Imperial College London

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:

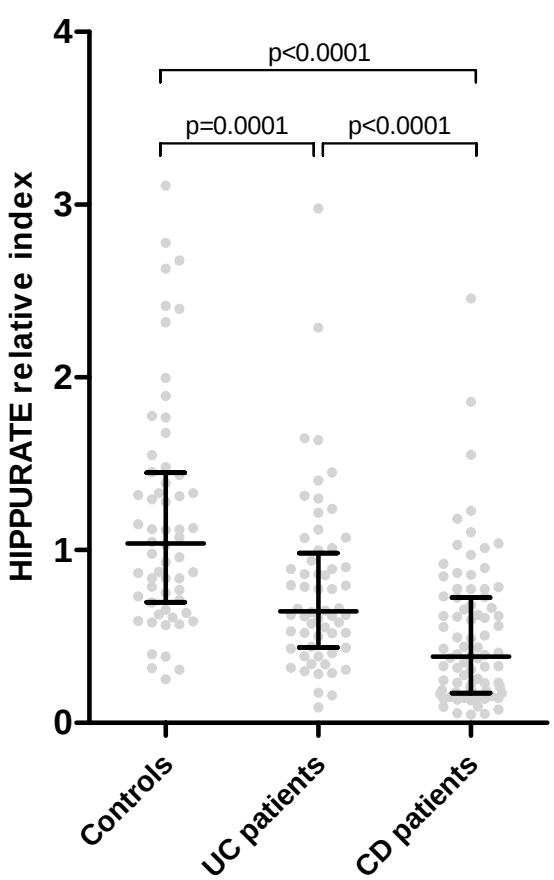
- Hippurate
- Formate
- 4-cresol sulphate
- Dimethylamine
- TMAO

