

Real-time, home-based analysis of microbiome metabolites with the OMED Health Breath Analyzer

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   Owlstone Medical



Introductions



**Dr Elizabeth (Liz)
Crone (PhD)**



Dr Rui Lopes (MD)



Dr Matt Kerr (PhD)

Today's Webinar



High-Level Introduction to Owlstone

Endogenous (human) metabolites



Microbial metabolites



Exogenous metabolites



Discover

Deploy

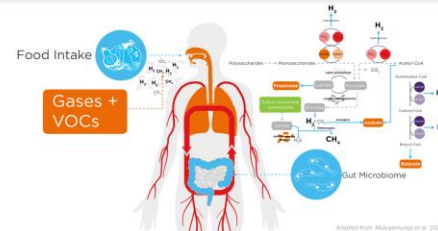


Breath Analyzer and App – How it Works

- Handheld breath analyzer to accurately measure hydrogen and methane in the home environment.
- Feasible to take multiple measurements per day (only 15 minute intervals needs between samples), over multiple days. No consumables needed.
- Companion mobile app to support breath sampling protocol, record breath measurements, as well as the logging of food/drink and tracking symptoms and lifestyle
- Secure, reliable cloud infrastructure for storage of Special Category data (UK/EU/US), secure APIs for integration and interoperability
- App to support research use (in development) should enable:
 - Compliance monitoring in trials
 - Detailed interrogation of data



Gas production from gut microbiota



Adapted from: Makymenko et al. 2023

Exploring emerging basic science indications for methane

Aims

- Summarise exciting, emerging, basic science research on methane.
- Focus on production and potential roles of methane outside of those already discussed.
- Address challenges associated with correlation vs causation in these settings.
- Highlight areas where easy access to epidemiological datasets could provide novel insights into existing hypotheses.



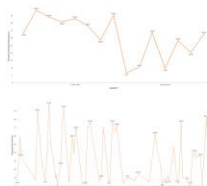
Inclusion or discussion of data or findings within this presentation does not constitute endorsement or agreement by OAM.

How Can the OMED Device Be Useful in Research: Dense Datasets and Longitudinal Monitoring

The OMED device makes it possible to build a detailed understanding of hydrogen and methane dynamics.

Repeated sampling is possible (without extra analysis cost), and can help average out sources of biological variability (e.g. diet, circadian rhythm or exercise) to allow you to better understand the true impact of your intervention/challenge. To give some potential examples for how this could be used:

- Better understanding of how patients respond to particular challenges (e.g. fibers)
- Better comparisons between different challenge substrates (e.g. different fibers)
- It could be possible to avoid a challenge altogether and instead build averages for a person natural hydrogen and methane levels pre and post intervention

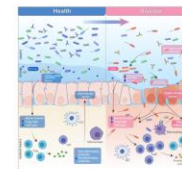


Microbiome in Health and Disease

The gut microbiome is not only an inert onlooker, but also dynamically affects different host functions.

Eubiosis: delicate ecosystem with symbiotic "healthy" relationship between gut microbiome community and host;

Dysbiosis: impairment of homeostasis, reflected by a change in composition, diversity, or metabolites of the microbiome from a healthy pattern to a pattern associated with disease or a predisposition to disease;



Hsu, K., et al. Microbiota in health and disease. (2022).

Hsu, K., Yin, Y., Kaplan, D., Sankaranarayanan, S., Valasek, C., Farnsworth, M., Mackay, C., Collins, C., Srinivasan, S., Chappell, S. Editors and authors. The Gut Microbiome. New Mexico: (2022 Jan 2022) p. 1-18. 978-1-953000-00-0.

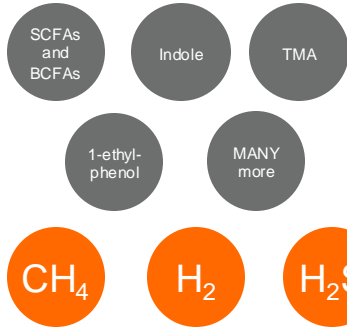
High-Level Introduction to Owlstone



Endogenous (human) metabolites



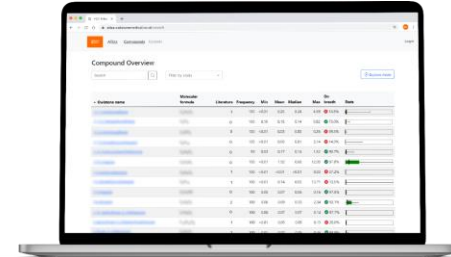
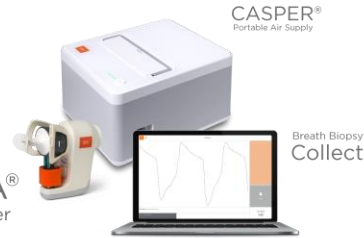
Microbial metabolites



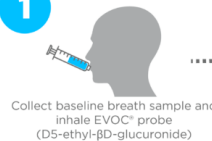
Exogenous metabolites



ReCIVA[®]
Breath Sampler



1



2



3

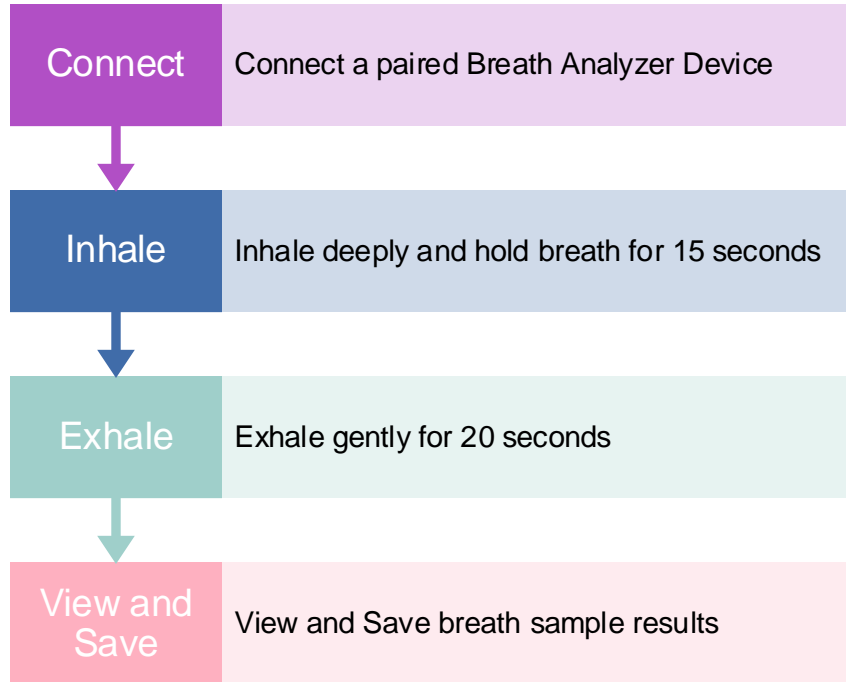


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Collect & Analyze Breath (< 3 minutes per sample)



Gas production from gut microbiota



Phylum	Genus/Species	Gas	Reference
Bacteroidetes	<i>Parabacteroides</i>	H ₂	Ezeji et al., 2021
	<i>Alistipes</i>	H ₂	Oliphant & Allen-Vercoe, 2019
	<i>Bacteroides</i>	H ₂	Smith et al., 2019
Firmicutes	<i>Enterococcus</i>	H ₂	Robert & Bernalier-Donadille, 2003
	<i>Dorea</i>	H ₂	Oliphant & Allen-Vercoe, 2019
	<i>Clostridium spp.</i>	H ₂	Steer et al., 2001
	<i>Roseburia intestinalis</i>	H ₂	Duncan et al., 2002
	<i>Ruminococcus</i>	H ₂	Zheng et al., 2014
	<i>Anaerostipes caccae</i>	H ₂	Schwartz et al., 2002
	<i>Eubacterium rectale</i>	H ₂	Duncan & Flint, 2008
	<i>Blautia</i>	H ₂	Suzuki et al., 2018
	<i>Veillonella</i>	H ₂	Aujoulat et al., 2014
	<i>Victivallis vadensis</i>	H ₂	Zoetendal et al., 2003
Proteobacteria	<i>Escherichia</i>	H ₂	Suzuki et al., 2018
Euryarchaeota	<i>Methanobrevibacter Smithii</i>	CH ₄	Weaver et al., 1986
	<i>Methanosphaera stadtmanae</i>	CH ₄	Fricke et al., 2006
	<i>Methanobrevibacter oralis</i>	CH ₄	Scanlan et al., 2006
	<i>Methanomassiliicoccus luminyensis</i>	CH ₄	Nkanga et al., 2017

- H₂ is the dominant gas produced by gut microbes.
- Approximately 70% of gastrointestinal microbial species in the Human Microbiome Project can generate H₂ (Wolf et al., 2016)
- Methane is produced via archaeal metabolism, specifically that of *M. smithii*, *M. stadtmanae*, and *M. luminyensis*, with *M. smithii* making up the vast majority of contributions (~94%)¹⁻⁴.

