INSPIRING PEOPLE

Stable Isotope Breath Tests: Applications, Challenges and Opportunities

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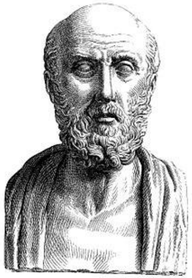
Breath tests – what's new?



O=C=O

460 – c. 370 BC

1743 –1794

1950

The First Glycine Metabolic Pool in Man

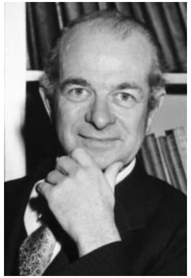
By R. W. E. WATTS and J. C. CRAWHALL
The Medical Unit, St Bartholomew's Hospital, London, E.C. 1

(*Received* 29 *January* 1959)

The number of reported investigations which have been designed to determine directly with the help of isotopically labelled intermediates whether a given precursor–product relationship operates in man *in vivo* are still relatively few. In connexion with our studies on the conversion of glycine into oxalate in subjects with primary hyperoxaluria (Scowen, Crawhall & Watts, 1958), we required to and the first glycine metabolic pool during a period of repetitive feeding with, and after a single dose of, isotopically labelled glycine.

Stable isotopes were used in the present work as in the subsequent clinical investigations to eliminate any slight risk of radiation injury. Some of the results presented here have been the subject of preliminary communications (Crawhall & Watts,

1959



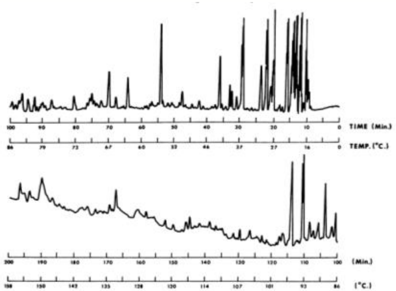


Fig. 2. Chromatogram of breath.

1971

Barriers to uptake of BT's

- Lack of sensitivity and specificity
  - **Lack of appropriate target**
  - **Lack of / cost of appropriate labelled substrate**
  - ➢ **Limits impact on clinical decision making**

- Need to define population reference ranges
  - **Ambiguous**
  - **Need to re-define for each new target population**

- Technology
  - **IRMS not a standard laboratory MS (Infrared options now available)**

- Ignorance
  - **Stable isotope tests "complicated" compared with other diagnostic tests**
  - **Requirement for additional infrastructure / expertise**

What do we want from breath (tests)?

1. Diagnostic accuracy / clinical decision making
2. Increased understanding of real physiology / function
3. Non-invasive / rapid
4. Field deployable

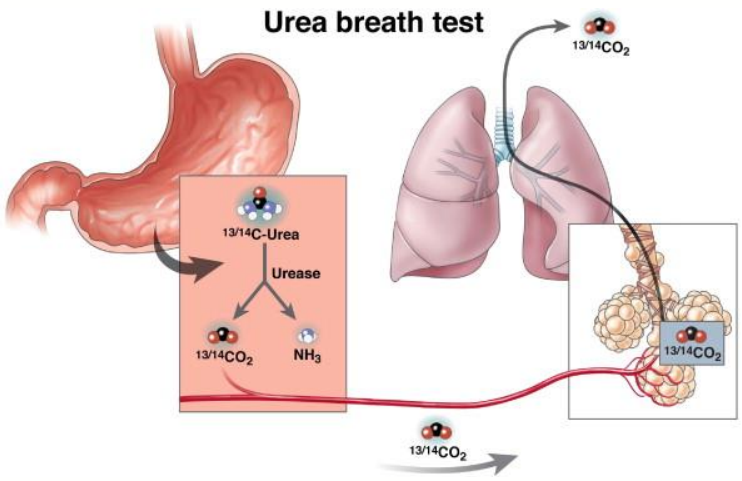
Adding an isotope tracer “targets” a specific **function**

Fingerprint (“breathome”) complicated but potentially useful

Breath tests in small intestinal disease

Test	sensitivity	specificity	Comments	Refs
GHBT	62 %	78 %	ROME CONSENSUS: H2-BREATH TESTING IN GI DISEASES. Glucose Breath Test is the most accurate hydrogen breath test for non-invasive diagnosis of SIBO.	Corazza et al. Gastroenterol. 1990
LHBT	52 %	86 %	SIBO	Gasbarrini et al, APT, 2009
SoHBT	71 %	46 %	Celiac Disease	Tveito et al. Scand. J. Gastroenterol, 2009
^{13}C SoBT	74 %	85 %	Celiac Disease	Tveito et al. Scand. J. Gastroenterol, 2009
^{13}C XBT	88 %	84 %	Celiac Disease	Tveito et al. Scand. J. Gastroenterol, 2010.
^{13}C SBT	98 %	94 %	chemotherapy-induced small intestinal damage, rats	Tooley et al, Cancer Chemother Pharmacol 2010

H. Pylori UBT: a paradigm in ^{13}C BT's



Cut-off	Sensitivity	Specificity	
10min	98.6%	98.6%	Mauro et al, 2006
30min	93.8%	99.1%	Cardinali et al, 2003

University of Glasgow

Challenge for diagnostic test: GI transit

European Journal of Nuclear Medicine

Original article

Increased accuracy of the carbon-14 D-xylose breath test in detecting small-intestinal bacterial overgrowth by correction with the gastric emptying rate

Chi-Sen Chang[1], Gran-Hum Chen[1], Chia-Hung Kao[2], Shyh-Jen Wang[2], Shih-Nen Peng[1], Chih-Kuen Huang[1], Sek-Kwong Poon[1]

[1] Division of Gastroenterology, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.
[2] Department of Nuclear Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.

Received 2 February and in revised form 13 April 1995

Test	sensitivity	specificity	Comments
^{14}C XBT	60 %	90 %	Uncorrected
	90 %	100 %	Corrected for Gastric emptying

