

Janus

Two faces of metabolomics

TNO | Knowledge for business



Elwin Verheij



Janus – God of gates & doors, beginnings and endings
Symbol of change, transition, growth, past and future

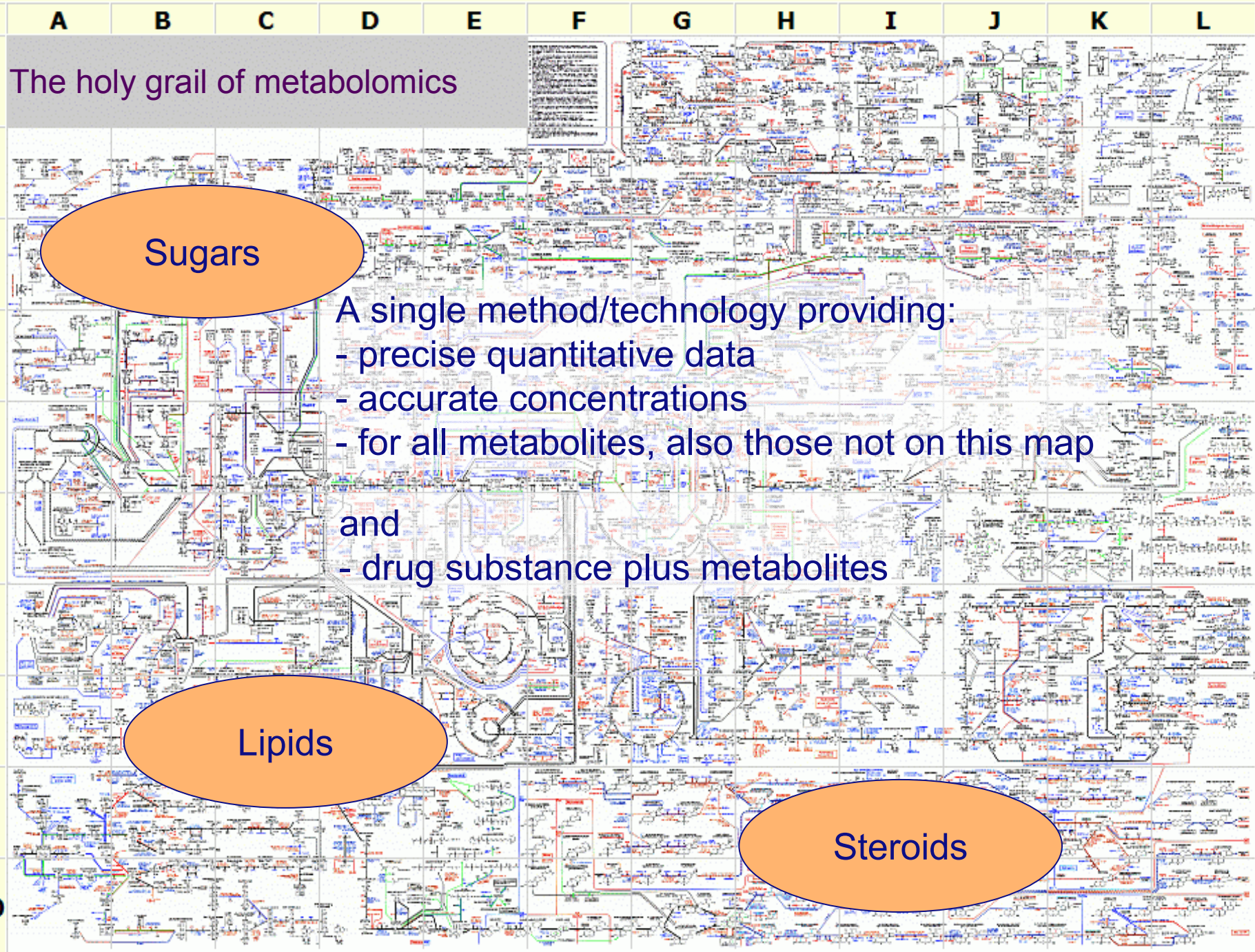
Analytical technology

Statistics
Data push
Identification
Hypothesis generation
Unbiased
Biomarker discovery



Biology

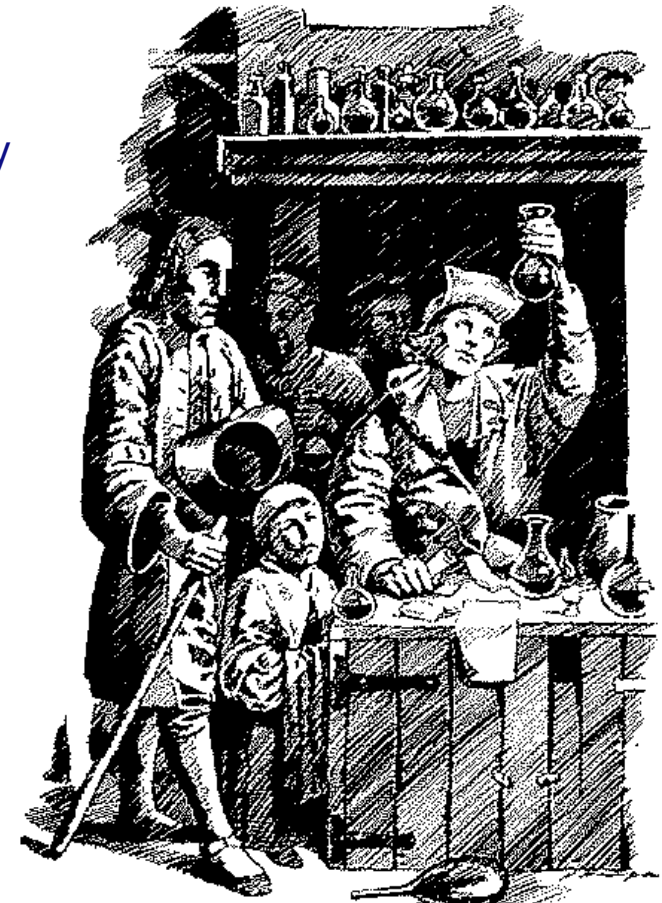
Bioinformatics
Information pull
Knowledge
Hypothesis testing
Targeted
Mechanisms



16th and 17th century 'biomarkers': Piskijkerij/Uroscopy



Urine watcher,
Joost van Craesbeeck,
first half of 17th century

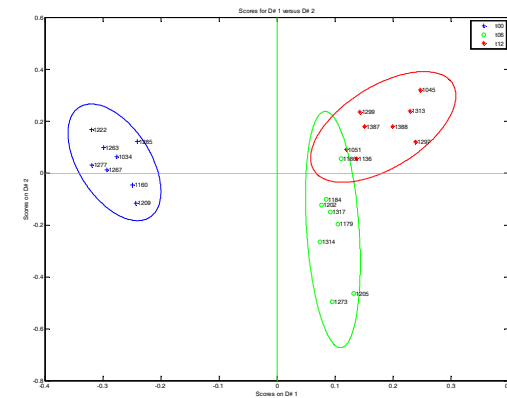
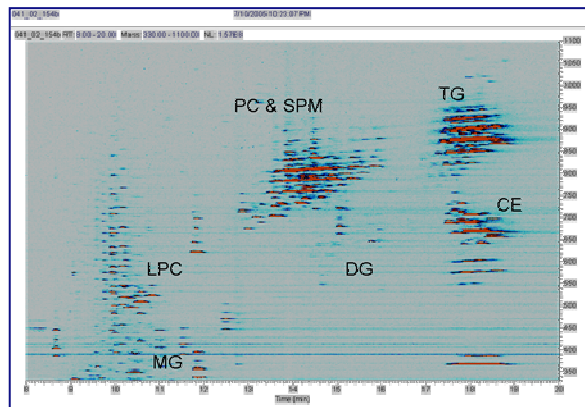
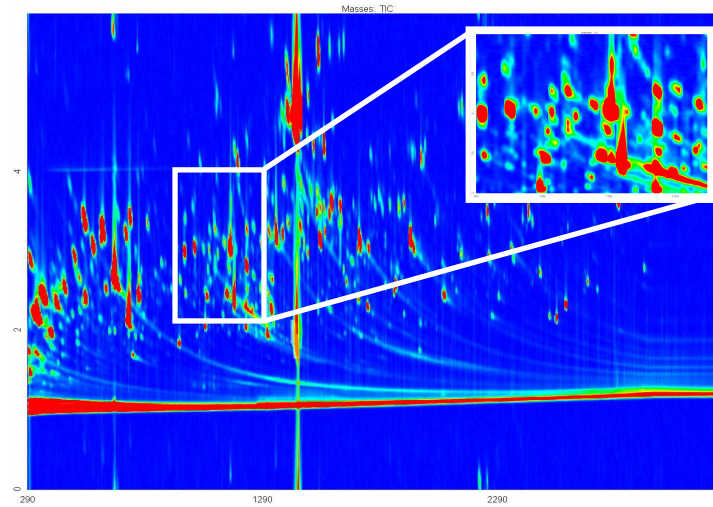
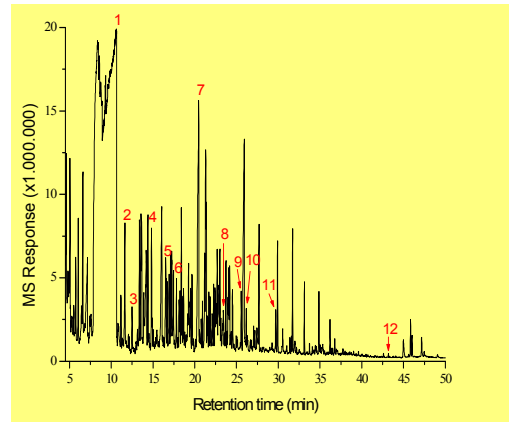
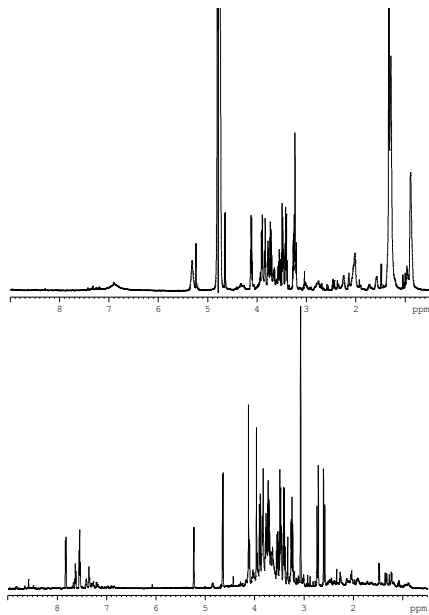