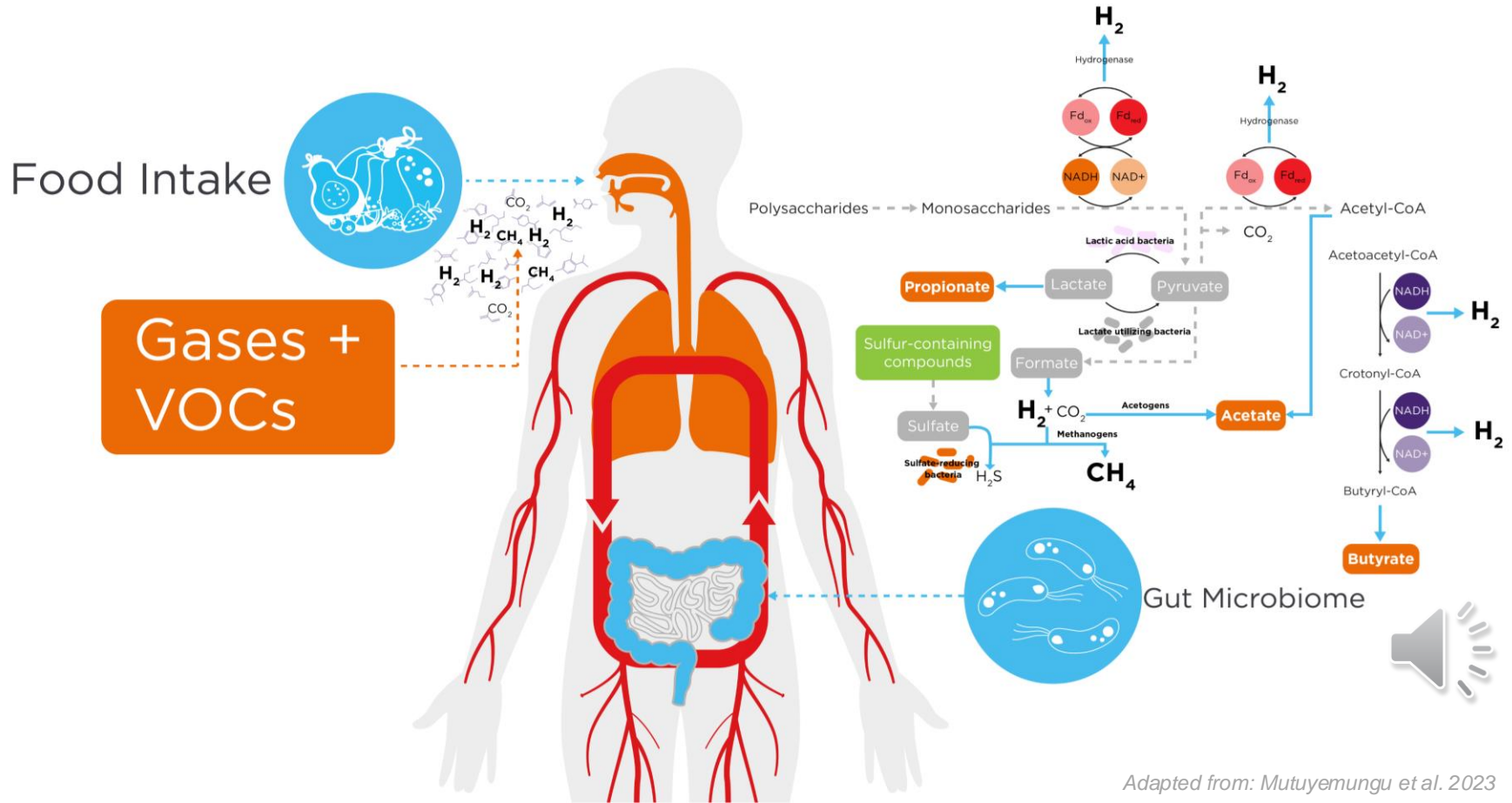
1 - Weaver GA, Krause JA, Miller TL, Wolin MJ. Incidence of methanogenic bacteria in a sigmoidoscopy population: an association of methanogenic bacteria and diverticulosis. Gut. 1986 Jun 1;27(6):698-704.

2 - Fricke WF, Seedorf H, Henne A, Krüger M, Liesegang H, Hedderich R, et al. The Genome Sequence of Methanosphaera stadtmanae Reveals Why This Human Intestinal Archaeon Is Restricted to Methanol and H₂ for Methane Formation and ATP Synthesis. J Bacteriol. 2006 Jan 15;188(2):642-58.

3 - Scanlan PD, Shanahan F, Marchesi JR. Human methanogen diversity and incidence in healthy and diseased colonic groups using mcrA gene analysis. BMC Microbiol. 2008 May 20;8(1):79.

4 - Nkanga VD, Henrissat B, Drancourt M. Archaea: Essential inhabitants of the human digestive microbiota. Hum Microbiome J. 2017 Mar 1;3:1-8.

Gas production from gut microbiota

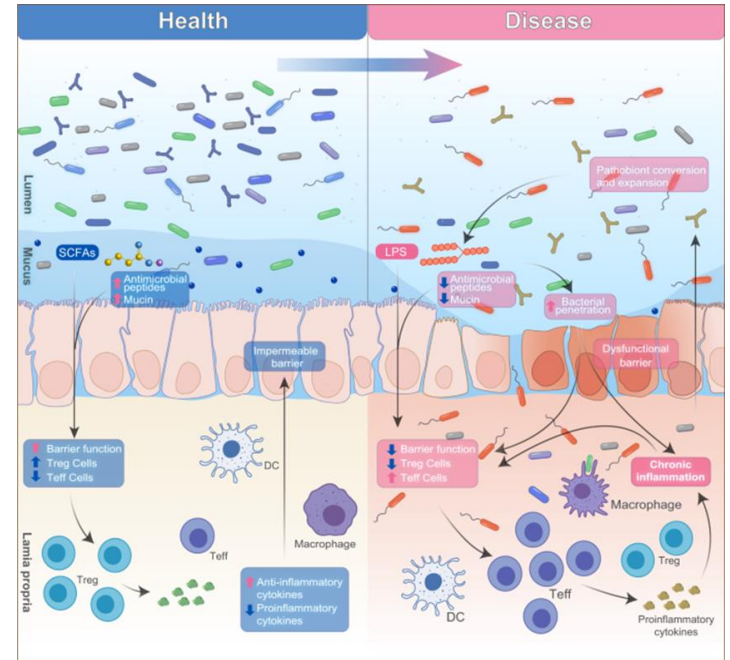


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Hou, K., et al. Microbiota in health and diseases. (2022).

Hydrogen and Methane in Eubiosis

Hydrogen (H₂) is produced when unabsorbed carbohydrates reach the colon and are fermented by commensals of the large intestine.

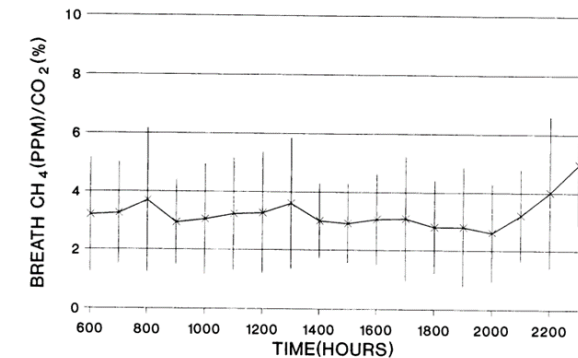
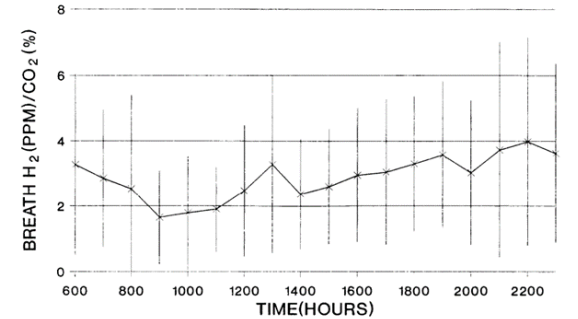
Methane (CH₄) is produced by utilising hydrogen to reduce CO₂. Methanogens are present in virtually all healthy adults' flora, but just one to two thirds of healthy adults have enough methanogens to produce detectable breath levels¹.

Hydrogen in breath follows a circadian pattern, influenced by diet (and particularly carbohydrate ingestion).

Breath methane is relatively constant throughout the day².

Baseline H₂: 6 (3-11) ppm³

Baseline CH₄: 4 (2-10) ppm³



Le Marchand L, et al (1992)

1. Pimentel, M., Mathur, R. & Chang, C. Gas and the Microbiome. *Curr Gastroenterol Rep* 15, 356 (2013). <https://doi.org/10.1007/s11894-013-0356-y>

2. Le Marchand L, Wilkens LR, Harwood P, Cooney RV. Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies: circadian patterns of excretion. *Environ Health Perspect*. 1992 Nov;98:199-202. doi: 10.1289/ehp.9298199. PMID: 1486849; PMCID: PMC1519616.

3. Alegre, E., Sandúa, A., Calleja, S. et al. Modification of baseline status to improve breath tests performance. *Sci Rep* 12, 9752 (2022). <https://doi.org/10.1038/s41598-022-14210-0>

Hydrogen and Methane in Dysbiosis

Hydrogen (H₂) may be produced in large amounts in malabsorption and if there is excess of bacteria in the small intestine. Recognised patterns in hydrogen breath tests (ie. Earlier peaks when small bowel colonised)^{1,2}

Methane (CH₄) producers may not exhale as much hydrogen as it is converted into methane. They traditionally have higher baselines with earlier methane rise.^{1,2}

High (≥20ppm) baseline H₂: 11%³
High (≥10ppm) baseline CH₄: 25%³

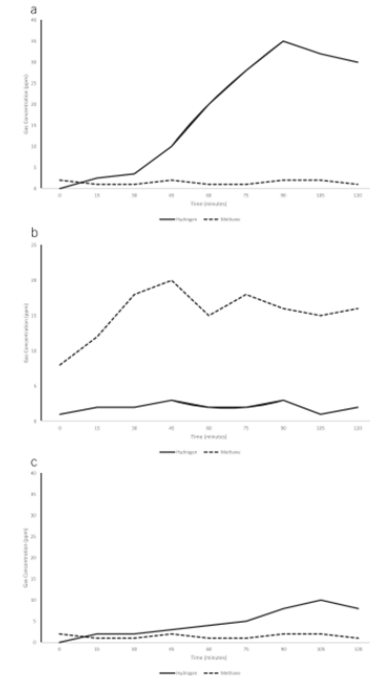


Figure 1. Breath test examples. (a) Hydrogen-positive breath test to suggest small intestinal bacterial overgrowth. (b) Methane-positive breath test to suggest intestinal methanogen overgrowth. (c) Normal breath test. ppm, parts per million.

