BSc Pharmacology and Translational Medicine

Module: Principles of Pharmacodynamics and Pharmacokinetics
Lecture: Application of Metabonomics to Toxicology & Pharmacology
Date: Friday 2nd November 2012

Toby Athersuch

Application of Metabonomics

Learning Objectives

By the end of the lecture and the associated learning activities the student should:

- Be able to give examples of the application of metabonomics in toxicology and other areas.

Lecture Outline

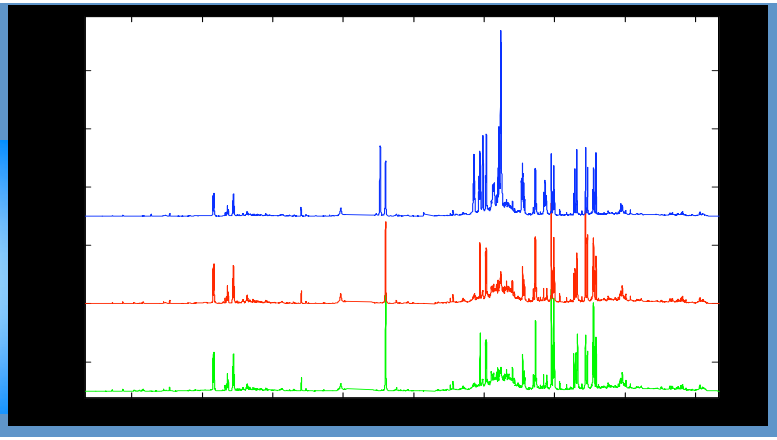
- Background to NMR-based metabolic profiling in toxicology & pharmacology
- Toxicological application example: COMET
- Summary

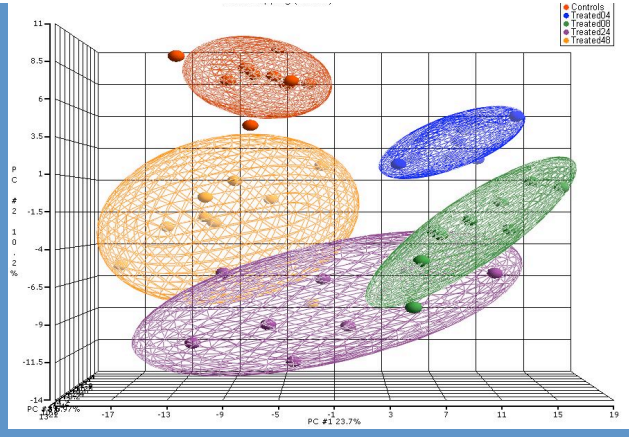
Background to Metabolic Profiling in Toxicology & Pharmacology

Background

Metabolic Profiling (Metabonomics / Metabolomics) – Systems Pharmacology

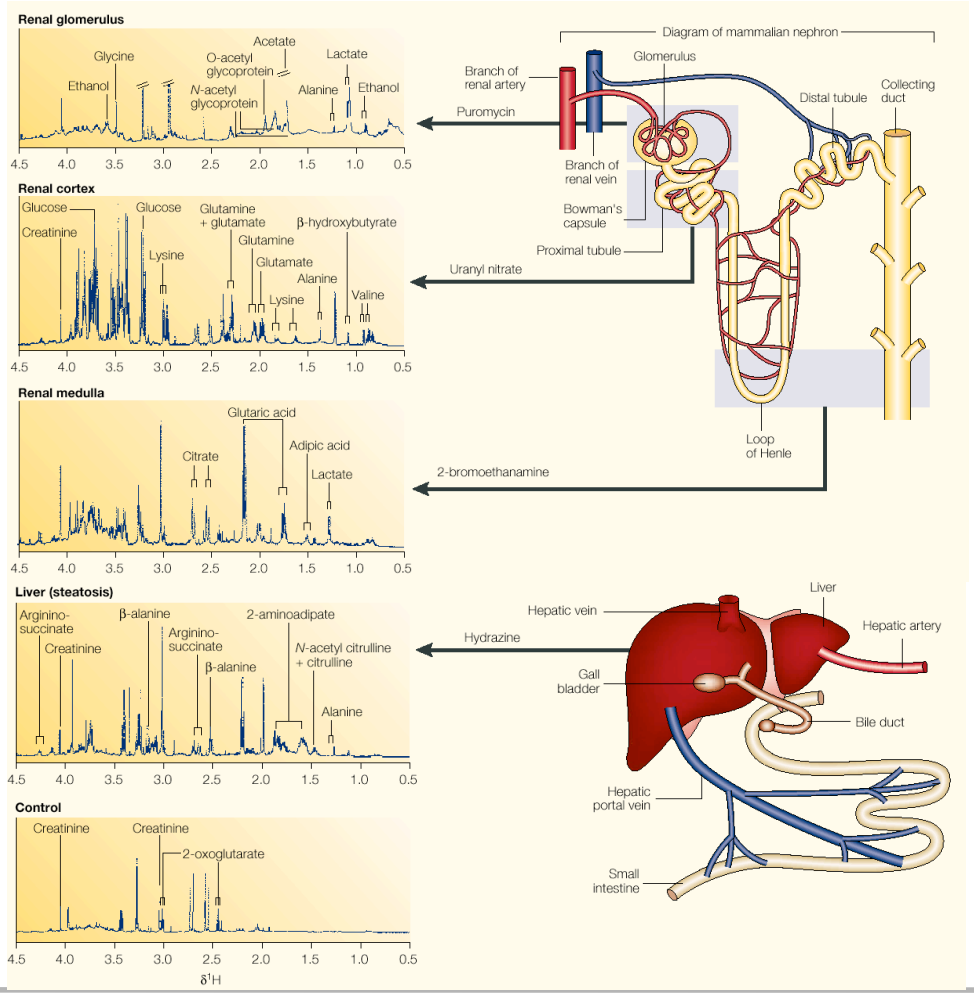
- Typically a non-targeted approach (‘agnostic’)
- Unique metabolic ‘fingerprints’
- Mechanistic insight / predictive ability - *pharmacometabonomics*
- Systems response – genetic / environmental – closest to phenotype



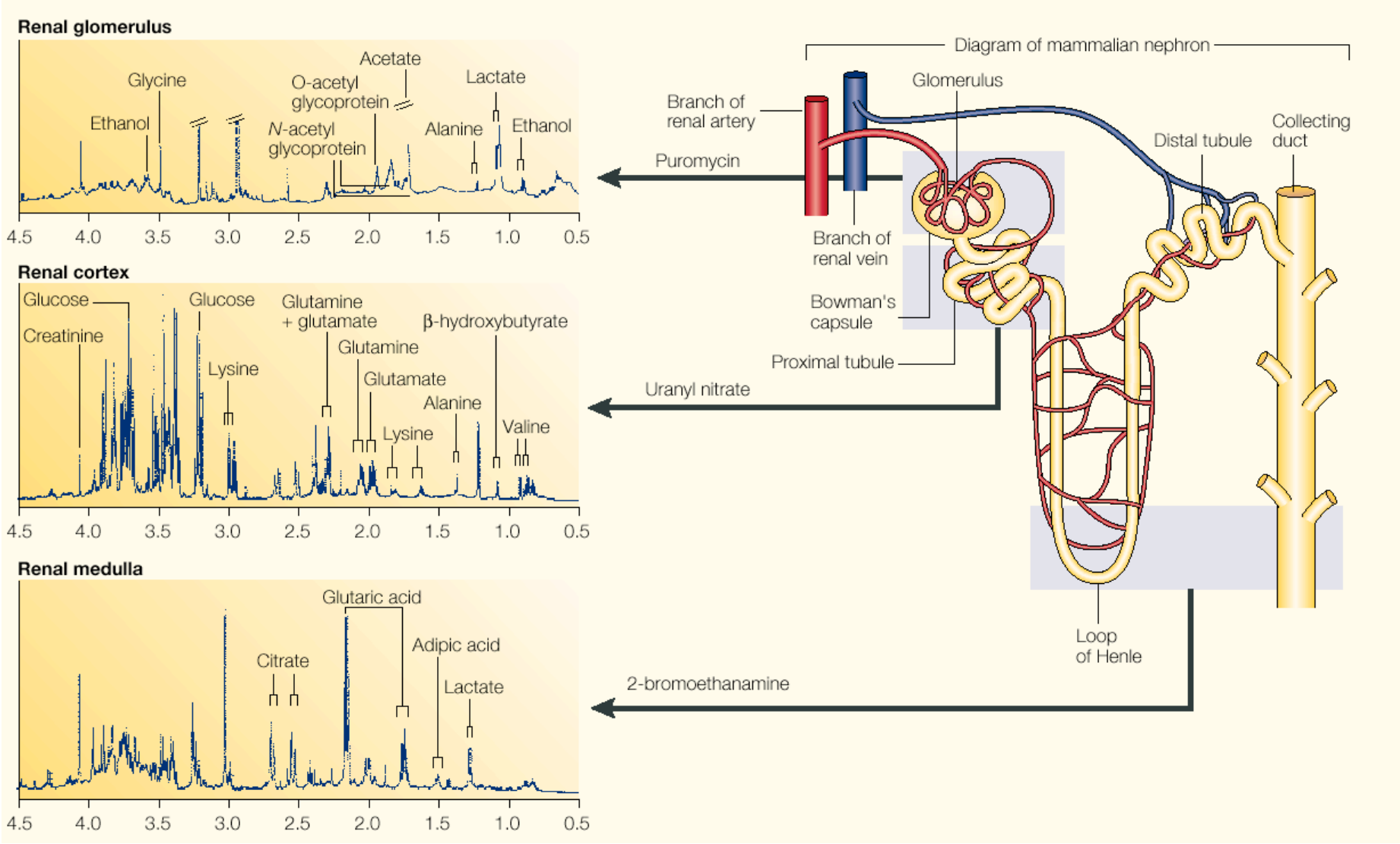


Metabolic Profiles Reflect Target Organ

- Partial 600 MHz ^{1}H NMR spectra of rat urine. Indicates organ specificity of metabolic fingerprint



