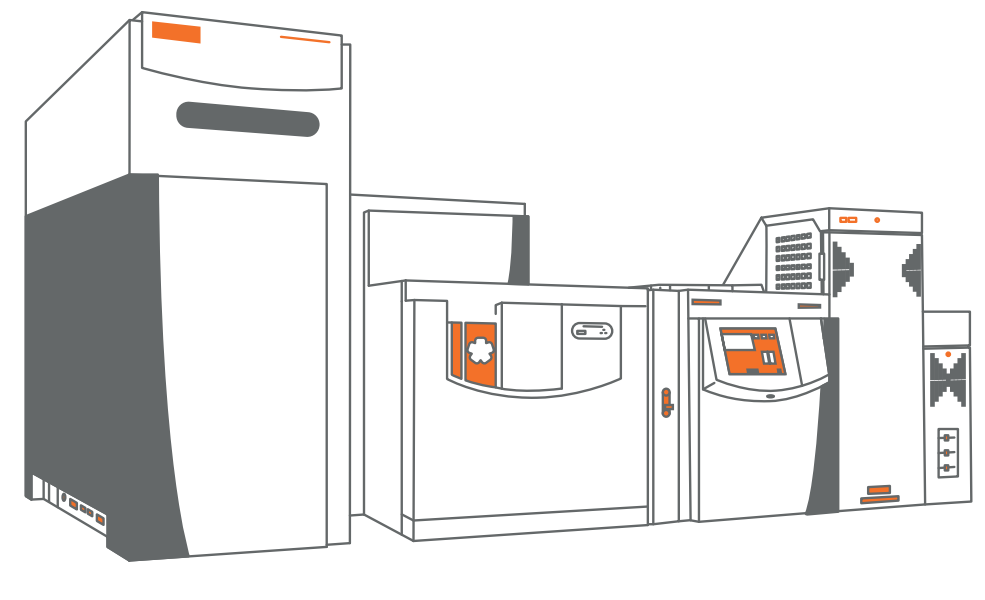
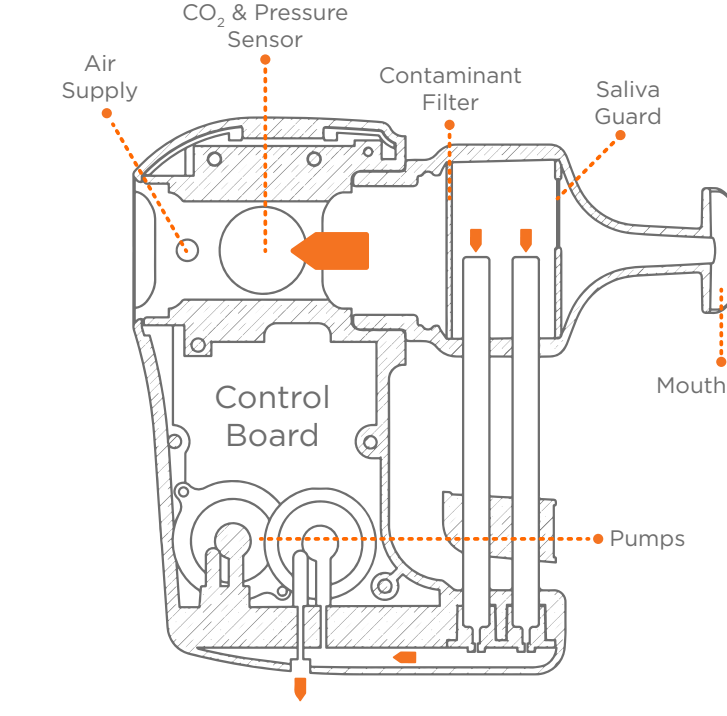


Asthmatic n = 9 (6 female, 3 male)
Average age = 36.8 ± 13.6



Exhaled breath sampling at baseline and post-challenge at 30 min, 1h, 3h, 5h, 6h, 7h and 24h.

Sample Processing



Collection

Analysis

Interpretation

- Breath collected using the Breath Biopsy Collection Station.
- GC-MS processed on high-resolution accurate Q Exactive Orbitrap system.
- Comparing VOCs profile of pre- (baseline) and post-allergen/diluent challenge (all time points).
- Targeted analysis (Table 1), i.e. looking at absolute concentration of named VOCs in exhaled breath and untargeted analysis, i.e. any possible VOC detected in exhaled breath.
- Principal Component Analysis (PCA) and statistical testing was used to find differences in VOC profiles.