Using stable isotopes to interrogate function *in vivo*

1. Overnutrition

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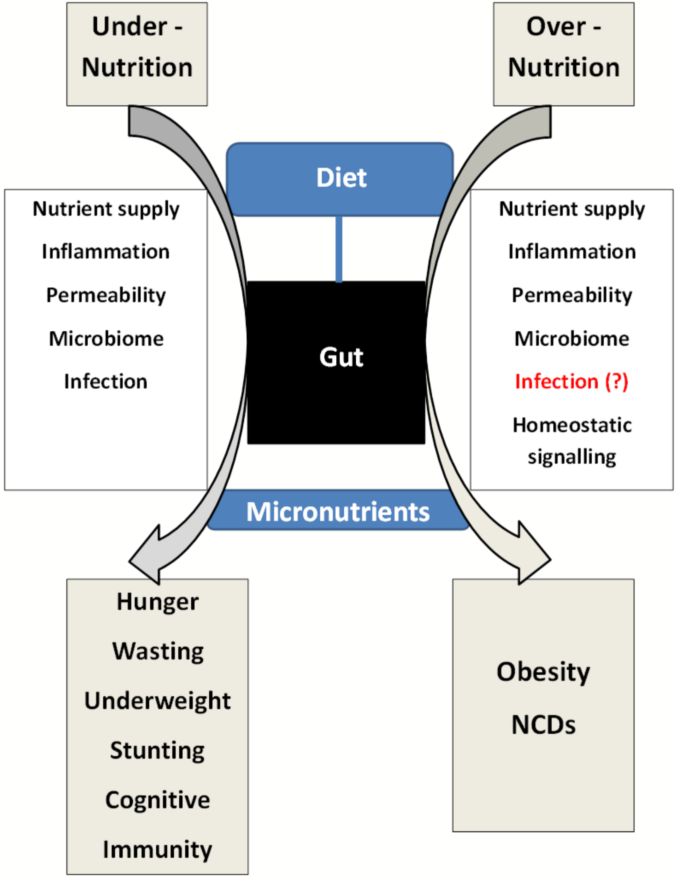
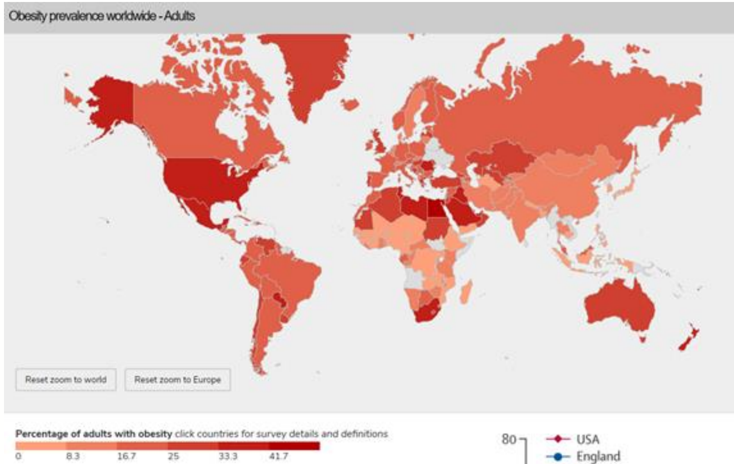
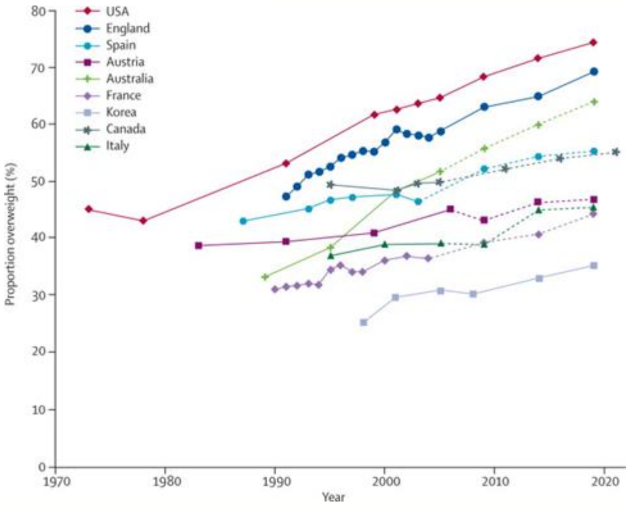
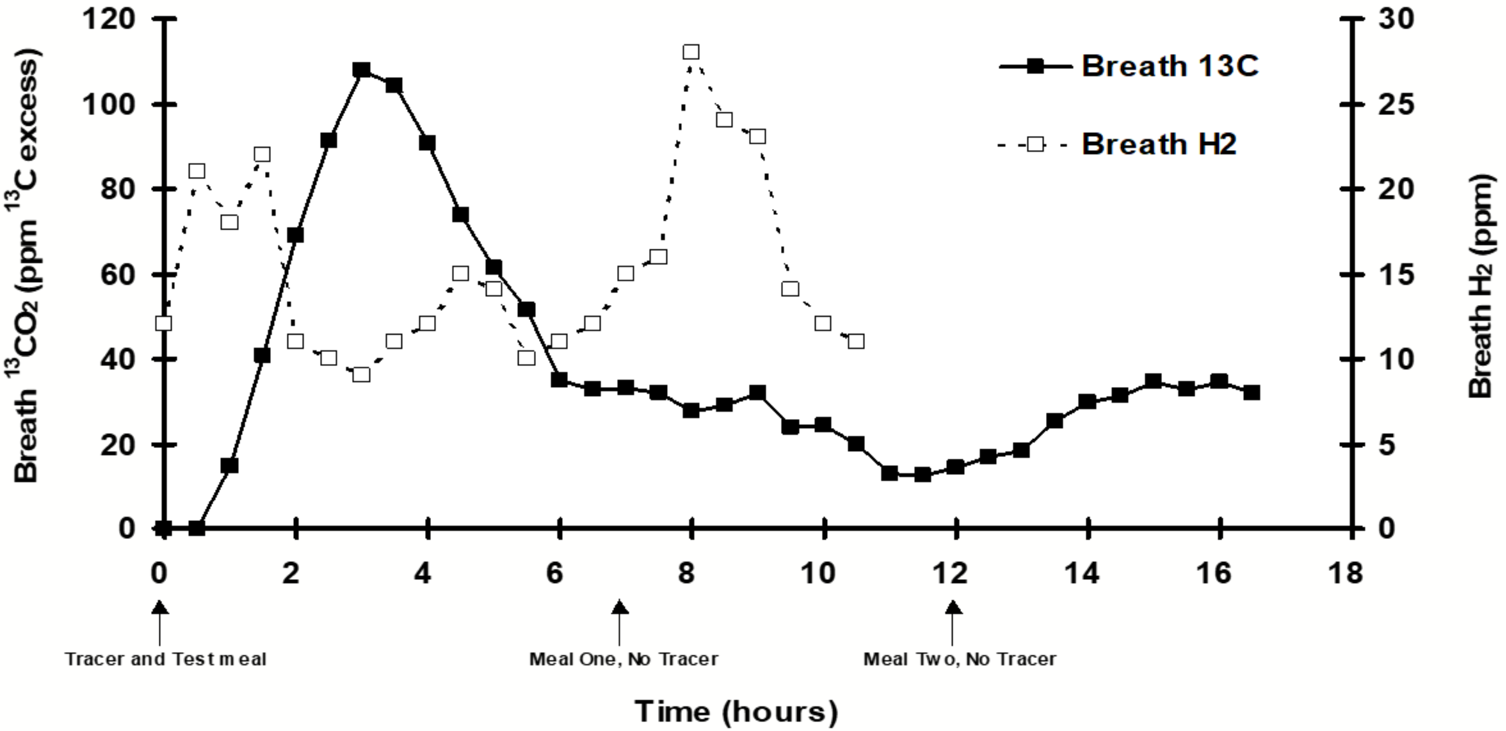
Under and over nutrition converge in the gut







Substrate utilisation – its complicated!