-MET CONSULT BIJ DEN PISKIJKER- NAAR OENGRAVING VAN H. VAN HAEFTEN



Comprehensive analysis - Analytical technologies

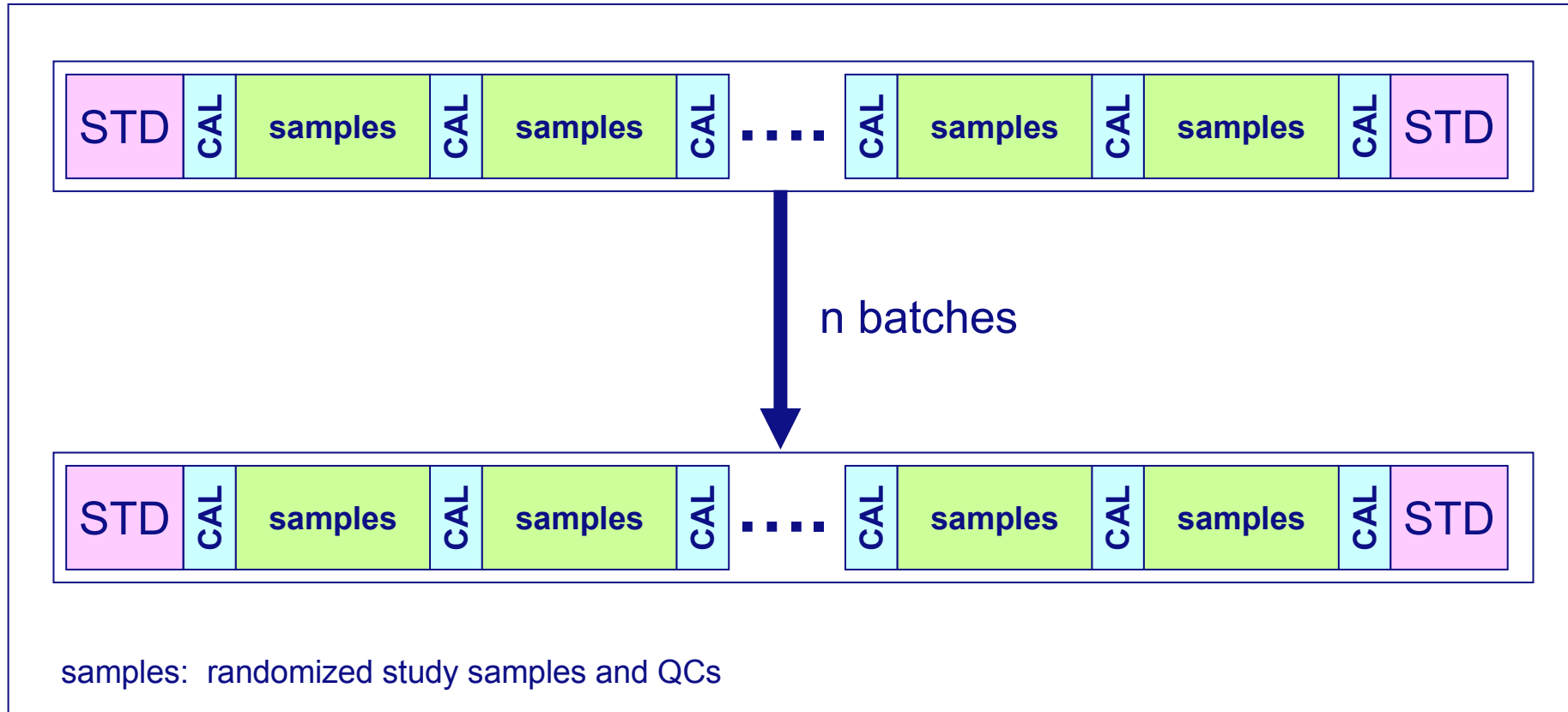
- NMR
- GC-MS
- GCxGC-MS
- LC-MS
- LC-IM-TOF
- CE-MS



