

From Breath to Nutritional Insights: High-Resolution Mass Spectrometry in NutriExhalomics

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ETH Zürich

Nachhaltigkeitstagung Agroscope

Mikroorganismen – fleissige Helfer vom Feld bis in
unseren Körper, 25.01.2024



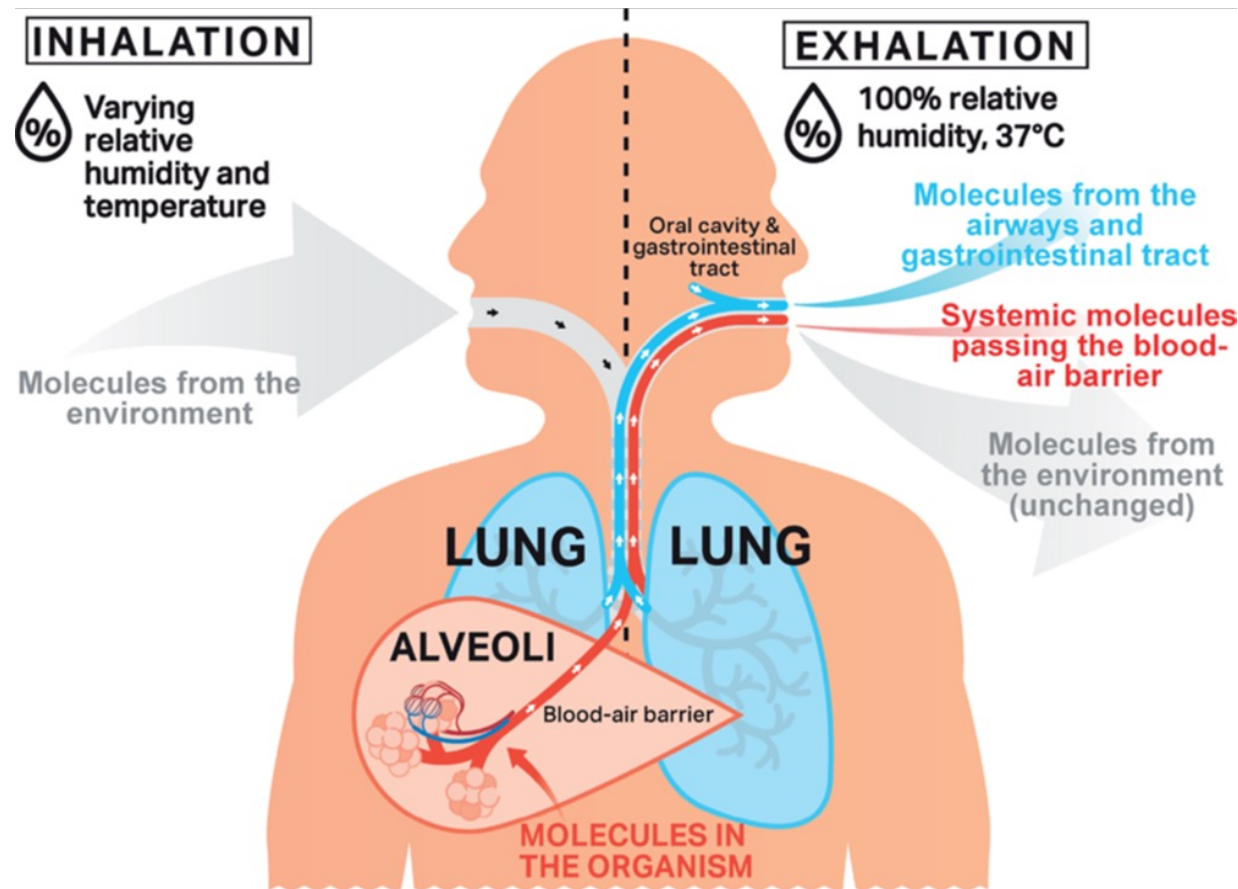
NutriExhalomics

Promote the analysis of breath metabolites in nutritional research.

- Characterize the human nutritional exhalome
- Characterize the impact of microbial foods and intestinal communities on the human exhalome
- Characterize the bovine exhalome



Why Breath Analysis?



Contains rich chemical information

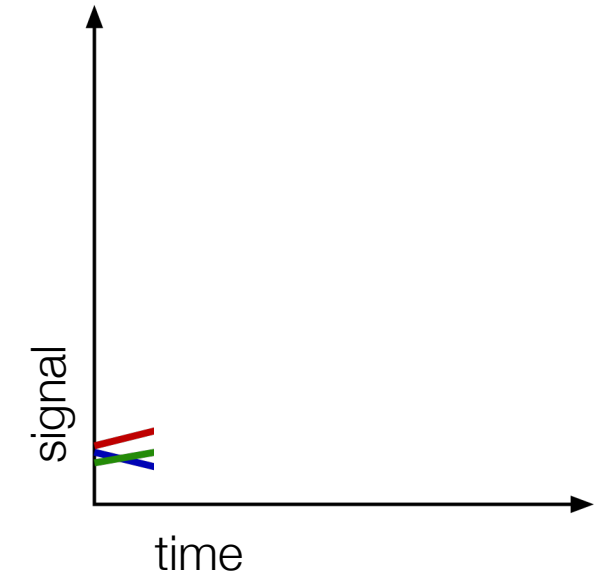
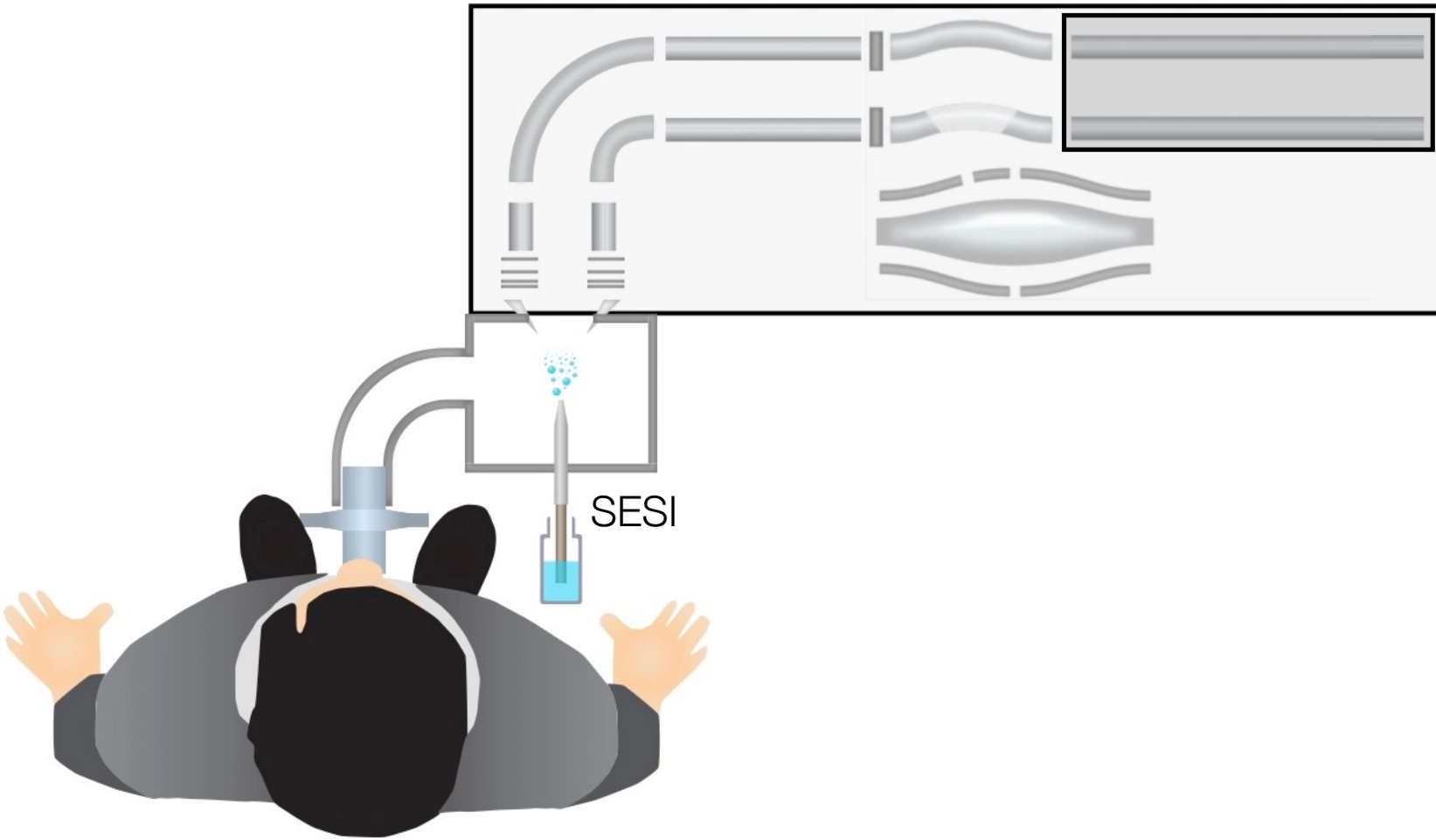
Non-invasive

