“Towards Microbial Fermentation Metabolites as Biomarkers for Health Benefits of Prebiotics"

Kieran Tuohy

for working group on Microbial Metabolism and Fermentation

SUPPORTED BY THE ILSI EUROPE PREBIOTICS TASK FORCE

ILSI Europe
International Life Sciences Institute

Expert group members

Prof Kristin Verbeke – **Chair**	Targid	BE
Dr Annick Bernalier	INRA	FR
Prof Alan Boobis	Imperial College London	UK
Prof Christine Edwards	University of Glasgow	UK
Dr Anne Franck	Cargill	BE
Prof. Michiel Kleerebezem	Wageningen University	NL
Dr Gunhild Kozianowski	Südzucker/BENEO Group	DE
Dr. Arjen Nauta	FrieslandCampina	NL
Prof. Jeroen Raes	Vrije Universiteit Brussels	BE
Dr Kieran Tuohy	Fondazione Edmund Mach	IT
Dr Ric van Tol	Mead Johnson	NL
Dr. Alessandro Chiodini	ILSI Europe	BE
(Dr. Tobias Recker	ILSI Europe	BE)

www.ilsi.eu

ILSI Europe

info@ilsieurope.be

Background

The ILSI Europe Prebiotic Task Force identified a particular need to explore available evidence for positive or negative physiological effects of microbial (faecal) metabolites on the host.

Aim

- Exploring how to define and impact digestive health

- Looking at the different gut functions that contribute to a healthy gastro-intestinal tract and thereby to overall health

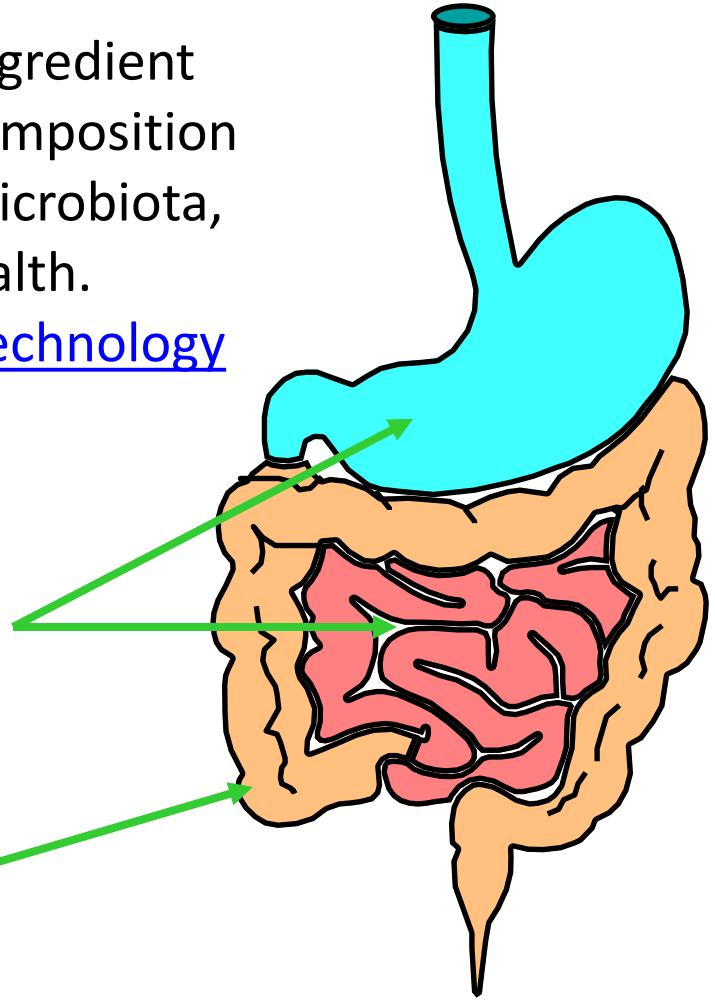
ILSI Europe

Prebiotic definition:

A **prebiotic** is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health. (Gibson et al. 2010. Food Science and Technology Bulletin: Functional Foods 7 (1) 1–19.)

Persist until the colon

Selective fermentation in the colon



Objectives and Methods:

A literature search was performed to address:

- Available evidence for the beneficial or harmful effects of known microbial metabolites including short chain fatty acids and protein fermentation products.
- The potential for functional analysis of faecal water
- The applicability of metabolome signatures and systems biology

ILSI Europe

Overview on different metabolites

Summarising available evidence

a. List of relevant metabolites

b. Highlighted metabolites

Integrated perspectives (harm+benefit) including:

- Pharmacological perspectives
- Toxicological perspectives
- Nutrition perspectives
- Normal ranges

c. More holistic approach: Profiles

- Functional analysis of faecal water (include genotoxicity)
- Metabolomics
- Metagenome analysis

