

Winter School



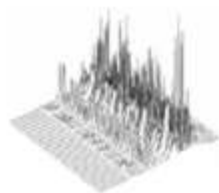
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Metabolomica e microbioma

Francesco Capozzi

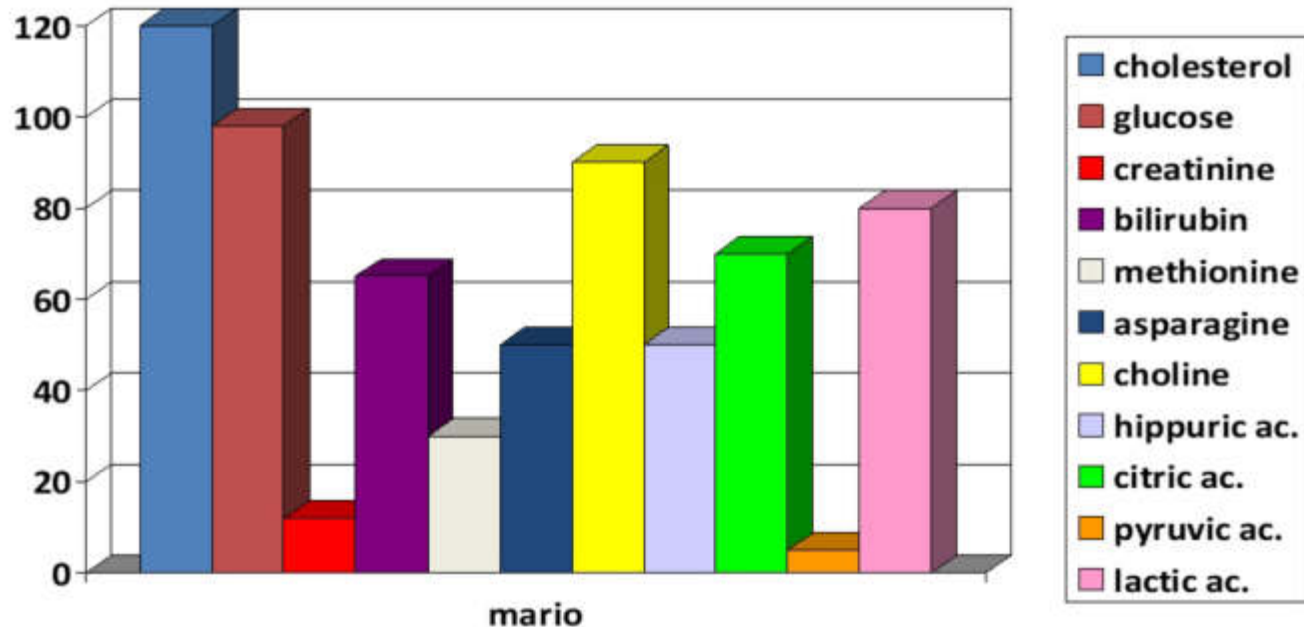
Department of Agricultural and Food Sciences
Alma Mater Studiorum - University of Bologna

Bologna, 22nd March 2022



- **Metabolites**: molecules and other chemical species of small size (< 1 kDa) present in biological systems by transfer from the external environment or produced by metabolic reactions.
- **Metabolic Profile**: description (tables, histograms, graphs, etc..) of a biological system based on the list of metabolites present in it, and their corresponding quantity.
- **Metabolome**: The complete set of metabolites present in a (super)organism or in a tissue/organ, including biological fluids, as described by holistic methods.

CONVENTIONAL METABOLIC PROFILE



METABOLOMICS O METABONOMICS ?

- **MetaboLomics**: studies the entire metabolome of an organisms through holistic techniques, including comparative analyses between species or groups of individuals. The purpose is to understand the metabolic phenotype arising from the specific genome of an individual.
- **MetaboNomics**: “quantitative measure of the dynamic multi-parametric metabolic response of living systems to patho-physiological stimuli or genetic modification” (J.K. Nicholson, Xenobiotica, 1999, 29, 1181). The purpose is to understand the metabolic perturbations relating to the interaction between the individual genome and its environment.
- **In both cases**: the basic requirement is the absence of *a priori* selection of the metabolites to be searched.





Detailed, comprehensive description of the whole metabolome, without emphasis on any particular metabolite

(even the number and length of blades of grass are meaningful for the complete description of the picture)



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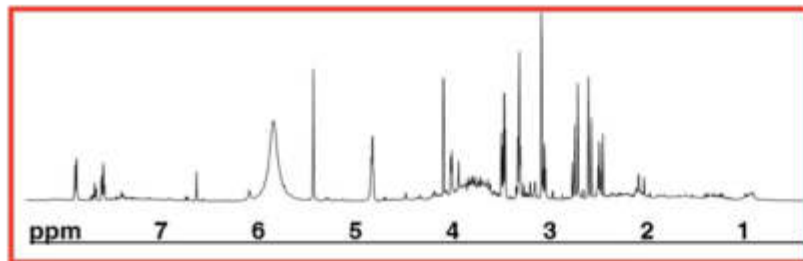
Appreciate all the detectable differences relating to an adaptation or a perturbation. It is based on the multivariate data analysis.



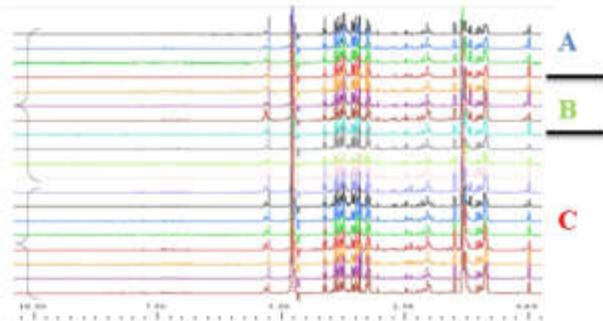
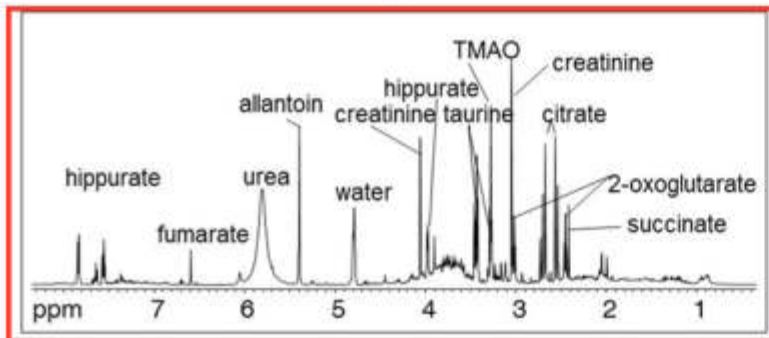
TWO ROUTES TO METABOLOMICS



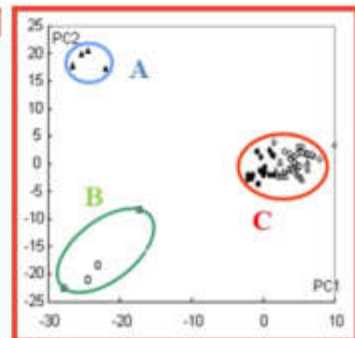
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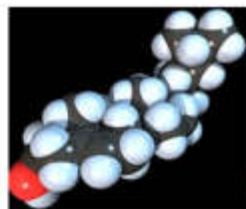
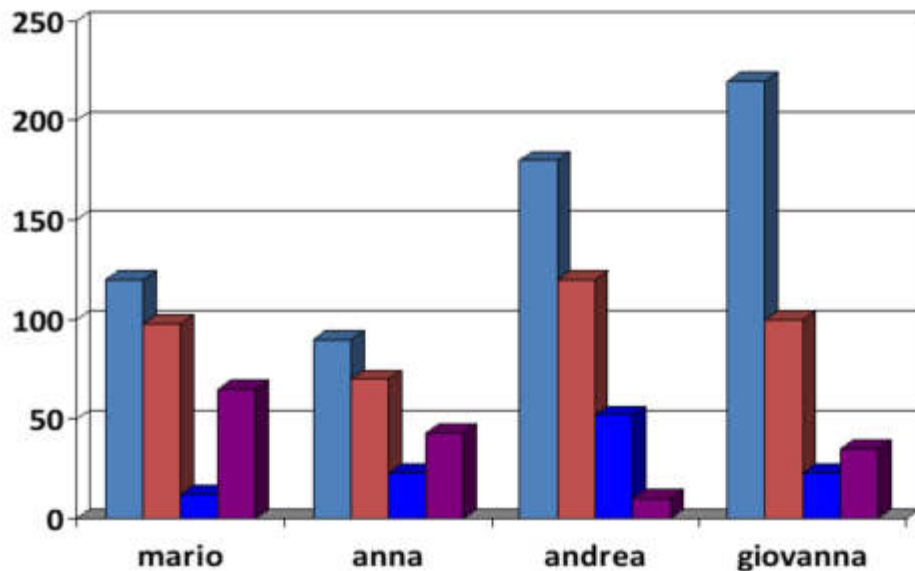
**Analytical Methods
(Target Analysis)**



**Chemometric Methods
(Pattern Analysis)**



CLASSIFICATION: FINDING GROUPS OF INDIVIDUALS



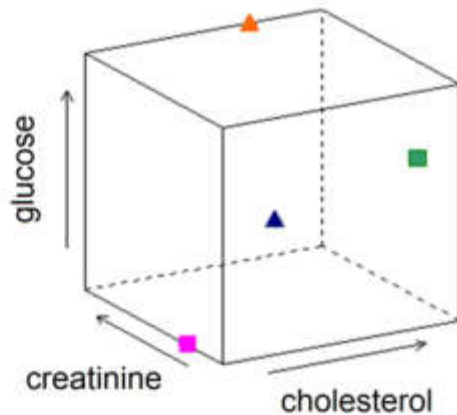
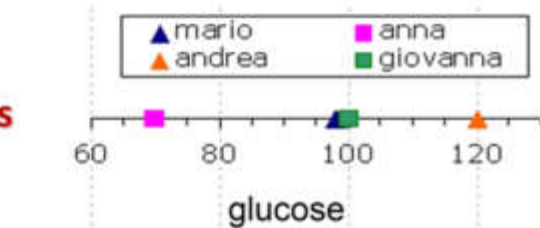
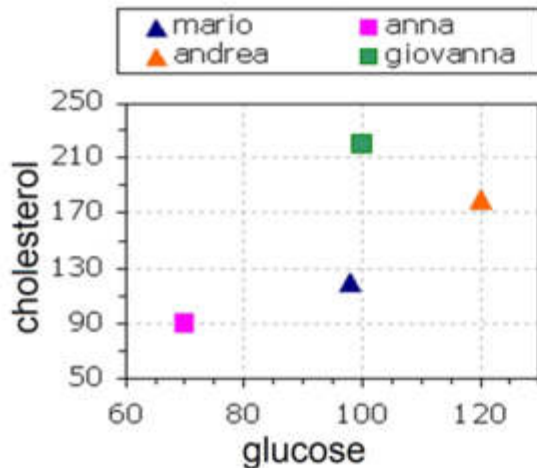
cholesterol
glucose
creatinine
bilirubin



Is there any cluster?