Unlimited availability

Continuous sampling

T. Bruderer, R. Zenobi, et al., *Chem. Rev.*, **2019**, 119, 10803

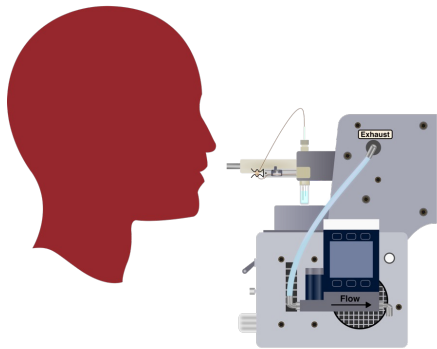
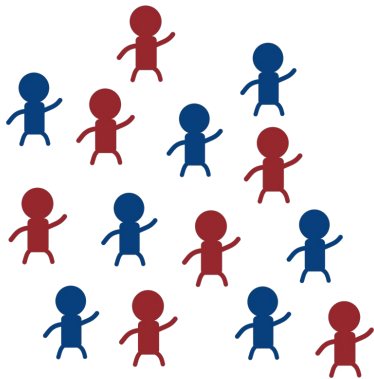
Secondary Electrospray Ionization Mass Spectrometry (SESI-MS)



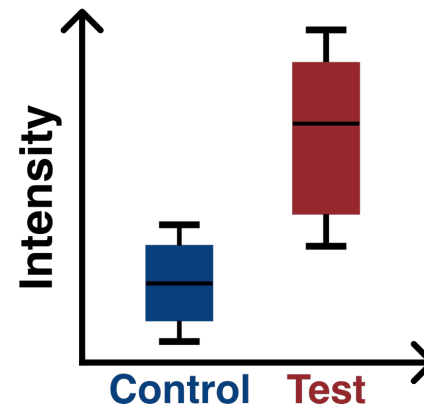
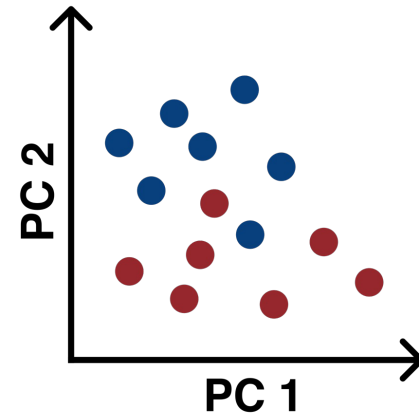
T. Bruderer, R. Zenobi, et al., *Chem. Rev.*, **2019**, 119, 10803

Biomarker Discovery with On-Line Breath Analysis

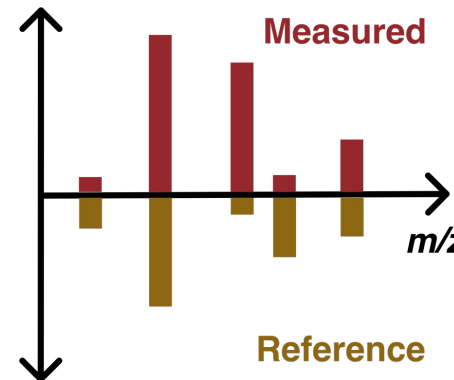
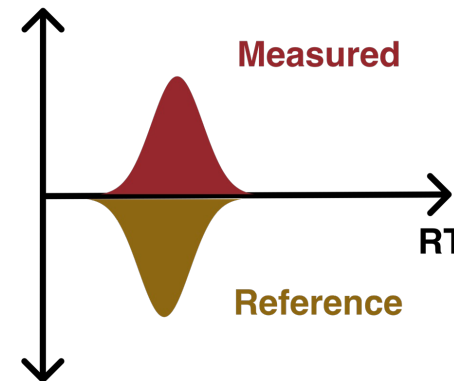
Measurements



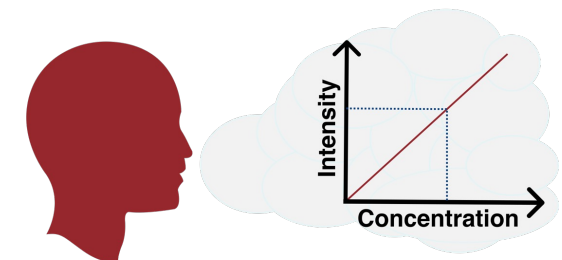
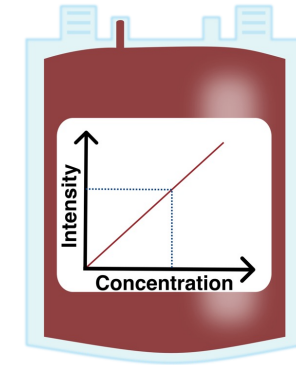
Statistics



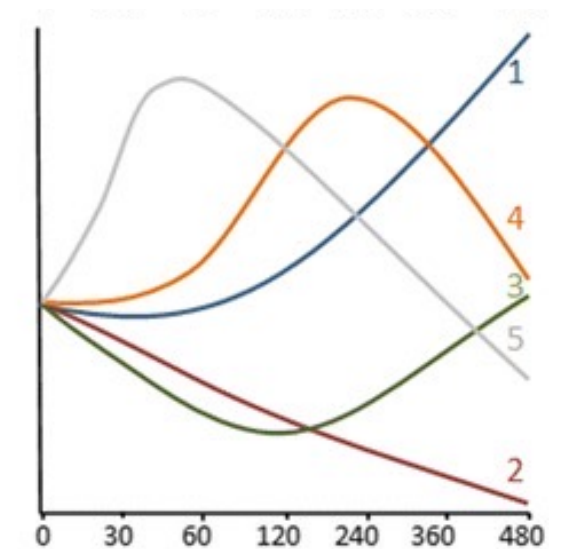
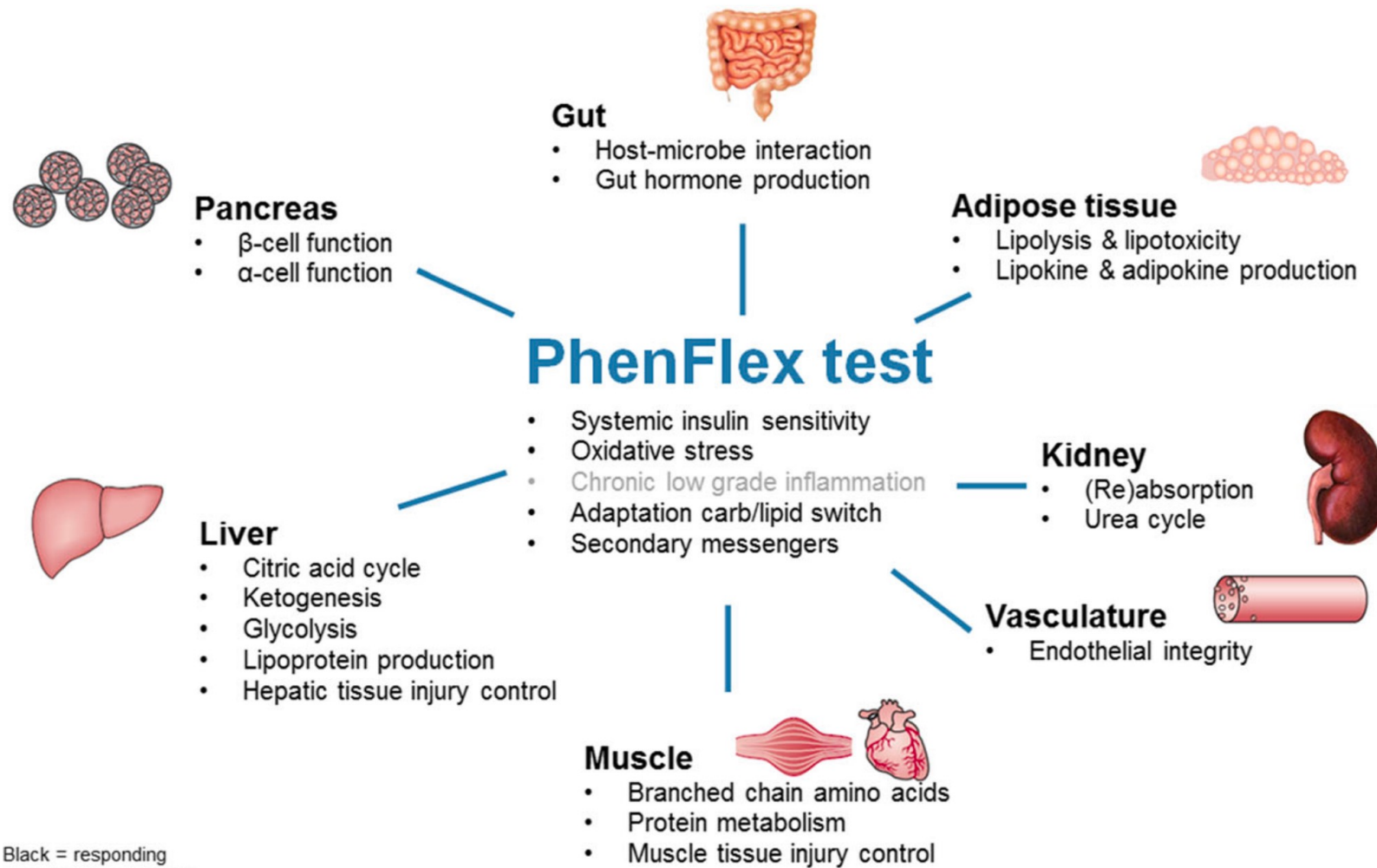
Identification (GC, LC, On-Line)



