Non-invasive Functional Assessment of the Microbiome from Exhaled Breath

7th February 2023

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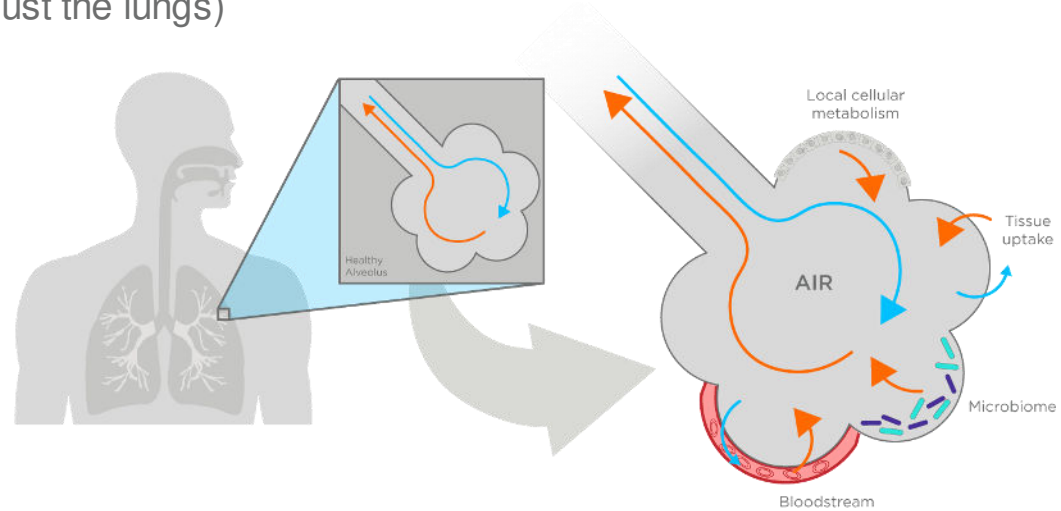
owlstonemedical.com

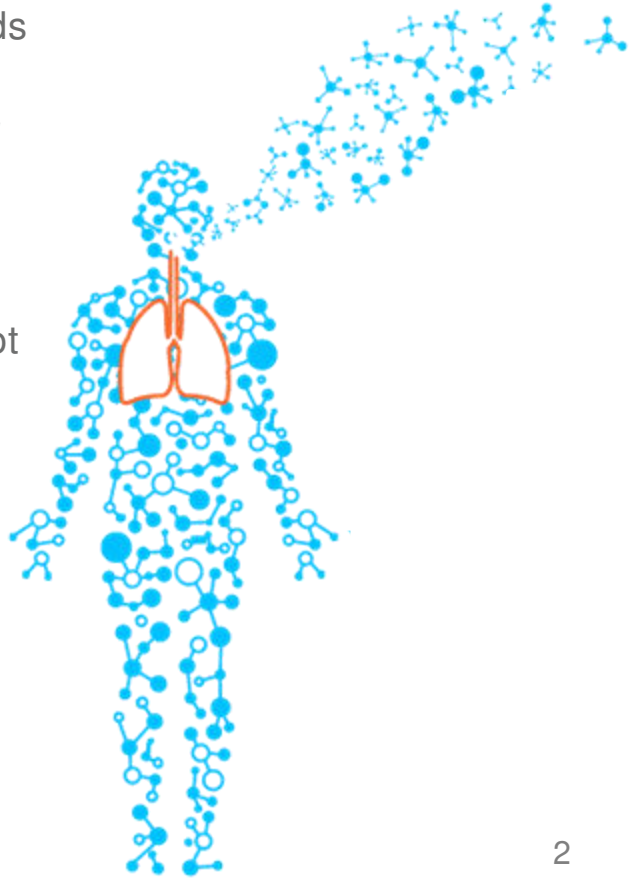
Owlstone Medical

Owlstone Medical Focus on Exhaled VOCs

Human breath contains hundreds of different volatile organic compounds (VOCs), that originate from a variety of sources. Many VOCs are metabolites from human or microbial / microbiome metabolism and thus reflect patient phenotype.

VOC metabolites in exhaled air can originate from both the airways and other tissues in the body, carried via the bloodstream and crossing the alveolar interface, and thus can reflect biology from around the body (not just the lungs)





Company Background

OWLSTONE INC. SPUN OUT FROM **CAMBRIDGE UNIVERSITY** 2004, **OWLSTONE MEDICAL** SPUN OUT MAR 2016

MULTIDISCIPLINARY TEAM OF ~200 PEOPLE HEADQUARTERED IN CAMBRIDGE, UK

>15 YEARS' EXPERIENCE IN VOC ANALYSIS IN A RANGE OF INDUSTRIES

OWLSTONE MEDICAL >$**150**M INVESTMENT, OVER-SUBSCRIBED $**58**M D ROUND CLOSED SEPT 2021

DEEP IP PORTFOLIO, 100+ PATENTS (GRANTED AND PENDING)

WORLD'S FIRST **HIGH VOLUME** BREATH BIOPSY **LAB**

BREATH BIOPSY **IN USE IN >100 CLINICS** GLOBALLY

>100 PEER REVIEWED PUBLICATIONS AND SCIENTIFIC POSTERS

RUNNING THE WORLD'S **LARGEST BREATH-BASED CLINICAL TRIALS**

Why Analyse VOCs in Breath for the Microbiome?

The microbiome is known to produce VOCs as primary metabolites e.g. SCFAs

Breath testing reports metabolic changes in real time and avoids VOC evaporation

Breath VOCs are concentrated from large volumes of breath providing higher sensitivity

Breath testing is fully non-invasive and patient friendly

Could be used to determine approximate gut location of microbes

Potential to enable home-based sample collection

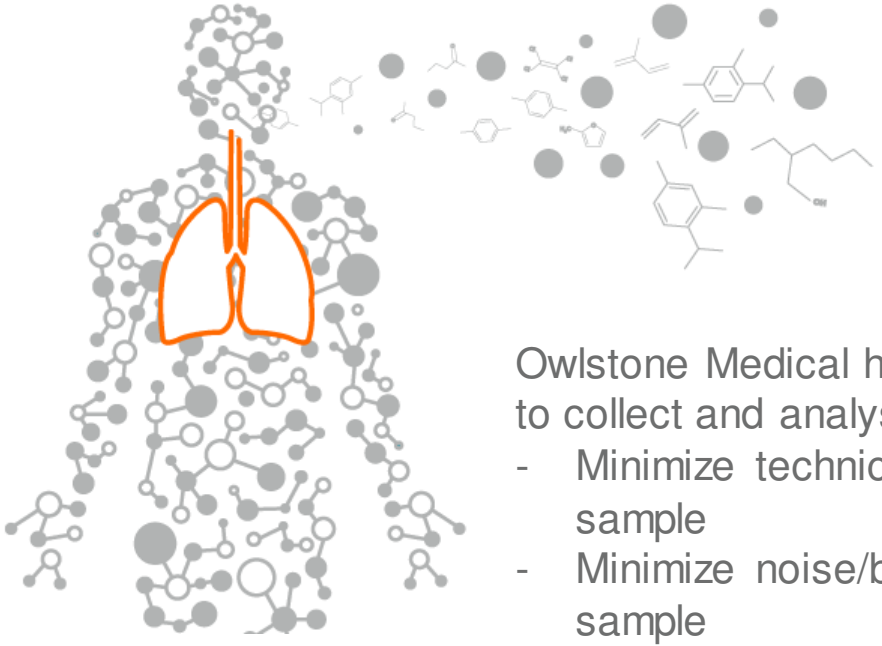
The Challenges of Breath and VOCs

How to standardize? Everyone breathes differently

VOCs also in environmental air people inhale

Diverse range of VOC concentrations

Diverse range of VOC chemistry



BREATH® BIOPSY

Owlstone Medical has developed methods to collect and analyse breath that:

- Minimize technical variability of a breath sample
- Minimize noise/background in a breath sample
- Analyse a broad range of VOCs
- In untargeted discovery – accurately identify VOCs for further validation and analysis

Breath Biopsy® OMNI® Platform

Breath Biopsy Collection Station enables reproducible breath sample collection and maximizes signal to noise ratio. Through ReCIVA, it collects and concentrates VOCs from large volumes of breath for high sensitivity and molecular diversity.

Collection

GC-MS analysis on high-resolution accurate mass (HRAM) Thermo Scientific™ Q Exactive Orbitrap systems further enhances analyzable molecular diversity, and reliable identification of VOCs.
Analysis includes deconvolution, feature extraction and normalization.

Analysis

Specialist data interpretation using NIST VOC Library and Breath Biopsy VOC library for high confidence VOC ID assignment. Reporting contains a complete feature table of scaled and normalized VOCs.

Interpretation

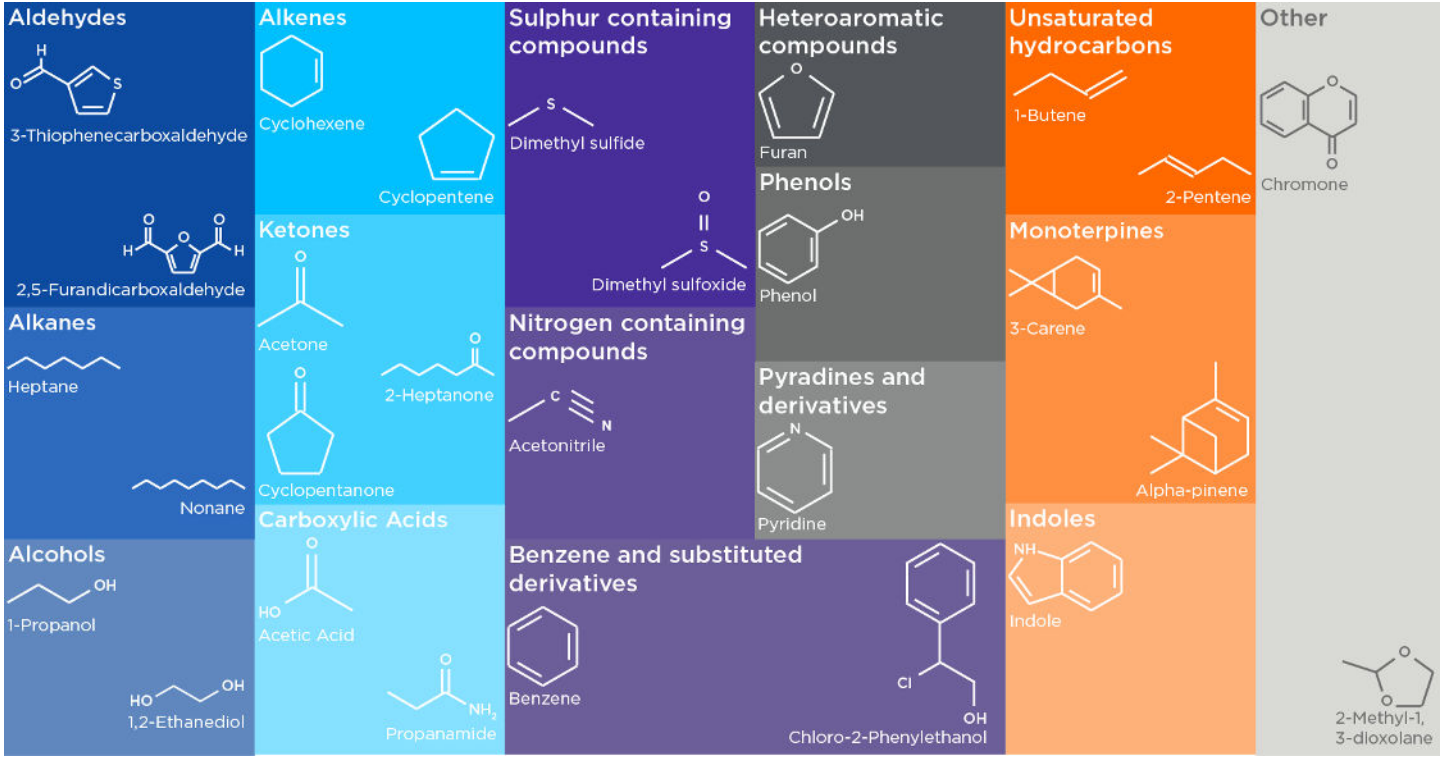
Breath Biopsy VOC Library

400 VOCs in HRAM Library and 150 VOCs in ATLAS

Retention time:
1.8 to 36.2 minutes

Molecular weight:
41 to 593

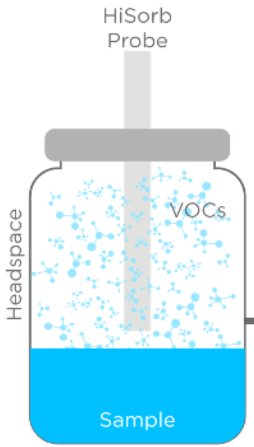
Polarity log kOW:
-1.4 to 8.2



Owlstone's *in vitro* Headspace Sampling Platform

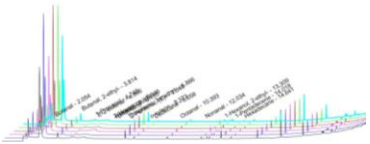
Cell/tissue culture or stool / biofluids collected at customer site