Pimentel M, et al. (2020)

1. Posserud I, Stotzer PO, Björnsson ES, et al. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut* 2007; 56:802.
2. Shah ED, Basseri RJ, Chong K, Pimentel M. Abnormal breath testing in IBS: a meta-analysis. *Dig Dis Sci* 2010; 55:2441.
3. Alegre, E., Sandúa, A., Calleja, S. et al. Modification of baseline status to improve breath tests performance. *Sci Rep* 12, 9752 (2022). <https://doi.org/10.1038/s41598-022-14210-0>

Hydrogen and Methane in GI Symptoms



Abnormal **quantity and location of gas production in the GI tract can result in adverse symptoms** for the patient (ie. Bloating and abdominal distention).¹

Hydrogen accumulation due to bacterial fermentation of carbohydrates in the small intestine **contributes to bloating and abdominal pain experienced by patients with SIBO.**²

High levels of methane have been linked to decreased intestinal motility and are associated with **constipation, especially in patients with IBS-C.**³ The rate of excretion of hydrogen and methane in IBS patients has been reported to be significantly higher compared to healthy individuals.⁴

1. Bendezú RA, et al. Intestinal gas content and distribution in health and in patients with functional gut symptoms. *Neurogastroenterol Motil.* (2015)

2. Azpiroz, F., et al. Abdominal bloating. In *Gastroenterology* (2005).

3. Kunkel, D., et al Methane on breath testing is associated with constipation: A systematic review and meta-analysis. *Digestive Diseases and Sciences*, (2011).

4. King, et al. Abnormal colonic fermentation in irritable bowel syndrome. *The Lancet* (1998).

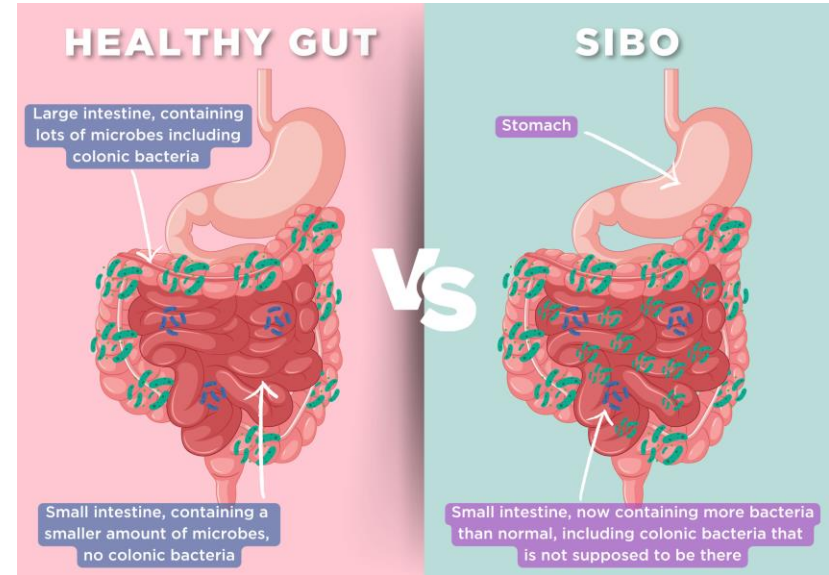
SIBO and Gastrointestinal Associations

Unclear prevalence, retrospective studies allude to 1 in 7.
SIBO is highly prevalent in various gastrointestinal disorders.

ETIOLOGY

- Functional and motility disorders (ie. IBS, pseudo-obstruction)
- Anatomic disorders (ie. Intestinal blind loops, small intestinal diverticulosis)
- Immune disorders;
- Hypochlorhydria (iatrogenic or primary)
- Systemic disorders;

The majority of patients with SIBO present with bloating, flatulence, abdominal discomfort, diarrhoea, or, in the case of intestinal methanogen overgrowth (IMO), constipation.

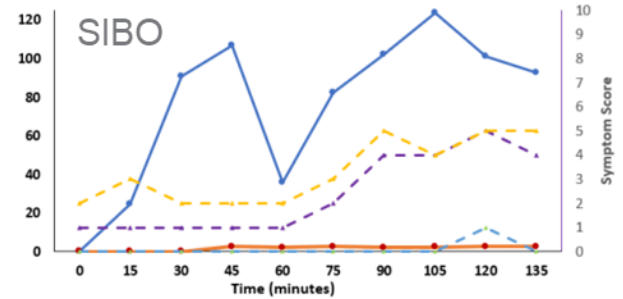
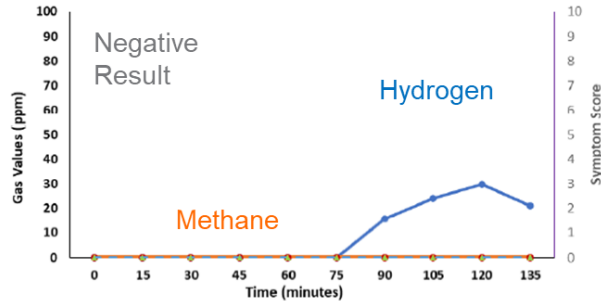


- Hydrogen and methane breath testing is a simple, non-invasive and safe way to start to unravel the biology behind an individual's GI symptoms
- HMBT is commonly performed in people with irritable bowel syndrome (IBS) to rule out other underlying conditions, or to identify potential causes of symptoms such as SIBO, IMO and Carbohydrate malabsorption.
- Based on biomarkers exclusive to the gut microbiome and founded on clear knowledge of the underlying biological processes and gut physiology, HMBT is backed by extensive scientific evidence¹
- HMBT challenge tests provide a practical way to study a person's microbial hydrogen and methane in a standardised way.
 - They generally require fasting ahead of the sampling, and often a controlled diet the night before to standardize between participants
 - Use of breath allows collection of multiple timepoints to explore how hydrogen and methane is changing – this is typically done in a clinical environment.

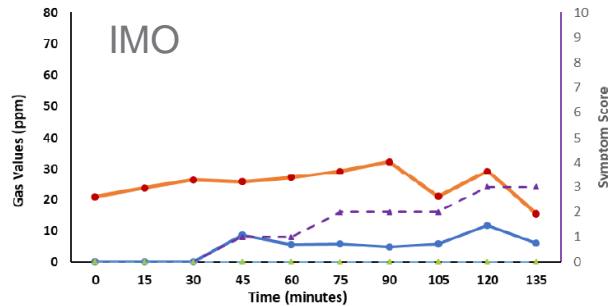
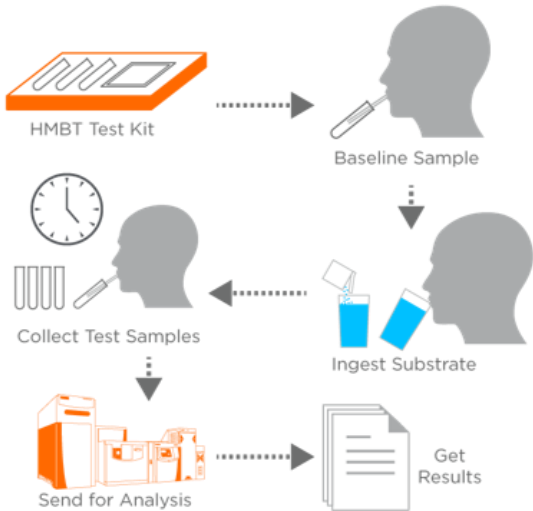
1. <https://pubmed.ncbi.nlm.nih.gov/17990113/>

2. https://journals.lww.com/ajg/Fulltext/2019/12000/Lactulose_Breath_Testing_as_a_Predictor_of.14.aspx

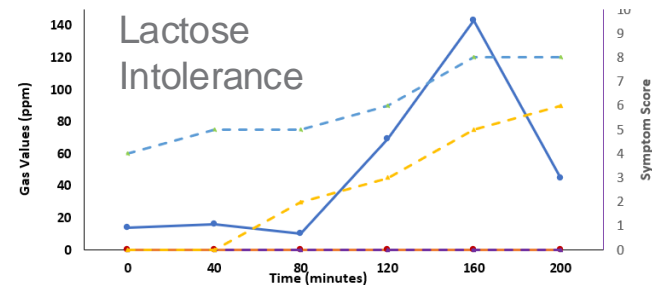
HMBT Challenge Tests



>20ppm in H₂ within 90 mins
 >10ppm in H₂ within 60 mins
 Double peak (lactulose only)



>10ppm in CH₄
 Typically elevated at baseline

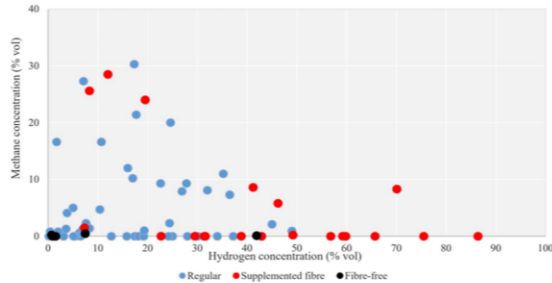


>20ppm in H₂
 Single peak

Challenges of Single Day Measurements

Significant variability not only across different users but also within each user across different days;

Breath hydrogen excretion has been consistently shown to be influenced by **diet** (particularly by carbohydrate ingestion), emotional factors (such as **anxiety** levels) and light exercise causing significant variation in baseline levels.



