Imperial College London

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NUGO, Wageningen

Using Metabolomics to Quantify Health

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Summary

- Background to technology and adaptations for modelling human data
- Characterizing metabolic consequences of food interventions
- Considering the mammalian ecosystem and complexity
- Defining biomarkers of health
- Future directions of metabolic phenotyping within the nutritional arena

Metabonomic Analysis Strategies

GC-MS

MS Strategy

Biofluids and Extracts

NMR

UPLC-MS

GC-MS

LC-MS^n

LC-MS^n-NMR

Other stuff

C

E

Biofluids

NMR Strategy ^1H, ^{13}C, ^{31}P

2 D methods

LC-NMR Etc.

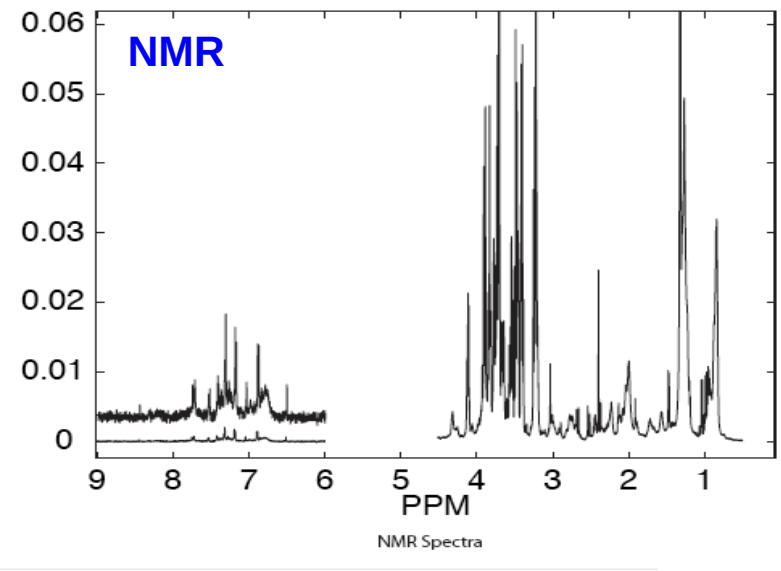
Diffusion analysis

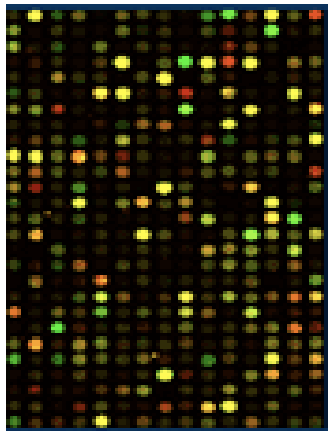
Intact Tissues (MAS)

Metabolite Identification/ Quantitation and Interactions

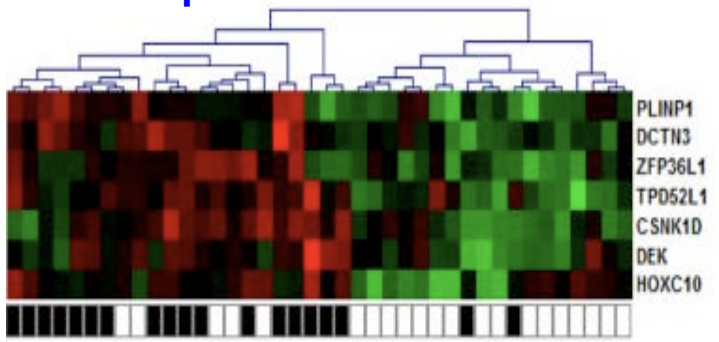
Chemometrics and Disease Modelling

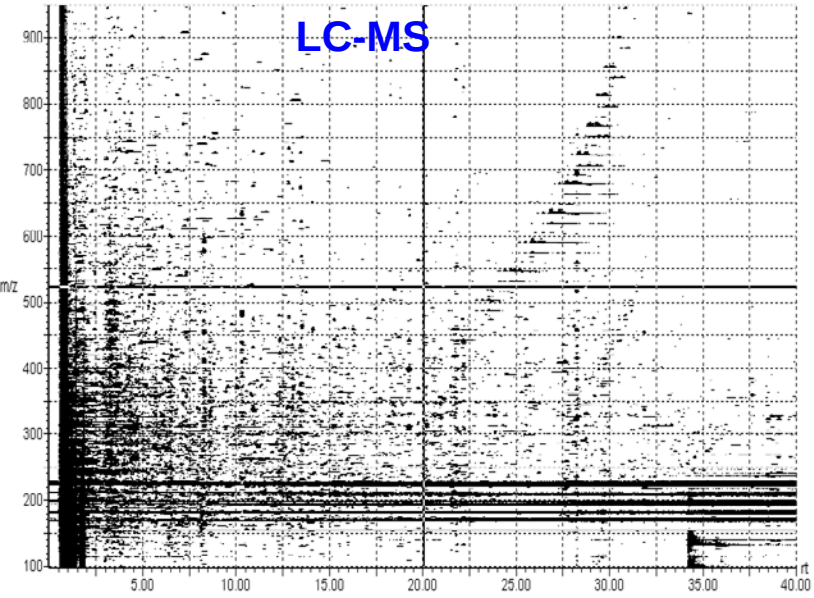
Omics data tend to be highly multivariate, may not be annotated completely, can have missing values, usually require aligning and standardizing in multiple dimensions and are all structured differently



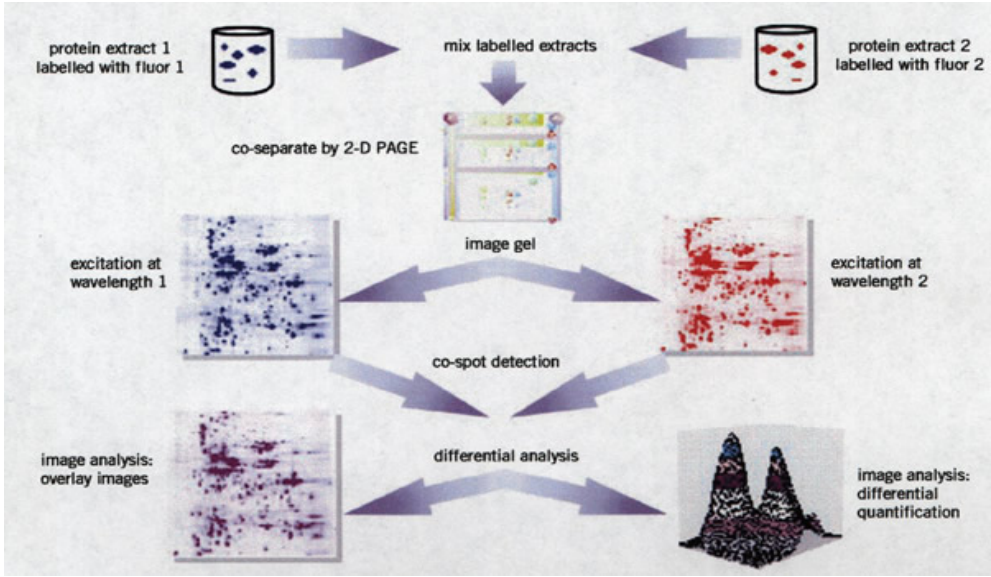


transcriptomic

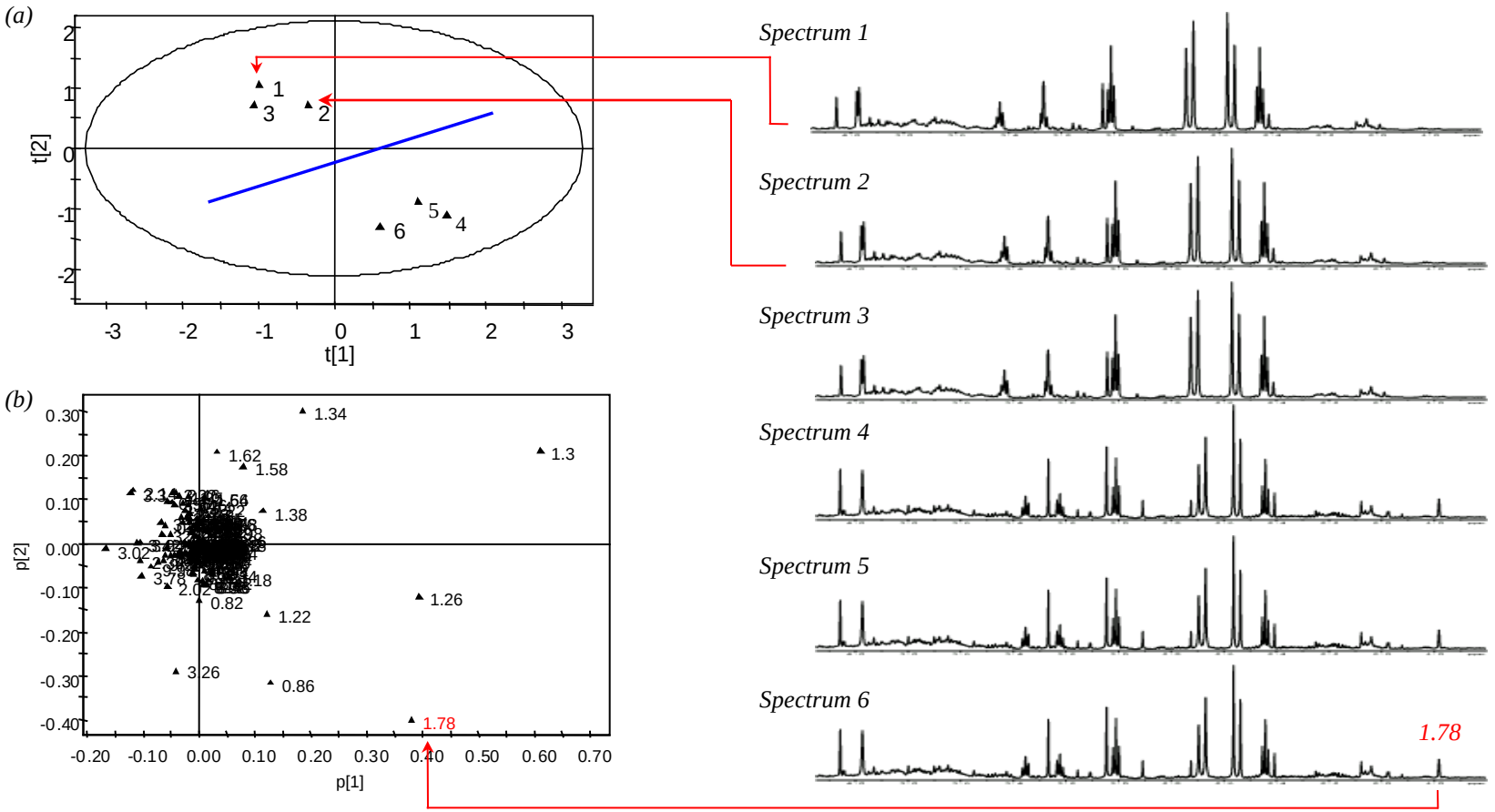




2D gel electrophoresis

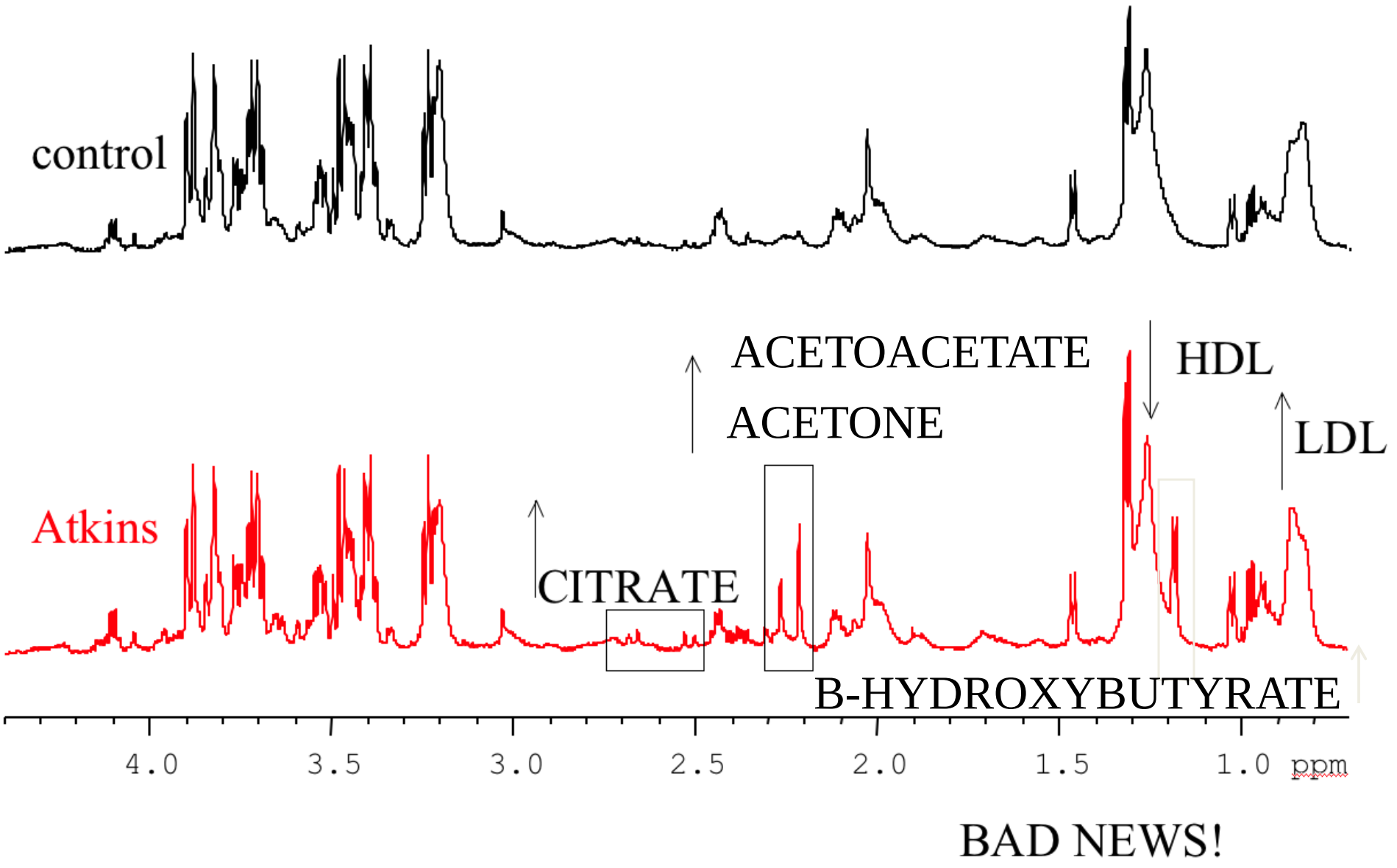


Example of PCA Analysis



Spectra → scores → loadings → spectra

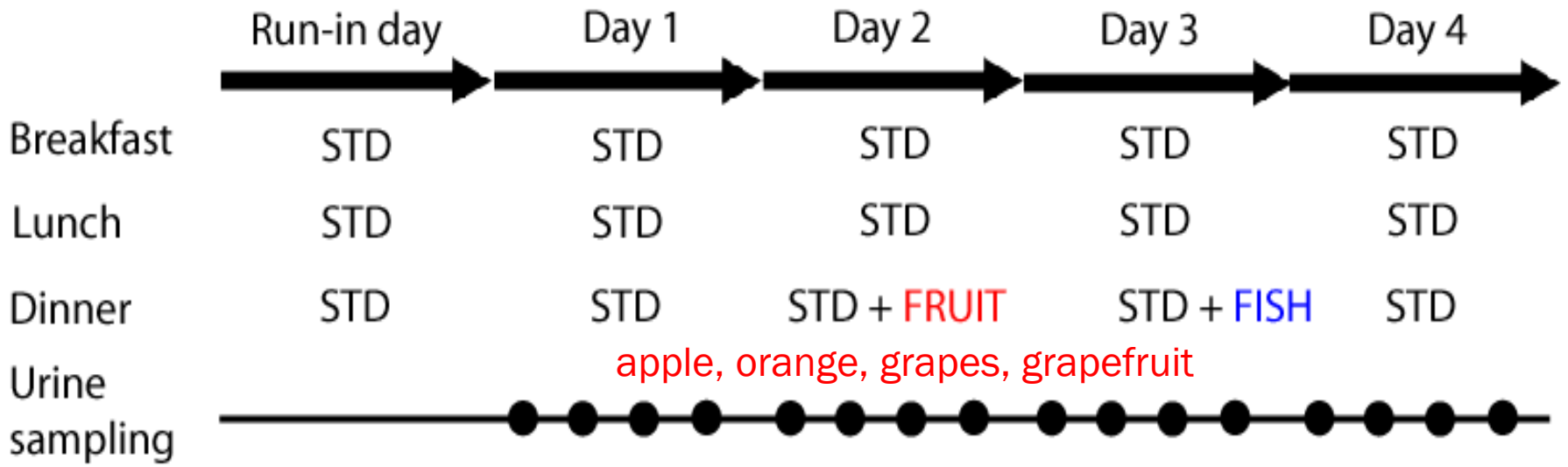
BLOOD PLASMA ANALYSIS FROM PATIENT BEFORE AND AFTER ATKINS DIET (6 WEEKS):



BAD NEWS!