Snap freeze samples for shipping and sent to Owlstone (Cambridge)



Incubation & Agitation

Automated Processing
(Centri)



TD-GC-MS

For more info: CLICK HERE

Development of Targeted Breath Tests for Routine Clinical Use

Tests for routine clinical use (e.g. past exploratory biomarkers) can be developed for specific VOCs of interest.

Appropriate test design will depend on:

1) The VOCs being targeted (concentration and chemistry)
2) The user requirements (e.g. clinical need, speed of results, collection site)
3) The commercial requirements (e.g. cost per use)

Owlstone are well positioned to support test development following VOC biomarker selection:

Owlstone already performs CE-marked digestive disease breath tests for NHS patients referred by specialists.

Tests are sent to patient's homes to collect breath samples, then shipped back to our Cambridge lab for analysis. This reduces hospital visits and supports diagnosis with expert interpretation of results.

Read More

The quickest route to market could be a medical device version of ReCIVA or a single use breath collector combined with a targeted GC-MS assay.

Additional technologies, focused on specific VOCs, are being investigated as part of our internal test development programmes. For example:

Single-use point of care breath sampler

GC-MS for laboratory use, high throughput

Point of care system designed for specific VOCs and patient use

Read More

Applications for Microbiome Research & Understanding Microbiota Function in Human Health

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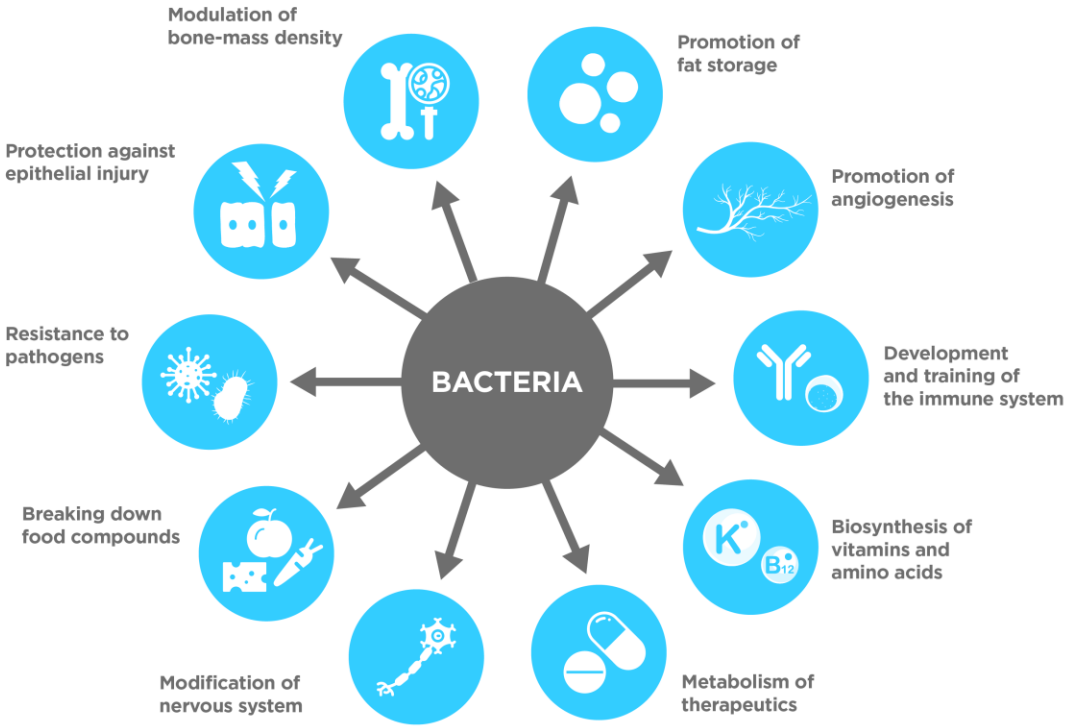
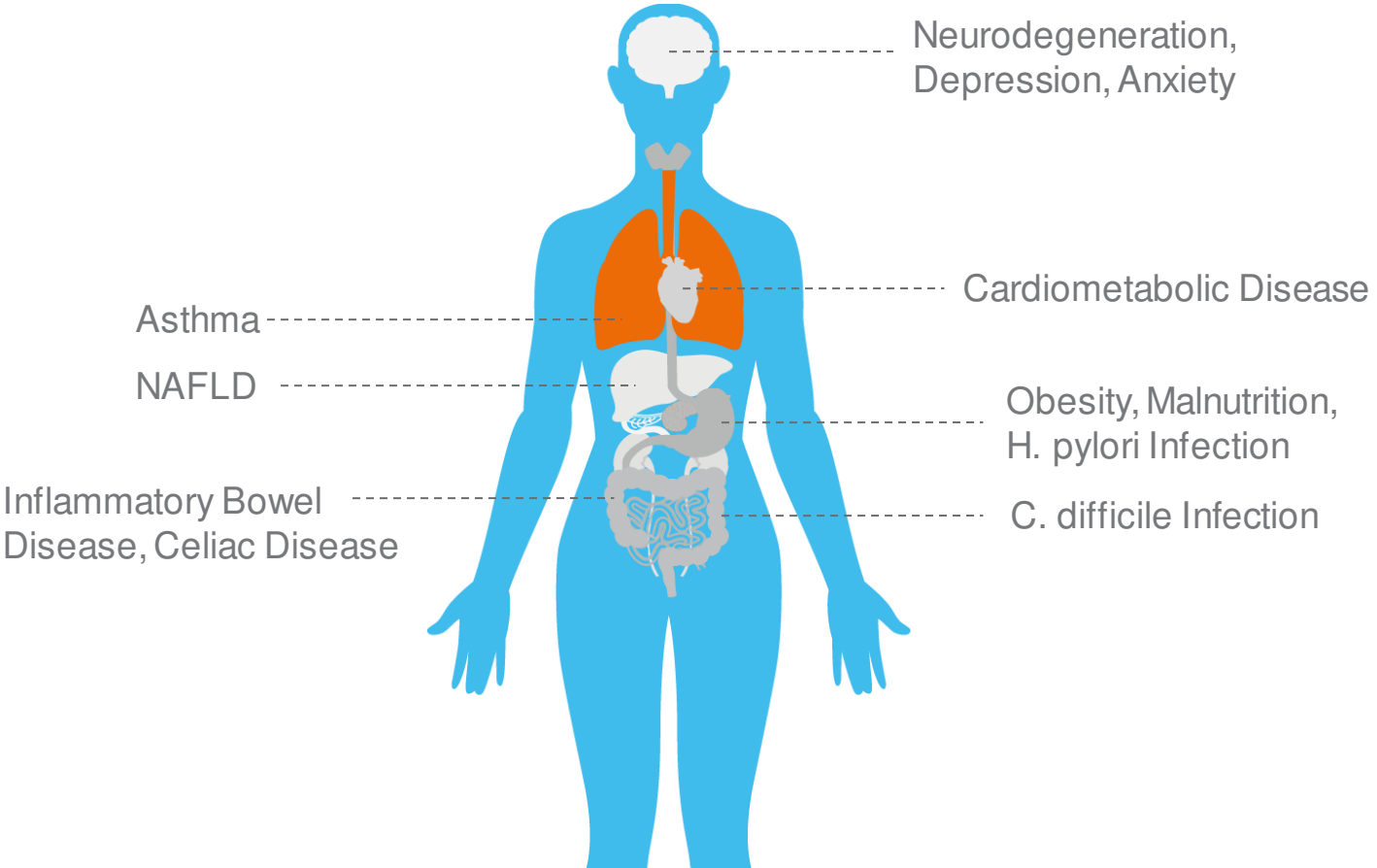
Microbiota Perform a Variety of Biological Functions

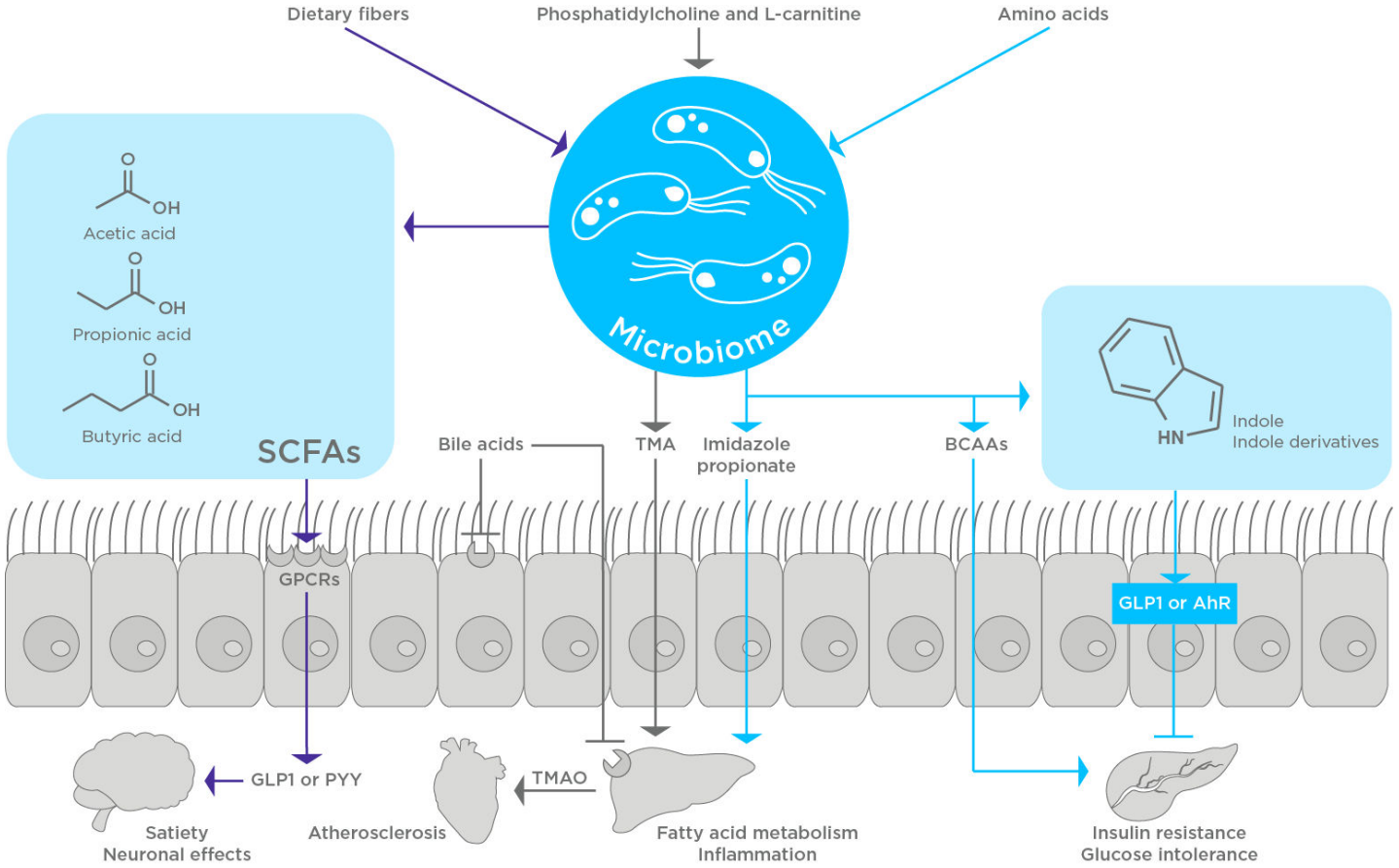


Laukens et al., *FEMS Microbiol Rev* **40**:117-32 (2016)

Gut Microbial Composition & Function Impact Health



Why Are Microbiome-derived Metabolites Important?

