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# Urinary metabolic profiling in inflammatory bowel disease

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# Background: Metabolic profiling

- Metabolic profiling or “metabonomics” describes the generation of metabolic information from biofluids or tissues
- NMR spectroscopy (NMRS)
  - simultaneous acquisition of multiple biochemical parameters
- Urinary metabolic profiling
  - study specific diseases based on underlying metabolic processes
  - no such application to IBD

# Rationale for metabolic profiling in IBD

**Gut microbiota** differ between CD, UC and healthy controls

**Gut microbiota** have important influences on specific urinary metabolites:

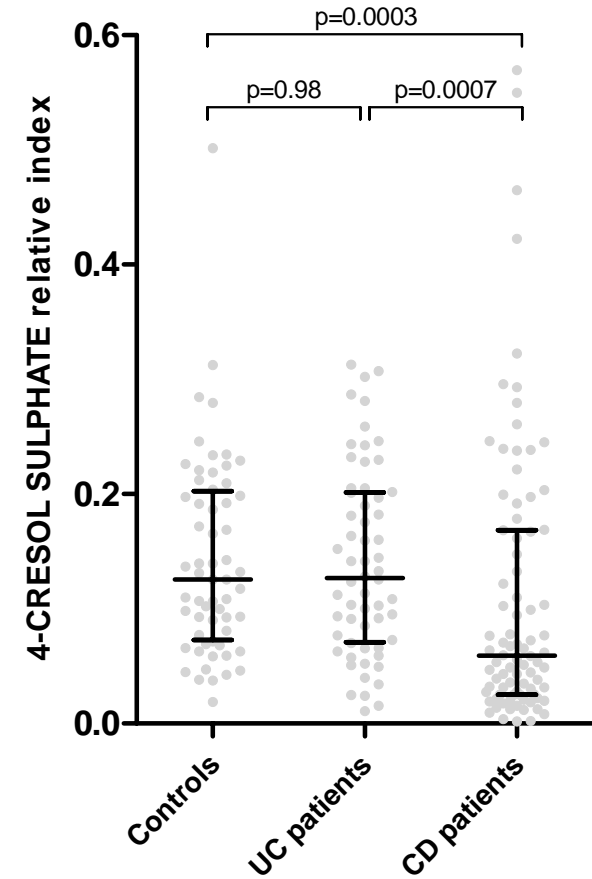
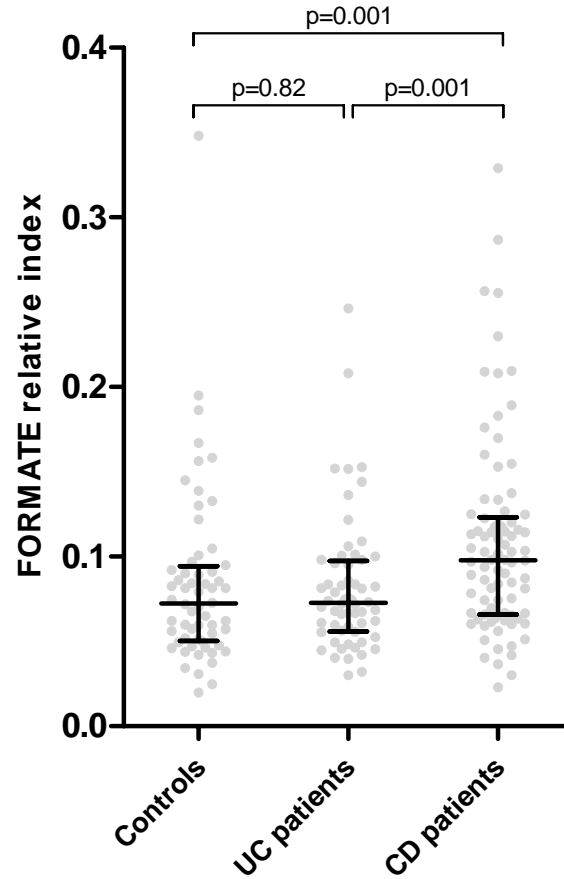
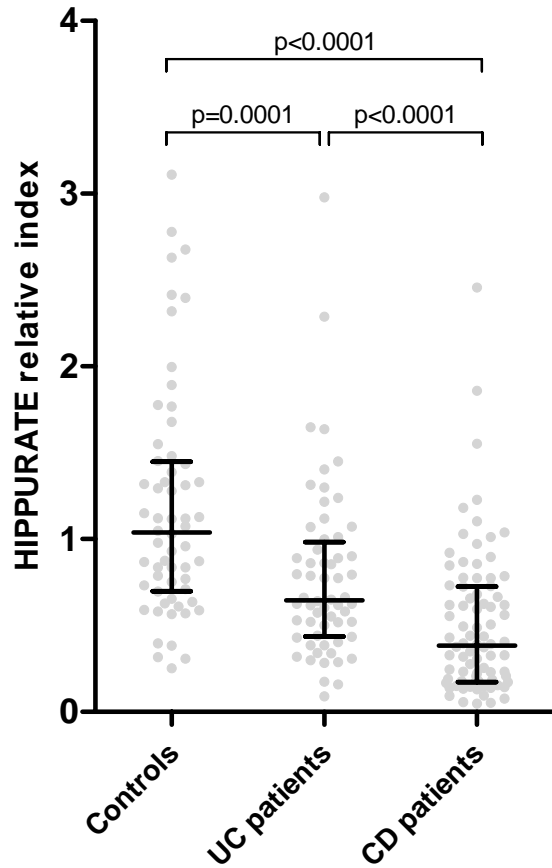
- Hippurate
- Formate
- 4-cresol sulphate
- Dimethylamine
- TMAO