Table 1. The list of targeted VOCs

2-methylfuran	E-2-Butenal	p-Xylene	Nonanal
Butanal	2-Hexanone	Cyclopentanone	Menthone
3-methylfuran	Hexanal	o-Xylene	2,4-Heptadienal
Pentanal	Undecane	Heptanal	2-ethyl-Hexanol
(E/Z)-1-methylthio-1-propene	(E)-2-methyl-2-butenal	Dodecane	Decanal
Decane	3-vinylfuran	D-Limonene	Pentadecene
(Z/E)-1-methylthio-1-propene	3-methyl-thiophene	2-pentylfuran	Hexadecane
1-propanol	E-2-Pentenal	Octanal	

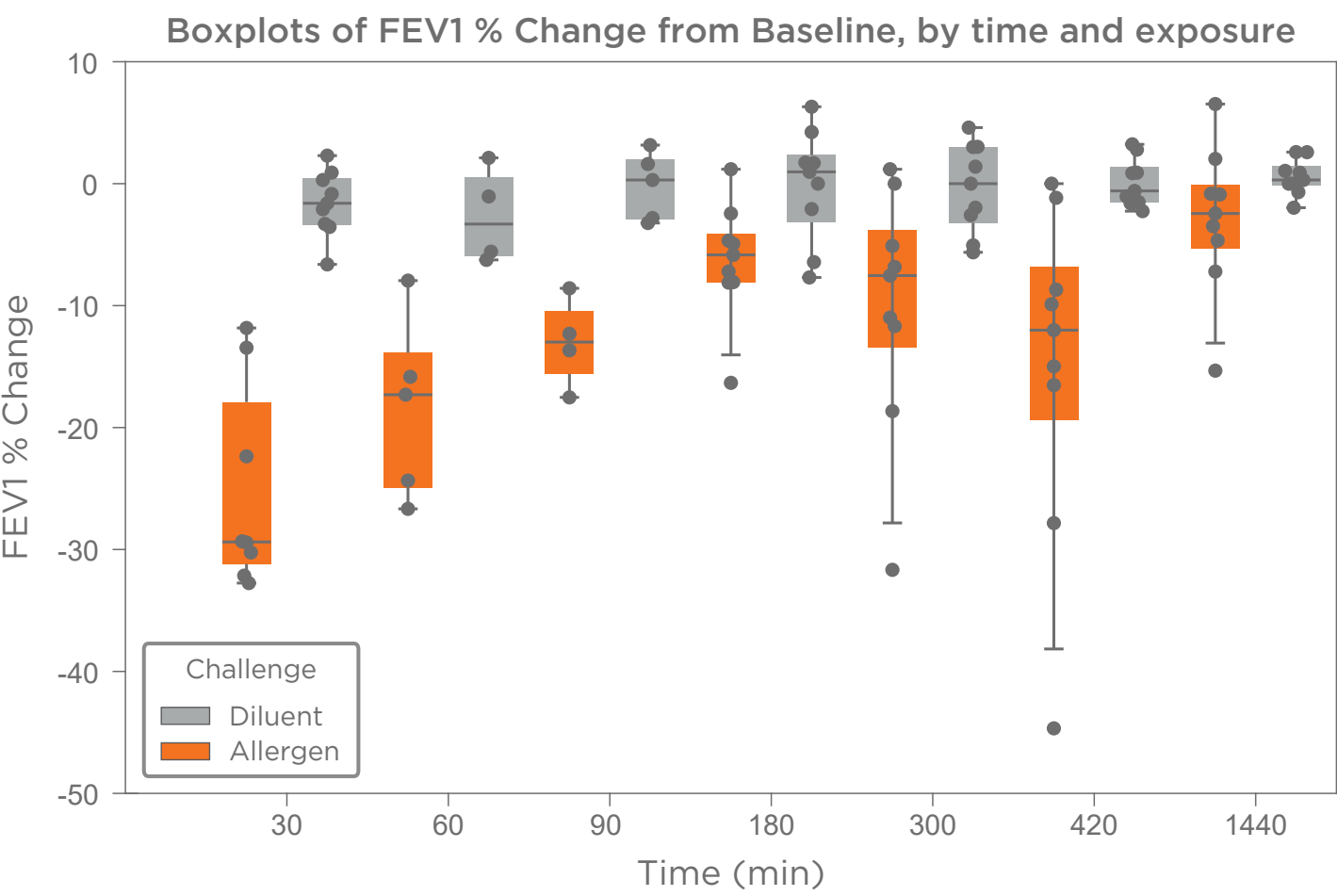


Figure 1. Percent change in FEV1 (y-axis) over time with allergen or diluent challenge. FEV1 % change pre-challenge is 0.

