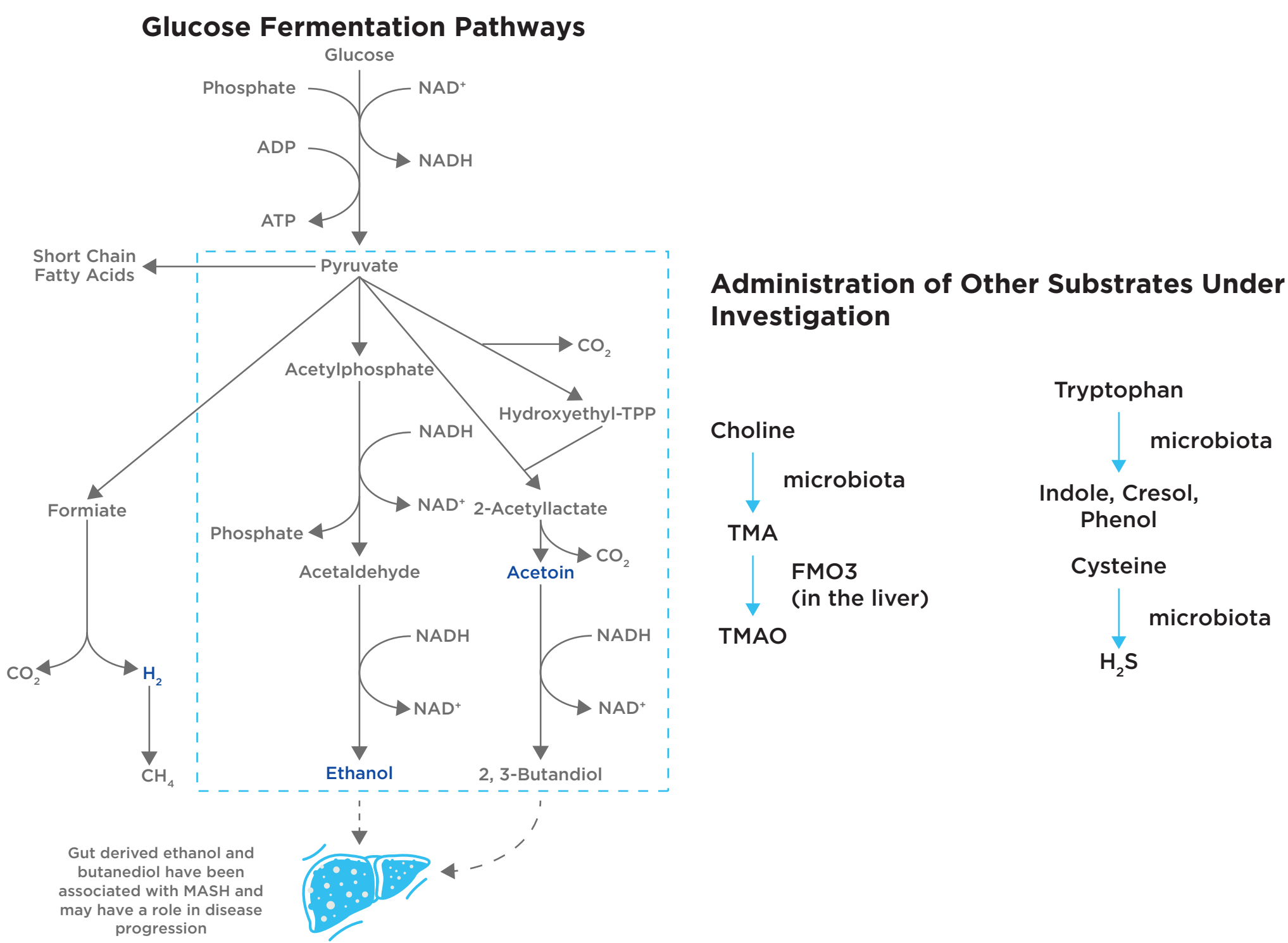


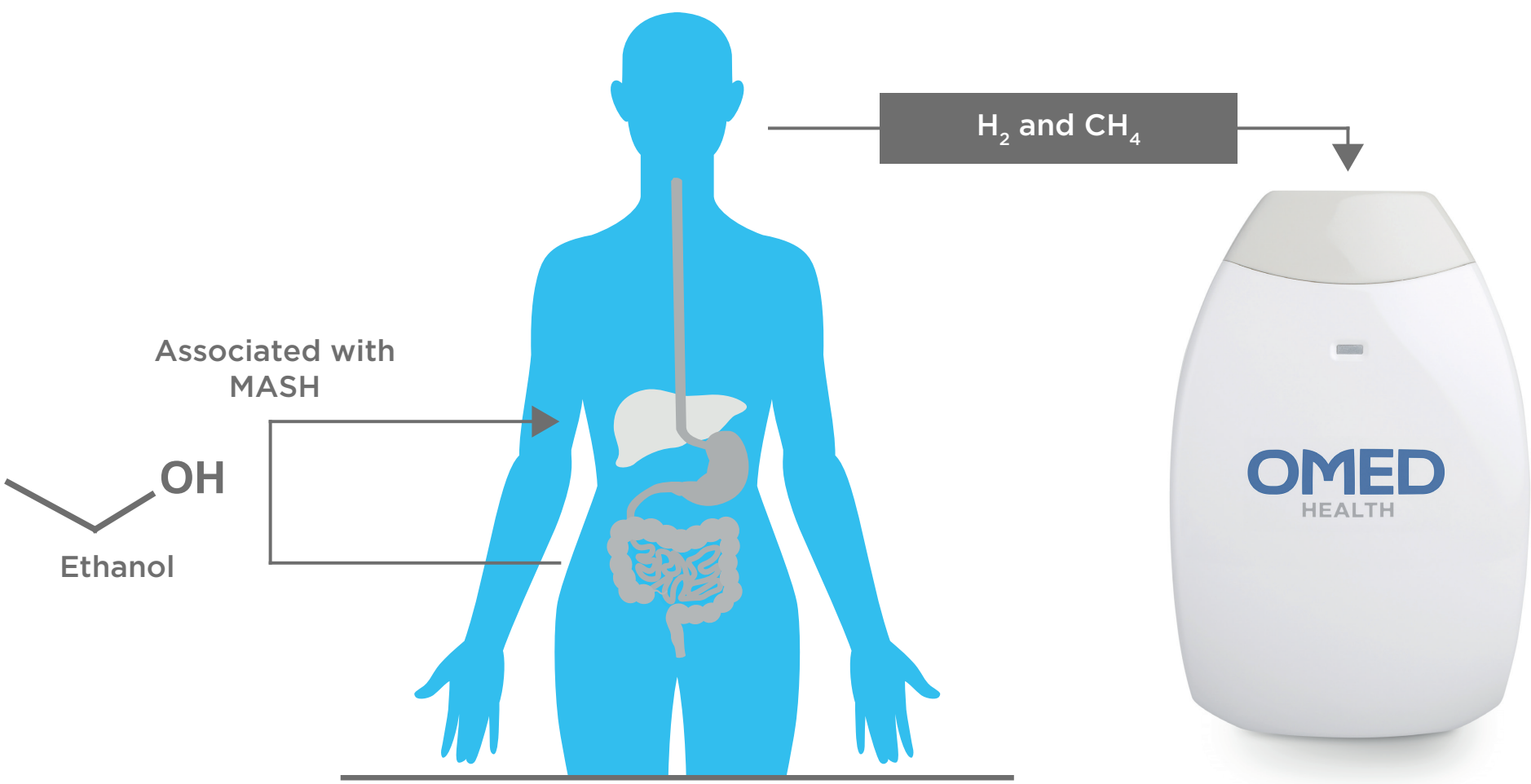
Key Findings

- Several metabolites generated by the microbiome in the digestive system and associated with disease can be measured in breath either at steady state, or after administration of certain substrates.
- Given ease of use of breath collection, this approach can be applied on large cohorts to better establish the effect of these metabolites on diseases.