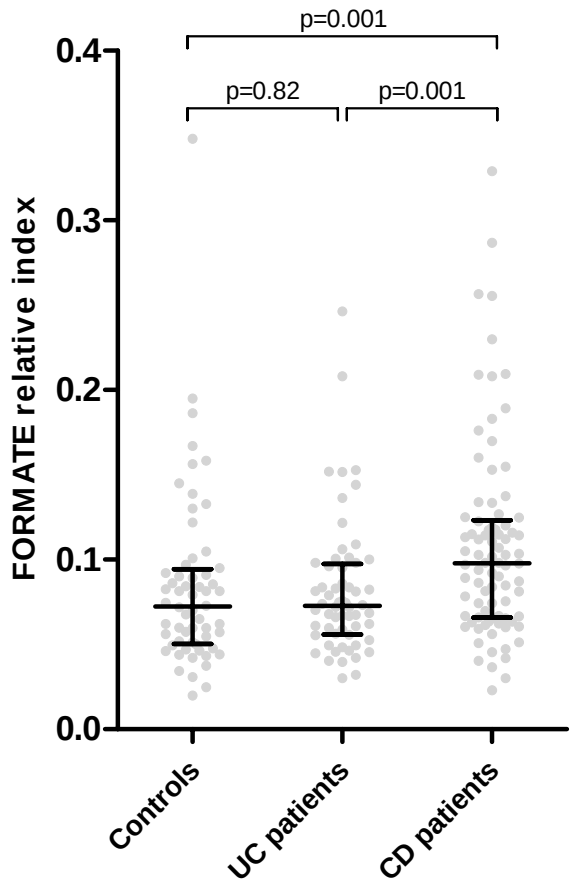
Experimental design: subject groups

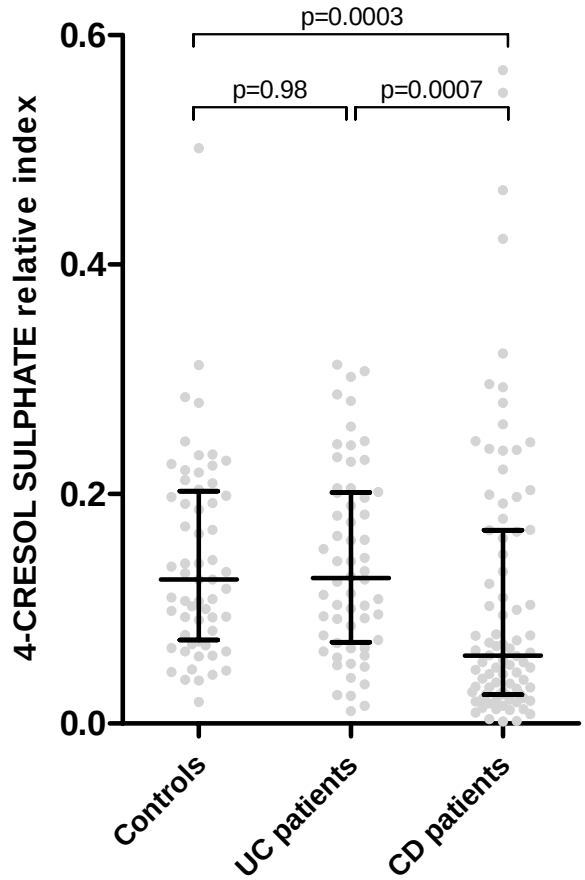
	Crohn's disease	Ulcerative colitis	Healthy controls
Number (Male/Female)	86 (47/39)	60 (30/30)	60 (30/30)
Median [range] age (years)	33 [16-66]	40 [17-66]	30 [18-61]
Disease location	L1: 18	E1: 14	-
	L2: 25	E2: 18	-
	L3: 43	E3: 28	-
Longitudinal Samples	35	14	26

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

Hypothesis-driven analysis





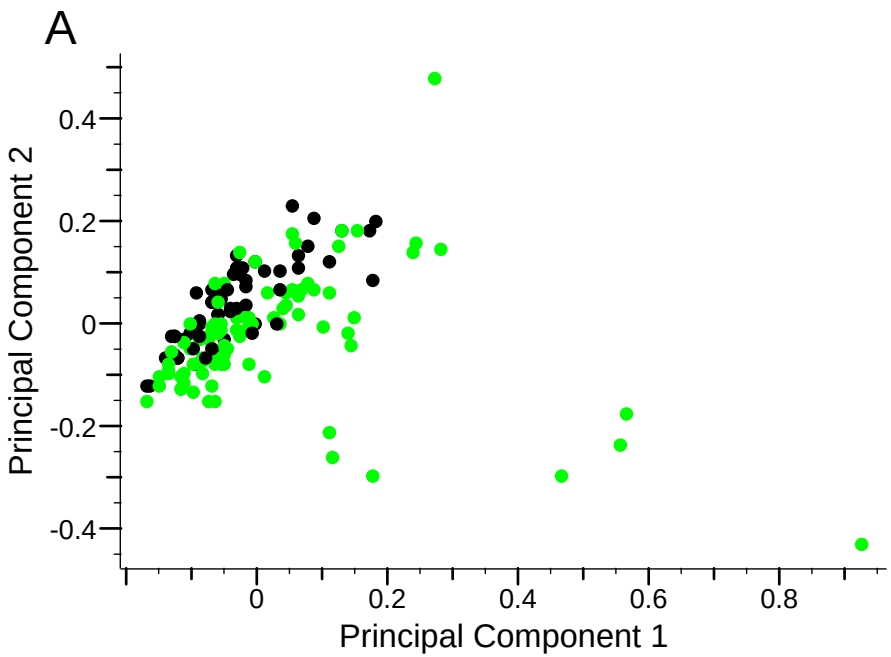


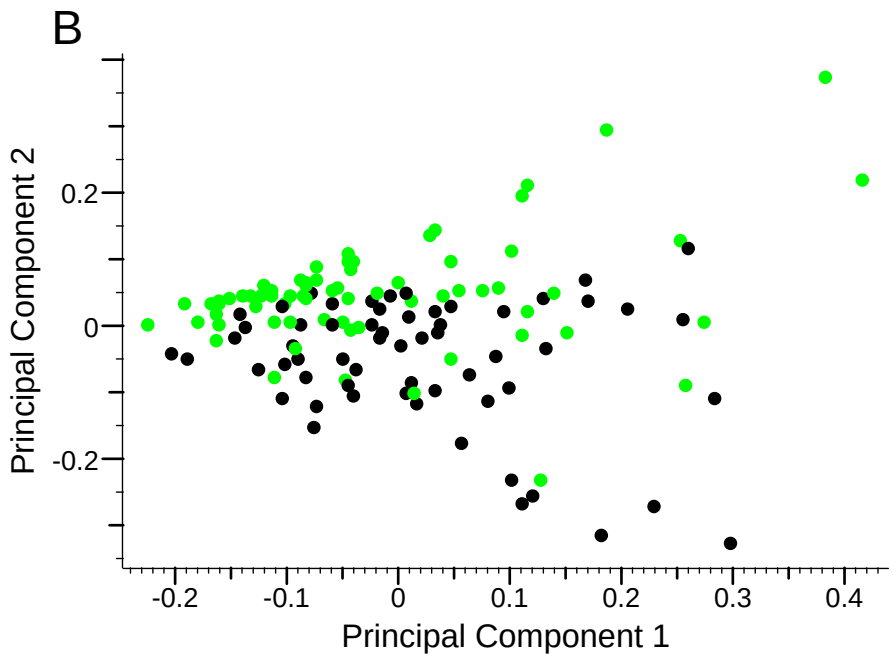
Multivariate factor analysis

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 co-ordinate 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).