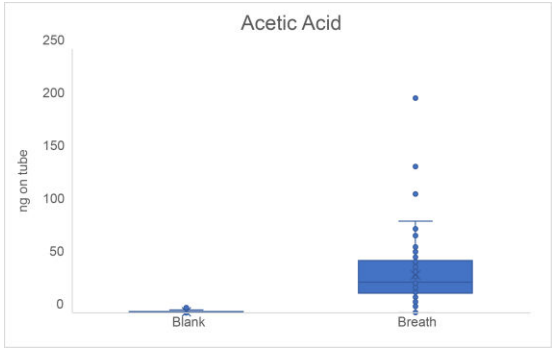


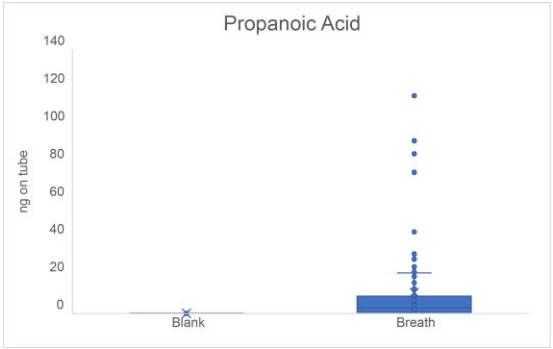
Example of VOCs Relevant to the Microbiome

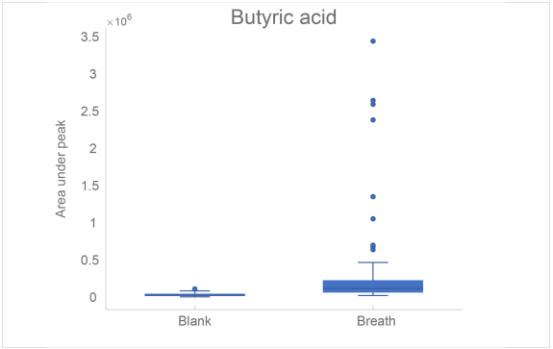
VOCs	Biology
Short-chain fatty acids (SCFAs) e.g. acetic acid, propanoic acid, butyric acid	Produced from anaerobic fermentation of indigestible polysaccharides. Different bacteria produce different types of SCFAs – e.g. clostridium, roseburia and eubacterium are likely butyrate producers. Roles in multiple signalling contexts, including CNS, gut, and immunity/ inflammation. (ref)
Branched-chain fatty acids (BCFA) e.g. isovaleric acid, isobutyric acid	Protein fermentation – products of branched-chain amino acid metabolism. Associated with *Bacteroides and Clostridium* (ref)
Aromatic amino acid metabolism products e.g. indoles, phenols, cresols,	Protein fermentation – products of aromatic amino acid metabolism. Different species produce different products e.g. indole associated with *E.coli, Lactobacillus, Enterococcus*, but not *Actinobacillus, Yersinia*. Indole also performs roles in regulation e.g. of biofilms (ref)
Trimethylamine (TMA), triethylamine	Fermentation of dietary nutrients such as choline, betaine, carnitine. TMA associated with multiple diseases atherosclerosis, CKD, NASH, obesity, Type 2 diabetes and colorectal cancer (ref)
Alcohols (e.g. propanol, propan-2-ol)	General fermentation of sugars
Aldehydes, alkanes, ketones	May be from metabolic conversion of alcohols by microbiome, but also associated with lipid peroxidation due to oxidative stress (commonly associated with inflammation and host response)

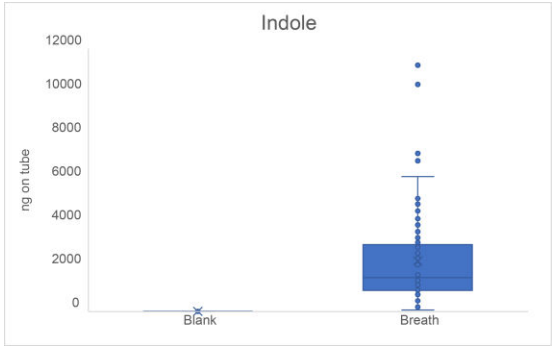
Owlstone's OMNI Platform Enables Measurement of Microbial VOCs in Exhaled Breath

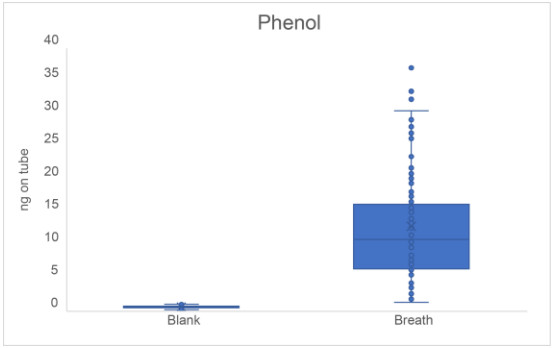
- Microbial VOCs are readily observed on human breath from healthy and disease subjects.
- They are observed at significantly elevated levels compared to control blanks (i.e. background air).
- Their levels vary in response to changes in diet, microbial composition, and activity.

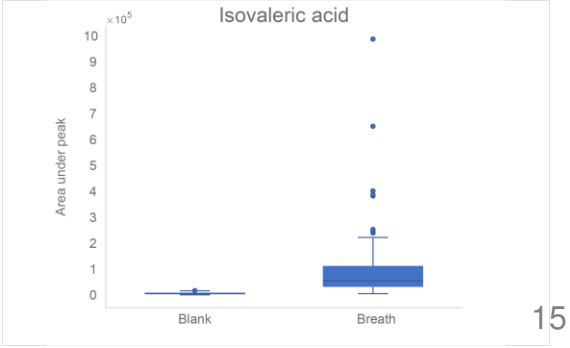






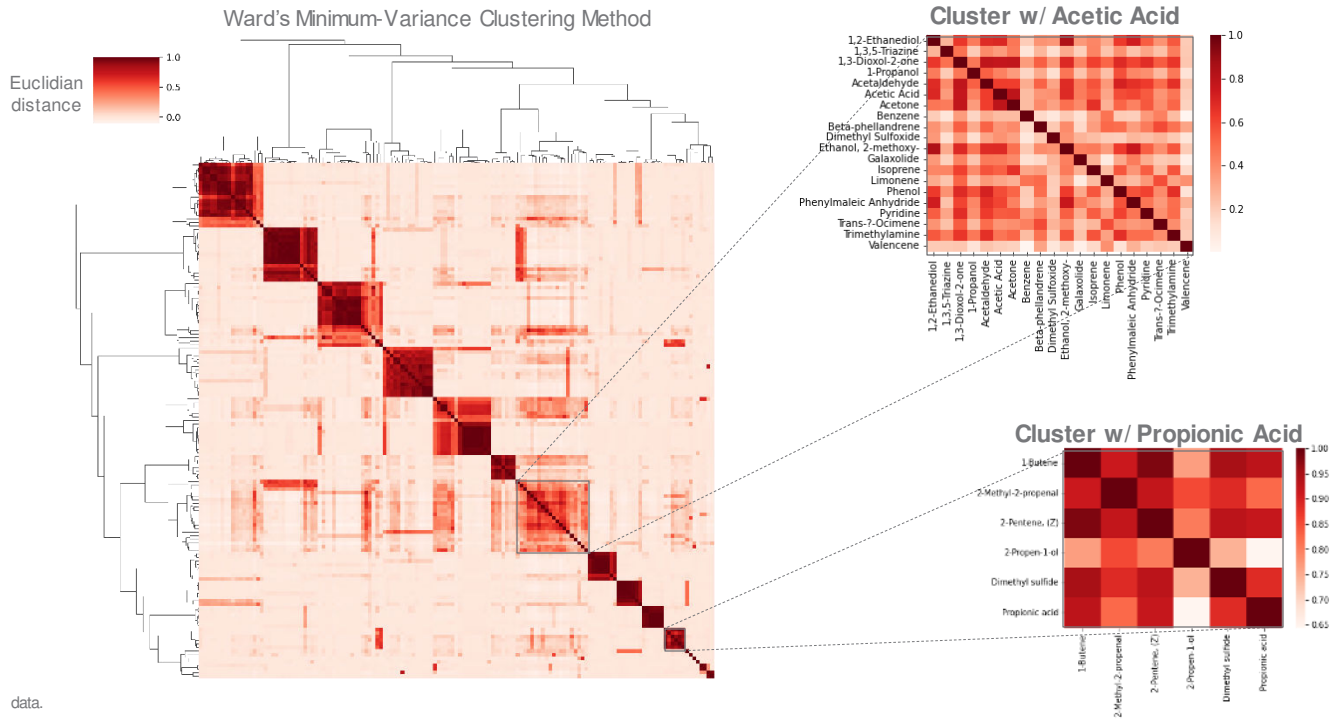






Owlstone's Technology Can Find Novel Microbiome-associated Compounds

- Untargeted analysis of on-breath VOCs from a normal, healthy population (90 subjects)
- Correlation analysis identified clusters of compounds that associate with microbiome-related VOCs (like SCFAs), indicating a possible association with the microbiome

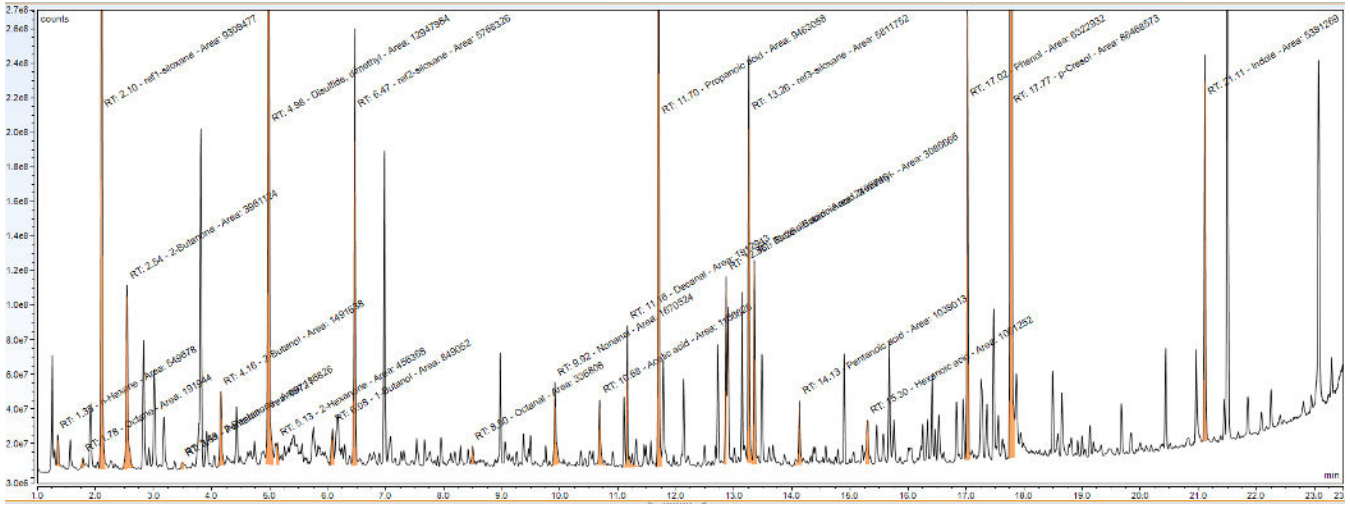


Owlstone Medical, unpublished data.