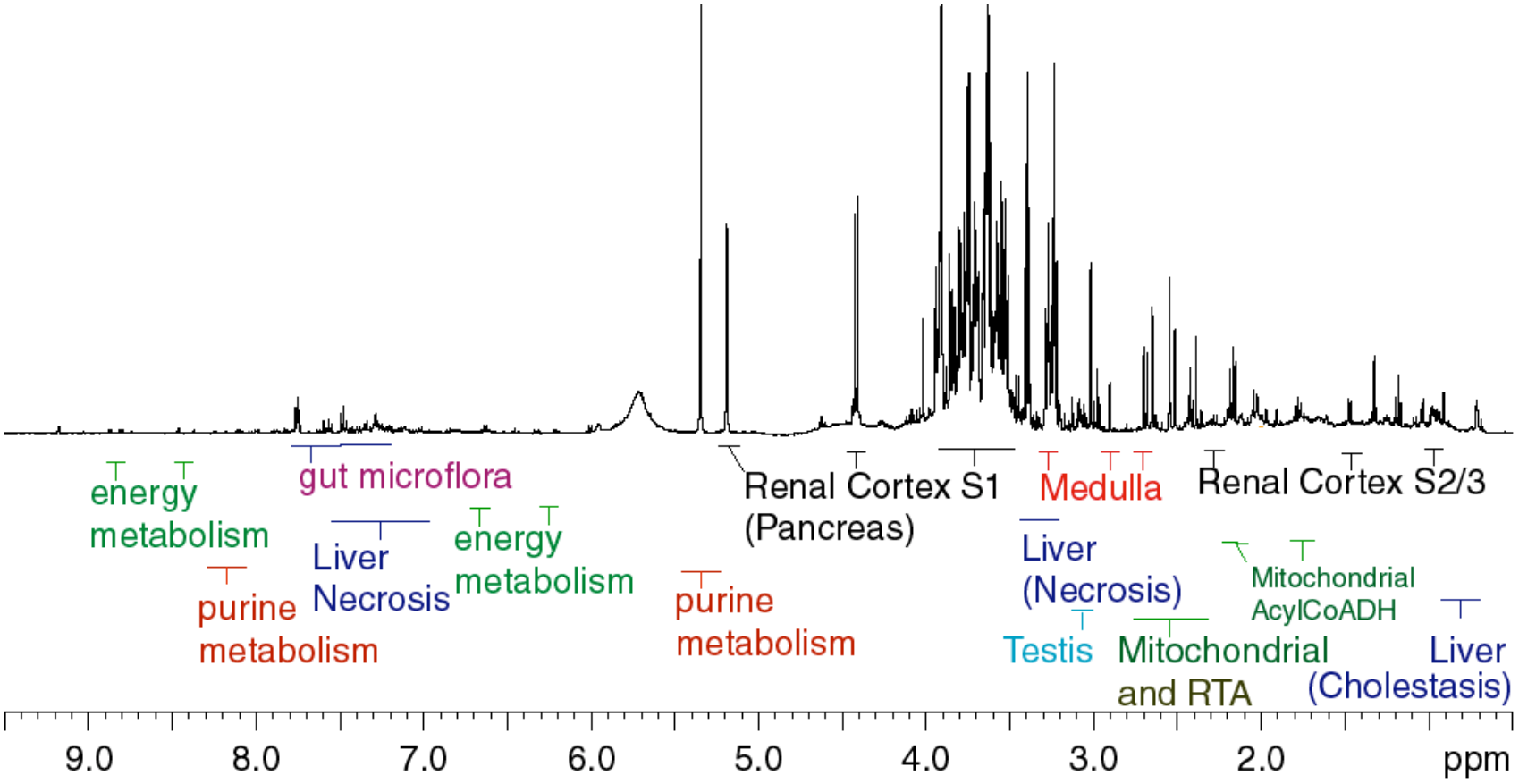
Metabolic Profiles Reflect Target Organ



Background

Metabolic Profiling as a "Functional Tool"

- ^{1}H NMR spectrum recorded at 600 MHz. Rat urine (abnormal), indicating regions reflecting selected biomarker regions/windows



Application of Metabonomics

Toxicological Applications:

COMET 1 / COMET 2 Projects

Consortium on Metabonomics in Toxicology (COMET)

- Research consortium comprised of 5 pharmaceutical companies, work steered by Imperial College over 3 years

- Pfizer Global R&D (Pharmacia Corporation)
  - Eli Lilly & Co Bristol-Myers Squibb
  - Novo Nordisk A/S F. Hoffmann-la Roche AG

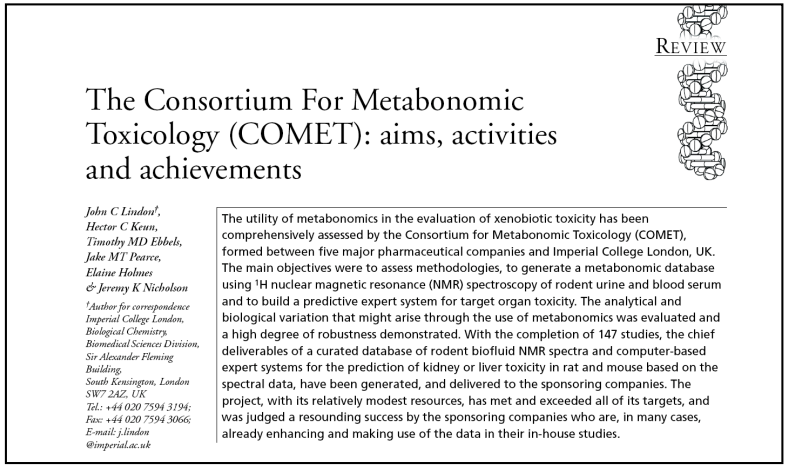
- Generation of a comprehensive database of NMR spectra of rodent biofluids after treatment with various toxicants (147 in total)
- Evaluation of chemometric models
- Predictive screening methodologies and development of novel biomarkers and methods for identifying them

- Now embarked on COMET-2 – mechanism studies

Toxicological Applications

The Consortium for Metabonomics Toxicology – COMET 1

- Academia (Imperial) and pharmaceutical companies
- 147 treatments including model toxins and dietary interventions
- 7 day single (low/high) and multi-dose toxicology studies in the rat and mouse
- Lindon *et al.*, Pharmacogenomics, 2005, **6**, 691
- Expert system developed - predictive models



REVIEW

The Consortium For Metabonomic Toxicology (COMET): aims, activities and achievements

John C Lindon†, Hector C Keun, Timothy MD Ebbels, Jake MT Pearce, Elaine Holmes & Jeremy K Nicholson

