Introducing the Breath Biopsy® Atlas: A List of Microbiome-Associated VOCs Related to Metabolic Responses Following Supplementation

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Key points

- Several metabolites generated by the microbiome in the digestive system that are associated with health and disease can be measured in breath either at steady state, or after administration of certain substrates.
- Given ease of breath collection, this approach can be applied to large cohorts to better establish the effect of these metabolites on health.
- Using the Breath Biopsy VOC Atlas, ranges for microbiome-associated, on-breath VOCs observed in nominally healthy and disease populations can be established.

1. Background and Objectives

Volatile organic compound (VOC) biomarkers in exhaled breath offer a promising avenue to safe, non-invasive diagnostics in multiple disease settings. Some of these VOCs are linked to the gastrointestinal (GI) microbiome and could provide insight into the response of the gut microbiome to health status as well as dietary and therapeutic interventions.

The gastrointestinal microbiome plays a crucial role in health and disease. It provides pathogen resistance, enforces barrier integrity, stimulates the immune system, and produces essential compounds for maintaining host health. Understanding the relationship between the gut microbiota and health is crucial for developing strategies like pre- and probiotics to optimize gut health to prevent or treat various conditions.

Microbial VOCs produced in the gut are transported via blood to the lungs where they are exhaled. However, currently a lack of standardization has been a barrier in translating breath analysis to clinically useful biomarkers.

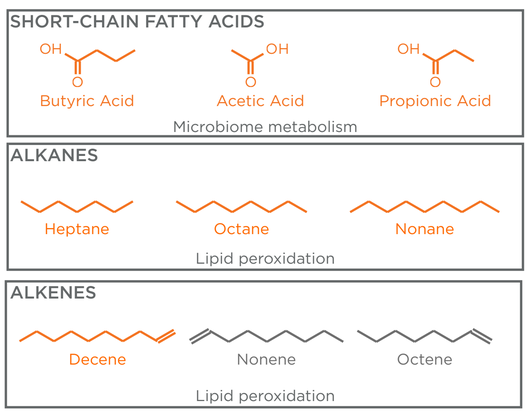


Figure 1: Example volatile compounds related to both health and disease. Those in orange are present in the VOC Atlas.

The Breath Biopsy® VOC Atlas is a curated database of detectable on-breath VOCs that can be used for confident biomarker identification and assessment. Alongside Breath Biopsy OMNI®, Owlstone Medical's breath analysis platform, this method is optimized for the identification and analysis of compounds in exhaled breath.

2. Method

Study Cohort and Breath Sample Collection:
In total, 94 adult volunteers were enrolled between 2022 and 2023 in Cambridge, UK. All volunteers provided written informed consent and breath samples were collected according to IRB-approved protocols at Owlstone Medical's Breath Biopsy Laboratory (Cambridge, UK).

Breath samples were collected from a demographically mixed cohort of volunteers, and 94 paired background samples were also collected. All breath samples were collected using Owlstone Medical's Breath Biopsy OMNI sample collection and analysis platform. All equipment background samples were generated by collecting room air.

Breath Sample Analysis:
Samples were analyzed using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) (Thermo Scientific, Waltham, MA, US). On-Breath Calculations: Molecular features were determined to be originating from breath or "on-breath" using three criteria. 1) Sample signal of at least 50% of breath samples > Mean + 3 standards deviations of background. 2) Paired t-test with p values <0.05 and fold change >2 and time-matched blank samples, with a mean fold change > 2. 3) ROC-AUC > 0.8 and a positive mean fold change between breath and blank. VOCs confirmed to be on-breath were identified using purified chemical standards.

Table 1: Study cohort displayed by age and sex.