Data quality

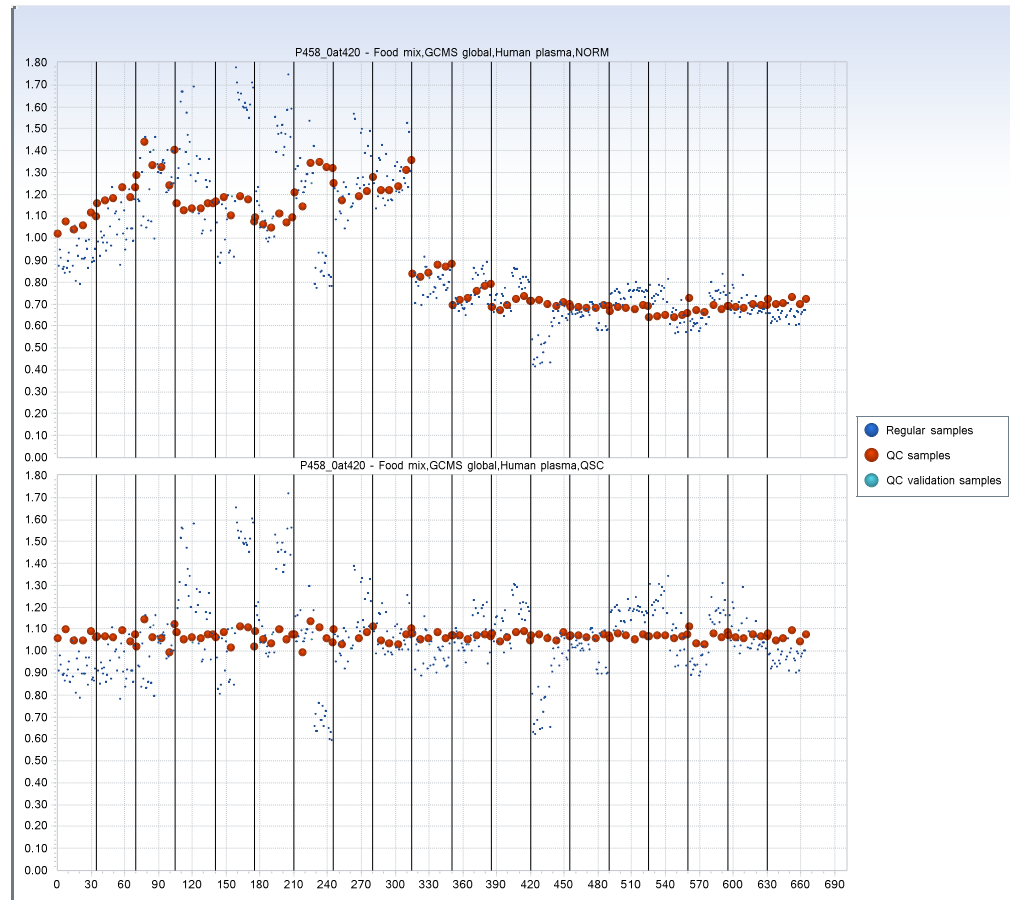


Standardized analysis scheme

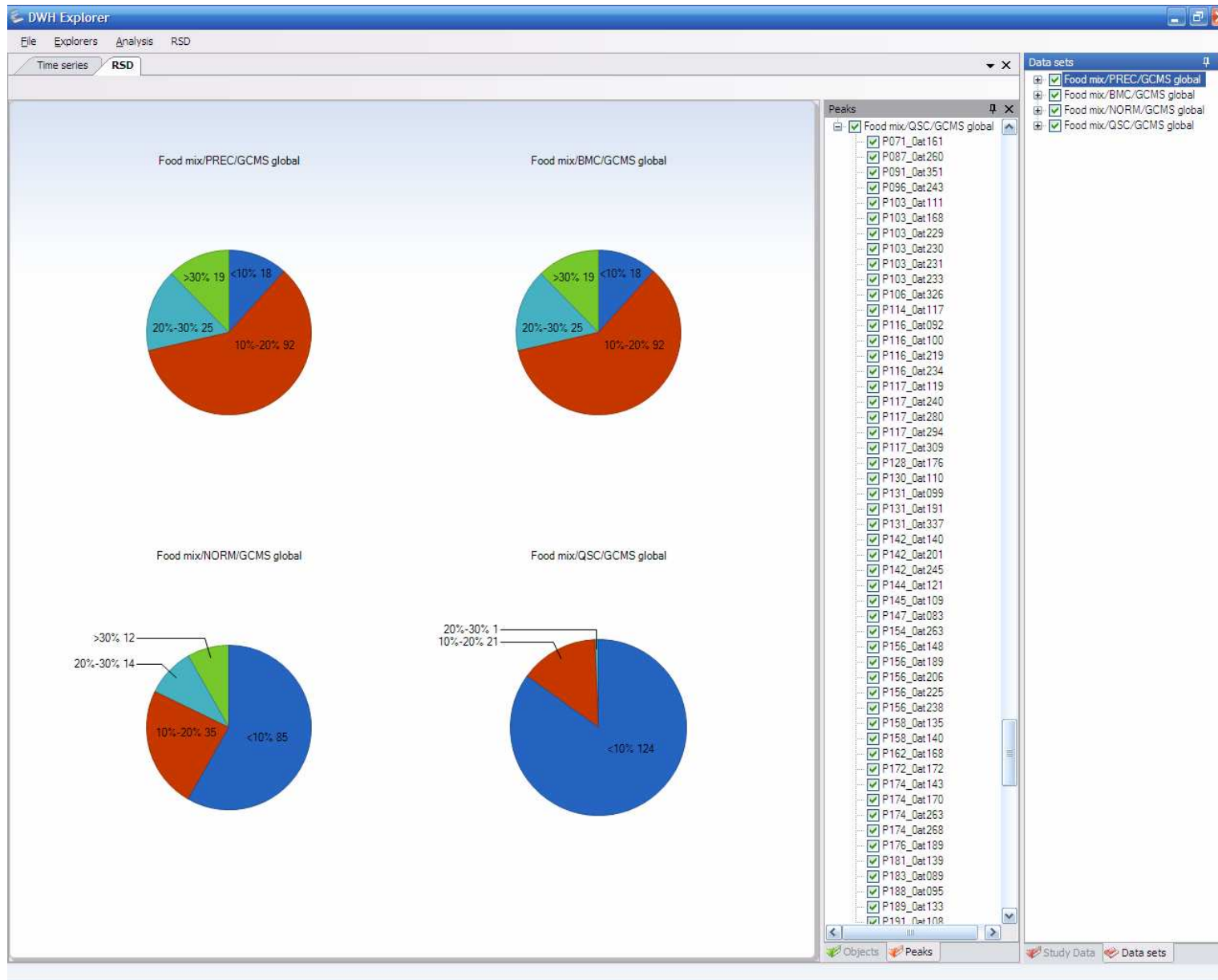


J. Proteome Research, Scheele Award Paper, 2007

Batch offset correction GC-MS

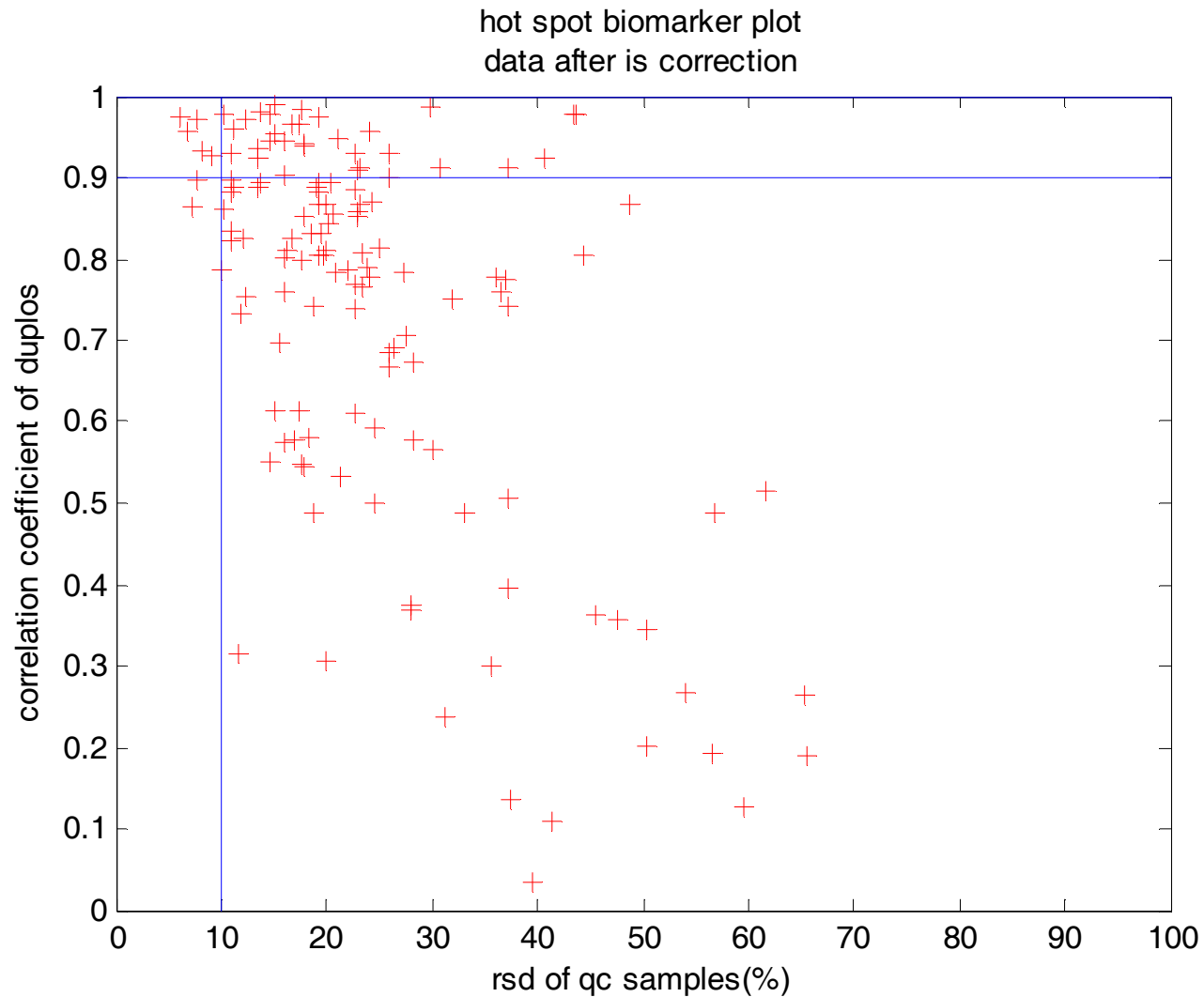


Metabolite RSD% distribution at different processing stages

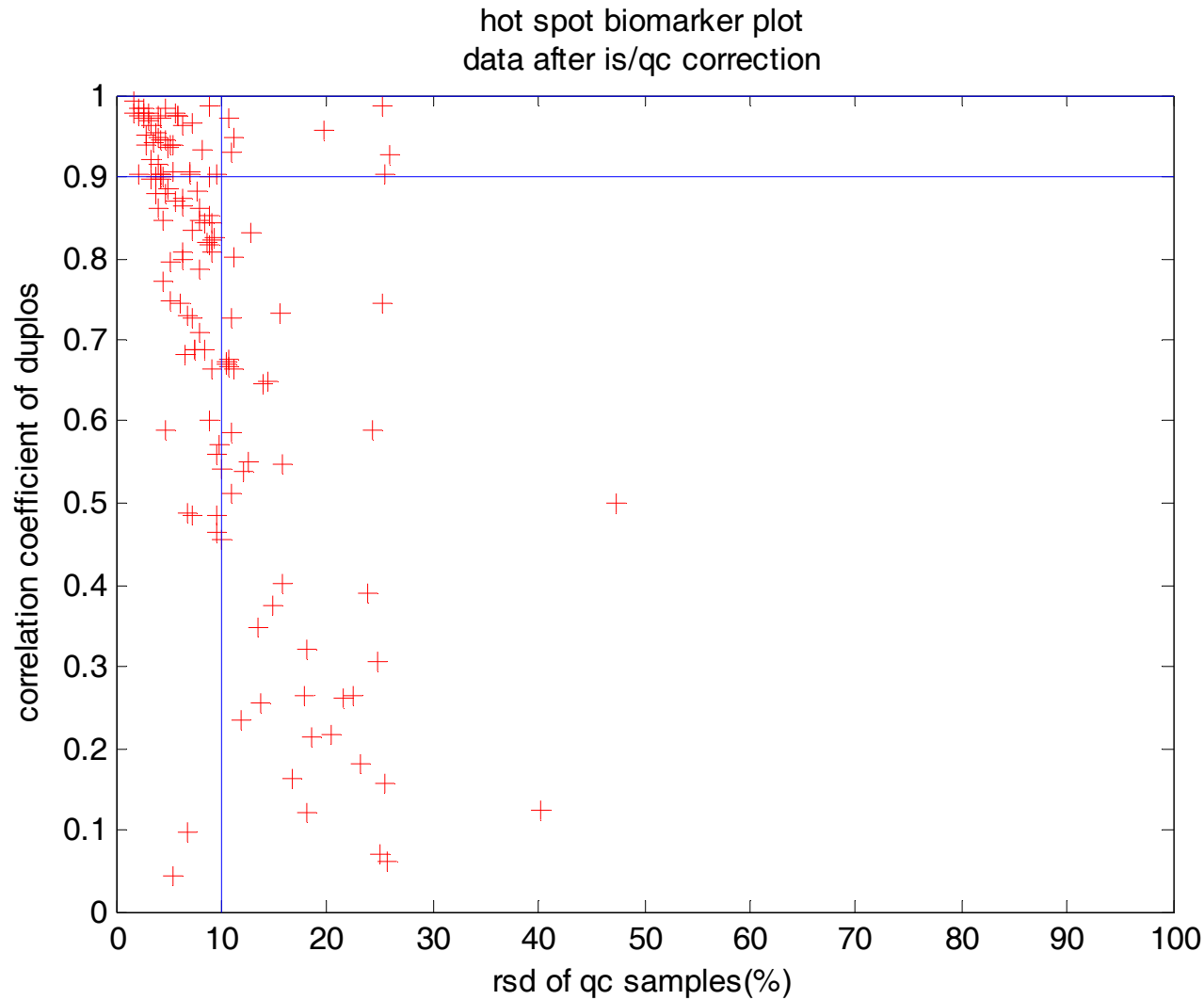


Biomarker discovery hotspot plot

GC-MS, IS corrected data