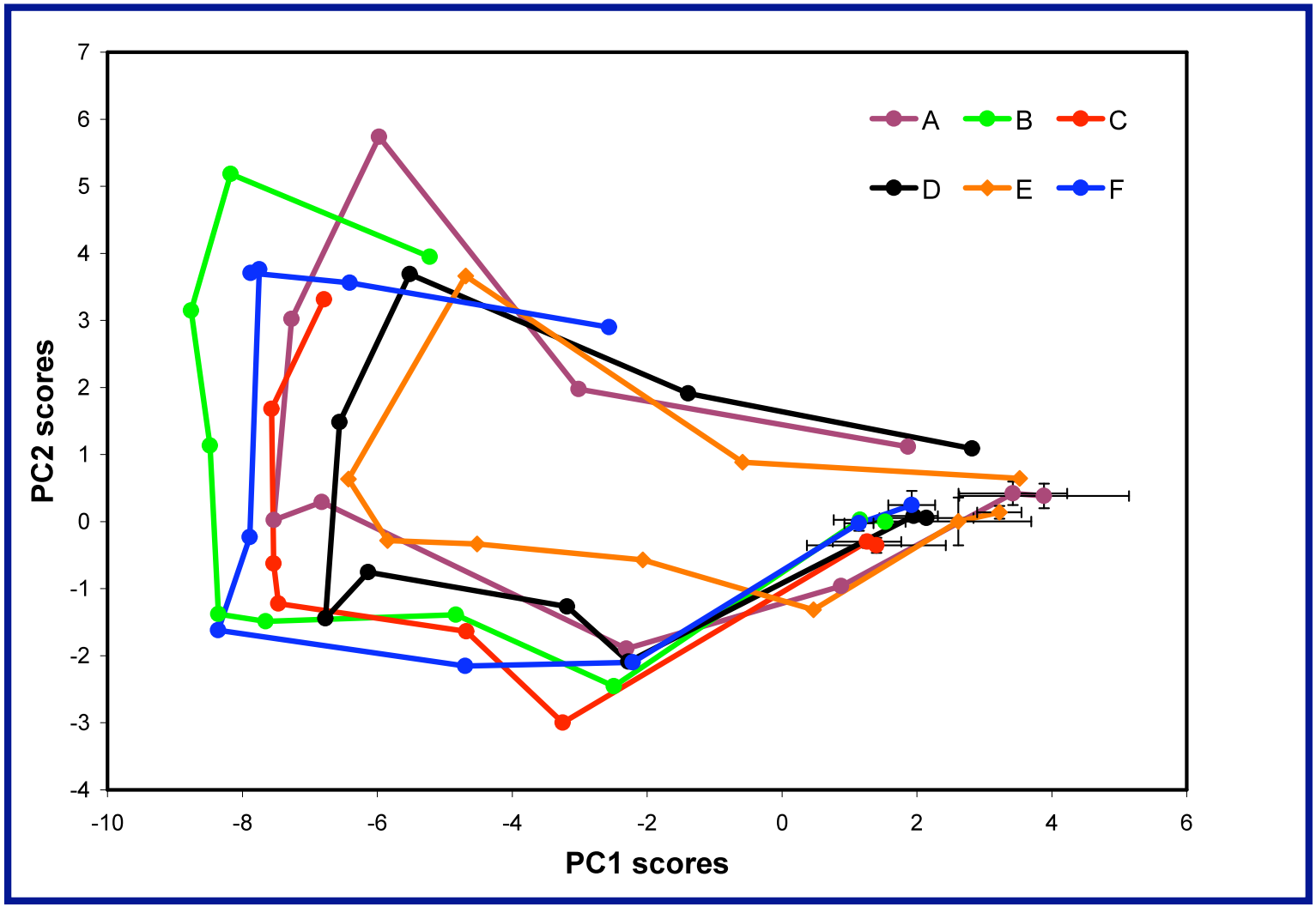
†Author for correspondence Imperial College London, Biological Chemistry, Biomedical Sciences Division, Sir Alexander Fleming Building, South Kensington, London SW7 2AZ, UK Tel.: +44 020 7594 3194; Fax: +44 020 7594 3066; E-mail: j.lindon @imperial.ac.uk

The utility of metabonomics in the evaluation of xenobiotic toxicity has been comprehensively assessed by the Consortium for Metabonomic Toxicology (COMET), formed between five major pharmaceutical companies and Imperial College London, UK. The main objectives were to assess methodologies, to generate a metabonomic database using ^{1}H nuclear magnetic resonance (NMR) spectroscopy of rodent urine and blood serum and to build a predictive expert system for target organ toxicity. The analytical and biological variation that might arise through the use of metabonomics was evaluated and a high degree of robustness demonstrated. With the completion of 147 studies, the chief deliverables of a curated database of rodent biofluid NMR spectra and computer-based expert systems for the prediction of kidney or liver toxicity in rat and mouse based on the spectral data, have been generated, and delivered to the sponsoring companies. The project, with its relatively modest resources, has met and exceeded all of its targets, and was judged a resounding success by the sponsoring companies who are, in many cases, already enhancing and making use of the data in their in-house studies.

Treatment
Sham surgery, partial hepatectomy, unilateral nephrectomy, phenobarbital, probenecid, pregnenolone-16α-carbonitrile, choline + methionine deficiency, insulin, food restriction, water deprivation,pentenoic acid, 2,4 dinitrophenol, acivicin, maleic acid + trichloroethylene, rosiglitazone (chron), rosiglitazone (acute), acetazoleamide, furosemide, carboplatin, ammonium chloride, methotrexate, sodium bicarbonate
Dexamethasone, streptozotocin, caerulein, 1-cyano-2-hydroxy-3-butene, L-arginine
Gadolinium chloride, lipopolysaccharide, galactosamine, clofibrate, clofibrate (duplicate), diethylhexylphthalate, sodium valproate, bromobenzene, methapyrilene, 1-fluoropentane, azathioprine, lithocholic acid, retinyl palmitate, 4-amino-2, 6-dichlorophenol, cyproterone, ANIT, allyl alcohol, BHT, chlorpromazine, ethionine, dimethylformamide, *N*-methyl formamide, $FeSO_4$, allyl formate, trichloroethylene, monocrotaline, DMN, lead acetate, buthinionine sulphoxide, aflatoxin, carbon tetrachloride, WY14,643, dichlorobenzene, phalloidin, WY14,643 - multidose, paraquat, ketoconazole, microcystin-LR, acetaminophen (acute), acetaminophen (chronic), dichloroethylene, rotenone, indomethacin, methylene dianiline, phenyl isothiocyanate, 1,2,3,4,5,6-hexa-chlorocyclohexane, phenyl diisothiocyanate
Cisplatin, puromycin, gentamycin, folic acid, cisplatin, puromycin, *N*-phenylanthranilic acid, D-limonene, 2-chloroethanamine, adriamycin, mercury chloride, hexachlorobutadiene, vancomycin, maleic acid, aurothiomalate, 2-bromoethanamine, cephaloridine, ethylene glycol, para-aminophenol, 2-bromophenol, 3,5-dichloroaniline-HCl, atractyloside, *N*-dichlorophenyl succinimide
Cadmium chloride, carbendazim, ethane-(dimethane sulfonate), 1,3-dinitrobenzene, cadmium chloride, methoxyacetic acid, di-n-pentyl phthalate
Ifosfamide
Phloracetophenone, chloroform, azaserine, amphotericin B, thioacetamide, cyclosporin A, potassium dichromate, mitomycin-C, S-(1,2-dichlorovinyl)-L-cysteine
Hydrazine (7 studies), hydrazine (in Wistar rats)

Metabolic Trajectories

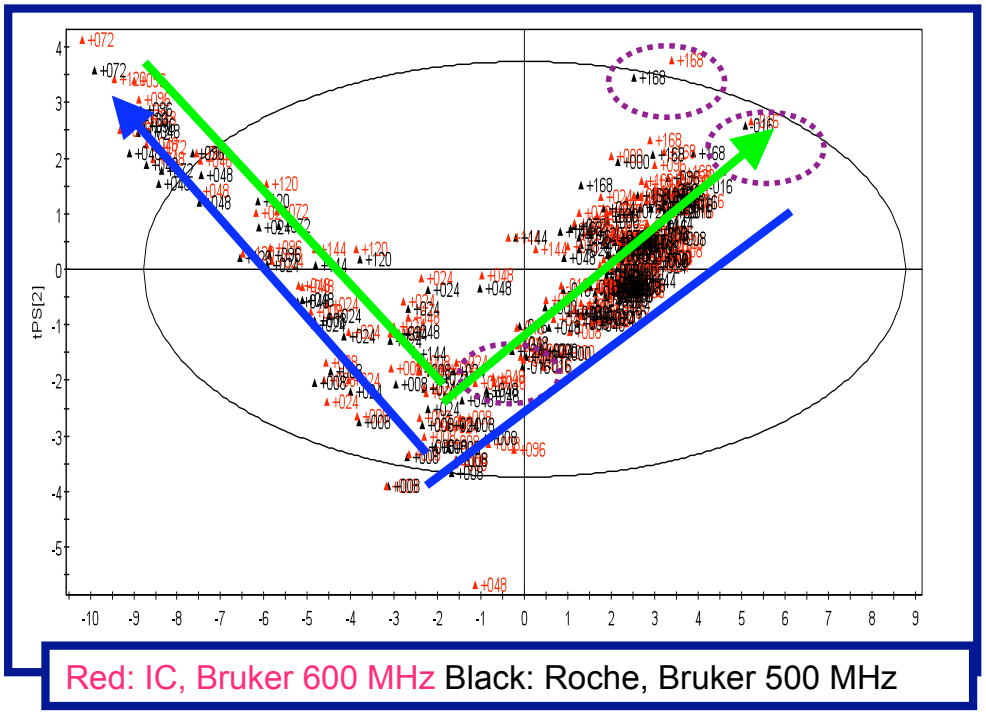
The Consortium for Metabonomics Toxicology – COMET 1



Testing Analytical and Biological Variation

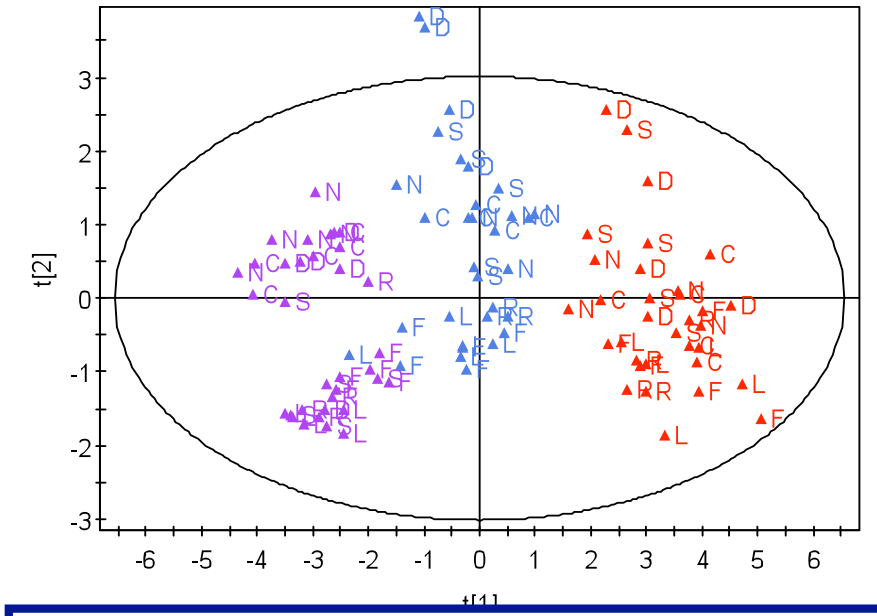
The Consortium for Metabonomics Toxicology – COMET 1