Age Group (Yr)	Male (n)	Female (n)
18-30	10	14
31-50	19	18
51-70	12	15
71+	4	2

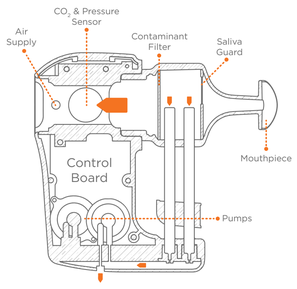
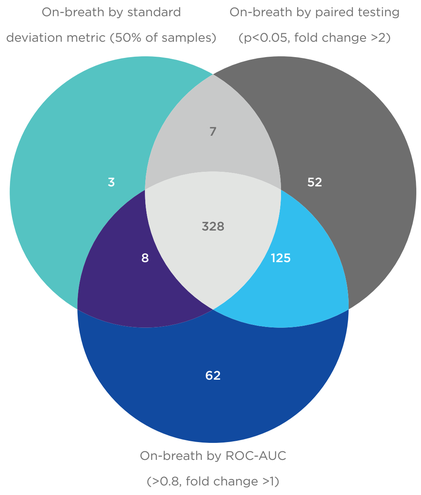


Figure 2: ReCIVA® Breath Sampler.

3. Results and Discussion

A: Study Cohort



B: Schematic Showing Signal Over Background

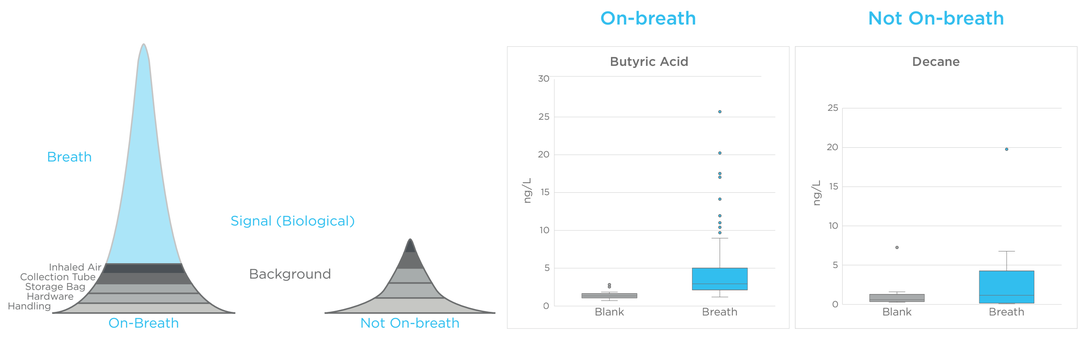


Figure 3: On-breath features (n) by each criterion. A) 1471 total features were identified in breath samples, of these 328 are on-breath by all 3 criteria. B) An on-breath compound must be distinguishable from background noise: (Individual) area under peak ≥ mean background + 3 x standard deviation background.

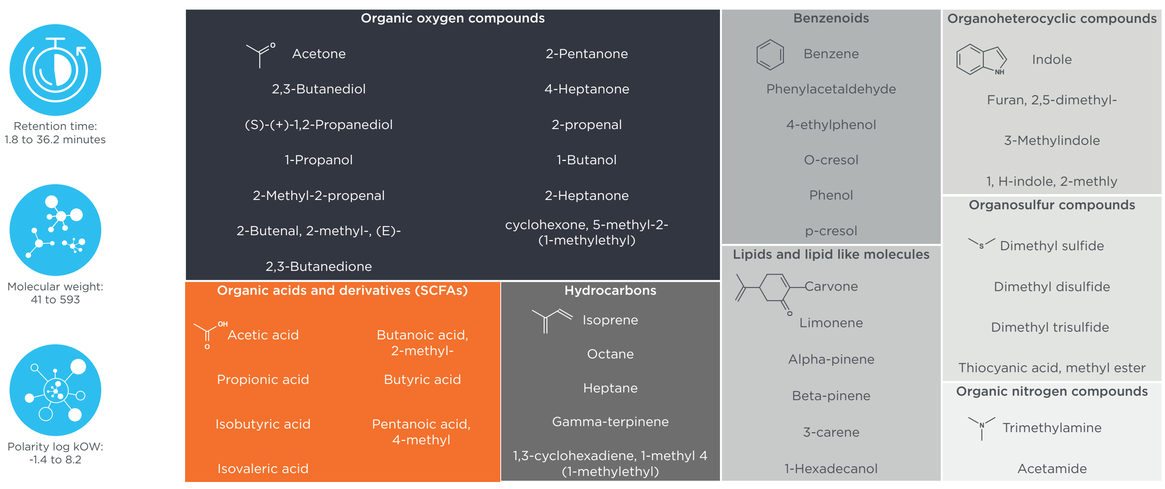


Figure 4: Summary of microbiome-associated Breath Biopsy Atlas contents. Currently, >180 identified VOCs, with 47 being microbiome-associated, have been identified using comparisons to purified chemical standard. On-breath VOCs reflect multiple chemical classes (right) and chromatographic properties (left). Categories will expand as additional VOCs are added to the Atlas.