A food intervention study to find food biomarkers *via* metabolic profiling.

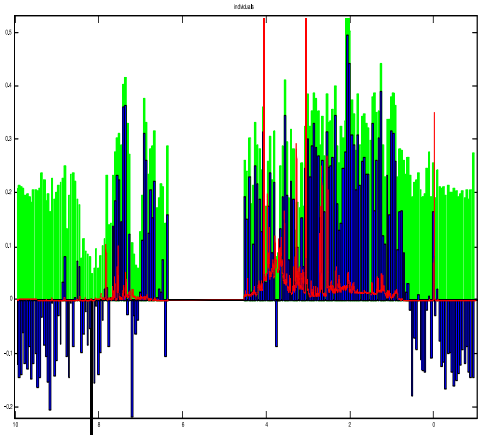
Compliance; quantitative intake; product quality / differences

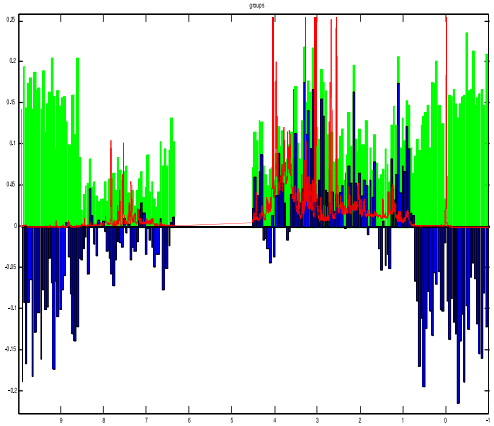


NOT ALL PARTS OF THE SPECTRA YIELD EQUAL INFORMATION AND DIFFERENT SPECTRAL AREAS REFLECT DIFFERENT SOURCES OF VARIANCE TO DIFFERENT DEGREES.

Inter-individual variation

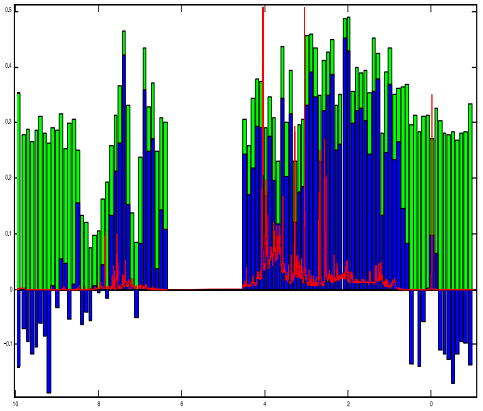
Diet-related variation

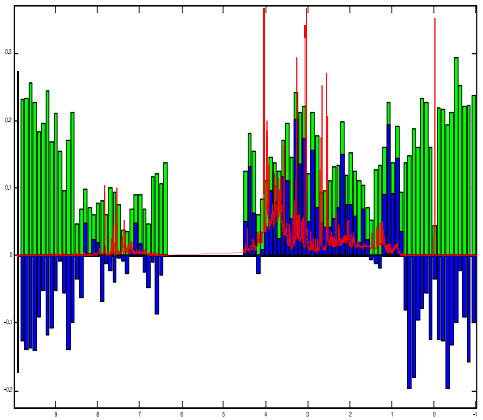




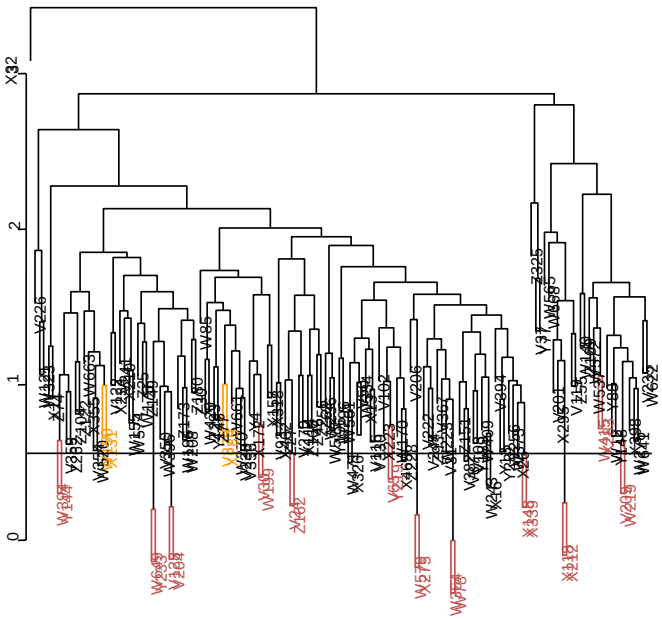
0.05 ppm

- Variance explained (R^2)
- Variance predicted (Q^2)





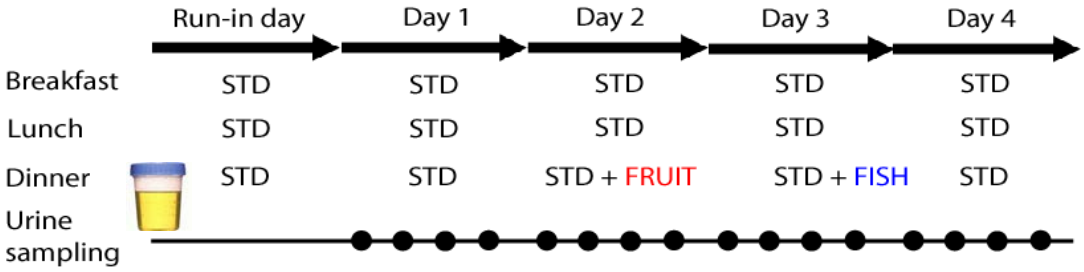
0.1 ppm



similarity dendrogram

Silke Heinzmann / Marc Dumas

Elucidation of potential food biomarkers from metabolic profiles with pattern recognition



Are biomarkers of healthy food intake equivalent to biomarkers of health?
ACUTE VS CHRONIC

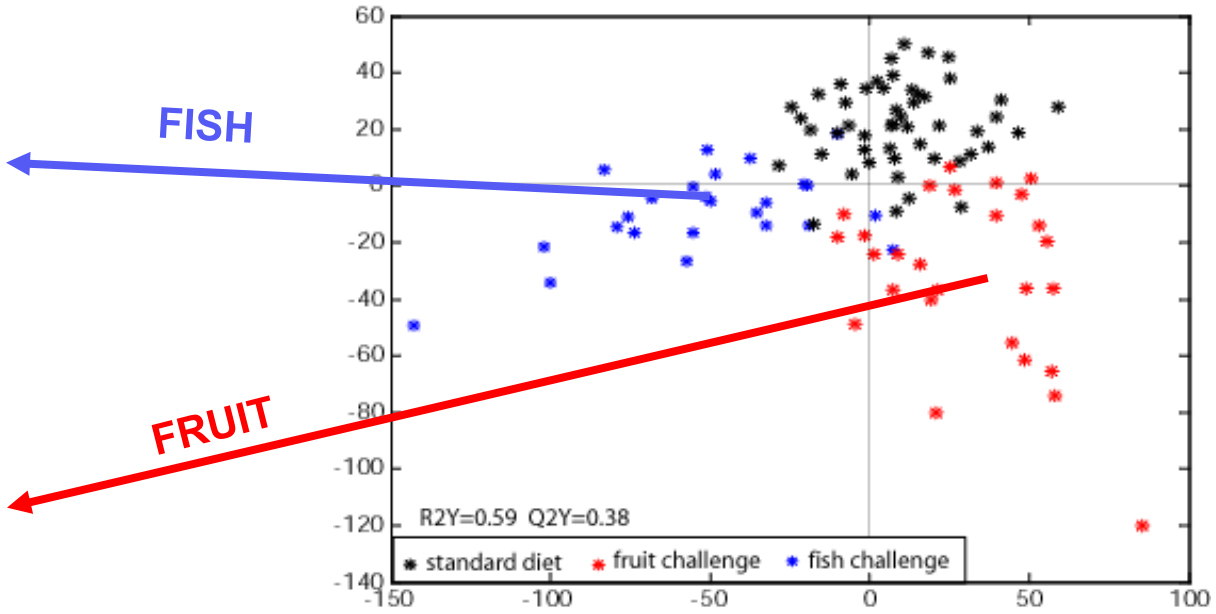
Increased excretion of:

- trimethylamine-*N*-oxide
- taurine
- methylhistidine
- creatine
- choline
- carnitine

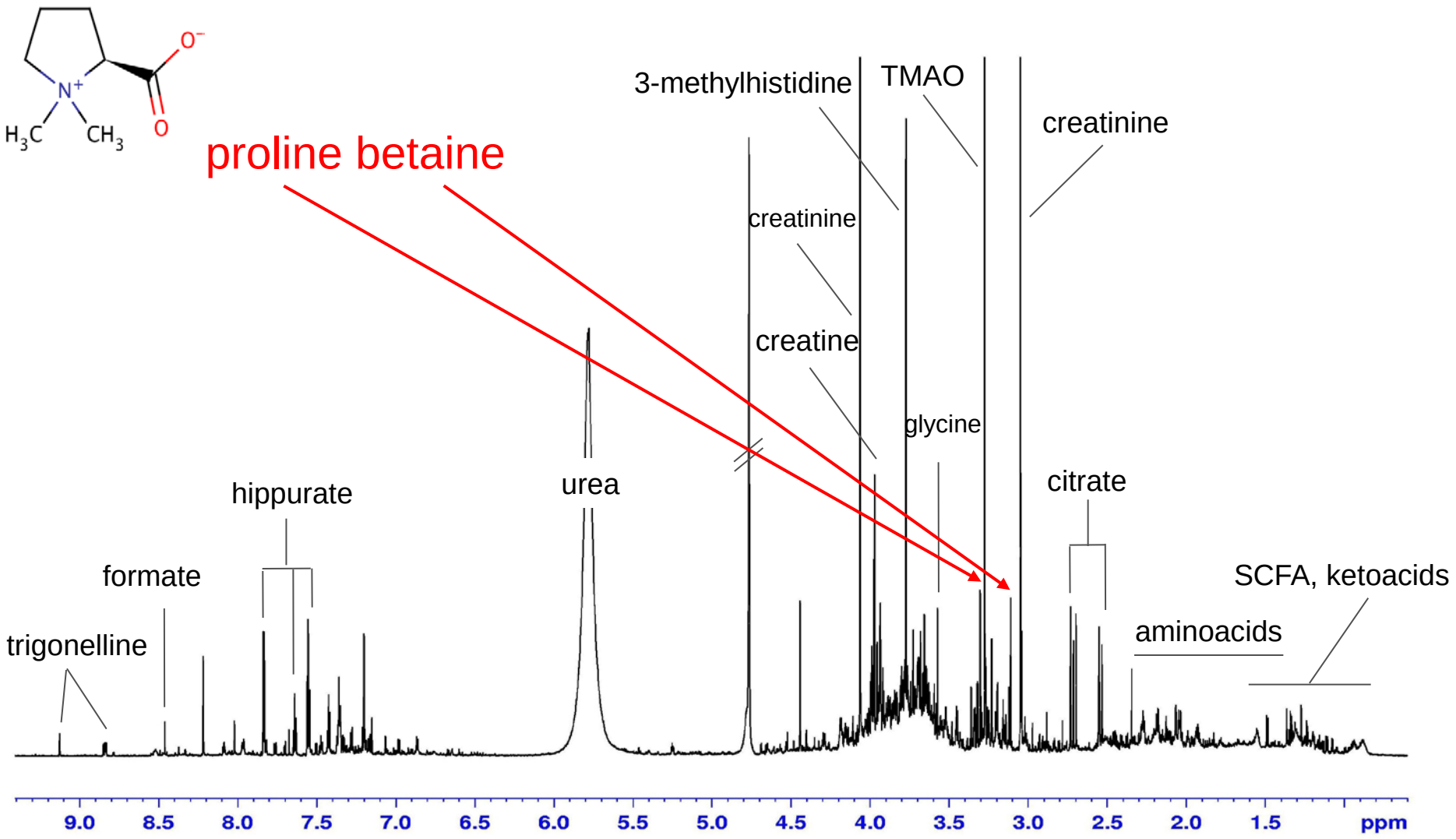
Increased excretion of:

- **proline betaine r=0.71**
- tartaric acid
- hippuric acid

Partial least squares discriminant analysis



Features of a good food biomarker: Detectable (relatively high concentration, low probability of overlap, more than one spectral feature); Long half life; not multiply metabolized



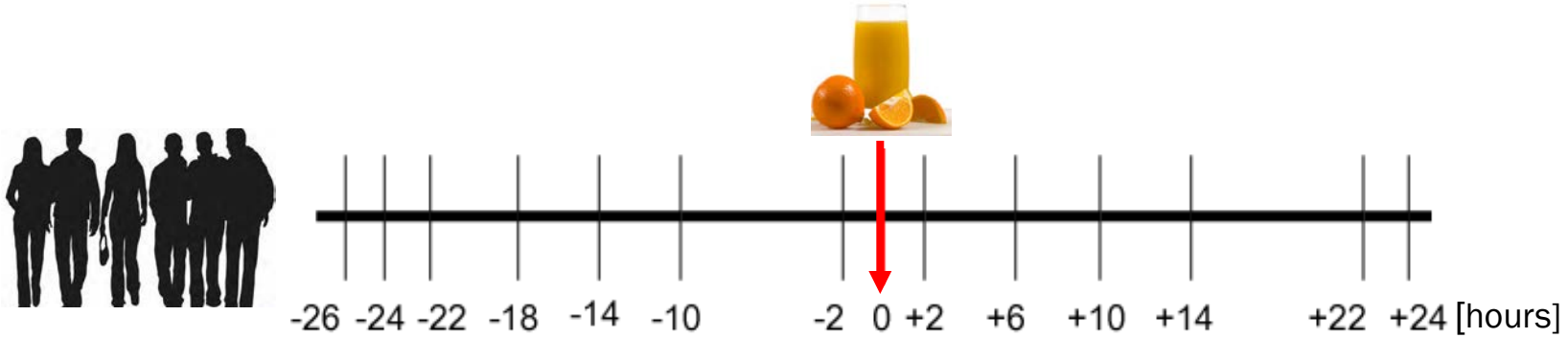
^{1}H NMR analysis to quantify proline betaine in fruit juices and fruits

Analysis of different fruits and fruit juices in a fully relaxed ^{1}H NMR experiment

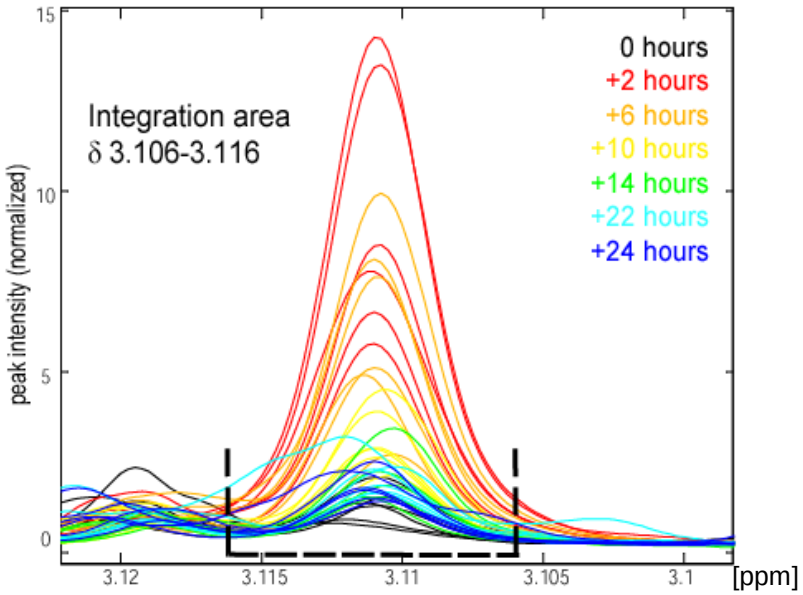
Juice / fruit description	Proline betaine (mg/L)
Citrus fruit juice (authentic)	
Orange juice, from concentrate	1316
Orange juice	1189
Orange juice, freshly squeezed	1062
Grapefruit juice	766
Citrus fruit juice (synthetic)	
Orange soft drink	216
Orange squash (20% orange juice from concentrate)	201
Orange squash	75
Citrus fruit	
Orange	786
Lime	651
Satsuma	486
Lemon	240