Inter-site NMR spectroscopy comparison using urine samples



Red: IC, Bruker 600 MHz Black: Roche, Bruker 500 MHz

Inter-site sample reproducibility comparison using diffusion-edited NMR spectra of serum



R-Roche, C-Covance, L-Lilly, N-Novo, D-Dupont/BMS, S-Pharmacia, F-Pfizer

48 h post dose

90 mg/kg, 30 mg/kg, control

Modeling Normality

The Consortium for Metabonomics Toxicology – COMET 1

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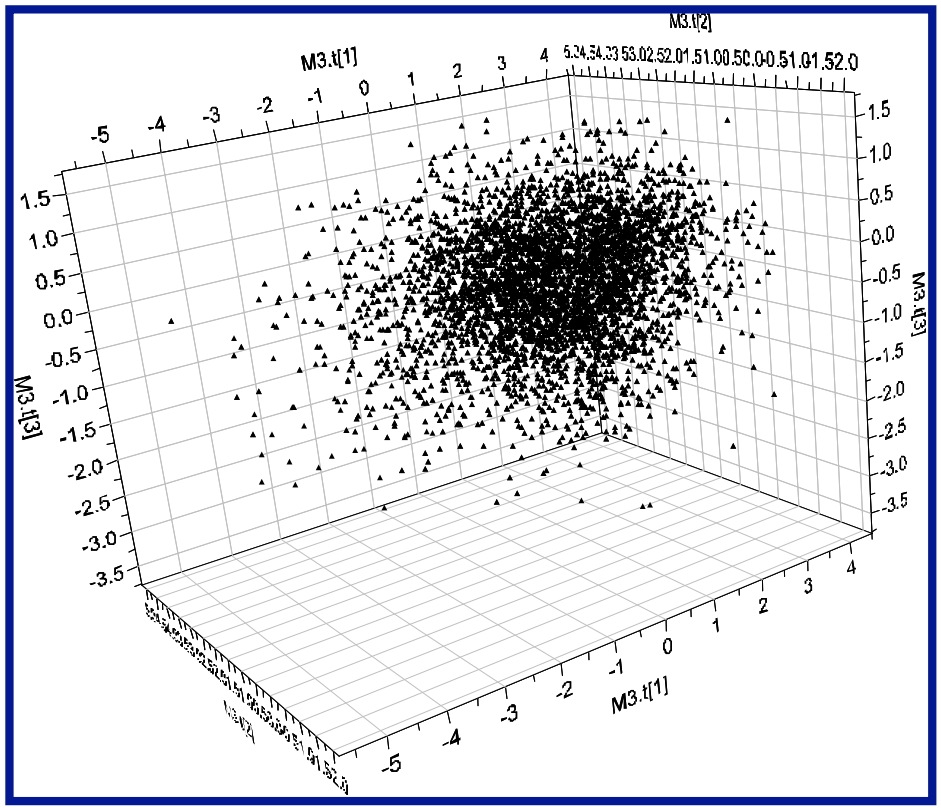
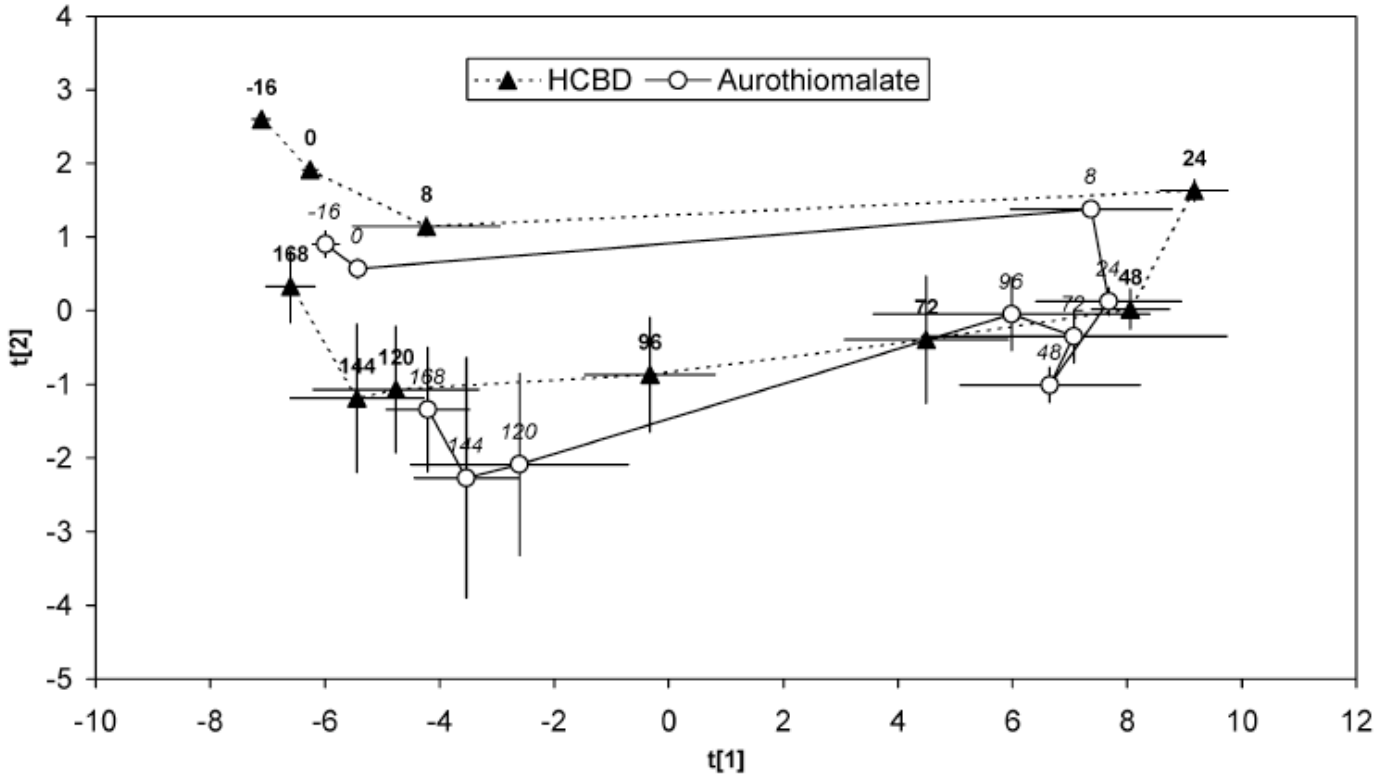


PCA of 5,000 600 MHz urine ^{1}H NMR spectra from control Sprague Dawley rats

Toxicological Applications

The Consortium for Metabonomics Toxicology – COMET 1

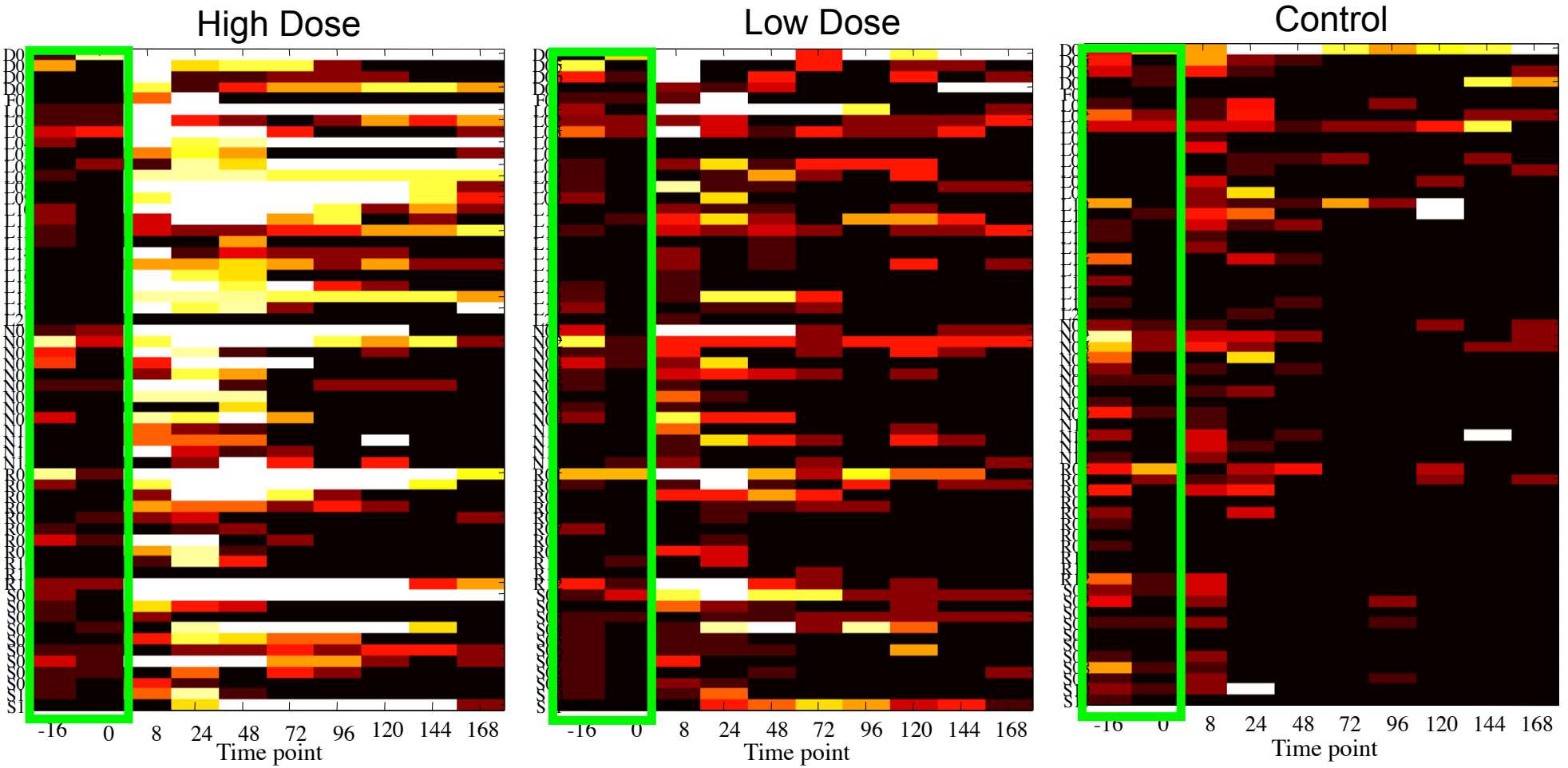
- Used ^{1}H NMR of rodent biofluids and tissue to derive an expert system for target organ toxicity
- 147 treatments including model compounds and dietary intervention
- Models of metabolic change over *time* (onset, progression, recovery)