Modesto, A., *et al.* Meta-Analysis of the Composition of Human Intestinal Gases. (2022).

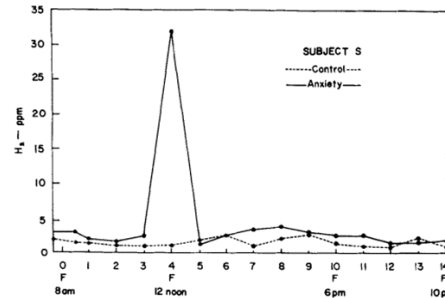
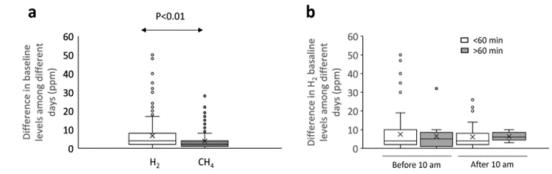


FIGURE 7. Effect of an anxiety-provoking interview on breath hydrogen concentration of a subject fed a formula diet.

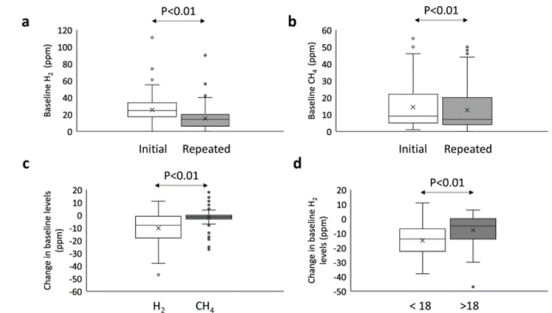
Calloway DH, et al. The use of expired air to measure intestinal gas formation. Ann NY Acad Sci. (1968)

Figure 2



(a) Indifference in H_2 and CH_4 baseline levels between different days. (b) Difference in H_2 baseline levels among different days depending on time of baseline sampling and whether sampling hours differ more than 60 min or not. Changes in baseline levels are represented in Box-Whisker plots.

Figure 3



Initial and repeated baseline levels of H_2 (a) and CH_4 (b). (c) Range of change in H_2 and CH_4 levels between initial and repeated baseline samples. (d) Range of change in H_2 levels according to patients' age. Baseline levels are represented in Box-whisker plots.

Alegre, E., *et al.* Modification of baseline status to improve breath tests performance. (2022).

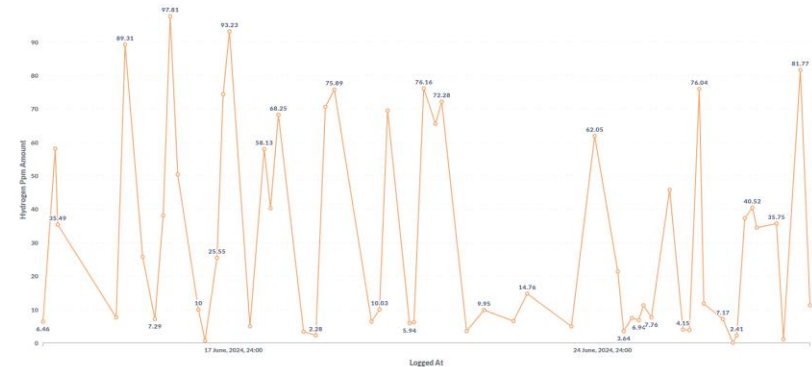
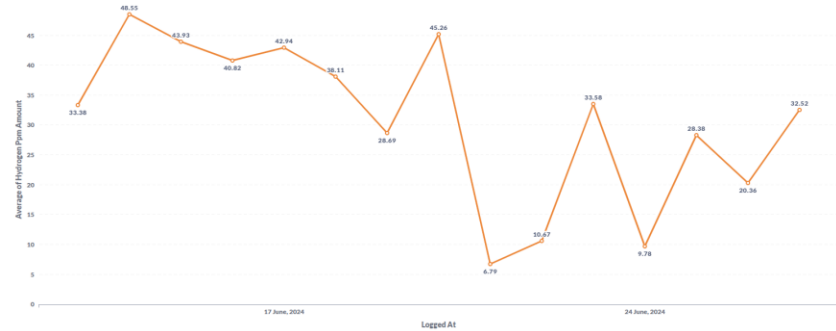
How Can the OMED Device Be Useful in Research: Dense Datasets and Longitudinal Monitoring



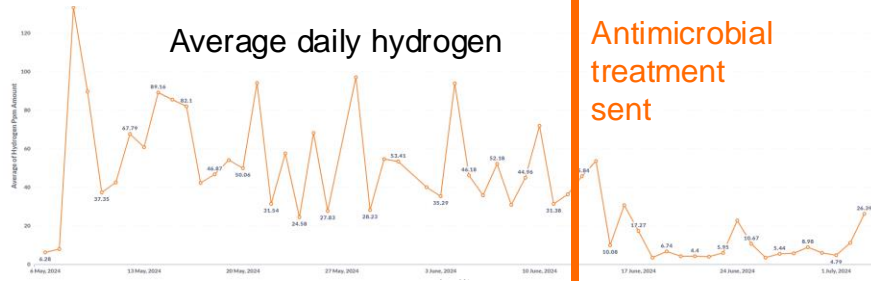
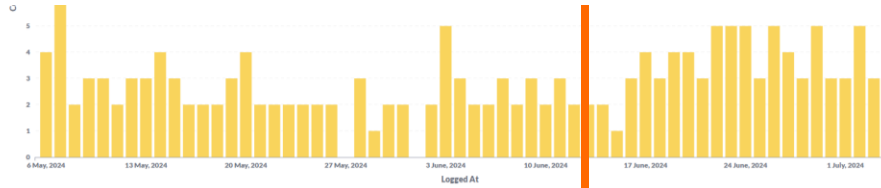
The OMED device makes it possible to build a detailed understanding of hydrogen and methane dynamics.

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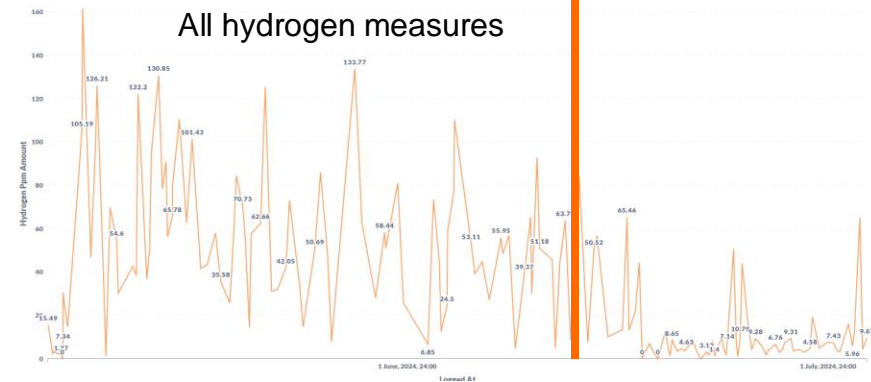
- Better understanding of how patients respond to particular challenges (e.g. fibers)
- Better comparisons between different challenge substrates (e.g. different fibers)
- It could be possible to avoid a challenge altogether and instead build averages for a person natural hydrogen and methane levels pre and post intervention



How Can the OMED Device Be Useful in Research: Example of Effects



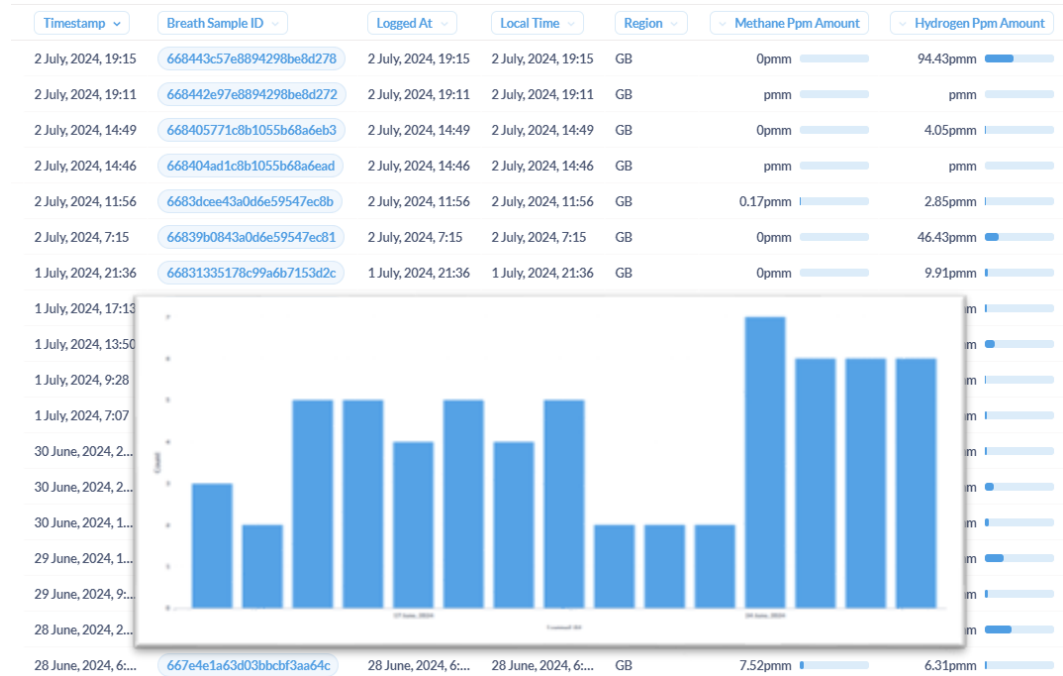
Antimicrobial
treatment
sent



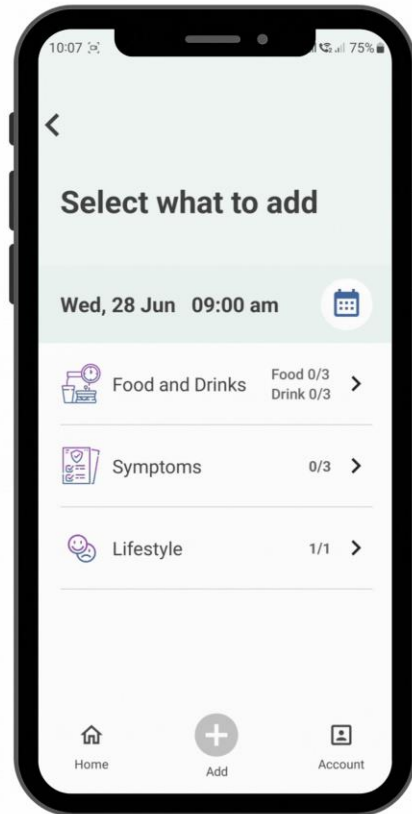
- Data provides an example of a treated patient with GI problems.
- Patient received antimicrobials on 13th June (unknown currently when they started taking them)
- Clear difference pre and post for hydrogen.