# Experimental design: subject groups

	<b>Crohn's disease</b>	<b>Ulcerative colitis</b>	<b>Healthy controls</b>
<b>Number (Male/Female)</b>	86 (47/39)	60 (30/30)	60 (30/30)
<b>Median [range] age (years)</b>	33 [16-66]	40 [17-66]	30 [18-61]
<b>Disease location</b>	L1: 18	E1: 14	-
	L2: 25	E2: 18	-
	L3: 43	E3: 28	-
<b>Longitudinal Samples</b>	35	14	26

- Urinary NMR Spectra acquired using JEOL 500MHz Eclipse+ NMR spectrometer
- Largest urinary metabonomic study in any disease to date

# Hypothesis-driven analysis

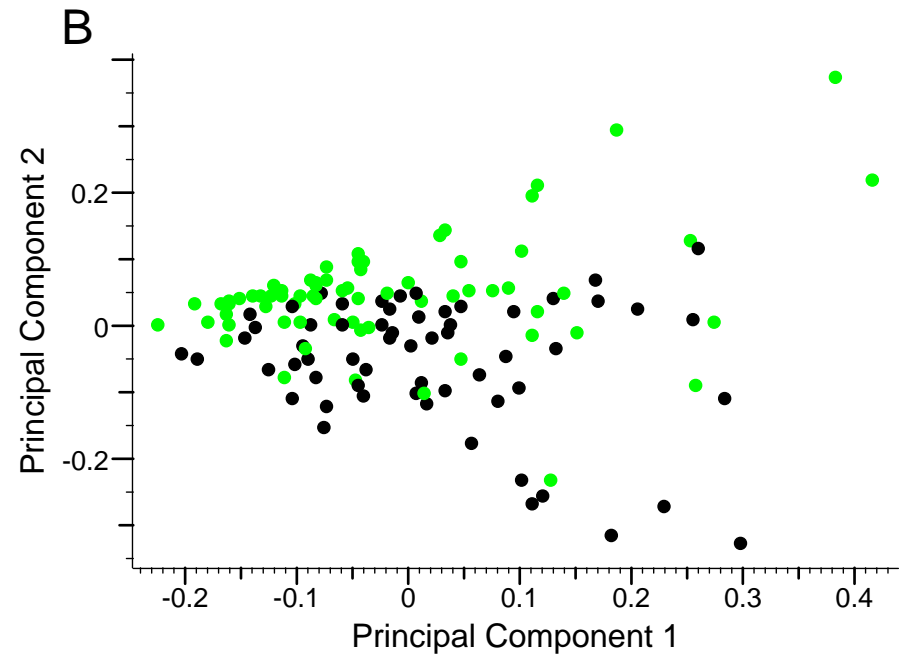
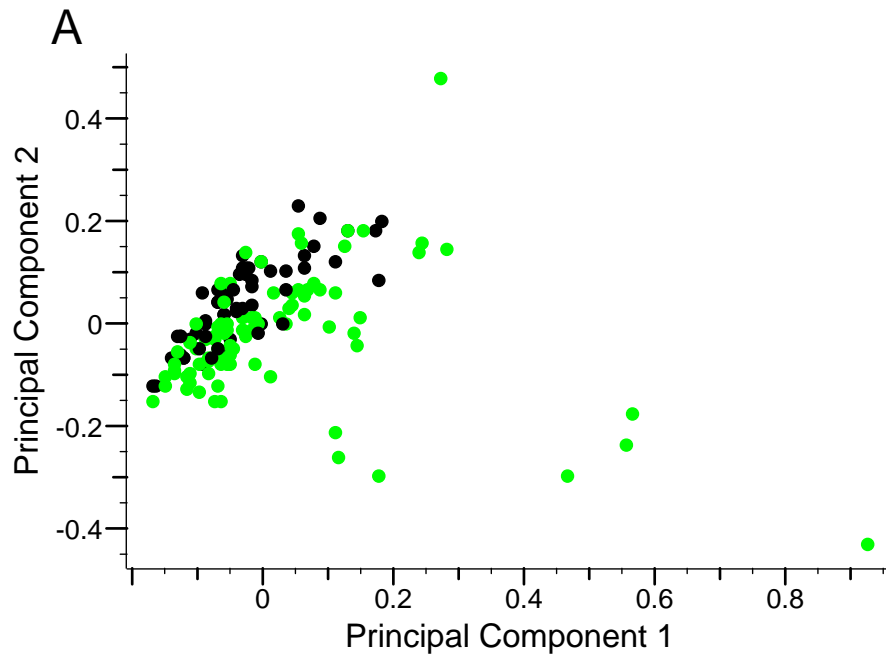