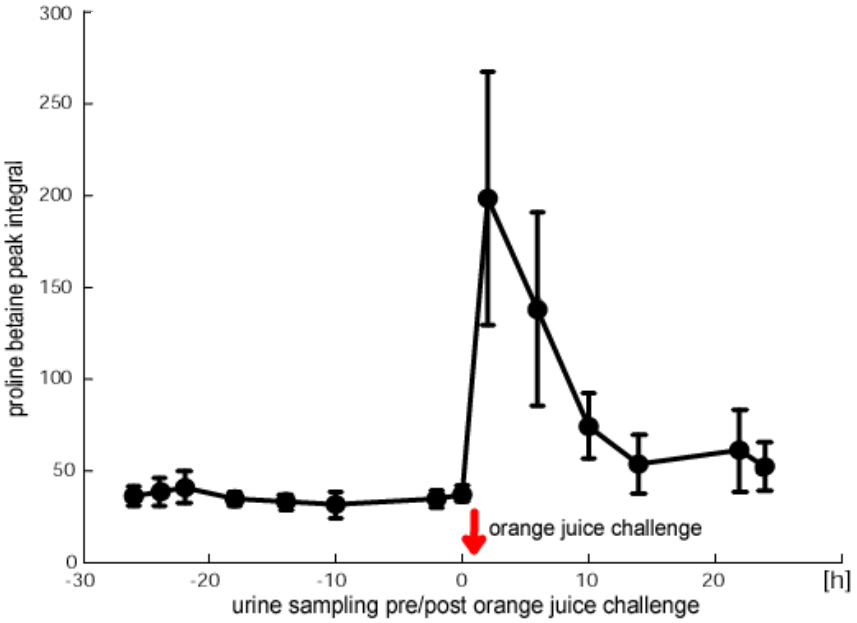
Juice / fruit description	Proline betaine (mg/L)
Other fruit juice	
Pineapple juice	57
Red grape and raspberry juice	46
Pomegranate and blueberry juice	18
Peach, mango, passionfruit juice	17
Apple juice	14
Blackcurrant juice	12
Other fruit	
Kiwi	66
Grape	51
Melon	34
Banana	28
Strawberry	22
Pear	14
Apricot	10

Proline betaine excretion in individual volunteers



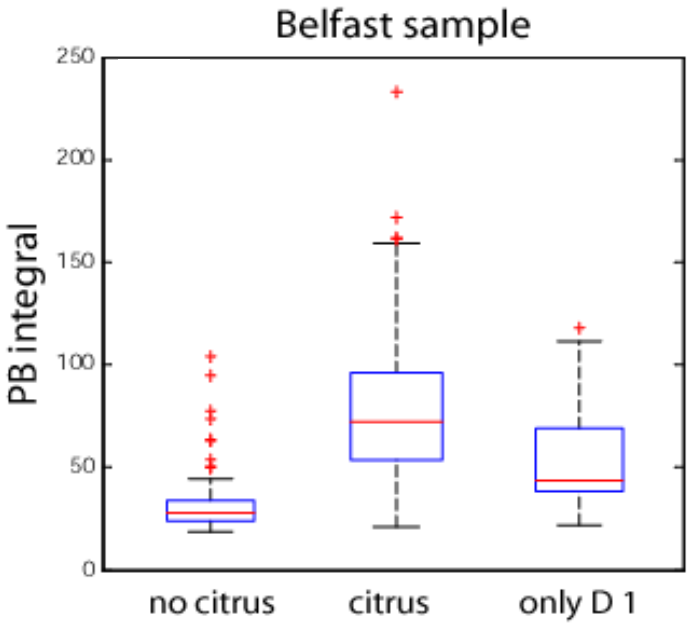
Kinetics study (N=6): orange juice consumption and urine sampling

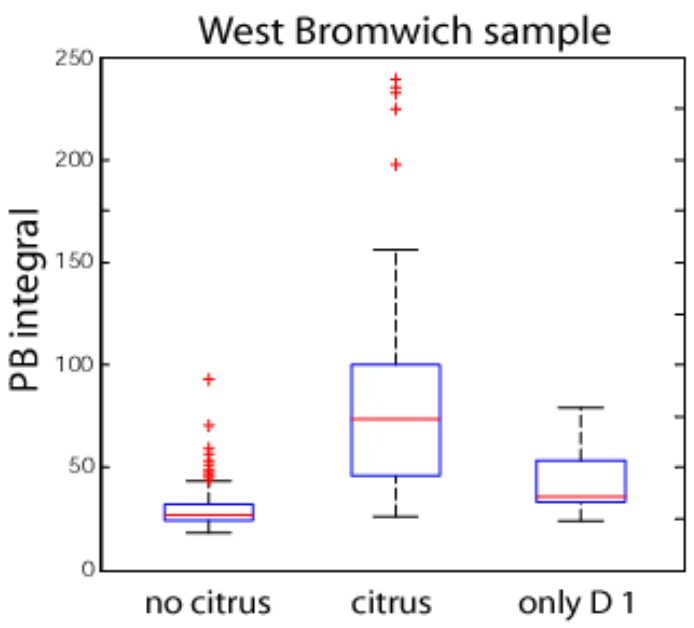




Establishing the relationship between proline betaine excretion and citrus consumption in population studies: Validation of biomarkers

- Quantify proline betaine concentrations in 24-hr urine samples
- Ascertain whether the recorded intake of citrus fruit matches the dietary questionnaires data

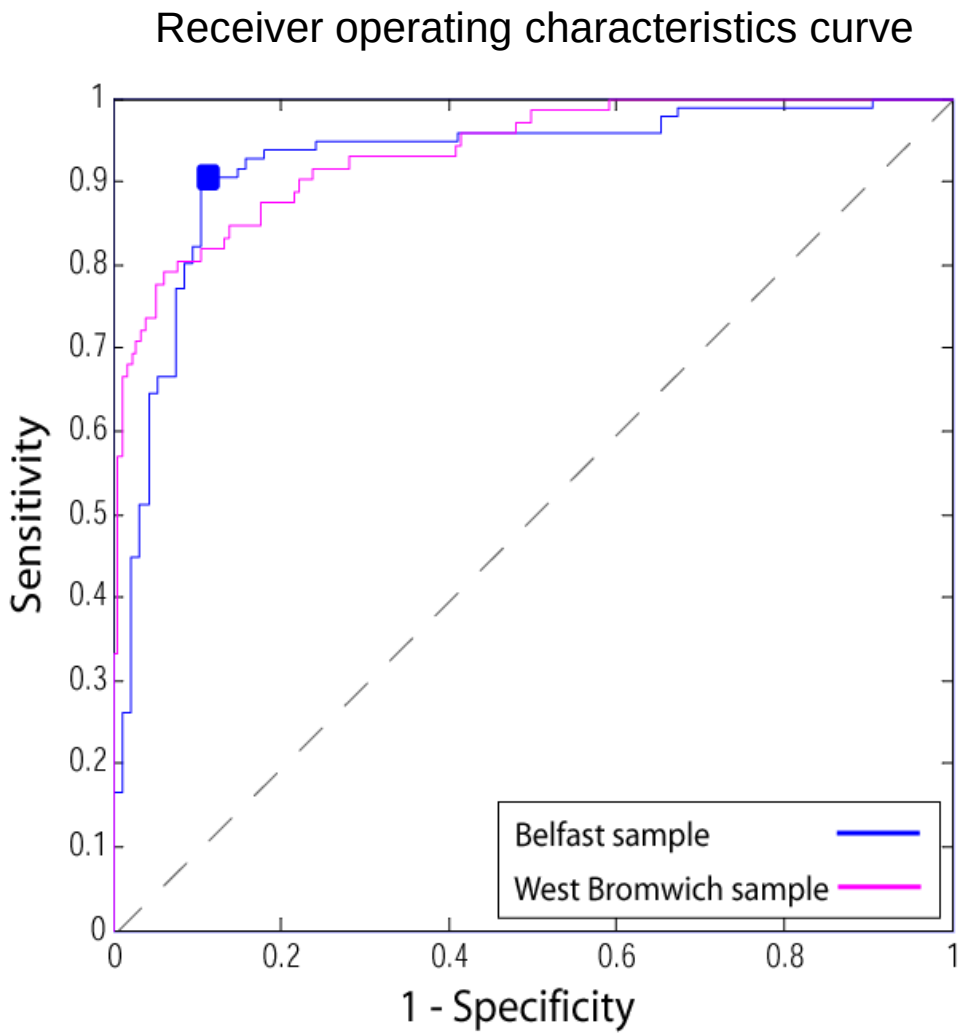




p-value = 1.2×10^{-24}

p-value = 8.8×10^{-35}

Good predictivity of the biomarker in a free-living population



Training Set:

AUC: 92.3%

90.6% specificity

86.2% sensitivity

Test Set:

AUC: 93.5%

92.3% specificity

80.6% sensitivity

Significant differences in nutrient intake of citrus consumers and non-consumers

Variable		*P value*
Energy, kcal/24-h	0.8%	*n.s.*
Total fat, % kcal	-9.4%	*<0.0001*
Total SFA, % kcal	-10.1%	*<0.0001*
Total MFA, % kcal	-9.9%	*<0.0001*
Total PFA, % kcal	-10.1%	*<0.0001*
Omega-3 FA, % kcal	-4.0%	*0.01*
Omega-6 FA, % kcal	-11.3%	*<0.0001*
Trans FA, % kcal	-11.8%	*<0.0001*
Cholesterol, mg/1,000 kcal	-11.0%	*<0.0001*
Keys Score‡	-8.1%	*<0.0001*
Total carbohydrate, % kcal	8.1%	*<0.0001*
Starch, % kcal	0.0%	*0.95*
Total sugars, % kcal	16.7%	*<0.0001*
Fructose, % kcal	44.4%	*<0.0001*
Galactose, % kcal	20.0%	*<0.001*
Glucose, % kcal	39.5%	*<0.0001*
Lactose, % kcal	3.7%	*n.s.*
Maltose, % kcal	-12.5%	*n.s.*
Sucrose, % kcal	2.0%	*n.s.*
Fibre, g/1,000 kcal	11.9%	*<0.0001*

lipids

carbohydrates

Variable		*P value*
Total protein, % kcal	-1.3%	*n.s.*
Vegetable protein, % kcal	7.4%	*<0.0001*
Animal protein, % kcal	-4.9%	*<0.001*
Vitamin A, IU/1,000 kcal	29.3%	*<0.0001*
Beta-carotene, µg/1,000 kcal	35.5%	*<0.0001*
Retinol, µg/1,000 kcal	9.1%	*n.s.*
Thiamine, mg/1,000 kcal	8.3%	*<0.0001*
Riboflavin, mg/1,000 kcal	4.5%	*<0.01*
Niacin, mg/1,000 kcal	1.8%	*n.s.*
Pantoth. acid, mg/1,000 kcal	8.3%	*<0.0001*
Vitamin B6, mg/1,000 kcal	11.0%	*<0.0001*
Vitamin B12, µg/1,000 kcal	0.0%	*n.s.*
Vitamin C, mg/1,000 kcal	126.4%	*<0.0001*
Vitamin E, mg/1,000 kcal	-2.2%	*n.s.*
Folic acid, µg/1,000 kcal	18.2%	*<0.0001*

protein

vitamins

Variable		*P value*
Urinary sodium, mmol/24-h	-3.2%	*0.05*
Urinary potassium, mmol/24-h	16.9%	*<0.0001*
Magnesium, mg/1,000 kcal	8.8%	*<0.0001*
Calcium, mg/1,000 kcal	4.6%	*<0.01*
Phosphorus, mg/1,000 kcal	2.1%	*<0.05*
Iron, mg/1,000 kcal	7.4%	*<0.0001*
Copper, mg/1,000 kcal	11.1%	*<0.0001*
Selenium, µg/1,000 kcal	-1.5%	*n.s.*
14-day alcohol, g/24-h	-10.8%	*n.s.*
Caffeine, mg/1,000 kcal	-29.7%	*<0.0001*
Body mass index, kg/m^2	-3.8%	*<0.0001*
Systolic BP, mm Hg	-1.4%	*<0.01*
Diastolic BP, mm Hg	0.3%	*n.s.*
Education, years	9.0%	*<0.0001*

minerals

Proline betaine excretion is correlated with:

Healthy eating patterns
Low BMI & BP

Non-consumers > consumers (stat. sign.)

Consumers > non-consumers (stat. sign.)

Not significant

The INTERMAP Population Study

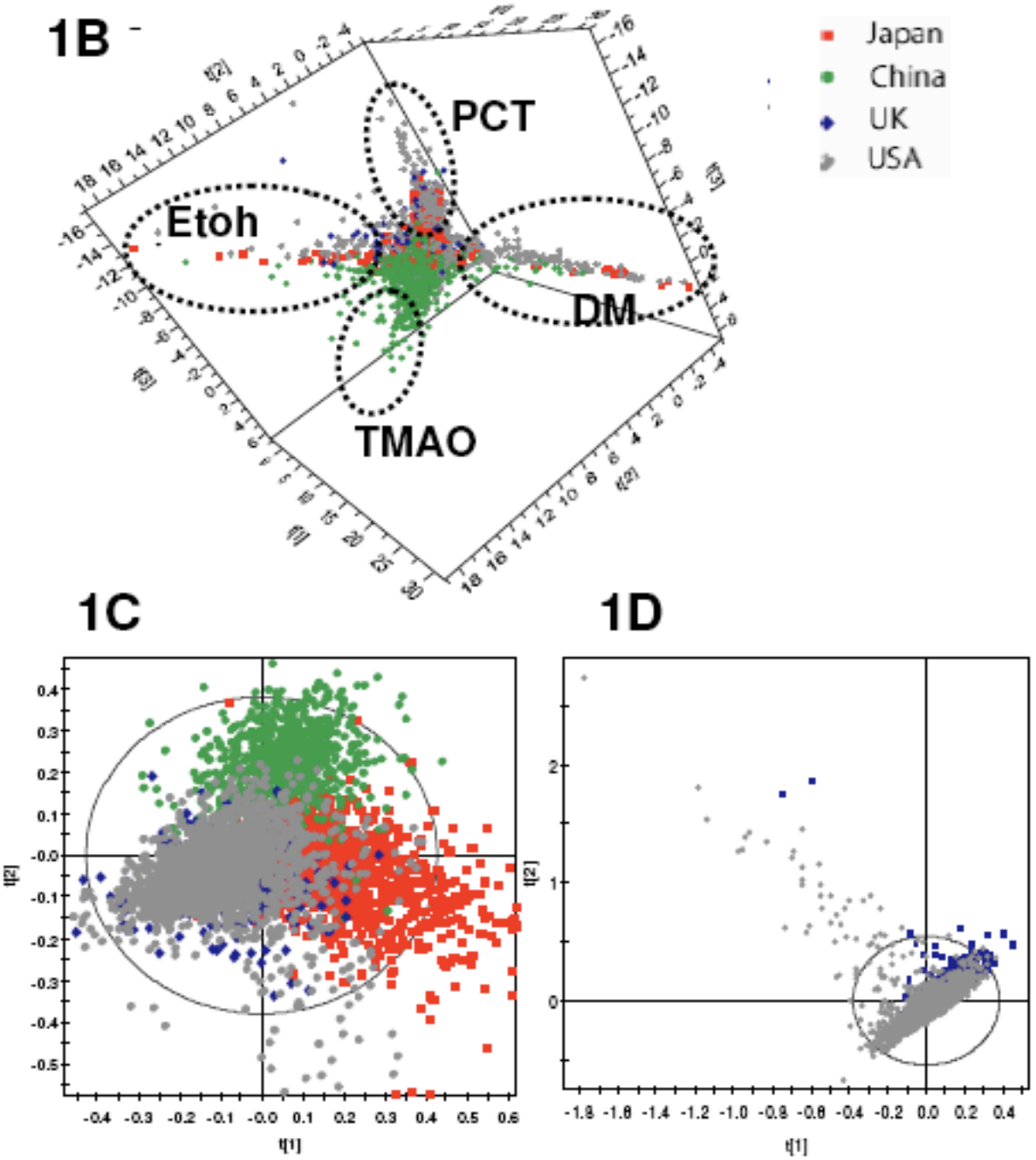
Investigating the effects of nutrition on adverse population blood pressure levels *via* metabolic profiling.