1. Background and Objectives

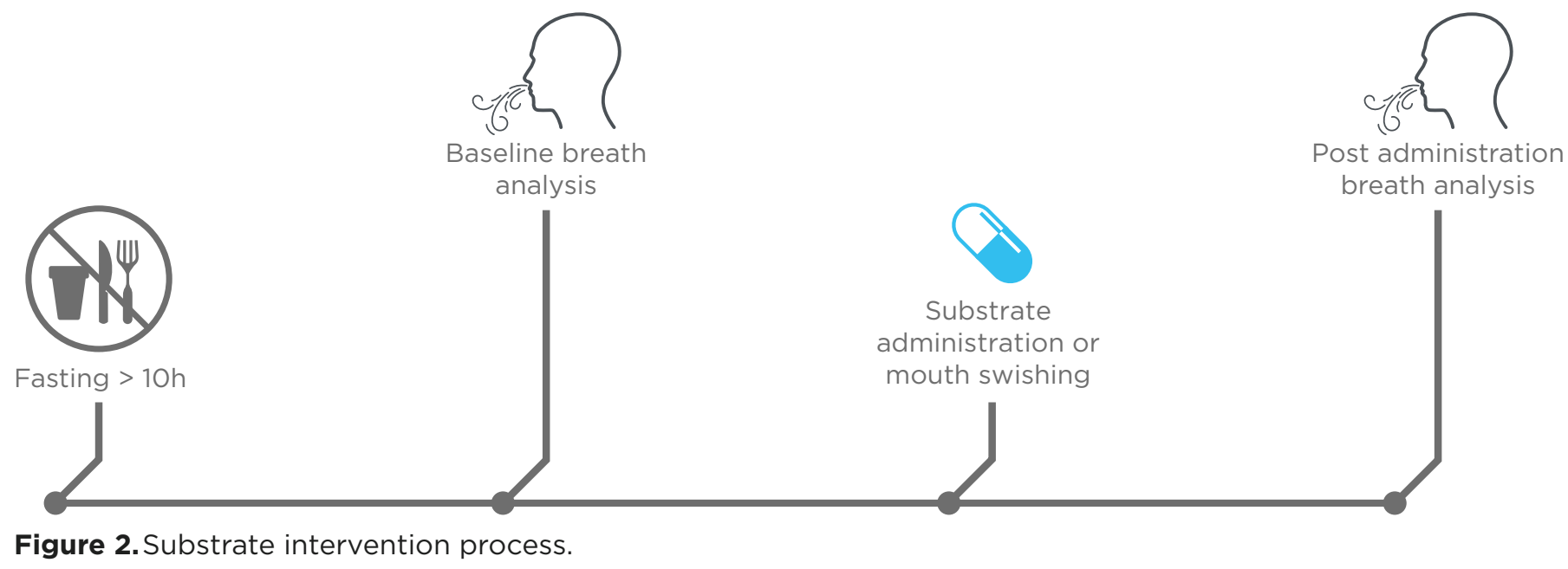
- Excessive production of metabolites produced by the microbiome of the digestive system may exacerbate certain diseases, for example gut ethanol production has been associated with metabolic dysfunction associated steatohepatitis (MASH).
- Many of these metabolites can be measured non-invasively on breath.
- We investigated the feasibility of quantification of these metabolites in breath with and without substrate intervention.



**Fig 1.** Disease-associated metabolites generated by the microbiome in the digestive system are detectable in breath allowing non-invasive at-home self testing. For example, the OMED Health Breath Analyzer is under development for at-home testing of hydrogen and methane to diagnose small intestinal bacterial overgrowth (SIBO) and carbohydrate malabsorption (CM).