# Multivariate factor analysis

## Principal Components Analysis (PCA)

- Assumes no *a priori* knowledge
- Overview of the samples, analysing the whole spectrum
- Principal components are linear combinations of variables (metabolites) accounting for the greatest variation within the dataset
- Scores plot: each sample represented in the new coordinate space
- Loadings plot: combination of metabolites responsible for the scores plot

# Multivariate factor analysis



A: PCA scores plot: 86 CD patients and 60 controls.

B: PCA scores plot: 68 CD patients and 60 controls (excluding outliers).

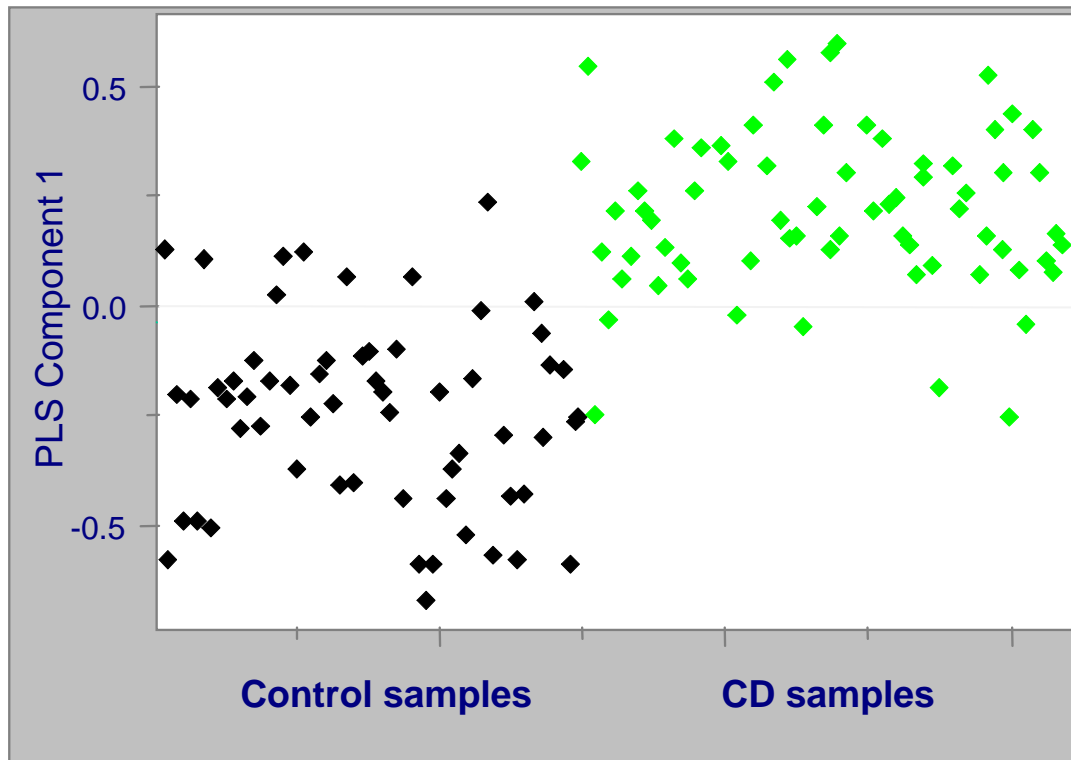


# Multivariate factor analysis

## **Partial least squares discriminant analysis (PLS-DA)**

- NMR spectroscopic variables (metabolites) are related to class membership