Capabilities to Perform Headspace Analysis of Fecal VOCs

Target	Average Conc. (ng/tube)	LoD (ng/tube)
p-Cresol	154.62	0.19
* Acetic acid	110.87	2.24
* Propanoic acid	64.63	1.01
* Butanoic acid	61.15	0.64
Disulfide, dimethyl	38.29	0.13
Butanoic acid, 3-methyl-	30.29	0.17
Phenol	24.58	0.42
* Pentanoic acid	16.64	0.18
Indole	7.96	0.02
Nonanal	6.05	0.25
Hexanoic acid	4.89	0.22
2-Butanone	3.40	TBD
2-Butanol	3.15	<0.01
1-Butanol	1.76	<0.01
Decanal	1.14	0.11
Octanal	0.74	0.02

*SCFAs (C2-C5)



- Reproducibility was assessed by comparing initial and recollected samples; 2 recollects
- Average RSDs across the 16 targeted VOCs was ~3%
- Correlation between the original and recollected samples was >0.99

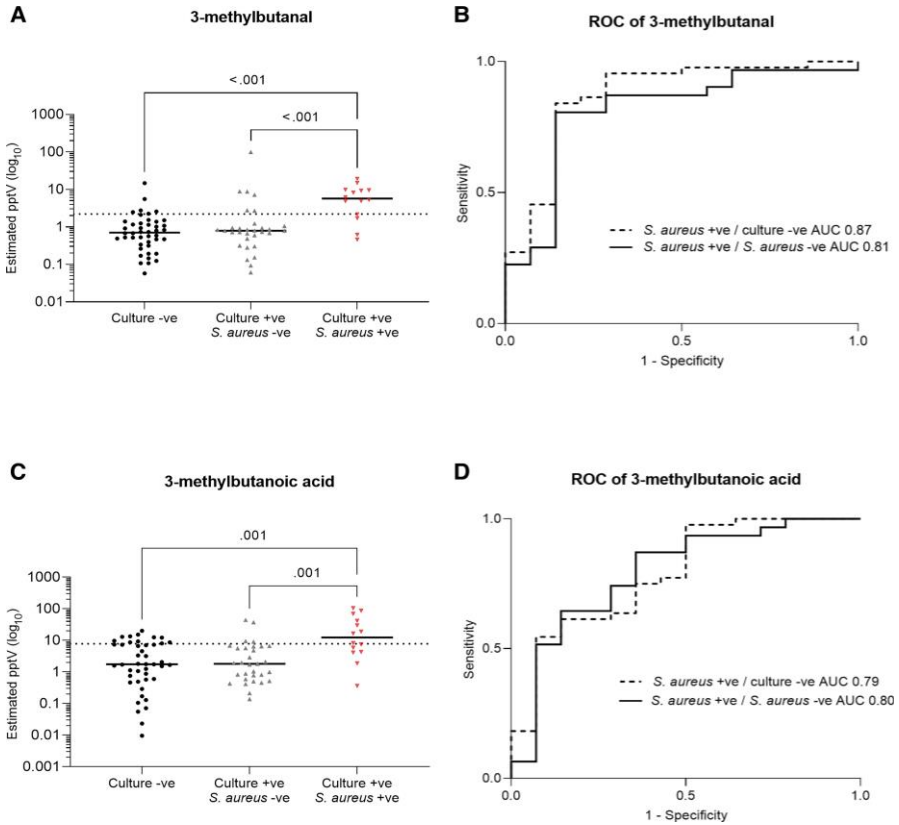
Microbial Volatiles are Associated with Specific Species and Translate from *In Vitro* to *In Vivo*

Clinical Infectious Diseases
MAJOR ARTICLE

Microbial Volatiles as Diagnostic Biomarkers of Bacterial Lung Infection in Mechanically Ventilated Patients

Waqar M. Ahmed,[1,a] Dominic Fenn,[2,3] Iain R. White,[1,4] Breanna Dixon,[1] Tamara M. E. Nijsen,[5] Hugo H. Knobel,[6] Paul Brinkman,[2] Pouline M. P. Van Oort,[7] Marcus J. Schultz,[8,9,10] Paul Dark,[1,11] Royston Goodacre,[12] Timothy Felton,[1] Lieuwe D. J. Bos,[2,3,9] and Stephen J. Fowler,[1] for the BreathDx Consortium[a]

- Identified VOCs that were enriched reference strain cultures of the most commonly observed respiratory pathogens (*S. aureus, P. aeruginosa, E. coli, and K. pneumoniae*)
- Several VOCs were unique to a single pathogen:
  - dimethyl sulfide & 2-aminoacetophenone from *P. aeruginosa*
  - ethyl acetate & 2-heptanone from *K. pneumoniae*
  - indole from *E. coli*
- The most significant result was a higher abundance of 3-methylbutanal and 3-methylbutanoic acid in cultures of *S. aureus* AND in exhaled breath from patients with confirmed *S. aureus* infections



Due to low numbers of *K. pneumoniae*-positive and *E. coli*-positive cultures (n=2 each), these samples were excluded from the analyses shown.

Ahmed et al., *Clin Infect Dis.* In Press (2022)

Monitoring Exercise-induced Inflammation in IBD

metabolites

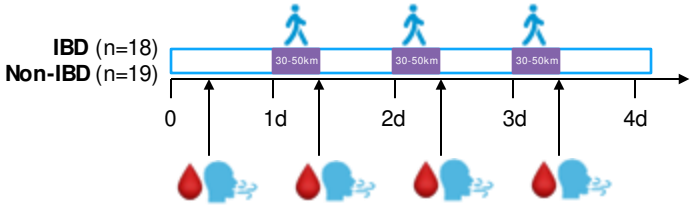
MDPI

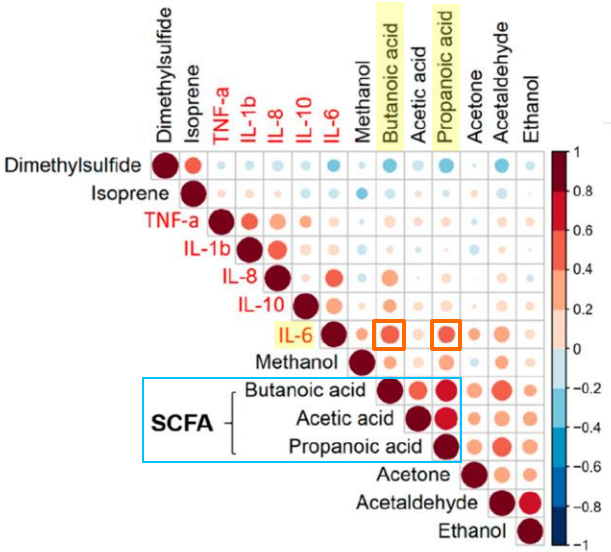
Article

Non-Invasive Monitoring of Inflammation in Inflammatory Bowel Disease Patients during Prolonged Exercise via Exhaled Breath Volatile Organic Compounds

Ben Henderson [1], Joris Meurs [1], Carlijn R. Lamers [2,3], Guilherme Lopes Batista [1], Dušan Materić [4], Carlo G. Bertinetto [1], Coen C. W. G. Bongers [5], Rupert Holzinger [4], Frans J. M. Harren [1], Jeroen J. Jansen [1], Maria T. E. Hopman [5] and Simona M. Cristescu [1,*]

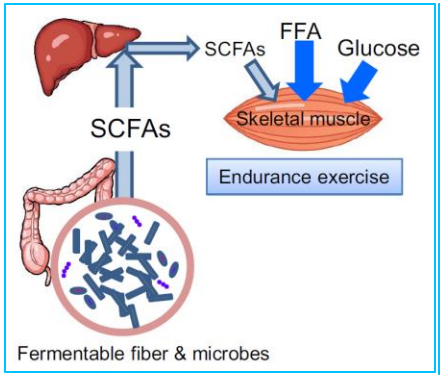
- IBD is a debilitating condition that is normally managed rather than cured; focus on relieving symptoms
- Regular exercise shows promise as a management tool for IBD, but moderate-intensity exercise can induce inflammation and changes in gut microbiota
- Henderson et al. investigated the potential of VOCs as non-invasive markers of exercise-induced inflammation in IBD patients
- Breath (VOCs) & plasma (cytokines) samples were collected at baseline and at 1, 2, and 3d after 30-50 km of walking

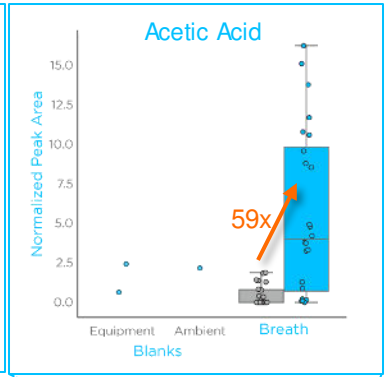


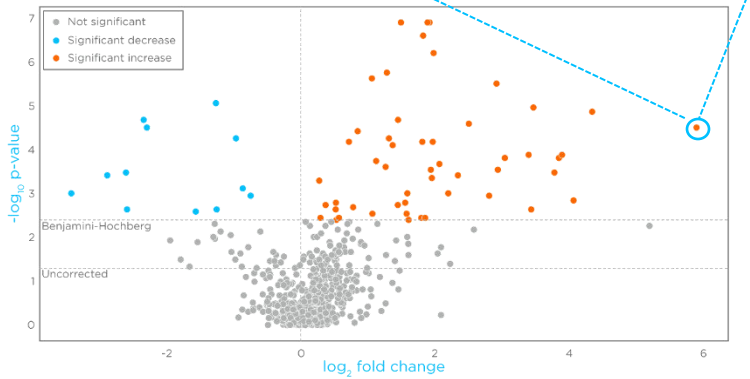


Henderson et al., *Metabolites* 12:224 (2022)

Owlstone Study: Significant Accumulation of the SCFA Acetic Acid after Exhaustive Exercise