Quantification (Breath, Blood)

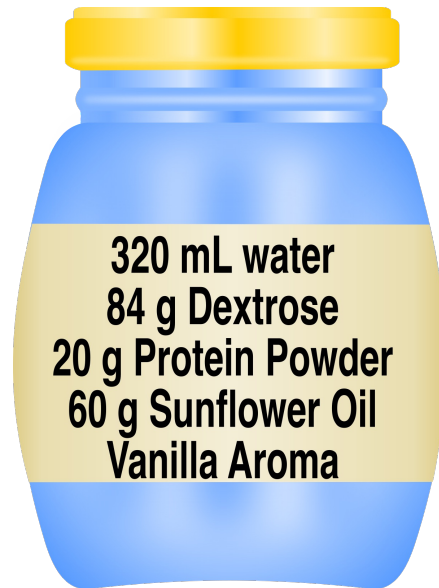


Nutritional Challenge

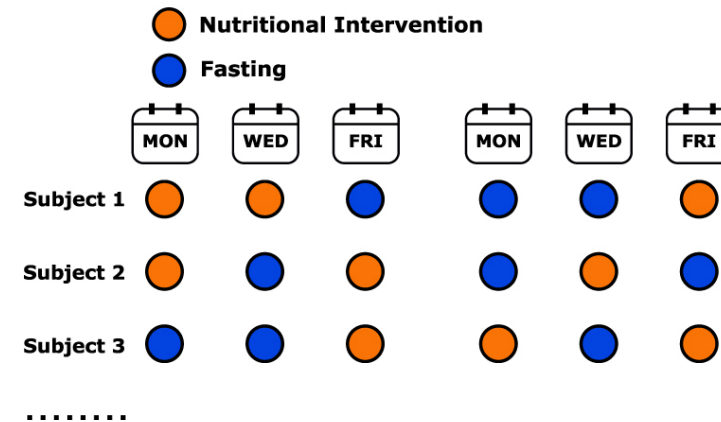
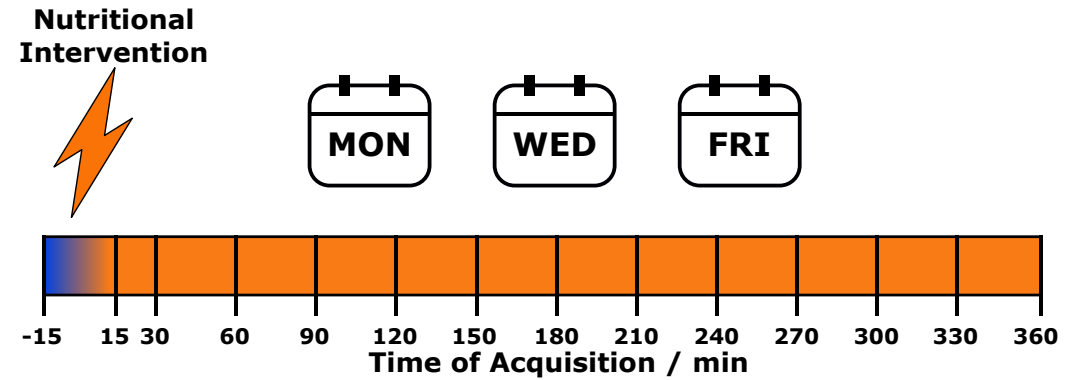