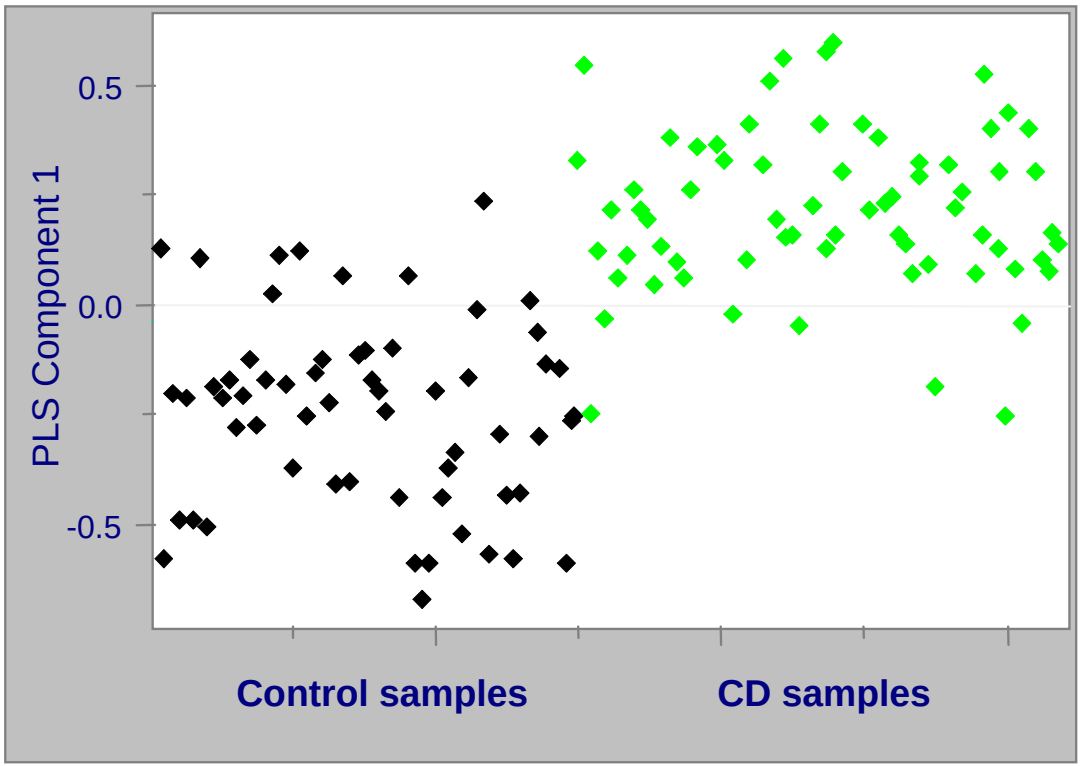
CD
Control

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**

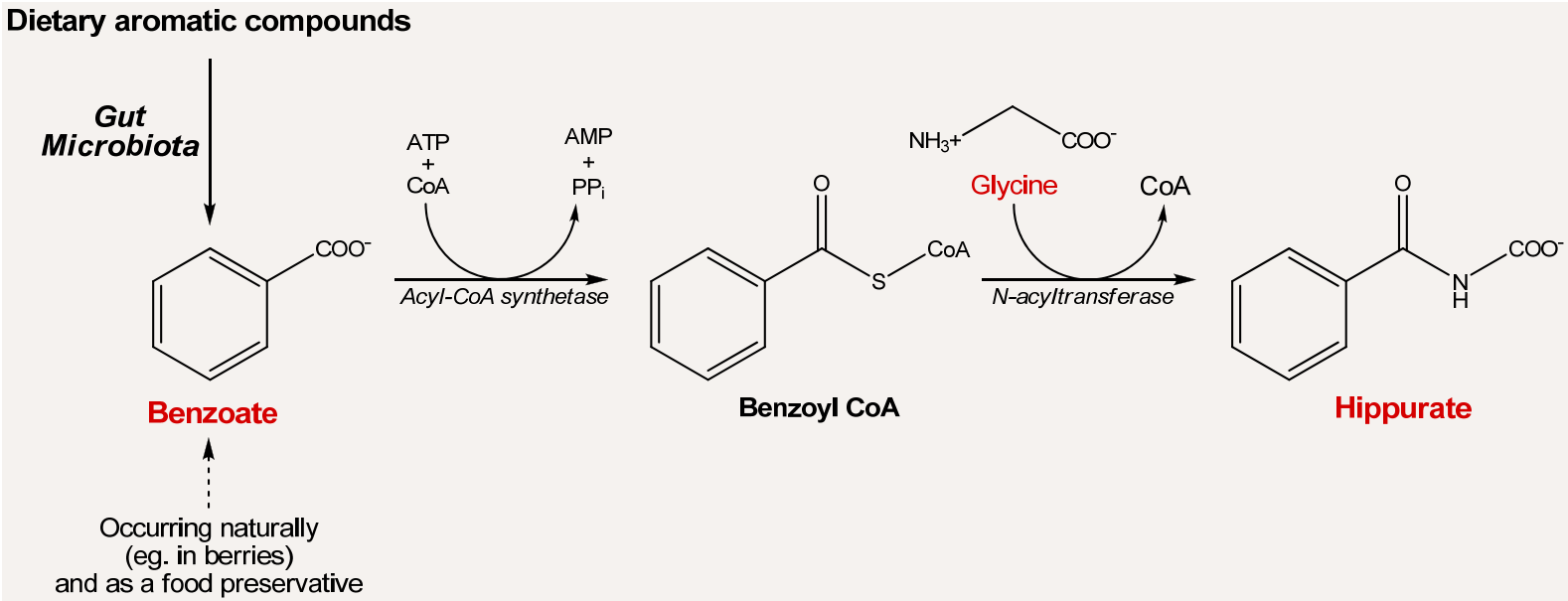
	All individuals		Individuals on no medication
	CD: UC	L2 CD: UC	CD: UC
Sensitivity: specificity	**86: 82**	**79: 83**	**82: 97**