The Microbiome

This Atlas currently contains >180 identified VOCs, with 47 being microbiome-associated, including short-chain fatty acids (SCFAs). In both populations studied here, SCFAs were reliably detected: 1.20 - 25.69 ng/L butyric acid (median= 2.92), 0.01 - 81.47 ng/L acetic acid (median= 11.73) and 0.01 - 46.20 ng/L propanoic acid (median= 1.2).

The dietary fibers which cannot be processed by human metabolism are fermented by microbes in the intestines, resulting in the production of VOCs such SCFAs. These VOCs have roles in several signaling contexts including the central nervous system, immunity, and inflammation[1]. A study from Smolinska *et al.* demonstrated the connection between exhaled breath VOCs and intestinal microbiota in subjects with Crohn's disease (CD)[2]. Samples were collected and analyzed in the same subjects during both active symptom flares and during remission. Acetate and propionate (both in the Atlas) correlated significantly with *Bifidobacteria* and several other microbes in the Firmicutes phylum in both disease states. Moreover, the microbial strains and the relative abundances of SCFAs both decreased in the active disease state[2].

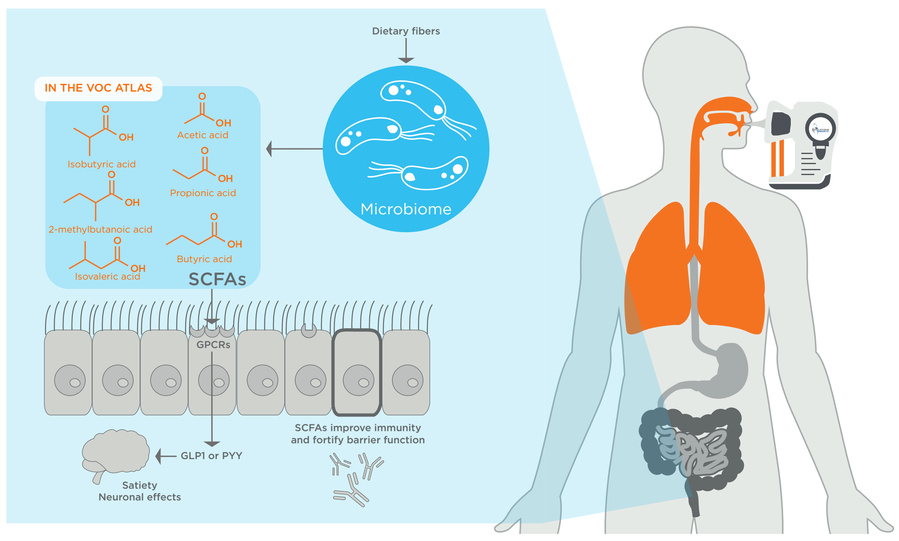


Figure 5: VOCs in the Breath Biopsy Atlas are potentially produced by microbiome activities thus on-breath VOCs could be a non-invasive source of microbiome biomarkers.

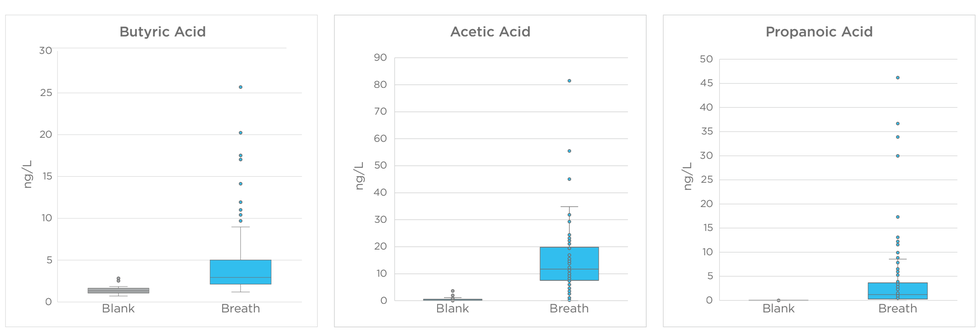


Figure 6: Butyric, acetic and propanoic acids are SCFAs that are present the in Breath Biopsy VOC Atlas. They can be detected at levels significantly above background signals in exhaled breath using Owlstone's technology. These SCFAs are notable products of microbiome metabolism that could inform on microbiome activity.