Assessment of temporal profile response similarity

Toxicological Applications

Heat map - key: black = control like, white = most abnormal



74% of studies correctly predicted to a single organ of effect correctly (liver or kidney), 20% are predicted to more than one organ of toxicity, 6% are predicted incorrectly but the classifications can be explained (J.C. Lindon *et al*. The Consortium for Metabonomic Toxicology (COMET): aims, activities and achievements. *Pharmacogenomics* 6, 691, (2005)

Toxicological Applications

The Consortium for Metabonomics Toxicology – COMET 1

- Used ^{1}H NMR of rodent biofluids and tissue to derive an expert system for target organ toxicity

- 147 treatments including model compounds and dietary intervention

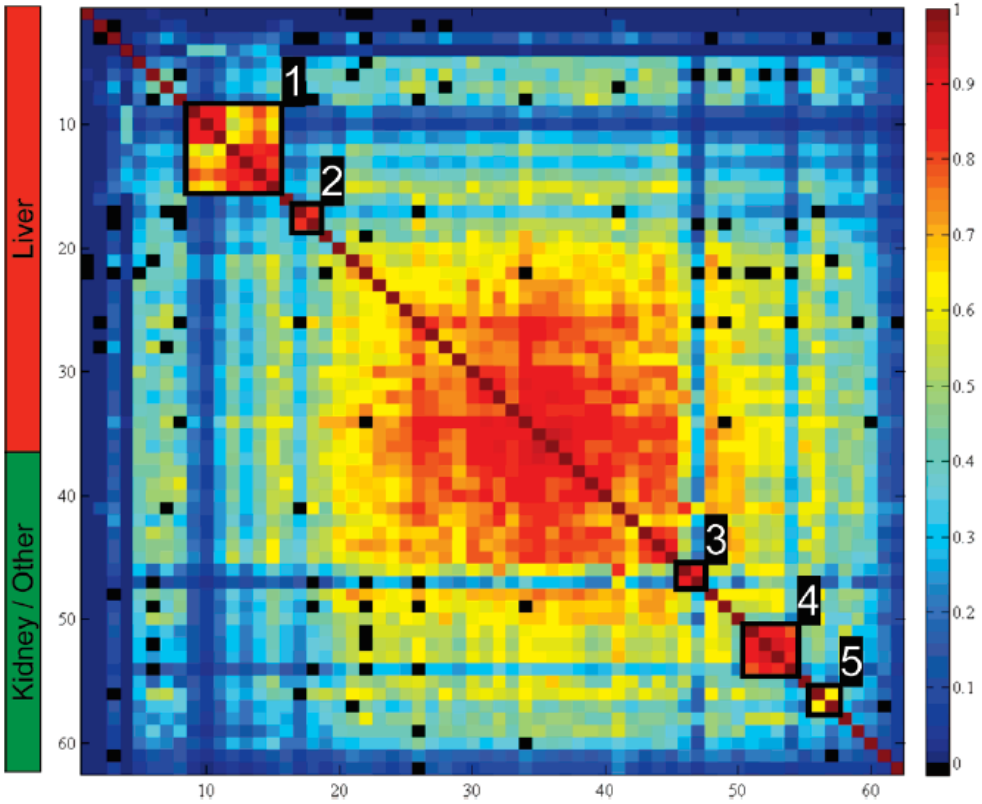
Block 1 – Hydrazine (replicate studies)

Block 2 – Paracetamol

Block 3 – endocrine toxins
(models for diabetes, increased urinary glucose)

Block 4 – tubular renal toxins (cisplatin, $HgCl_2$)

Block 5 – only 2 papillary toxins (BEA and CEA)



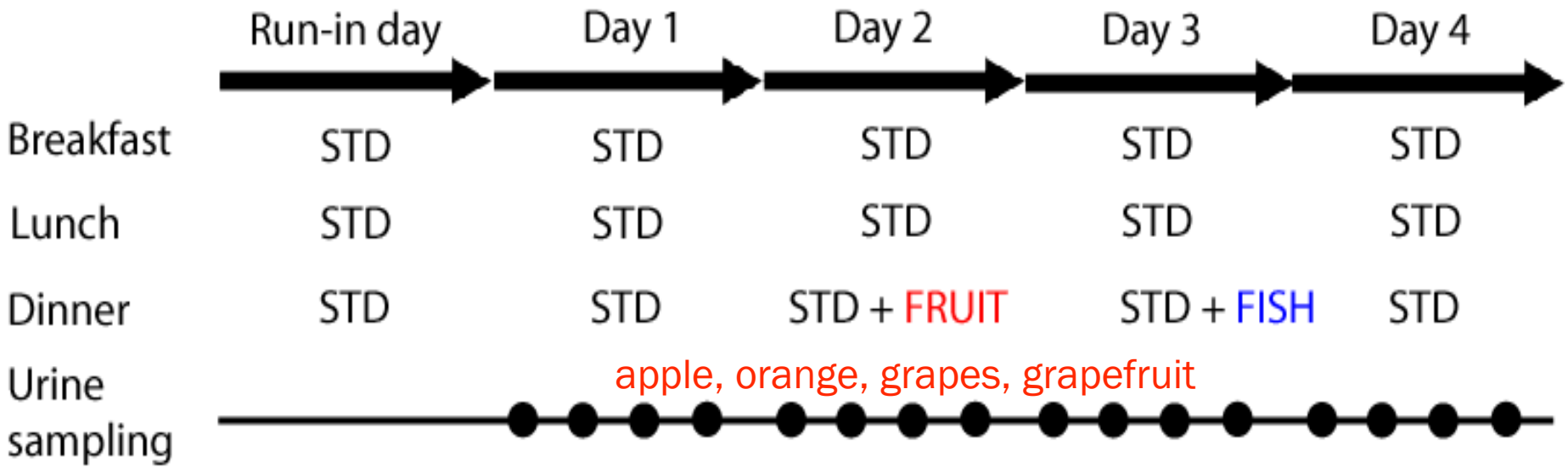
Clustering compound effects by similarity of urine profile response

Other Applications

Nutritional / Exposure Biomarkers

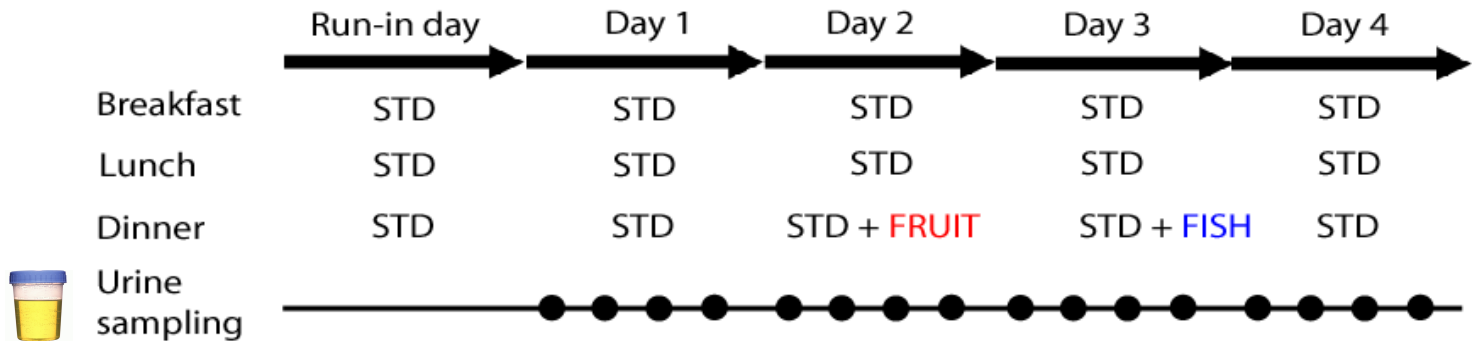
Biochemical indicators of nutrient ingestion / absorption
A food intervention study to find food biomarkers (of exposure/consumption) *via* metabolic profiling