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

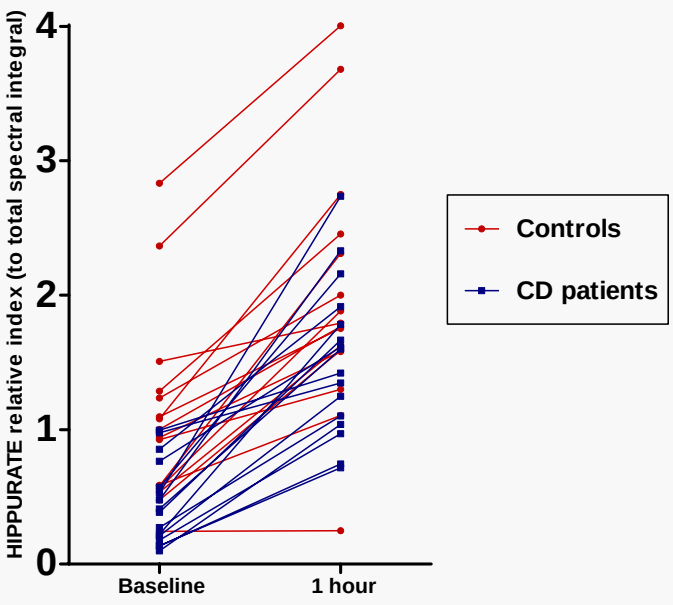


Investigating Hippurate Metabolism in IBD

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

Results

A



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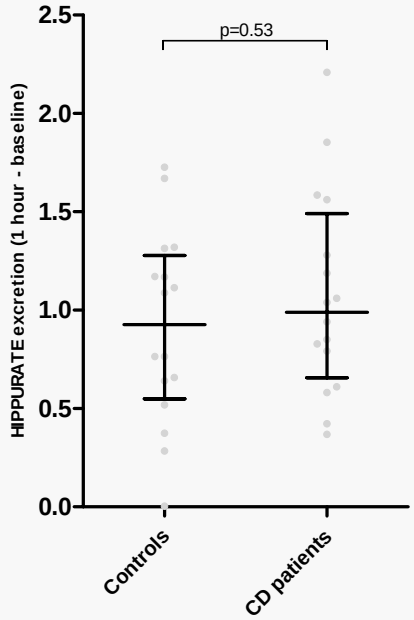