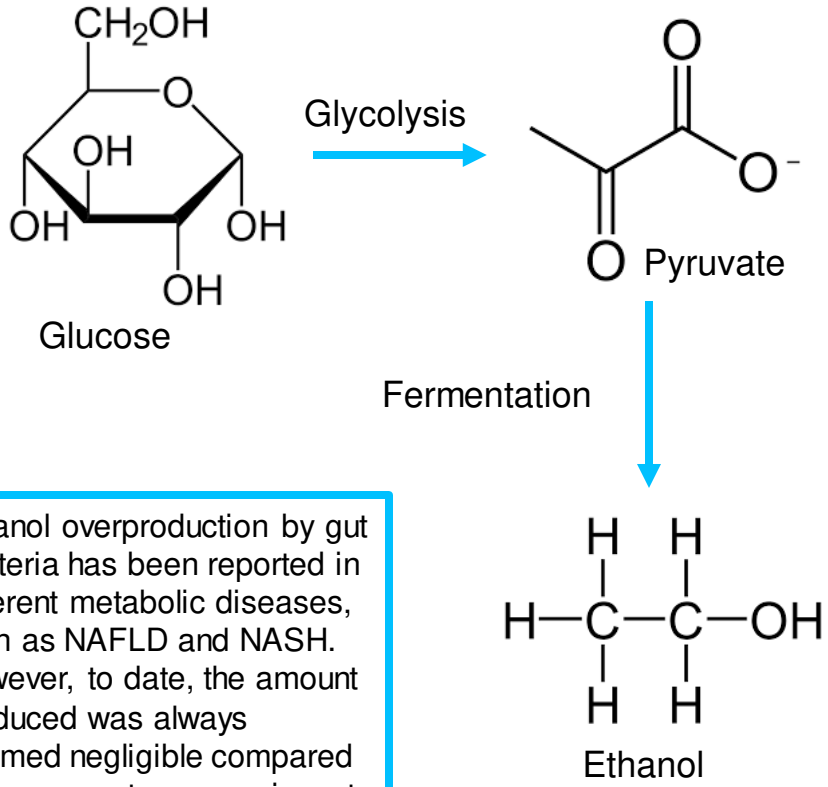


- Project in partnership with Bruce Johnson at Mayo Clinic
- Exhaustive exercise, typified by ultra-marathons, triggers unique physiological responses, providing an opportunity to identify markers of inflammation and physical & metabolic stress
- Of ~800 VOCs that were identified, 63 were significantly altered between pre- and post-exhaustive exercise
- The SCFA acetic acid was shown to increase significantly after exhaustive exercise

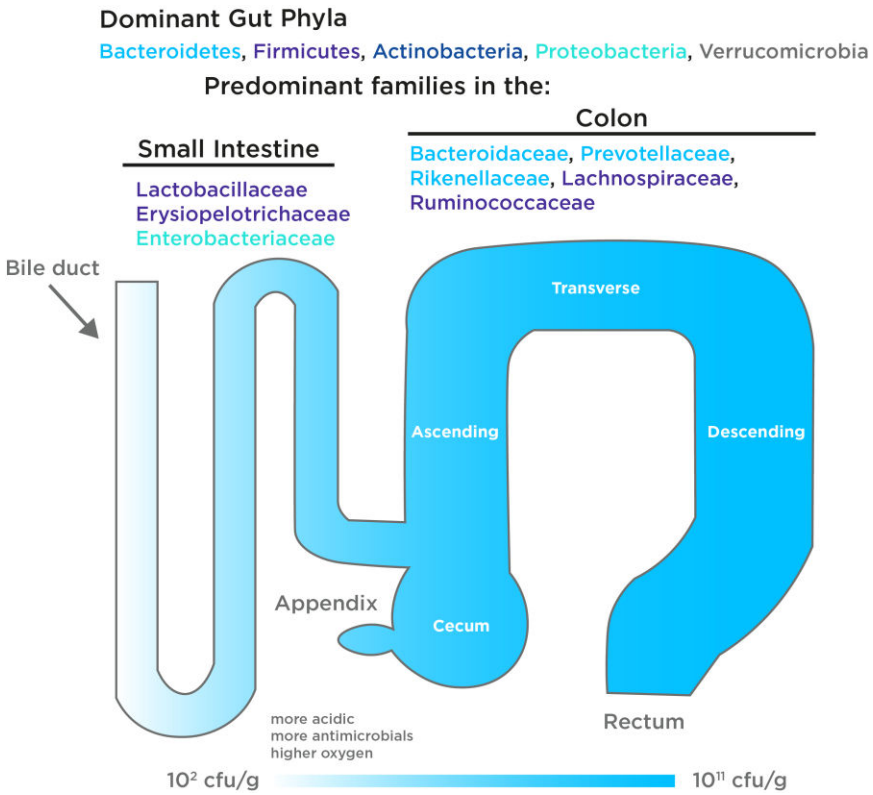
Okamoto et al., *Am J Physiol Endocrinol Metab* ***316***:E956-E966 (2019)

Bruce Johnson, Mayo Clinic, unpublished results.

No Test Available to Monitor for Ethanol Produced from Gut Bacterial Fermentation



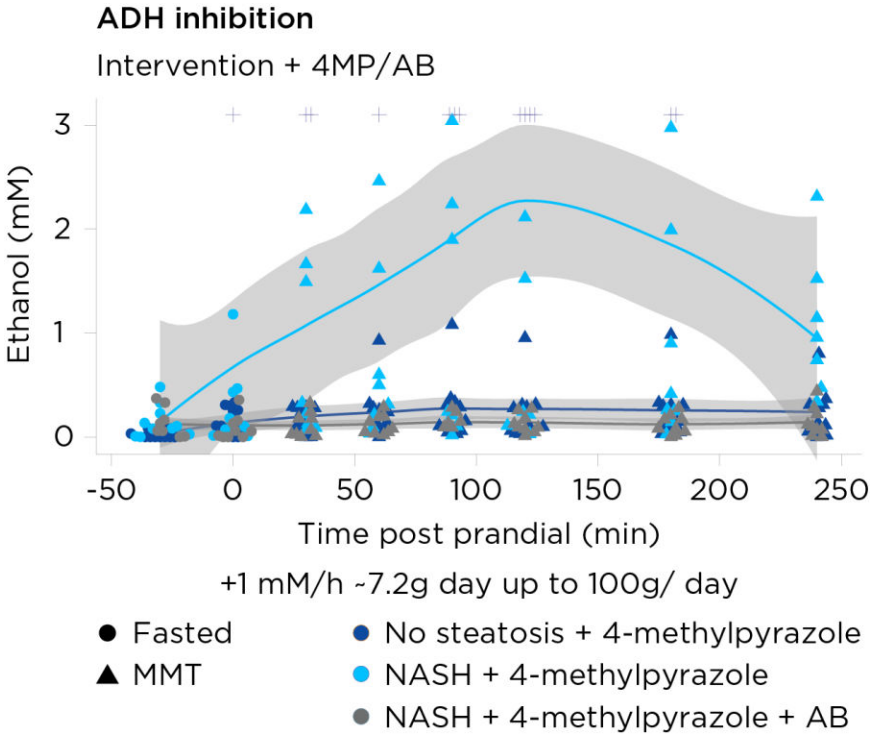
Ethanol overproduction by gut bacteria has been reported in different metabolic diseases, such as NAFLD and NASH. However, to date, the amount produced was always deemed negligible compared to the amount one can ingest



Donaldson GP et al. Gut biogeography of the bacterial microbiota. Nature Reviews Microbiology. 2016;14:20-32

Estimated that Individuals with NASH May be Exposed to up to 100 g/day of Ethanol Without Alcohol Consumption

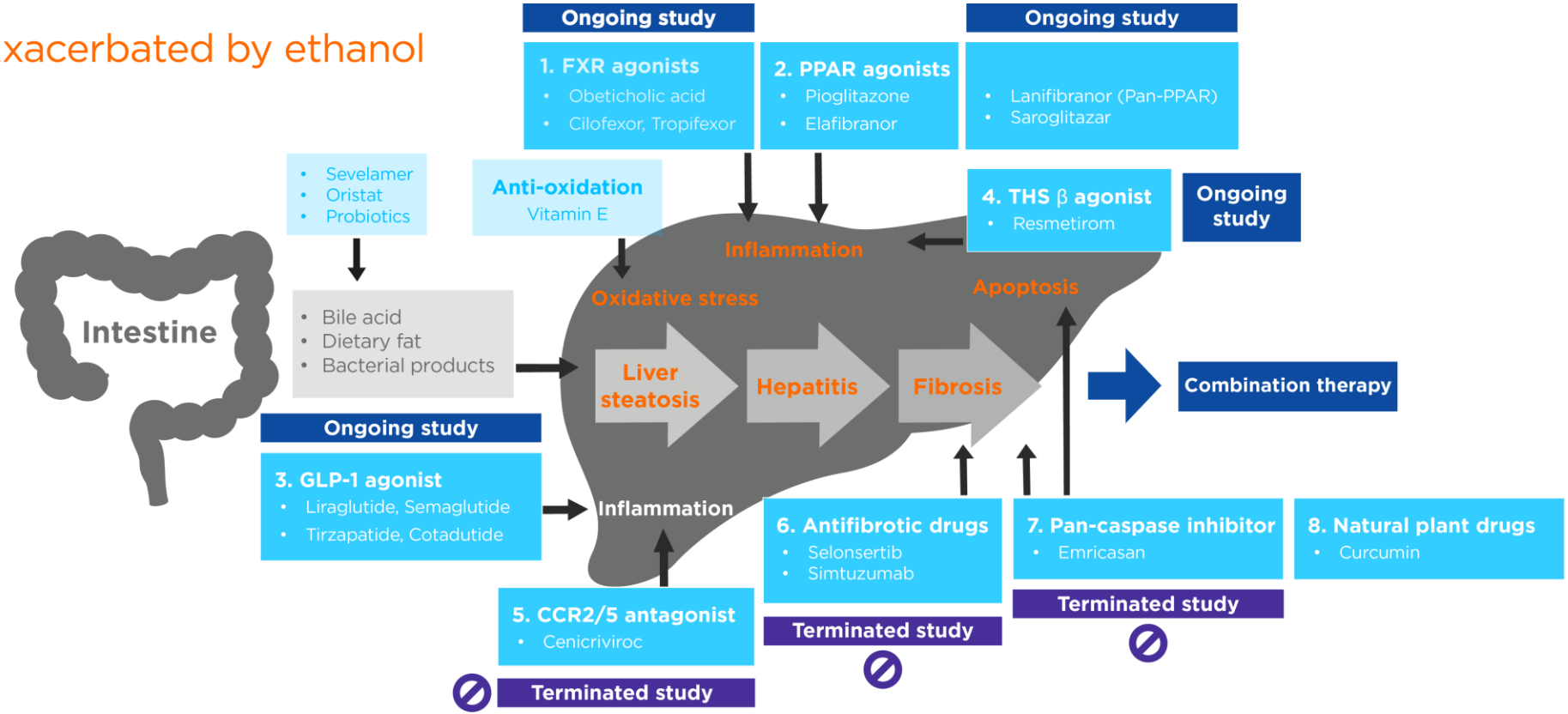
- Subjects were treated with 4-methylpyrazole, an ADH inhibitor
- Ethanol was measured before and at different timepoints after a carbohydrate meal
- NASH subjects were given an antibiotic and the experiment repeated
- Estimates indicate that NASH subjects produced between 7.2 and 100 g/day of ethanol. Therefore, some subjects produced more ethanol than the thresholds for a diagnosis of NASH (<20 W and <30 M g/day)



From talk of Prof. S. Meijnikman ILC 2022

Meijnikman, A.S. et al. Nature Medicine 2022

Chronic Ethanol Exposure Conflicts Mechanistically with NASH Experimental Drugs



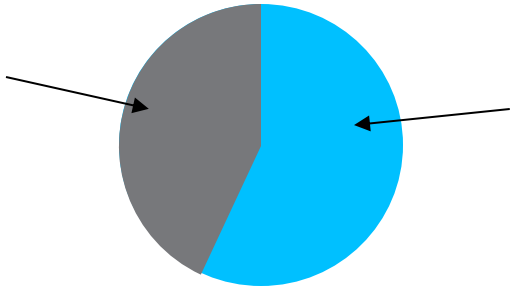
*Liu, J. et.al, WJG, 2014

Prasoppokakorn, T. et.al, JCTH, 2021

Uncontrolled Gut Ethanol Production May Reduce Probability of Establishing Efficacy of a NASH Drug