ILSI Europe

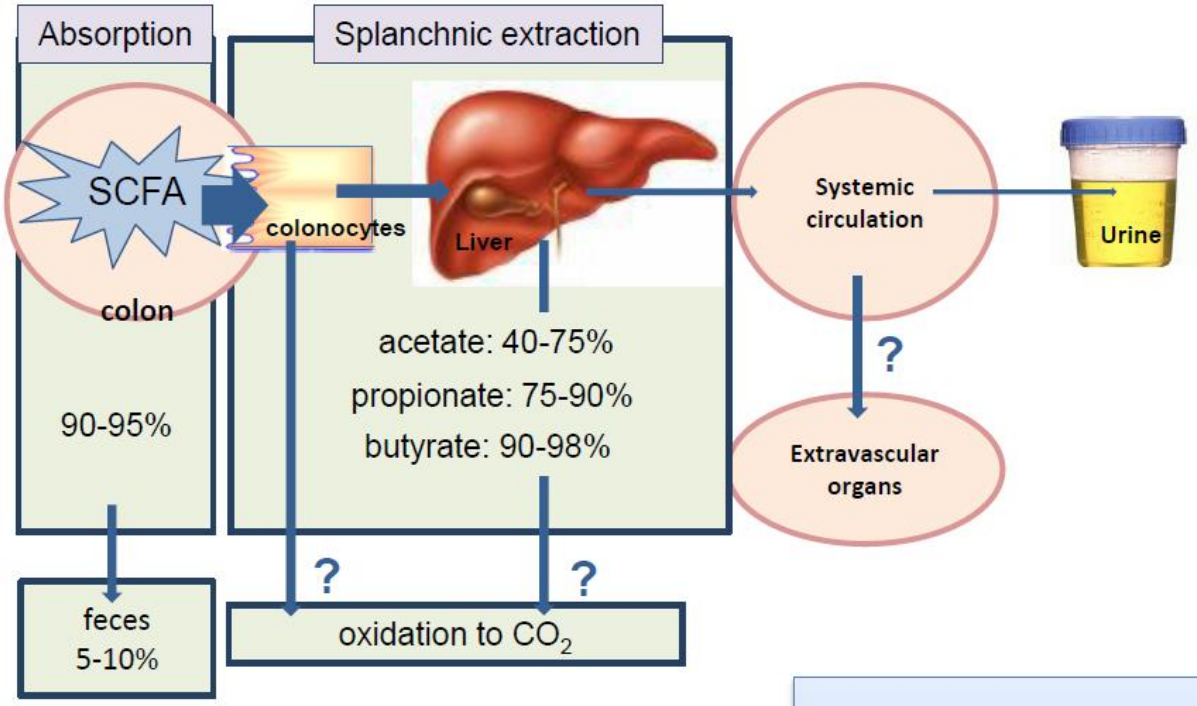
Colonic Microbial Metabolites

Carbohydrate (Dietary fibre)	Protein	Plant Polyphenolics	Fat and related bile acids

• Short chain fatty acids
  ○ Acetate
  ○ Propionate
  ○ Butyrate
• Lactate
• Succinate
• Alcohols
• Gasses:
  ○ Hydrogen
  ○ Methane
  ○ Carbondioxide
	• Ammonia
	• Hydrogen Sulphide
	• Phenols
	• p-cresol
	• Indoles
	• Branched chain fatty acids
		• Large range of phenolic compounds and acids including:
		• Simple phenols
		• Glycinated benzoic acids
		• Derivatives of benzoic acid
		Derivatives of
		  ○ Phenyl acetic acid
		  ○ Phenylpropionic acid
		  ○ Mandelic acids
		  ○ Cinnamic acids
		  ○ Equols
			• Hydroxy fatty acids
			• Secondary bile acids
			• Long chain aldehydes

ILSI Europe

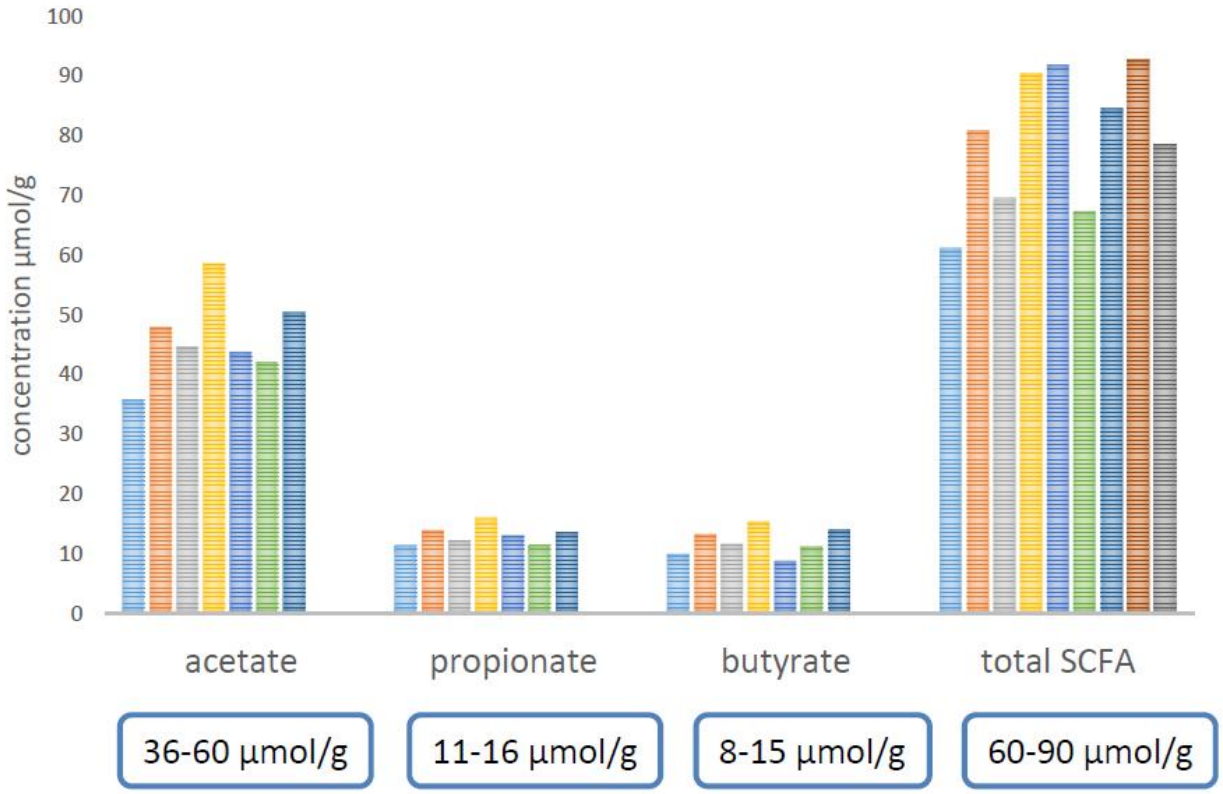
Metabolism of SCFA



- Inaccessibility of appropriate body compartment
  - ⇨ leaves faeces, plasma, urine
  - ⇨ not representative
- Where / when to measure
- Large normal variations – diet and other factors