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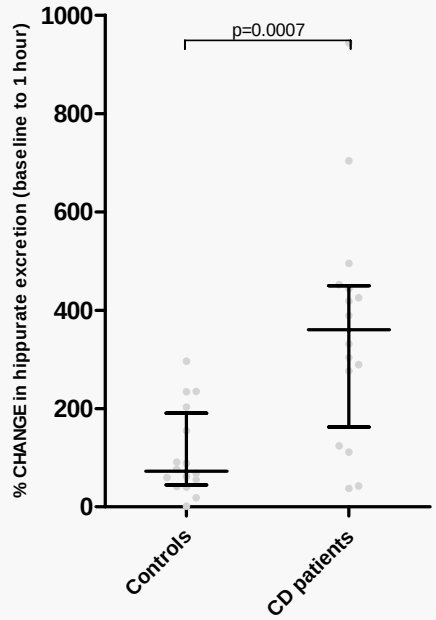

Discussion

- 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

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- Collaborators/Researchers
  - Dr. Bernard North
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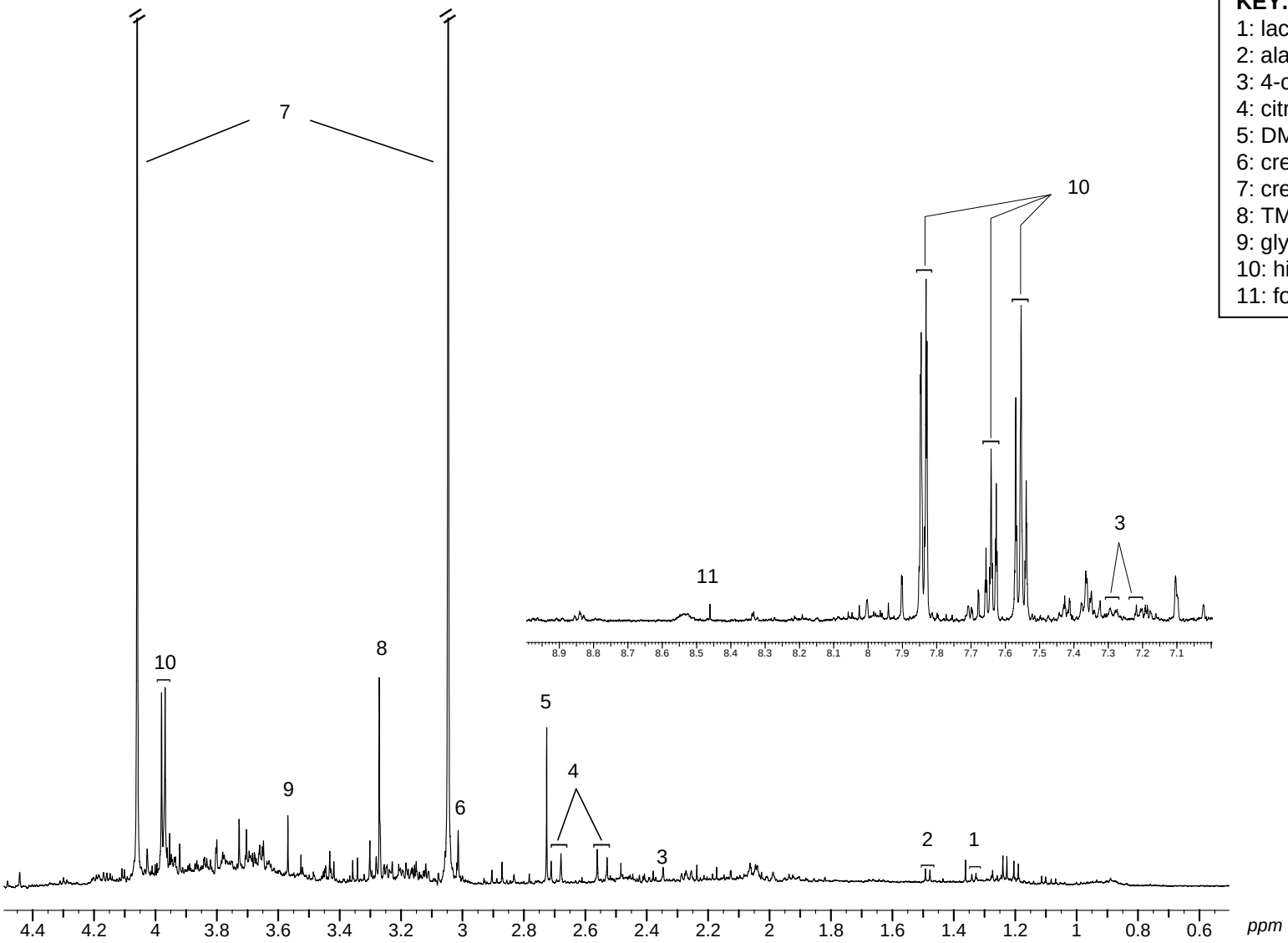
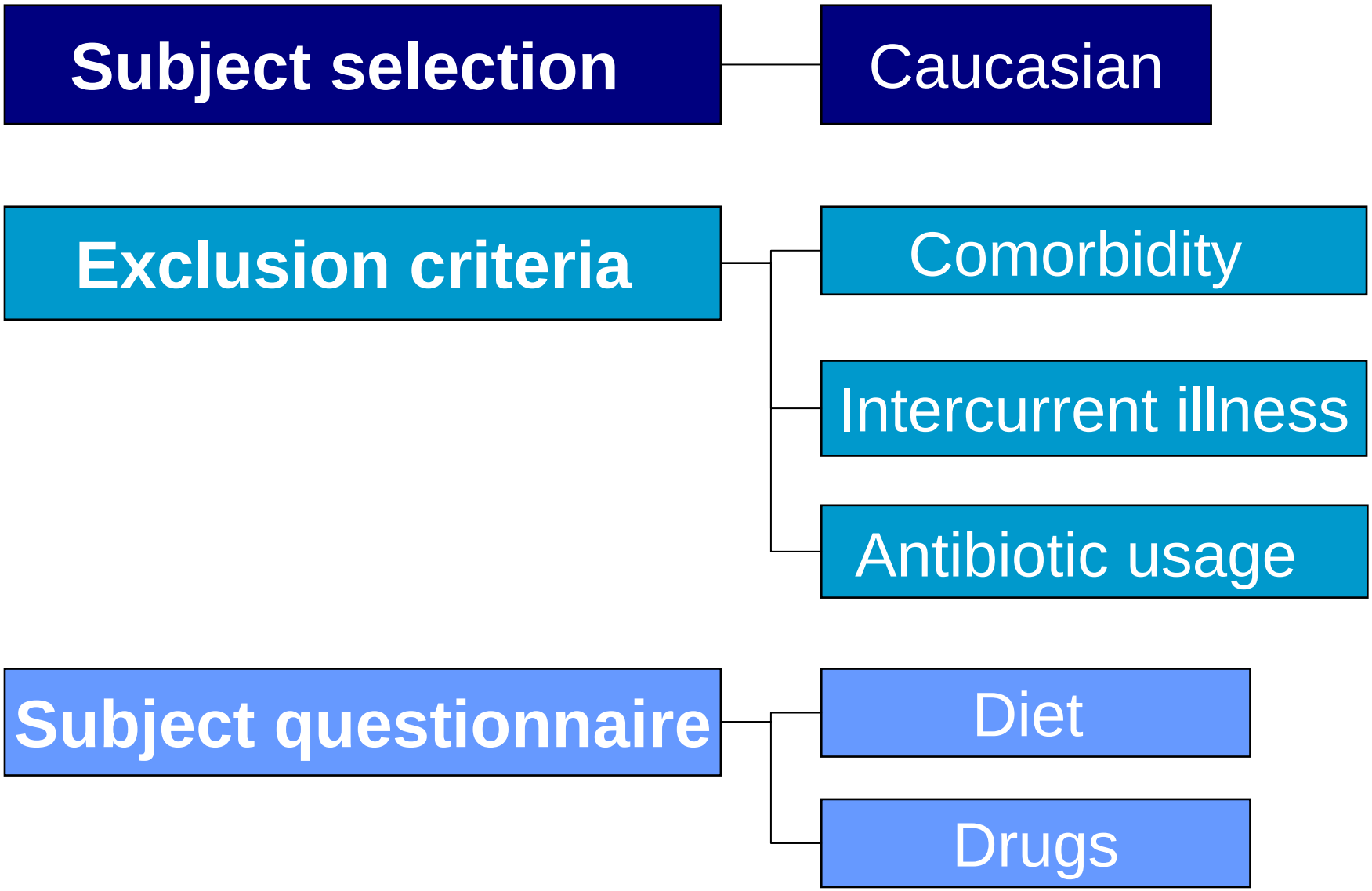
  - Dr. Simon Jakobovits

Urine NMRS from male healthy control



Addressing potential confounders



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 ± 3 hours