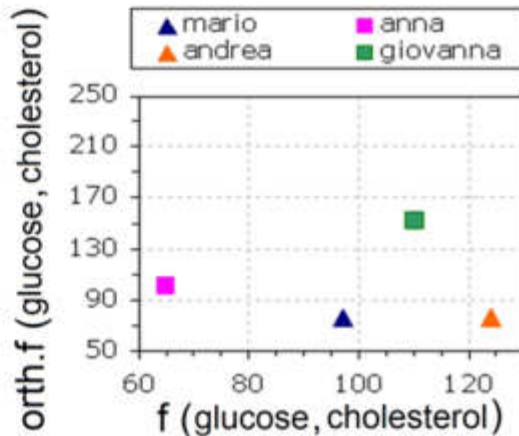
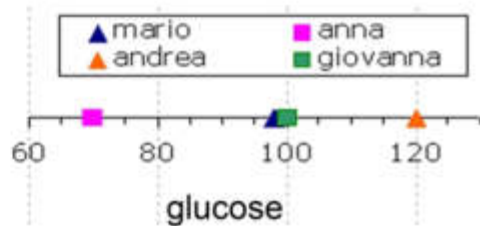
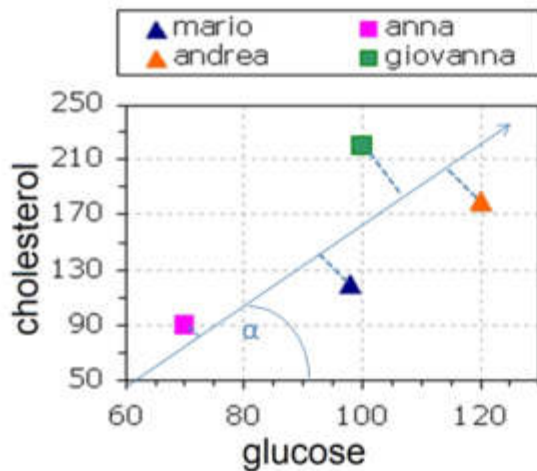
REPRESENTING DIFFERENT INDIVIDUALS (1,2,3-D)

First step: measure the distance between individuals



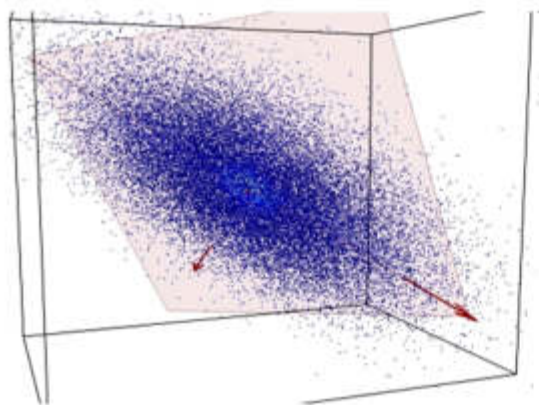
REDUCING THE DIMENSIONALITY OF REPRESENTATIONS

(2-D \rightarrow 1-D)

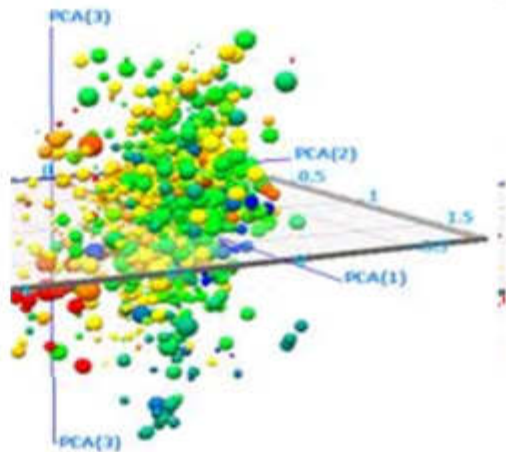


$$Y = P \cdot X$$

$$y_{12} = (p_{11}, p_{12}, \dots, p_{1m}) \text{ dot-product } (x_{12}, x_{22}, \dots, x_{m2})$$



Mathematical transformations to reduce the space to a smaller number of dimensions



MS vs. NMR COMPARISON

M.Jacob et al. Metabolomics toward personalized medicine. *Mass Spec Rev.* 2019; 38: 221–238.

Direct Infusion-Tandem Mass Spectrometry

	NMR	GC-MS	DI-MS
Sample prep time	30 -120 min/20 samples	30 -120 min/20 samples	3-4 h for 96 samples
Run time	20 -90 min/sample	30-60 min/sample	7 min/sample
Data Analysis	30-60 min / sample	30-60 min / sample	1-2 h for 96 samples
Limit of Detection	~ 5 μ M	~ 100 nM	~ 5 nM
No. of metabolites	~ 20 - 50	~20 -50	~ 100-180
Overlapping Metabolites	10-15	10-15	10-15
Cross-checking	10-30 %	10-30 %	10-30 %



WHY NMR ?

Advantages	Disadvantages
Nondestructive	Not very sensitive ($>1-5 \mu\text{M}$)
Fast	Expensive Instrumentation
Quantitative	Require large samples ($>500 \mu\text{l}$)
Requires no Separation	
Detects all organic classes	

NMR spectroscopy is expensive: thus find a true reason to use it!

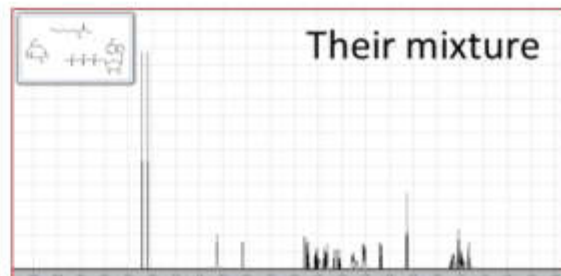
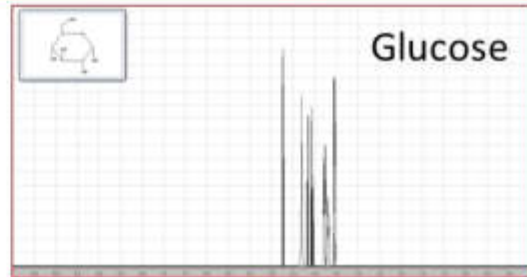


- **Organisms or tissues:** NMR can analyze heterogeneous mixtures, either solid or semisolid, without any particular preparation.
- **Biological Fluids:** NMR can analyze and return the intact samples of CSF, serum, plasma and urine (centrifugation is only required).
- **Cell extracts:** NMR can analyze, without further manipulation, homogeneous mixtures whose composition differ depending on the extraction solvent.
- **Feces:** simple preparation consisting of homogenization by stirring for few minutes with buffer or 95% ethanol, then centrifuged for 2 h at $35\ 000 \times g$ at $4\ ^\circ\text{C}$.

Fecal material contains both **endogenous and exogenous metabolites** with variant polarity, e.g., nonpolar fatty acids, triglycerides, and phosphoglycerolipids, and polar compounds, such as **short chain fatty acids** (SCFAs), amino acids, bile acids, and carbohydrates. In addition, feces contain both microbial and mammalian cells. Fecal samples provide direct information about **interactions between host and gut microbiota** since they carry numerous biochemical compounds derived from the host, the host's microbiota, and food residuals.