2. Methods

- For each intervention healthy subjects were enrolled after overnight fasting. Breath was analyzed before and after substrate administration.
- Breath analysis was performed using selected ion flow tube mass spectrometry (SIFT-MS) with direct sampling.



**Figure 2.** Substrate intervention process.

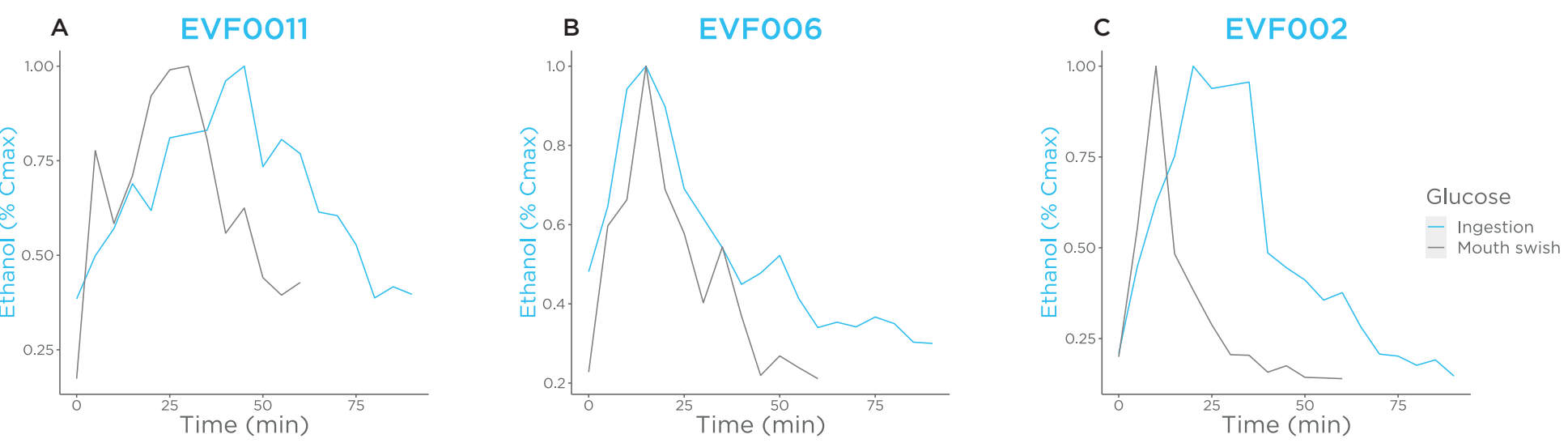
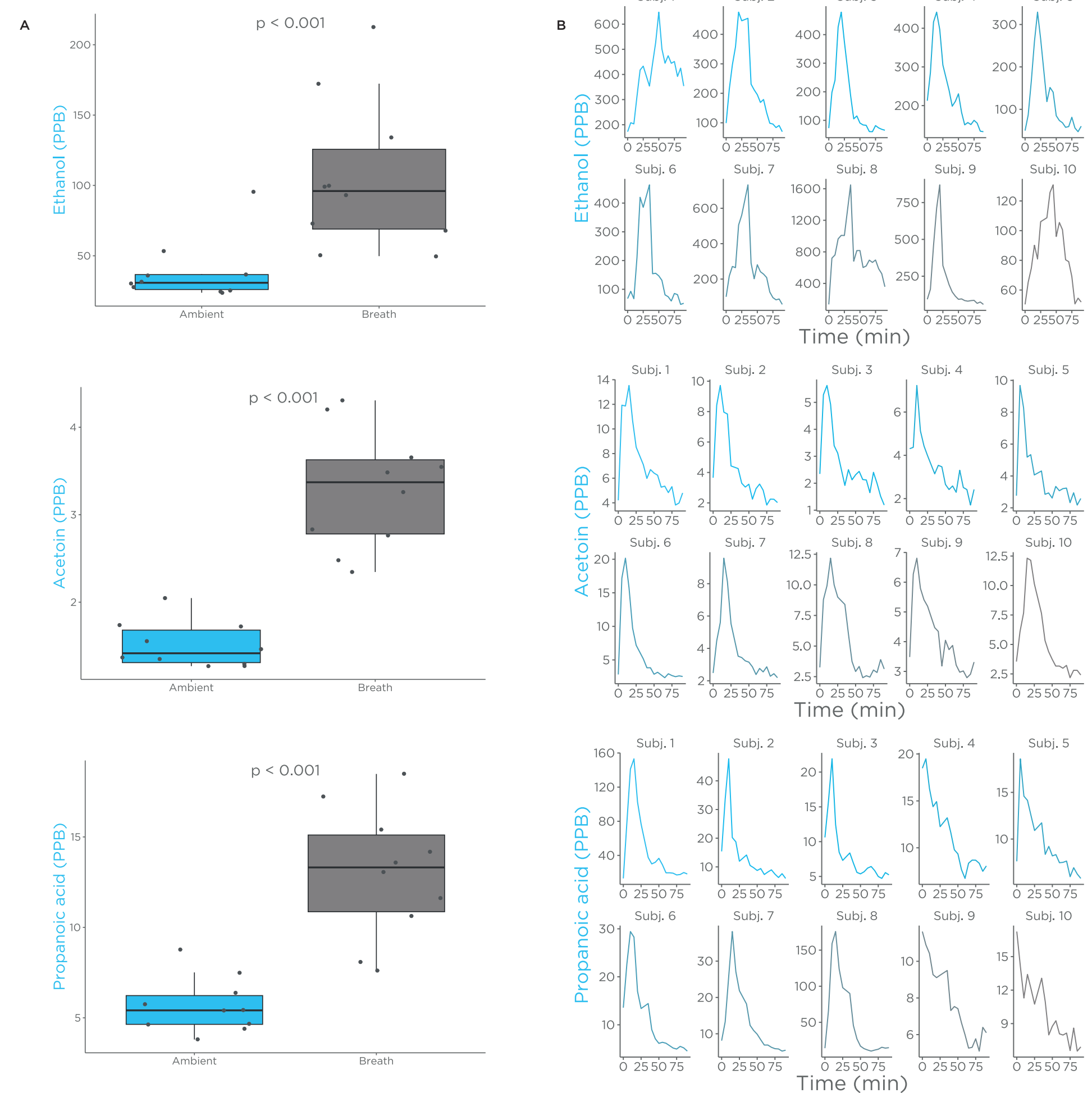
**Table 1.** Subject characteristics for glucose (75g) intervention.