Wopereis *et al.*, *Genes Nutr.* **2017**, 12, 21

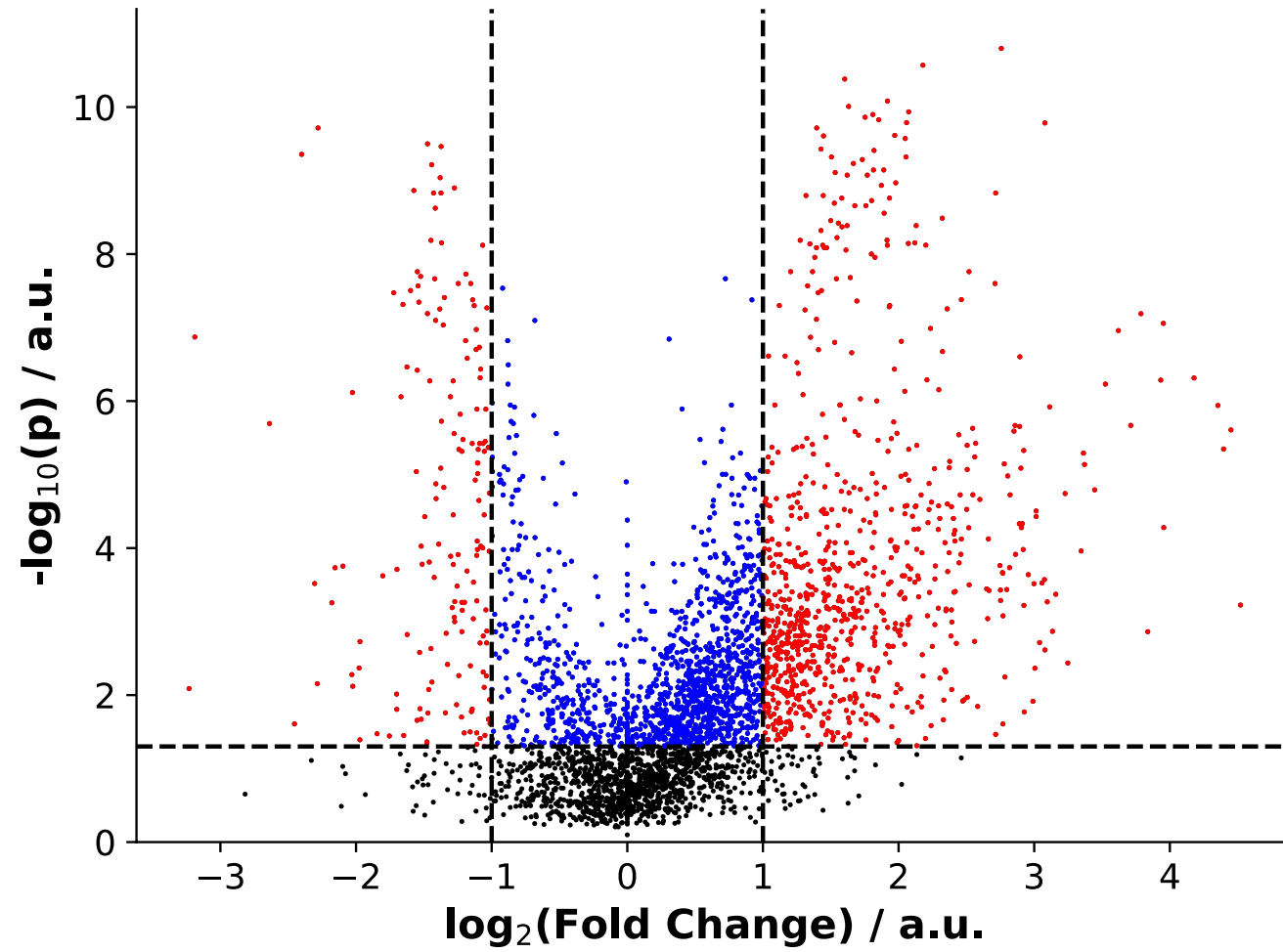
Methodology



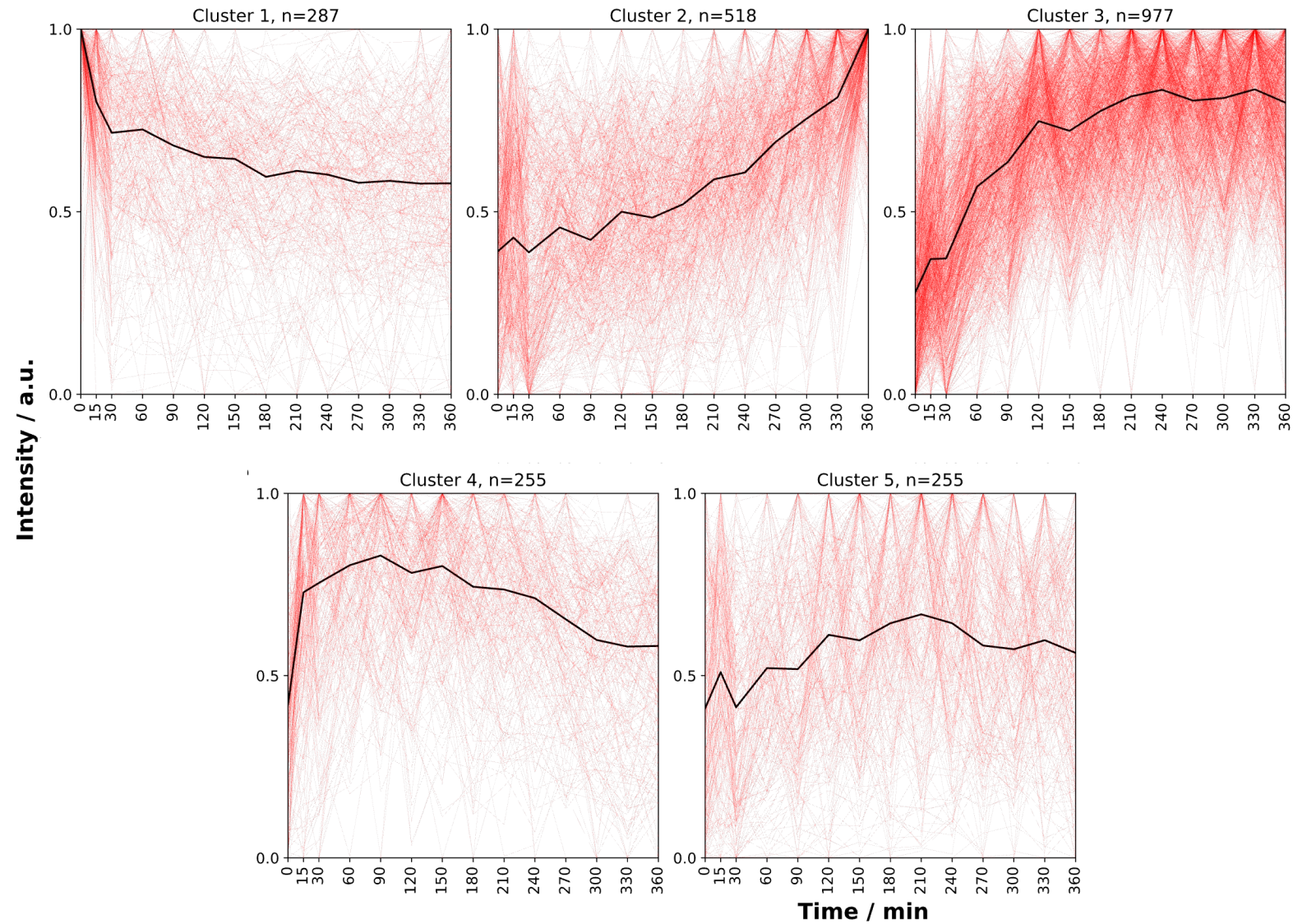
3915 kJ / 945 kcal



Results

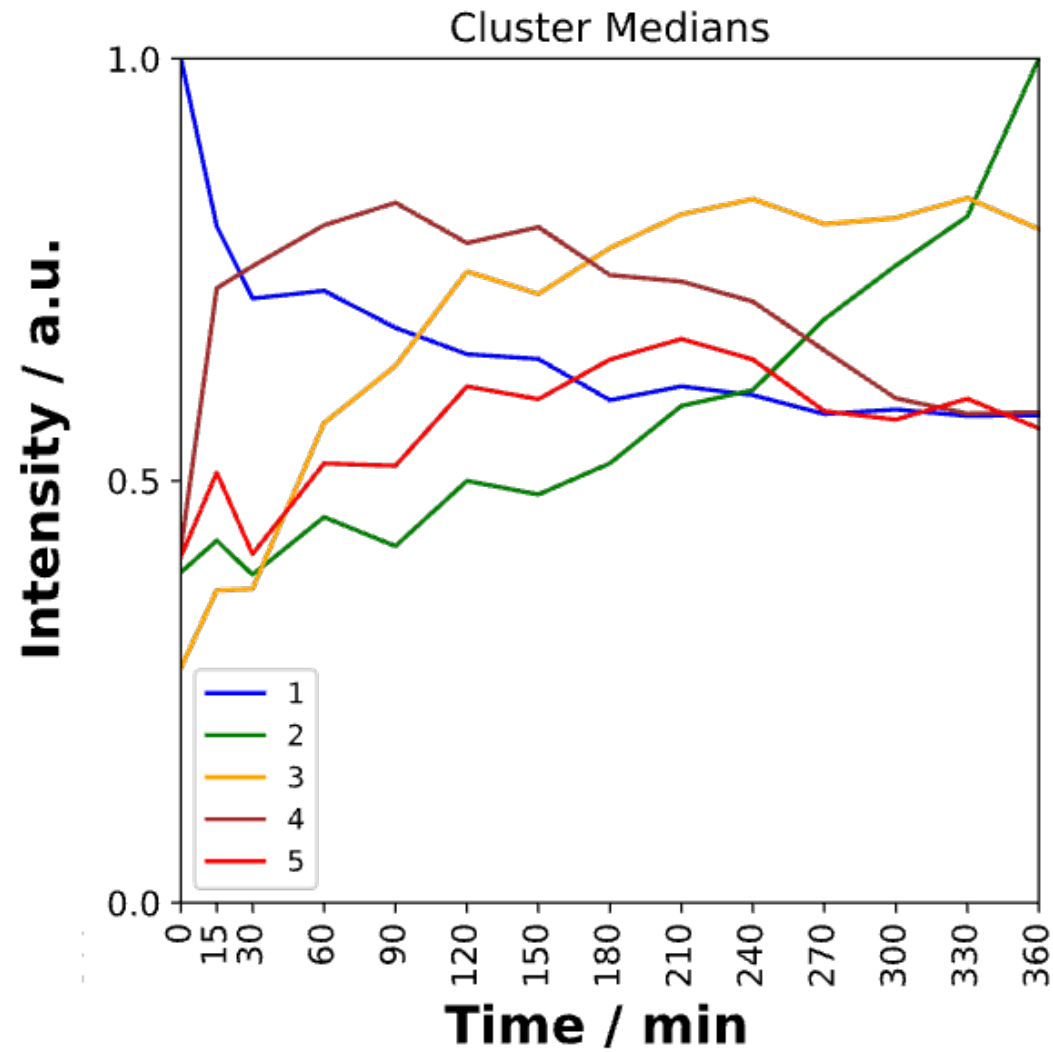


Results



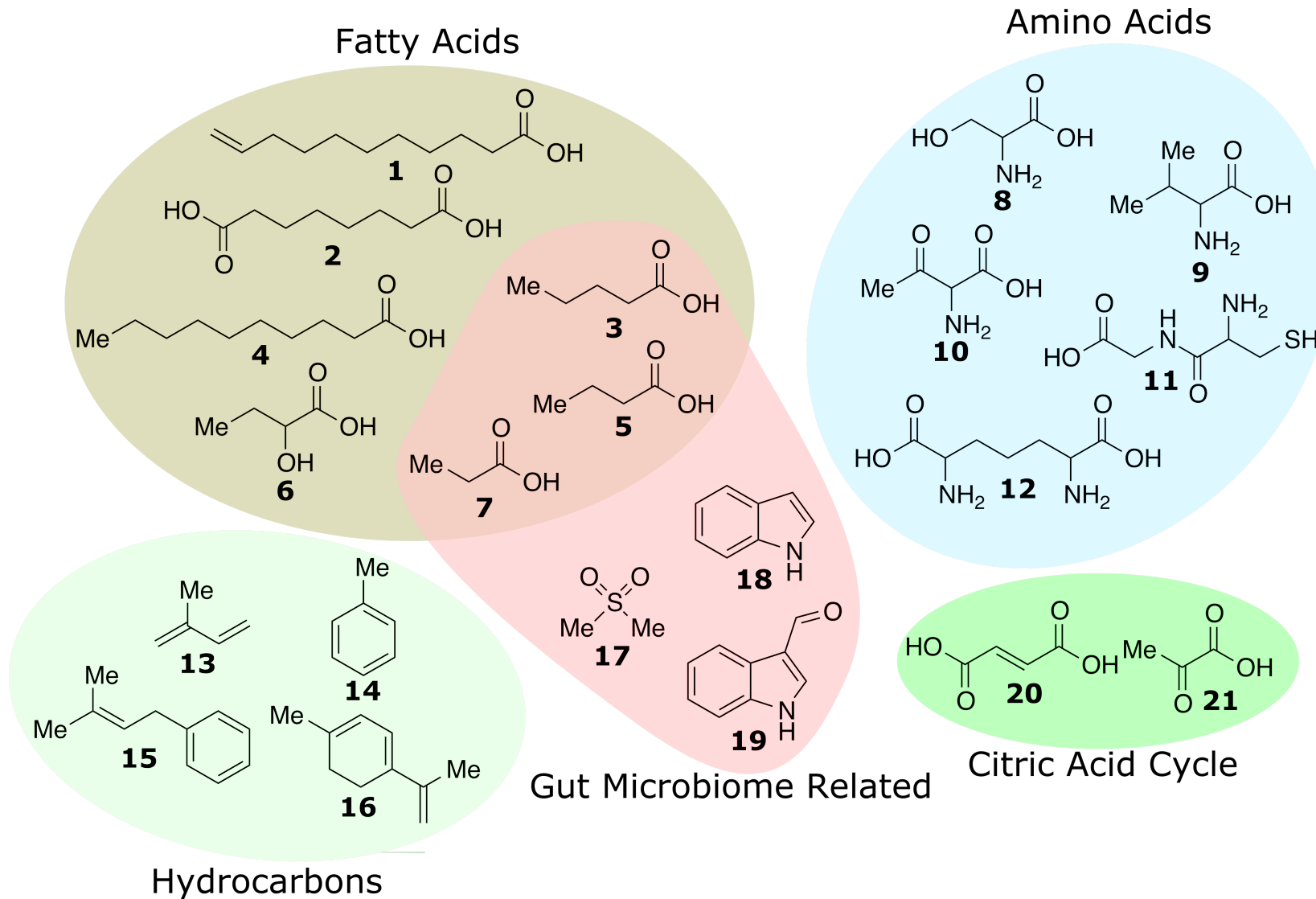
C. Wüthrich *et al.*, *J. Breath Res.* **2022**, *16*, 046007

Results



C. Wüthrich *et al.*, *J. Breath Res.* **2022**, *16*, 046007

Compound Annotation



Quantitation of Breath Metabolites with SESI-MS



CHEM, chemical industries except petrochemical and pharmaceutical



Acetylene	Dimethylamine	n-Pentane
Aminomethane	2,2-Dimethylpropane	iso-Pentane
Ammonia	Epoxyethane	Phosgene
Boron trichloride	Ethanamine	Phosphine
Boron trifluoride	Ethane	Propadiene
Bromoethene	Ethanedinitrile	Propane
1,3-Butadiene	Ethene	Propylene
n-Butane	Ethyl chloride	Propyne
iso-Butane	Ethyl formate	Silicon tetrachloride
1-Butene	Fluorine	Silicon tetrafluoride
cis-2-Butene	1,1,1,2,3,3,3-Heptafluoropropane	Sulfur dioxide
iso-Butene	Hexafluoroethane	2,3,3,3-Tetrafluoro-1-Propylene
trans-2-Butene	1,1,1,3,3,3-Hexafluoropropane	trans-1,3,3,3-Tetrafluoro-1-Propylene
1-Butyne	Hydrogen	Tetrafluoromethane
Carbon dioxide	Hydrogen bromide	Trichlorosilane
Carbon monoxide	Hydrogen chloride	Trifluoroethane