How Can the OMED Device Be Useful in Research: Decentralised Testing

Decentralized testing. Compliance monitoring feasible as data can be seen in real time. Alerts can be set up.



How Can the OMED Device Be Useful in Research: Centralised Additional Data



Record food and drink details quickly and easily

- Select from your 'Favourite' list
OR
- Add entries with keypad and 'Add to Favourite' for next time



Set the date/time as required for your entry

- Default is current date/time
OR
- Enter time retrospectively over last 14 days

How Can the OMED Device Be Useful in Research: Centralised Additional Data



Use the slider bars to record key symptoms severity



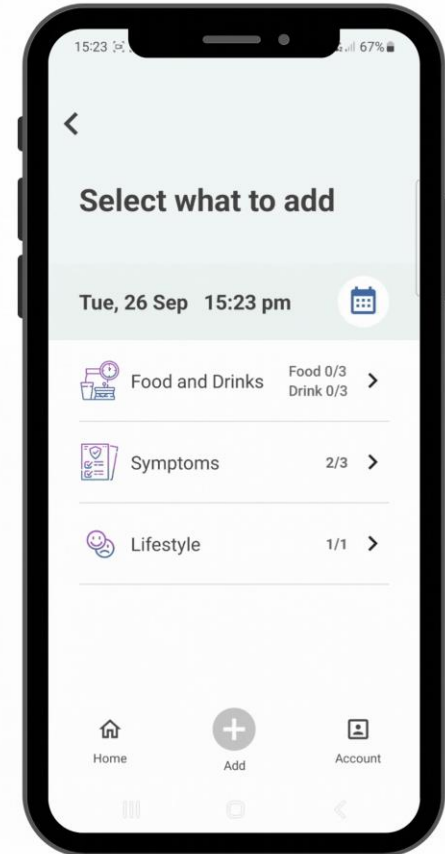
0-10 scores for Bloating, Abdominal Pain, Nausea and Flatulence



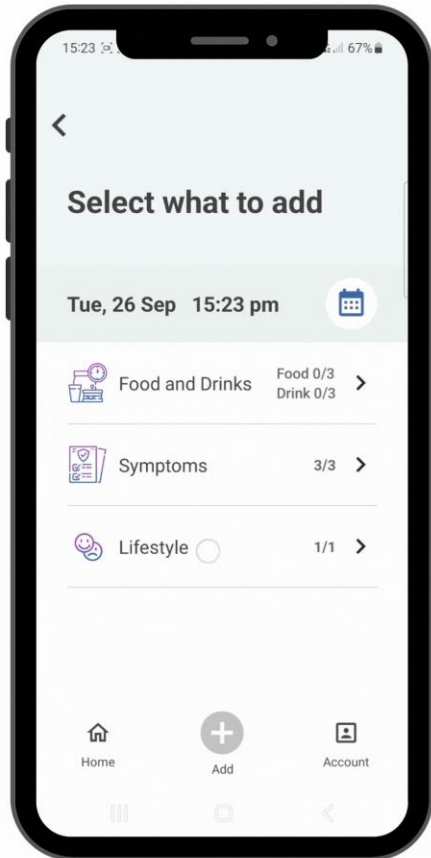
Bristol Stool Scale for Stool records



Set the date/time as required over last 14 days



How Can the OMED Device Be Useful in Research: Centralised Additional Data



Use the slider bars to record lifestyle activities



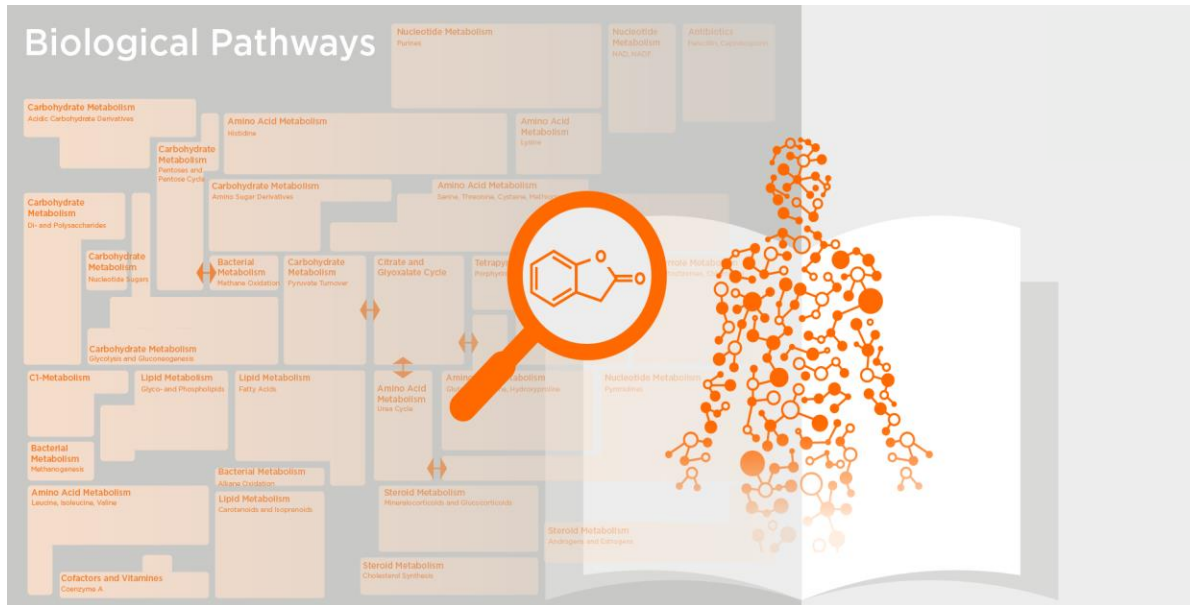
0-10 scores for Stress, Sleep Quality and Exercise



Set the date/time as required over last 14 days

Continued Development of Breath: The Breath Atlas

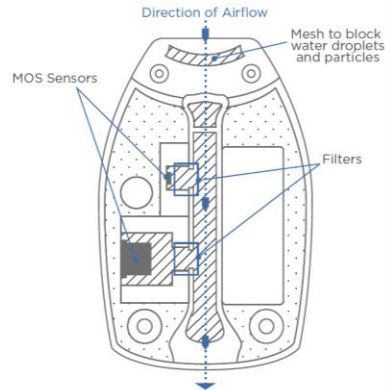
A searchable database of chemically identified **VOCs** (growing list of over 200 entries) proven to **exist on-breath** (differentiated from background contamination) with **quantitative data** from previous human studies including cohorts of healthy and diseased subjects



Allows **data-mining across studies** to enable:

- Understanding of underpinning **biological pathways**
- ***In silico* cross-validation of results** via comparison with previous studies to accelerate biomarker development
- Links to results from peer-reviewed **Literature** for individual VOCs
- Informed choices on **sampling and analytical methods**
- **Faster optimisation** of VOC analysis platforms and assays

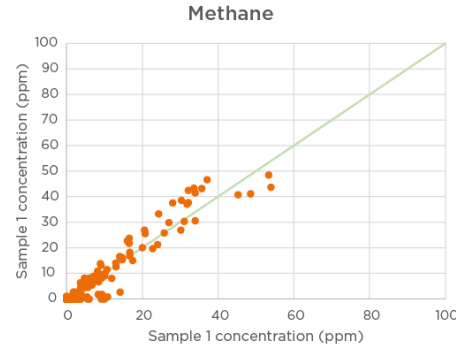
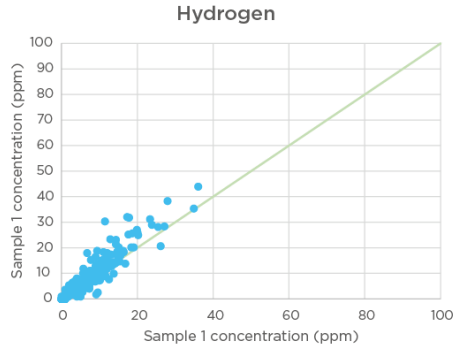
Technology in the OMED Device



- As breath passes through the device, active carbon components filter out any interferent gasses that may be present
- The hydrogen and methane gasses make contact with two metal oxide sensors creating electronic signals dependent on each gas concentration
- The signals are passed to an onboard processor where gas concentrations are calculated by a machine learning algorithm trained with hundreds of precise synthetic breaths
- The device sends the gas concentration results to a paired app for review and to inform any action to be taken

