Analyzing Metabolic Responses to a Standardized Meal Challenge using the Breath Biopsy® OMNI® Platform

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1. Introduction

- Volatile organic compounds (VOCs) in exhaled breath can reflect metabolic responses, and understanding the intricate connection between human metabolism and different physiological states in a non-invasive manner is crucial for future clinical applications.

- In this study, we aim to elucidate the impact of dietary intake on exhaled breath VOCs, highlighting the capability of our OMNI® breath collection and analysis platform to detect metabolically relevant volatile compounds. Non-invasively detecting diet-induced metabolic changes in breath VOCs will facilitate future disease-related research.

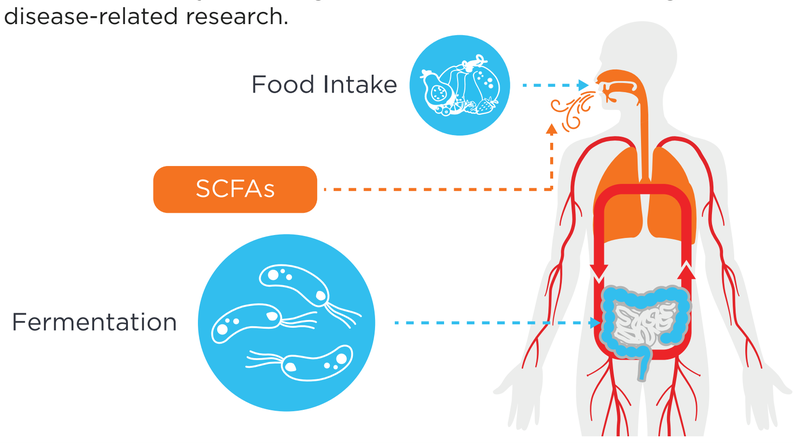

Figure 1: After food intake, short-chain fatty acids (SCFAs) are produced via fermentation of indigestable fiber by the microbiome. These travel through the bloodstream before diffusing into the lungs where they are detectable in breath.

2. Methods

- 20 volunteers underwent an overnight fast followed by a standardized meal challenge comprising a balanced 400 kcal meal composed of 19g of fat, 34g of carbohydrate (4g sugars), 20g of protein, and 6g of fiber. Two breath samples were collected in the fasted state and two at 20 mins and 1h after the meal. Equipment blank samples were also collected to rule out background noise from ambient air.

- Using thermal desorption gas chromatography-mass spectrometry (TD-GC-MS) with the Breath Biopsy OMNI Platform, we analyzed the collected breath samples and equipment blanks. The untargeted data were matched to our internal High Resolution Accurate Mass (HRAM) library. Statistical analyses were performed to distinguish breath compounds from ambient air, and the main groups (fasted sample 1/sample 2 vs. fed sample 1/sample 2) were compared using repeated measures ANOVA and Wilcoxon tests.

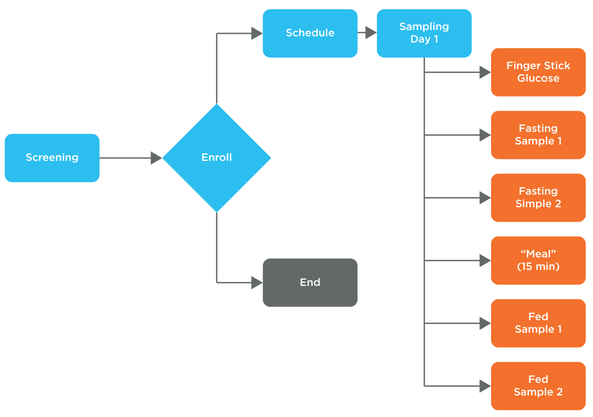

Figure 2: 20 healthy volunteers (non-smokers without chronic illness) were recruited for the study. Breath samples were collected while the volunteers were in a fasted state (8+ hours without food or liquids other than water) and after consuming a liquid meal to induce a fed-state metabolic transition. Two samples were taken before the meal (fasted 1 and fasted 2) and post-meal (fed 1 at 20 minutes after the meal and fed 2 at 1 hour after the meal). An equipment blank sample was collected each sampling day for each volunteer. The breath and equipment blank samples were compared to identify compounds present on breath. Volunteer-matched fed and fasted samples were compared to identify VOCs associated with the metabolic-state transition.