8 volunteers (healthy, 18-45, non-smokers, BMI 18-25, no medication use)
Standardised breakfast, lunch and dinner according to diet group
Urine collections 4/day



Other Applications

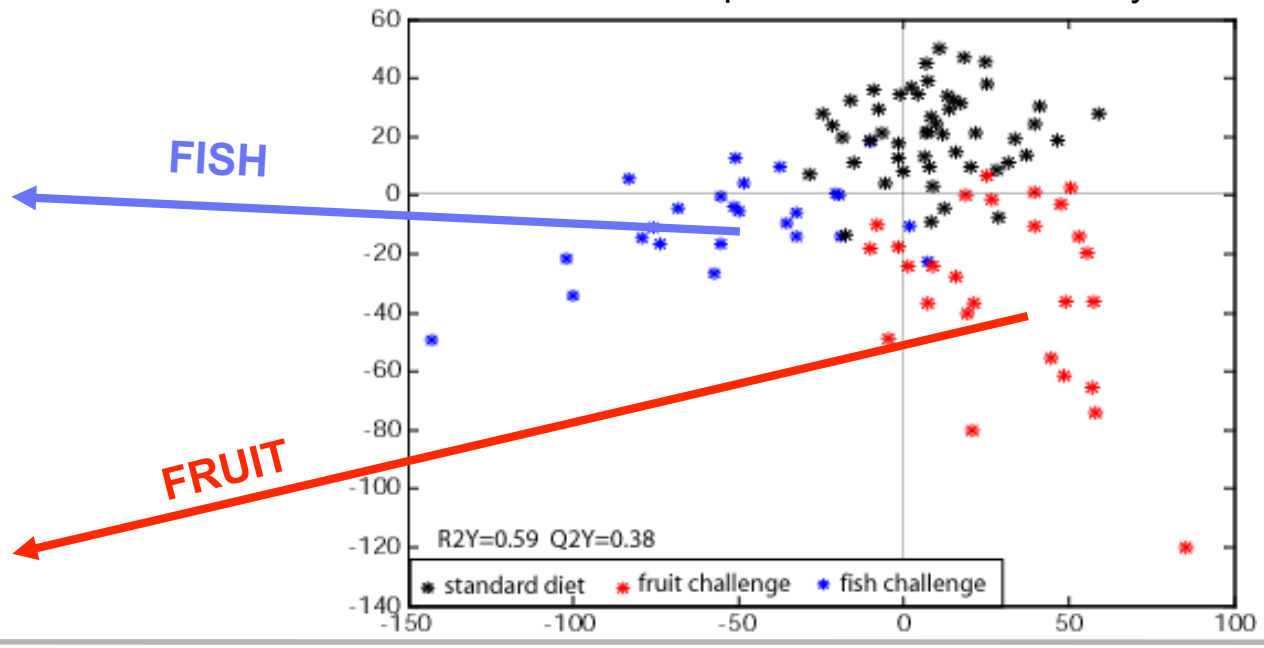
Elucidation of potential food biomarkers from metabolic profiles with pattern recognition



Increased excretion of:
- trimethylamine-*N*-oxide
- taurine
- methylhistidine
- creatine
- choline
- carnitine

Increased excretion of:
- proline betaine
- tartaric acid
- hippuric acid

Partial least squares discriminant analysis

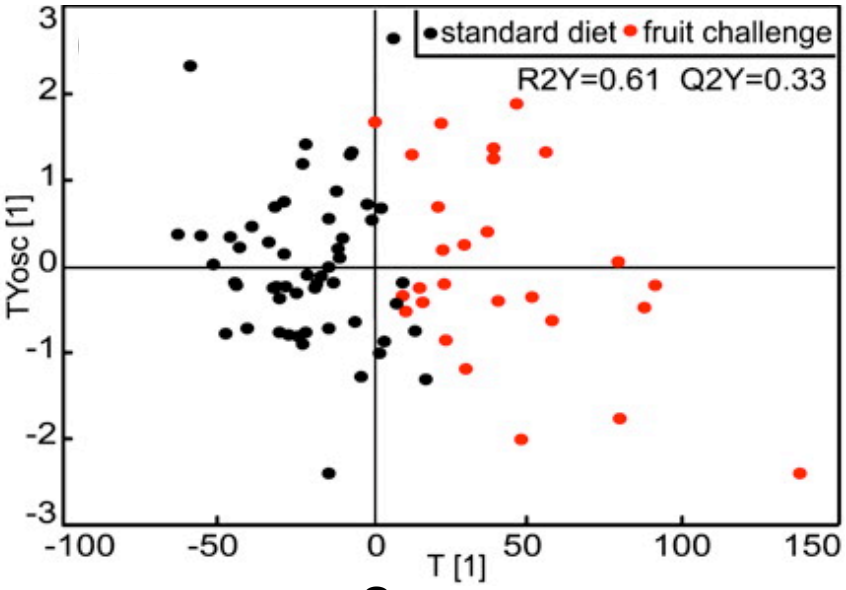


Other Applications

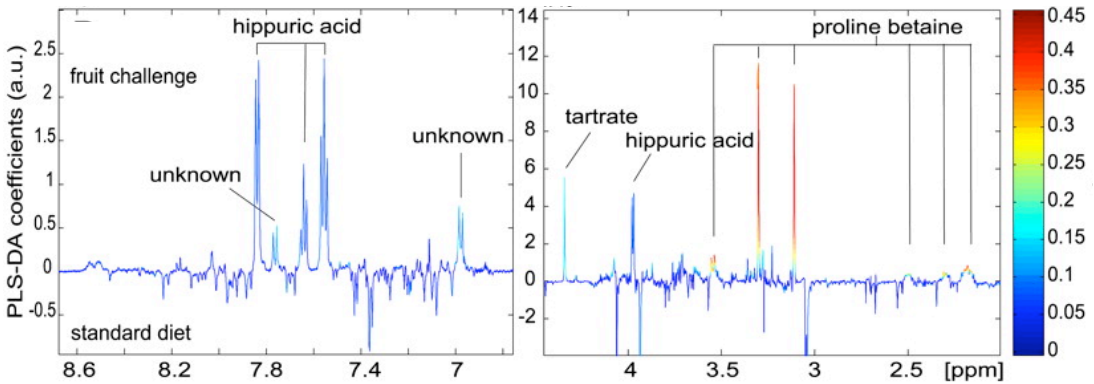
Focus on urinary composition with fruit challenge

Pattern recognition indicates clearly regions of the spectrum that discriminate between the challenge groups

Visualisation of the model coefficients on a spectral plot is useful for spectroscopists for assignment