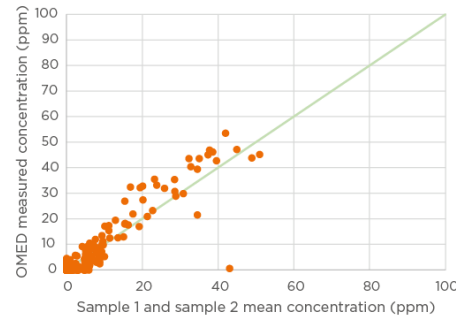
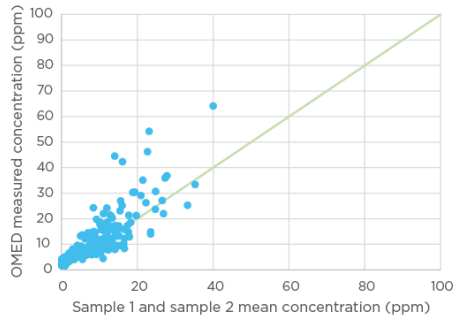
Evaluation on real breath samples and comparison against in clinic instrument

GG2 versus GG1

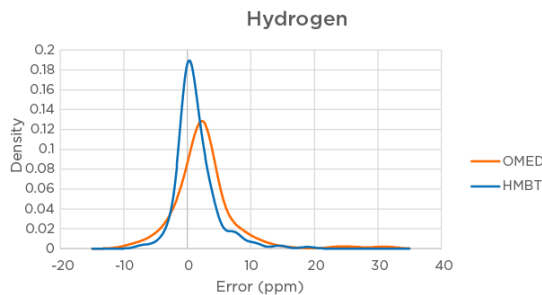
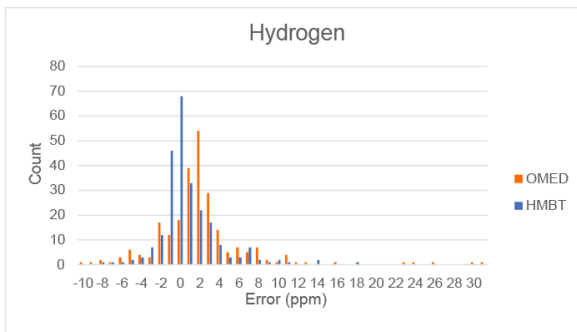


- **243** breath samples from **54** participants
- Back-to-back sampling (3min interval) with mean of Gastrogenius measurements (GG1, GG2) as assumed ground truth

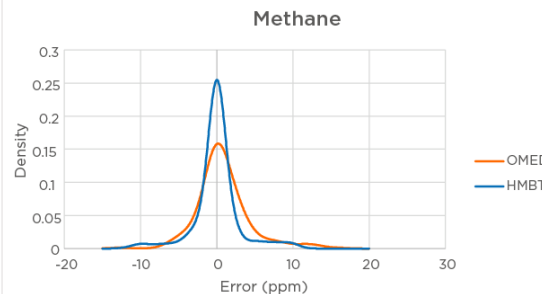
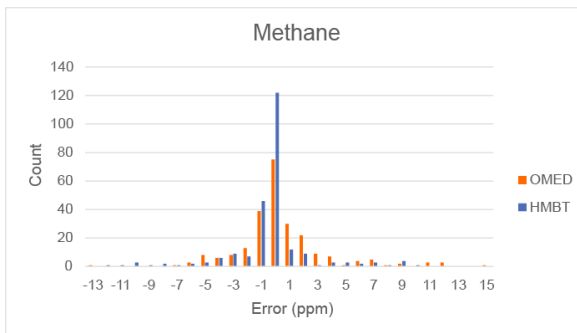
OMED versus mean of GG1 & GG2



Evaluation on real breath samples and comparison against in clinic instrument



- **243** breath samples from **54** participants
- Back-to-back sampling (3min interval) with mean of Gastrogenius measurements (GG1, GG2) as assumed ground truth



Exploring emerging basic science indications for methane

Aims

- Summarise exciting, emerging, basic science research on methane.
- Focus on production and potential roles of methane outside of those already discussed.
- Address challenges associated with correlation vs causation in these settings.
- Highlight areas where easy access to epidemiological datasets could provide novel insights into existing hypotheses.



Gastrointestinal
mobility

Investigating the link between methane generation and decreased gastrointestinal motility.



Methane and
metabolic status

Exploring the connection between methane emission and body mass index.



Infection

Examining the potential for non-microbial methane formation and the contexts in which this may be significant.

Gastrointestinal transit time is positively correlated with methane production



- Methane production is associated with significant elevations in gastrointestinal transit time across a variety of transit metrics¹.
- Notably, this increased transit time can be corrected through administration of rifaximin².

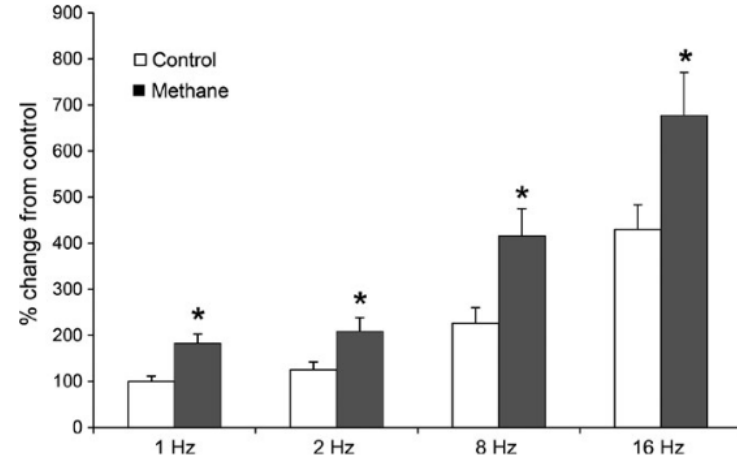
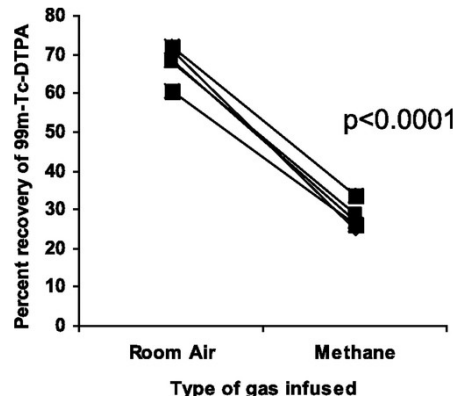
Study	Measure of transit	Controls	Methane producers	P value
Stephen <i>et al.</i>	Whole gut transit time (h)	48.6 ± 6.6	84.6 ± 11.7	0.05
Cloarec <i>et al.</i>	Orocecal transit time (m)	68 ± 24	111 ± 52	0.005
Rumessen <i>et al.</i>	Orocecal transit time (m)	60	75	0.1
Oufir <i>et al.</i>	Whole gut transit time (h)	50	95.5	0.05
Soares <i>et al.</i>	Colonic transit time (h)	61	80.5	0.05
Levitt <i>et al.</i>	Stool frequency (bm/day)	1.11 ± 0.06	1.03 ± 0.08	0.1
Morken <i>et al.</i>	Whole gut transit time (h)	95 ± 8	141 ± 14	0.005
Chatterjee <i>et al.</i>	Stool frequency (bm/day)	1.96 ± 1.4	1.17 ± 0.86	0.05

1 - Kunkel D, Basseri RJ, Makhani MD, Chong K, Chang C, Pimentel M. Methane on breath testing is associated with constipation: a systematic review and meta-analysis. *Dig Dis Sci.* 2011 Jun;56(6):1612-8. doi: 10.1007/s10620-011-1590-5. Epub 2011 Feb 1. PMID: 21286935.

2 - Ghoshal UC, Srivastava D, Misra A. A randomized double-blind placebo-controlled trial showing rifaximin to improve constipation by reducing methane production and accelerating colon transit: A pilot study. *Indian J Gastroenterol.* 2018 Sep;37(5):416-423. doi: 10.1007/s12664-018-0901-6. Epub 2018 Nov 8. PMID: 30406392.

Prolonged GI transit time can be re-created through administration of methane acutely

- Methane administration via intestinal fistulae increased GI transit times by 59% in dogs¹.
- Acute methane infusion significantly increased (guinea pig) ileal contraction under electrical field stimulation².



1 - Pimentel M, Lin HC, Enayati P, van den Burg B, Lee HR, Chen JH, Park S, Kong Y, Conklin J. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. *Am J Physiol Gastrointest Liver Physiol.* 2006 Jun;290(6):G1089-95. doi: 10.1152/ajpgi.00574.2004. Epub 2005 Nov 17. PMID: 16293652.

2 - Park YM, Lee YJ, Hussain Z, Lee YH, Park H. The effects and mechanism of action of methane on ileal motor function. *Neurogastroenterol Motil.* 2017 Sep;29(9). doi: 10.1111/nmo.13077. Epub 2017 Apr 18. PMID: 28417537.

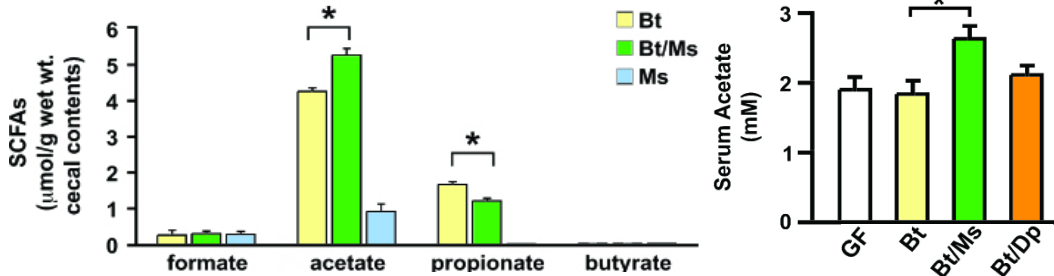
Methane production is associated with elevations in BMI



Methane production observed to correlate with obesity (in patients with a BMI > 30).