Study Design

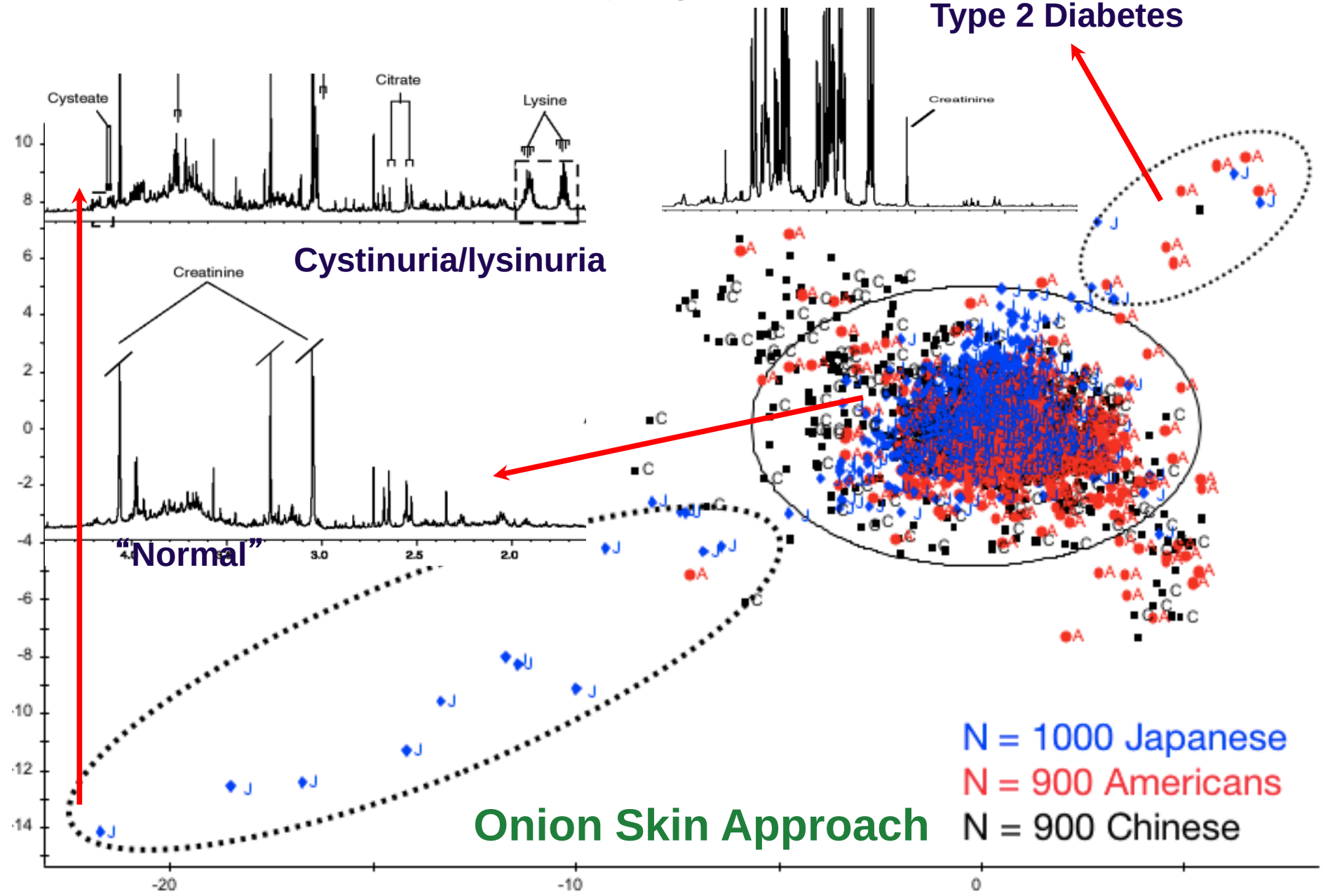
- **2 x 24 h urine samples collected 3-6 weeks apart from 17 centres spanning Peoples Republic of China (PRC), Japan, US and UK (4,680 people = ca 10,000 samples including quality control split samples). NMR spectra were acquired for each sample**
- **4 visits were made by each person**

Measurements:

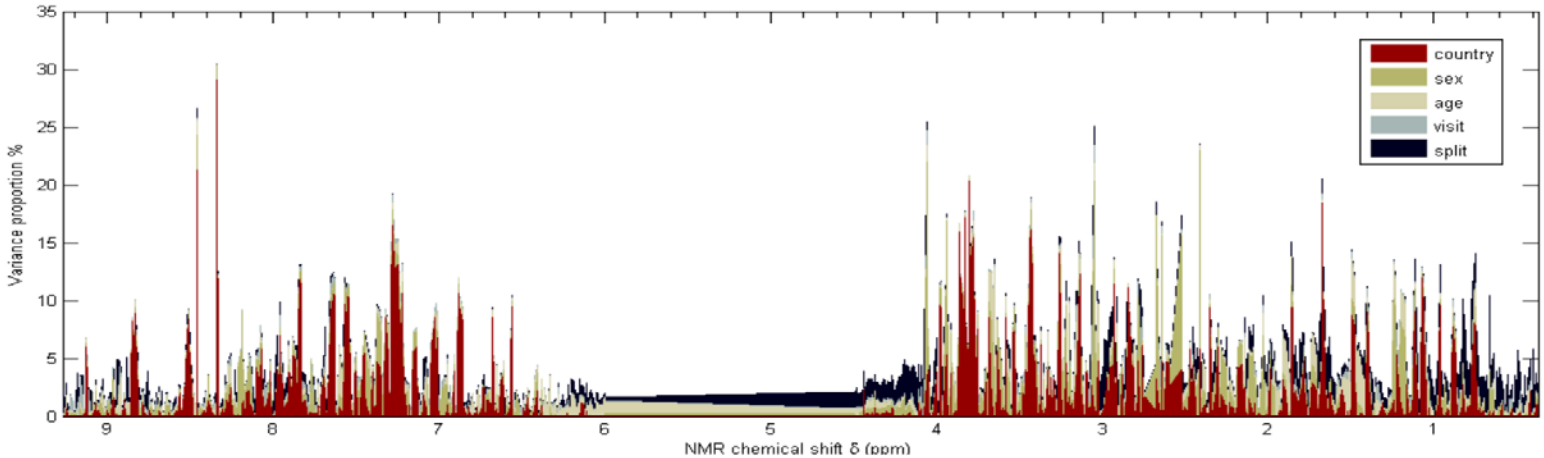
blood pressure - seated (2 x for 4 visits)
Height (2 visits)
weight (2 visits)
daily alcohol over previous 7 days questionnaire (2 visits)
Dietary intake (including vitamin/mineral supplements) recorded using 24 hr recall method (4 visits)
Demographic and medical history questionnaire (1 visit)

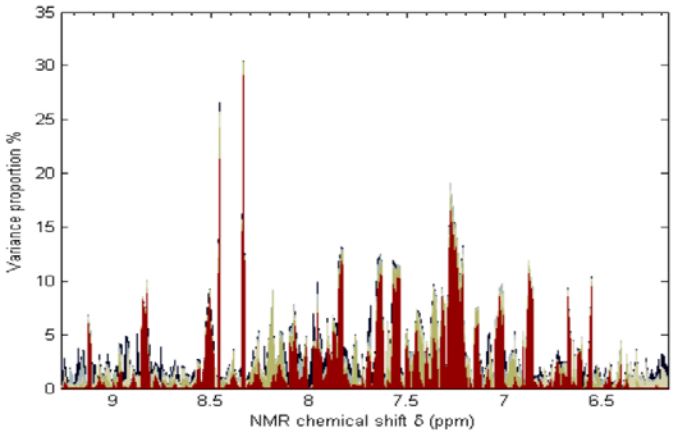

1B
Japan
China
UK
USA
PCT
Etoh
DM
TMAO
1C
t[2]
t[1]
1D
t[2]
t[1]

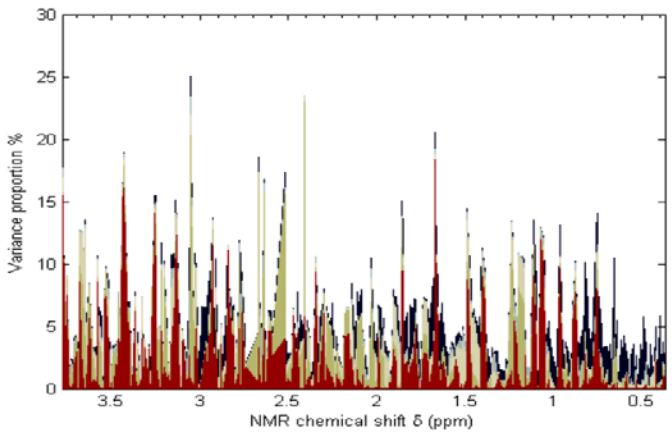
Large Scale Metabonomic Screening of Human Populations: Identifying Outliers



DIFFERENT PATTERNS OF VARIATION IN HUMAN METABOTYPE



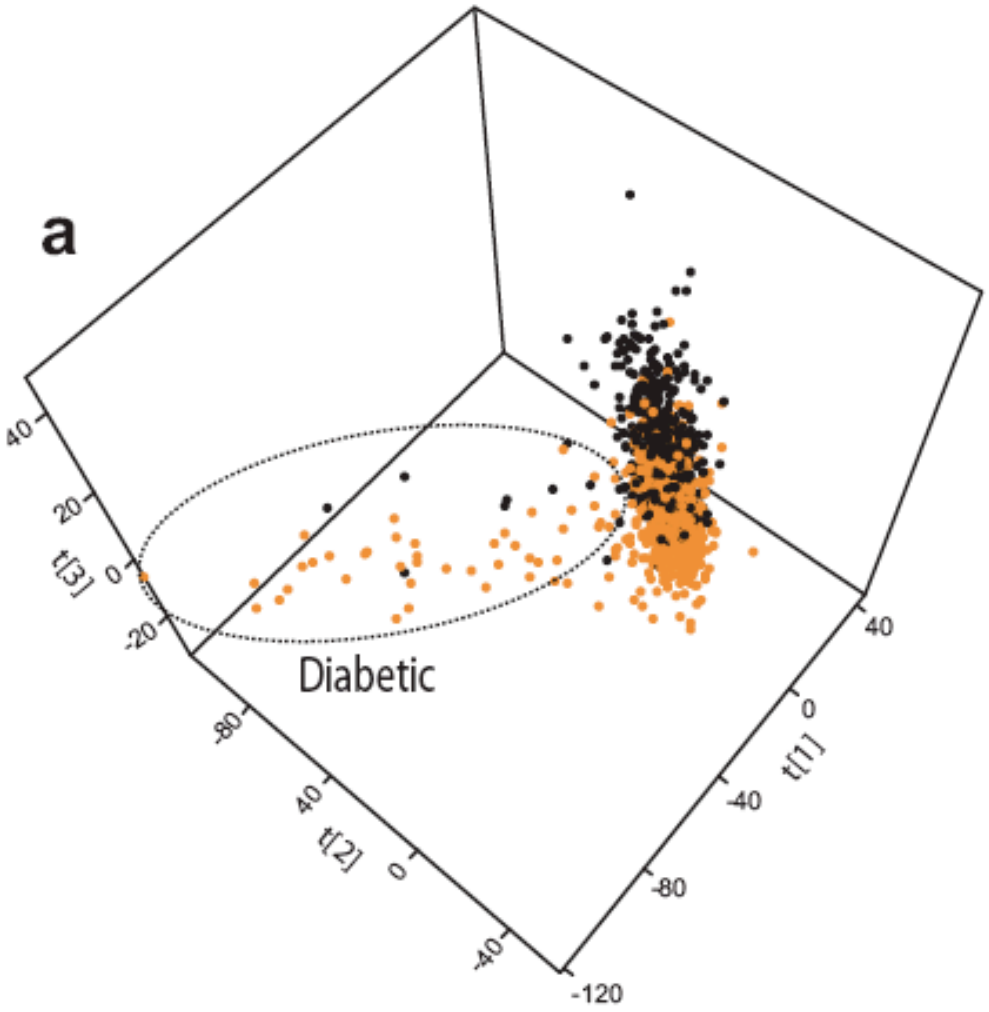


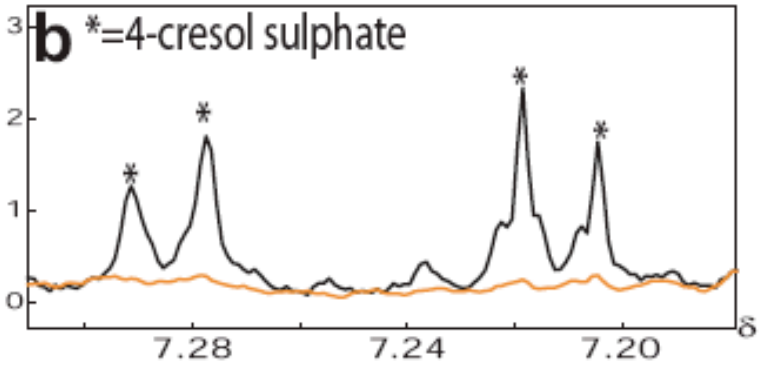


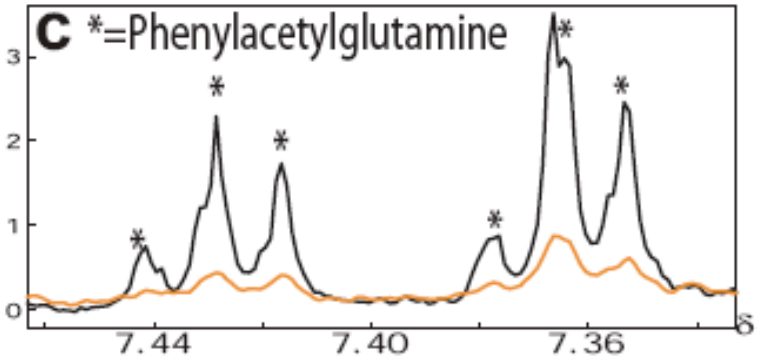
Factor	Df	Wilks Lambda	approx. F	P-value
Country	2	0,025	47,613	0
Sex	1	0,335	17,611	0
Age	1	0,752	2,921	0