Example of cohort stratification

Effect size in ethanol non-producer must be massive to meet endpoint



Overall NASH cohort

*Up to 60% ethanol producers: Range, e.g., 10 to 100 g/day + daily intake within guidelines limits, may be non/low-responders

*Yuan J et al. Cell Metabolism 2019;30:675-88

Controlling for overall ethanol exposure (gut production + intake) may help meet endpoint in phase 2 and enrol better cohorts in phase 3 increasing chances of success

Summary of Need for a High Sensitivity Test for Gut Produced Ethanol in NASH

- Level of ethanol intake forms part of the diagnostic protocol for NASH (<20g/day W, <30g/day M), and represents an exclusion criteria for clinical trials
- It has been shown that subjects with NASH have microbiome ethanol production up to 100g/day independent of alcohol intake
- High amounts of gut ethanol production may ablate beneficial effects of experimental drugs and variability in the amount of EtOH produced represents an unaccounted for confounder
- Drugs tested in ongoing NASH clinical trials aim to correct metabolic processes that are exacerbated by chronic ethanol exposure. For this reason, ethanol intake is strictly controlled but gut produced ethanol affecting the liver is not taken into account.
- A diagnostic test for gut ethanol production would help to assess and control for this confounder with the potential to identify a subset of patients more likely to respond to therapy in clinical trials increasing the potential to bring new NASH drugs to market

nature medicine

Article

https://doi.org/10.1038/s41591-022-02016-6

Microbiome-derived ethanol in nonalcoholic fatty liver disease

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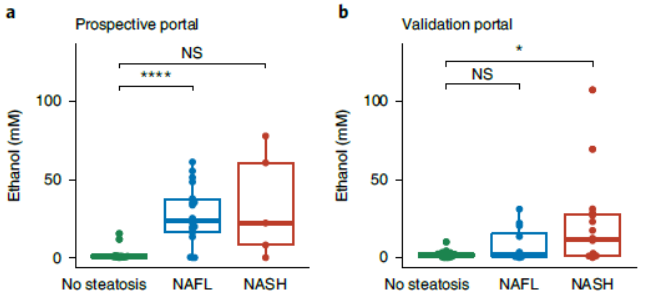
Check for updates

Abraham S. Meijnikman[1,2], Mark Davids[1], Hilde Herrema[1], Omrum Aydin[1,2], Valentina Tremaroli[3], Melany Rios-Morales[1], Han Levels[1], Sjoerd Bruin[2], Maurits de Brauw[2], Joanne Verheij[4], Marleen Kemper[5], Adriaan G. Holleboom[1], Maarten E. Tushuizen[6], Thue W. Schwartz[7], Jens Nielsen[8], Dees Brandjes[1], Eveline Dirinck[9], Jonas Weyler[10], An Verrijken[9], Christophe E. M. De Block[9], Luisa Vonghia[10], Sven Francque[10], Ulrich Beuers[11], Victor E. A. Gerdes[1,2], Fredrik Bäckhed[3,7], Albert K. Groen[1] and Max Nieuwdorp[1,2]



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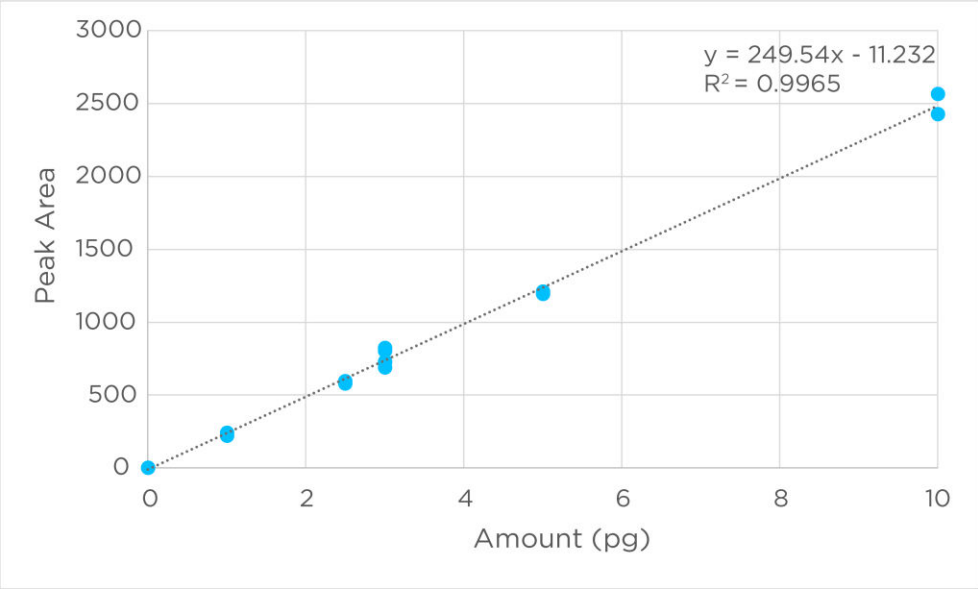
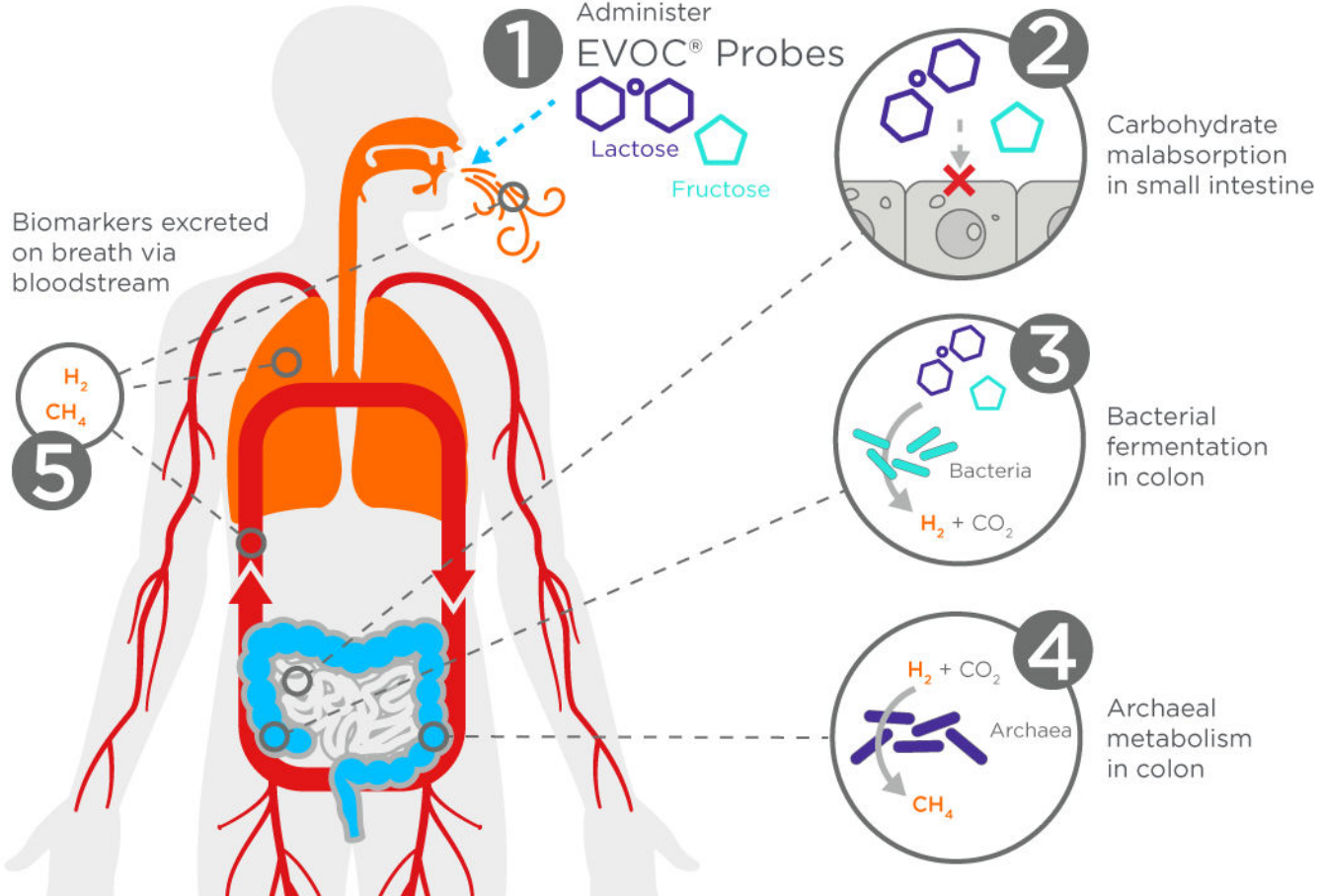
Owlstone Medical is Well Positioned to Fill this Diagnostic Need

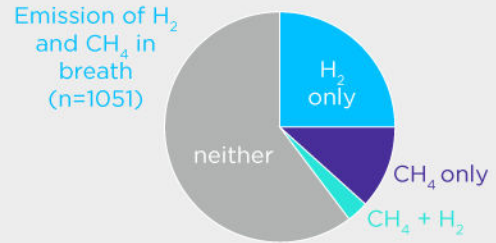
- Expected breath levels of ethanol from gut fermentation are < 20 PPB v/v (hepatic masking effect)
- Commercially available breathalysers have a sensitivity of > 100 PPB v/v
- Owlstone has developed a breath sampling device and analytical method able to detect ethanol in the PPQ range v/v
- The device could be used for at home collection allowing identification (and potential exclusion) of subjects before their first visit

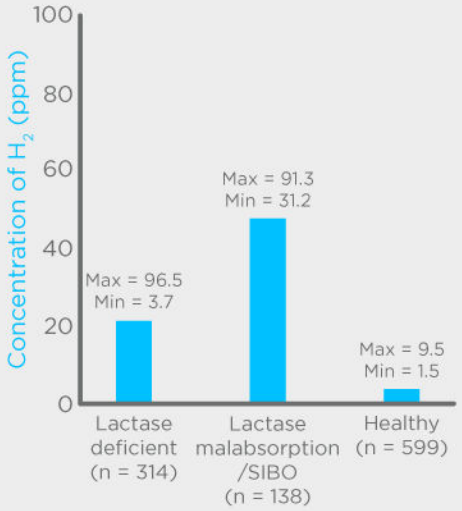


Picograms (pg) on tube at these levels equates to < 100 part per quadrillion (ppq v/v) levels on breath