NMR SPECTRA OF METABOLITES AND THEIR MIXTURES

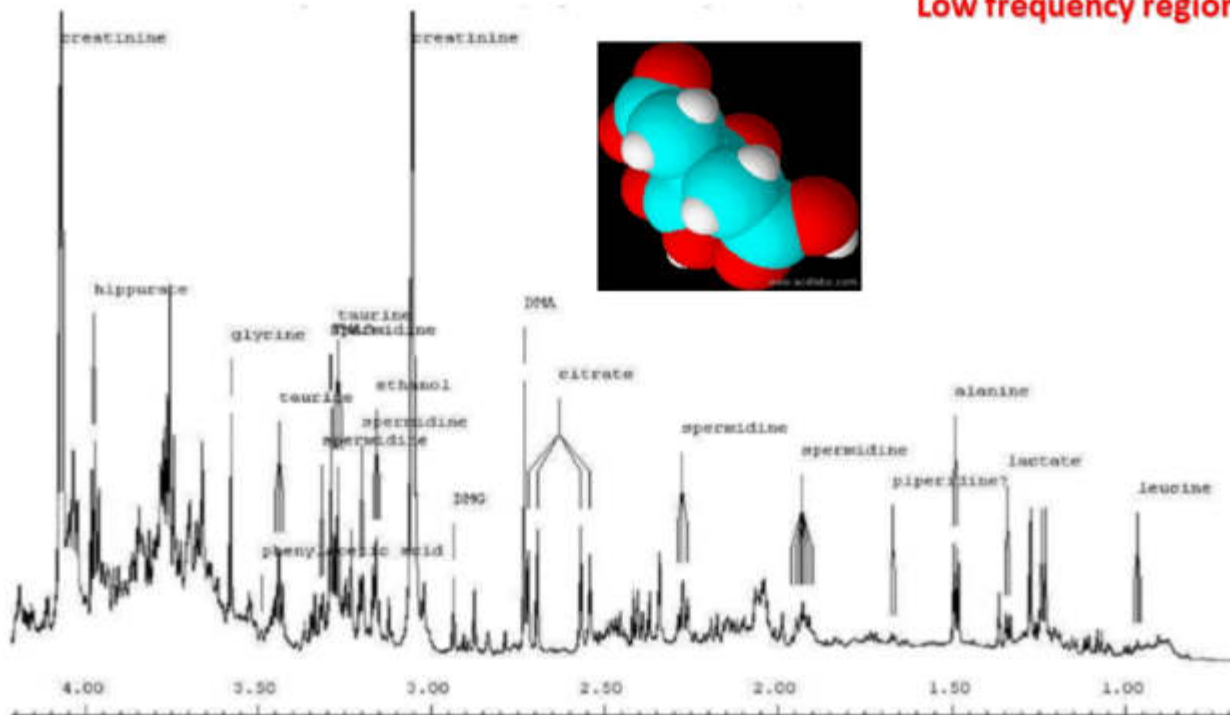


$^1\text{H-NMR}$ SPECTRUM OF HUMAN URINE

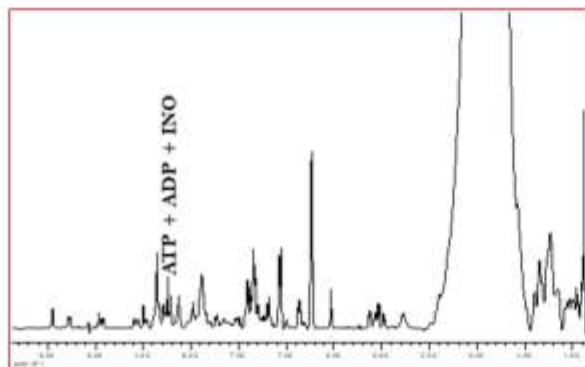


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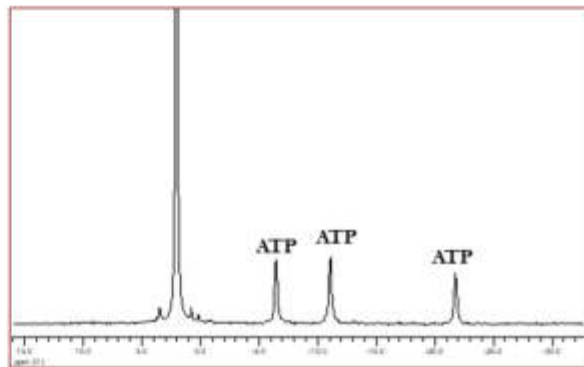
Low frequency region



Acquisition of other nuclei may resolve the overlapping signals corresponding to different metabolites



^1H NMR



^{31}P NMR

NMR Spectroscopic Data

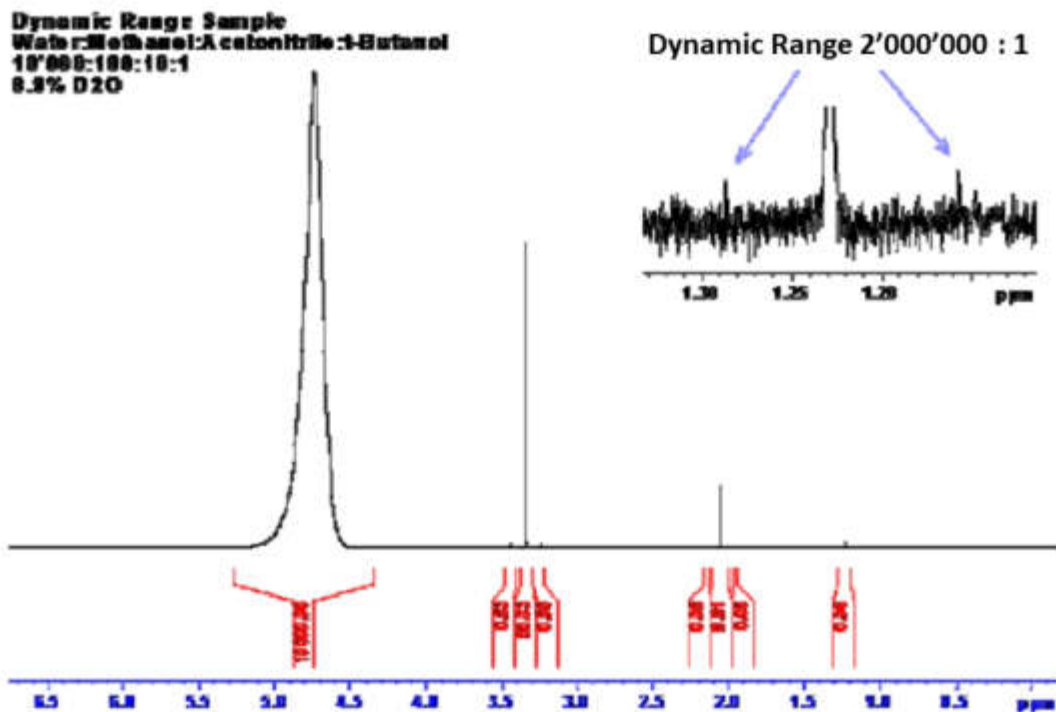
The NMR spectrum is directly useable for representing each individual, because the NMR spectra acquired, over time, on a kinetically stable sample are repeatable and perfectly superimposable.

HPLC-MS or GC-MS Data

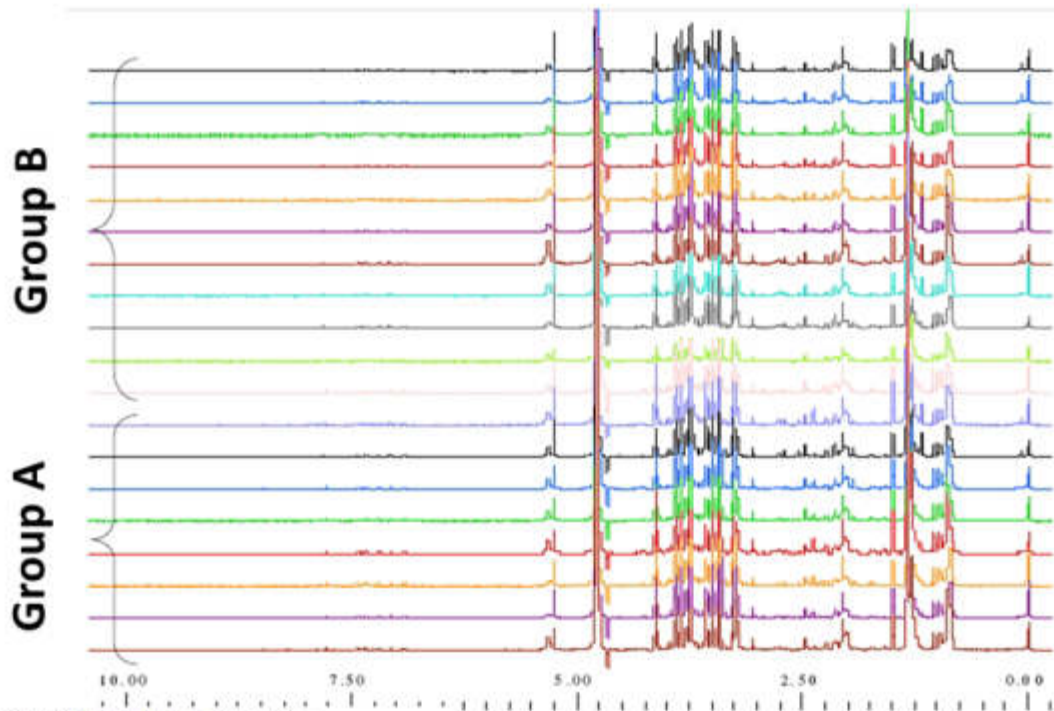
The metabolome is represented by derived data, organized in tables, because the chromatogram trace is not sufficiently repeatable even for the same kinetically stable sample.