- This correlation remained despite corrections for antidepressant usage and constipation.

	Methane not detected (n=46)	Methane present (n=12)	
Subject characteristics	Mean±SE	Mean±SE	P-value*
Demographics			
Age (years)	41.9±1.6	41.6±3.3	.933
Height (in)	66.5±0.7	67.7±1.4	.373
Weight (lbs)	242.3±7.1	299.0±22.7	.002
BMI (kg/m ²)	38.5±0.8	45.2±2.3	.001



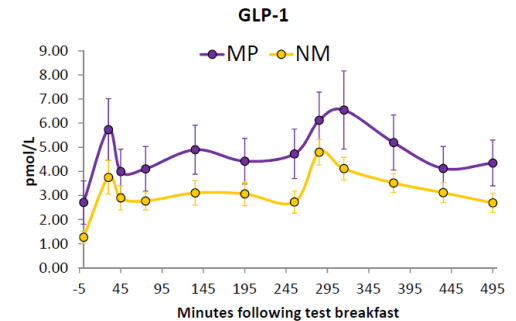
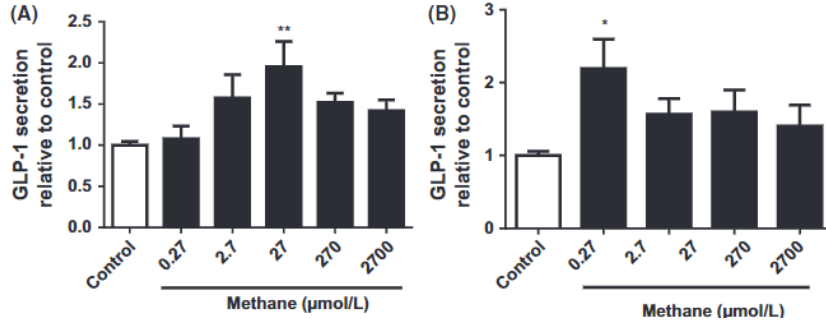
Methanogen (*M.smithii*) co-colonisation with *B.thetaiotaomicron* significantly increased acetate formation, with elevations in acetate observed in the blood.

1 - Basseri RJ, Basseri B, Pimentel M, Chong K, Youdim A, Low K, Hwang L, Soffer E, Chang C, Mathur R. Intestinal methane production in obese individuals is associated with a higher body mass index. Gastroenterol Hepatol (N Y). 2012 Jan;8(1):22-8. PMID: 22347829; PMCID: PMC3277195.

2 - Samuel BS, Gordon JL. A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. Proc Natl Acad Sci U S A. 2006 Jun 27;103(26):10011-6. doi: 10.1073/pnas.0602187103. Epub 2006 Jun 16. PMID: 16782812; PMCID: PMC1479766.

Methane production may provide some protective effects in obesity

- In some instances, methane production correlates with improved outcomes;
 - One of the most conserved microbial pathways downregulated in type 2 diabetes (T2D) is bacterial methanogenesis¹.
 - Visceral fat was found to be lower in high methane producers².
- Methane was found to directly stimulate secretion of GLP-1, in both mouse (GLUTag) and human (NCI-H716) L cells³, a finding which correlates with elevated GLP-1 levels observed in methane producers compared to methane non-producers following a meal⁴.



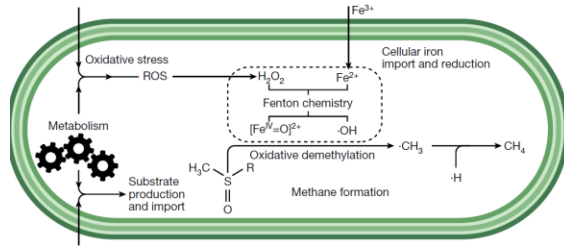
1 - Wu H, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, Gummesson A, Perkins R, Bergström G, Bäckhed F. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based Cross-Sectional Study. *Cell Metab.* 2020 Sep 1;32(3):379-390.e3. doi: 10.1016/j.cmet.2020.06.011. Epub 2020 Jul 10. PMID: 32652044.

2 - Ozato N, Saito S, Yamaguchi T, Katashima M, Tokuda I, Sawada K, Katsuragi Y, Kakuta M, Imoto S, Ihara K, Nakaji S. Association between breath methane concentration and visceral fat area: a population-based cross-sectional study. *J Breath Res.* 2020 Feb 25;14(2):026008. doi: 10.1088/1752-7163/ab61c6. PMID: 31835267.

3 - Laverdue R, Mezouari A, Carson MA, Basiliko N, Gagnon J. A role for methanogens and methane in the regulation of GLP-1. *Endocrinol Diabetes Metab.* 2017 Dec 1;1(1):e00006. doi: 10.1002/edm.2.6. PMID: 30815543; PMCID: PMC6353219.

4 - The Etiology and Pathophysiology of Insulin Resistance and Mediating Factors from the Gut - ProQuest

Reactive oxygen species can cause methane production *in vitro*



Non-enzymatic routes for methane production (to accompany microbial enzymatic routes of production) have been proposed since the early 2000's. Recently Ernst *et al.*¹ outlined a ROS-driven Fenton based scheme for this non-enzymatic route.

This supports earlier findings that this can occur both fully *in vitro* (a) as well as *ex vivo* (b and c) in response to elevations in ROS from either exogenous (b) or endogenous (c) sources.

Methyl containing exogenous compounds, such as N-methylethanolamine, generate methane when exposed to *in vitro* hydroxyl radicals².

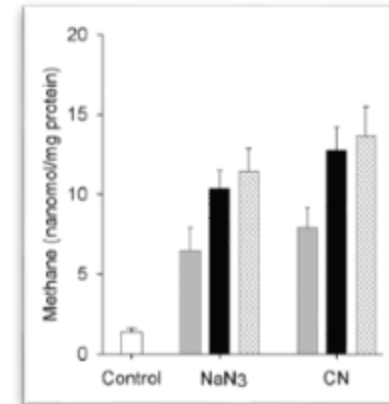
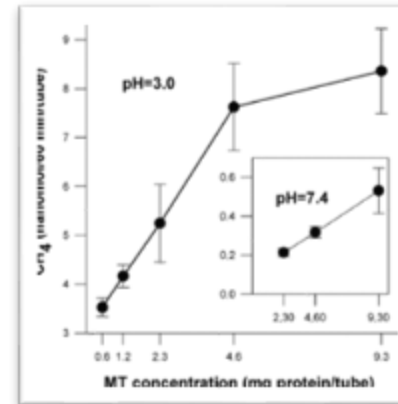
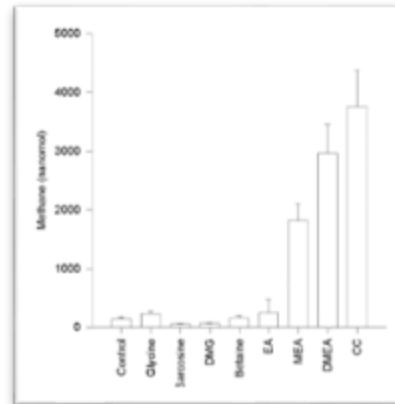
Application of exogenous ROS to isolated mitochondria results in the production of methane, with a linear relationship to mitochondria protein levels³.

Mitochondrial inhibitors (and associated ROS elevations) lead to methane production in primary aortic endothelial cells².

1 - Ernst L, Steinfeld B, Baraye U, Klintzsch T, Kurth M, Grimm D, Dick TP, Rebelein JG, Bischofs IB, Keppler F. Methane formation driven by reactive oxygen species across all living organisms. *Nature*. 2022 Mar;603(7901):482-487. doi: 10.1038/s41586-022-04511-9. Epub 2022 Mar 9. PMID: 35264795.

2 - Ghyczy M, Torday C, Kaszaki J, Szabó A, Czóbel M, Boros M. Hypoxia-induced generation of methane in mitochondria and eukaryotic cells: an alternative approach to methanogenesis. *Cell Physiol Biochem*. 2008;21(1-3):251-8. doi: 10.1159/000113766. Epub 2008 Jan 16. PMID: 18209491.

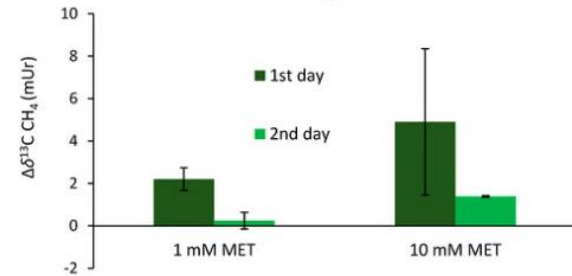
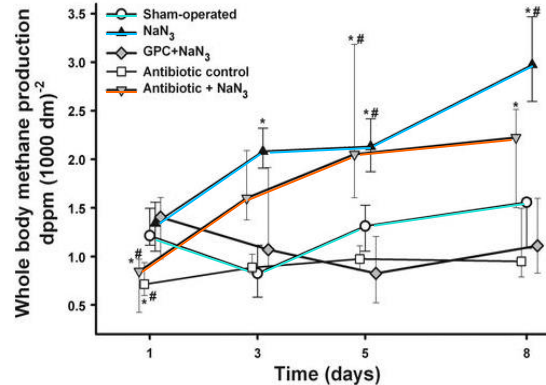
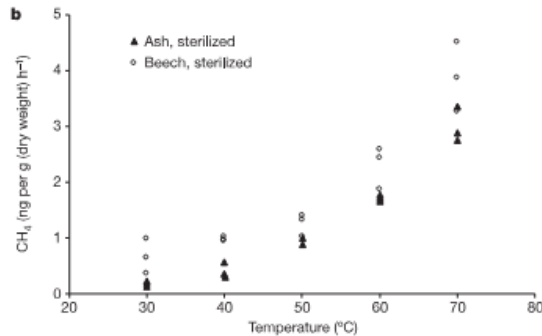
3 - Ghyczy M, Torday C, Boros M. Simultaneous generation of methane, carbon dioxide, and carbon monoxide from choline and ascorbic acid: a defensive mechanism against reductive stress? *FASEB J*. 2003 Jun;17(9):1124-6. doi: 10.1096/fj.02-0918fje. Epub 2003 Apr 8. PMID: 12692080.