Edwards et al, 2002

University of Glasgow

Using pea genetics and stable isotopes to probe gut physiology

Exploiting a natural mutation in peas (Pisum sativum) starch branching enzymes

RR

rr

^{13}C label

↓

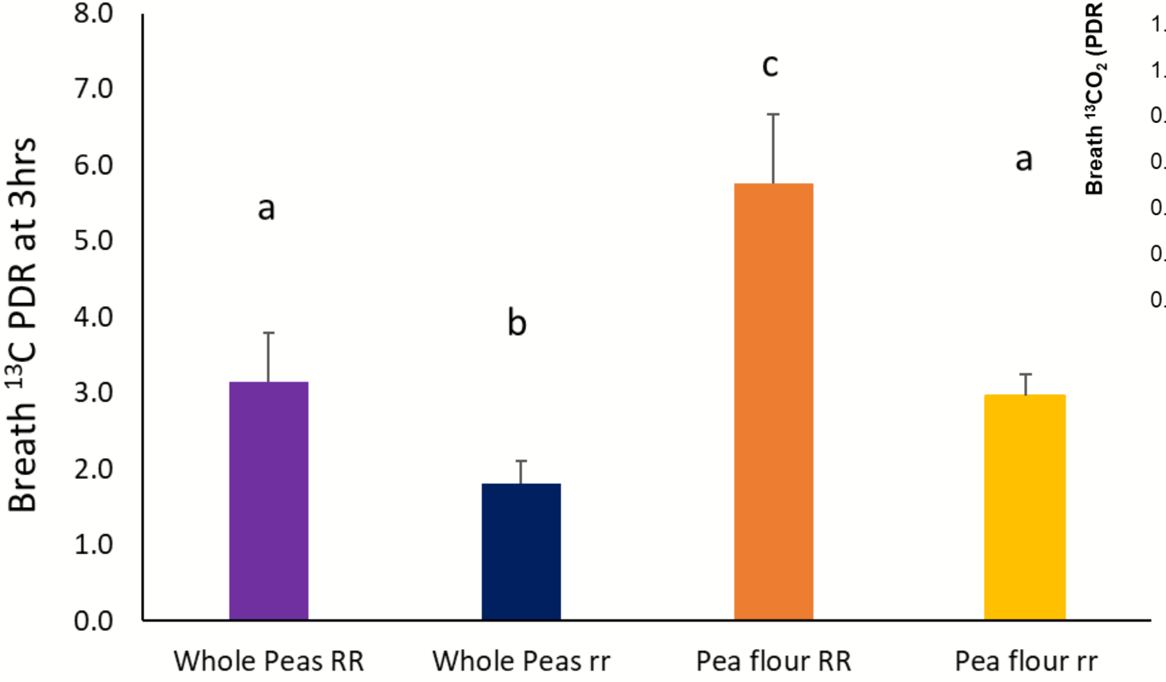
Human feeding studies

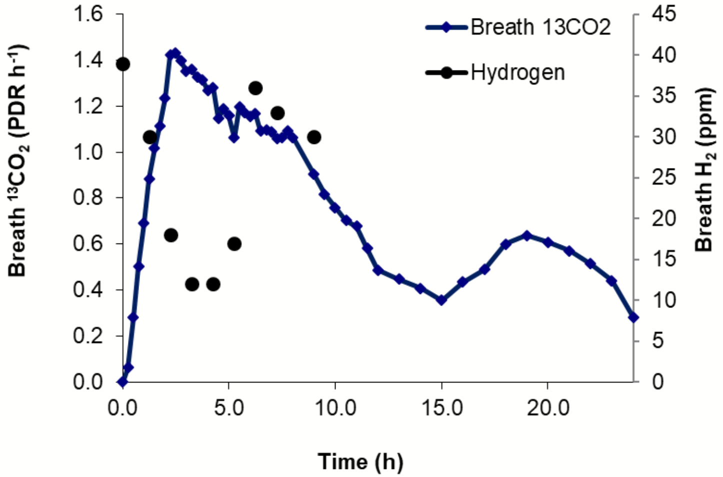
Quadram Institute
Science Health Food Innovation