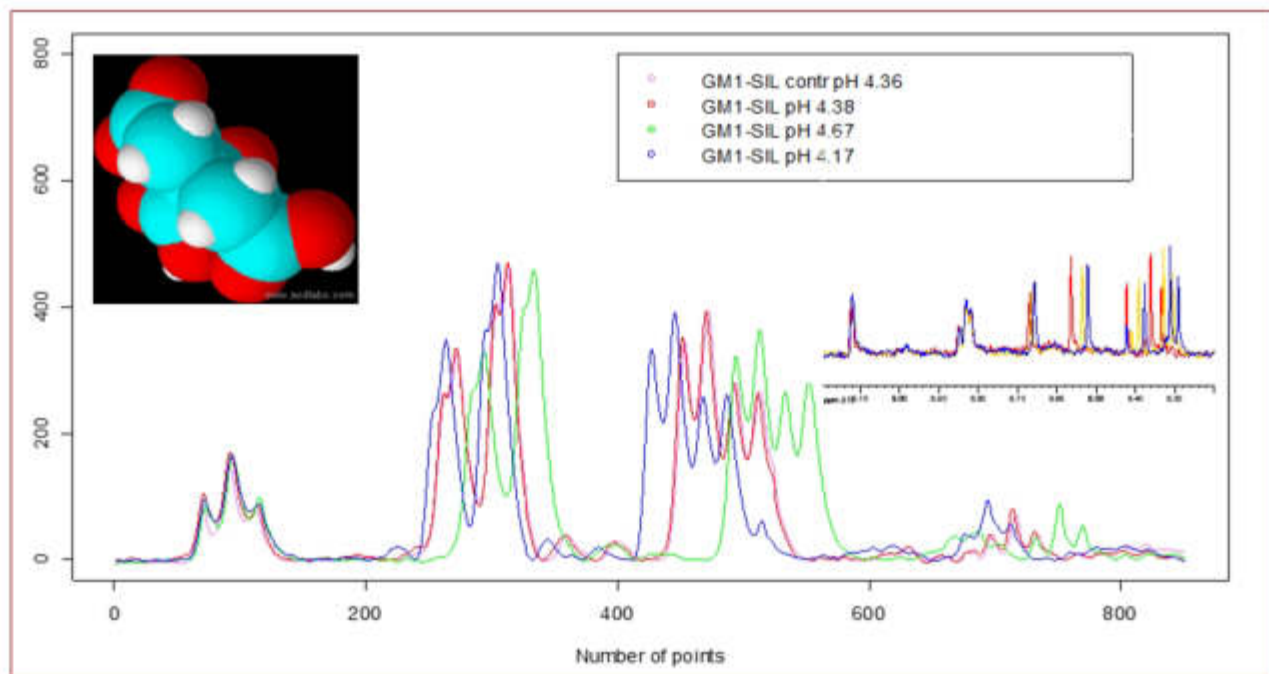
SIGNAL/NOISE @ 600MHz



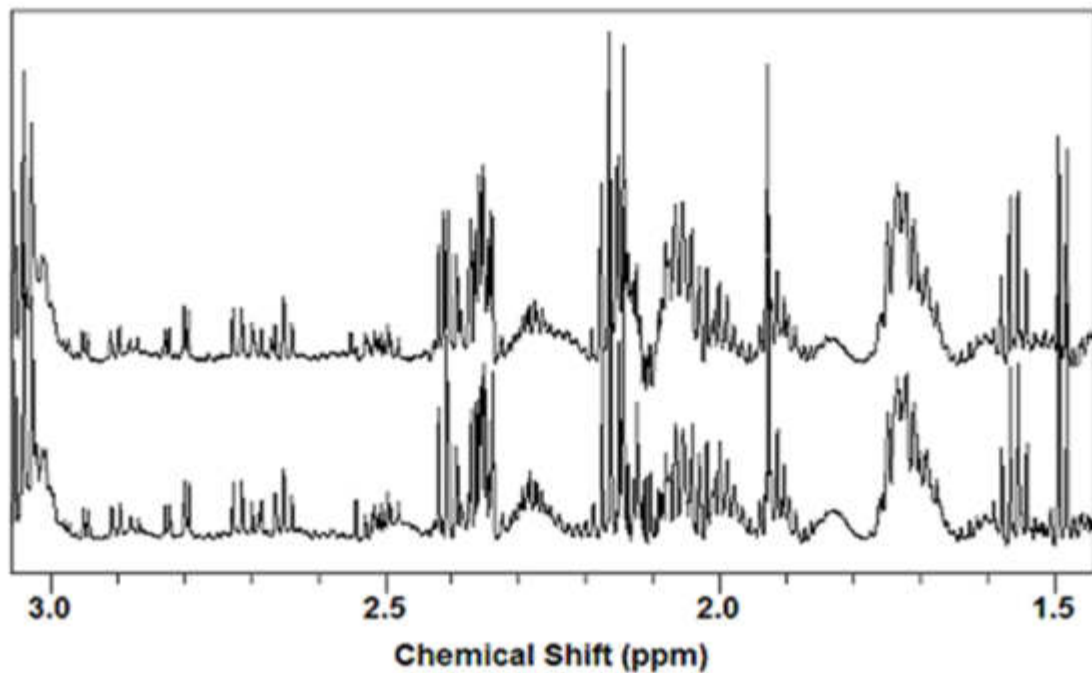
^1H -NMR SPECTRA OF HUMAN URINE



THE EFFECTS OF pH, T & IONIC STRENGTH



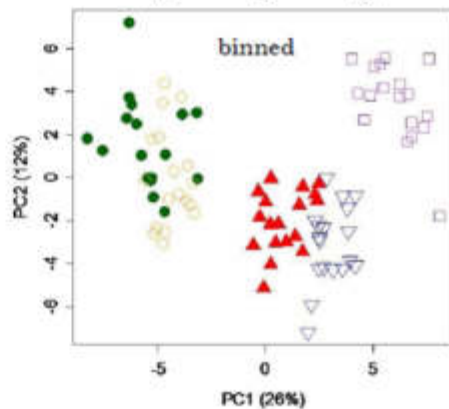
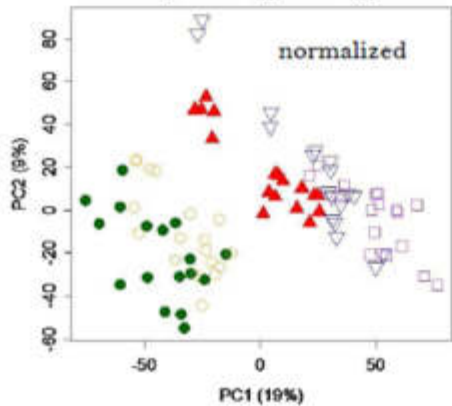
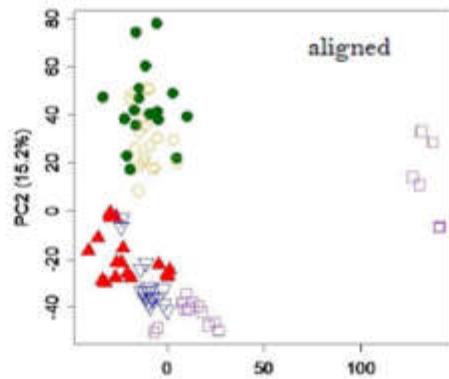
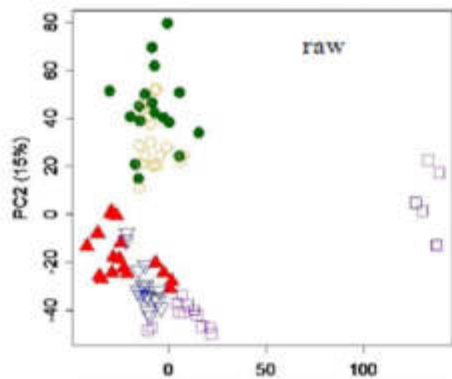
PROFILES COMPARISON



THE ABILITY TO EXTRACT INFORMATION FROM RAW DATA



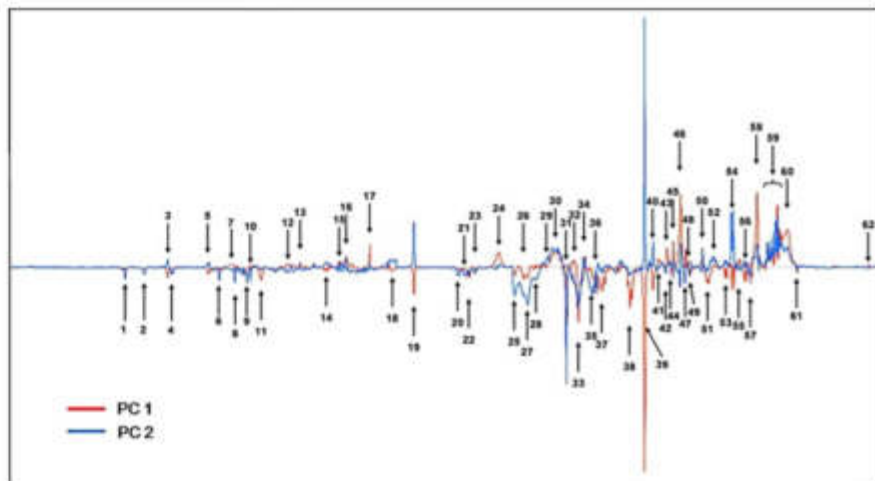
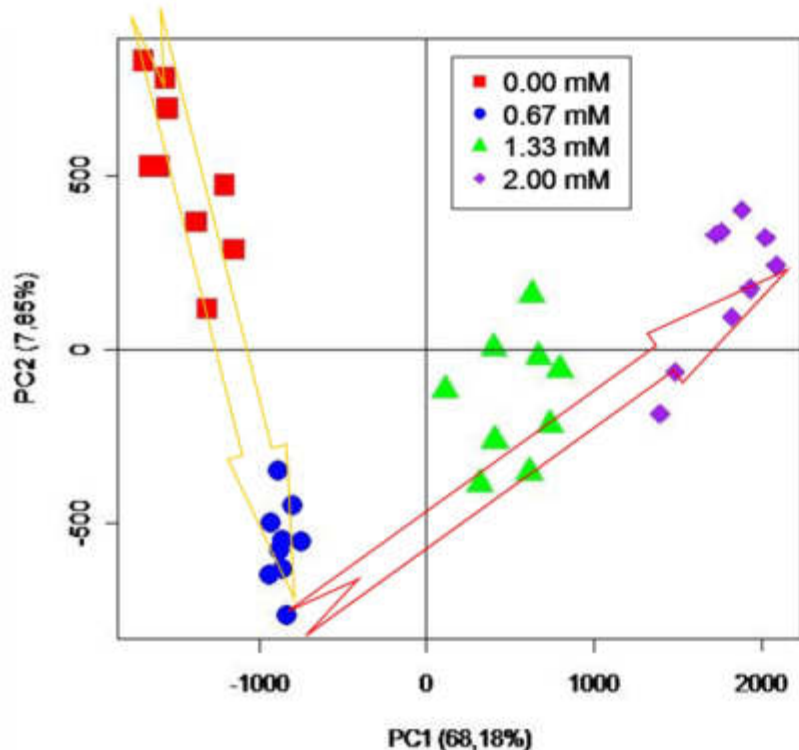
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THE METABOLIC SWITCH



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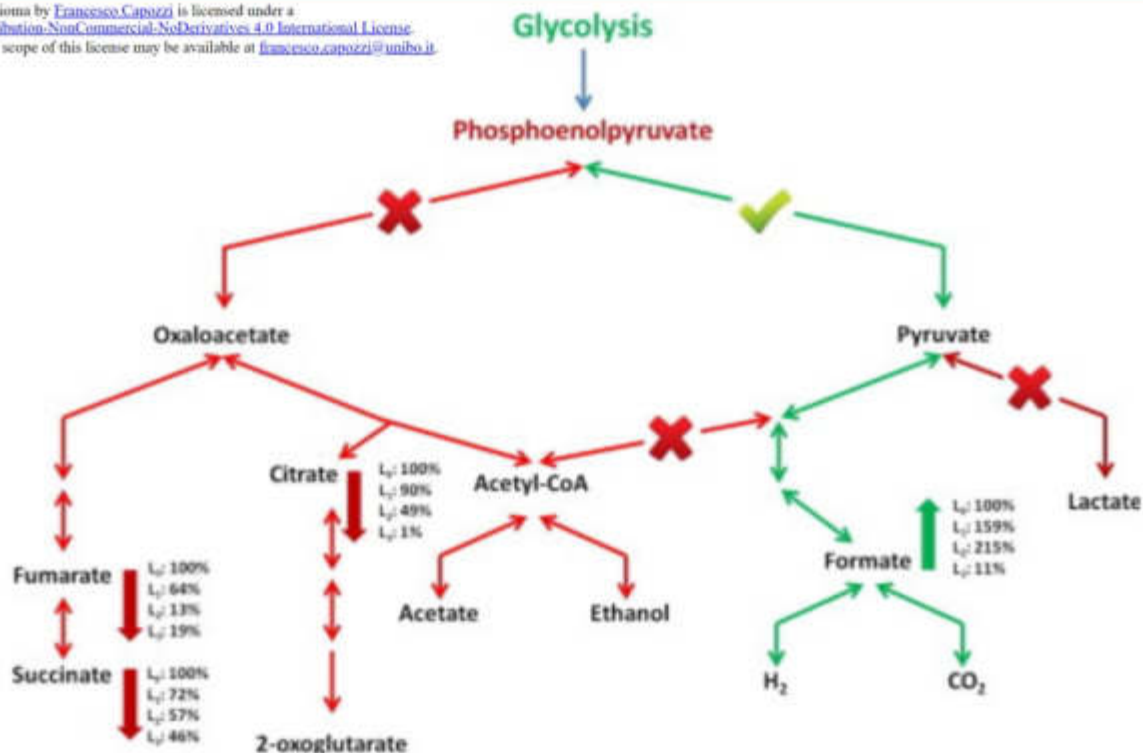
- 19 Fumarate
- 31 Unknown, possible glycolate derivative
- 38 Citrate
- 39 Succinate
- 40 Pyruvate
- 54 Alanine