- Scores plots generated
- Loadings plots and regression vector: identification of spectral regions (metabolites) responsible for separation between groups
- Orthogonal Signal Correction (OSC)
- Rigorous validation techniques

# Multivariate factor analysis



	CD	Control
CD	61	7
Control	10	50

**Sensitivity: 90%**

**Specificity: 83%**

◆ **CD**  
◆ **Control**

# PLS-DA models: CD vs. UC

	All individuals		Individuals on no medication
	CD: UC	L2 CD: UC	CD: UC
<b>Sensitivity: specificity</b>	<b>86: 82</b>	<b>79: 83</b>	<b>82: 97</b>

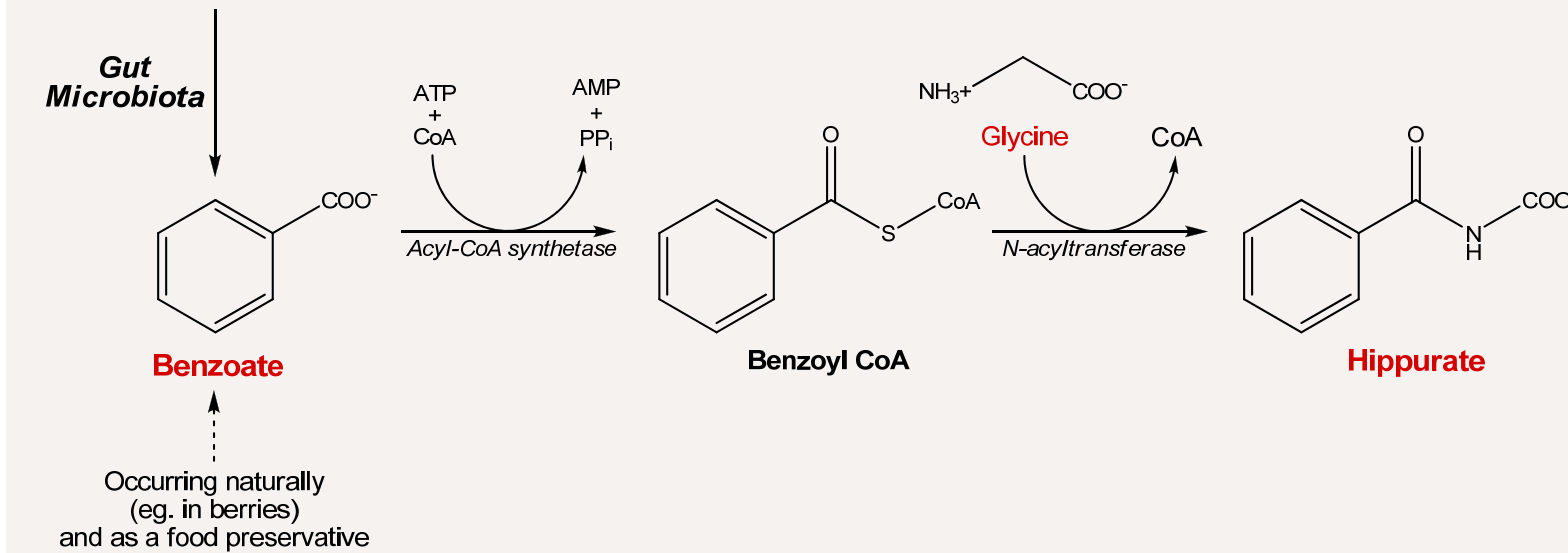
Results compare favourably with predictive abilities of ASCA / pANCA