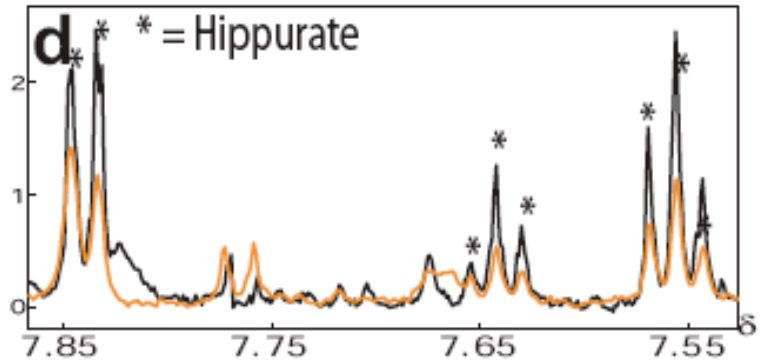
MANOVA

• BMI 18.5 - 24.9 kg/m^2 • BMI ≥30kg/m^2

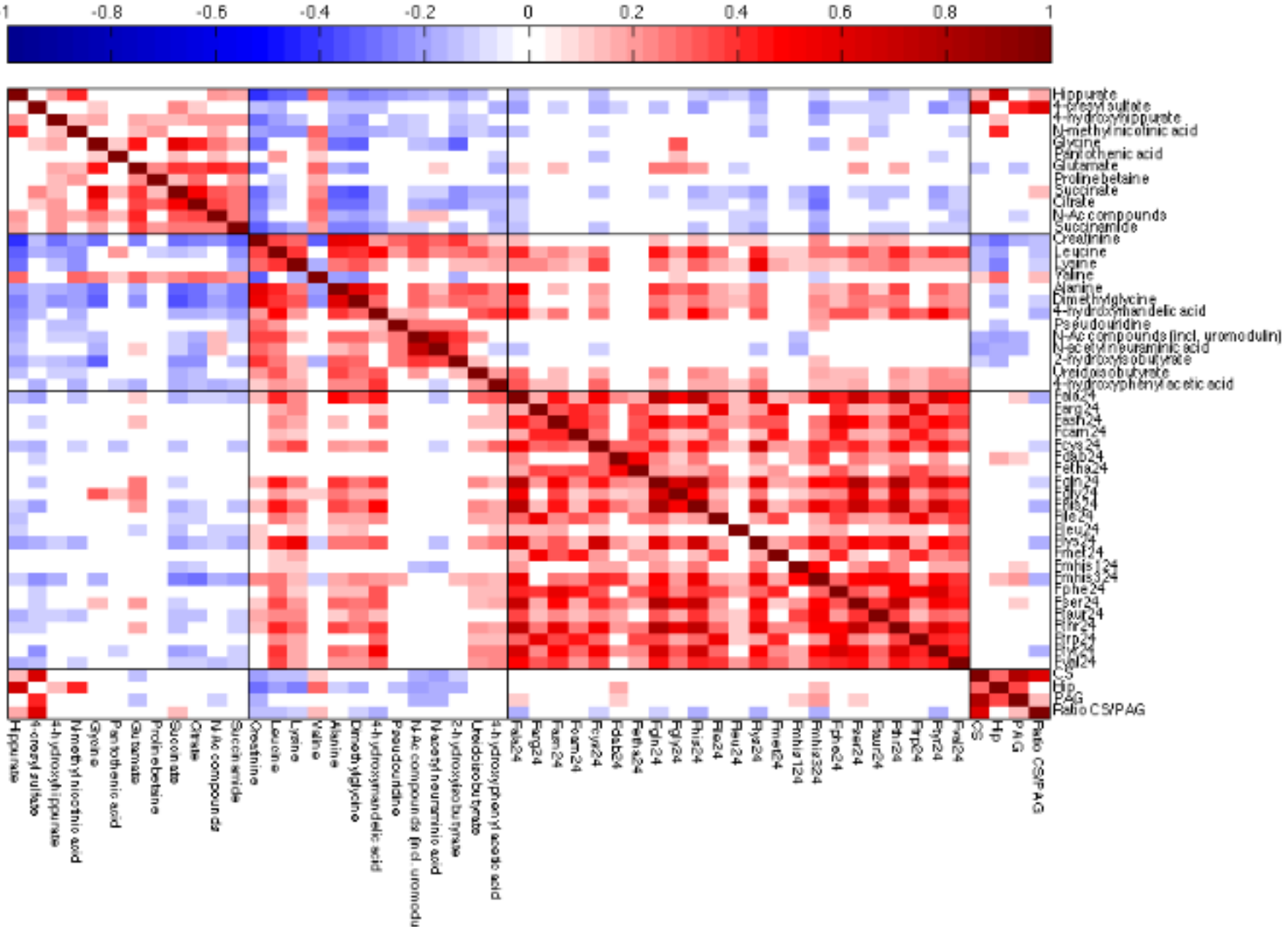








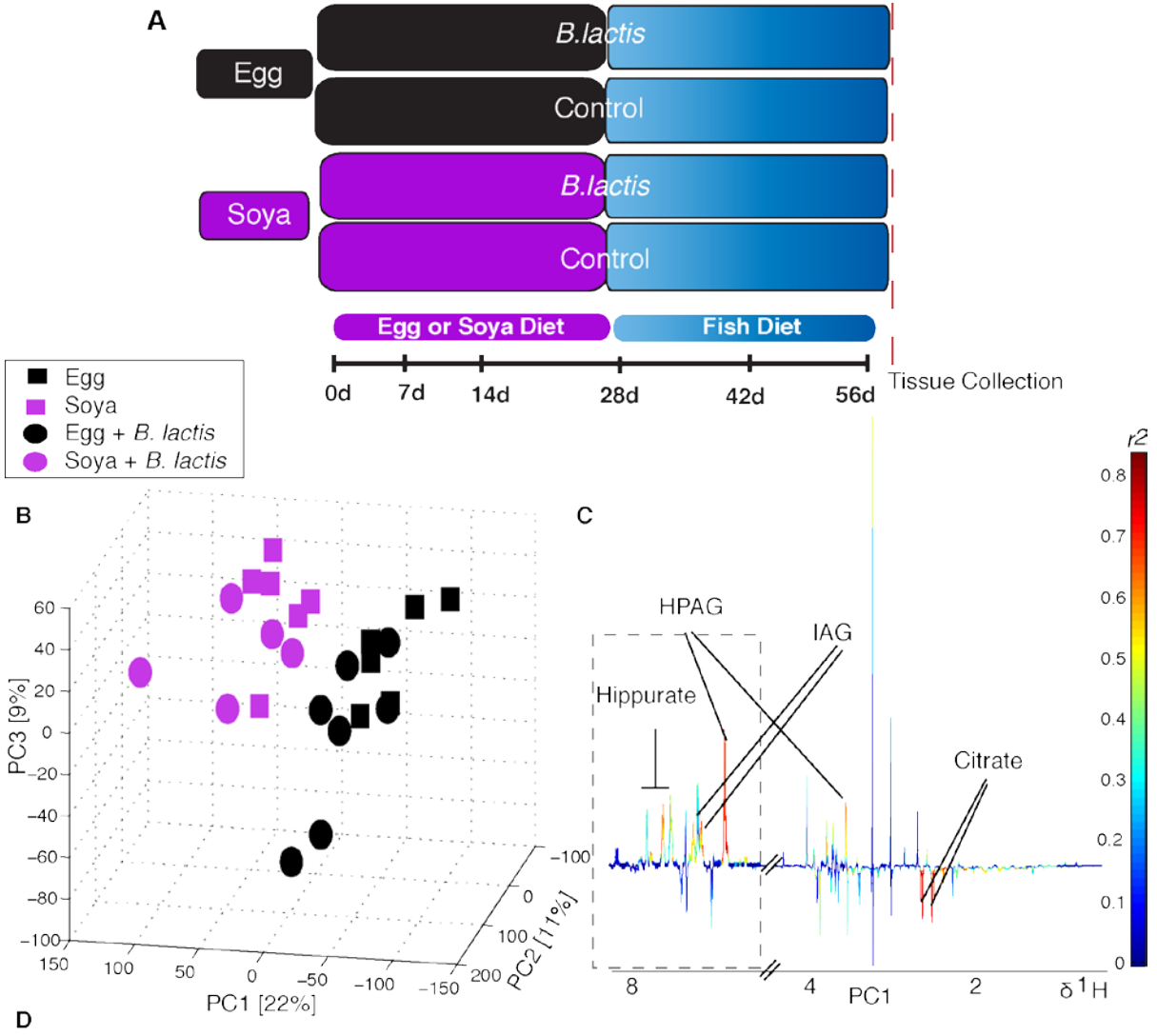
Correlation between dietary input, metabonomic profiles and ion exchange data that discriminate normal vs clinically obese



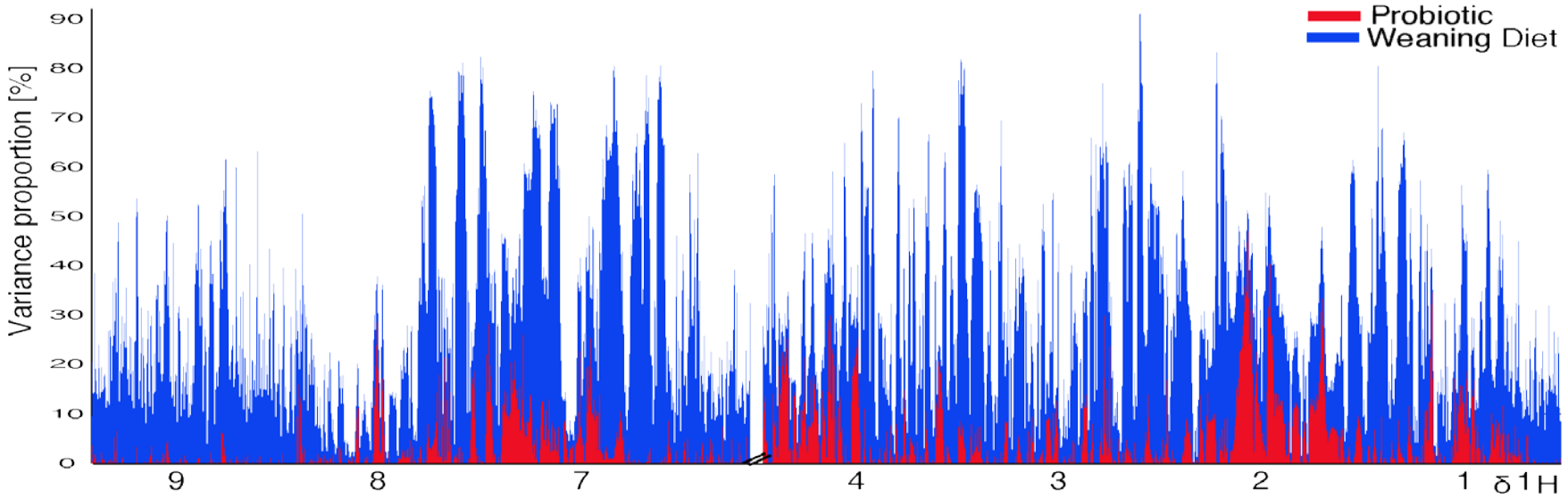
Mapping and Understanding The Integrated System Response to Interventions.

Can we measure "lifestyle interventions" metabolically- and if so what do they mean?"

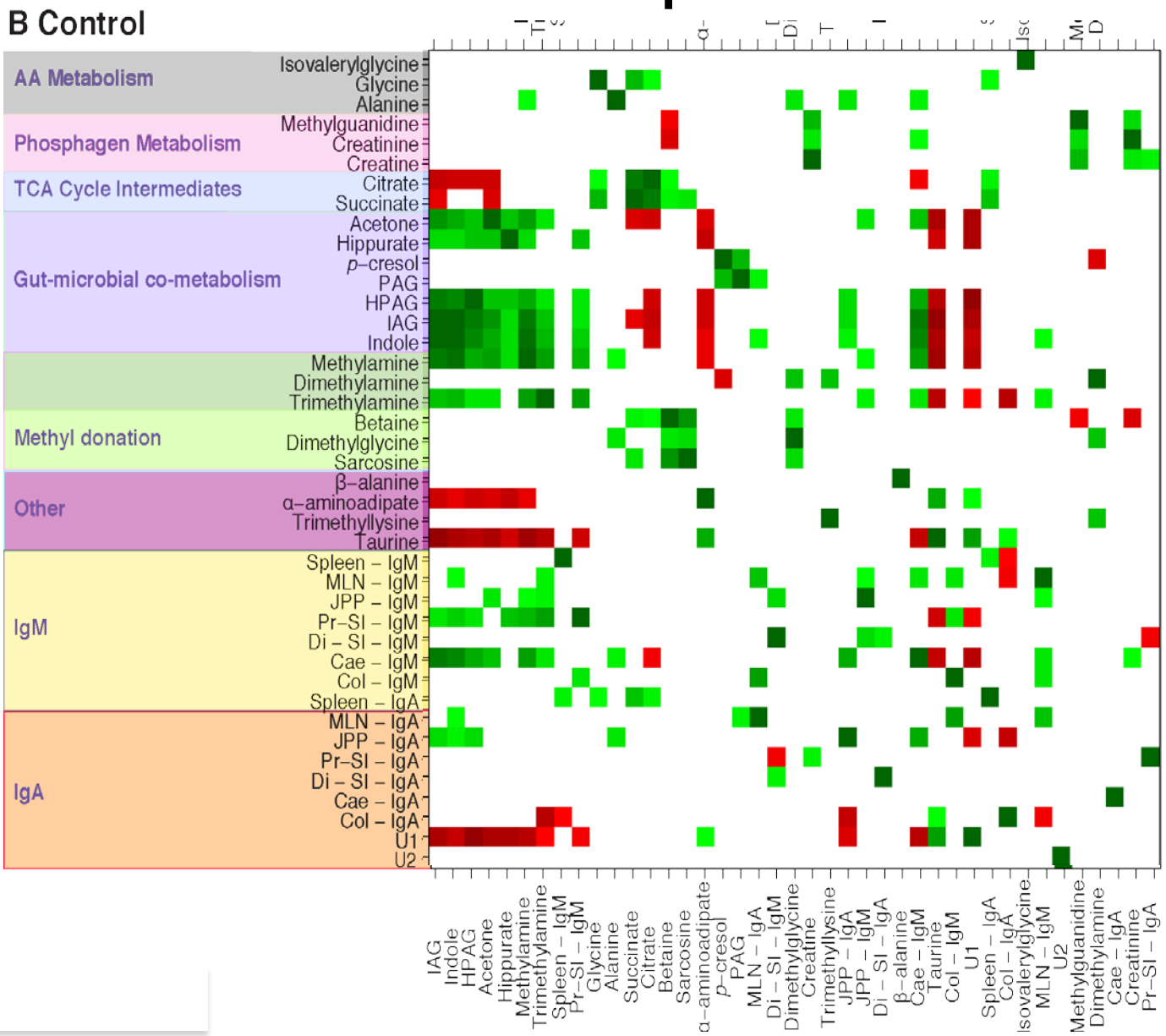
Porcine model of weaning diet and probiotic to explore shaping of the metabolome, immune system and microflora



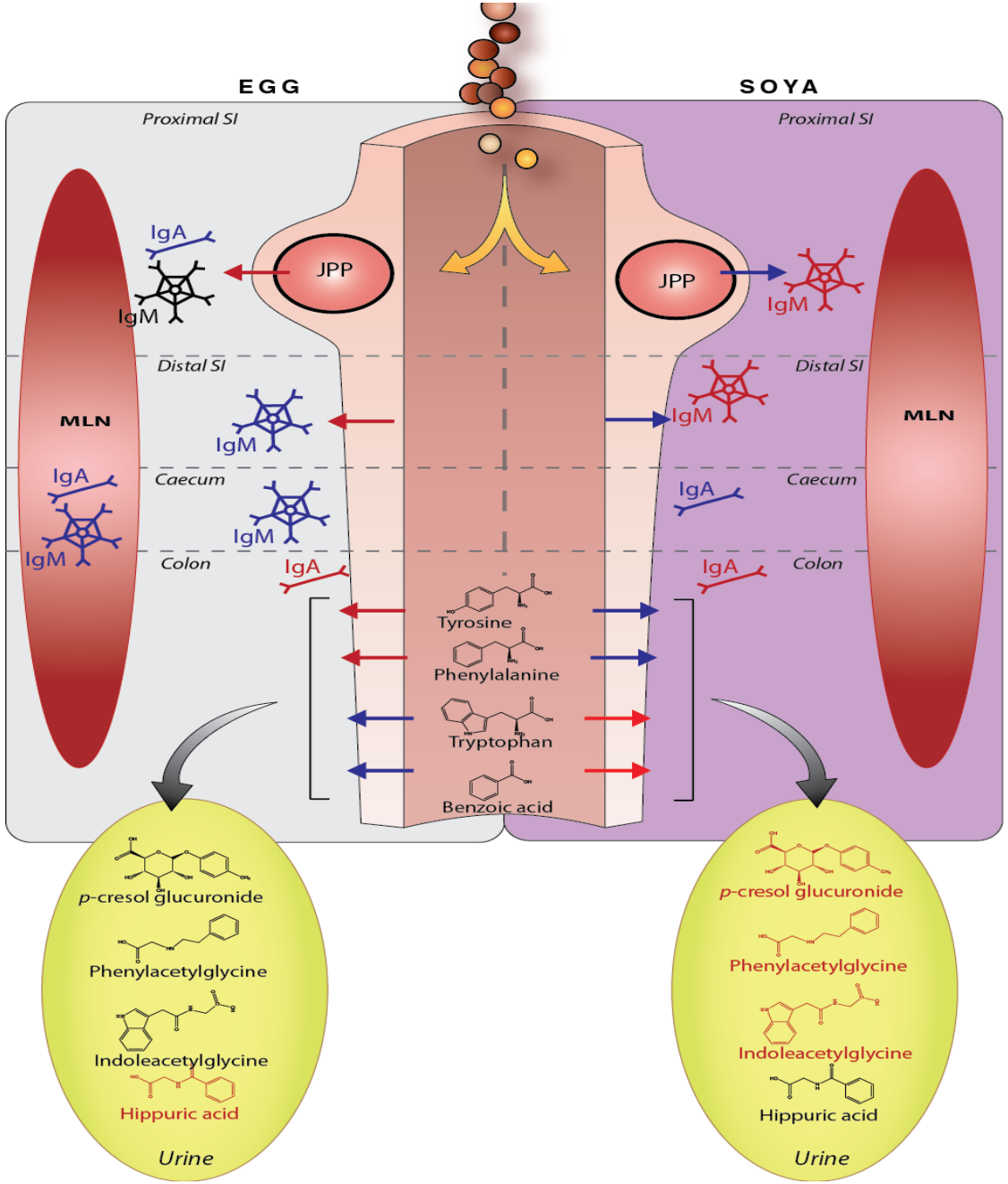
Differential spectral patterns associated with weaning diet and probiotic intervention.



Correlation map showing relationship between immune and metabolic parameters.