Exogenous (EVOC) Probes for SIBO & Carbohydrate Malabsorption







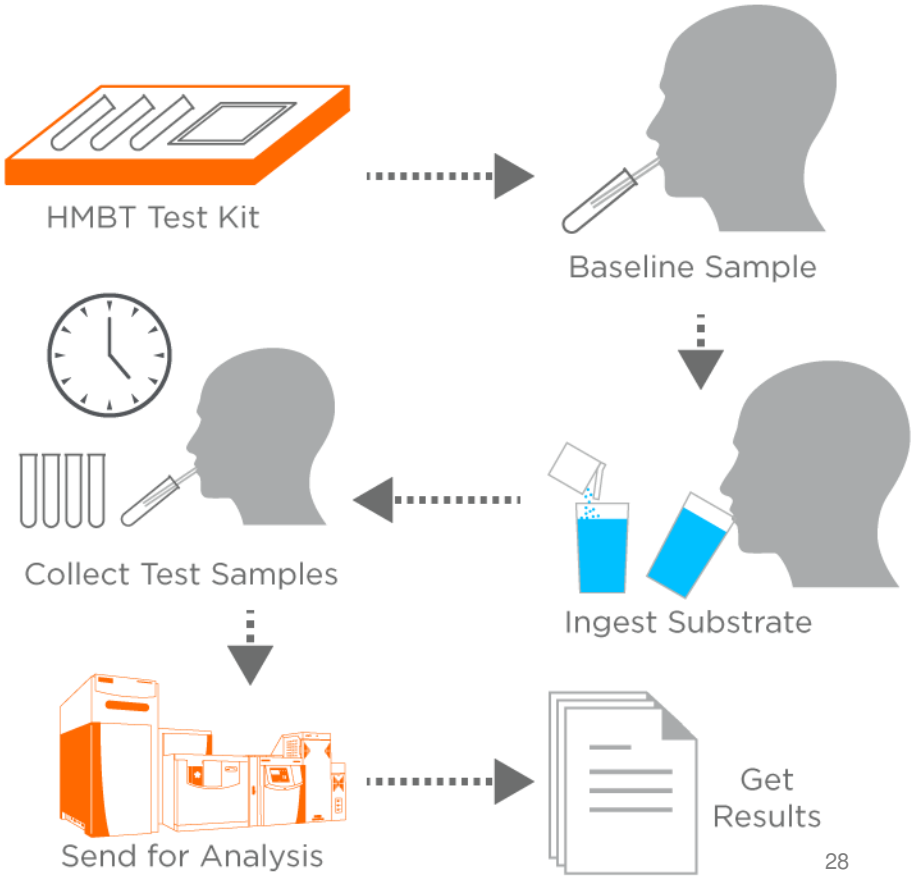
Lactose Breath Test Results (n = 1051)

Houben et al. (2015) *Nutrients*

Hydrogen and Methane Breath Test (HMBT) – Translation to Home Testing

HMBT is now available through Breath Biopsy

- HMBT is provided as a separate kit and does not use the ReCIVA Breath Sampler or Breath Biopsy Collection Station
- HMBT sampling kits are easy to use and can be distributed to clinics or for home use
- Each subject provides multiple samples over a period of time to measure response to substrate
- A range of tests are available for different research needs – e.g. SIBO



Changes in exhaled volatile organic compounds following iron supplementation in self-reported healthy adults

BREATH BIOPSY

functional GUT CLINIC

Changes in exhaled volatile organic compounds following iron supplementation in self-reported healthy adults.

Rory Stallard[1], Ahmed Tawfike[1], Federico Ricciardi[1], Agnieszka Smolinska[1,3], Liz Thompson[1], Amerjit Kang[1], Kirk Pappan[1], Sarah Bloor[2], Anthony Hobson[2], Max Allsworth[1], Nabeetha Nagalingam[1]

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Aims

- To investigate changes in VOCs (volatile organic compounds) due to iron supplementation between Day 1 and Day 28 of the study. This will assess VOC changes due to iron supplementation.
- To investigate changes in VOCs following lactulose ingestion. This will assess VOC changes along the gastrointestinal tract.

1. Background and Objectives

Iron deficiency anaemia (IDA) affects approximately >1.2 billion people worldwide[1,2,3]. In the UK, it can be the reason for up to 13% of referrals to gastroenterologists[4]. Furthermore, The World Health Organisation recognizes IDA as one of the most expensive diseases due to its negative impact on productivity.

IDA can be treated with both oral supplements or IV infusions which are both effective at restoring iron levels in patients. Unabsorbed iron can have unintended side-effects such as enriching intestinal bacteria that result in bloating due to production of gases. These gases can diffuse into the lungs via the blood and are then detectable on exhaled breath.

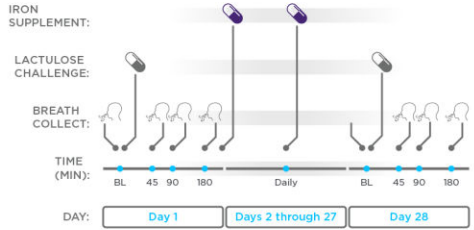
Hydrogen and methane are two gases that have been associated with IDA following consumption of the fermentable carbohydrate, lactulose[5]. This research aims to extend this knowledge by exploring whether other gases, volatile organic compounds (VOC), are associated with oral iron supplementation using the lactulose test.

2. Methods

This project was based on VOC changes caused by 28 days of iron supplementation in healthy volunteers.

Owlstone Medical and The Functional Gut Clinic (FGC) were interested in identifying novel breath biomarkers that change in response to oral iron supplementation, and whether production of these biomarkers are related to intestinal geography.

Samples were collected at multiple time points before and after the iron supplementation process, thus each subject served as their own control. Breath samples were analysed at Owlstone Medical Inc. using SIFT-MS technology. Targeted analyses were performed, and compounds deemed statistically significant if they were more than two standard deviations from the lab ambient



Day	Description	Sampling Time Point (n)			
		T=0 Baseline Before Lactulose Challenge	45 min	90 min	180 min
1	Post-iron suppl.	N=25	N=25	N=25	N=25
28	Post-iron suppl.	N=25	N=25	N=25	N=25

Figure 1: Experimental Design: This was a single-centre, longitudinal study with a population of healthy volunteers monitored before and after exposure to iron supplementation [ClinicalTrials.gov identifier (NCT number): NCT04705662]. 25 adult healthy volunteers were recruited for breath sampling for breath collection using polyvinylidene difluoride (PDVF) breath bags. The site of volunteer induction and sample collection was The Functional Gut Clinic, Manchester. Each volunteer underwent sampling on day 1 before and after administration of lactulose to measure baseline of fermentation levels. After the day 1 visit, volunteers took iron supplements daily and kept a record of any gastrointestinal (GI) tract symptoms experienced. Each volunteer underwent sampling on day 28 ± 2d or sooner if GI symptoms were severe (follow-up clinic visit) before and after administration of lactulose to measure follow-up levels of fermentation.

3. Results

From the 25 healthy volunteers that participated in this study, 2 were excluded due to incomplete samples. Ambient (blank) samples were collected, but not for all patients/visits/timepoints. Data was symmetrically distributed therefore further mathematical transformation was unnecessary.

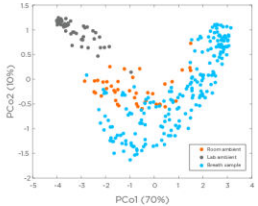


Figure 2: PCA analysis showing breath samples are distinct from both lab and room ambient samples using targeted compounds. Pentanoic, butanoic, propanoic, acetic 3-methylnutanoic and hexanoic acids, ethanol, hydrogen sulphide, methane, indole, isoprene, cresols, 2,3-butanedione, trimethylamine, acetone, limonene and phenol were selected in targeted analysis. Lab ambient and room ambient samples show divergent composition, with room ambient resembling the composition of breath samples more than lab samples.

Compound	Adjusted difference	p-value
3-methylbutanoic acid	0.675	0.017
butanoic acid	15.486	0.047
propanoic acid	4.707	0.026
2,3-butanedione	9.793	0.045
limonene	1.307	0.007
hydrogen sulfide	-22.667	0.026
cresol	0.194	0.005

Table 1: Table shows compounds that significantly change after iron supplementation. Linear Mixed models were fitted to evaluate the evolution overtime of the compounds' intensities. These models allow evaluation of both the effect of iron supplementation and that of lactulose challenge, also accounting for the observations' dependence due to repeated measurements from the same HV. For iron supplementation, the model shows that baseline, i.e. time pt. 0, levels of several compounds are significantly different between Day 1 and Day 28.

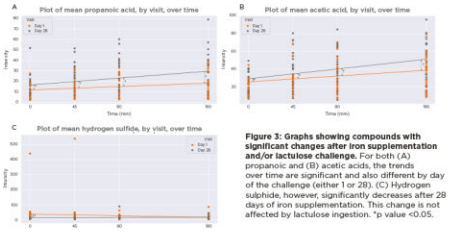


Figure 3: Graphs showing compounds with significant changes after iron supplementation and/or lactulose challenge. For both (A) propanoic and (B) acetic acids, the trends over time are significant and also different by day of the challenge (either 1 or 28). (C) Hydrogen sulphide, however, significantly decreases after 28 days of iron supplementation. This change is not affected by lactulose ingestion. *p value <0.05.

4. Conclusions

Some short chain fatty acids (SCFAs), butanoic, propanoic and acetic acids, increased after 28 days of iron supplementation following lactulose ingestion: Increases in SCFA has been linked to increased gut health[6]. They have been shown to maintain colonocyte development, promote metabolic health and speculated to play a key role in neuro-immunoendocrine regulation[6,7]. The significant increase in these SCFAs indicate a positive effect of iron supplementation in this cohort.

SCFAs propanoic and acetic acids are associated with geography specific fermentation: Relatively higher levels of these compounds were observed at 180m post lactulose ingestion indicating colonic fermentation[8]. These findings are supported by previous evidence showing SCFAs are the main metabolites produced in the colon by bacterial fermentation[9].

Hydrogen sulphide (H2S) was significantly decreased after 28 days of iron supplementation following lactulose ingestion. H2S is considered to be detrimental to gut health thus decreases in this compound is beneficial[10]. Please see talk 'The past, present and future of breath testing for bacterial overgrowth' at BBCon 2022 to hear more about the effects of H2S and its role in this study.

It should also be noted that a limitation of this study was that blank measurements were done at the clinic site by drawing ambient air into a bag via a syringe. This air may have atmospheric contamination due to cleaning agents, perfumes etc. Thus, the room ambient and breath samples may be noisy. The statistically significant changes were calculated as two standard deviations from lab ambient.

Another limitation of this study was that not all subjects were healthy. After filling out clinical questionnaires, it was determined that subjects showed signs of small intestinal bacterial overgrowth (SIBO) or irritable bowel syndrome (IBS). These underlying conditions would have likely impacted VOCs produced.

References

1- Mathers, C., Fat, D.M., Boerma, J.T. and World Health Organization eds., 2008. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization.

2- Schrier, S.L. and A., 2018. Treatment of iron deficiency anaemia in Adults. [online] Available at: <https://www.uptodate.com/contents/treatment-of-iron-deficiency-anemia-in-adults> [Accessed 2 Nov. 2018].

3- Camaschella, C., 2019. Iron-deficiency. Blood, The Journal of the American Society of Hematology, 133(1), pp.30-39.

4- Goddard, A.F., James, M.W., McIntyre, A.S., Scott, B.B. and on behalf of the British Society of Gastroenterology, 2011. Guidelines for the management of iron deficiency anaemia. Gut, 60(10), pp.1309-1316.

5- Rezaie A et al. Rimatori M. Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus. Am J Gastroenterol. 2017 May;112(5):775-784. doi: 10.1038/ajg.2017.46. Epub 2017 Mar 21. PMID: 28323273; PMCID: PMC5449659.

6- Martin-Gallausiaux C, et al. SCFA: mechanisms and functional importance in the gut. Proc Nutr Soc. 2021 Feb;80(1):37-49. doi: 10.1017/S0029665120006916. Epub 2020 Apr 3. PMID: 32238208.

7- Blaak EE, et al. Short chain fatty acids in human gut and metabolic health. Benef Microbes. 2020 Sep 1;11(5):411-455. doi: 10.3920/BM2020.0057. Epub 2020 Aug 31. PMID: 32865004.

8- Ghoshal UC. How to interpret hydrogen breath tests. J Neurogastroenterol Motil. 2011 Jul;17(3):312-7. doi: 10.5056/jnm.2011.17.3.312. Epub 2011 Jul 14. PMID: 21860825; PMCID: PMC3155063.