PATHWAY: MIXED-ACID FERMENTATION



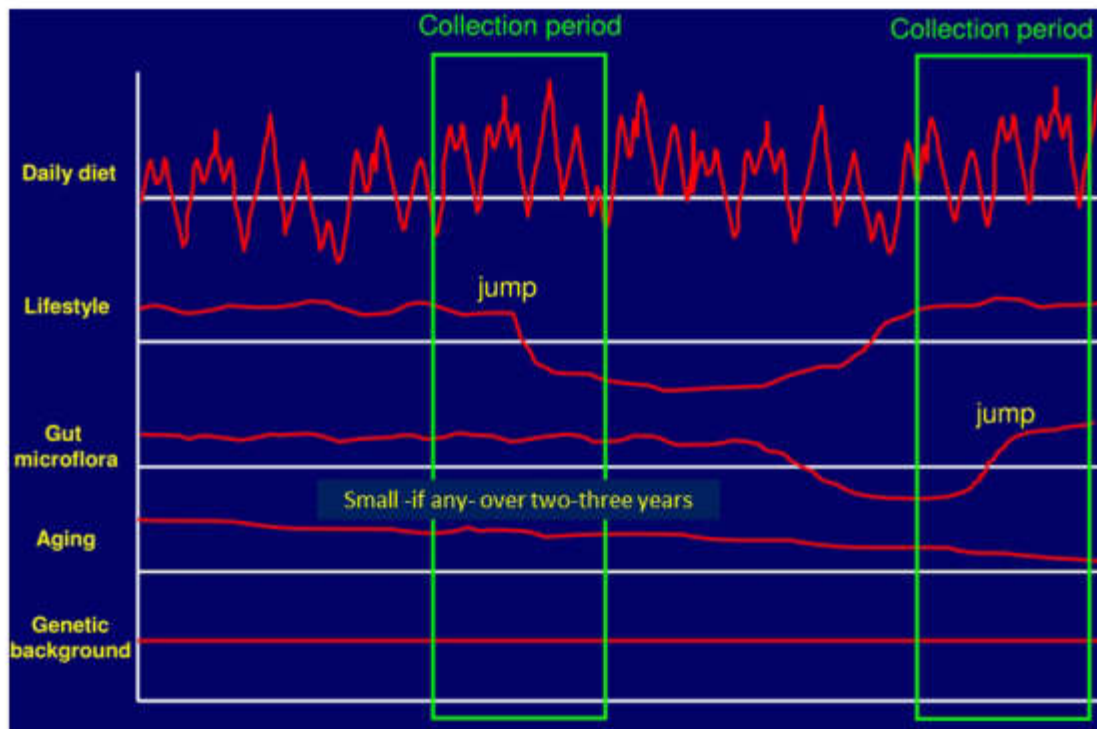
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VARIABILITY IN INTRA-INDIVIDUAL TIME-SERIAL DATA



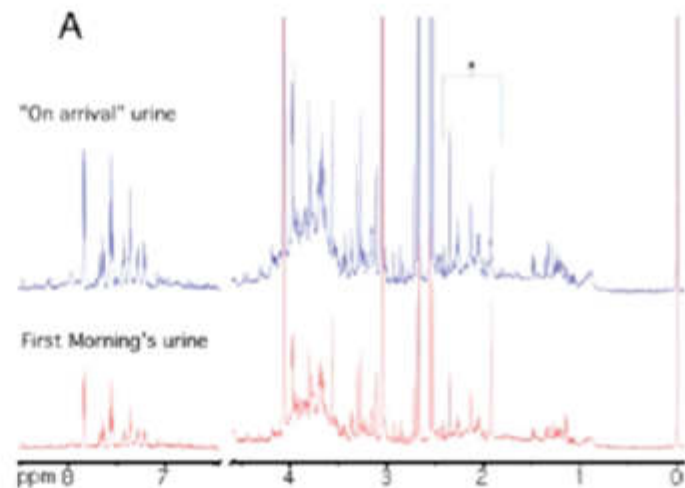
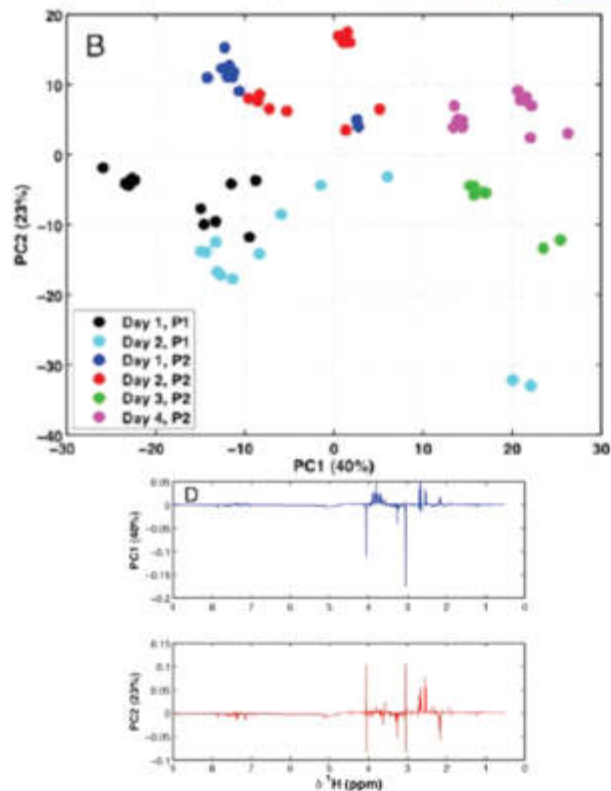
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IS IT THE URINE A CONSTANT BIOLOGICAL FLUID ?



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The discrimination identifiable by PCA and O-PLS-DA analysis of the entire data set (Figure 1) indicated that the major source of metabolite spectral variation was at the level of the participant, followed by day of collection, and then time of day of collection.

Jeremy K. Nicholson

Anal. Chem. 2007, 79, 5204–5211

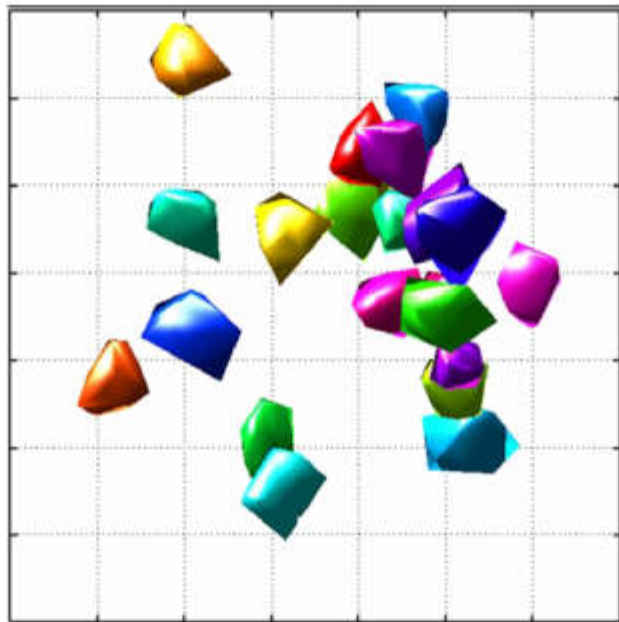


A PRETTY CONSTANT URINE METABOTYPE



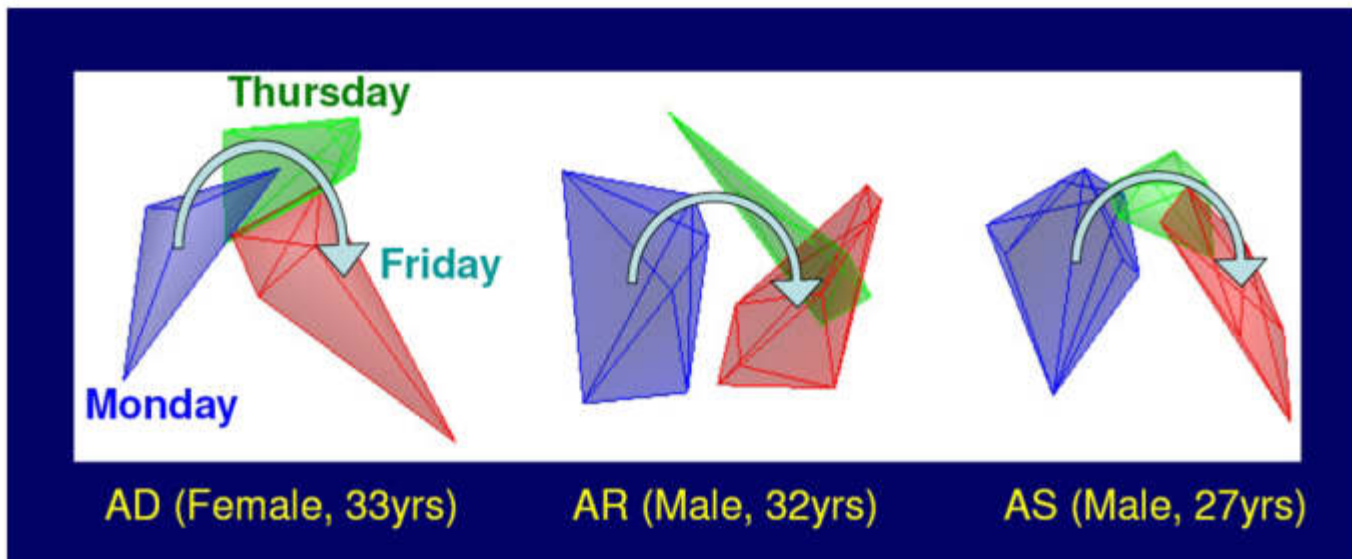
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Convex hulls of 22 donors in the three most significant PCA-CA dimensions