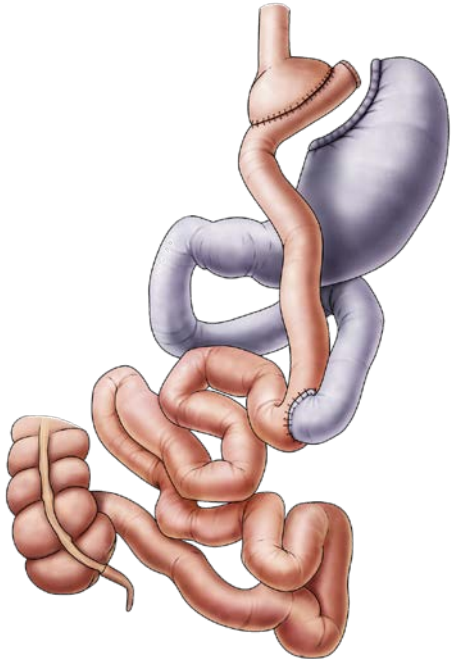


Diet & Prebiotic



Bariatric Surgery in a Rat model

Jia Li, Nigel Gooderham
Collaborators: Prof. Darzi, Mr. Athanasiou and Mr. Ashrafian Dr Marchesi



Surgery & Weight loss

Groups:

Sham (N=18)

Roux-en-Y gastric bypass (N=18)

Time points:

Pre-op, 2, 4, 6 and 8 weeks post-op

Samples:

Urine and faeces

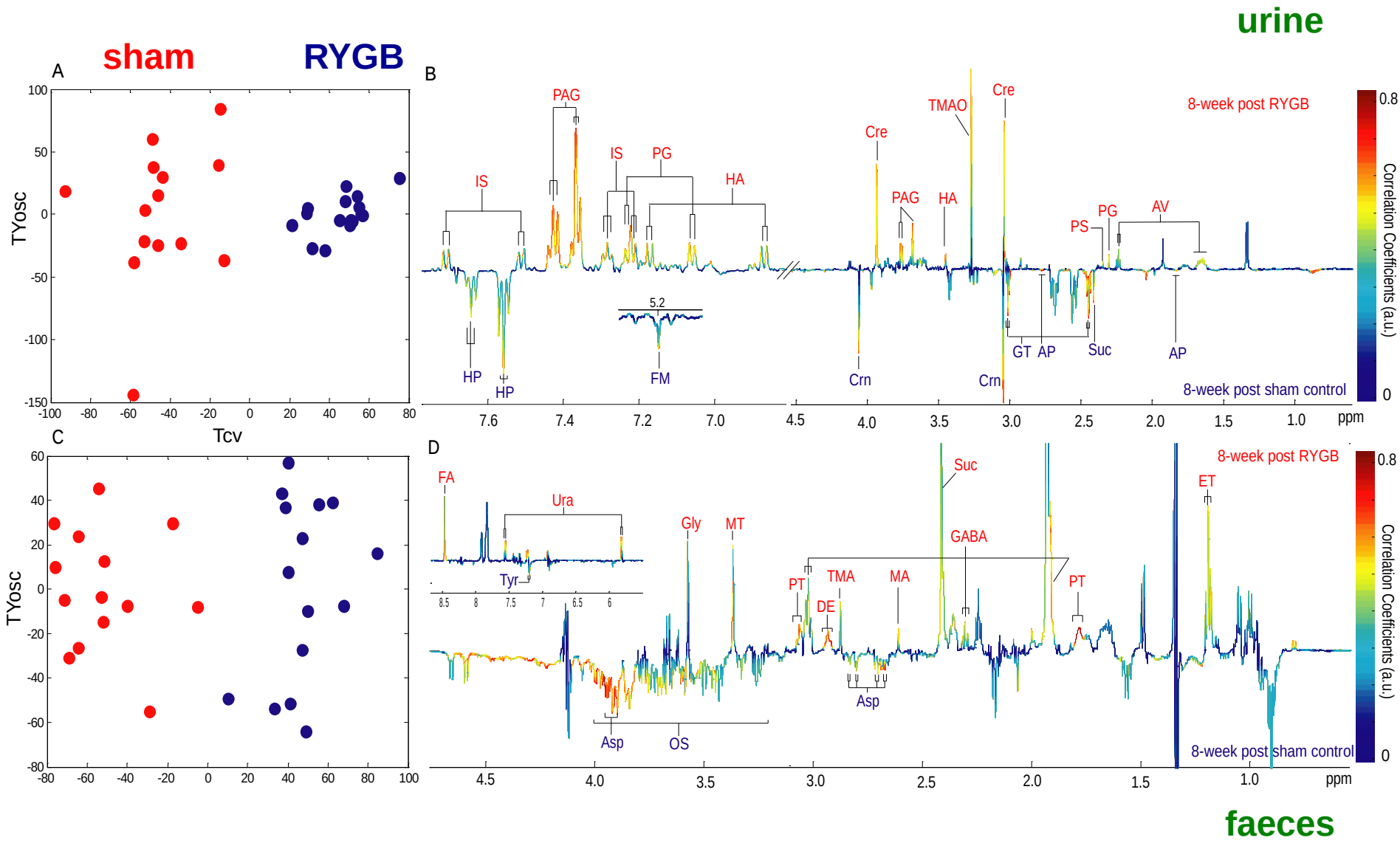
Methods:

NMR-based metabolic profiling

UPLC-MS analysis on faecal bile acids

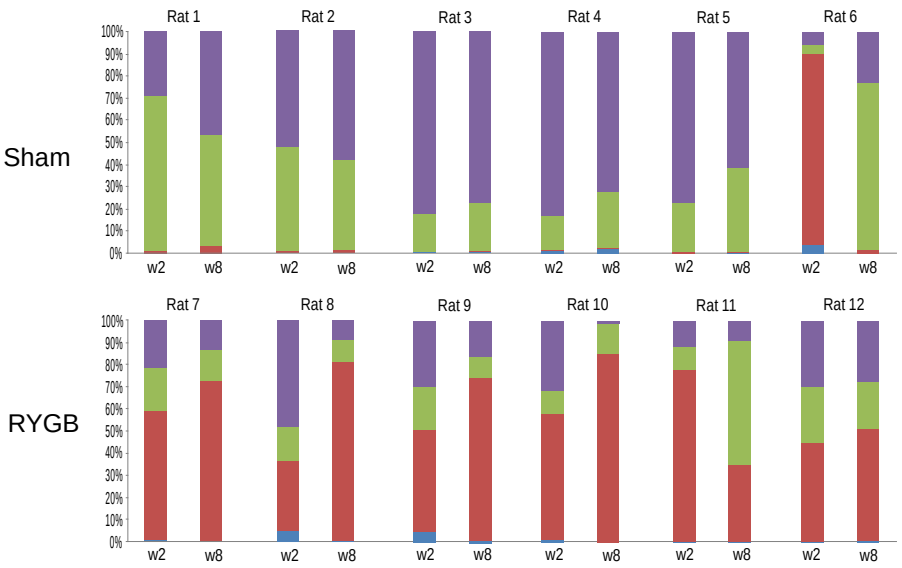
454 Sequencing system for microbial composition analysis

Bariatric Surgery: RYGB in non-obese rats



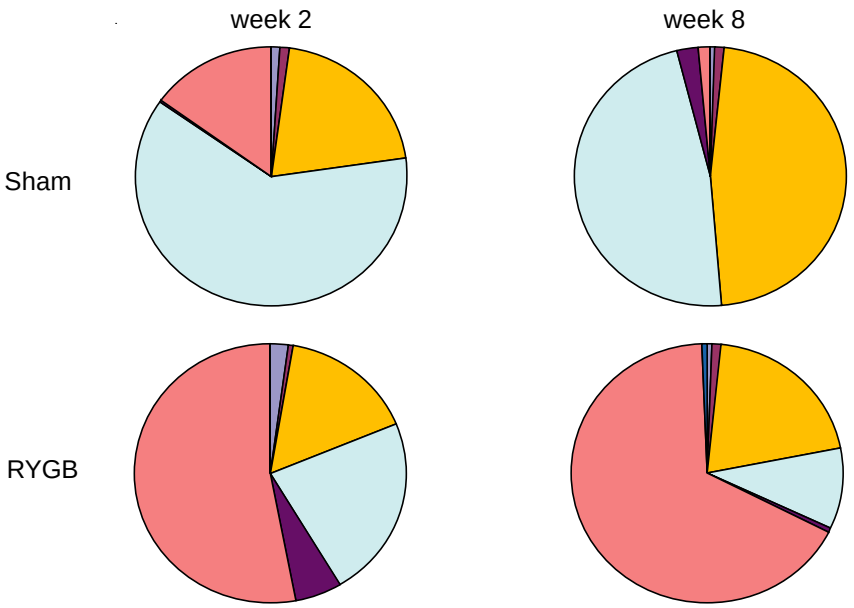
Bariatric Surgery: 454 sequence data

A



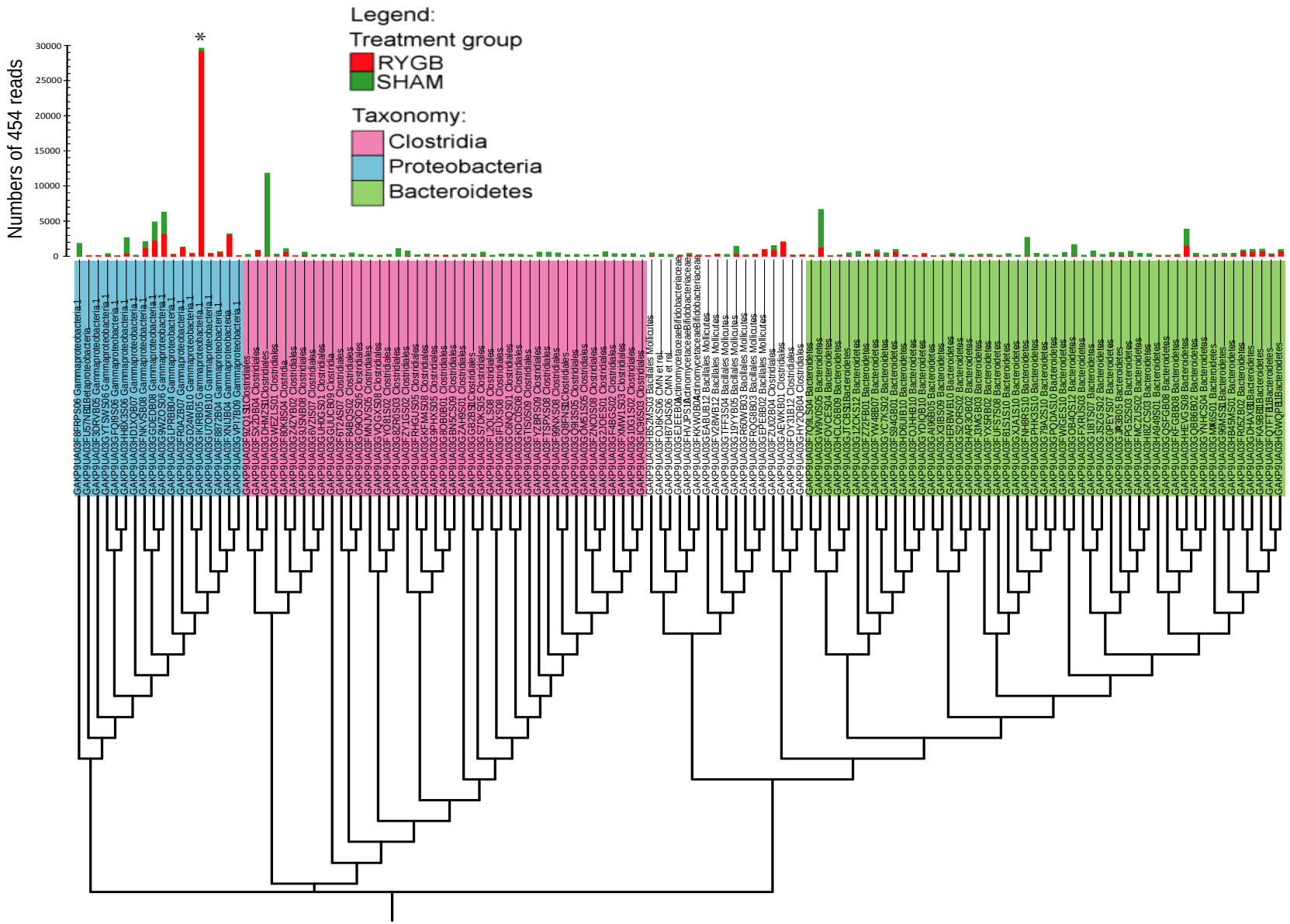
	p (sham *vs.* RYGB)	
	w2	w8
Firmicutes	0.089	0.0006***
Bacteroidetes	0.26	0.079
Proteobacteria	0.037*	<0.0001***
Actinobacteria	0.41	0.96

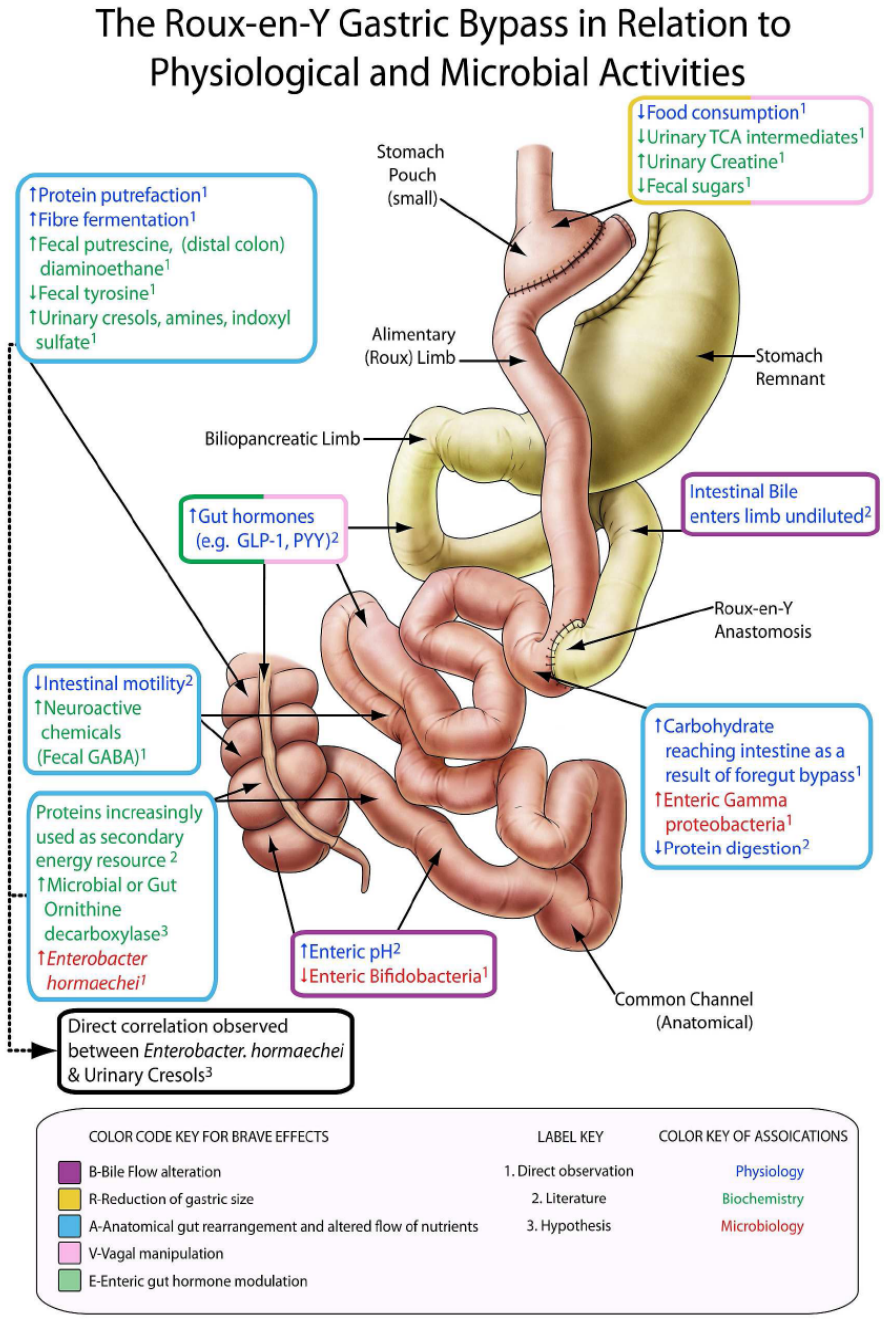
B



	p (sham *vs.* RYGB)	
	w2	w8
Actinobacteria	0.41	0.99
Bacilli	0.41	0.90
Bacteroidetes	0.47	0.03*
Clostridia	0.012**	0.0048***
Erysipelotrichi	0.11	0.39
Gammaproteobacteria	0.036*	<0.0001***
Others	–	–

Phylogenetic relationships in RYGB/sham operated rats





The Roux-en-Y gastric bypass (RYGB) operation is a bariatric procedure that achieves its physiological benefits through B.R.A.V.E effects:

- **Bile flow alteration**
- **Reduction of gastric size**
- **Anatomical gut rearrangement and altered flow of nutrients**
- **Vagal manipulation**
- **Enteric gut hormone modulation**

Ashrafian *et al.* 2010 Obes Rev. Sep 29

Summary

(1) A shift from Firmicutes to Proteobacteria, especially gamma-proteobacteria post RYGB.

(2) Upregulation of TCA cycle indicates increased energy expenditure.

(3) Increased biogenesis of urinary *p*-cresol and related compounds and host-microbial co-metabolites.