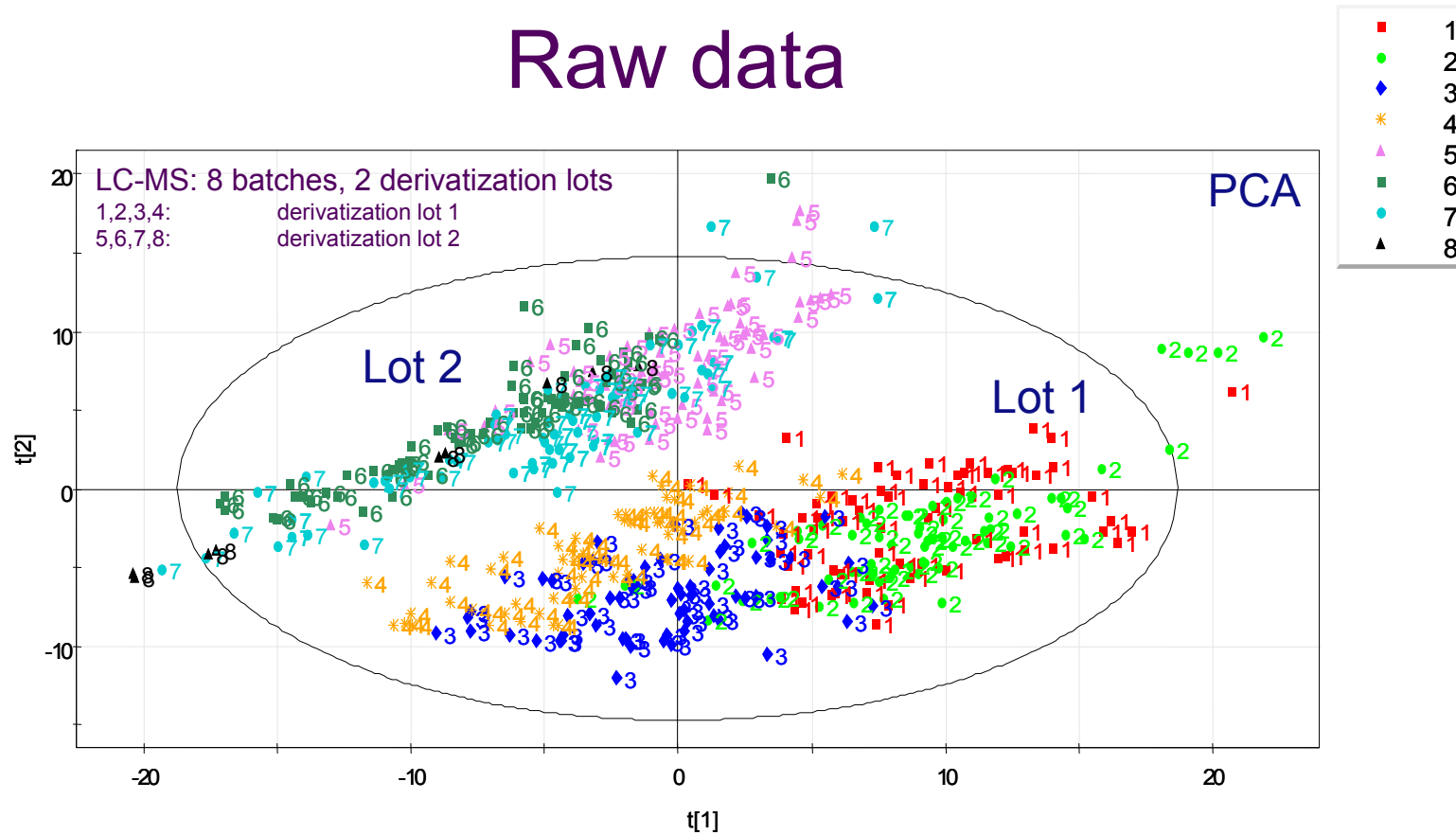
Biomarker discovery hotspot plot GC-MS, QC corrected data



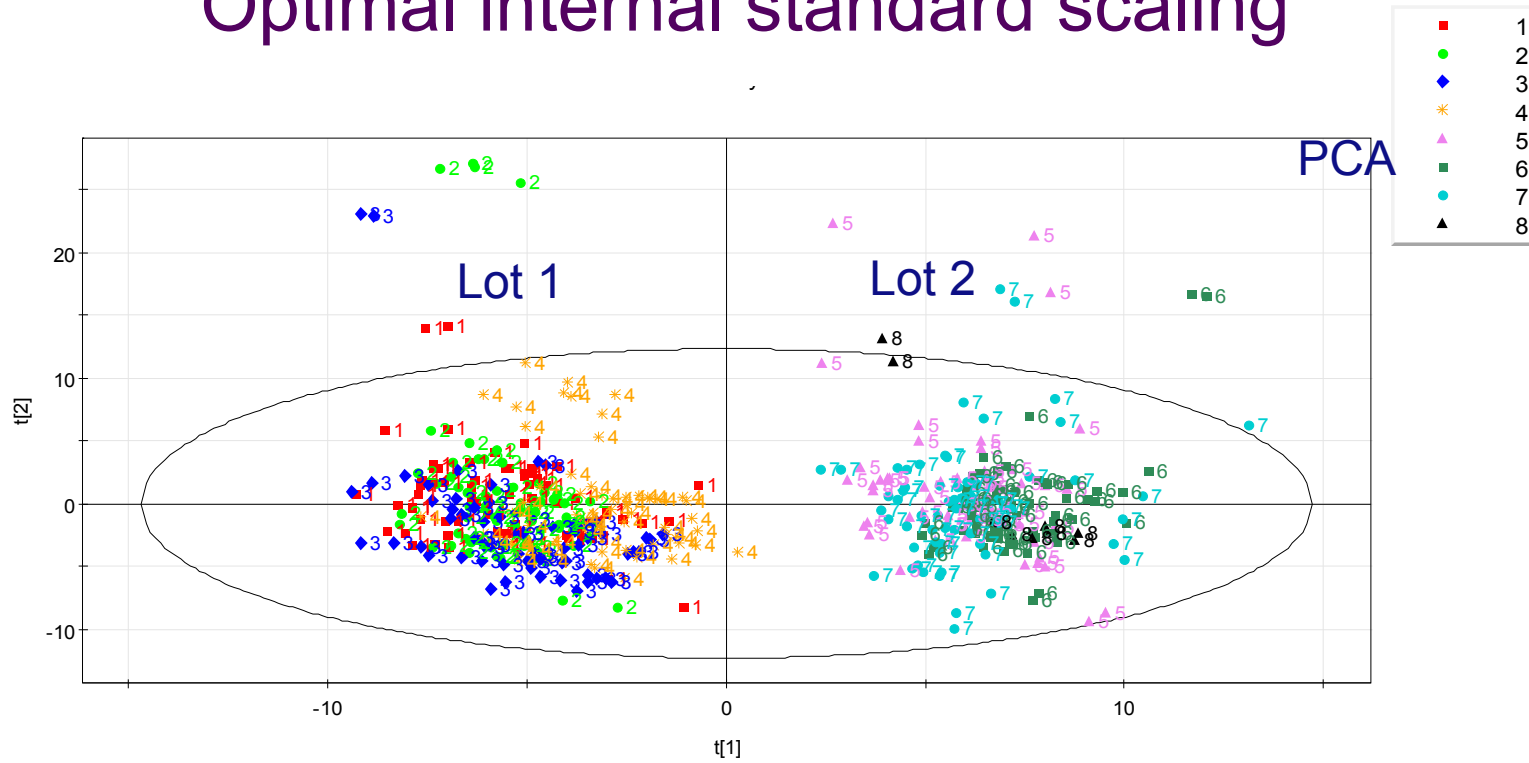
Example LC-MS derivatized

Clinical samples – predisposition markers side effects

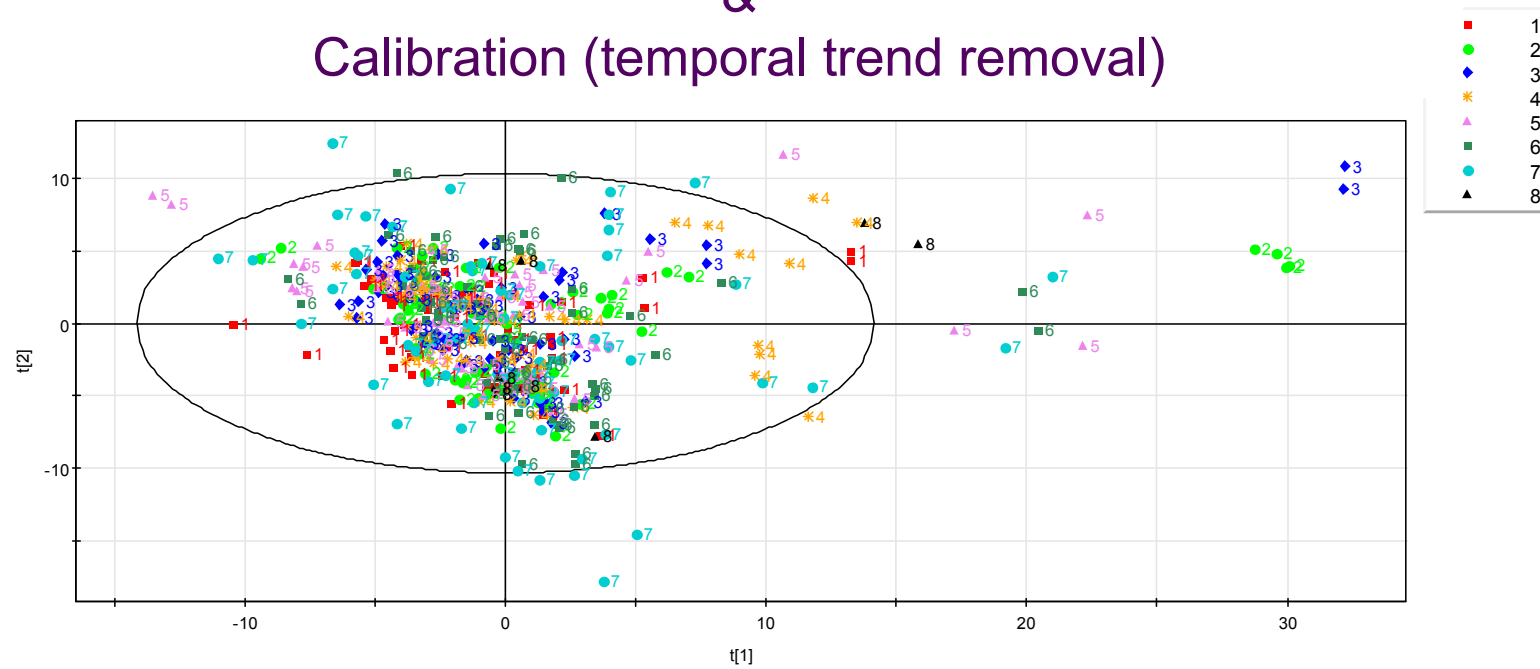
Raw data



Optimal internal standard scaling



Optimal internal standard scaling & Calibration (temporal trend removal)