3. Results

Exhaled air profile is different from ambient samples.

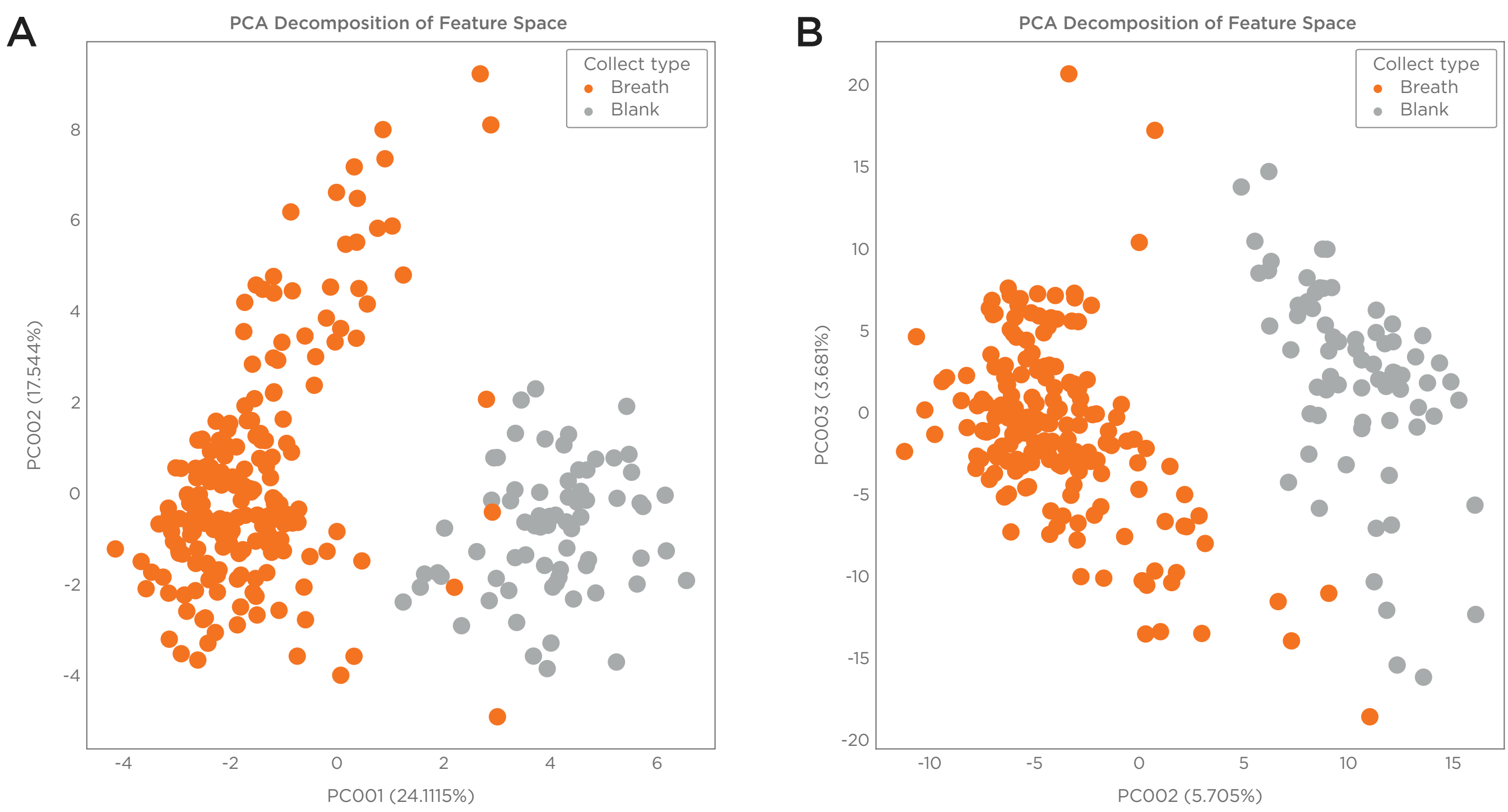


Figure 2. PCA score plot of 144 exhaled breath and 32 ambient blank samples; (A) consisting of 31 targeted VOCs (Table 1) and (B) 858 VOCs from untargeted analysis. Clear separation between breath samples and ambient blanks was demonstrated.