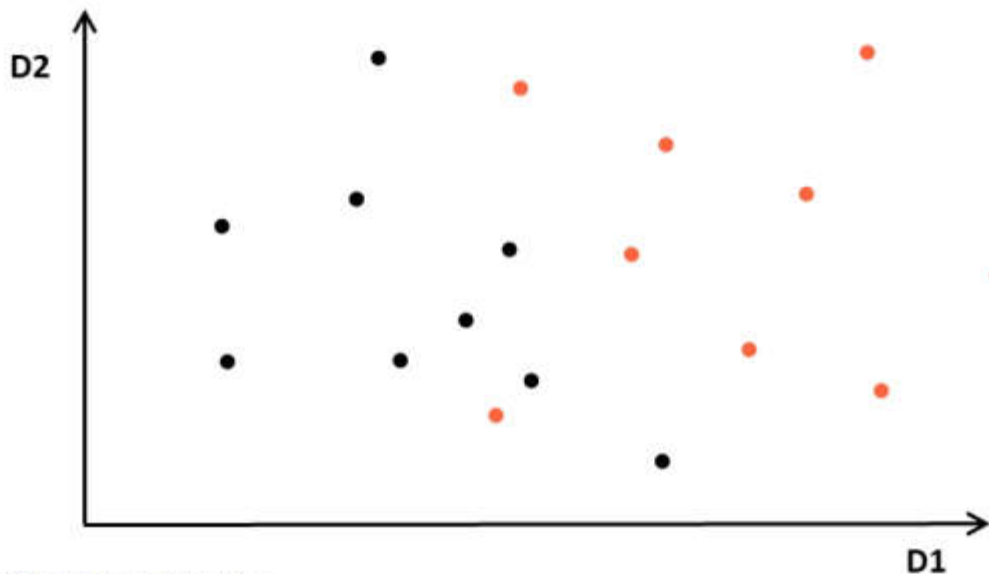


URINE IS SENSITIVE TO THE WEEKDAY

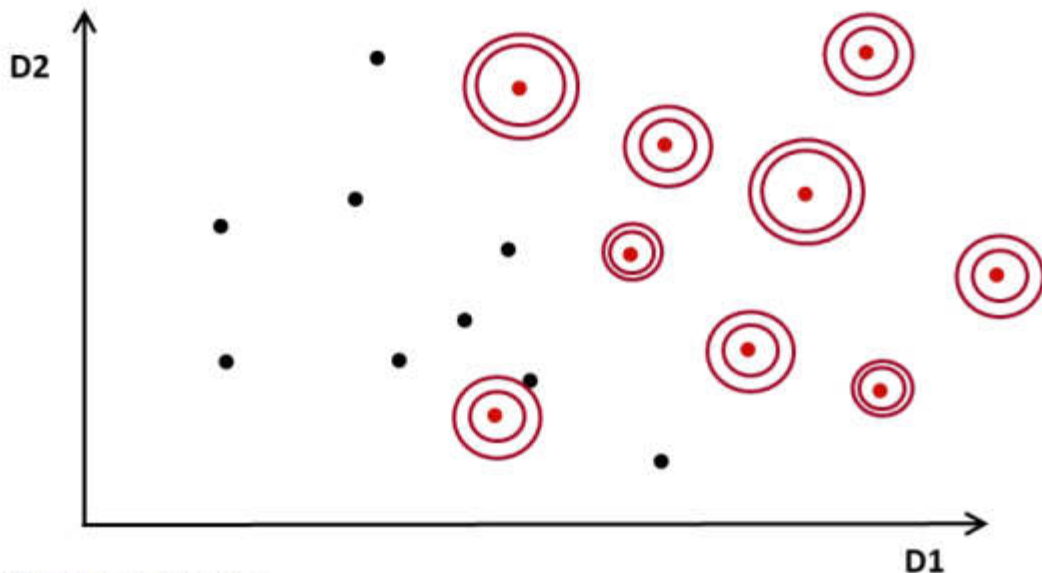
Weekday variation of 3 donors' urine, expressed as convex hulls in the three most significant PCA/CA dimensions



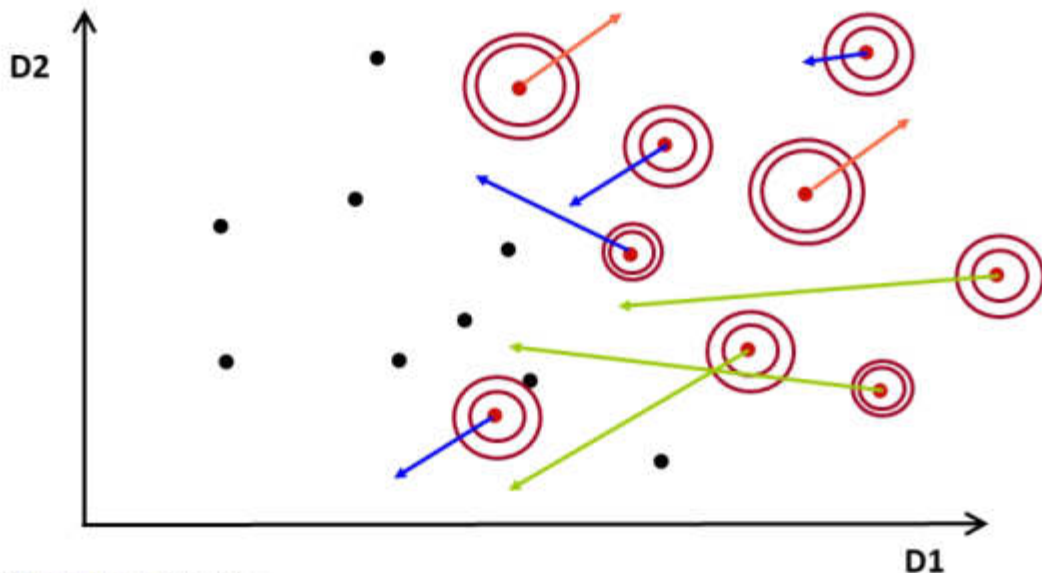
The main sources of variance must relate to the expected classification target



The individual variability must be assessed before the intervention studies



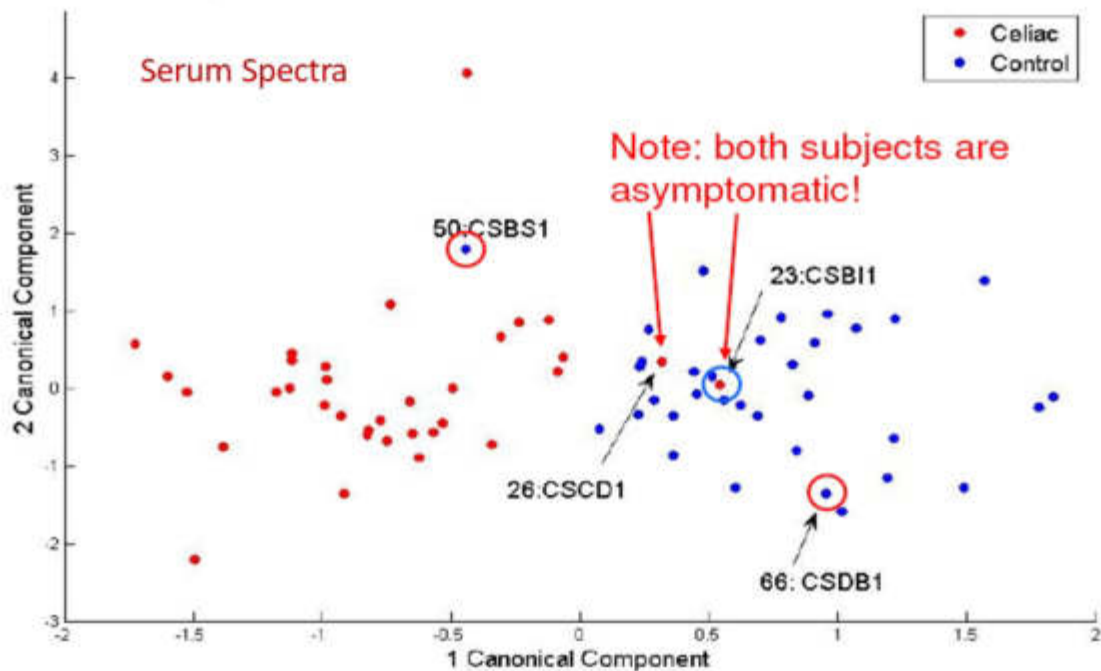
Metabolic directions in the multivariate space: *same intervention but different individual responses*

