Breath Biopsy® OMNI® Demonstration - Identification of Breath Biomarkers on Diabetic Subjects

Hsuan (Tina) Chou, Liam Grimmett, Ibrahim Karaman, Oliver Dean, Julia Greenwood, Billy Boyle, Max Allsworth

Owlstone Medical, Cambridge, UK

Aim

- The purpose of this study is to demonstrate the capability of the Breath Biopsy® OMNI® platform.
- The clinical aim is to identify volatile metabolites associated with type 2 diabetes (T2D) disease progression, in addition to blood metabolites linked to T2D that have been established in literature.

1. Background and Objectives

Type 2 diabetes is a complex metabolic disorder defined by continuation of elevated blood glucose levels (BGL > 126mg/dL at fasting or HbA1c > 48mmol/mol (6.5%)). It is related to long-term trends such as high calorie intake, associated changes in the gut microbiota and a sedentary lifestyle. T2D is progressed from prediabetes (PD) (BGL 100-125mg/dL; HbA1c 36-48mmol/mol) and requires continuous therapy with drugs and blood glucose monitoring to ensure control and prevent development of associated co-morbidities (e.g., renal impacts).

Breath is a complex matrix containing volatile organic compounds (VOCs) from several potential sources. VVolatile compounds in breath can be inhaled from the outside air, or they can be volatile metabolites originating from within the body. Once generated in the body, these volatile metabolites can diffuse from their point of origin into the blood, and they are readily exchanged from the blood into the air in the lungs at the alveolar membrane. Volatile compounds that originate from endogenous processes represent metabolic processes occurring at distal sites, including liver, gastrointestinal space, heart, muscle, and brain. To date, the challenge of breath sampling for the field is the development of standardized, sensitive, and reproducible methods to quantify breath-based VOCs.

In this study, the objective was to evaluate VOCs in exhaled breath that differ in abundance among T2D, PD and healthy control (HC) subjects utilizing Breath Biopsy OMNI (Owlstone Medical). Breath Biopsy OMNI is a platform developed and optimized specifically for the confident identification and analysis of compounds from exhaled breath. The goal was to utilize the platform to identify breath volatile compounds for which the association between blood metabolites and T2D has been established in literature in addition to new potential breath biomarkers.

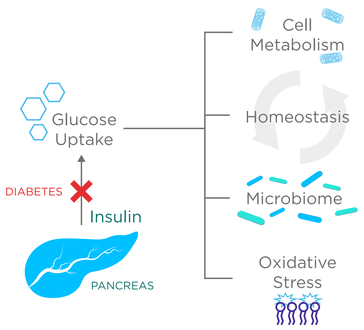

Figure 1: Physiological processes underlying the complexity of metabolic disorders in diabetes

2. Methods

- 76 volunteers participated in this study, with gender and age well balanced across different groups (Table 1).
- Breath Biopsy samples were collected using the ReCIVA® Breath Sampler (Figure 2), developed by Owlstone Medical.
- Samples were analyzed with thermal desorption gas chromatography mass spectrometry (TD-GC-MS) using the Breath Biopsy OMNI Platform.
- 157 VOCs were removed from the analysis due to low presence across samples. Semi-present VOCs were imputed with 80% of the minimum value of that specific VOC and 1110 compounds were moved forward for identification and statistical analysis.
- Compound ID was assigned using three different tiers: validated tier 1 IDs (with reference standards run either in the same sequence or equivalent sequence), putative tier 2 IDs (assigned through comparison to Owlstone Medical's HRAM library), and tentative tier 3 IDs (assigned through comparison to the NIST library).

Inclusion Criteria	Variable	HC	PD	T2D
	N	29	20	27
30-65	Age	46.7 (9.9)	48.2 (8.9)	50.5 (7.6)
> 25	BMI	29.7 (4.2)	33.5 (6.6)	36.8 (7.7)
	Sex (Female)	52% (n=15)	65% (n=13)	52% (n=14)
Fast for > 2h	Hrs since last meal	5.9 (4.9)	8.3 (5.5)	7.4 (5.1)
	Hrs since last drink	6.5 (6.3)	9.2 (5.6)	6.4 (5.3)
	Smoking	6% (n=2)	0%	22% (n=6)
Glucose test onsite	Glucose level	5.2 (0.5)	6.1 (0.8)	7.8 (1.8)

Table 1: Overview of study inclusion criteria and demographics in different subject groups. Ages and gender were well balanced in this study. While the inclusion criteria for BMI was >25 in this study, BMI remained a significant covariate as well as smoking status.