Scores

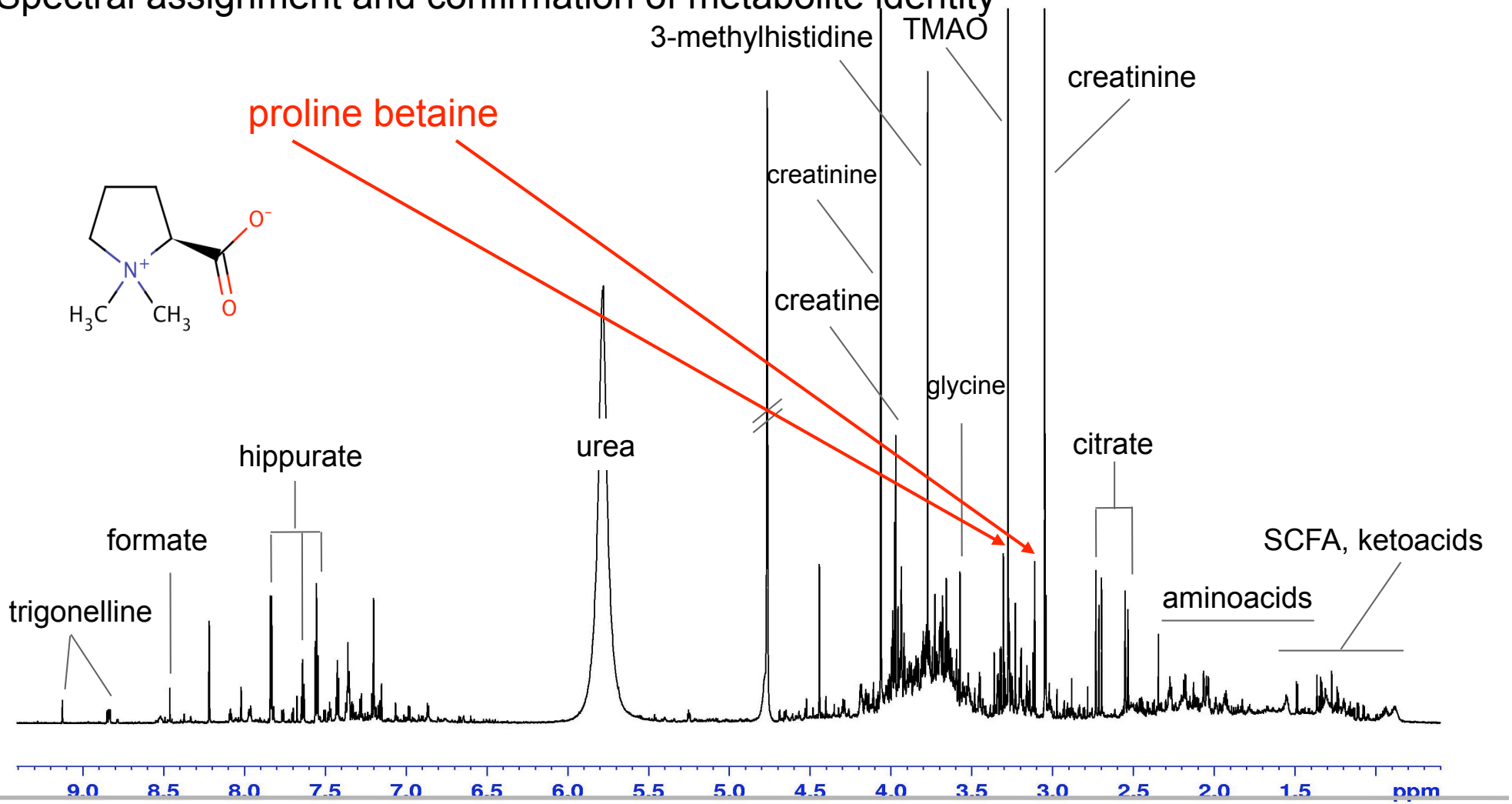


Loadings

Other Applications

Biomarker elucidation by ^{1}H NMR spectroscopy

Spectral assignment and confirmation of metabolite identity



Other Applications

Molecular Epidemiology - The INTERMAP Population Study

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

Study Design
Urine samples 2 x 24 hr
Collected 3-6 weeks apart
17 centres including Peoples Republic of China (PRC), Japan, US and UK
Total of 4,680 people
~ 10,000 samples including quality control split samples
4 visits were made by each person

^{1}H NMR spectra were acquired for each sample

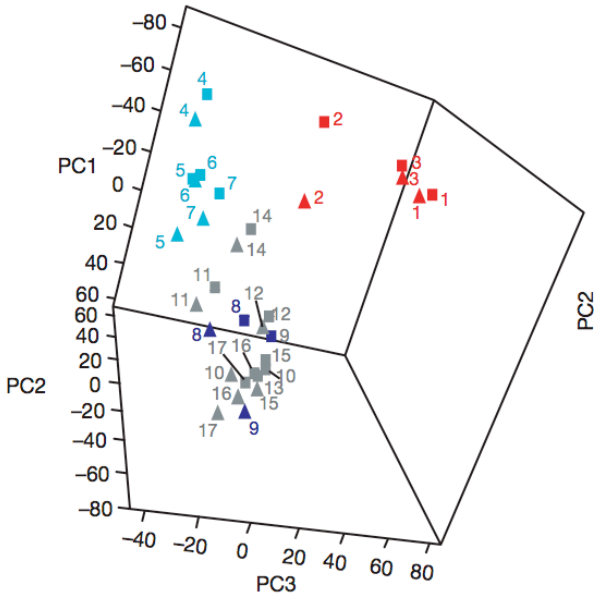
Measurements:
Blood pressure - seated (2 x for 4 visits)
Height and weight (2 visits)
Daily alcohol over previous 7 days questionnaire (2 visits)
Dietary intake (including vitamin/mineral supplements)
    Recorded using 24 hr recall method (4 visits)
Demographic and medical history questionnaire (1 visit)

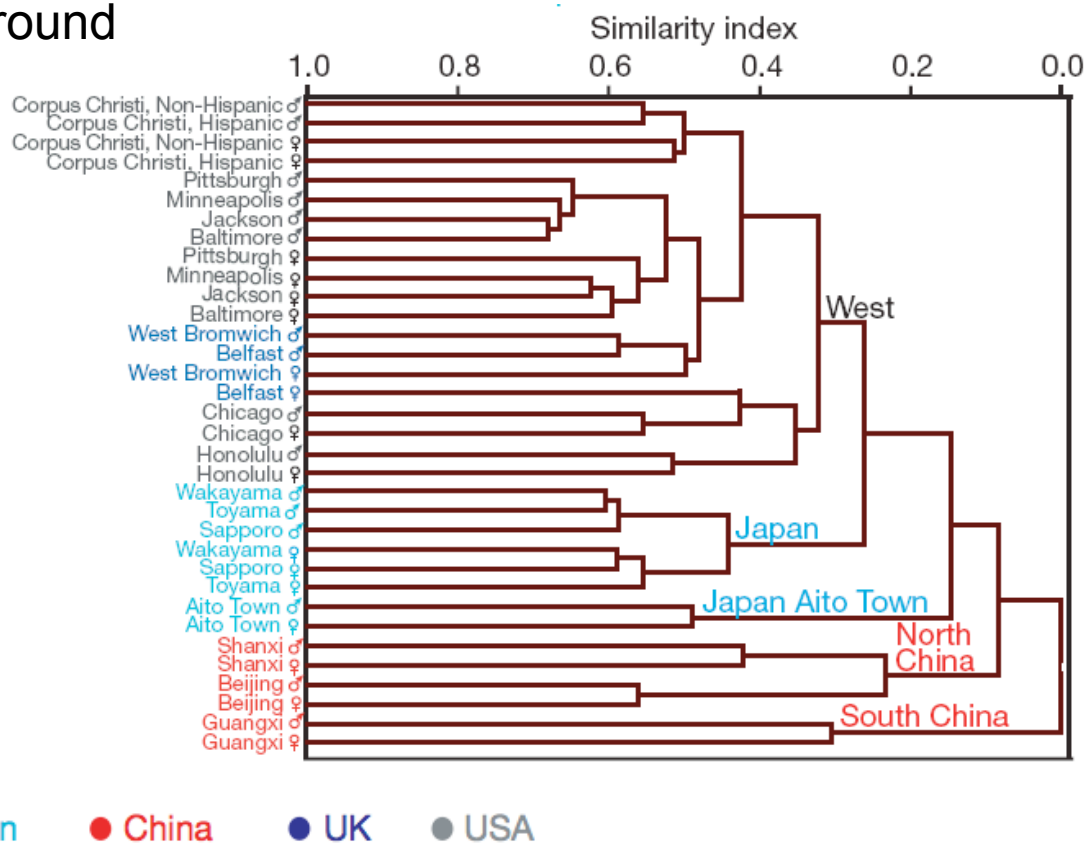
Other Applications

Molecular Epidemiology - The INTERMAP Population Study

Metabolites responsible for discriminating populations and diet, also showed significant associations to blood pressure in individuals

Differences NOT just genetic background





● Japan ● China ● UK ● USA

PCA HCA

Holmes E *et al.* 2008. Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature* 453 396-400.

Other Applications

Molecular Epidemiology - The INTERMAP Population Study

Metabolites responsible for discriminating populations and diet, also showed significant associations to blood pressure in individuals

Differences NOT just genetic background

Table 1 | Estimated mean differences in systolic and diastolic BP

Urinary metabolite	A*				B†			
	Not adjusted for BMI‡		Adjusted for BMI‡		Not adjusted for BMI‡		Adjusted for BMI‡	
	Systolic blood pressure (mm Hg)							
Alanine	2.69	(6.06)	0.40	(0.92)	2.66	(5.54)	1.13	(2.43)
Formate	−1.19	(−2.62)	−1.42	(−3.29)	−1.94	(−3.92)	−1.04	(−2.20)
Hippurate	−2.10	(−4.85)	−1.63	(−3.95)	−1.72	(−3.70)	−0.82	(−1.83)
N-methylnicotinate	−0.09	(−0.21)	0.20	(0.49)	0.00	(0.00)	0.65	(1.53)
	Diastolic blood pressure (mm Hg)							
Alanine	1.57	(5.17)	0.17	(0.55)	1.58	(4.77)	0.61	(1.90)
Formate	−0.90	(−2.96)	−1.02	(−3.49)	−1.41	(−4.22)	−0.86	(−2.65)
Hippurate	−0.98	(−3.33)	−0.71	(−2.50)	−0.77	(−2.42)	−0.23	(−0.73)
N-methylnicotinate	−0.07	(−0.25)	0.09	(0.32)	−0.01	(−0.03)	0.37	(1.27)

Systolic and diastolic blood pressure differences per +2 s.d. difference in each of four quantified urinary metabolites (mean of two 24-h urine values). Numbers in parentheses are *Z* scores, that is, regression coefficient divided by standard error (Z-score ≥ 1.96, $P < 0.05$; ≥ 2.58, $P < 0.01$; ≥ 3.29, $P < 0.001$; ≥ 3.89, $P < 0.0001$). 2 s.d. difference for alanine = 0.34 mmol per 24 h ($n = 4{,}232$); formate = 0.29 mmol per 24 h ($n = 4{,}147$); hippurate = 3.55 mmol per 24 h ($n = 4{,}184$); *N*-methylnicotinate = 0.41 mmol per 24 h ($n = 4{,}081$) (chemical shifts used for quantification: alanine, δ 1.48; formate, δ 8.45; hippurate, δ 7.85 and *N*-methylnicotinate, δ 4.44). Regression coefficients for individuals are pooled across countries (Methods); there is no evidence for cross-country heterogeneity in size of coefficients.