John Innes Centre
Unlocking Nature's Diversity

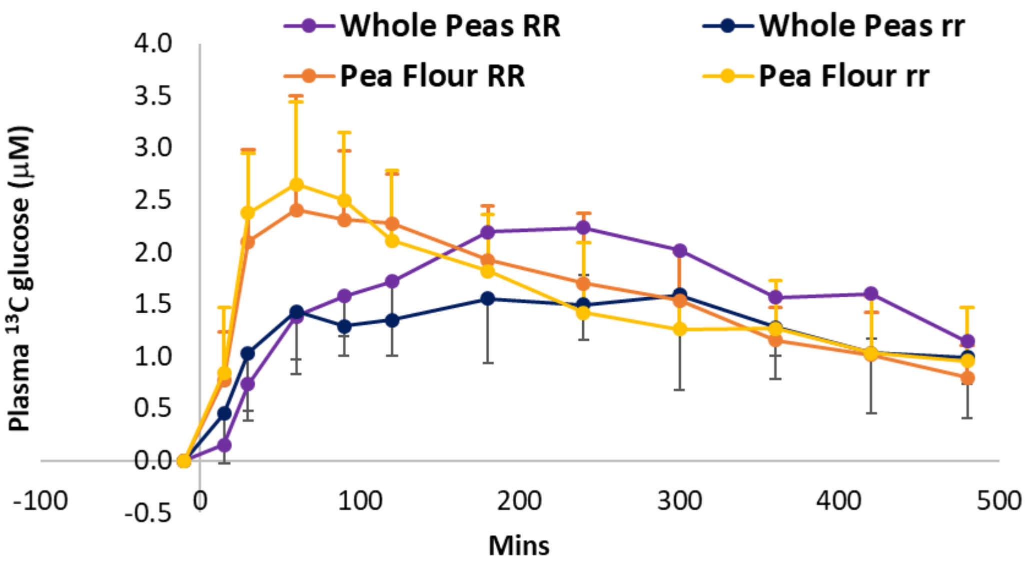
Imperial College London

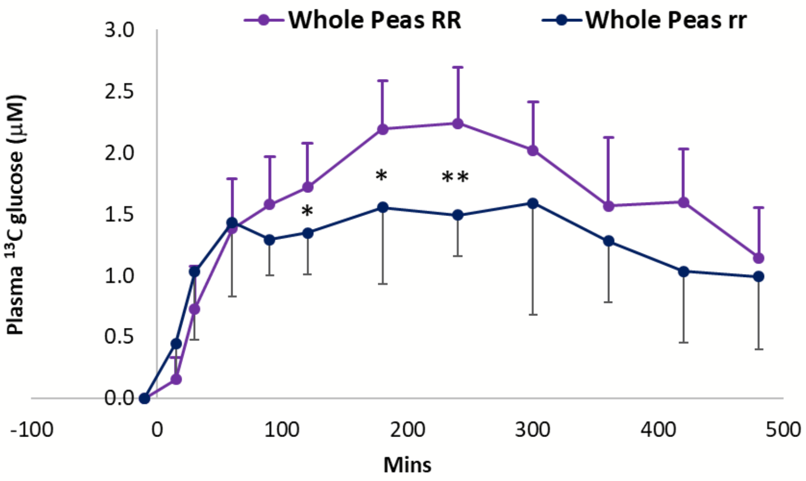
Breath $^{13}CO_2$

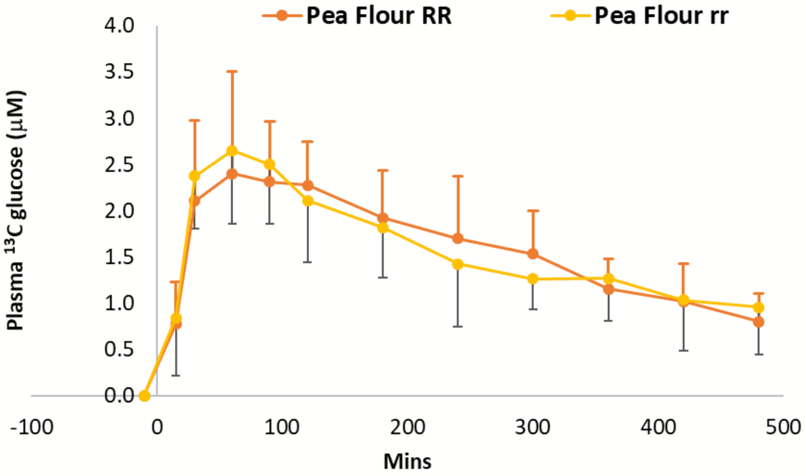




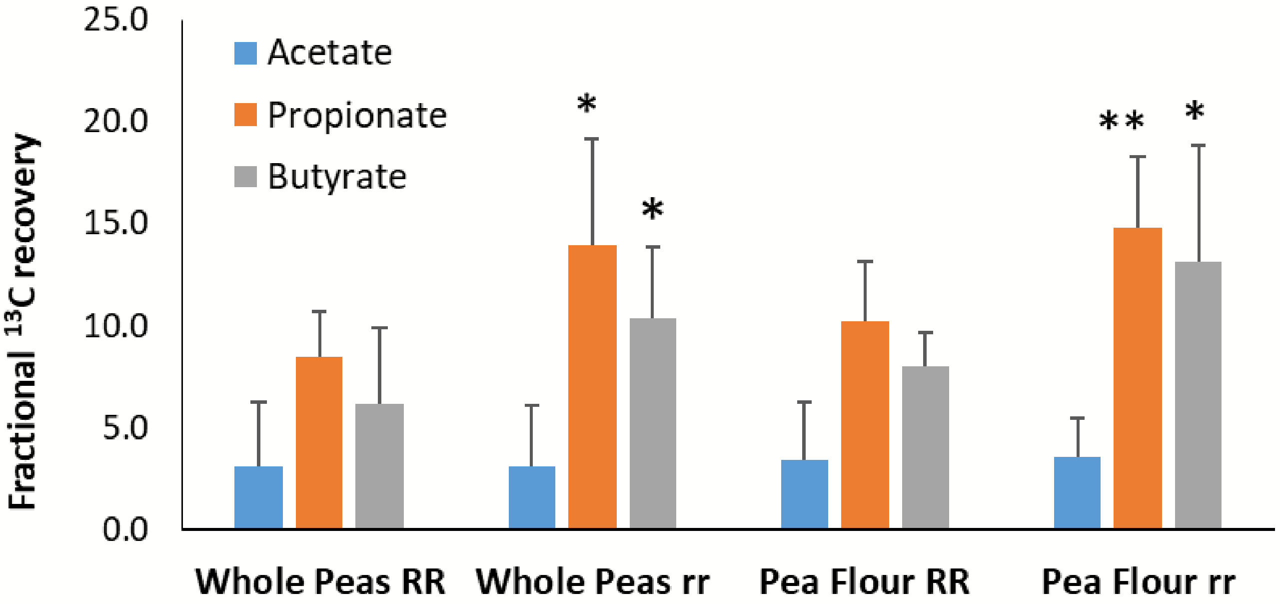
Plasma ^{13}C Glucose



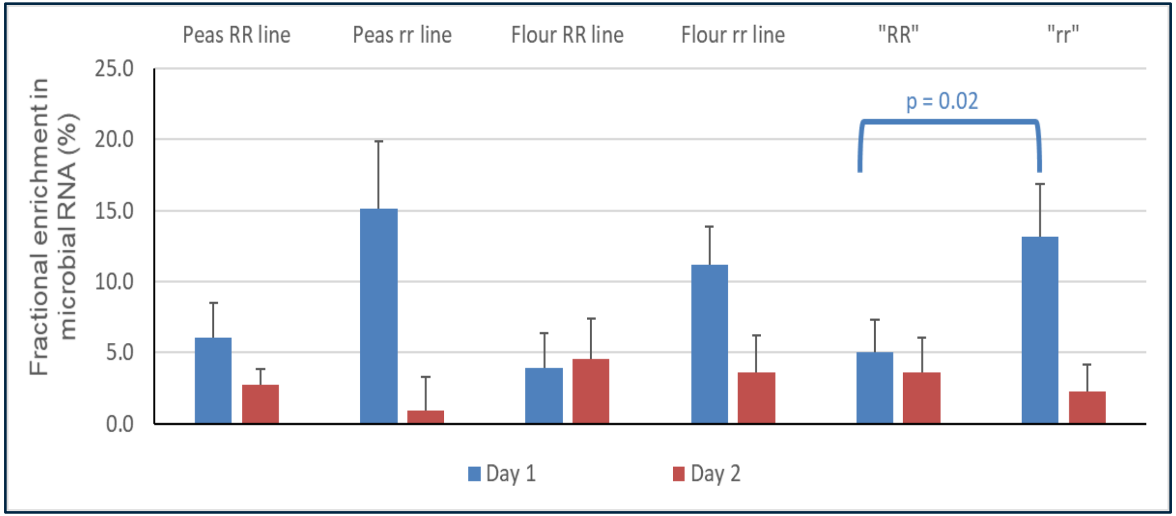




Urinary ^{13}C SCFA



^{13}C appearance in gut microbial RNA



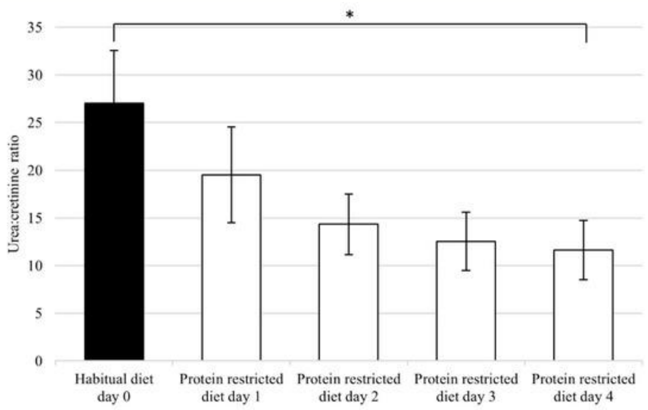
Total ^{13}C appearance in stool

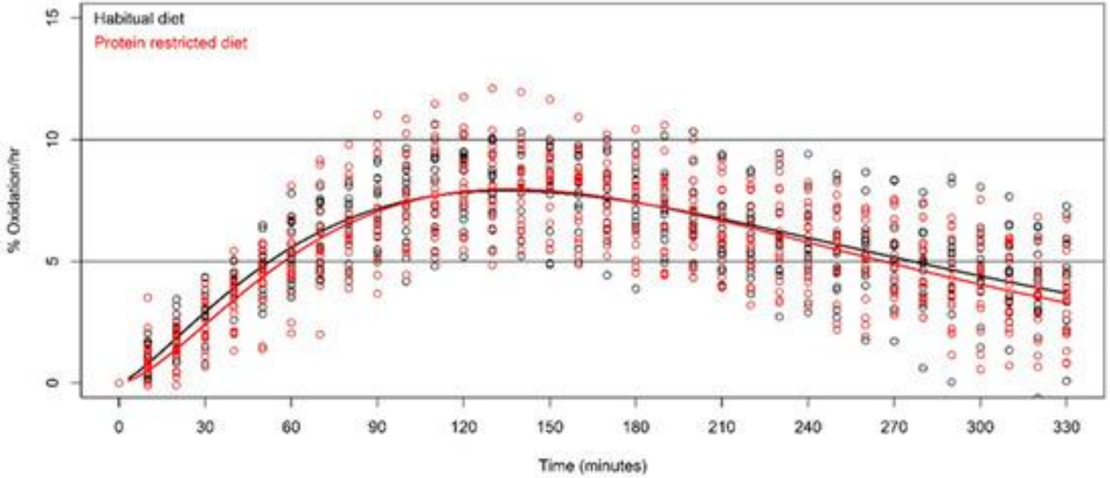
Group	Treatment	% recovered
A	Whole peas RR	11.2
B	Whole peas rr	11.0
C	Pea flour RR	4.6
D	Pea flour rr	9.9

- Rapid digestion of carbohydrate is associated with higher post-prandial glucose
- Shifting carbohydrate load to the colon is associated with improved glycaemia

Protein oxidation

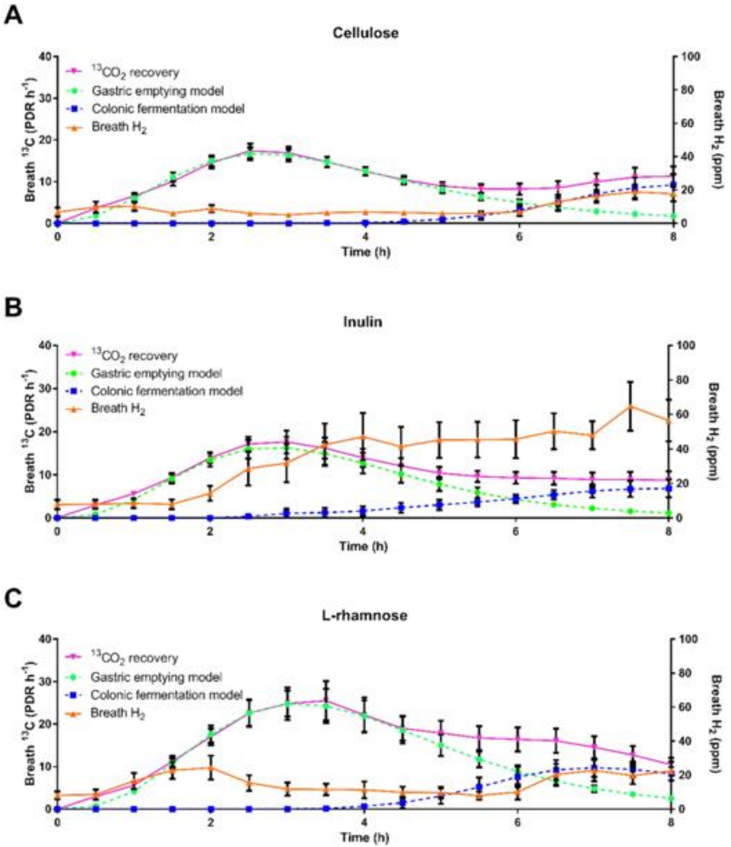
4 day restriction of protein intake has no effect on protein oxidation of ^{13}C milk protein in healthy males



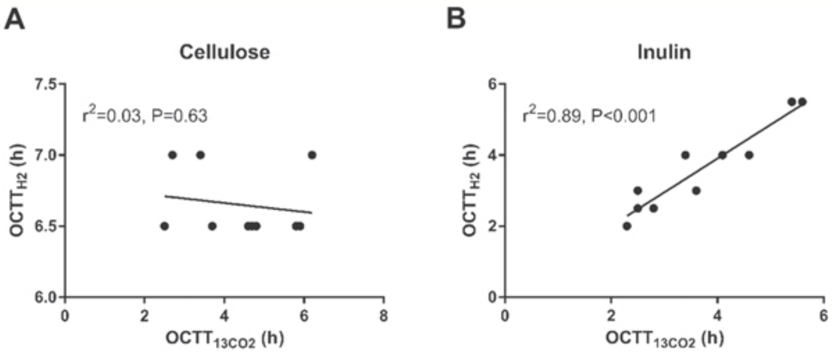


Reckman et al, 2019

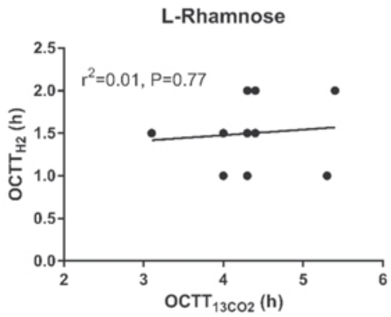
Combining BTs to understand gut physiology