Table 1: Cohort characteristics. The distribution of the population is balanced across genders (female 60% and male 40%), with comparable average age, body mass index (BMI) and fasting blood glucose levels.

	Age	BMI	Blood Glucose mmol/L
Total	28.9	24.7	5.0
Female (60%)	29.3	23.6	4.9
Male (40%)	28.3	26.4	5.1

3. Results

- Our untargeted approach yielded a final dataset of 1,157 molecular features, of which 687 passed the sparsity criteria, and 217 were identified as on-breath, as illustrated in Figure 3. A VOC is defined as on-breath if it exceeds 3 standard deviations above the mean of blanks for that VOC and surpasses this threshold in 50% of samples. Alternatively, a feature is considered on-breath if the p-value is less than 0.05 when a t-test is applied between blank and breath samples, or if the ROC-AUC is greater than 0.8 between breath and blank samples.

- As expected, several microbiome-related and exposure compounds (deriving from food or flavoring) changed from fasting to refeeding, as shown in Table 2. Specifically, Figure 4 highlights the most significant changes in microbial metabolites just 20 minutes after meal intake. Short-chain fatty acids (SCFAs) such as butyric acid, propionic acid, and acetic acid showed an upward trend following refeeding. Other microbiome-related metabolites, such as 1-propanol and 2,3-butanedione, also increased, while indole and 3-methylindole, products of microbial fermentation of tryptophan, decreased or remained unchanged after food intake.

- Additionally, other VOCs linked to exposure, commonly found in food or used as flavoring agents, such as valencene, limonene, and terpenes such as alpha-pinene and beta-pinene, also significantly increased 20 minutes after food consumption and stabilized at the 1-hour timepoint, as depicted in Figure 5.

- Indole is a product of the bacterial breakdown of the amino acid tryptophan. Various gut bacteria, including *Escherichia coli*, can produce indole. It has multiple roles, including signaling within the gut and influencing host physiology. Additionally, dimethyl disulfide, p-cymene, and 2,3-butanedione are notable microbiome-related compounds that change from fasting to refeeding.

- Of interest, butyric and propionic acids are SCFAs produced by the fermentation of dietary fibers by certain gut bacteria, such as members of the *Firmicutes* phylum (e.g., *Clostridia* species). These acids play a crucial role in maintaining gut health and have anti-inflammatory properties. Indole and 3-methylindole are products of the bacterial breakdown of the amino acid tryptophan. Various gut bacteria, including Escherichia coli, produce indole, which has multiple roles, including signaling within the gut and influencing host physiology.

References:

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2. Neyrinck AM, et al. Noninvasive monitoring of fibre fermentation in healthy volunteers by analyzing breath volatile metabolites: lessons from the FiberTAG intervention study. Gut Microbes. 2021 Jan-Dec;13(1):1-16. doi: 10.1080/19490976.2020.1862028.
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Figure 3: 217 VOCs were identified as on-breath. 54 passed by paired t-test, 7 had 50% > mean + 3SD and paired t-testing, 92 had all three.

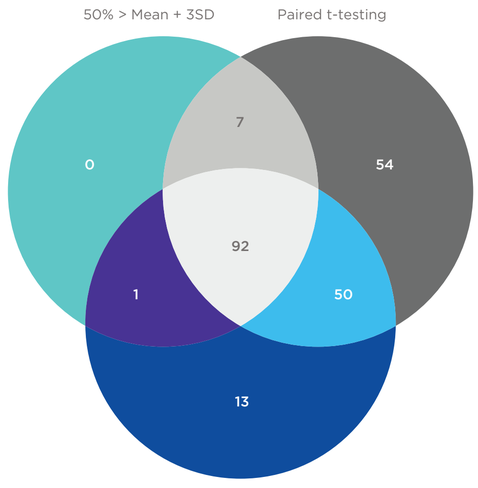


Table 2: Several VOCs in breath vary between fasting and fed states, including significant microbiome metabolites and those linked to exposure.