* A: Adjusted for age, sex, sample, special diet, supplement use, cardiovascular disease or diabetes mellitus diagnosis, physical activity (h per 24 h moderate or heavy activity), family history of high blood pressure.

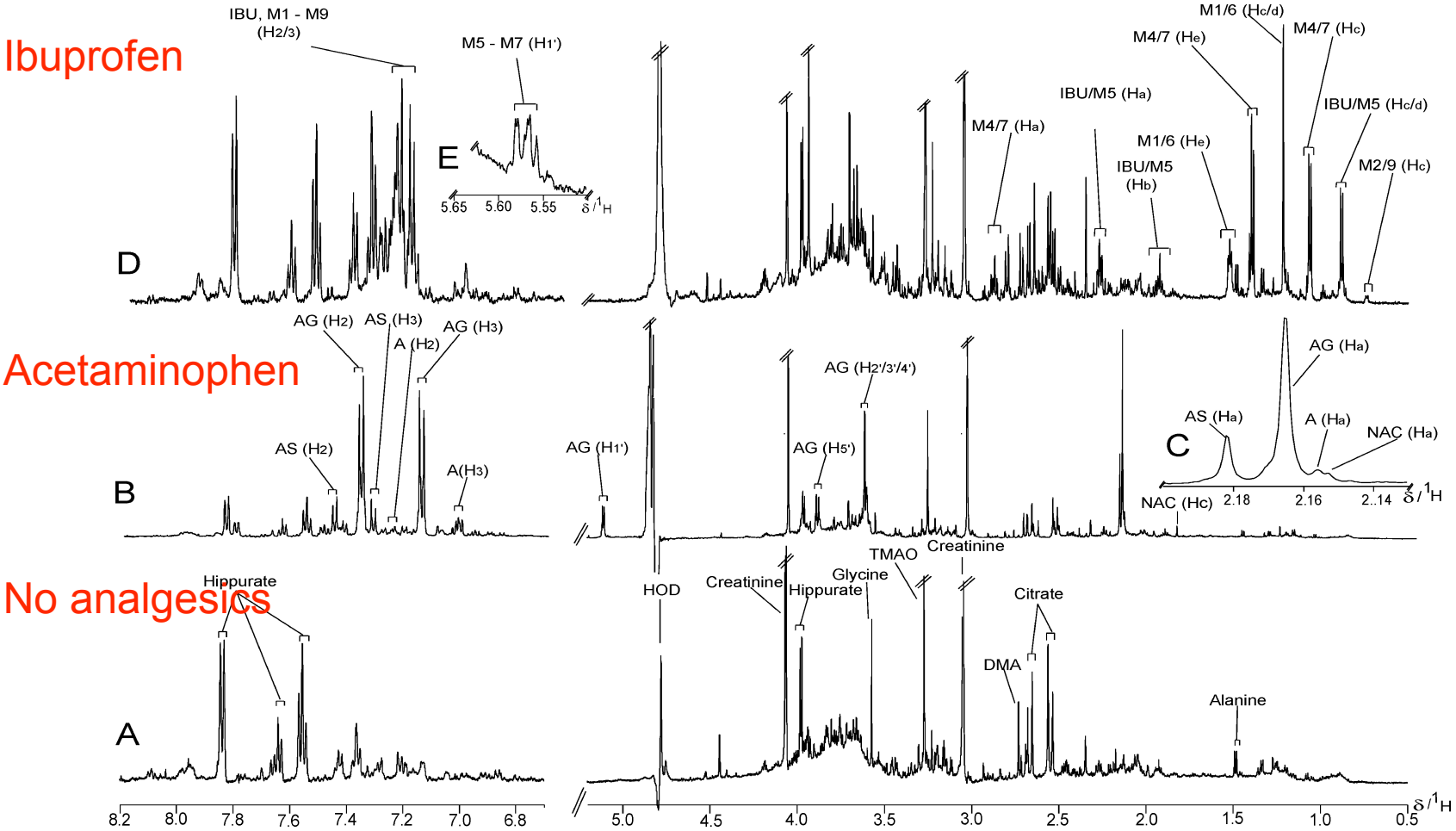
† B: A + 7-day alcohol (g per 24 h) + urinary Na^+ (mmol per 24 h) + urinary K^+ excretion (mmol per 24 h).

‡ Body mass index ($kg\,m^{-2}$).

Holmes E *et al.* 2008. Human metabolic phenotype diversity and its association with diet and blood pressure. *Nature* 453 396-400.

Other Applications

Molecular Epidemiology - The INTERMAP Population Study

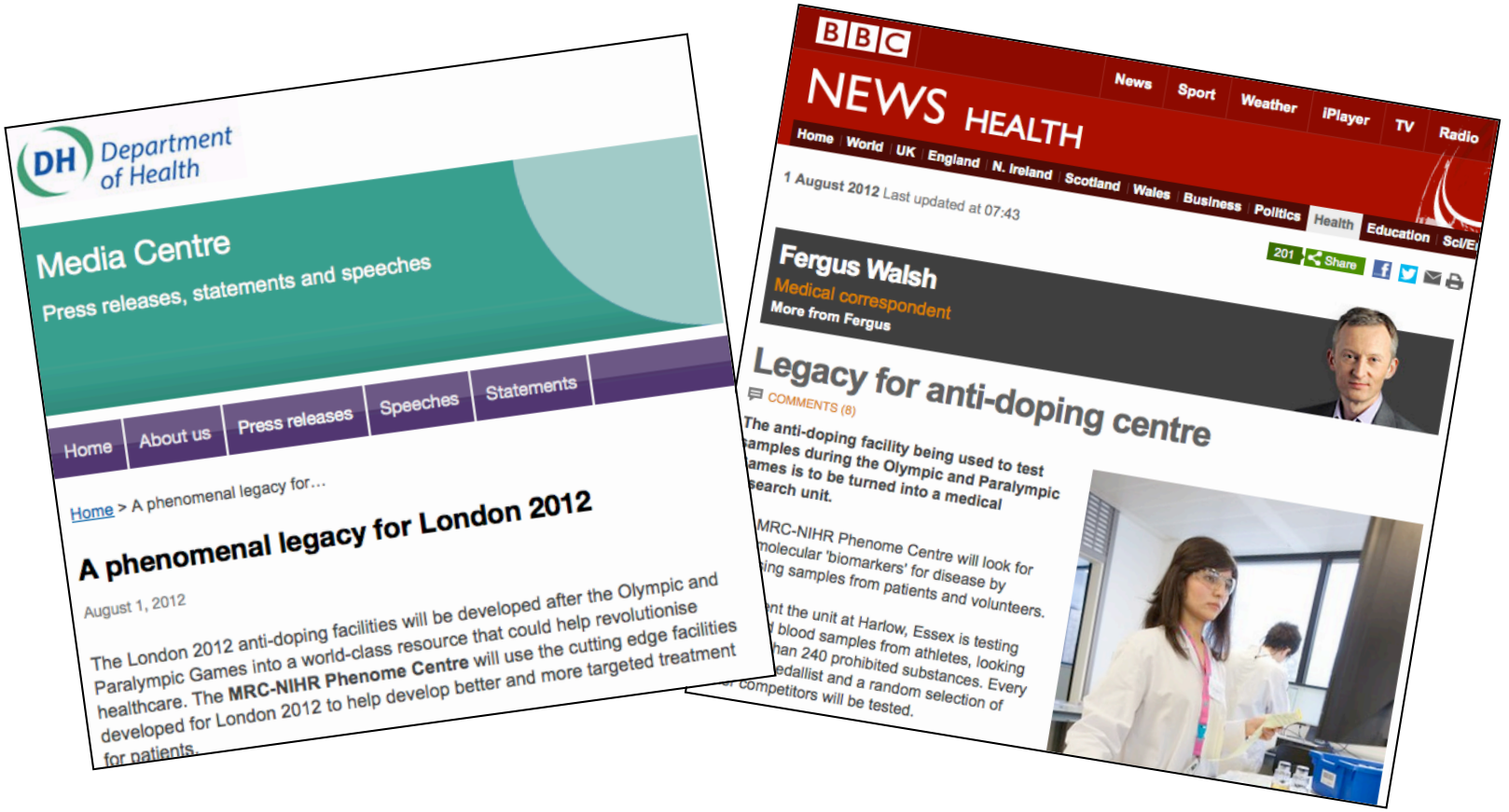


Typical 1H NMR spectra of urine from participants: (A) no analgesic intake; (B) after ingestion of acetaminophen; (C) expansion on acetaminophen region at δ 2.16; (D) after ingestion of ibuprofen; (E) showing the characteristic of the anomeric proton signal of the ibuprofen glucuronide rings. For a key to the identity of ibuprofen metabolites, refer to Figures 1 and 6.

Recent Developments and Future Perspectives

A National Phenome Centre for the UK

The London 2012 Olympic and Paralympic Games required a large biospecimen testing facility. As part of the legacy, this facility will be repurposed for metabolic profiling of large samples set, principally from epidemiological cohorts.



Application of Metabonomics

References & Further Reading

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