9- Hosseinkhani F, Heinken A, Thiele I, Linsen HW, Stams AJM, Abdul Hamid MI, Harms N, Harmsen HJM. The contribution of gut bacterial metabolites in the human immune signaling pathways non-communicable diseases. Gut Microbes. 2021 Jan-Dec;13(1):1-22. doi: 10.1080/19490976.2021.1882612. PMID: 33651651; PMCID: PMC7928018.

10- Dordević D, et al. Hydrogen sulfide toxicity in the gut environment: Meta-analysis of sulfate-reducing and lactic acid bacteria in inflammatory processes. Journal of Advanced Research, Volume 27, 2021, Pages 69-68, ISSN 2090-1232, https://doi.org/10.1016/j.jare.2020.05.005.

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Background, Objectives & Design

- Iron deficiency Anaemia (IDA) affects approximately 1.2 billion people worldwide and is treated with oral supplements or IV infusions.
- Unabsorbed iron can have unintended side effects like enriching intestinal bacteria that result in bloating, gas and constipation.
- Hydrogen and methane are two gases that have been associated with IDA following consumption of the fermentable carbohydrate, lactulose. These gases can diffuse into the lungs via the blood and are then detectable on exhaled breath.
- This research aims to investigate if other gases, volatile organic compounds (VOCs), are associated with oral iron supplementation using lactulose testing.
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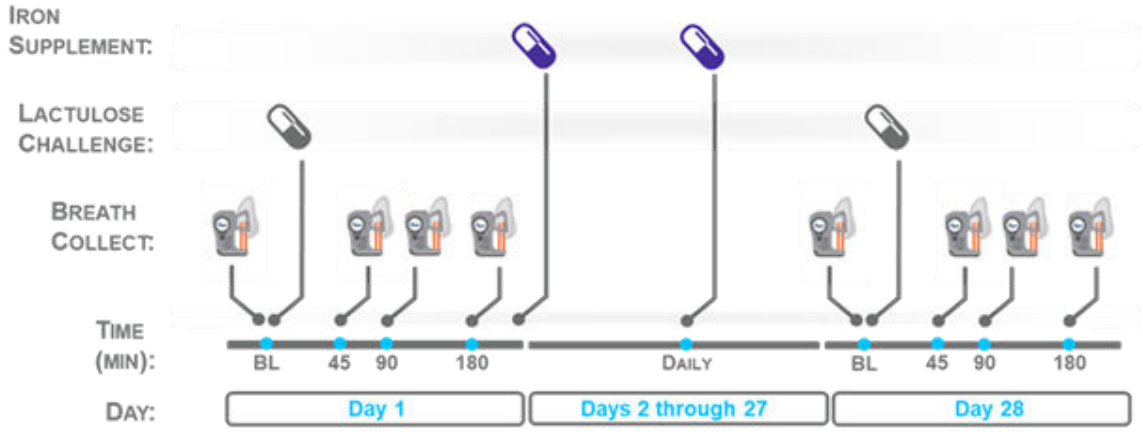

Results

- Pentanoic, butanoic, propanoic, acetic 3-methylnutanoic and hexanoic acids, ethanol, hydrogen sulphide, methane, indole, isoprene, cresols, 2,3-butanedione, trimethylamine, acetone, limonene and phenol were selected in targeted analysis.
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Table shows compounds that significantly change after iron supplementation.

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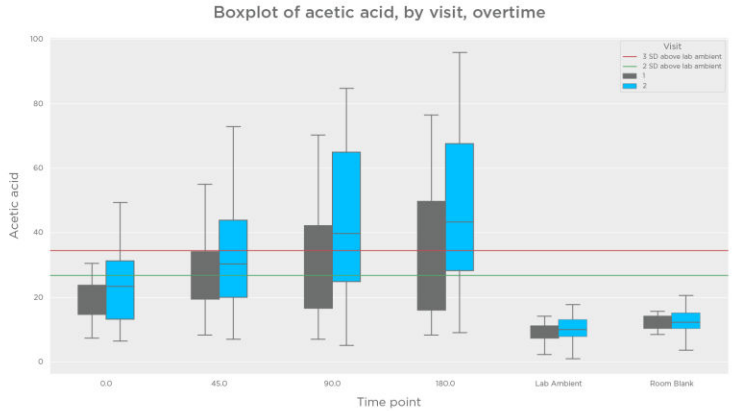
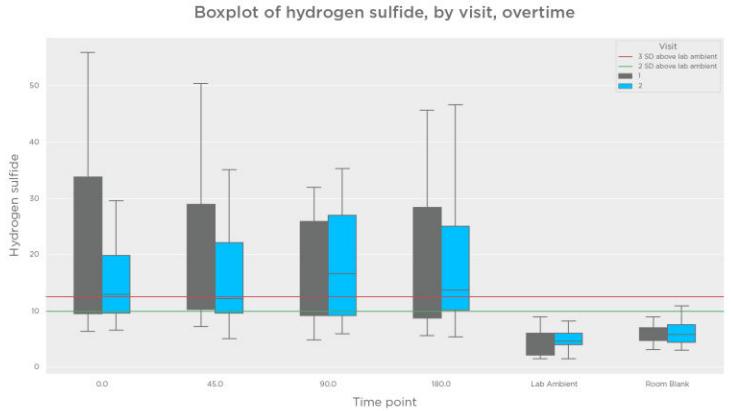
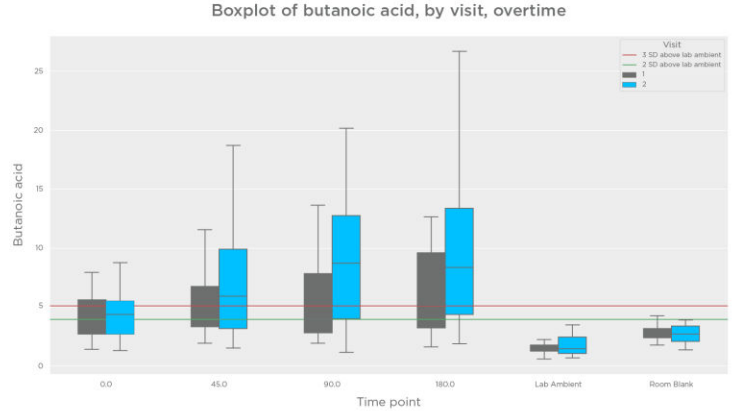
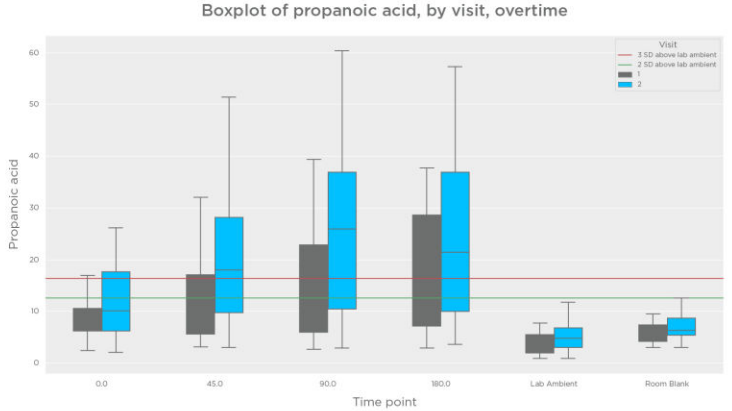
- (C) Hydrogen sulphide, however, significantly decreases after 28 days of iron supplementation.
- This change is not affected by lactulose ingestion. *p value <0.05.

Results from targeted VOC analysis – Hydrogen Sulphide, Acetic Acid, Butanoic Acid, Propanoic Acid







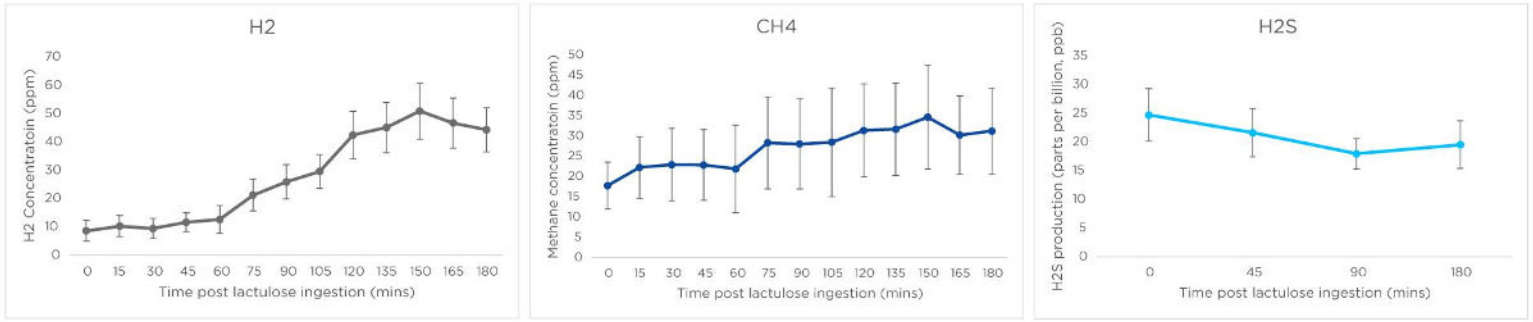


Detection of Hydrogen Sulphide in Breath

- Hydrogen sulfide (H2S) is a gas produced by certain gut bacteria, including sulphate reducing bacteria (SRB).
- H2S acts as a gut health regulator, influencing motility, ischemia, and reperfusion, and can promote or inhibit inflammation depending on its concentration - Singh, S. and Lin, H., 2015.
- However, excessive levels of SRB in the gut can lead to small intestinal bacterial overgrowth (SIBO) or colonic dysbiosis.
- Alterations in H2S levels have been linked to gastrointestinal disorders such as ulcerative colitis, Crohn's disease, and irritable bowel syndrome (IBS) - Banik, G et al., 2016.
- In healthy individuals, H2S is observed at a rate below 1.2ppm in the breath.
- H2S levels >1.2ppm are clinically significant and correlate with diarrhoea, urgency, and abdominal pain - Fowler, H et al., 2020.

Detection of Hydrogen Sulphide in Breath

- We completed an assessment of H2S levels in healthy subjects aged 18-60 using lactulose breath testing.
- Participants provided baseline breath samples before ingesting 10g lactulose. Further breath tests were collected at 15-minute intervals until 180 mins had passed, for hydrogen and methane analysis, and at 45-, 90-, and 180-minutes post lactulose ingestion for hydrogen sulphide analysis.
- Whilst hydrogen and methane levels increased over the course of the study as the lactulose was fermented, hydrogen sulphide production decreased over the time course of the breath test.
- Hydrogen sulphide levels were consistently below 50ppb which allows us to establish the normal range that can be used in future clinical studies.



Mean hydrogen (H_2), methane (CH_4) and hydrogen sulphide (H_2S) production in parts per million (ppm) for hydrogen and methane, and parts per billion (ppb) for hydrogen sulphide over a 3hr lactulose breath test.

What Can Owlstone Offer?

- A robust platform for breath VOC collection and analysis
- Proposed list of putative biomarkers based on literature and biology
- Quantify targeted panel in human breath and *in vitro* (bottom-up)
- Run non-targeted broad-based analysis of other VOCs in human breath and *in vitro* (top-down)
- Study consultation & project management, collection, analysis, statistics, biological interpretation to validate biomarkers
- Ability to develop point-of-care / home-based solutions for decentralized clinical trials and screening applications

Discover & Validate → Deploy Home Collect → Deploy Home Collect & Analyze

THANK YOU

Interested in applying this to your work?

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