Matched Compound	Origin	Effect Size	p value
1-Propanol	Microbiome Fermentation	2.238474638	1.91E-06
Propinoic acid	Microbiome Fermentation	2.148193466	1.91E-06
Indole	Microbiome Fermentation	-2.51086657	2.67E-05
Butyric acid	Microbiome Fermentation	1.948055356	0.000261
3-Methylindole	Microbiome Fermentation	-0.71522331	0.000483
2,3-Butanedione	Microbiome Fermentation	1.577482726	0.00639
Acetic acid	Microbiome Fermentation	1.216365075	0.036234
Limonene	Exposure - food and flavoring	3.74845005	1.91E-06
2-Pentylfuran	Exposure - food and flavoring	3.437482291	1.91E-06
Valencene	Exposure - food and flavoring	3.297065551	1.91E-06
2-Propenal, 2-methyl-3-phenyl-	Exposure - food and flavoring	3.258943355	1.91E-06
alpha-Pinene	Exposure - food and flavoring	2.72710383	1.91E-06
2,4-Dimethylfuran	Exposure - food and flavoring	2.420229989	1.91E-06
Octane	Exposure - food and flavoring	2.690181083	3.81E-06
beta Pinene	Exposure - food and flavoring	2.514595331	5.72E-06
gamma Terpinene	Exposure - food and flavoring	1.480385675	1.34E-05
p-Cymene	Exposure - food and flavoring	1.708236756	0.000261

Figure 4: Microbiome related VOCs that changed after refeeding

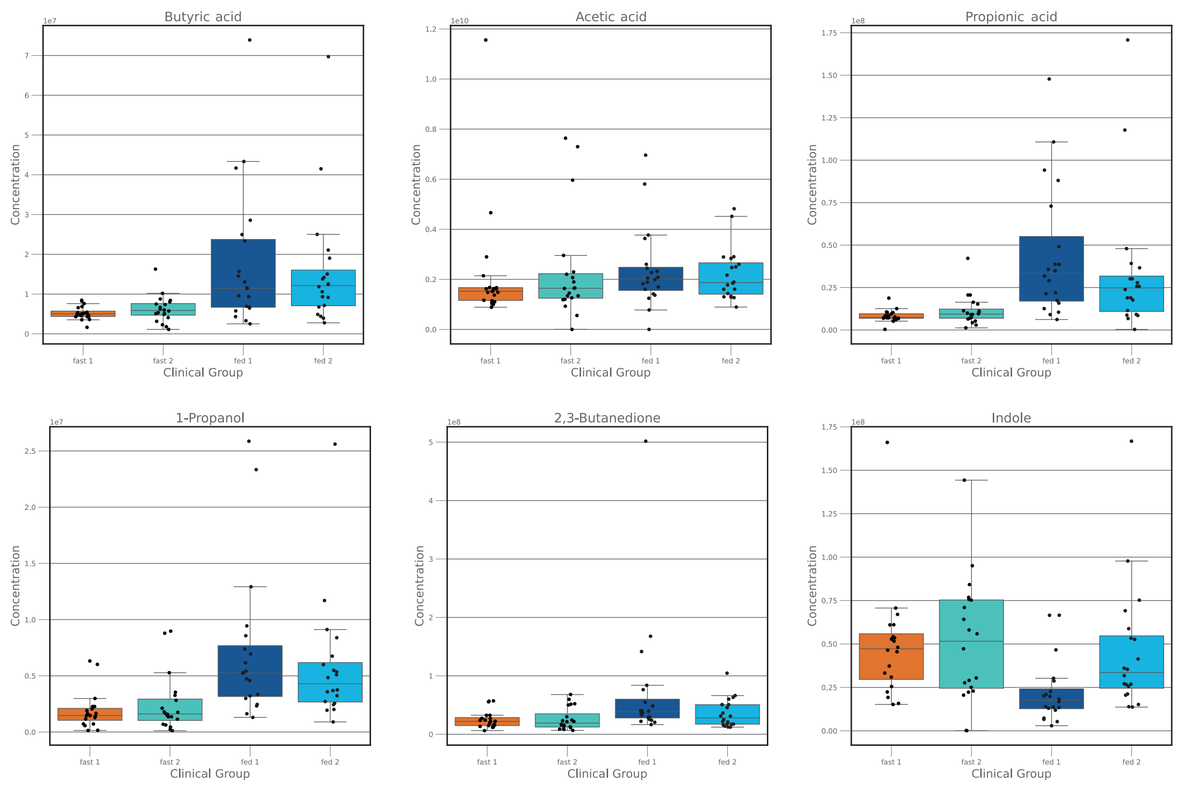
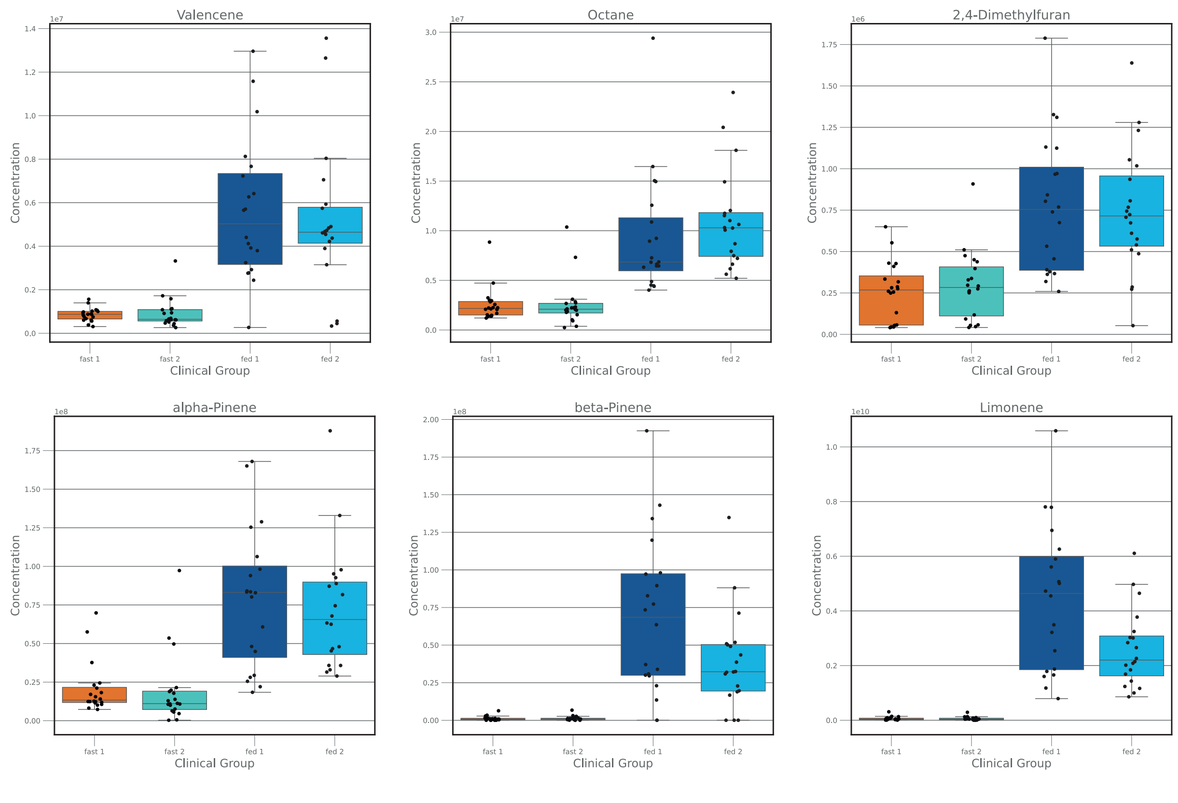


Figure 5: Exposure related VOCs that changed after refeeding



4. Conclusions

An interesting finding is that the upregulation of SCFAs only 20 minutes after refeeding was not observed in previous literature using a similar protocol and SIFT-MS approach (1-2). However, another study found that the SCFA producer *Faecalibacterium* showed differing fasting responses between healthy individuals and those with metabolic syndrome but exhibited consistent growth upon refeeding in both groups (3). This suggests that SCFA profiles can change during fasting (4) and may respond to peristalsis stimuli and colon contractions following a liquid meal, contributing to the observed changes. Additionally, another study observed a negative correlation between exhaled indole and blood glucose when breath samples were collected for six hours after type 1 and type 2 diabetes patients ingested a standardized meal (5), consistent with our current data.

In conclusion, this study underscores the promising utility of the Breath Biopsy OMNI Platform in advancing our understanding of metabolic responses to dietary stimuli. Notably, the platform demonstrated its ability to identify established nutrition and microbiome-associated metabolites such as SCFAs documented in existing literature, while also revealing novel candidate breath biomarkers associated with food digestion. Moreover, it sets the stage for future investigations aimed at further validating the platform's suitability and refining non-invasive diagnostic methods in both health and disease using breath analysis.