(4) A shift from protein degradation to putrefaction

Metabolic Surgery and Cancer

Cancer

Article first published online: 29 NOV 2010

DOI: 10.1002/cncr.25738

Protective Effects of Bariatric Procedures

Hutan Ashrafian, MRCS; Kamran Ahmed, MRCS; Simon P. Rowland, BSc(Hons); Vanash M. Patel, MRCS; Nigel J. Gooderham, PhD; Elaine Holmes, PhD; Ara Darzi KBE, FMedSci; and Thanos Athanasiou, MD, PhD, FETCS

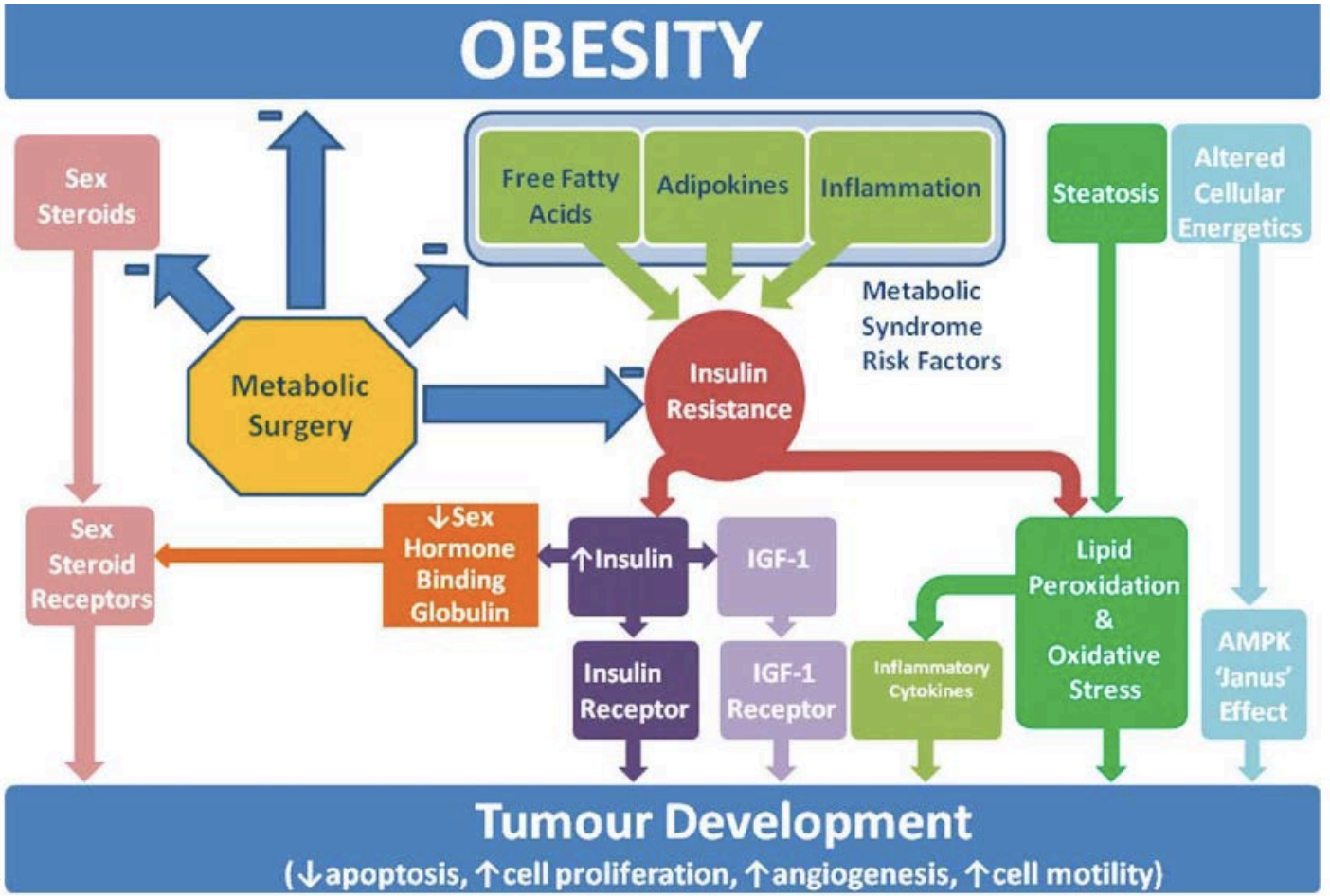
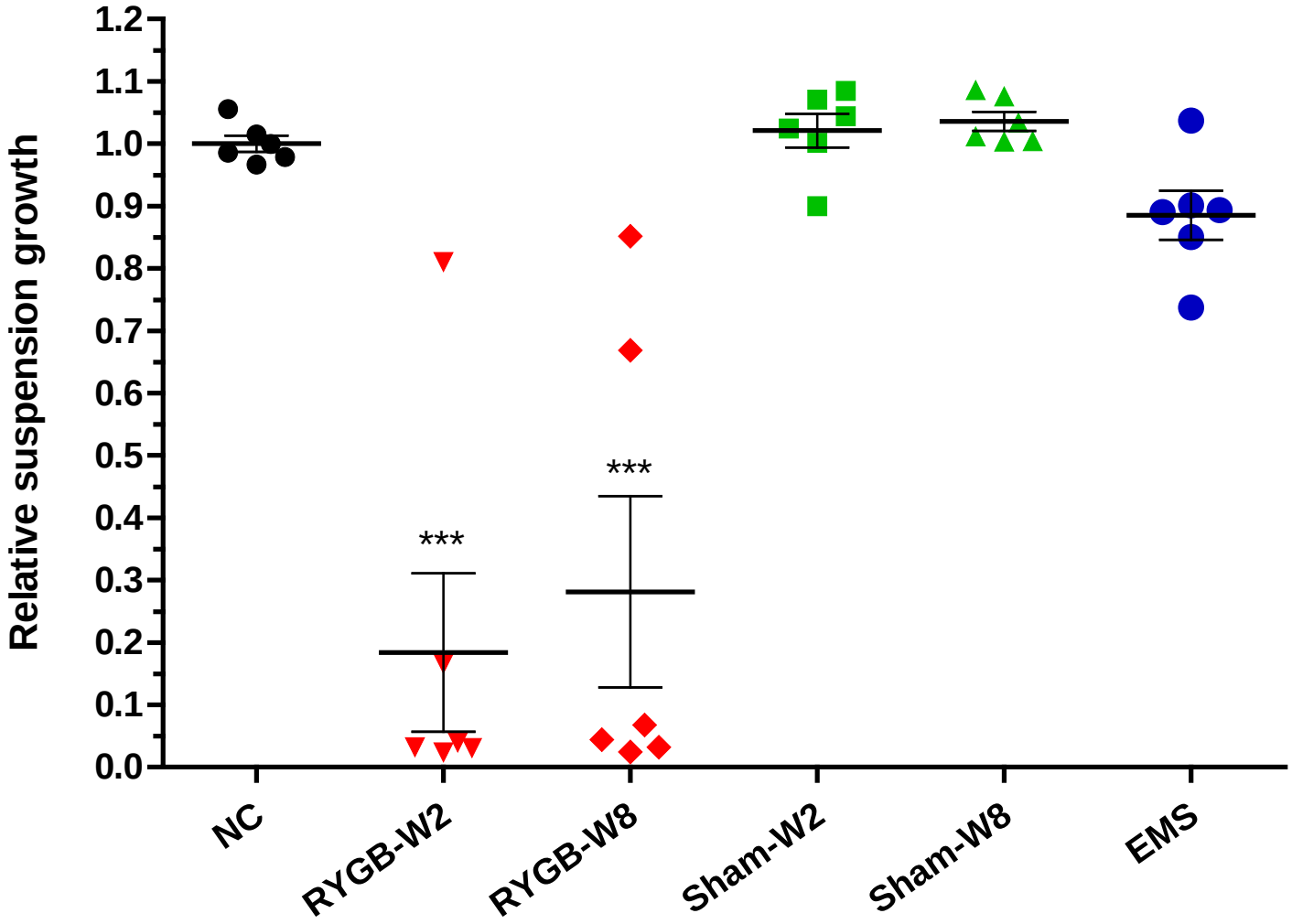
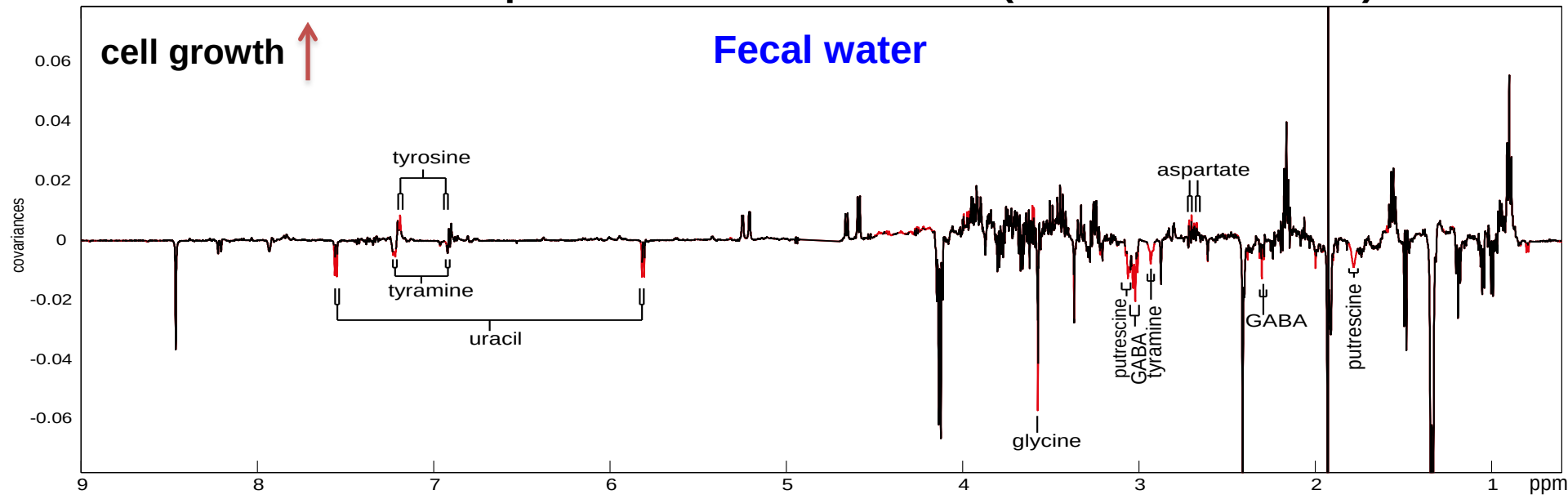


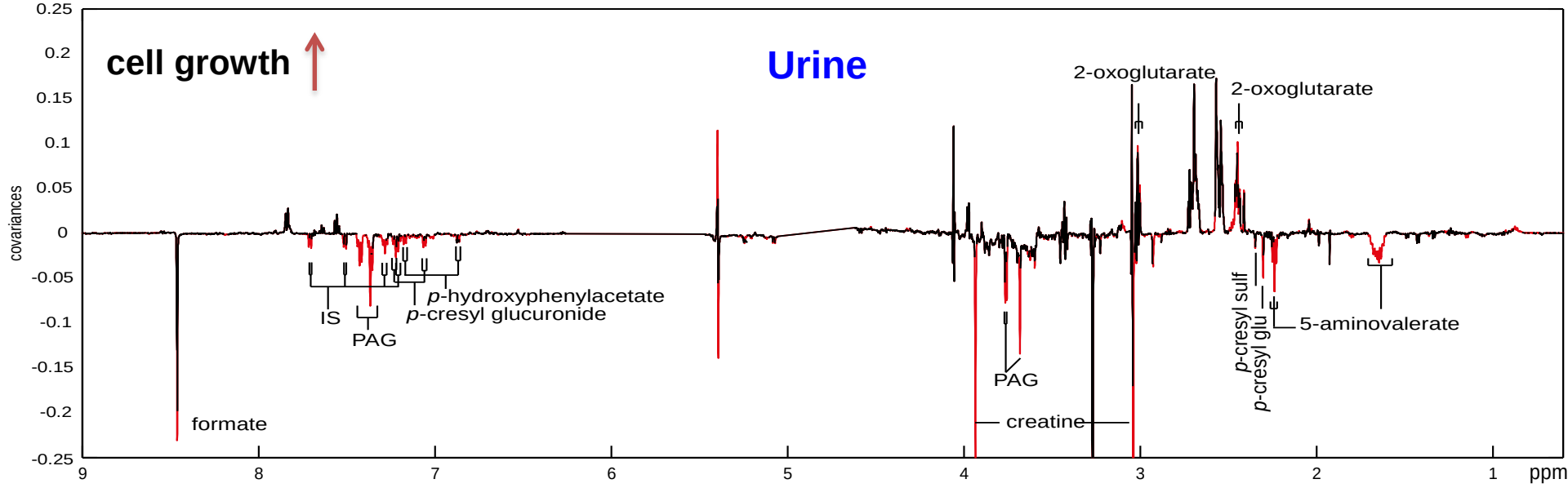
Figure 1. Mechanisms of decreased cancer risk by metabolic surgery are depicted. IGF-1 = Insulin-like Growth Factor 1, AMPK = 5′ adenosine monophosphate-activated protein kinase.

Determination of the relative suspension growth as a measure of cytotoxicity of fecal water extracts following treatment of L5178Y mouse lymphoblastoid cells for 24 h (mean ± S.E.M of 6 independent samples).

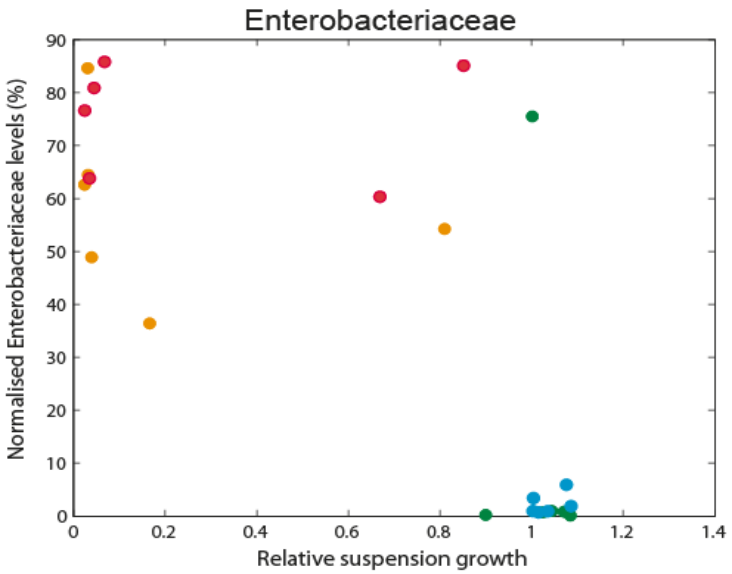


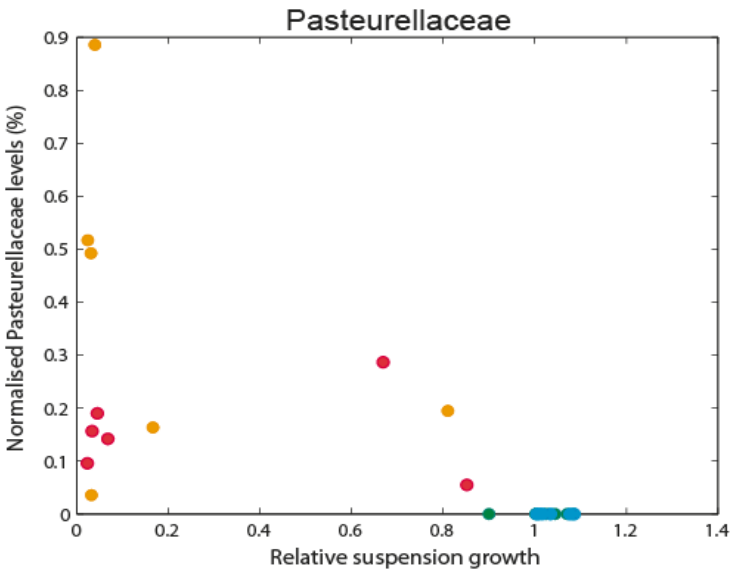
O-PLS regression analyses of fecal water and urine extracts against relative suspension growth values obtained from a 24h treatment of L5178Y cells with sham or RYGB operated rat faecal extracts (week 2 and week 8)

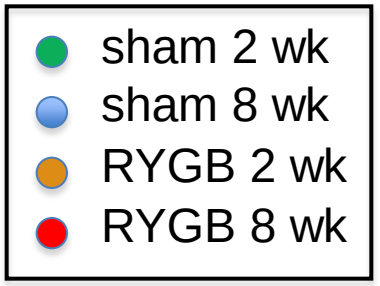


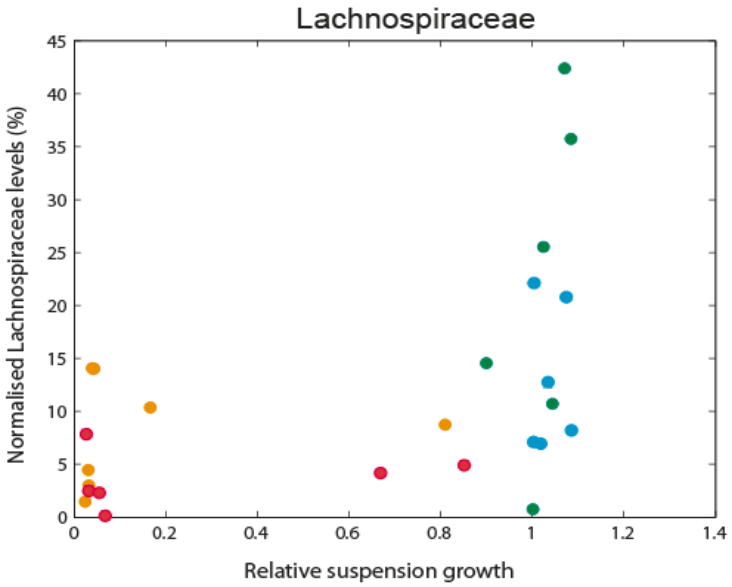


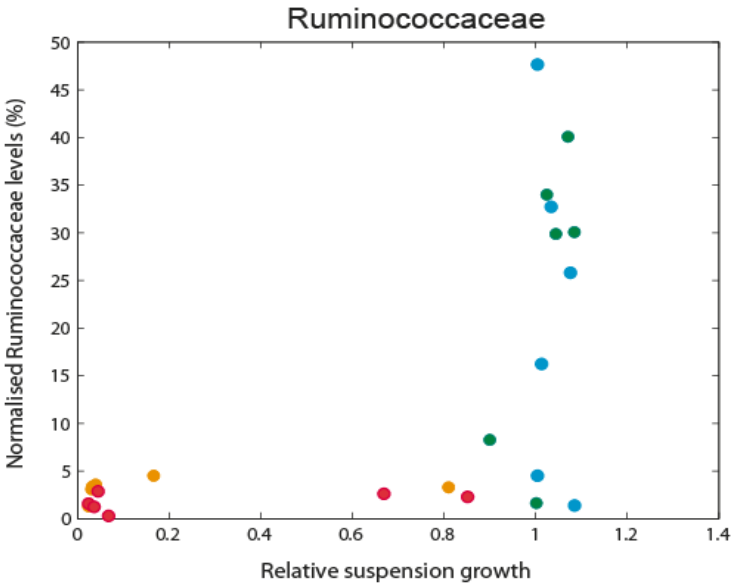
Scatter plots of normalised bacterial levels and relative suspension growth of L5178Y mouse lymphoblastoid cells exposed to fecal water extracts











Early influences on the development of the gut microbiome

Infection

Delivery Method

Antibiotics

Microbiology. 2011 Feb 17. [Epub ahead of print]

Determinants of the human infant intestinal microbiota after introduction of first complementary foods in five European centres.