Carbon oxyfluoride	Hydrogen cyanide	Trimethylamine
Carbonyl sulfide	Hydrogen fluoride	Xenon
Chlorine	Hydrogen iodide	40% Hydrogen, Balance Helium
1-Chloro-1,1-difluoroethane	Hydrogen sulfide	10% Methane, Balance Argon
1-Chloro-1,2,2,2-tetrafluoroethane	Methane	5% Methane, Balance Argon
Chlorodifluoromethane	Methoxyethene	1% Oxygen, Balance Nitrogen
Chloroethene	Methoxymethane	20 ppm Carbon Monoxide, Balance Air
Chloropentafluoroethane	Methyl bromide	450 ppb Nitric Oxide, Balance Nitrogen
Cyanoic chloride	Methyl chloride	450 ppb Nitrogen Dioxide, Balance Air
Cyclopentane	Methyl formate	450 ppb Sulfur Dioxide, Balance Air
Cyclopropane	Methyl mercaptan	90 ppm Methane, Balance Nitrogen
Deuterium	Nitric oxide	5 ppm Ammonia, Balance Nitrogen
Diborane	Nitrogen	5 µ/m³ Mercury, Balance Nitrogen
1,1-Dichloro-1-fluoroethane	Nitrogen dioxide	10 ppm Hydrogen Sulphide, Balance Nitrogen
2,2-Dichloro-1,1,1-trifluoroethane	Nitrogen trifluoride	1.1% Propane, Balance Air
Dichlorodifluoromethane	Nitrous oxide	2.5% Methane, Balance Air
Dichlorosilane	Oxygen	2% Hydrogen, Balance Air
1,1-Difluoroethane	Pentafluoroethane	40% Hydrogen, Balance Nitrogen
1,1-Difluoroethene	1,1,1,3,3-Pentafluoropropane	



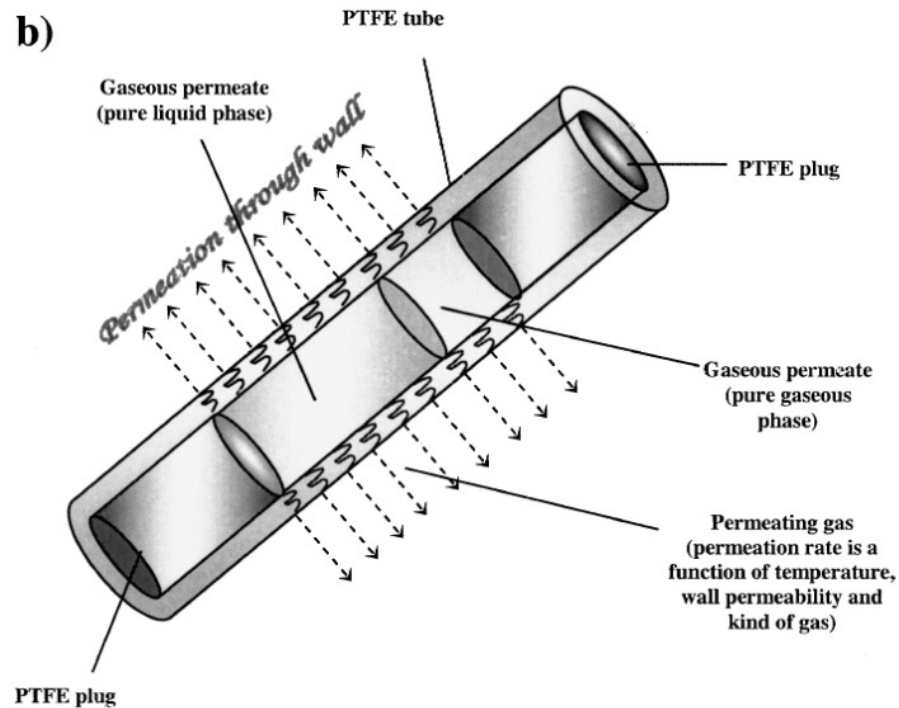
Acetone 5 PPM
Calibration Gas
Balance Air in a
552 Liter Cylinder
CGA590