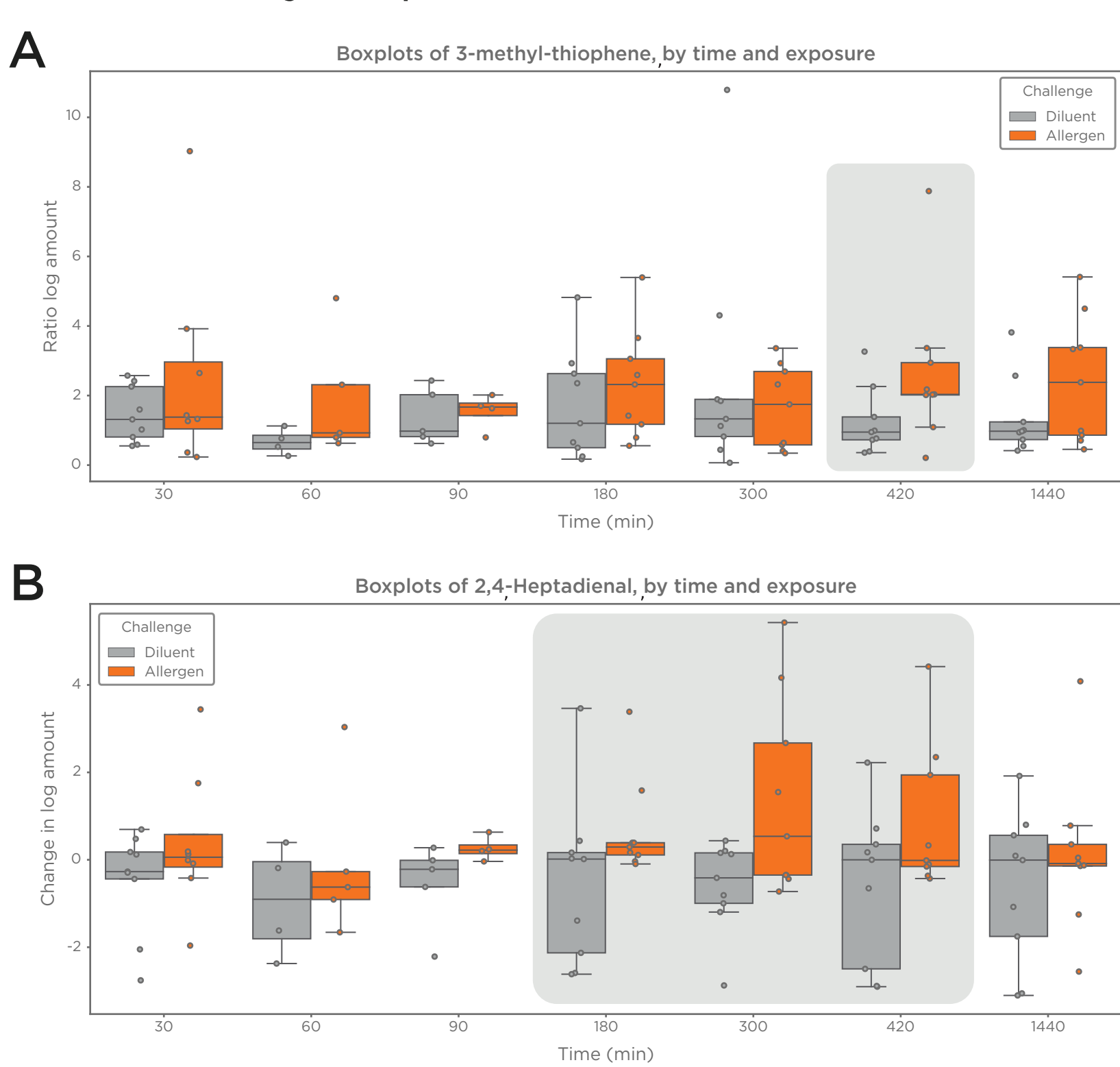


Figure 3 A-B. Examples of compounds significantly increased by allergen challenge compared to diluent. Largest differences occurred during the late airway response.