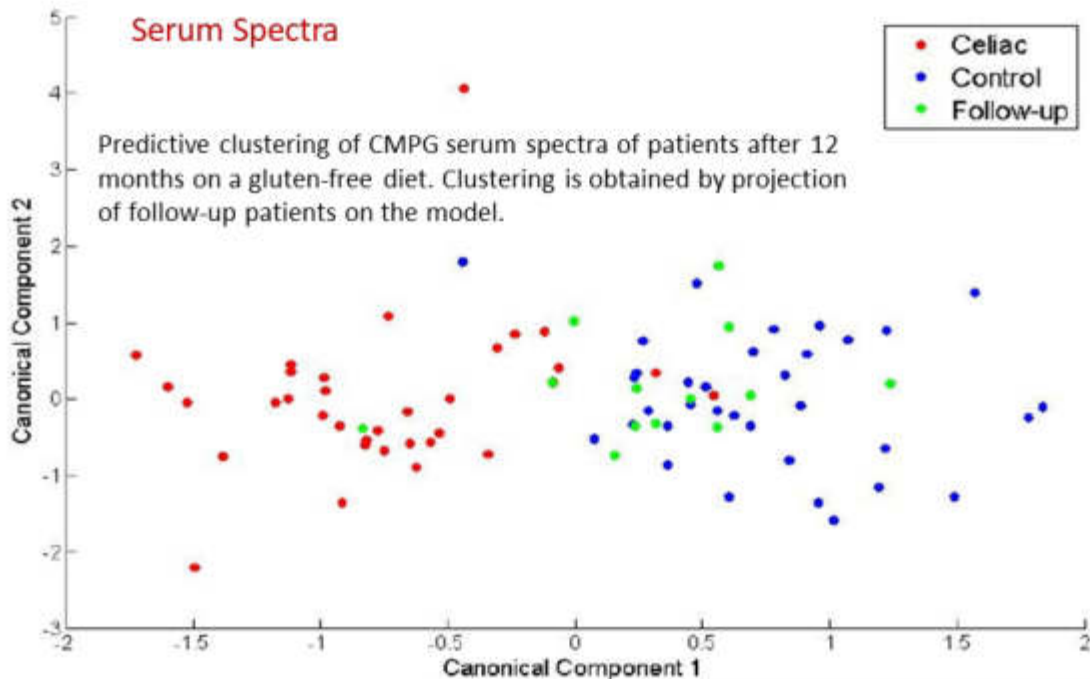




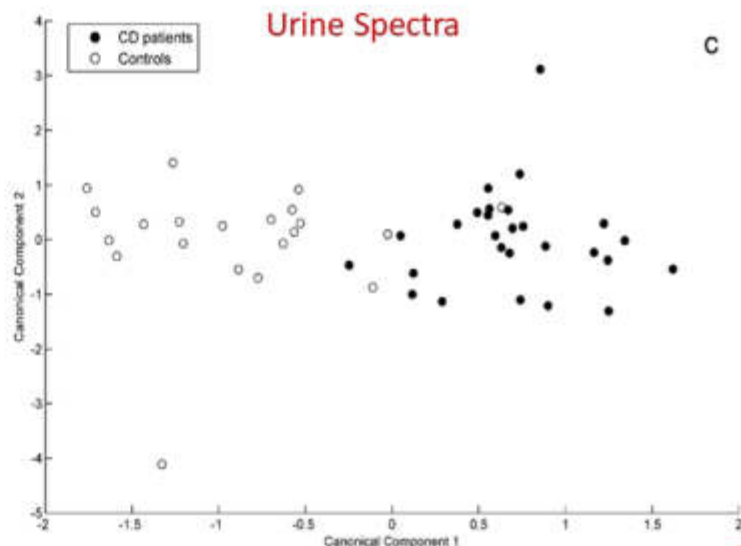
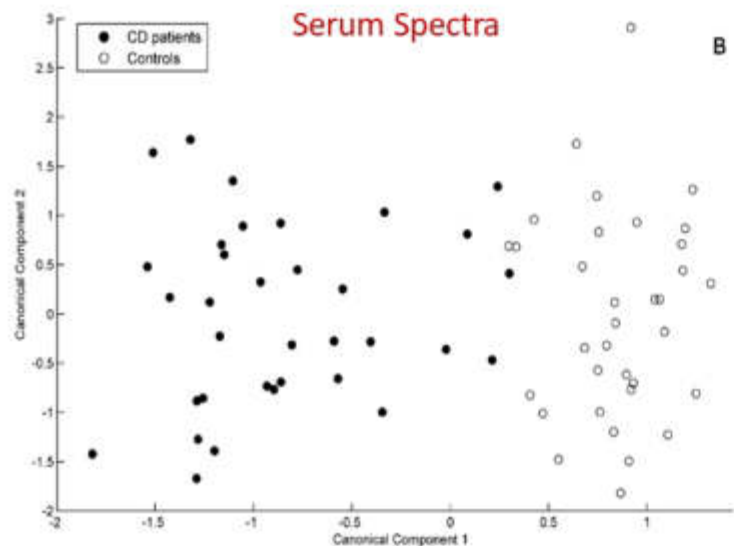
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The clustering model is built on 34 untreated patients and 34 controls spectra.





THE COMPARISON BETWEEN SERUM AND URINE



THE CLASSIFICATION RESULTS FOR SERUM AND URINE

	CD patients	HS	sensitivity	specificity	accuracy
Serum CPMG Spectra					
CD patients	32	2	94.1%		94.1%
HS	2	32		94.1%	
Serum NOESY Spectra					
CD patients	30	4	88.2%		92.6%
HS	1	33		97.2%	
Urine NOESY Spectra					
CD patients	24	3	88.9%		83.3%
HS	5	16		76.2%	

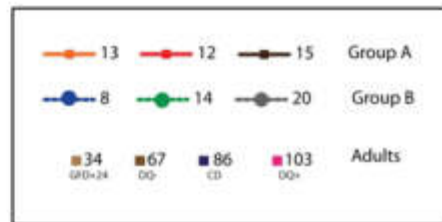
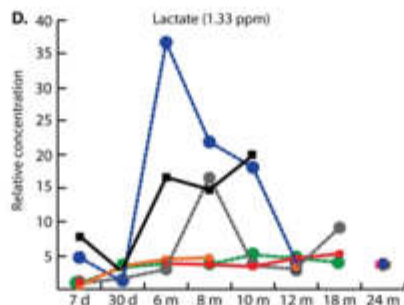
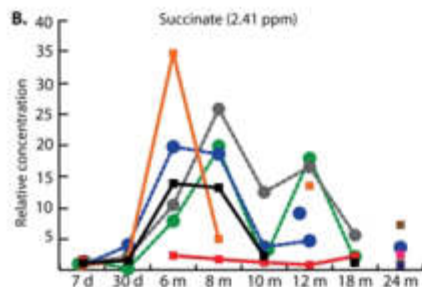
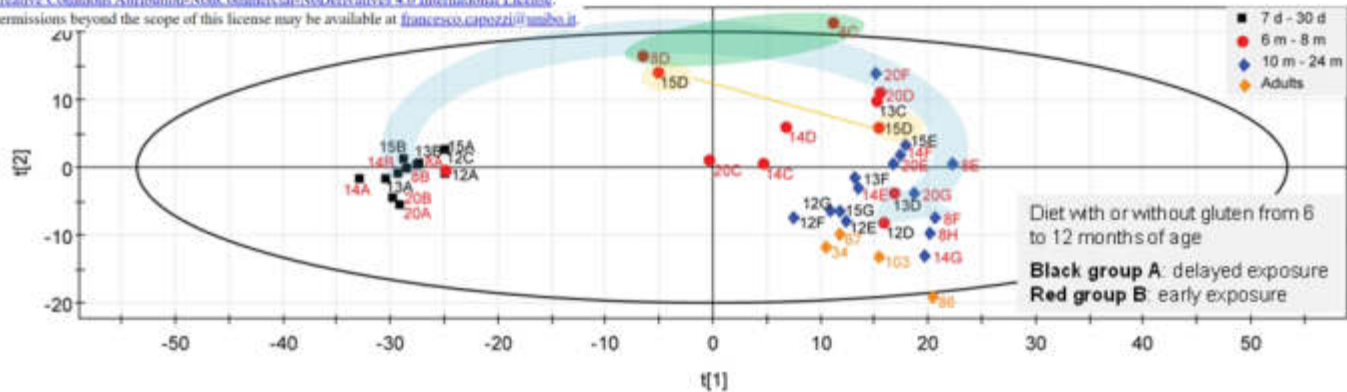
HS: healthy subjects



PCA APPLIED TO $^1\text{H-NMR}$ METABOLIC PROFILES OF FECAL SAMPLES from infants genetically susceptible to celiac disease



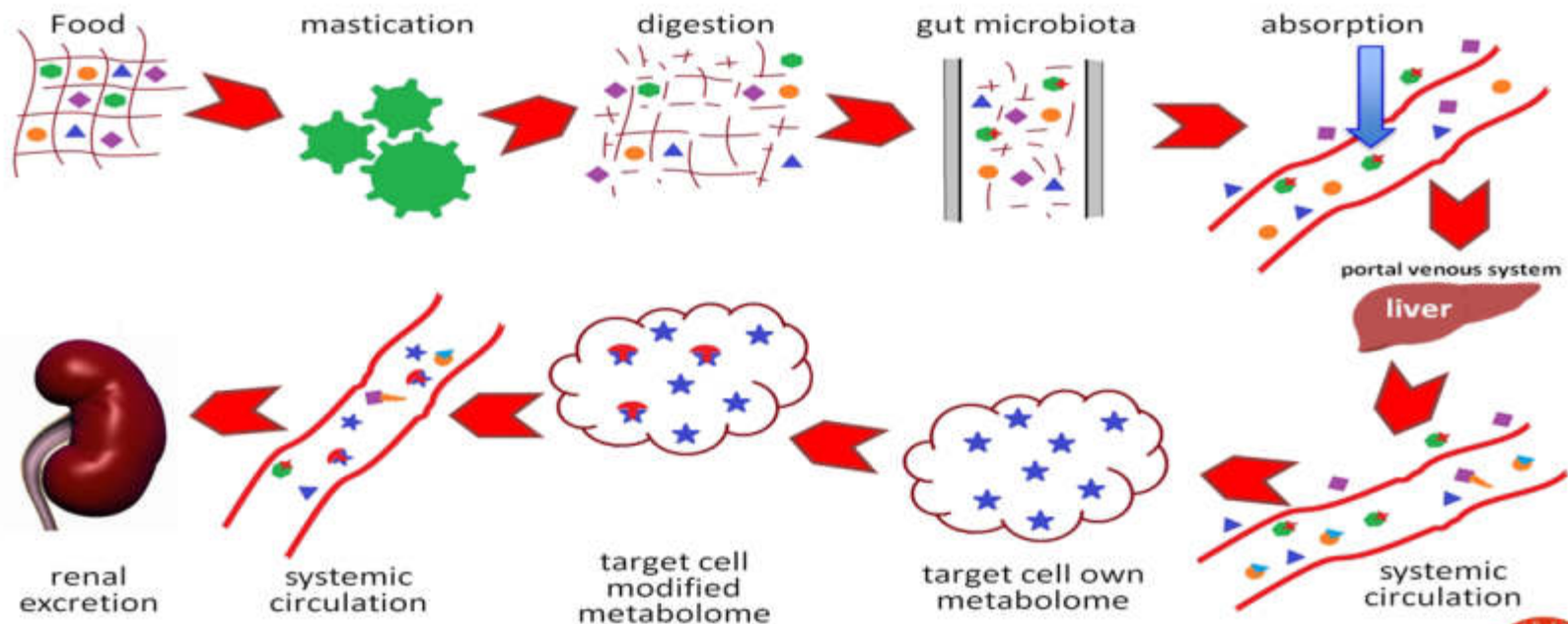
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FROM BIOACCESSIBILITY TO EXCRETION