Unbiased metabolomics

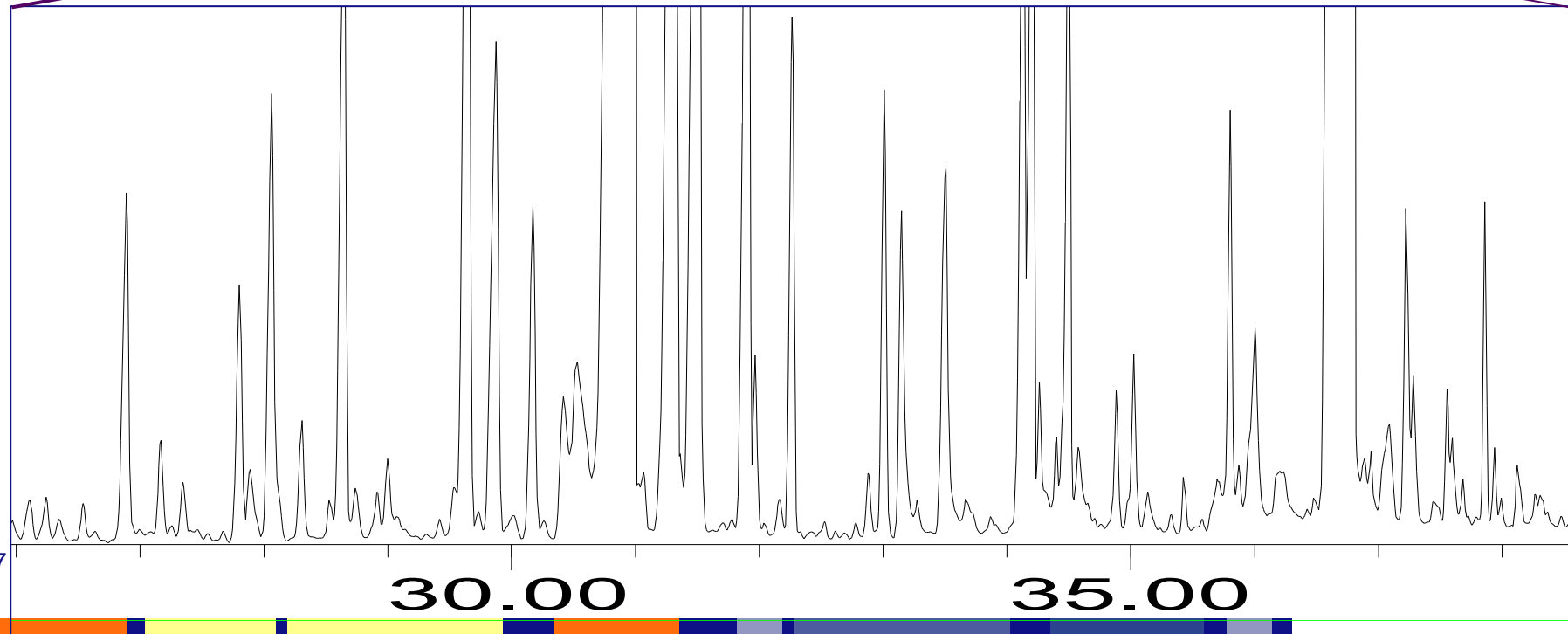
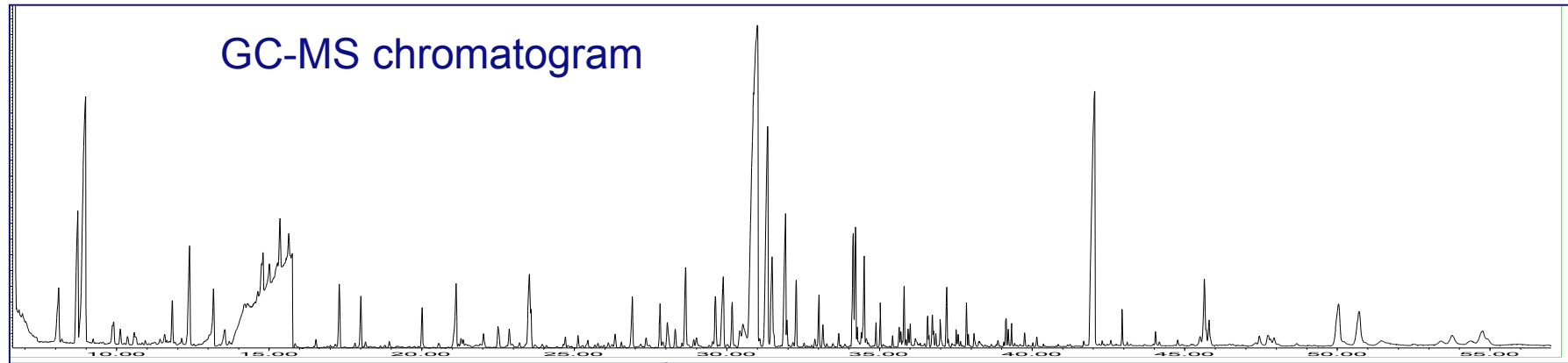
- Tons of data
- Complex multivariate statistics for finding differences
- Poorly understood multivariate models
- Too many unknowns
 - Frustrated clients
 - No/limited biological interpretation
 - Relatively poor method performance and data quality
 - Identification is time and money consuming effort

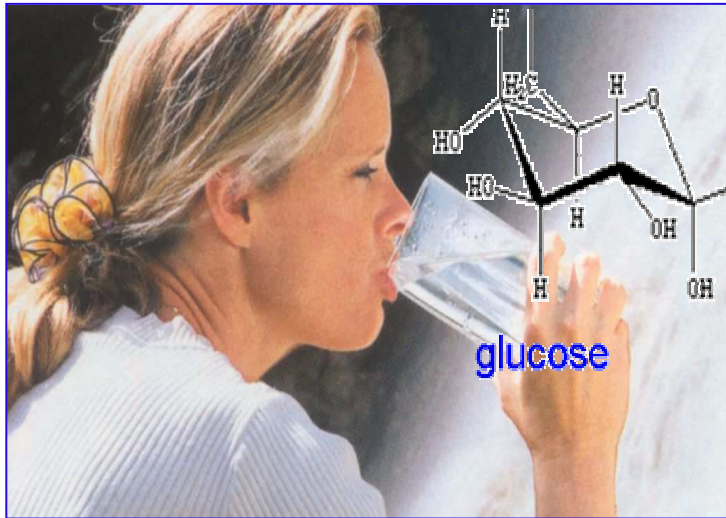
The relationship between metabolic status and inflammatory status

Characterization of 19 overweight males (BMI 28), by analysis the “omics” response to an oral glucose tolerance test

- **Transcriptome** analysis of Peripheral Blood Mononuclear Cells
- **Proteome** analysis of 80 inflammation & metabolism related proteins
- OGTT plasma **metabolomics** at t=0, 15, 30, 45, 60, 90, 120, 180 minutes
 - Lipidome analysis at all time points
 - Free Fatty Acids
 - Inflammation related lipids (oxylipids)
 - All TG, LPC, PC, Cholesterol esters, SPM
 - Metabolome analysis at all time points

Metabolomics: ~ 350 metabolites quantified in human plasma



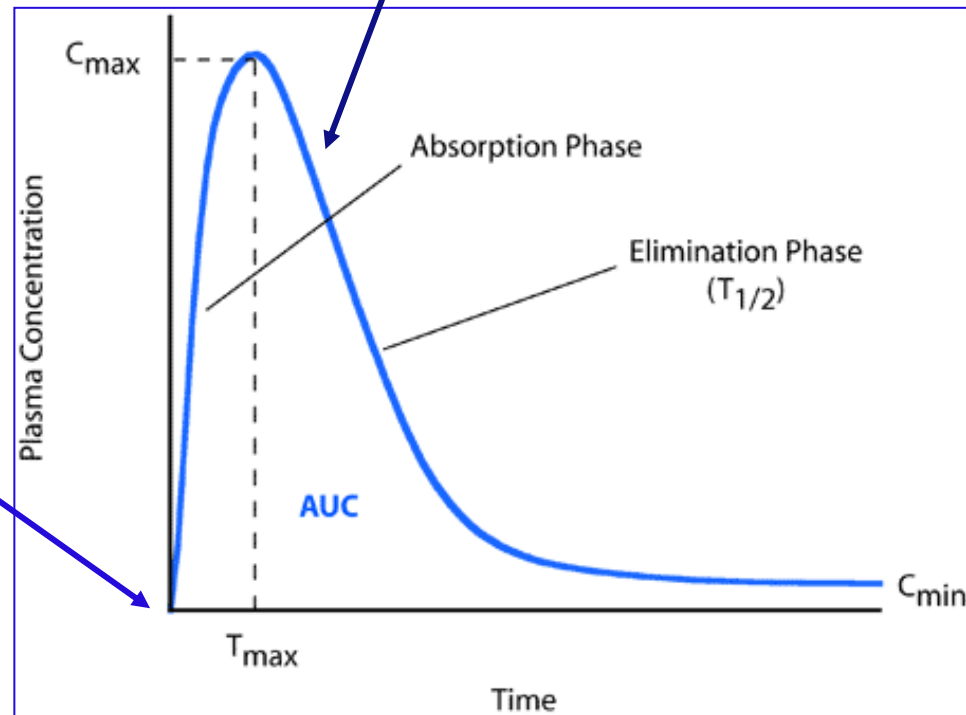


Metabolite plasma concentration curve after glucose dose

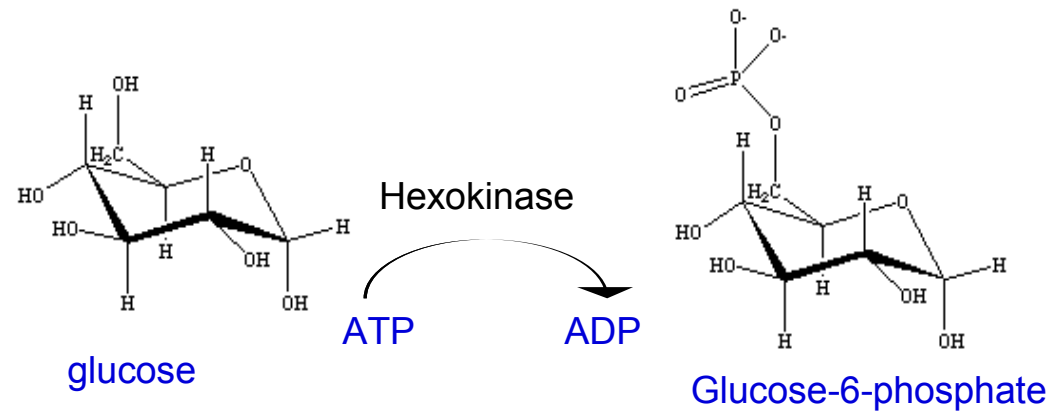
- GCMS and LCMS (251 polar metabolites)
- LCMS lipidome (74 metabolites)