\$552.00

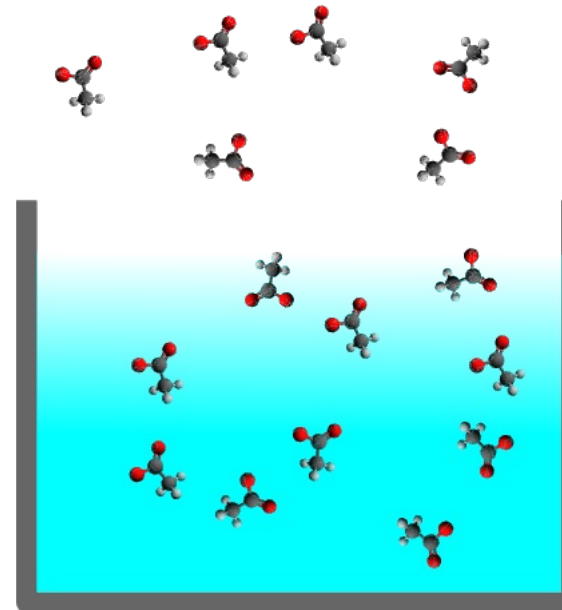
Availability and Price

Generation of Low-Concentration Gas Standards

Permeation



Evaporation



$$H = c * p$$

H : Henry's constant
 c : concentration
 p : pressure

Naganowska-Nowak *et al.*, *Crit. Rev. Anal. Chem.* **2005**, 35, 31–55.

Generation of Low Concentration Gas Standards through Evaporation

Aqueous Stock Solution

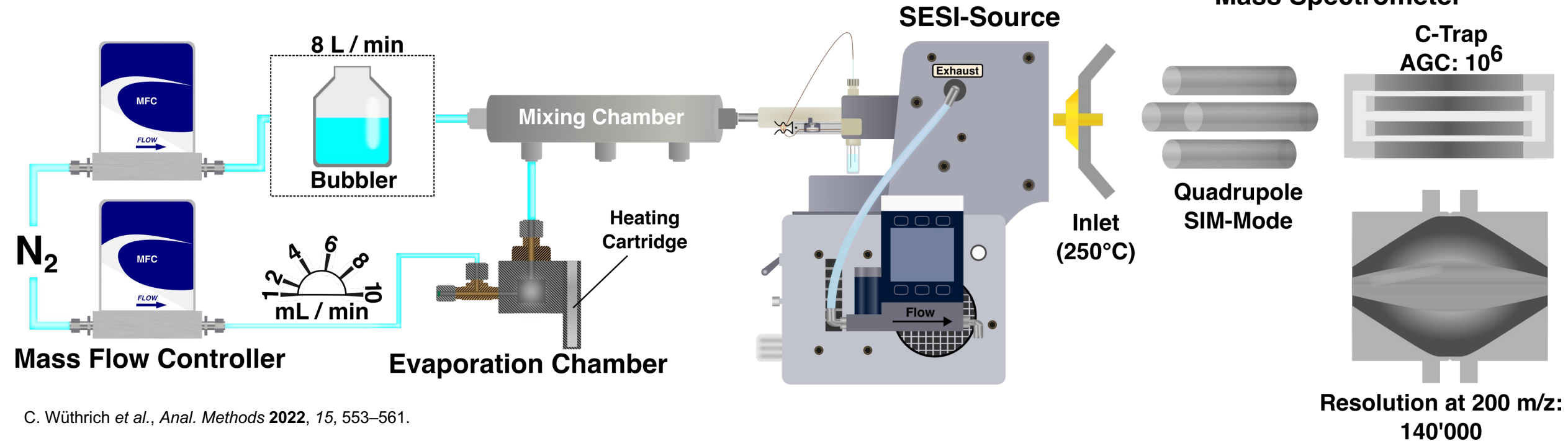
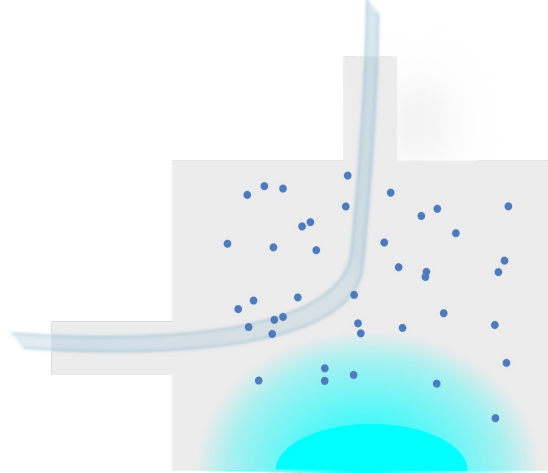
Henry's Constant