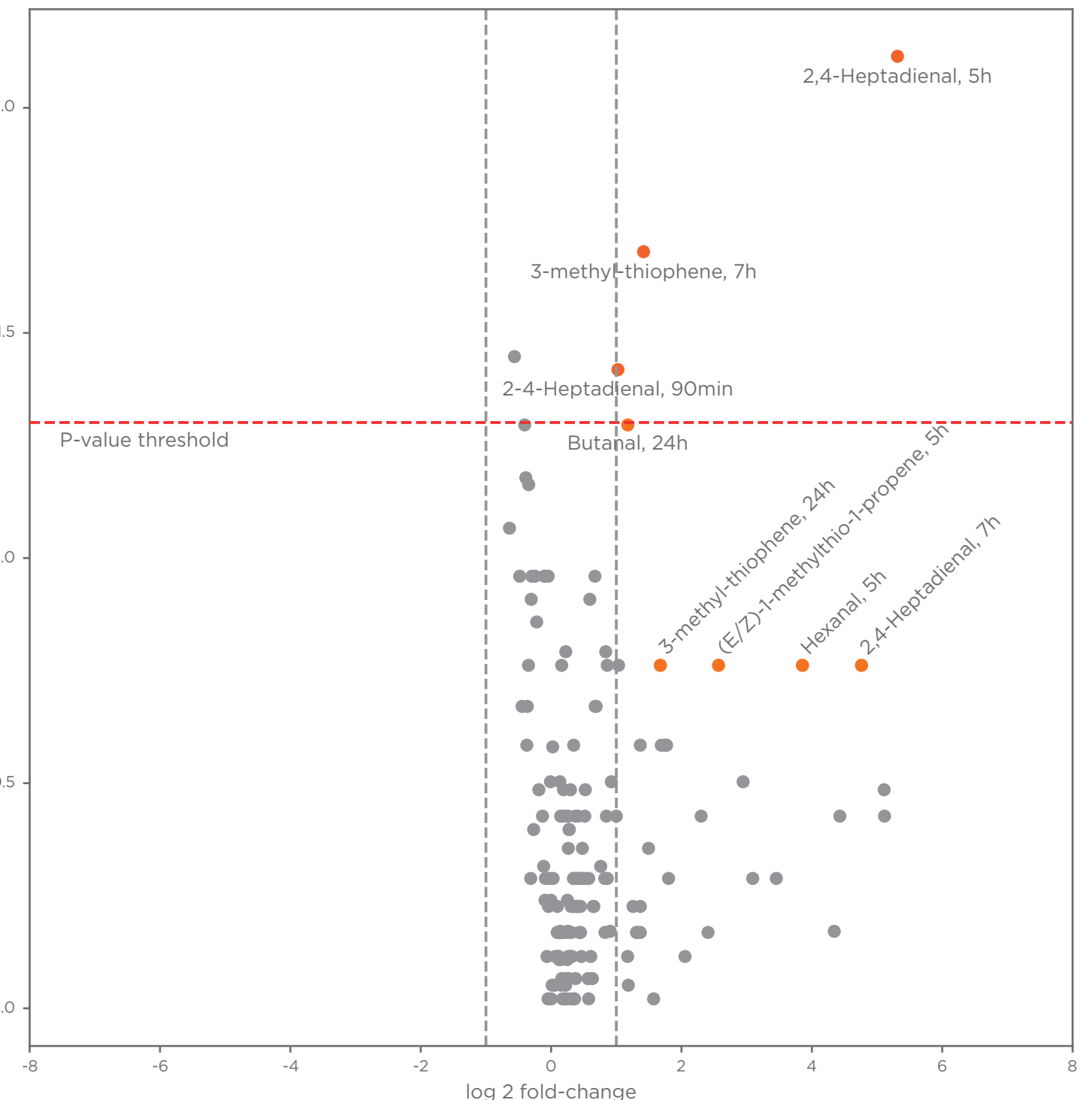


Figure 4. The volcano plot of results from Wilcoxon Signed-Rank tests.

Figure 5. Correlation map between targeted VOCs (Table 1) and lung function test, blood/sputum parameters at 7h and 24h post-allergen administration. The VOCs in white represent significant correlation and defined as on-breath (i.e. amount in breath is higher than 3 standard deviation of amount in ambient blanks).

FEV1: Forced exhalation volume in one second in litres (raw). BLDEOS: Blood eosinophils (%). SPUTEEO: Sputum eosinophils (%). BLDTC: White blood cells x 10⁶ per mL. MCHPC20: Methacholine PC20 (mg/mL) wrt baseline.

4. Conclusions

- Significant differences in VOC profiles between baseline and post-allergen challenge; as well as between allergen and diluent (p-value<0.05).
- The present study shows that the allergen challenge induces inflammation in the lung and can be associated with changes in VOCs profile observed at different time points, which are linked to early (30 min - 3h) and late (5 - 7h) airway responses to the allergen.
- The exhaled breath profile after 24h - post allergen does not show significant differences between diluent and allergen, indicating resolving late airway response presence.
- Several VOCs related to inflammation show significant correlation with blood and/or sputum markers. These may provide valuable insights into the underlying biological processes and interactions between the immune system and metabolic pathways.
- The statistically significant differences in compound abundance are mostly related to ROS and inflammation as seen with elevated aldehyde and ketones.