## **Metabolites primarily responsible for distinguishing CD and UC:**

- HIPPURATE, citrate, methylhistidine, guanidoacetate, 4-cresol sulphate

# Hippurate Metabolism

## Dietary aromatic compounds

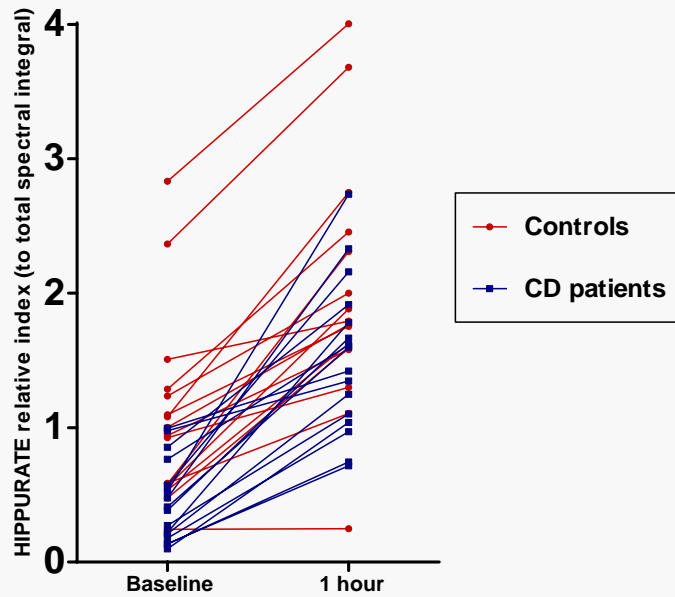


## Investigating Hippurate Metabolism in IBD

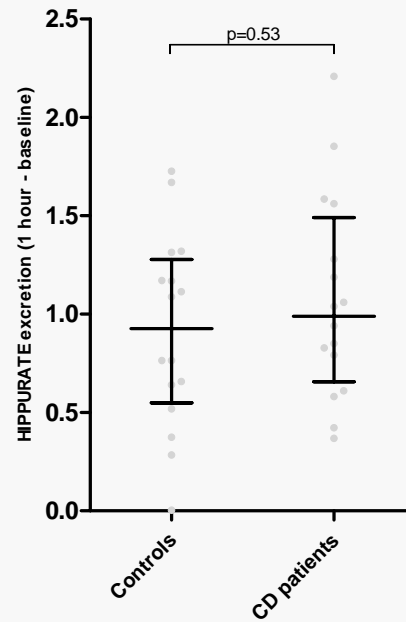
- 16 CD patients, 16 healthy controls
- Low benzoate diet
- Administered 5mg/kg sodium benzoate.