Total Number	10
Sex (female/male) n	7/3
Age, median [IQR] (years)	29[28-37]
Weight, median [IQR] (Kg)	62[54-70]
BMI, median [IQR] (Kg/m <sup>2</sup> )	23[22-25]

3. Results

- Fermentation products associated with metabolic dysfunction-associated fatty liver disease (MAFLD) and gut discomfort showed a spike on breath after substrate administration.
- Some subjects had a different profile when the substrate was ingested, compared to when the substrate was swished around the mouth without ingestion.
- Additional metabolites associated with neurodegenerative diseases were detected.

Glucose Administration



**Fig 4.** Ethanol breath profile of three subjects shown as % of Cmax, when glucose was ingested or when glucose was swished in the mouth and spat out.

**Table 2.** Summary of microbiome-derived metabolites measured on breath.

Phenol	Acetone	Ammonia	Ethanol
p-Cresol	Ethyl phenol	Hydrogen sulfide	Acetoin
Acetaldehyde	Dimethyl sulfide	Propanoic acid	Methyl mercaptan
Indole	2,3 butanediol	Pentane	1,2 Propandiol
Methanol	Propanol	Methane	Trimethylamine
Propanol	Acetic acid		

4. Conclusions

- Many metabolites generated by the microbiome of the digestive system and associated with different diseases are detectable in breath.
- This non-invasive method can replace the current need for blood collection allowing scaling to large cohort populations.