Other microbiome-associated VOCs

Other interesting microbiome-associated VOCs present in the Breath Biopsy Atlas are related to inflammation such as indole, trimethylamine (TMA) and isoprene. Indole has been linked to maintaining the biological barrier of the human intestine[3]. TMA is linked to heart disease via trimethylamine N-Oxide (TMAO)[4]. TMAO can promote the release of inflammatory cytokines, enhance monocyte adhesion to endothelial cells, and promote oxidative stress[4], and isoprene-derived aerosols have been found to alter inflammatory/oxidative stress genes[5]. These three VOCs are present in the Breath Biopsy Atlas. TMA, indole, cresols, and phenols are also products of aromatic amino acid metabolism and have demonstrated associations with metabolic responses.

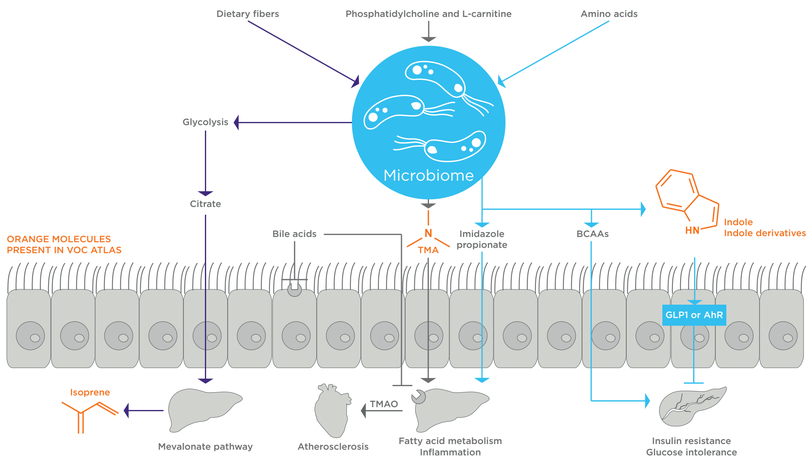


Figure 7: VOCs in the Breath Biopsy Atlas are potentially associated with the activity of the fatty acid oxidation pathway. Additional experimentation will elucidate mechanistic associations between on-breath VOCs.

4. Conclusions

Pairing reliable detection of microbiome-associated VOCs with the Breath Biopsy VOC Atlas could provide greater insight into the normal levels of these metabolites, and allow for targeted study designs to investigate the response of the gut microbiome to health status as well as dietary and therapeutic interventions. For more information on the effects of supplementation on health and disease, please see our other posters titled 'Monitoring changes in exhaled volatile organic compounds following iron supplementation for anemia treatment' and 'Breath-based monitoring of microbiome metabolic responses before and after ingestion of nutrients'.

References

1. Nogal, A, et al. (2021) 'The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health.' Gut Microbes. 13(1), DOI:10.1080/19490976.2021.1897212
2. Smolinska, A., et al. (2018). "Volatile metabolites in breath strongly correlate with gut microbiome in CD patients." Anal Chim Acta.1025: p. 1-11.DOI:10.1016/j.aca.2018.03.046
3. Ye X et al. (2022). "Dual Role of Indoles Derived From Intestinal Microbiota on Human Health." Front Immunol. 17;13:903526. doi:10.3389/fimmu.2022.903526. PMID: 35784338; PMCID: PMC9248744.
4. Liu, Y et al. (2020). "Trimethylamine N-Oxide Generated by the Gut Microbiota Is Associated with Vascular Inflammation: New Insights into Atherosclerosis." Mediators Inflamm. Article ID 4634172, 15 pages. https://doi.org/10.1155/2020/4634172
5. Eaves, LA et al.(2020). "Isoprene-Derived Secondary Organic Aerosol Induces the Expression of MicroRNAs Associated with Inflammatory/Oxidative Stress Response in Lung Cells." Chem Res Toxicol. 33 (2), 381-387 DOI: 10.1021/acs.chemrestox.9b00322