ILSI Europe

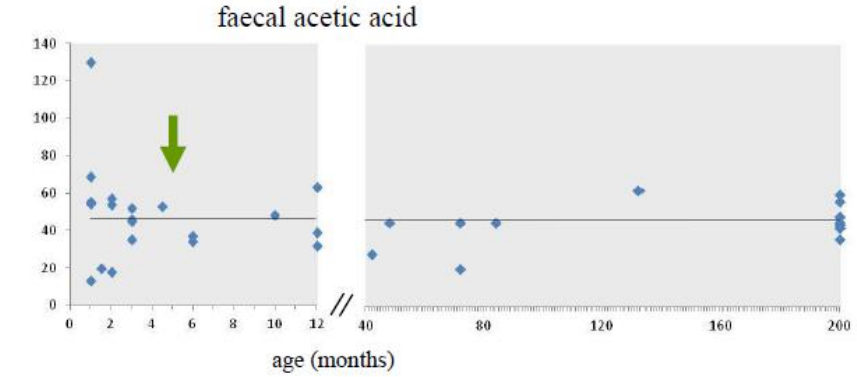
Faecal SCFA concentrations

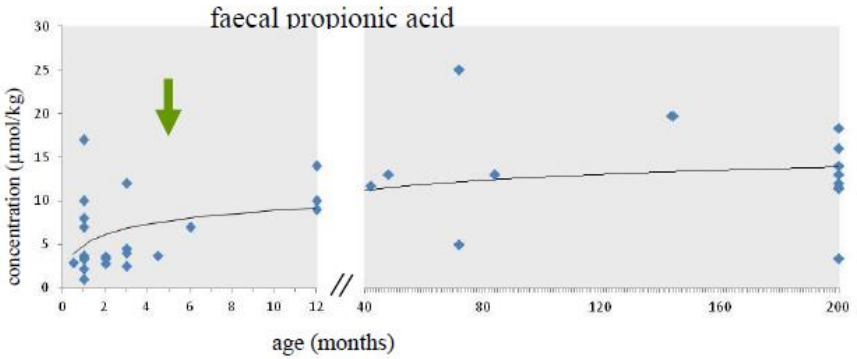


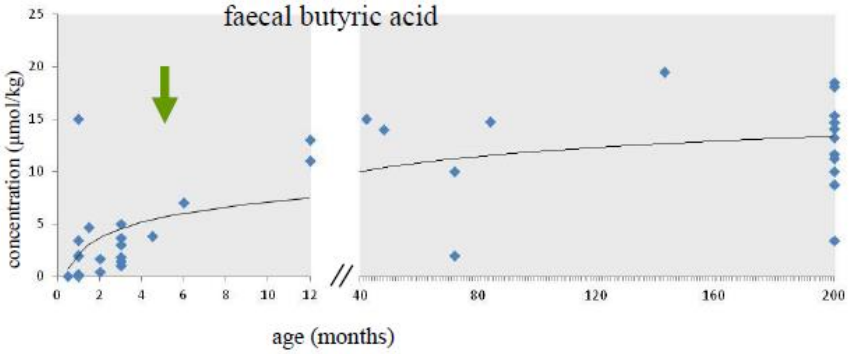
Reimer *et al.* J Hum Nutr Diet 2012, 25, 373-377
Fernando *et al.* Benef Microbes 2010, 1, 197-207
McOrist *et al.* J Nutr 2011, 141, 883-889
Nemoto *et al.* Dig Dis Scie 2012, 57, 2955-2964
Tiihonen *et al.* Br J Nutr 2010, 103, 1070-1078
McOrist *et al.* Br J Nutr 2008, 100, 138-146
Hylla *et al.* Am J Clin Nutr 1998, 67, 136-142
Schwiertz *et al.* Obesity 2010, 18, 190-195

ILSI Europe

Faecal [SCFA] depend on age







Edwards *et al*. Acta Paediatr 1994, 83, 459-462
Midtvedt *et al*. J Pediatr Gastroenterol Nutr 1992, 15, 395-403
Parrett *et al*. Am J Clin Nutr 1997, 65, 927-933
Tjellstrom *et al*. Microb Ecol Health Dis 2013, 24, 20905
Wang *et al*. Dig Dis Sci 2012, 57, 2096-2102
De Filippo *et al*. Proc Natl Acad Sci U S A 2010, 107, 14691-14696
Guerin-Danan *et al*. J Pediatr Gastroenterol Nutr 1997, 25, 281-289
Siigur *et al*. Acta Paediatr 1993, 82, 536-538
Holscher et al. J Parenter Enteral Nutr 2012, 36, 95s-105s
Norin *et al*. Microb Ecol Health Dis 2004, 16, 8-12
Bakker-Zierikzee *et al*. Br J Nutr 2005, 94, 783-790
Samuelsson *et al*. Diabet Med 2004, 21, 64-67
Parrett *et al*. Arch Dis Child 1997, 76, 249-253