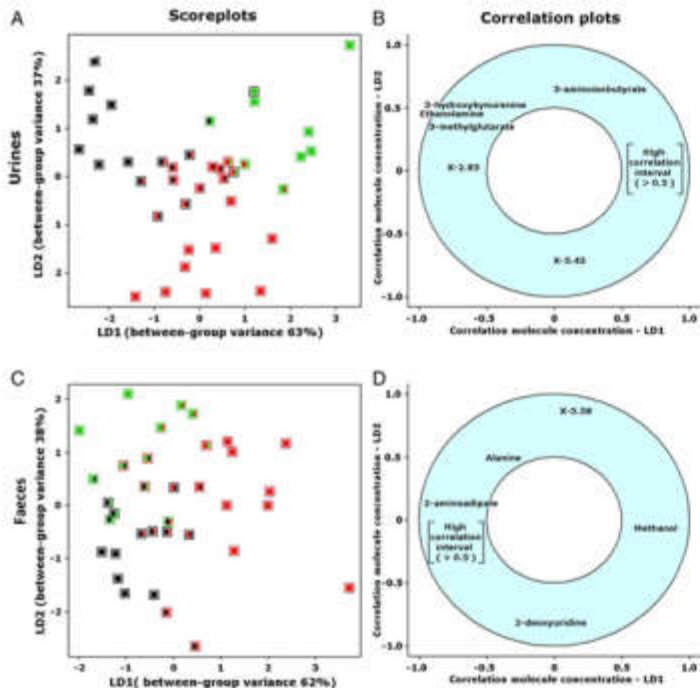
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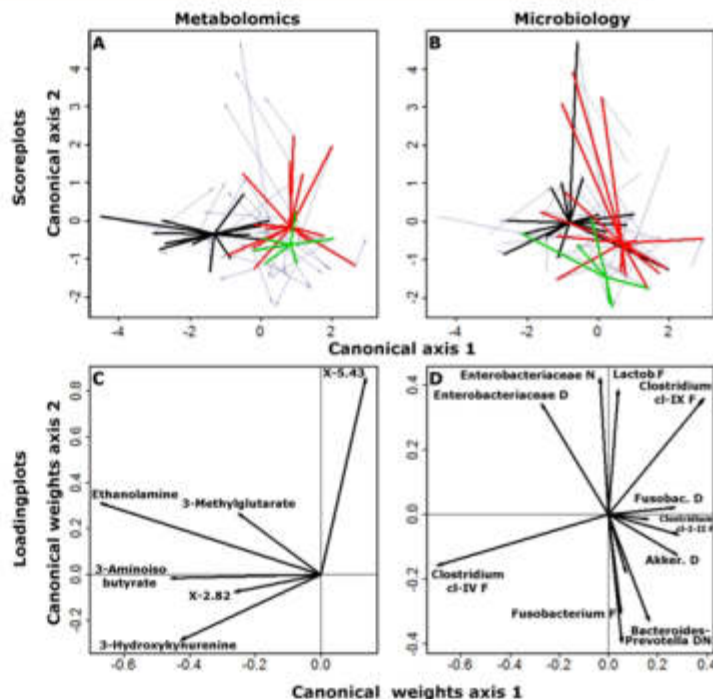
THE LINK BETWEEN METABOLOME AND MICROBIOME



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CTRL: asymptomatic control subjects without diverticula of the colon
 DIVERT: asymptomatic patients with diverticula of the colon
 SUDD: symptomatic uncomplicated diverticular disease



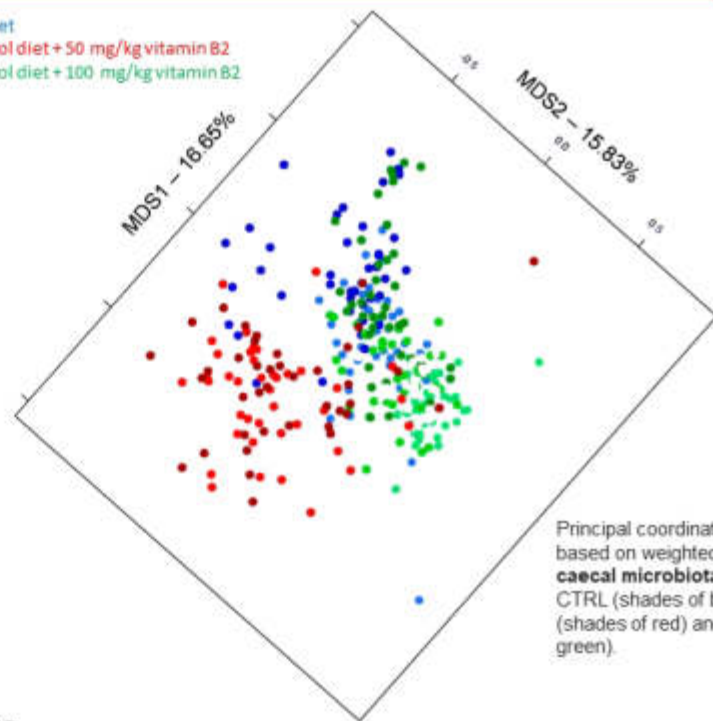
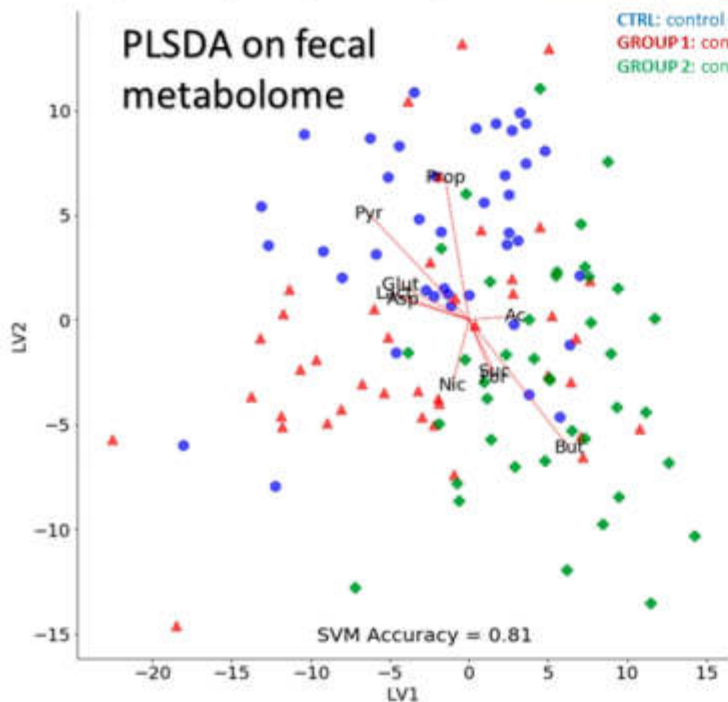
Canonical weights axis 1



THE SUPPORT VECTOR MACHINE CLASSIFIER



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Principal coordinates analyses (PCoA) based on weighted UniFrac distances of **caecal microbiota profiles** in broilers in CTRL (shades of blue), GROUP 1 (shades of red) and GROUP 2 (shades of green).

Ac: acetate, Pyr: pyruvate, Asp: aspartate, Lact: lactate, Nic: nicotinate, For: formate, Glut: glutamate, But: butyrate, Suc: succinate, Prop: propionate.

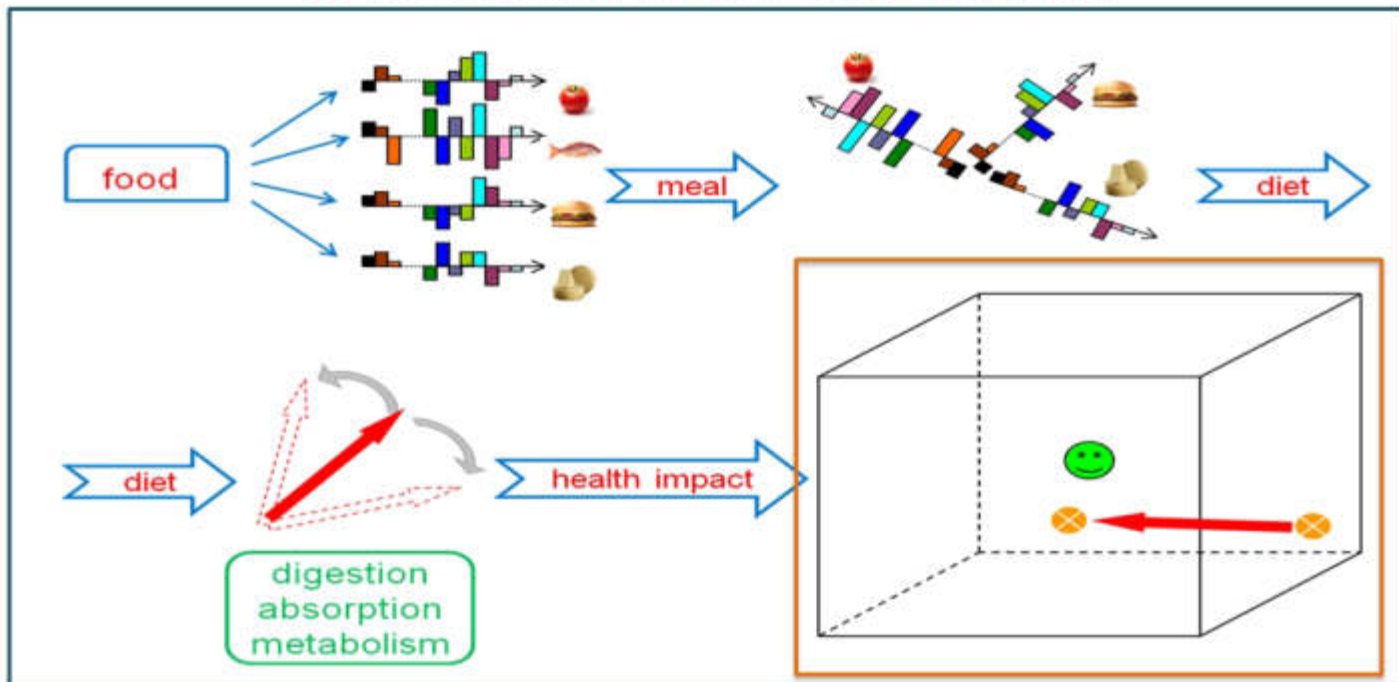


OBJECTIVE: FINDING THE LINK...



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... between food and health*



*Alessandra Bordoni and Francesco Capozzi. "Foodomics for healthy nutrition." *Current Opinion in Clinical Nutrition & Metabolic Care* 17.5 (2014): 418-424.





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Thank you for your attention !

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