Dilution Flows

Flexible

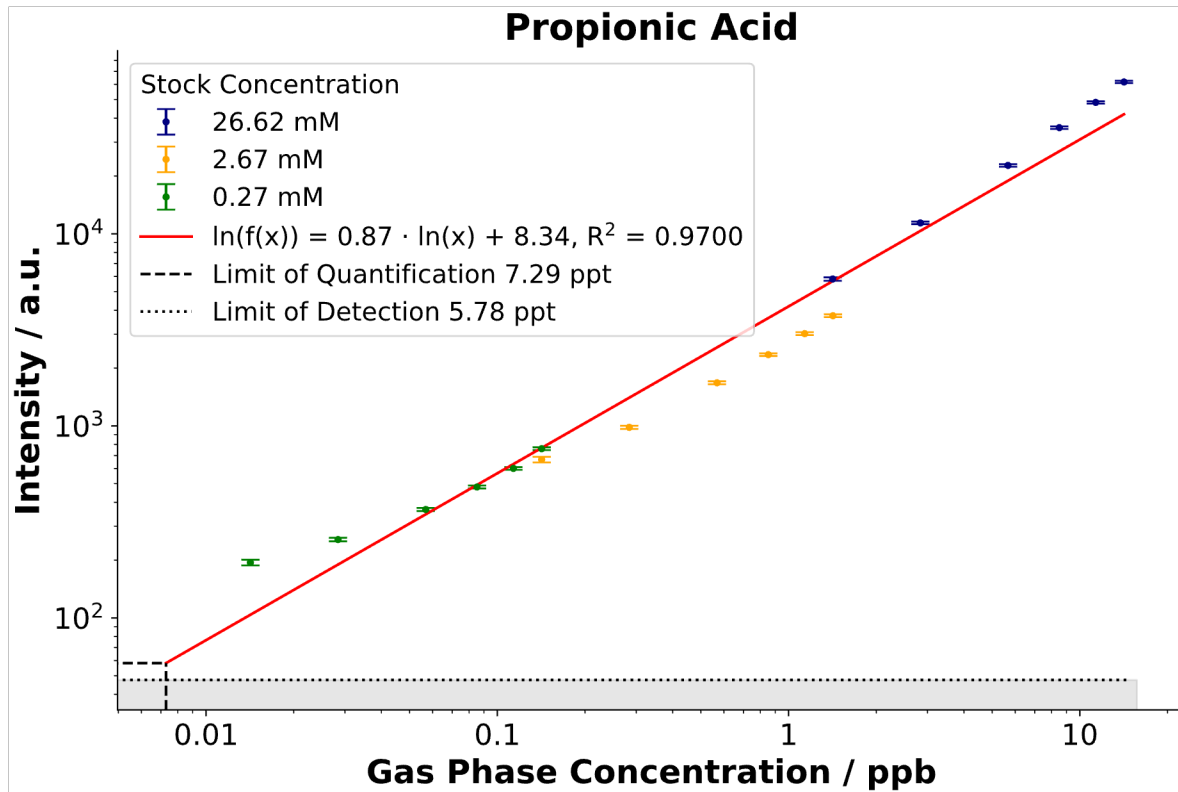
Parallelization

Wide Range



C. Wüthrich *et al.*, *Anal. Methods* **2022**, *15*, 553–561.

External Calibration with Evaporation Chamber System



Low Concentrations

Several Magnitudes

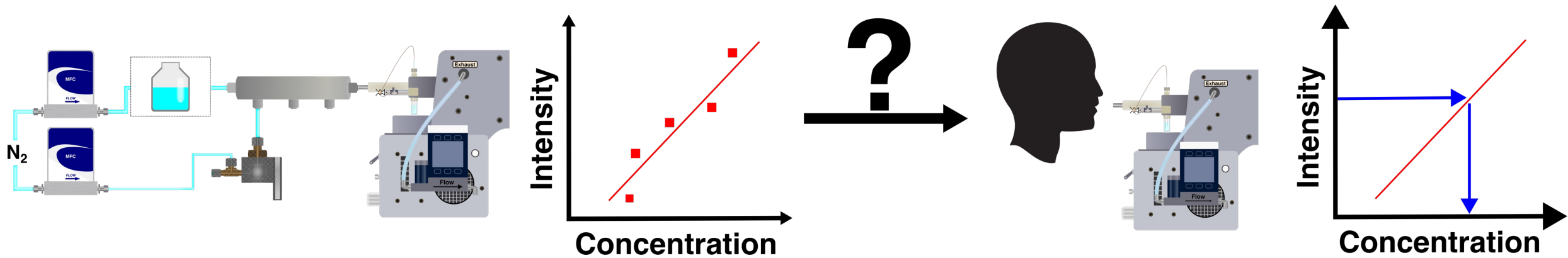
Dry and Humid

Similar to Breath

C. Wüthrich *et al.*, *Anal. Methods* **2022**, *15*, 553–561.

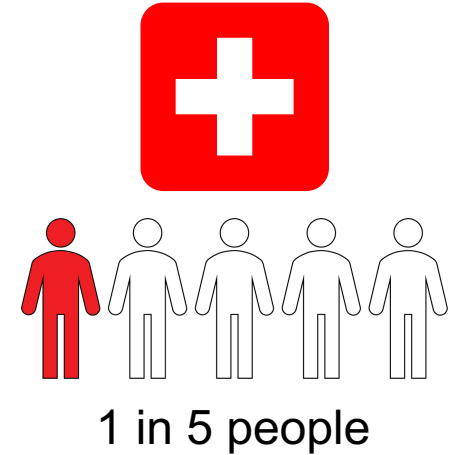
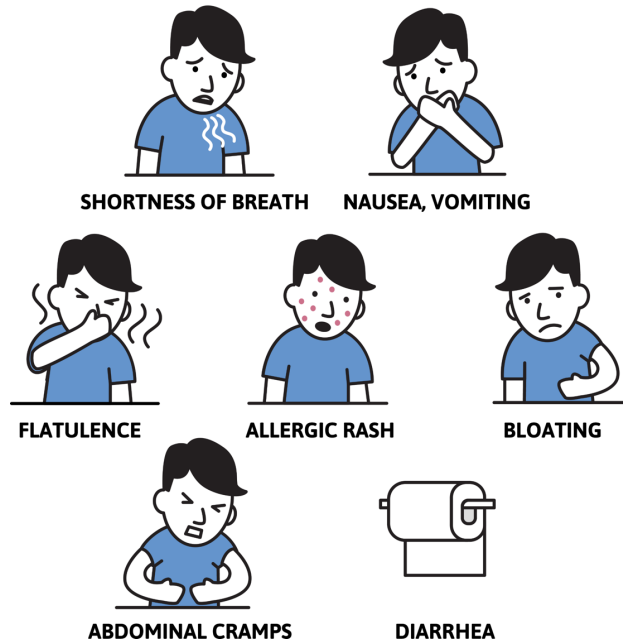
	0 % RH			95 % RH		
Compound	R ²	LOD (ppt)	LOQ (ppt)	R ²	LOD (ppt)	LOQ (ppt)
Propionic acid	0.9789	5.78	7.29	0.9915	3.40	5.27
Butyric acid	0.9721	1.85	2.32	0.9838	0.71	1.00
Pentanoic acid	0.9702	2.53	3.16	0.9890	21.89	28.13
Hexanoic acid	0.9971	40.63	47.42	0.9802	53.67	66.81

External Calibration with Evaporation Chamber System



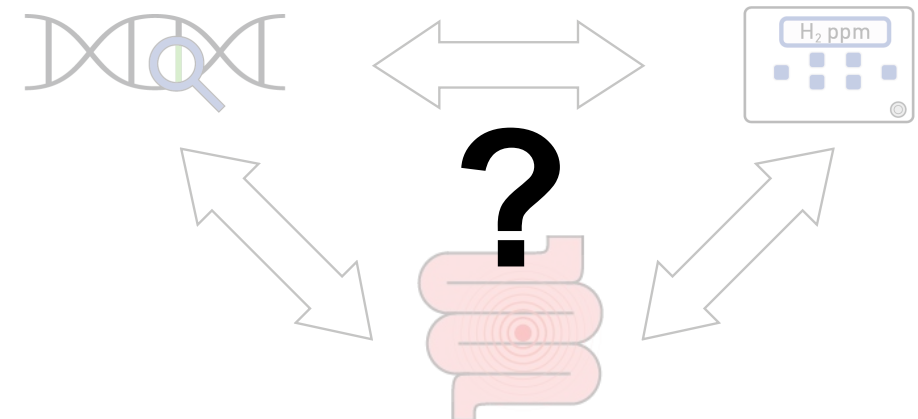
Lactobreath: A Pilot Study to Diagnose Lactose Intolerance based on the Exhaled Breath Metabolome - Lactose Intolerance / Malabsorption

SYMPTOMS

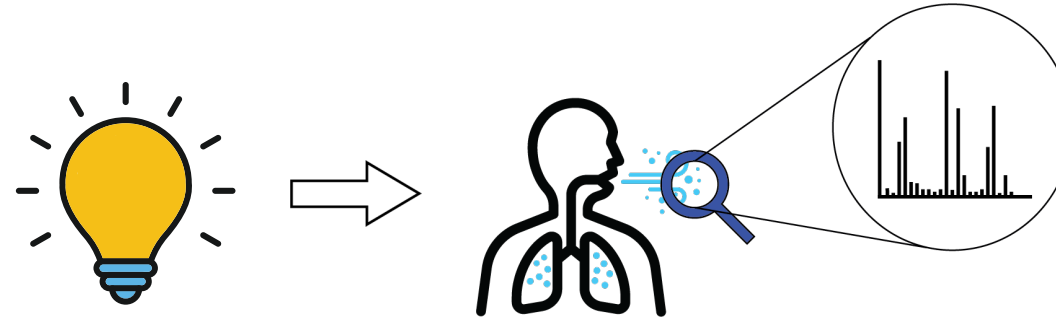


! Current tests have limited sensitivity and specificity for assessing LM symptoms

- **Lactose Malabsorption (LM):** Failure to digest and/or absorb lactose in small intestine
- **Lactose Intolerance (LI):** Clinical symptoms resulting from LM