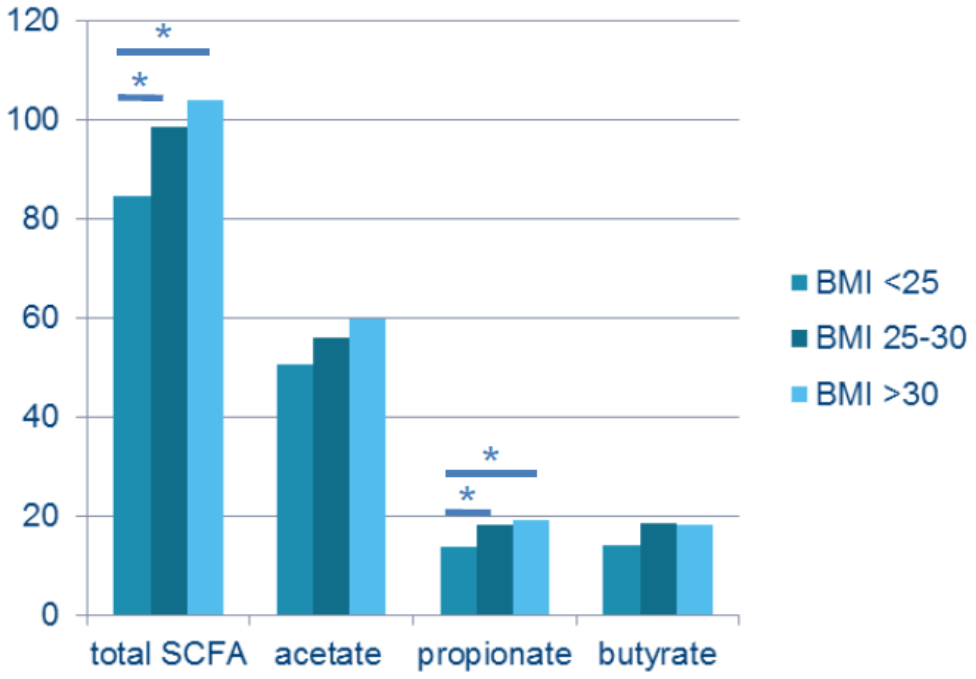
ILSI Europe

Faecal [SCFA] differ in lean v obese

Microbiota and SCFA in Lean and Overweight Healthy Subjects

Andreas Schwiertz[1], David Taras[2], Klaus Schäfer[2], Silvia Beijer[3], Nicolaas A. Bos[3], Christiane Donus[4] and Philip D. Hardt[4]

Obesity (2009) **18,** 190–195. doi:10.1038/oby.2009.167



ILSI Europe

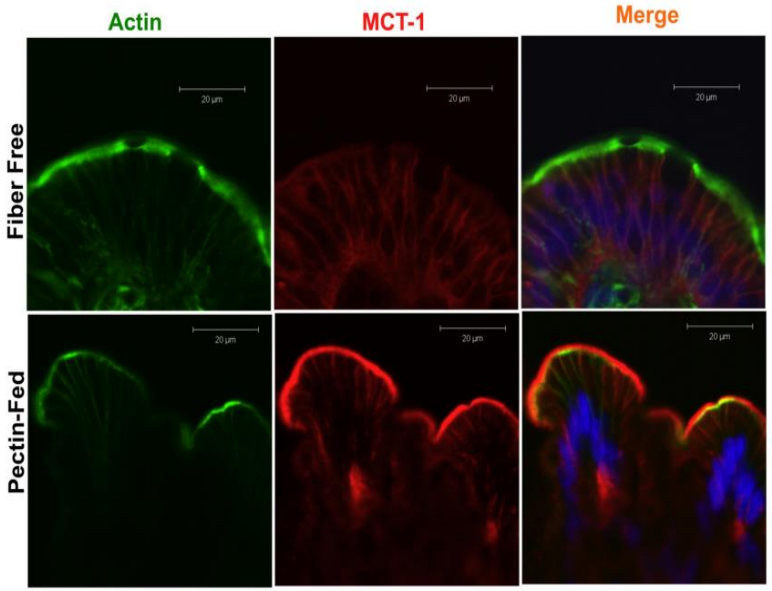
Faecal [SCFA] poor biomarker of gut health

Increased faecal SCFA excretion due to decreased SCFA uptake?

MCT transporters expression and apical location is promoted by luminal SCFA

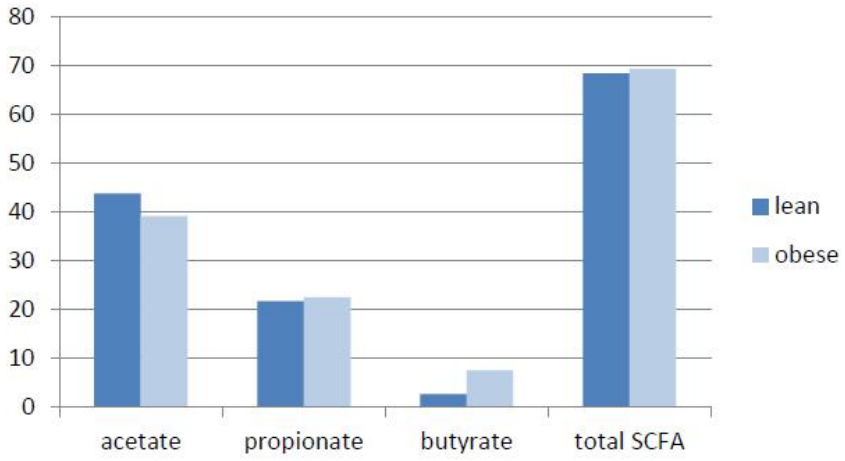
Increased faecal SCFA excretion could be due to decreased MCT active uptake in high fat/low CHO diets

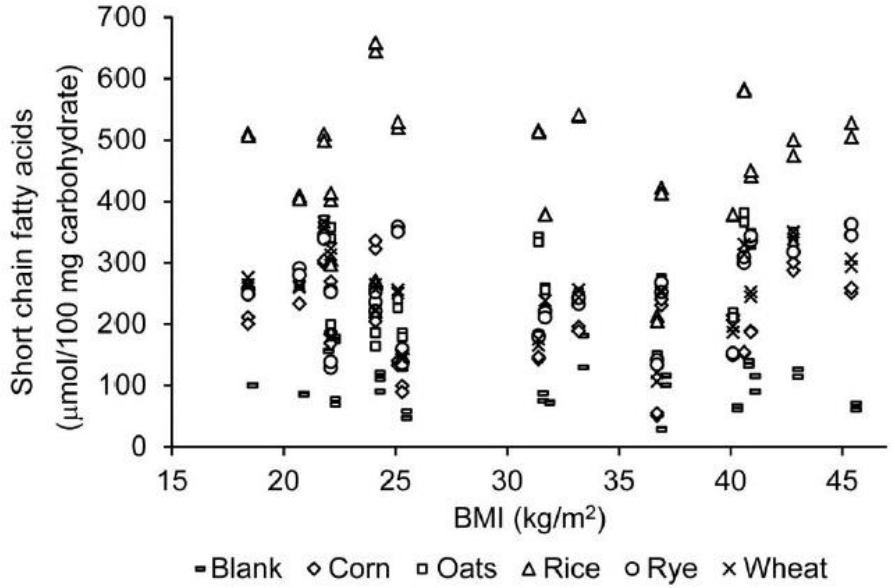
Bile salt CDCA and *E. coli* EPEC inhibit butyrate uptake