Homeostatic concentration

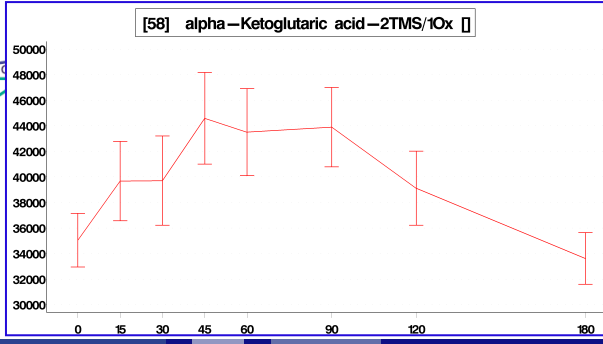
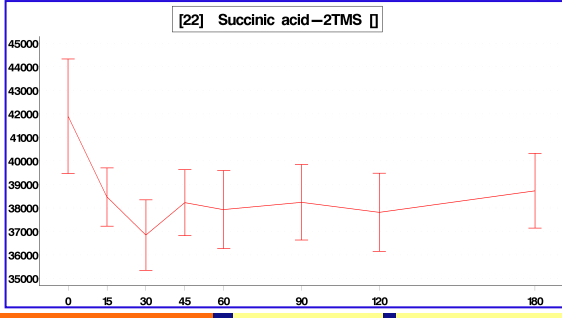
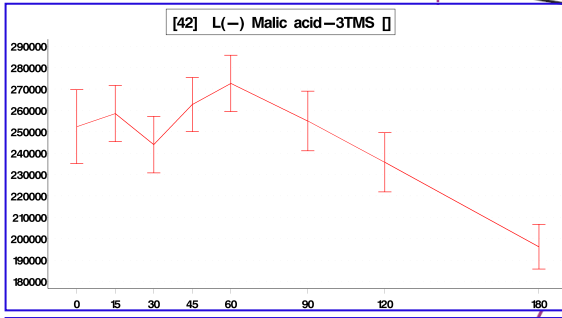
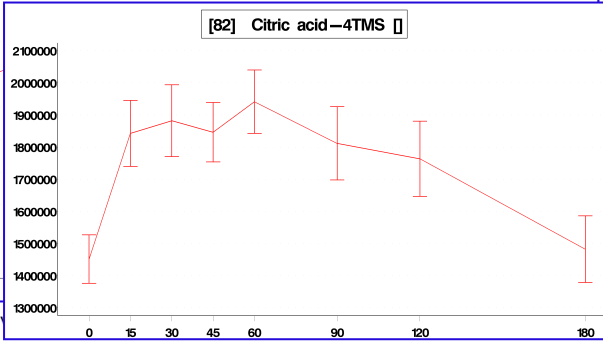
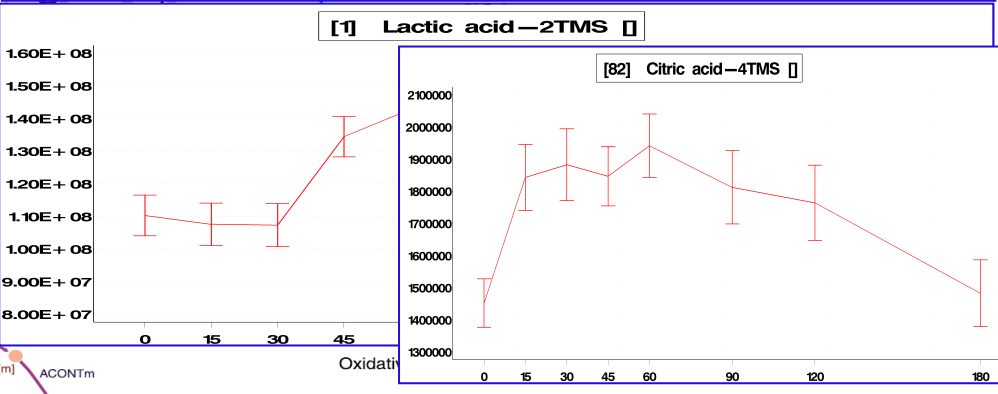
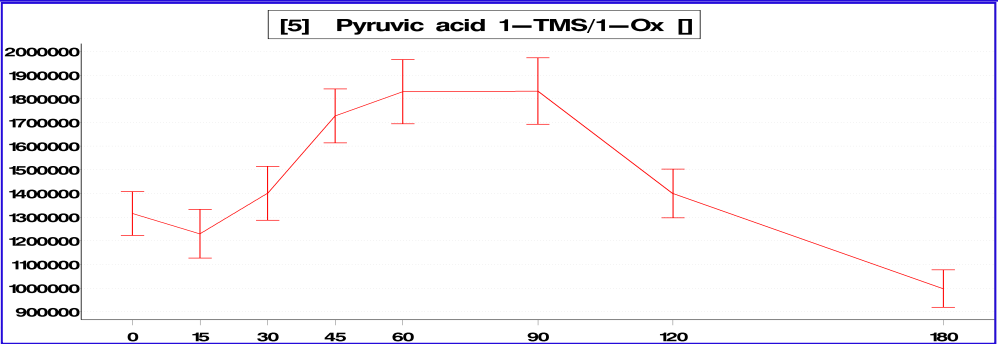
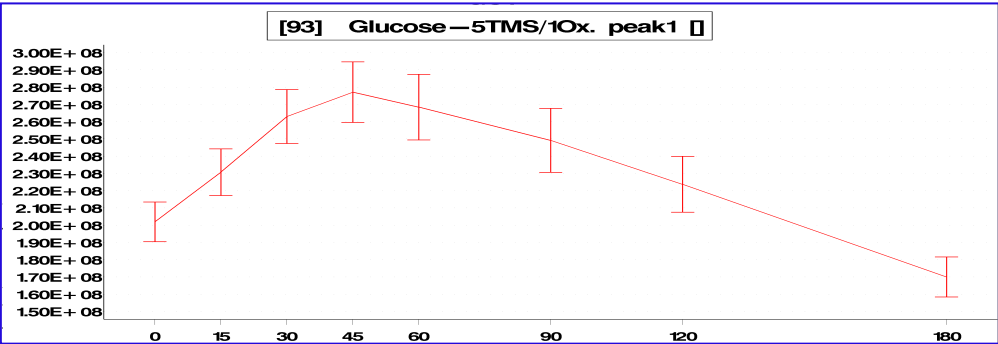
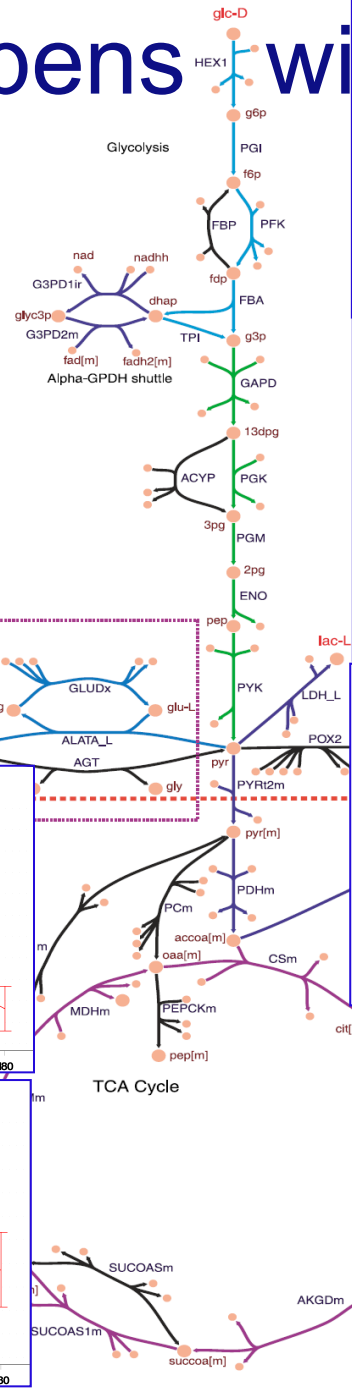
- PBMC transcriptome
- plasma proteome
- plasma oxylipids



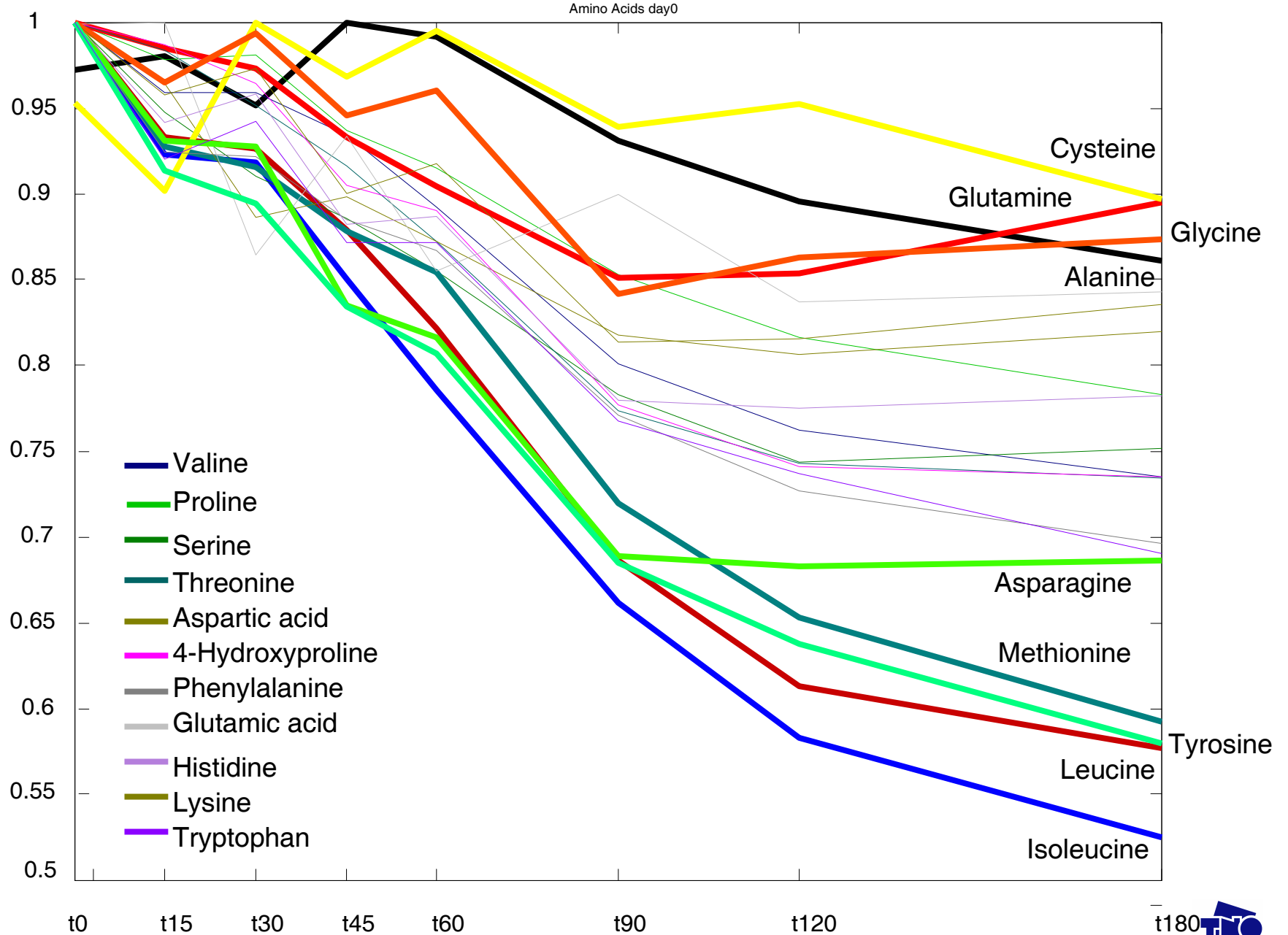
What happens with the glucose?