^{13}C acetate, ^{13}C lactose ureide, breath H_2



C



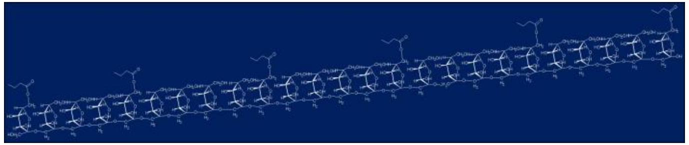
- Microbial degradation of rhamnose in SI
- ^{13}C BTs can be combined (in some cases)

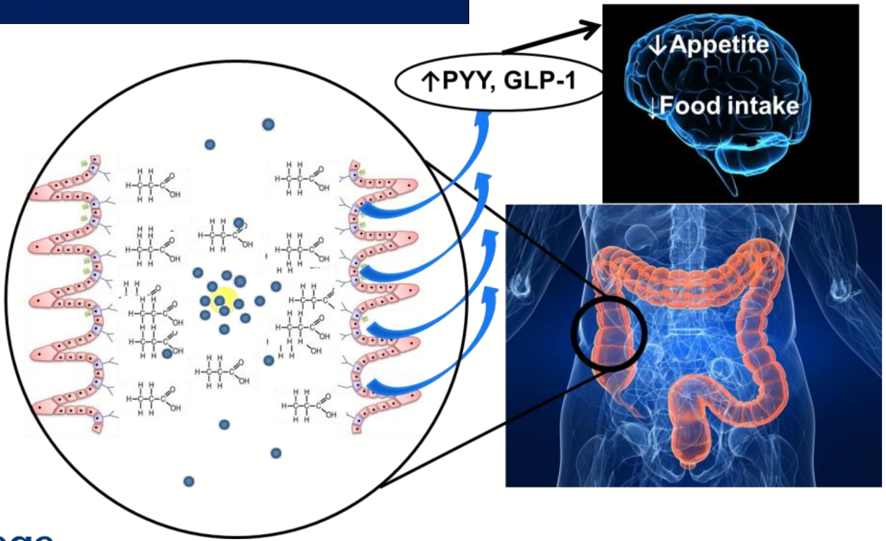
Byrne et al, 2018

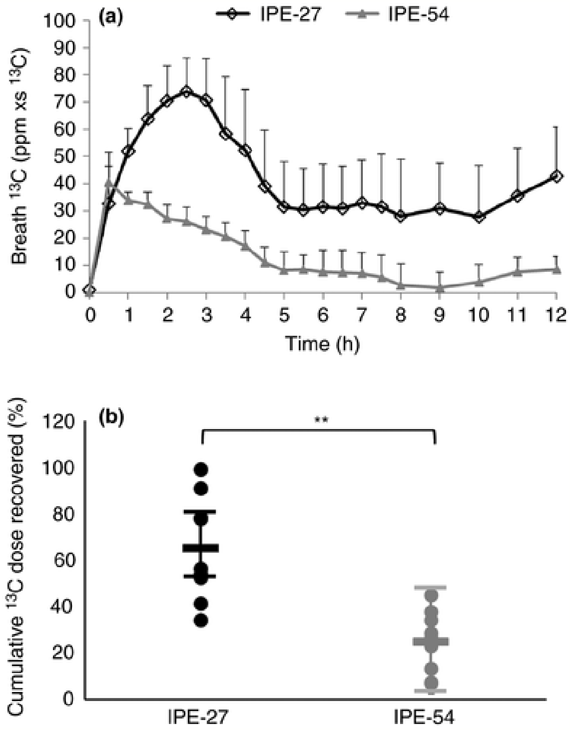
University of Glasgow

Using isotopes to target regions of the gut in obesity

Optimising inulin propionate ester formulation







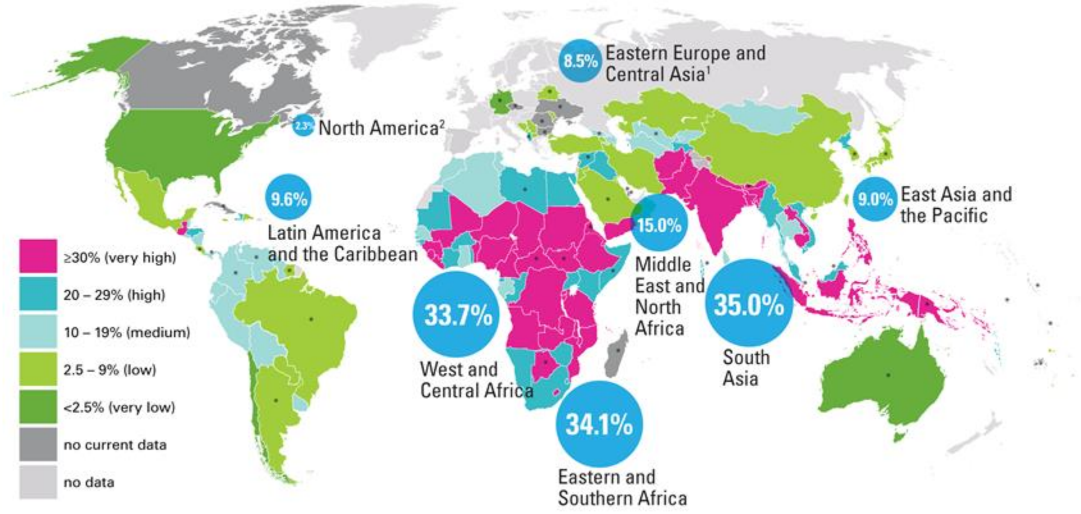
Imperial College London

Polyviou et al, 2016

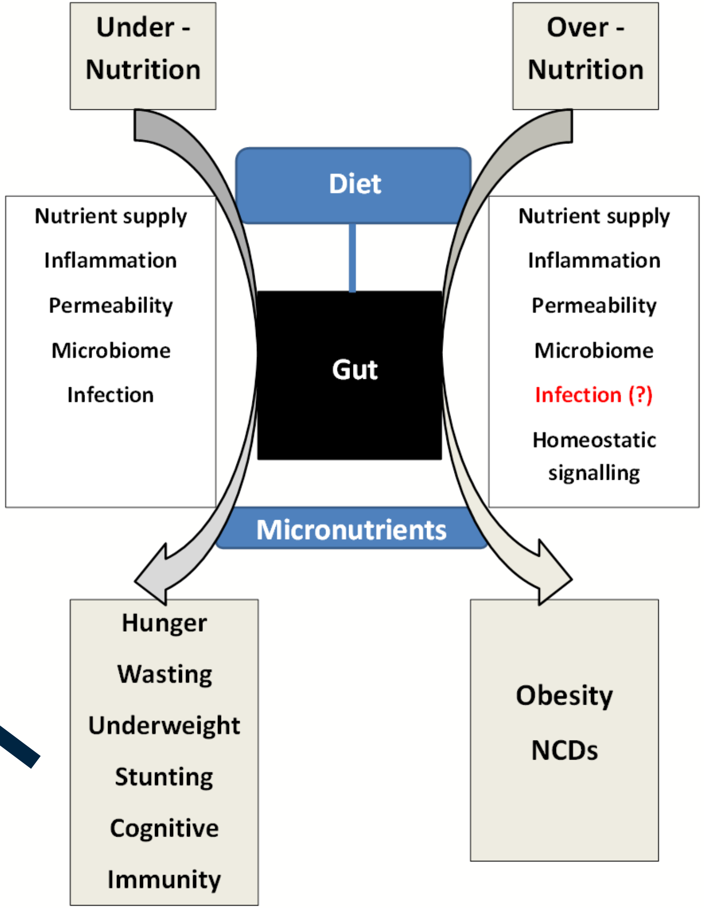
Using stable isotopes to interrogate function *in vivo*