Goncalves et al. J Cell Biochemistry 2012, 113, 2937-2947
Borthakur et al. Am J Physiol Gastrointest Liver Physiol 2012, 303, G1126-G1133

Little difference in SCFA production *in vitro* between lean and obese microbiota





ILSI Europe

Sarbini et al. Br J Nutr 2013, 109, 1980-1989
Yang et al. J Med Food 2013, 16, 862-867

Faecal [SCFA] in disease states

- IBD: lower levels of faecal SCFA
- celiac disease: increased levels of total SCFA and acetate
- allergy: lower faecal levels of propionate and butyrate

⇨ causative to disease?

⇨ markers of disease?

ILSI Europe

Huda-Faujan et al. Open Biochem 2010, 4, 53-58
Treem et al. J Pediatr Gastroenterol Nutr 2012, 18, 159-164
Tjellstrom et al. Scand J Gastroenterol 2012 47, 1454-1459
Tjellstrom et al. Microb Ecol Health Dis 2013, 24
Tjellstrom et al. Scand J Gastroenterol 2010, 45, 1059-1062
Tjellstrom et al. Am J Gastroenterol 2005, 100, 2784-2788
Bottcher et al. Clin Exp Allergy 2000, 30, 1590-1596

SCFA may beneficially impact on the following mammalian processes

- Apoptosis
- Cellular proliferation in non-cancer cells
- Inflammation
- Recruitment of immune cells to intestine
- Neutraphil activity/oxidative burst
- Adipose tissue – adipokine production, fat storage
- Tight junction control
- Expression of incretins/gut peptides, and regulation of food intake
- Intestinal motility
- Cholesterol production/lipogenesis
- Glucogenesis
- Cellular energy metabolism
- Thermogenesis
- Inhibits tumorigenesis
- DNA miss-match repair

Note: most mechanistic data from in vitro or animal studies – rodents, pigs, chickens

ILSI Europe

Faecal minor organic acids

- Lactate, succinate
  - do not accumulate much in faeces in health
  - cross-feeding between bacteria leads to formation of main SCFA
  - Lactate:
    - considered as a marker of dysbiosis
    - cosubstrate for sulphate reducing bacteria ⇨ promote sulphide generation
  - Succinate:
    - may act as a signal of inflammation
    - Increased levels have been linked to inflammatory bowel diseases (IBD)

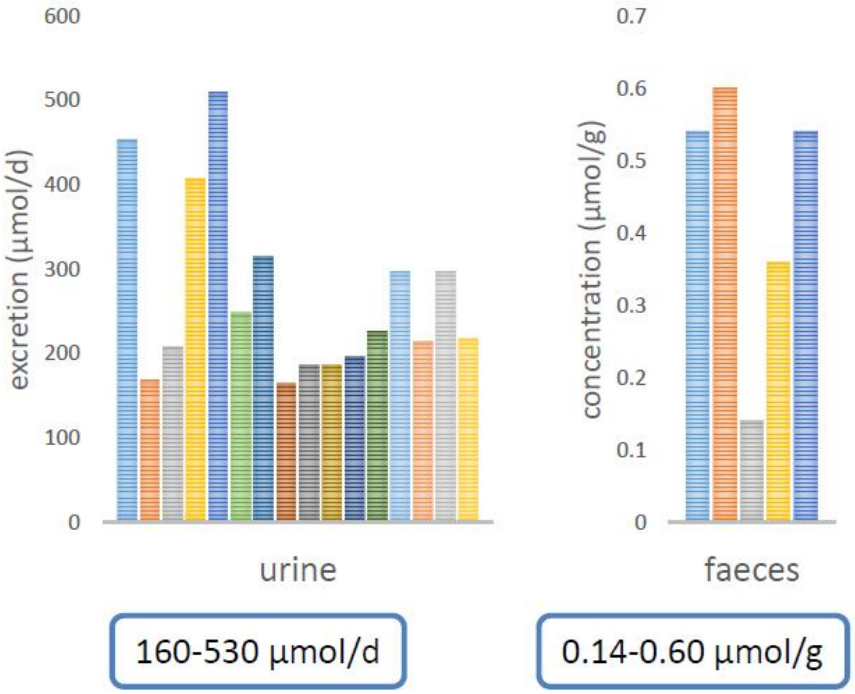
ILSI Europe

Metabolites of protein fermentation

Metabolite	Origin
Ammonia	• Bacterial degradation of amino acids • Hydrolysis of urea
Phenols	• Major metabolites of bacterial fermentation of aromatic amino acids • Rapidly absorbed by colonic mucosa and excreted in urine • Do not accumulate in healthy subjects
• p-cresol	< tyrosine
• phenol	< phenylalanine
• indole	< tryptophan

ILSI Europe

Urinary and faecal levels of p-cresol

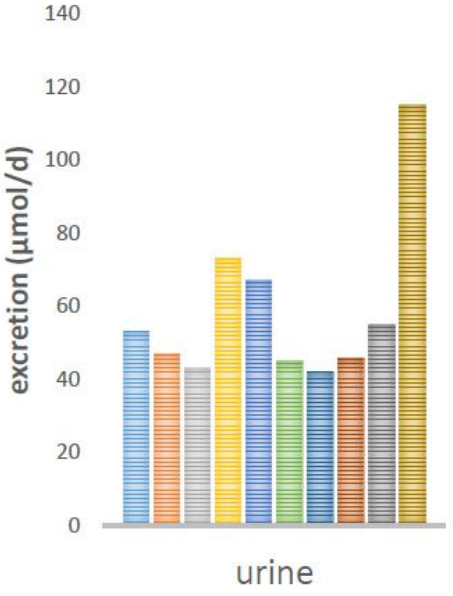


- Higher urine levels in obese than in normal weight subjects
- Urine p-cresol levels increase in very old subjects