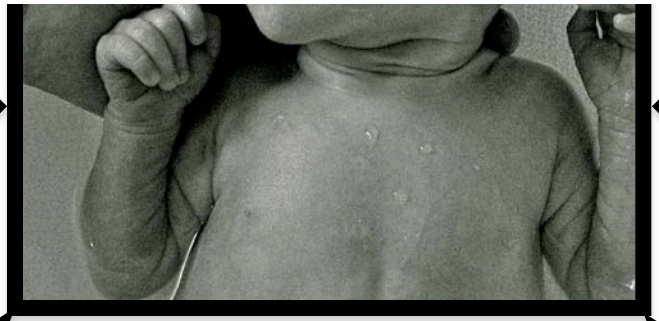
Fallani M, Amarri S, Uusijarvi A, Adam R, Khanna S, Aguilera M, Gil A, Vieites JM, Norin E, Young D, Scott JA, Dore J, Edwards C, Team TI.

Ayacucho 7101, Amazonas, Venezuela; and [2]The Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80305

Bre

lpol

Weaning Age



Exposure to Pets

Weaning Strategy

Travel

Hygiene

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Premature Birth and Later Insulin Resistance

Paul L. Hofman, M.B., Ch.B., Fiona Regan, M.B., B.S., Wendy E. Jackson, M.B., Ch.B., Craig Jefferies, M.B., Ch.B., David B. Knight, M.B., B.S., Elizabeth M. Robinson, M.Sc., and Wayne S. Cutfield, M.D.

(*Circulation*. 2006;114:1687-1692.)

Epidemiology

Low Birth Weight, a Risk Factor for Cardiovascular Diseases in Later Life, Is Already Associated With Elevated Fetal Glycosylated Hemoglobin at Birth

Thiemo Pfab, MD; Torsten Slowinski, MD; Michael Godes, MD; Horst Halle, MD, PhD; Friedrich Priem;; Berthold Hocher, MD, PhD

Clinical Epidemiology

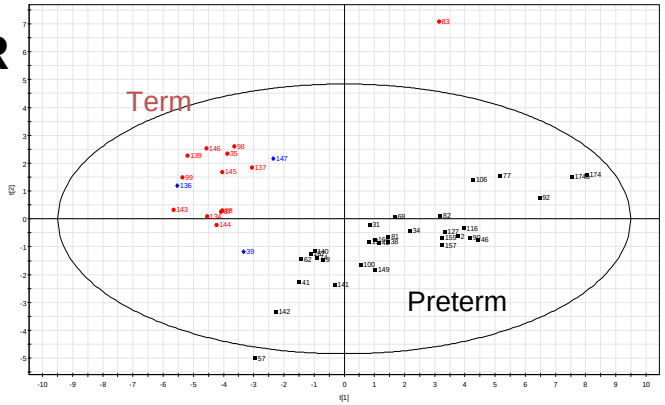
Low Birth Weight Increases Risk for End-Stage Renal Disease

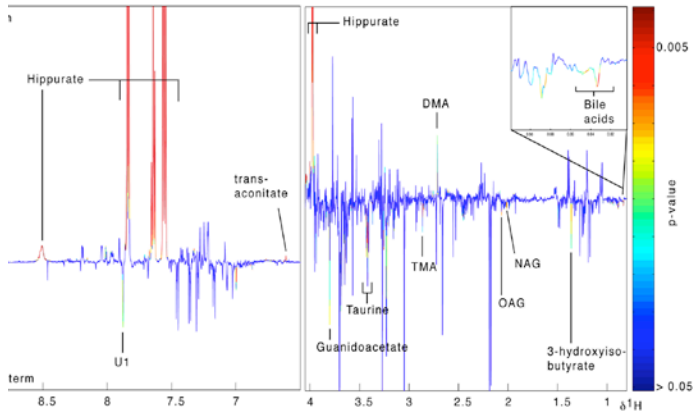
Bjørn Egil Vikse[*], Lorentz M. Irgens[‡], Torbjørn Leivestad[||], Stein Hallan[¶,] and Bjarne M. Iversen[*]**

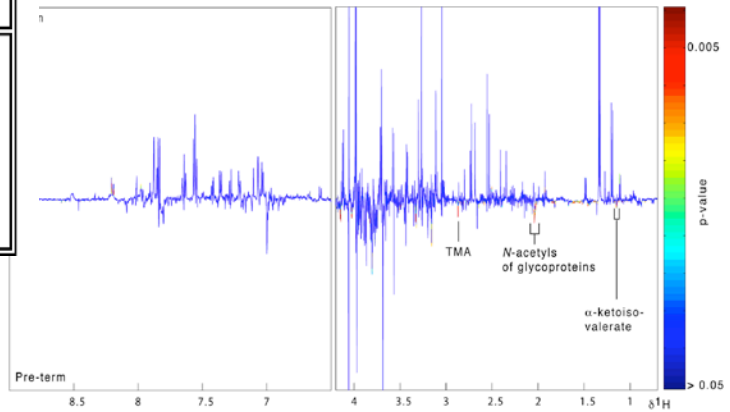
Differential biomarkers associated with preterm birth

PCA scores and loadings plots based on urine NMR profiles showing metabolic differentiation of young adults (18-25 yrs) in preterm vs ‘normal’ birth

Group	Metabolites	Direction of change	Chemical shift [ppm]	Multiplicity	p value
Male preterm	bile acids	+	0.83-0.85	m	0.0004
	3-hydroxyisobutyrate	+	1.36	s	0.014
	O-acetyl fragments of glycoproteins	+	2.066	s	0.012
	dimethylamine	-	2.71	s	0.03
	trimethylamine	+	2.86	s	0.009
	guanidoacetate	+	3.8	s	0.017
	t-aconitate	-	6.61	s	0.003
	hippurate	-	7.64	t	0.003
Female preterm	α-ketoisovalerate	+	1.145	d	0.03
	N-acetyl fragments of glycoproteins	+	2.04	s	0.007
	trimethylamine	+	2.86	s	0.0009







Neena Modi, Mathew Hyde, Jim Parkinson

Pediatrics International (2010) **52**, 44–50 doi: 10.1111/j.1442-200X.2009.02887.x

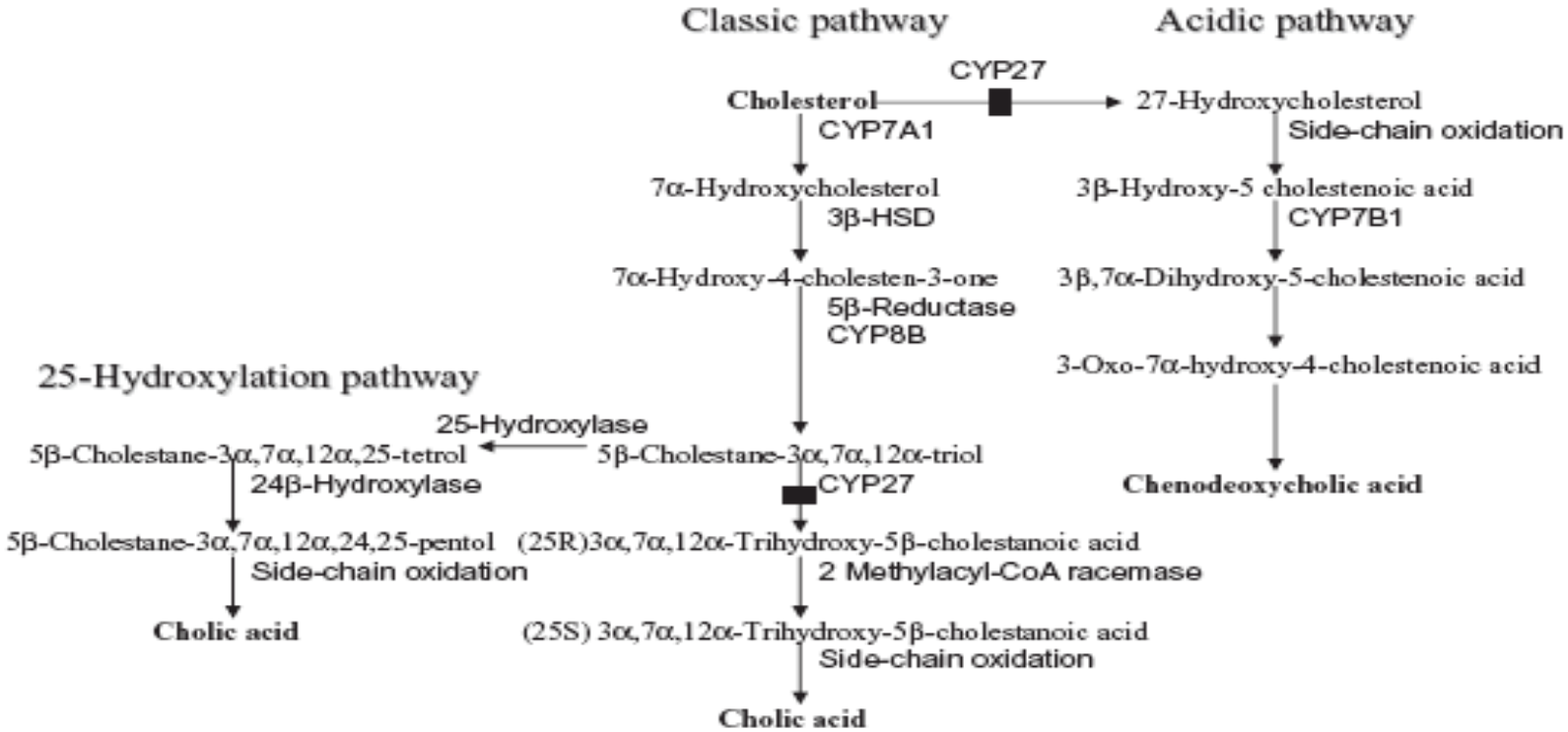
Original Article

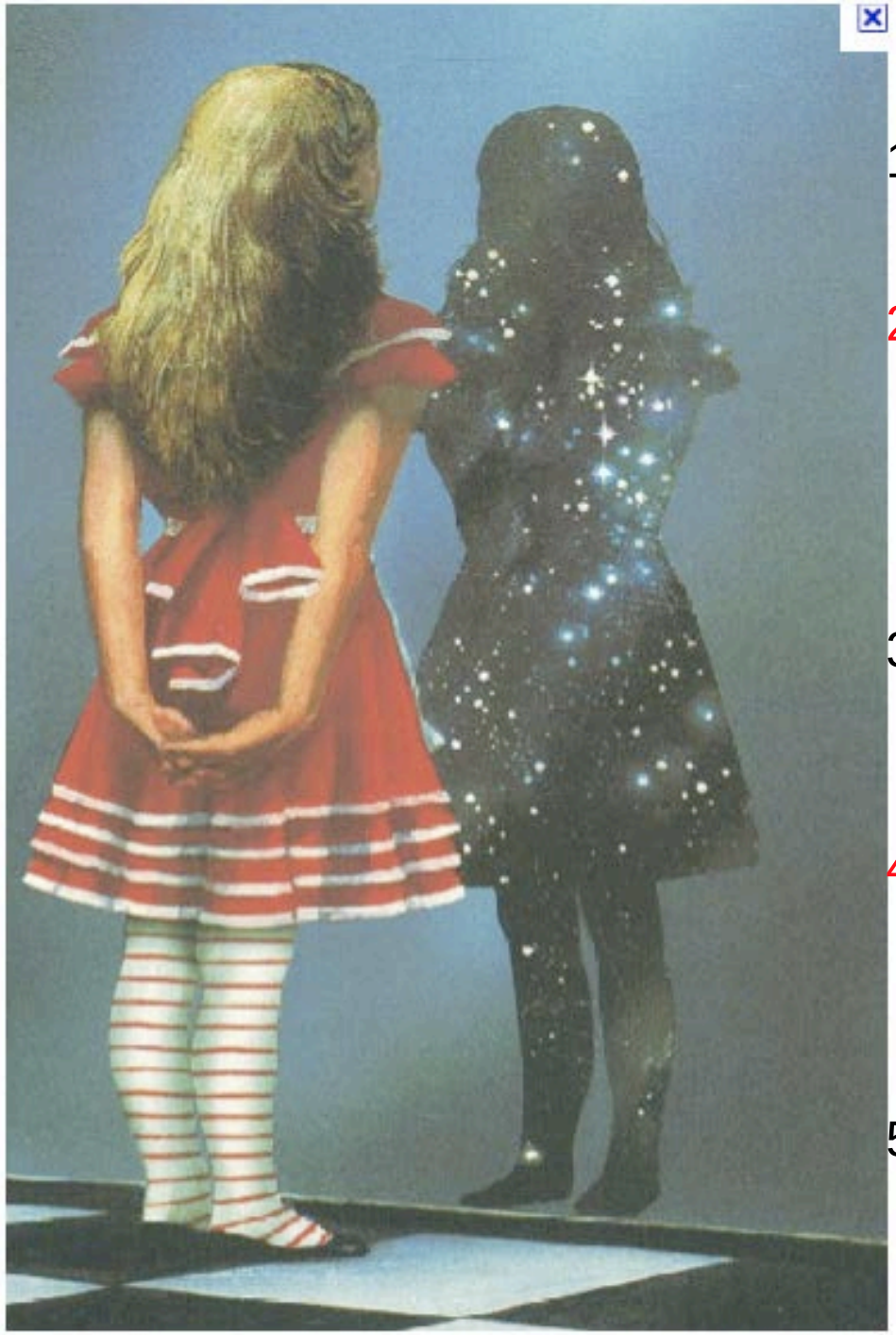
Developmental pattern of urinary bile acid profile in preterm infants

Hiroshi Nishiura,[1] Akihiko Kimura,[1] Yasuhiko Yamato,[1] Kumiko Aoki,[2] Takahiro Inokuchi,[2] Takao Kurosawa[3] and Toyojiro Matsuishi[1]

[1]*Department of Pediatrics and Child Health and* [2]*Research Institute of Medical Mass Spectrometry, Kurume University School of Medicine, Kurume, Fukuoka and* [3]*Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido, Japan*

Conclusion: Physiological cholestasis in preterm infants persists longer than in full-term infants. Moreover, as large amounts of cholic and 1β,3α,7α,12α-tetrahydroxy-5β-cholan-24-oic acids were detected in urine from preterm infants during this study, the 25-hydroxylation pathway may be particularly important for bile acid synthesis in early preterm infants.





Concluding Remarks

1. Advances in metabolic profiling methods allow its application in a wide variety of biomedical fields.
2. Statistical spectroscopic & chemometric analysis of spectroscopic and related data allow biomarker structure, pathway elucidation and mechanistic insights.
3. Can identify biomarkers of nutrient intake, quantify, and monitor effects on health in animals and humans.
4. Variation in human populations need not be an insurmountable obstacle and can even carry valuable information – Personalized Medicine
5. The metabolic looking glass provides a ‘systems biology’ window on an organism -provides a framework for stratification and

ACKNOWLEDGEMENTS

Pediatrics

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Imperial College

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Nigel Gooderham
Jia Li
Ivan Yap
Jonathan Swann
Will Edmands
Silke Heinzmann
Claire Merrifield

Cardiff University

Julian Marchesi

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