ANALYZING METABOLIC RESPONSES TO A STANDARDIZED MEAL CHALLENGE USING THE BREATH BIOPSY® OMNI® PLATFORM

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1. Background and Objectives

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.

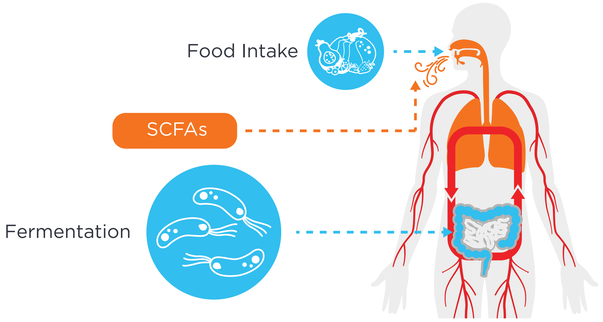

Figure 1: After food intake, short-chain fatty acids (SCFAs) are produced via fermentation of simple carbohydrates and amino acids by the microbiome. These travel through the bloodstream before diffusing into the lungs where they are detectable in breath.

2. Method

A total of 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.

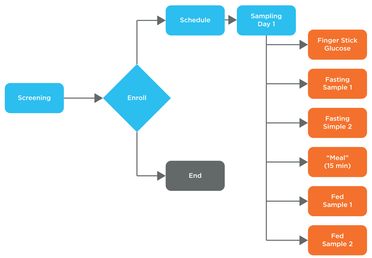

Figure 2: Twenty healthy, non-smoking volunteers participated in the study. Breath samples were collected after an 8+ hour fast (only water allowed) and following a liquid meal. Two samples were taken before the meal (fasted 1 and 2), with glucose monitoring in between, and two samples after the meal (fed 1 at 20 minutes and fed 2 at 1 hour). Equipment blanks were collected at the end of the day. The samples were analyzed to identify compounds and compare VOCs related to metabolic-state changes.

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.

	Average Age	Average BMI	Average 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 anticipated, several exposure-related compounds—derived from food or flavoring—changed between fasting and refeeding, as shown in Table 2. Figure 4 illustrates that VOCs linked to exposure, frequently found in food or used as flavoring agents, such as valencene, limonene, and terpenes like alpha-pinene and beta-pinene, significantly increased 20 minutes after food consumption and stabilized by the 1-hour mark.

Interestingly, Figure 5 highlights the most notable changes in microbial metabolites detected in breath just 20 minutes after eating. Short-chain fatty acids (SCFAs) such as butyric acid, propionic acid, and acetic acid exhibited an upward trend following refeeding. Other microbiome-related metabolites, including 1-propanol and 2,3-butanedione, also increased, while indole and 3-methylindole—products of microbial fermentation of tryptophan—either decreased or remained unchanged after food intake.

Indole is produced from the bacterial breakdown of the amino acid tryptophan, which is prevalent in the HUEL drink. Various bacteria, such as *Escherichia coli*, can generate indole, influencing host physiology. Additionally, microbiome-related compounds like dimethyl disulfide, p-cymene, and 2,3-butanedione also shift from fasting to postprandial state.

Notably, butyric and propionic acids are SCFAs produced by the fermentation of dietary sugars and amino acids by oral microbiota, including members of the Firmicutes phylum (e.g., *Clostridia* species) and Proteolytic bacteria, such as *Prevotella*. These microbial products are vital for maintaining oral health, possess anti-inflammatory properties, and act as intermediates in the catabolism of amino acids such as valine and isoleucine, as well as in the conversion of propionyl-CoA (tricarboxylic acid cycle intermediates, pyruvate, or coenzyme A-linked SCFA precursors) into propionic acid and other SCFAs.

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.

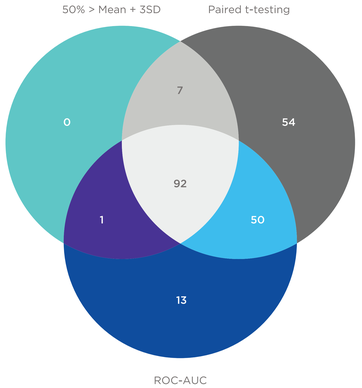

Table 2: Several VOCs in breath vary between fasting and fed states, including significant microbiome metabolites and those linked to exposure.

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

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Figure 4: Exposure related VOCs that changed after refeeding

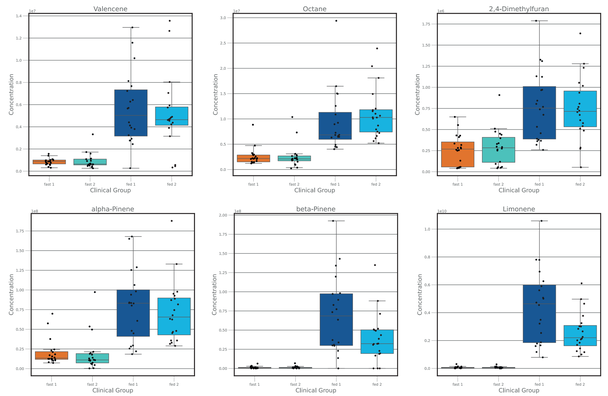
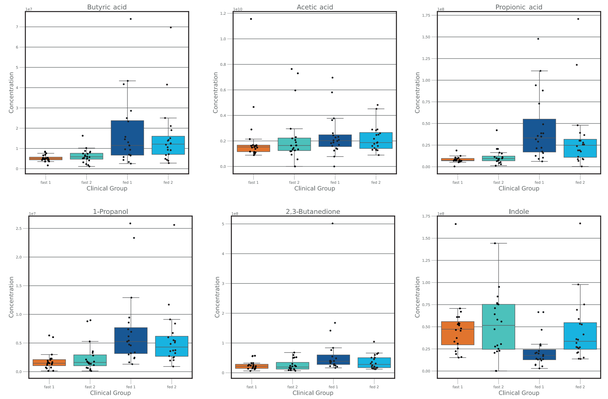


Figure 5: Microbiome related VOCs that changed after refeeding



4. Conclusions

Proteolytic and amino acid-degrading bacteria, such as *Prevotella* and *Porphyromonas* species, break down proteins and peptides into amino acids and further metabolize them through specific pathways to produce SCFAs, ammonia, sulfur compounds, and indole/skatole (1). In our study, the increase in SCFAs observed just 20 minutes after eating was not reported in previous studies using similar protocols and SIFT-MS methods (2-3). However, other research has demonstrated that the SCFA producer *Faecalibacterium* exhibited different fasting responses between healthy individuals and those with metabolic syndrome but consistently grew upon refeeding in both groups (4). This suggests that SCFA profiles can change during fasting (5) and may be influenced by the oral microbiome and intestinal peristaltic movements following a liquid meal, leading to the observed variations. Additionally, another study found a negative correlation between exhaled indole and blood glucose levels when breath samples were collected for six hours after a standardized meal in patients with type 1 and type 2 diabetes (6), which is consistent with our current findings.

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.