Birkett *et al*. Am J Clin Nutr 1996, 63, 766-772
Damen *et al*. J Nutr 2012, 142, 470-477
Ling *et al*. J Nutr 1992, 122, 924-930
Patel *et al*. Clin J Am Soc Nephrol 2012, 7, 982-988
Renwick *et al*. Hum Toxicol 1988, 7, 267-272
De Preter *et al*. Br J Nutr 2004, 92, 439-446
De Preter *et al*. J Am Coll Nutr 2007, 25, 541-549
De Preter *et al*. Am J Physiol Gastrointest Liver Physiol 2007, 292, G358-G368
Cloetens *et al*. Br J Nutr 2010, 103, 703-713
Cloetens *et al*. J Am Coll Nutr 2008, 27, 512-518
Windey *et al*. PlosOne 2012, 7, Article Number: e52387
Gostner *et al*. Br J Nutr 2006, 95, 40
Adams *et al*. Lancet 1985, 2, 1313-1313
Heavey *et al*. Br J Nutr 2003, 89, 509-515

ILSI Europe

Urinary and faecal levels of phenol



40-115 µmol/d

0.04-0.7 µmol/g

Birkett *et al*. Am J Clin Nutr 1996, 63, 766-772
Damen *et al*. J Nutr 2012, 142, 470-477
Ling *et al*. J Nutr 1992, 122, 924-930
Renwick *et al*. Hum Toxicol 1988, 7, 267-272
De Preter *et al*. J Am Coll Nutr 2007, 25, 541-549
Cloetens *et al*. Br J Nutr 2010, 103, 703-713
Gostner *et al*. Br J Nutr 2006, 95, 40
Adams *et al*. Lancet 1985, 2, 1313-1313
Heavey *et al*. Br J Nutr 2003, 89, 509-515
Clarke *et al*. Am J Clin Nutr 2011, 94, 1276-1283

ILSI Europe

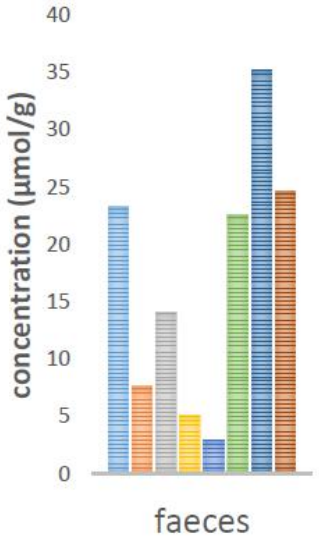
Effects of phenolic derivatives of protein fermentation

- Effects on epithelial cells mainly determined using *in vitro* incubation tests
  - Decreased viability
  - Reduced epithelial barrier function
- No systemic toxicity in healthy subjects
- Accumulates in serum in chronic kidney disease - uremic toxin
  - Contributes to endothelial disfunction
- Very limited data on toxicity of indole

ILSI Europe

Pedersen *et al*. Scand J Gastroenterol 2002, 37, 74-79
Mccall *et al*. Toxicol Appl Pharmacol 2009, 241, 61-70
Hughes *et al*. Nutr Cancer-An Int J 2008, 60, 259-266
Meijers *et al*. Am J Kidney Dis 2009, 54, 891-901

Faecal [ammonia]



3-35 µmol/g

- Comparable levels in overweight and normal weight subjects
- Higher levels in children with autism spectrum disorders (42 µmol/g)

Birkett *et al*. Am J Clin Nutr 1996, 63, 766-772
Heavey *et al*. Br J Nutr 2003, 89, 509-515
Clarke *et al*. Am J Clin Nutr 2011, 94, 1276-1283
Slavin *et al*. Food & Function 2010, 2, 72-77
Bianchi *et al*. Br J Nutr 2010, 104, 1500-1507
Tiihonen *et al*. Br J Nutr 2010, 103, 1070-1078
Nemoto *et al*. Dig Dis Scie 2012, 57, 2955-2964
Shinohara *et al*. Anaerobe 2010, 16, 510-515

ILSI Europe

ILSI Europe

Effects of intestinal ammonia

- Effects on epithelial cells:
    - alters nucleic acid synthesis
    - changes the morphology and intermediary metabolism of intestinal cells
    - reduces the lifespan of cells

- No adverse effects upon oral admintration

- Hepatic encephalopathy

ILSI Europe

Visek. Am J Clin Nutr 1978, 31, S216-S220
Blachier et al. Amino Acids 2007, 33, 547-562

Table 6. List of microbial catabolites of common plant polyphenols and their putative health effects[155]