1. Undernutrition

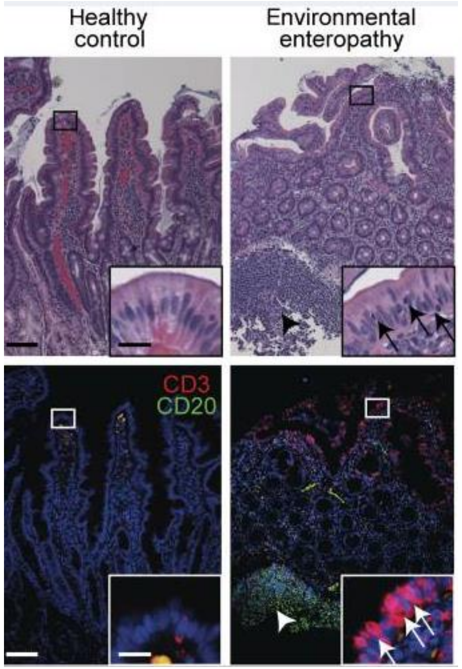
Under and over nutrition converge in the gut

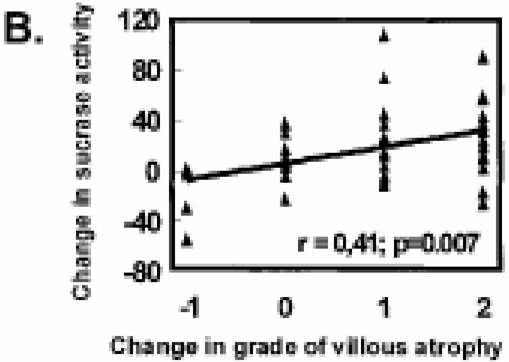


Percentage of children under 5 who are stunted, 2017
Source: UNICEF, WHO, World Bank Joint Child Malnutrition dataset, May 2018



Intestinal brush border functionality

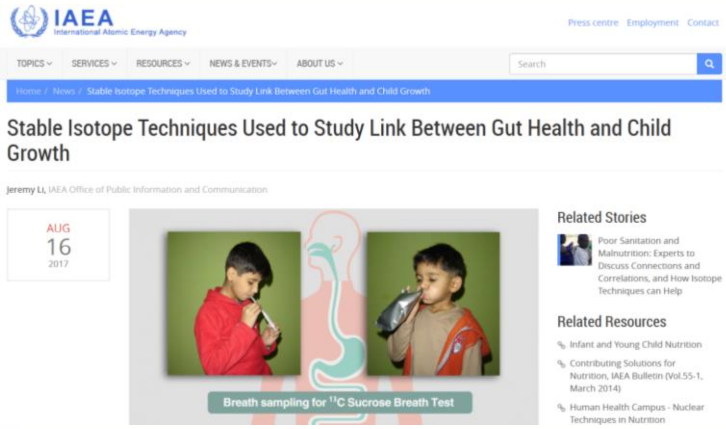


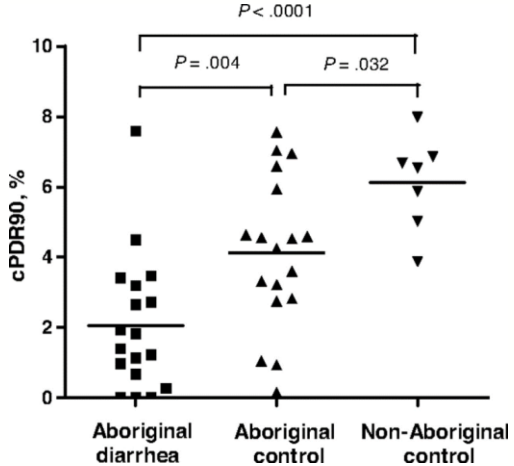


ORIGINAL ARTICLE

Duodenal Disaccharidase Activities in the Follow-up of Villous Atrophy in Coeliac Disease

U. Nieminen, A. Kahri, E. Savilahti & M. A. Färkkilä
Helsinki University Hospital, Dept. of Medicine, Division of Gastroenterology, Helsinki, Finland; Helsinki University Hospital, Hospital for Children and Adolescents and Helsinki University, Dept. of Pathology, Helsinki, Finland

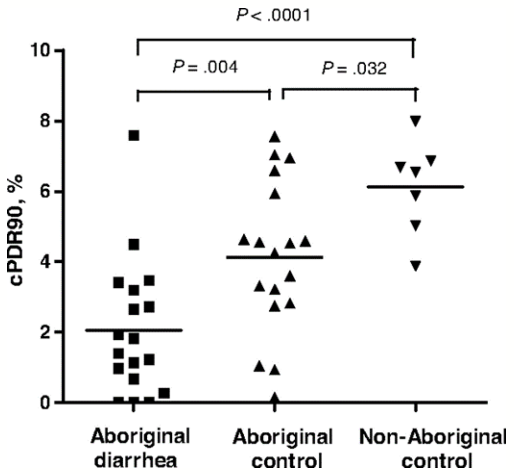




Syed et al, 2018

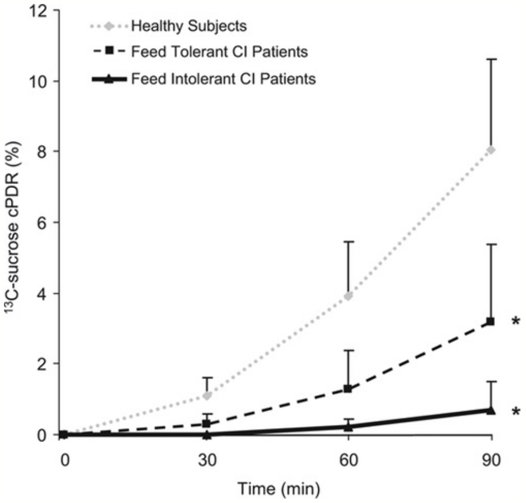
^{13}C-sucrose breath test in EED

"EED" (Ritchie et al, 2009)



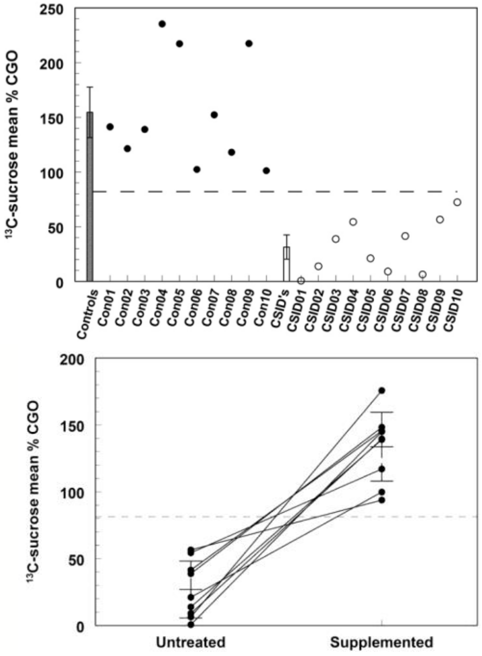
2g/kg or 20g C_4sucrose

Critical illness (Burgstad et al, 2013)



- 20g C_4sucrose
- Higher L/R in CI
- normal sucrase levels
- no relationship with mucosal damage

CSID (Robayo-Torres et al, 2009)



20mg ^{13}C-sucrose / 20mg ^{13}C-glucose

Need for a new ^{13}C sucrose BT

- “naturally enriched” sucrose (maize) does not yield sufficient signal to noise
- Large dose (20g) to achieve breath signal is not feasible in children

- Highly enriched ^{13}C-sucrose confers many advantages ….but
  - Which labelled variant?
  - What is the correct dose?
  - Flooding dose vs. breath biopsy?
  - Does it report on villous atrophy in children at risk of EED?