# Results

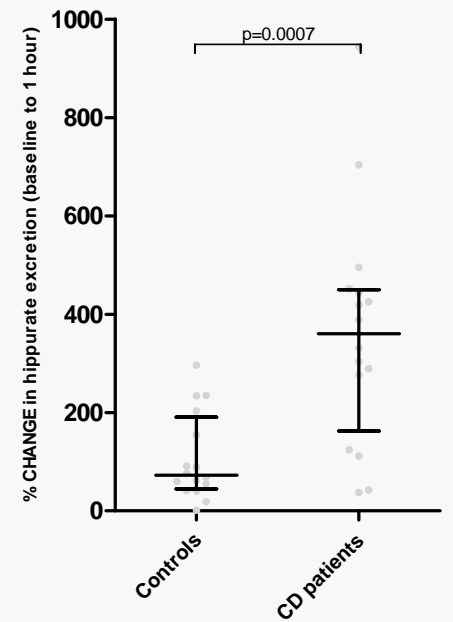
A



B



C



# Discussion

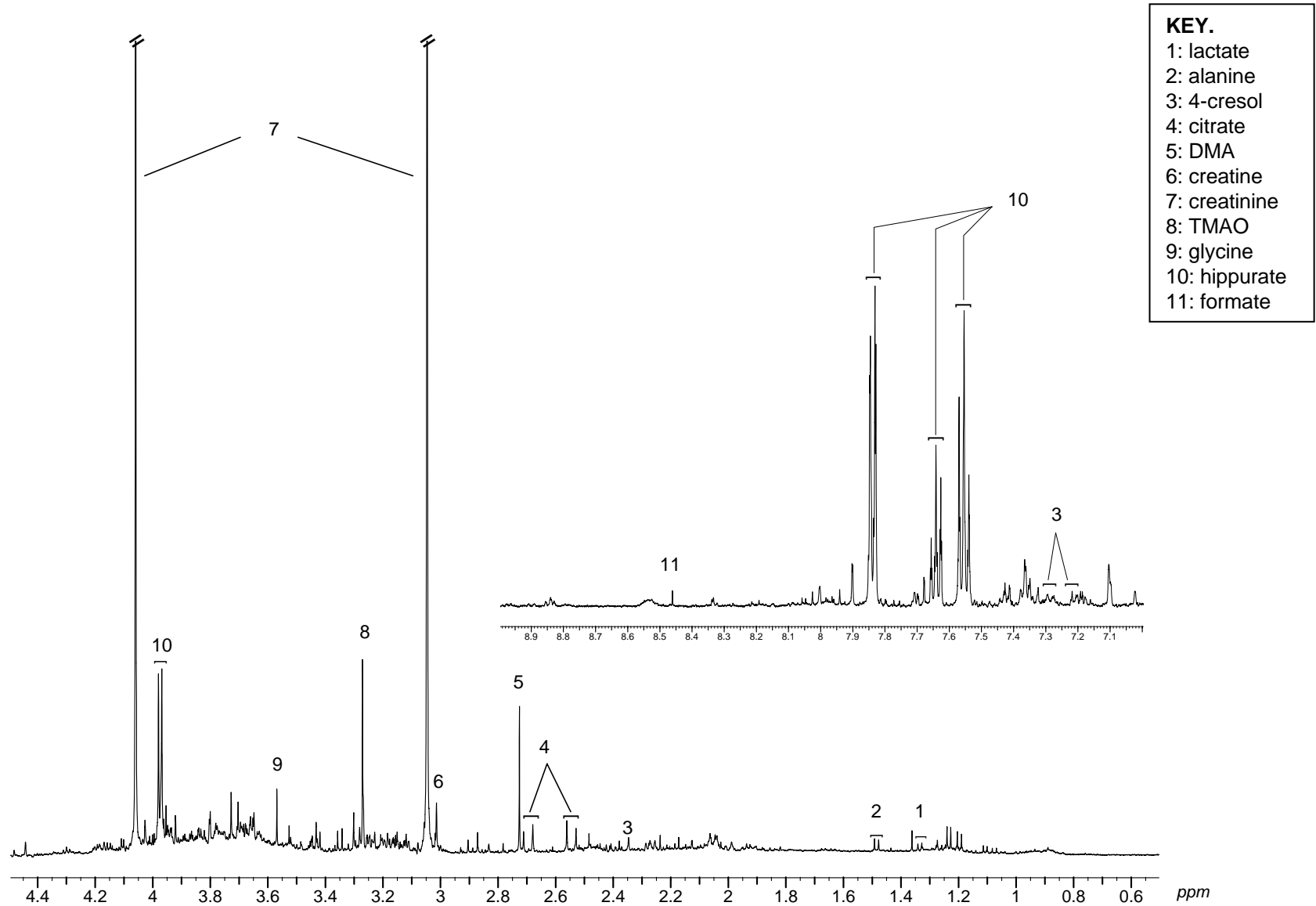
- Significant differences in IBD for specific urinary metabolites whose concentration is modulated according to make-up of the intestinal microbiota:
  - Hippurate, formate, 4-cresol sulphate
- Multivariate analysis (PLS-DA modelling):
  - Able to distinguish between cohorts, even colonic CD vs UC
  - Hippurate of major importance
- Elucidation of hippurate metabolism in IBD
  - Gut microbiota implicated in the lower levels found in CD

# Acknowledgements

- **Broad Medical Research Program**
- **Collaborators/Researchers**
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  - Miss. Venisha Patel
  - Dr. Simon Jakobovits



# Urine NMRS from male healthy control



# Addressing potential confounders

**Subject selection**

Caucasian

**Exclusion criteria**

Comorbidity

Intercurrent illness

Antibiotic usage

**Subject questionnaire**

Diet

Drugs

# Subject groups

- No significant differences between groups in terms of dietary constituents, EtOH intake, exercise, smoking
- Female subjects matched for reproductive status and use of hormonal therapies

# Addressing potential confounders

- Recent studies:
- Inter-individual variation > intra-individual variation
- Good reproducibility between individuals
- First void urine samples exhibit greatest variability
- Random urine samples,  $13.00 \pm 3$  hours