Plant polyphenol	Microbial catabolite	Possible health effects	References
(−)-Epicatechin	4-Hydroxyphenylacetic acid	Antimicrobial/antimycotic activity *in vitro*	Alakomi (2007)[254] Ko (2009)[255] Roowi (2010)[256]
	3-(3-Hydroxyphenyl)propionic acid	Antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilisation	
	5-(3,4-Dihydroxyphenyl)-γ-valeric acid	?	
	(-)-5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	?	
(−)-Epigallocatechin	4-Hydroxyphenylacetic acid	Antimicrobial/antimycotic activity *in vitro*	Roowi (2010)[256]
	(-)-5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone		
(−)-Epigallocatechin-3-*O*-gallate	Pyrocatechol		Ko (2009)[255] Roowi (2010)[256] Okello (2012)[257] Ni (2008)[258] Taguri (2006)[259]
	Pyrogallol	Antibacterial activity (especially against Gram-negative enterobacteria) An acetylcholinesterase inhibition greater than gallic acid parent Inhibition of *Vibrio* spp. quorum sensing	
	4-Hydroxyphenylacetic acid	Antimicrobial/antimycotic activity *in vitro*	
	(-)-5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	?	
Daidzein	Equol	Phyto-oeotrogen important for heart and bone health, and possible colon cancer protectants	Jackman (2007)[260] Ishimi (2009)[261] Davis (2009)[262] Selma (2009)[263]
Daidzein	*O*-demethylangolensin	Oestrogenic and/or anti-oestrogenic activity	Larrosa (2006)[264] Selma (2009)[263]
Quercetin	2-(3,4-Dihydroxyphenyl)acetic acid		Selma (2009)[263]
	2-3-(3-Hydroxyphenyl)acetic acid		
	3,4-Dihydroxybenzoic acid		
	Phloroglucinol		
	3-(3,4-Dihydroxyphenyl)propionic acid		
	3-(3-Hydroxyphenyl)propionic acid		
Kaempferol	2-(4-Hydroxyphenyl)acetic acid		Selma (2009)[263]
Naringenin	3-(4-Hydroxyphenyl)propionic acid	Antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilisation	Selma (2009)[263]
	Phloroglucinol		
Isoxanthohumol	8-Prenylnaringenin	?	Selma (2009)[263]
Catechin and epicatechin	3-(3-Hydroxyphenyl)propionic acid		Alakomi (2007)[254] Selma (2009)[263]
	5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone		
	5-(3′-Hydroxyphenyl)-γ-valerolactone		
	3-Hydroxyhippuric acid pyrogallol		
	5-(3,4-Dihydroxyphenyl)valeric acid		
	5-(3-Hydroxyohenyl)valeric acid		
	3-(3,4-Dihydroxyphenyl)propionic acid	Antimicrobial activity against Gram-negative enterobacteria via outer membrane destabilisation	
	5-(3-Methoxyohenyl)valeric acid		
	3-(3,4-Dihydroxyphenyl)propionic acid		
	5-(3-Methoxyohenyl)valeric acid		
	2,3-Dihydroxyphenoxyl 3-(3′,4′-dihydroxyphenyl) propionic acid		

Possible solution

A more holistic approach

ILSI Europe

Functional analysis of faecal water

- Functional analysis of faecal water provides an integrated measure of the overall contribution of the compounds present to a defined biological endpoint.

- Provides no information on compounds responsible for the functional effect

ILSI Europe

Available assays

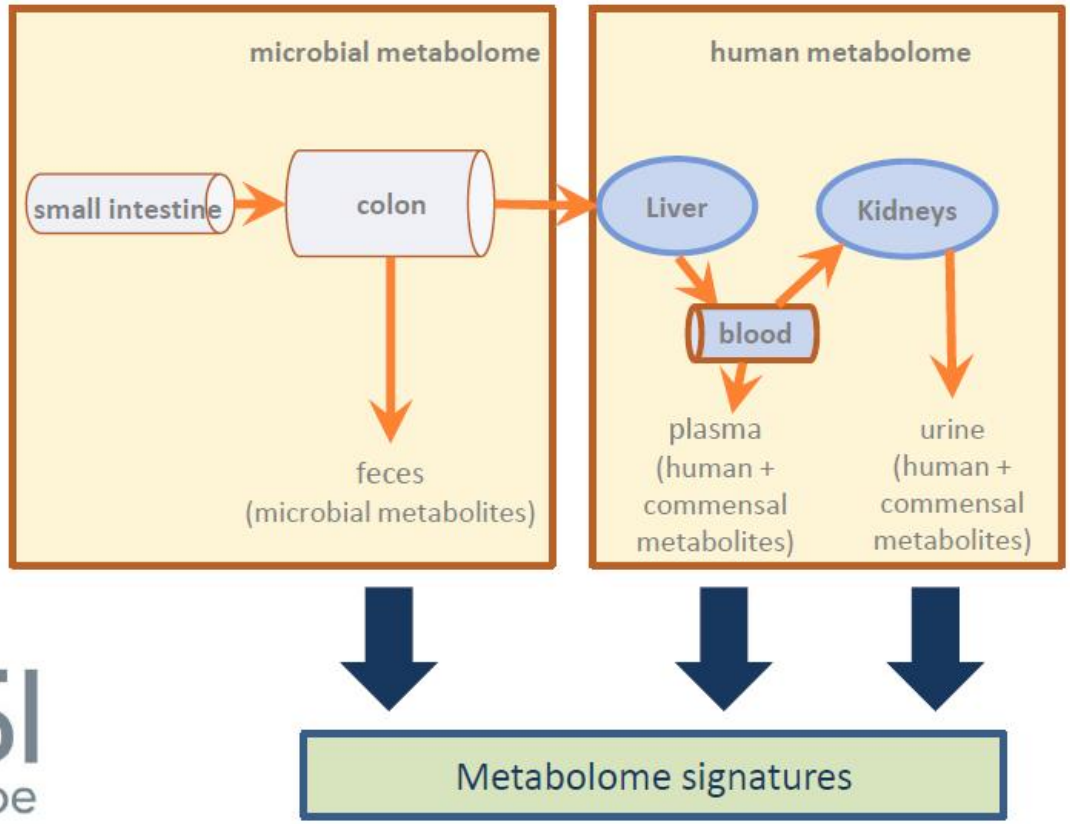
Endpoint	Assay
Genotoxicity	Bacterial mutagenicity
	Comet assay in mammalian cells
Cell toxicity	Cytotoxicity
	Barrier function
	Invasive potential
	Red cell lysis
Cell proliferation	Cell number
	Cell cycle analysis
Immune modulation	Expression of inflammatory markers
Gene expression	AP-1, COX-2,

- Limitation: lack of standardisation in target cells and sample preparation
- Few studies applied the approach to evaluate prebiotic effects