Phase 1

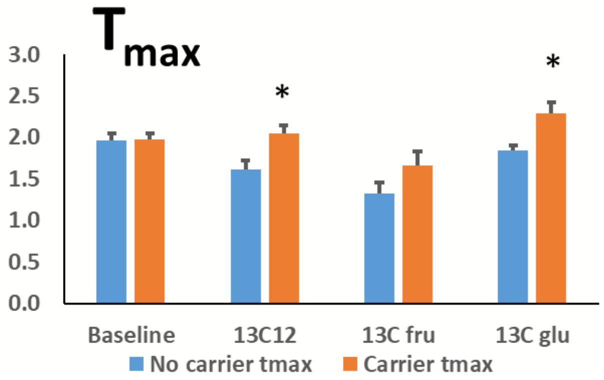
WP1: Optimising a New ^{13}C Sucrose Breath Test Protocol in Adults (Glasgow)

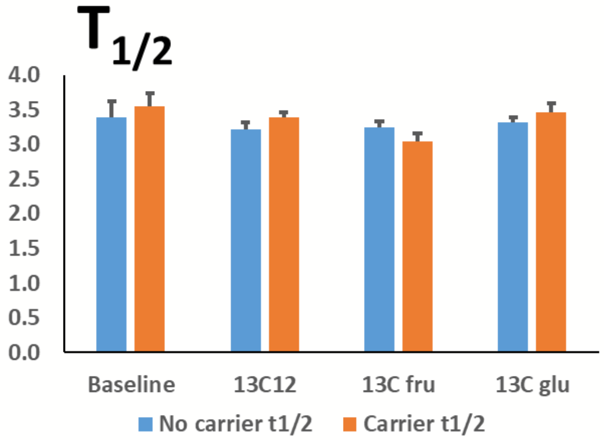
WP2: Validation of ^{13}C Sucrose Breath Test Against Intestinal Biopsies in Paediatric Coeliac Associated Enteropathy in Australia (Adelaide)

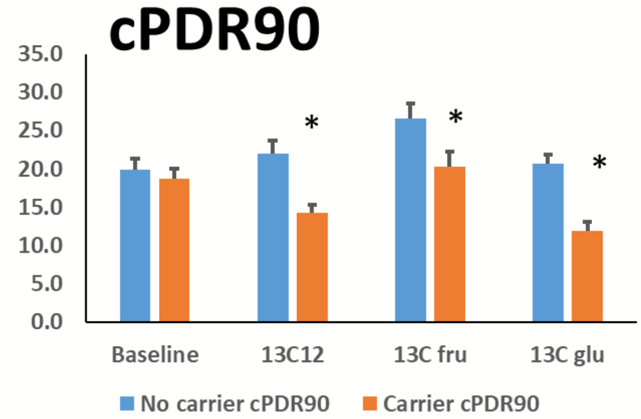
WP3: Validation of ^{13}C Sucrose Breath test Against Intestinal Biopsies in Environmental Enteropathy in Zambia (Lusaka)

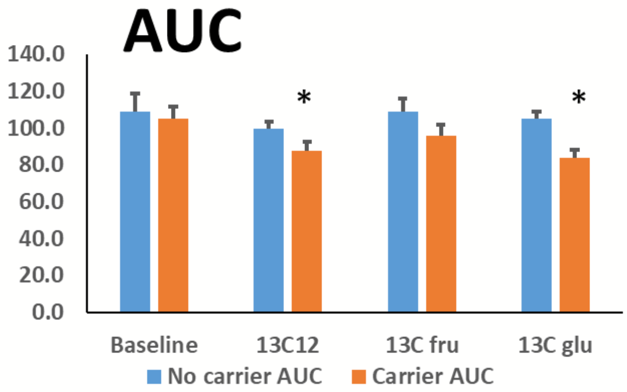
WP4:Validation of the ^{13}C Sucrose Breath Test Against L:R Ratio and Kynurenine:Tryptophan Ratios in Peruvian Children Living in High EED Risk Area. (Lima)

Preliminary data - Carrier vs. No Carrier









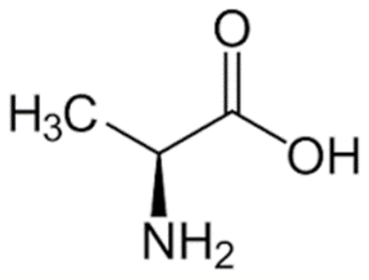
University of Glasgow

Phase 2 plan

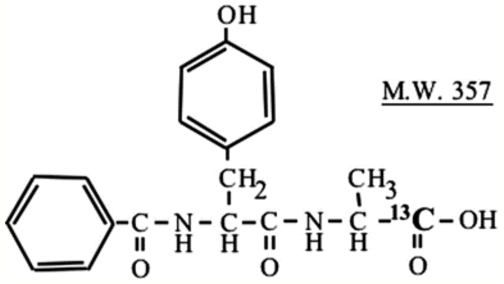
Site	Readouts	Population	Aim
UK	^{13}C SBT, L/R,	Adults (n=20)	Protocol
ZAM1	^{13}C SBT, L/R, biopsy	Adults (n=40, +/- EED)	Adult EED, biopsy
AUS	^{13}C SBT coeliac, L/R, biopsy	Children (n=60, +/- CD, remission)	Coeliac vs. healthy, biopsy
BGD	^{13}C SBT, L/R	Children (n=100)	^{13}C SBT vs. biomarkers, anthropometric
IND	^{13}C SBT, L/R	Children (n=100)	^{13}C SBT vs. biomarkers, anthropometric
JAM	^{13}C SBT, L/R	Children (n=100)	^{13}C SBT vs. biomarkers, anthropometric
KEN	^{13}C SBT, L/R	Children (n=100)	^{13}C SBT vs. biomarkers, anthropometric
PER	^{13}C SBT, L/R	Children (n=40)	^{13}C SBT vs. biomarkers, anthropometric
ZAM2	^{13}C SBT, L/R	Children (n=100)	^{13}C SBT vs. biomarkers, anthropometric

Can we probe specific functions in the gut?

Pancreatic exocrine function / Intestinal protease activity



Benzoyl L-tyrosyl L-[1-^{13}C]alanine



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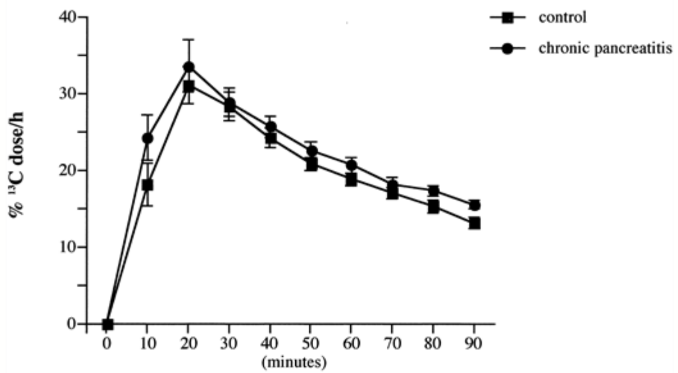
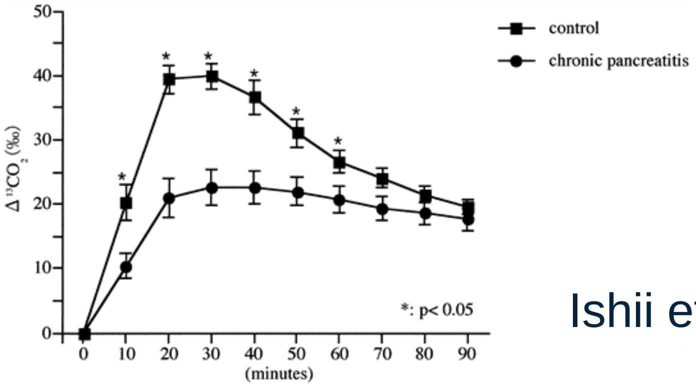




Ishii et al, 2007

Looking ahead

Targeting gut function

1. Hypothesis driven – isotope labelled substrates

2. Hypothesis generating – “omics”

Understanding the role of diet

1. Macronutrient utilisation – multi-isotope approaches

Hypothesis driven: targeted approaches

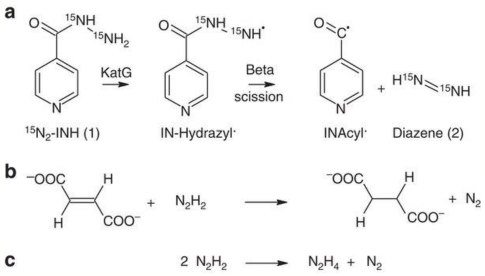
Exploiting 'omics' to develop targeted tests: a TB paradigm