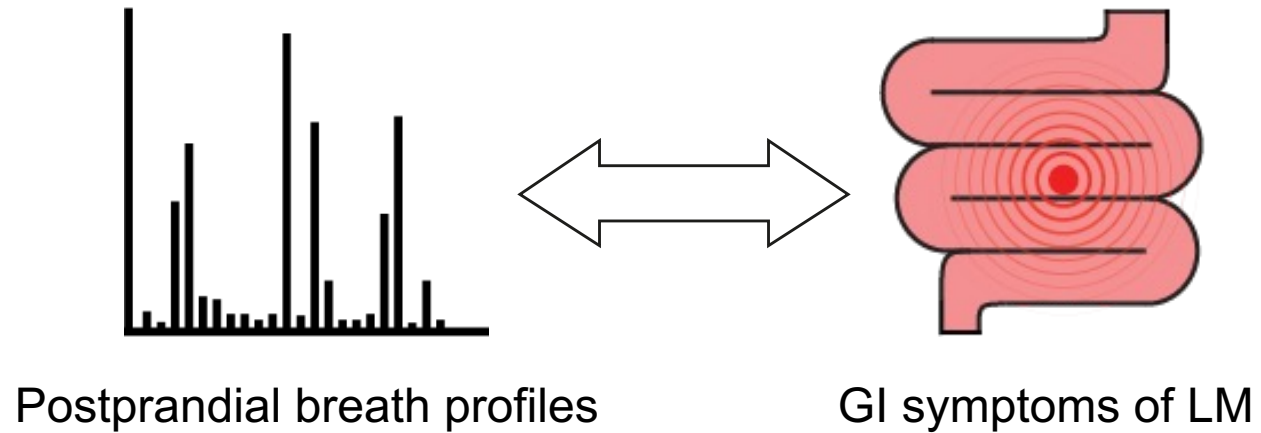


Lactobreath

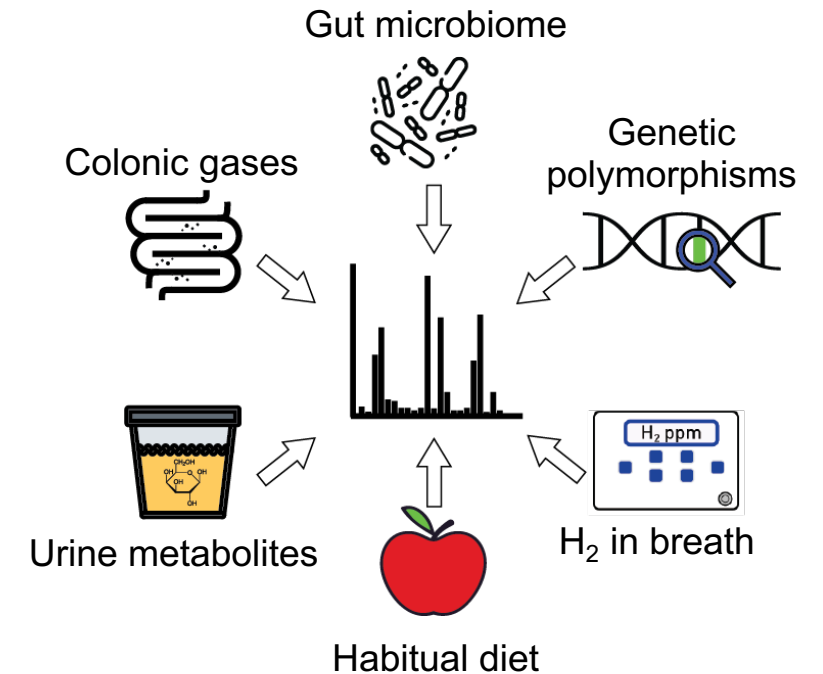


Diagnostic tool based on exhaled breath analysis with mass spectrometry

Primary Goal



Secondary Goal



Recruitment

3 groups (120 Participants total):



Genetically LP
without GI symptoms
 $n = 24$

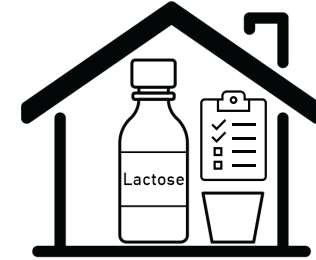


Genetically LNP
without GI symptoms
 $n = 24$

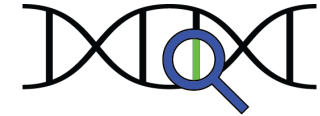


Genetically LNP
with GI symptoms
 $n = 72$

LP = Lactase persistent; LNP = Lactase non-persistent



Home Screening



Genetic screening
→ Variants of MCM 6

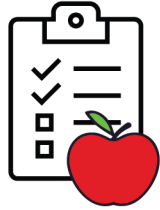


- Men and women aged 18-65
- Swiss resident
- Abstain from treatment
- Informed consent,...



- Allergic to milk
- Diabetes Mellitus
- GI diseases
- Drug abuse, Dysphagia,...

Baseline Analyses



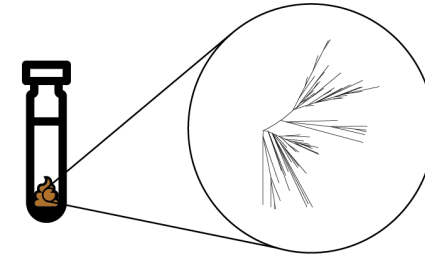
Habitual dietary
intake assessment

myfood²⁴

- 24h recall
- 4 consecutive days
- Digital form/App
- Picture to assess portion size



-30 to -7 d



Stool metagenomics



Centre hospitalier
universitaire vaudois

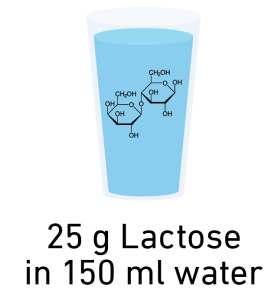
- Faecal microbiome analysis
- Shotgun sequencing
- 3x: +1, +2, +3 days after 1st recall

Intervention

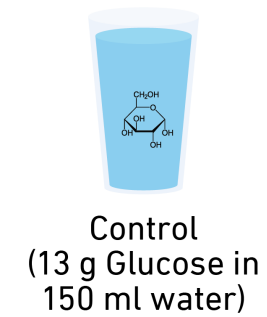


Controlled Diet

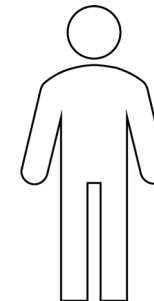
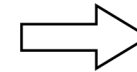
- 3 days prior to intervention
- Low in FODMAPs
- Low in added flavourings
- Portion sizes individually calculated



or

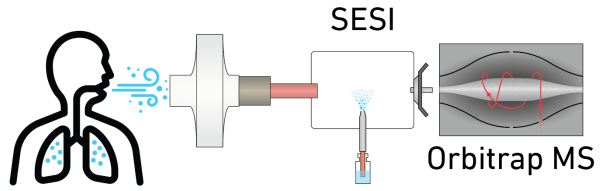


**Randomized controlled
Lactose Challenge**

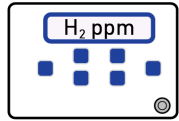


Postprandial Analyses

Biomarker discovery
Quantification



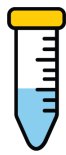
On-line exhaled breath metabolomics



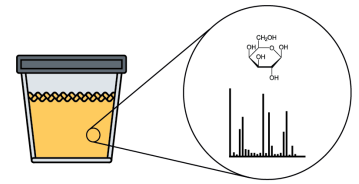
QuinTron BreathTracker

Hydrogen in Breath

LC-MS & GC-MS
Untargeted
Metabolite Identification



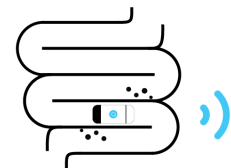
Exhaled breath condensate



GC-MS
Targeted (known metabolites)

Urine

Ingestible Atmo® Gas Capsule
H₂, O₂, CO₂, CH₄



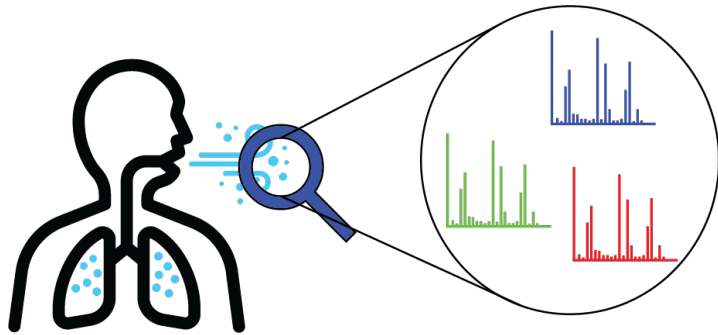
Intestinal gases



Standardized aCPQ questionnaire
(Digital)

GI symptoms

Expected results



→ Find distinct metabolic breath profiles for each of the 3 phenotypically different groups

→ Associate breath profiles with mechanisms specific to lactose metabolism

→ Gain deeper understanding of the metabolic pathways that are differently active across the 3 groups and may be responsible for GI symptoms of LM

Outlook

➡ For other FODMAPs

➡ Food Intolerances in general

➡ Personalized Nutrition

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Roxane Guillod