ILSI Europe

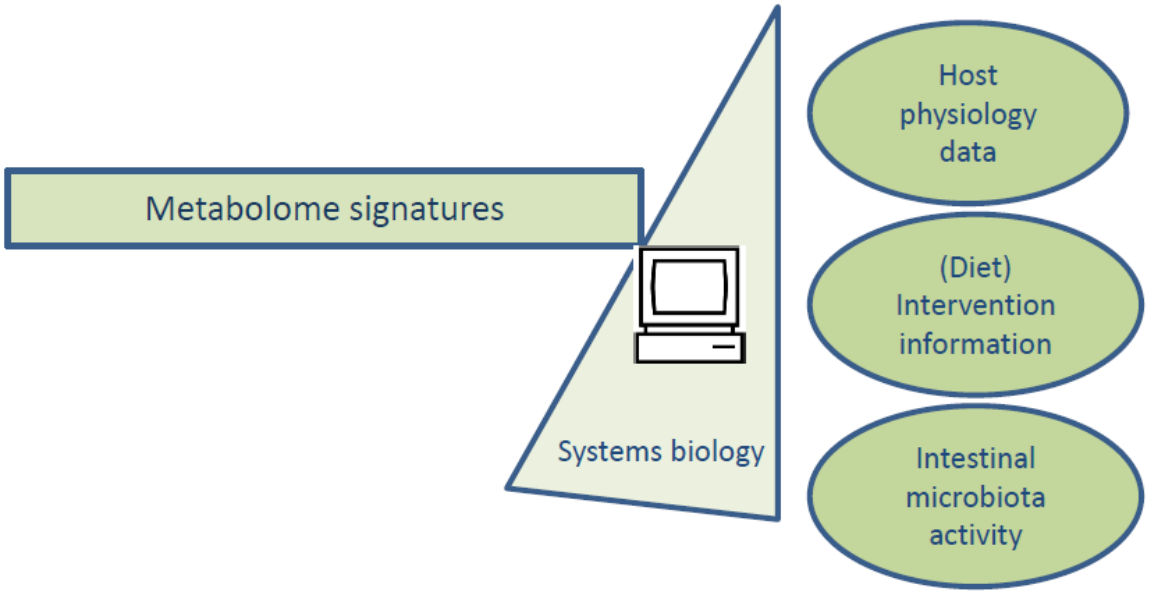
Metabolomics

- simultaneous monitoring of changes in a wide range of metabolites
  ⇨ No need for an *a priori* hypothesis
- ^{1}H-NMR, LC-MS, GC-MS
- matrix: feces, urine, plasma, tissue homogenates



ILSI Europe

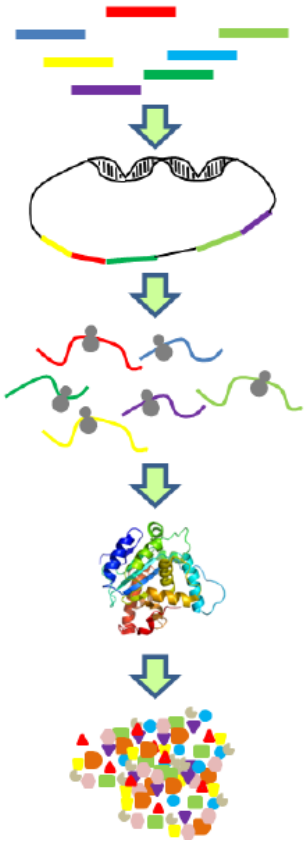
Applicability of systems biology



- ⇨ Metabolome signatures are analysed in the context of overall biochemistry of the tissue or sample
- ⇨ "Biochemical connectivity"
- ⇨ Enables to analyse the overall impact of the microbiota on host-biochemistry and -function

ILSI Europe

Tools for stools!



Quantified microbiota species (OTU) composition (16S rRNA based)

Quantified microbiota function profile (metagenome)

Quantified microbiota gene expression profile (metatranscriptome)

Quantified microbiota protein expression profile profile (metaproteome)

Quantified microbiota metabolite profiles (metametabolome)

Availability of methods

✓

✓

emerging

emerging

✓

ILSI Europe

Conclusions

- No formal systematic reviews evaluating the physiological or toxicological properties of bacterial fermentation metabolites were found.
- End products of saccharolytic fermentation, SCFA, may have varying effects on colonic health, host physiology, lipoprotein metabolism and appetite.
- Comprehensive reviews and experimental studies indicated that protein fermentation metabolites (phenol, p-cresol, indole, ammonia), typically considered as harmful metabolites, occur at concentration ranges in the colon such that no toxic effects are expected either locally or following systemic absorption.

ILSI Europe

Conclusions

- There is insufficient data published to support the use of any individual bacterial metabolite as faecal biomarker of gut health.

- Way to go:
  - Profiling of metabolites in the context of overall tissue biochemistry
  - correlation of (multivariate) metabolome signatures with microbial, dietary and physiological data

    ⇨evaluation of the overall impact of the microbiota on host health and gut function

- Current limitation
  - the bioinformatics integration and interpretation of the data
  - the lack of studies measuring metabolite fluxes in different body compartments or biofluids to provide an accurate picture of colonic metabolite nutrikinetics.

ILSI Europe

Thank you!

Nutrition Research Reviews

Nutrition Research Reviews (2015), **28**, 42–66 doi:10.1017/S0954422415000037

Towards microbial fermentation metabolites as markers for health benefits of prebiotics

Kristin A. Verbeke[1], Alan R. Boobis[2], Alessandro Chiodini[3]*, Christine A. Edwards[4], Anne Franck[5], Michiel Kleerebezem[6], Arjen Nauta[7], Jeroen Raes[8], Eric A. F. van Tol[9] and Kieran M. Tuohy[10] on behalf of the ILSI Europe Prebiotics Task Force Expert Group 'Microbial metabolism and fermentation'

ILSI Europe

Acknowledgements

Prebiotic Task Force, ILSI Europe