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).

3. Results

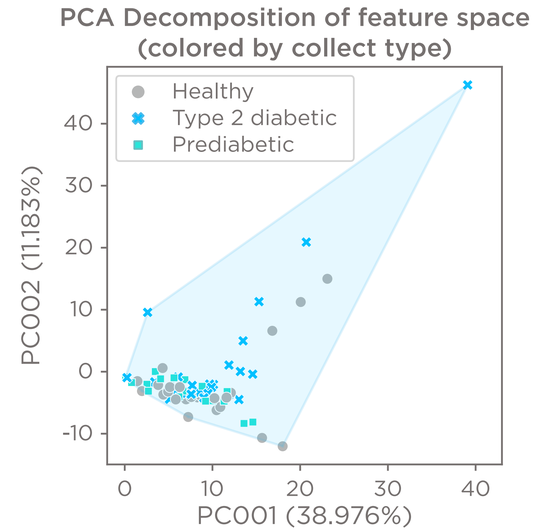

Figure 3: Principal component analysis (PCA) of breath samples suggested a modest metabolic differences in breath VOCs among the different groups. While there was no obvious separation observed, individual VOCs found to be significantly different in further analysis showed interesting results with disease relevance.

Model	Avg. Accuracy
Baseline	51.7%
Decision Tree	80.35%
KNN Classifier	73.2%
Multinomial Regression	76.8%

Figure 4: Multivariate analysis of breath profile- three different prediction models were tested to determine if significant VOCs identified in a subgroup of subjects with over eight hours fasting could train a predictive model for diagnosing diabetes. In comparison to baseline accuracy, all three models performed well, with the decision tree exhibiting the best performance, achieving an average accuracy at 80.4%.

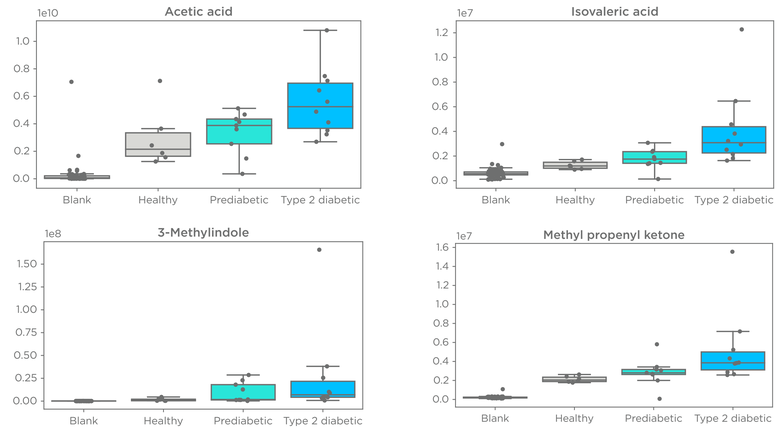

Figure 5: In this study, a total of 96 VOCs reached statistical significance among T2D, PD and HC groups. After excluding compounds that are not on-breath due to high background and those without a consistent trend between T2D, PD and HC, several interesting VOCs were found with biological relevance. For example, three gut microbiome associated VOCs and one NAFLD/UC associated VOC were found increased in T2D subjects compared to HC.

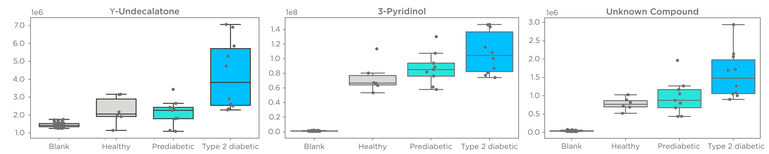

Figure 6: Additional VOCs found significantly increased in T2D include the tentatively identified γ-undecalatone, 3-pyridinol and an unknown compound. The biological relevance of these three compounds remain unknown.

4. Conclusions

In this study, the prediction models suggest that the significant breath VOCs found are capable of distinguishing different cohorts. The differences in exhaled breath volatile compounds among T2D, PD and HC potentially reflect various interesting physiological associations to the complex metabolic disorder of diabetes, such as metabolic processes, inflammation, the gut microbiome and exposure. The findings indicate that the Breath Biopsy OMNI Platform has the capability of detecting disease-associated metabolites already established in literature in addition to new potential breath biomarkers. For future metabolic disease studies, a larger number of participants fasted overnight will ensure better baseline status. Similarly, a population of treatment-naïve subjects will serve as a better cohort for identifying biomarkers for disease diagnosis and prediction.

References:

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