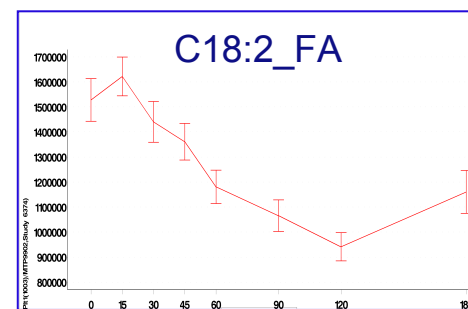
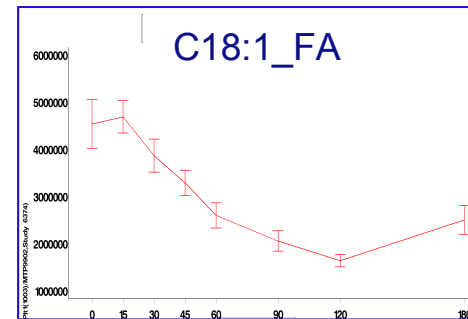
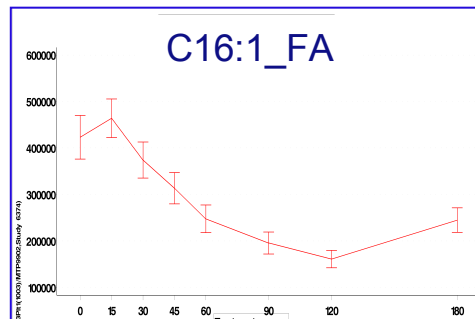
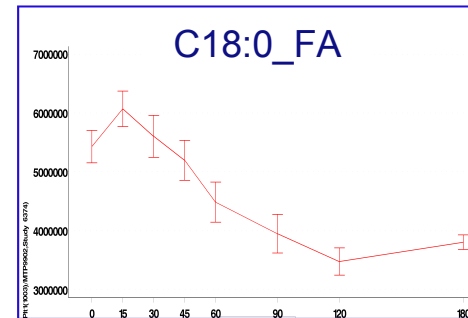
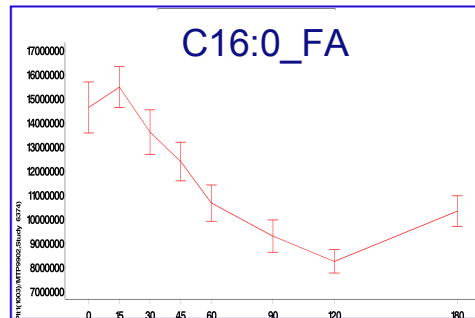
What happens with



Plasma Amino Acid time course in response to glucose challenge



OGTT response of Free Fatty Acids



2D GC×GC-MS

- Why?
- Use GC×GC-MS to increase metabolome coverage
 - 2 different column types improve separation power
 - Reduce co-elution
 - Improve quantification of metabolites eluting close to high abundant metabolites
 - Lower detection limits compared to 1D-GC-MS

Analytical performance (I): standard deviations of internal standards

Compound	Standard deviation of MS response	
	1D-GC-MS	2D-GCxGC-MS
Alanine-d4	9	8
Leucine-d3	5	8
Glutamic acid-d3	6	8
Phenylalanine-d5	12	7
Cholic acid-d4	4	6

RSD's of internal standards for 2D-GCxGC-MS comparable with 1D-GC-MS

Analytical performance (II): Detection limits

Detection limits in pg on-column

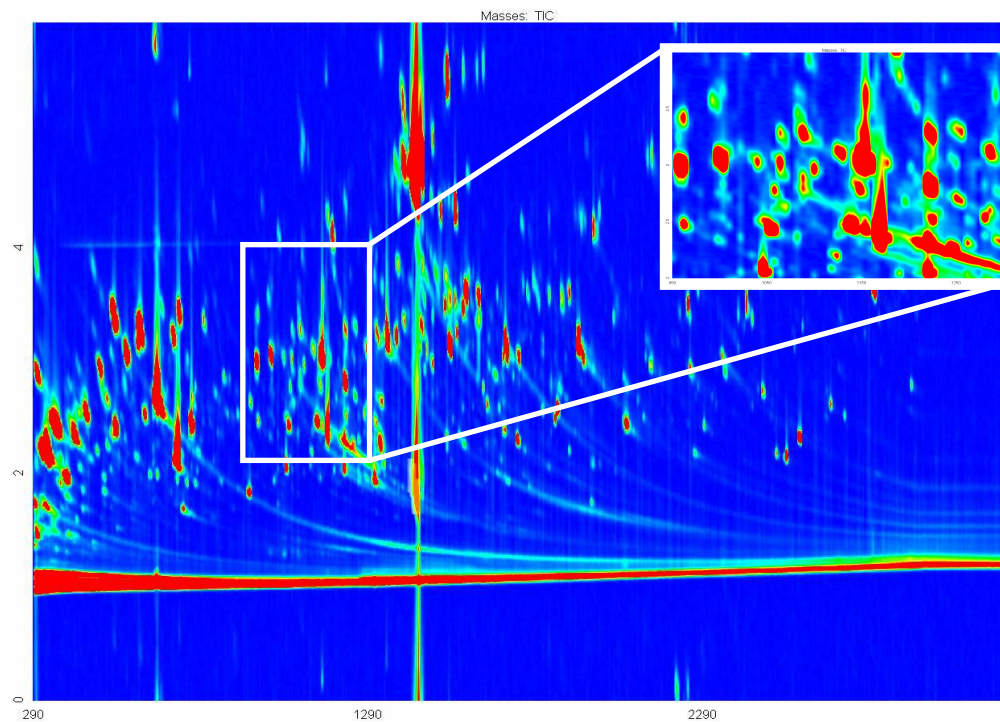
Metabolite	1D GC-MS	2D GC×GC-MS
Succinic acid	25	2
4-Amino-butanol	1	0.2
Citric acid	50	3
Fructose	15	7
Glutamine	120	110
Lysine	3	1
Pentadecanoic acid	30	0.5
9-Octadecenoic acid	35	2
Fructose-6-phosphate	55	15
11-Amino-undecanoic acid	6	0.5
Cholic acid	120	5

Analytical performance: metabolite coverage

Example: mouse liver study

1D GC-MS 300 compounds (S/N > 10)

2D GC×GC-MS 2000 compounds (S/N > 50)



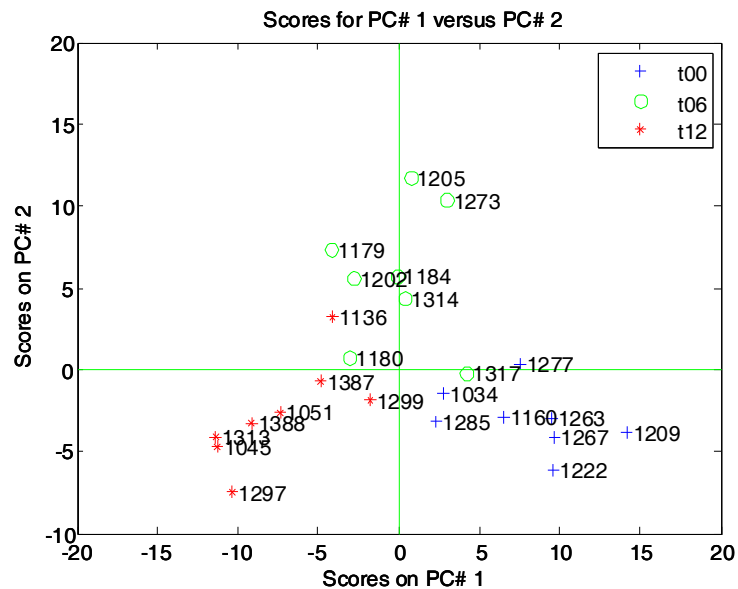
PCA

Goal: study the way metabolic dietary (high fat) stress induces insulin resistance.

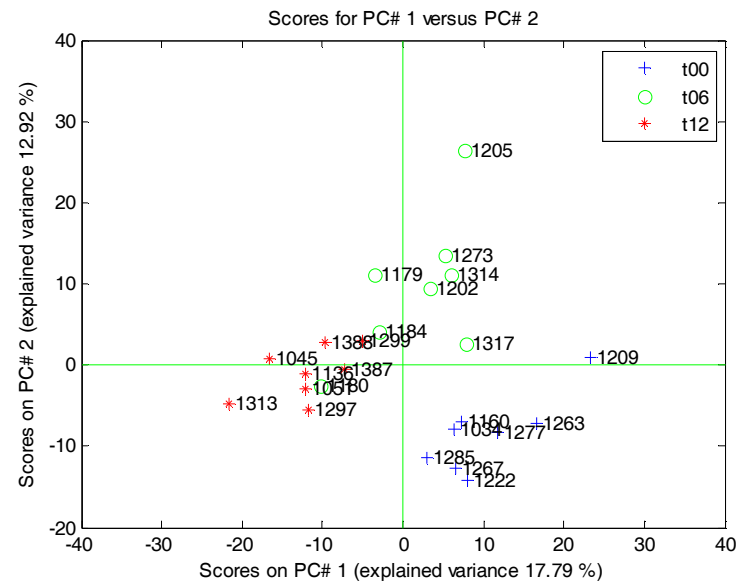
Study design: 24 mice on high fat diet. Liver samples collected on three time points after start of diet (0, 6 and 12 weeks).