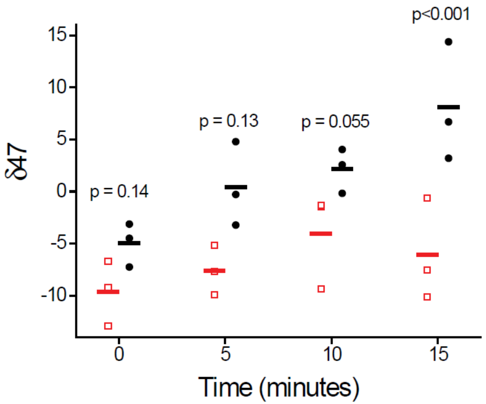
"An extensive genomic and metabolomic search supported the hypothesis that the enzyme CO dehydrogenase (CODH) might provide a suitable and highly specific metabolomic route to enable TB detection." $CO + H_2O >> CO_2 + 2H+ \; 2e^-$

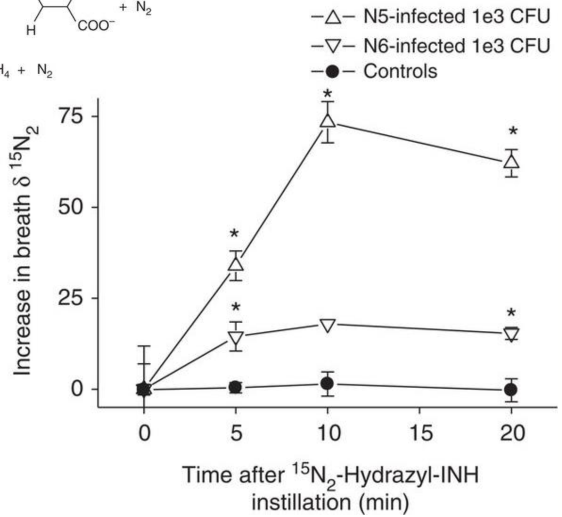
TABLE 1 Common lung pathogens expressing urease do not express CODH[a]

Pathogen	Expression of: Urease (source)	CODH
Pseudomonas aeruginosa	Yes (38)	No
Acinetobacter baumannii	Yes (8)	No
Klebsiella pneumoniae	Yes (9)	No
Haemophilus influenzae	Yes (39)	No
Staphylococcus aureus	Yes (40)	No

[a] Data are collected from prior work discussing genes or enzymatic activity. In this work, only genes were tested, and none of the tested strains expressed CODH.



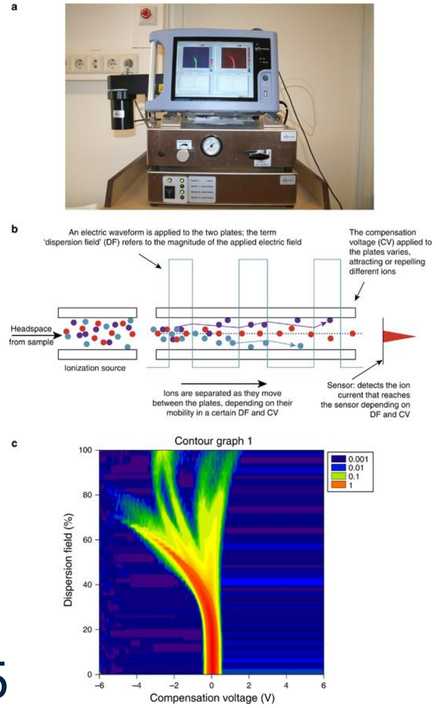




Maiga et al, mBio 2014
Choi et al, Nat. Commun. 2014

Hypothesis generating: Untargeted approaches

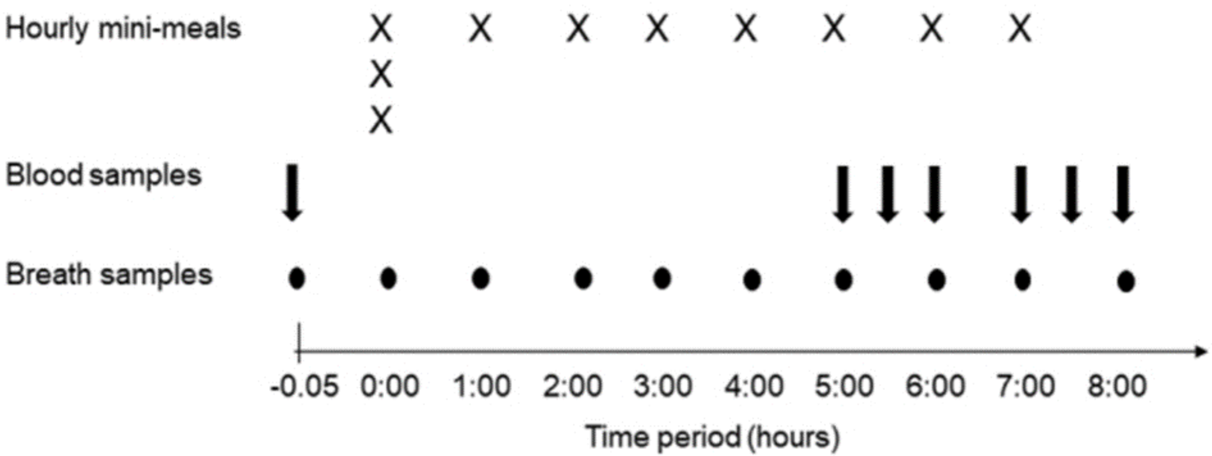
GCMS and field Asymmetric Ion Mobility Spectrometer (FAIMS) for VOCs



Bomers et al, 2015

Macronutrient utilisation – multi-isotope approaches

Spirulina digestibility : U-^{13}C-spirulina + U-^{2}H labeled amino acid mixture + $^{13}C_6$-phenylalanine + legume + rice
Legume digestibility : U-^{13}C-spirulina + $^{13}C_6$-phenylalanine + ^{2}H labeled legume + rice



Devi et al, 2018

Technology drivers

1. New isotope tests

- non-invasive, repeatability
- sensitivity / specificity
- tracer costs / sources
- instrumentation

IRMS, δ^2H, $\delta^{15}N$, $\delta^{13}C$ & $\delta^{18}O$

Mid-IR, $\delta^{13}C$ & $\delta^{18}O$ in CO_2

FTIR, δ^2H_2O

2. Usability

- point of care
- field deployability
- instrumentation / diagnostic capability

Fiber-Enhanced Raman Multigas Spectroscopy: A Versatile Tool for Environmental Gas Sensing and Breath Analysis

Stefan Hanf,[†] Robert Keiner,[†] Di Yan,[†] Jürgen Popp,[†,‡,§] and Torsten Frosch*[,†,‡]

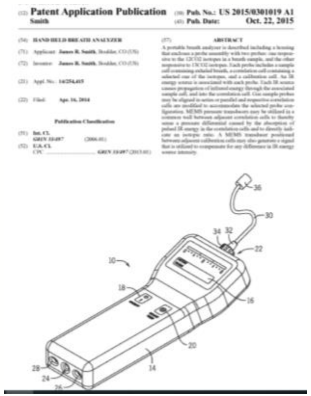
[†]Leibniz Institute of Photonic Technology, Jena, Germany
[‡]Institute for Physical Chemistry, Friedrich-Schiller University, Jena, Germany
[§]Abbe School of Photonics, Friedrich-Schiller University, Jena, Germany

Applied Physics B
April 2018, 124:62 | Cite as

A broadband Tm/Ho-doped fiber laser tunable from 1.8 to 2.09 μm for intracavity absorption spectroscopy

Authors | Authors and affiliations

Peter Fjodorow, Ortwin Hellmig, Valery M. Baev



Summary

- Diagnostic accuracy of ^{13}C BTs reduced beyond stomach
- SI labelled probes excellent for probing function (gut and other organs)
- Combining SI probes can be a powerful tool to gain mechanistic insight
- Some ^{13}C BTs have potential in field settings
  - Correct tracer, correct technology
- Other isotopes beyond ^{13}C can be useful (even in breath)

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BBSRC bioscience for the future
MRC Medical Research Council Centenary 1913-2013
Yorkhill children's charity
CICRA children with crohns and colitis
NIHR National Institute for Health Research
The Scottish Government
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