Extra-microbial methane production can occur *in vivo*, and is stimulated by mitochondrial stress

Kepler *et al.*¹, provided the first extension of these *in vitro* findings *in vivo*, demonstrating that plant material generated methane. This was then translated into a rodent setting by Tuboly *et al.*² through application of a mitochondrial inhibitor, known to elevate levels of ROS production leading to increased levels of rodent methane production.

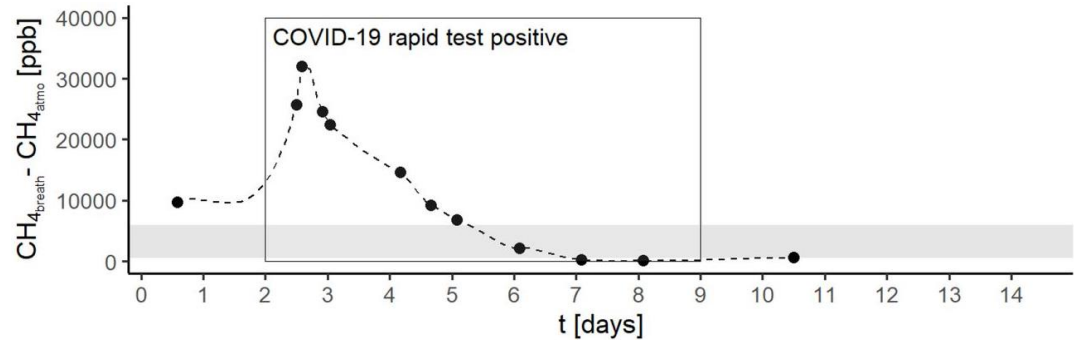
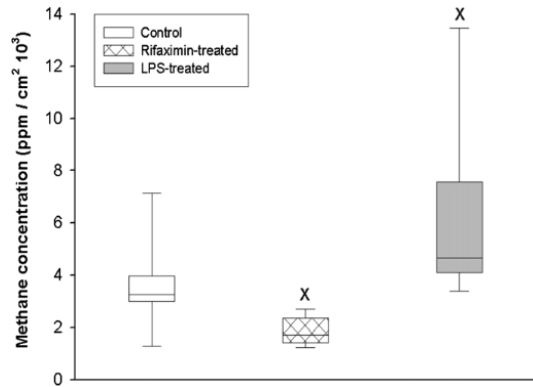
Importantly, both settings demonstrated independence of effects from microbially derived methane, with Kepler *et al.* irradiating plant matter prior to testing, and Tuboly *et al.* co-administering antibiotics.



The first data that this process could also occur with human material came in 2023 from Kepler *et al.*³ who demonstrated headspace methane production when human blood was incubated with radiolabelled methionine.

Methane production may increase in response to settings of inflammatory stress

Work from Tuboly *et al*¹. provided the first indications that endogenous responses to exogenous inflammation could also increase levels of methane production, demonstrating a 2-3-fold increase in methane in an LPS-based inflammatory model.



Further data from Polag *et al*². provided a preliminary insight into the potential for this phenomena to also occur in humans.

It should be noted that as of yet there have been no studies with sufficient power, or appropriate design to a) verify this elevation in breath methane following immune response in humans, b) provide data as to the magnitude of any elevation in methane and the ability to differentiate this from baseline dynamics and c) examine the uniformity of this response across the population, including high and low baseline methane producers.

Summary: Owlstone Provide Tools to Use Breath in Research, and Support Translation to Clinical Use



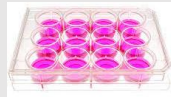
DISCOVER AND CHARACTERIZE



OMED for characterisation of hydrogen and methane

BREATH
BIOPSY

CASPER®
Portable Air Supply

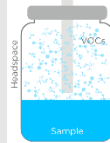


ReCIVA®
Breath Sampler



Breath Biopsy®
Collect

HiSorb
Probe



Comprehensive volatile metabolite collection and analysis

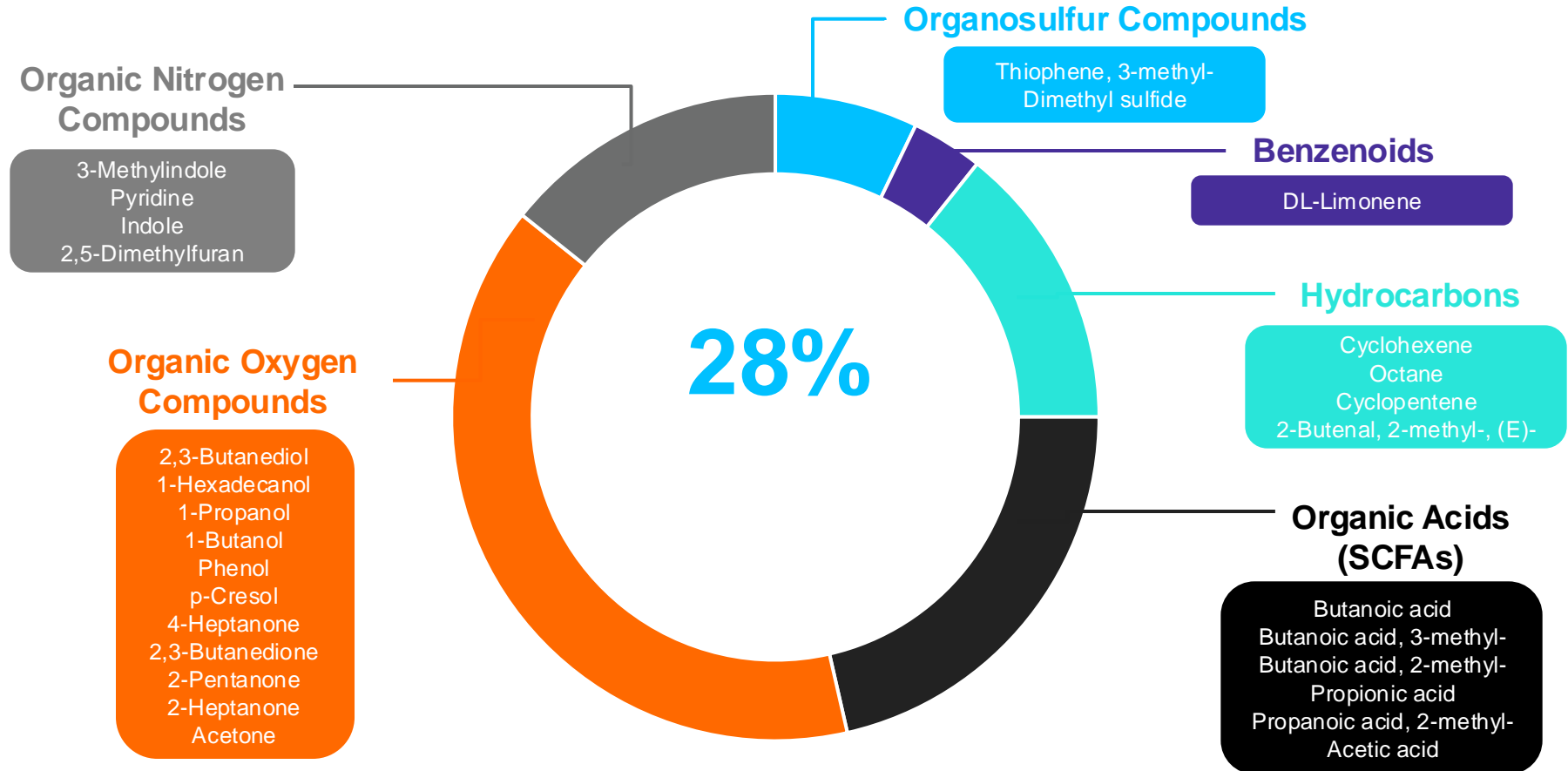


BREATH
BIOPSY

VOC Atlas

Defining and characterising the breath metabolome

Metabolites Related to the Microbiome Able to be Precisely Measured On Breath (Growing List) with OMNI



Summary: Owlstone Provide Tools to Use Breath in Research, and Support Translation to Clinical Use

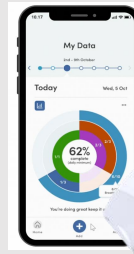


DISCOVER AND CHARACTERIZE



OMED for characterisation of hydrogen and methane

DEVELOP AND DEPLOY



OMED for SIBO and GI management



LIBRA test for liver cirrhosis



Comprehensive volatile metabolite collection and analysis



Defining and characterising the breath metabolome



Detection of pre-symptomatic infections



BILL & MELINDA GATES foundation

PRESS RELEASES

Owlstone Medical Secures \$6.5 Million to Support Development of Breath-based Diagnostics for Infectious Disease

28 APR 2024



Test development in partnerships

- We should be prepared for someone to ask us how our device compares to food marble, with an appropriate tactical answer.

Things to potentially include somewhere



- <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-023-01565-3> - finding that butyrate production is increased in high hydrogen concentrations.



THANK YOU



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