Insulin resistance measured in liver.

1D GC-MS



2D GC×GC-MS



Bile acids

- Are complex metabolic integrators and signalling factors
- Number of bile-acid-activated signalling pathways are becoming therapeutic agents for metabolic disorders
 - Obesity
 - Type 2 diabetes
 - Atherosclerosis
 - Non-alcoholic steatohepatosis
- Markers of hepatobiliary and intestinal disease
- Essential for digestion
- Important for cholesterol homeostasis
- Markers of liver toxicity

Thomas Ch. et al. Nature Reviews, Drug discovery, Volume 7, August 2008, 678-693

Bile acids analysis

- No SPE
- Straightforward sample preparation
- Low sample amounts needed
 - 50 μl plasma/serum
 - 20 μl urine
 - 5 mg liver
- Specificity based on accurate mass and retention time
- Detection limit: 10-50 pg on column

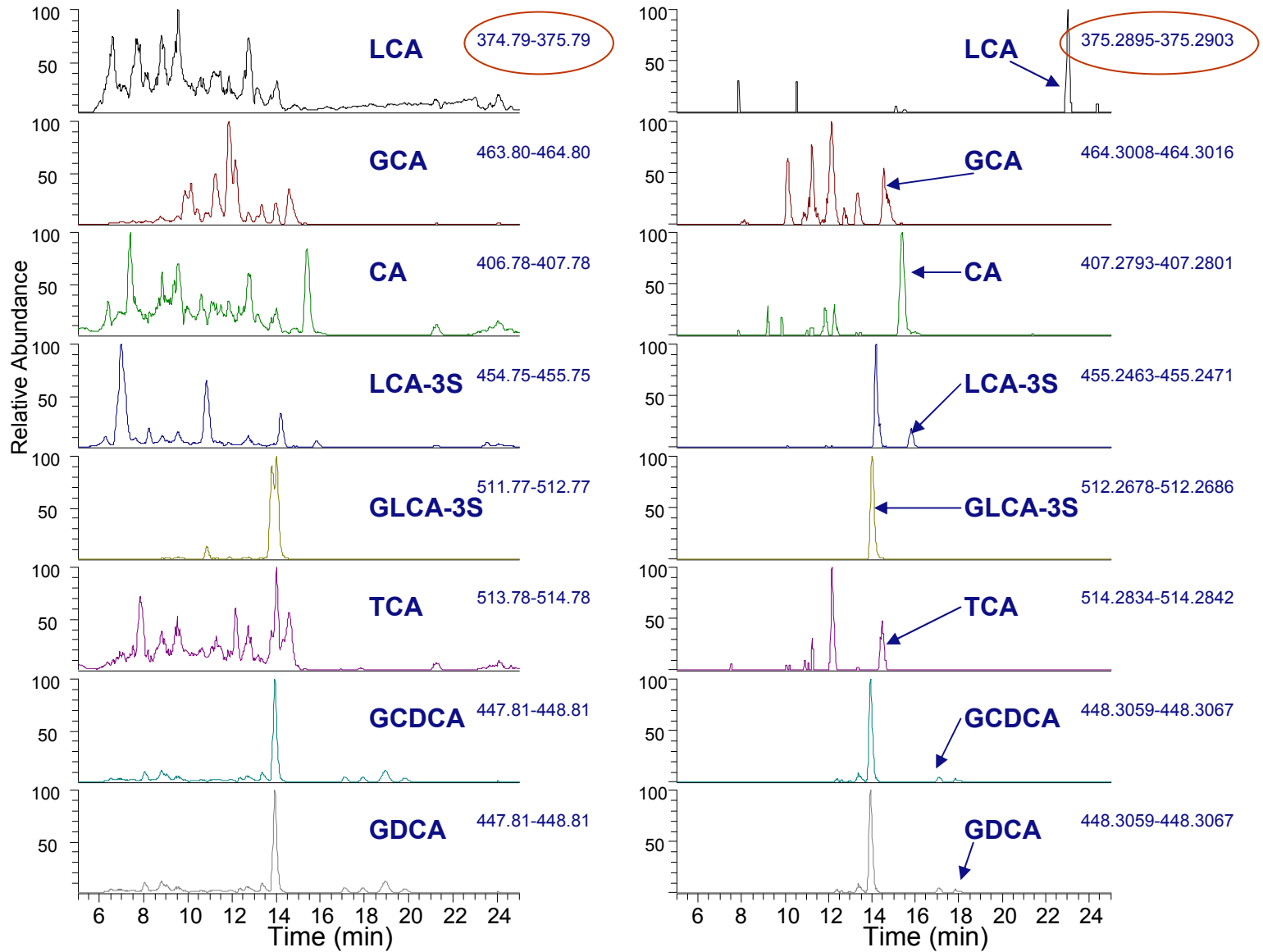


I. Bobeldijk et al. Journal of Chromatography B, 871 (2008) 306–313

Human urine sample

A: 1000 mDa window

B: 2 mDa window

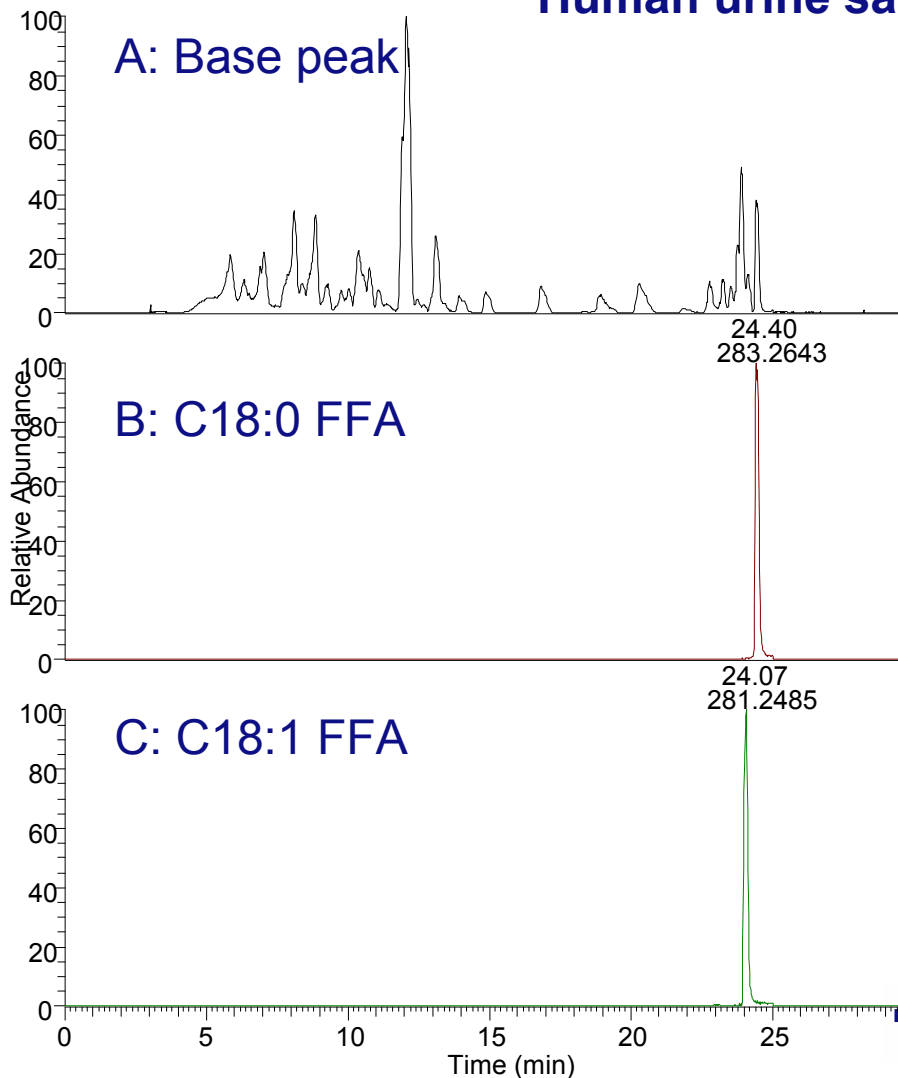


Bile acids

	bile acid
CA	Cholic_acid
CDCA	Chenodeoxycholic_acid
DCA	Deoxycholic_acid
GCA	Glycocholic_acid
GCDC	Glycochenodeoxycholic_acid
GDCA	Glycodeoxycholic acid
GLC-3S	Glycolithocholic acid 3-sulfate
HDCA	Hyodeoxycholic acid
LCA	Lithocholic acid
LCA-3S	Lithocholic acid 3-sulfate
MCA	Muricholic acid
TCA	Taurocholic_acid*
TCDC	Taurochenodeoxycholic_acid
TDCA	Taurodeoxycholic_acid
TLCA	Taurolithocholic acid
TUDCA	Tauroursodeoxycholic_acid
UDCA	Ursodeoxycholic_acid

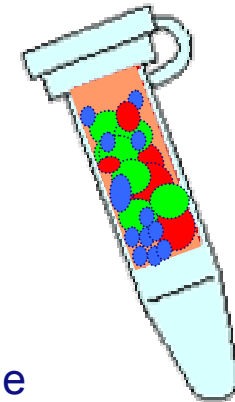
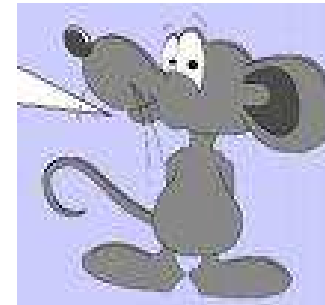
Other metabolites

Human urine sample



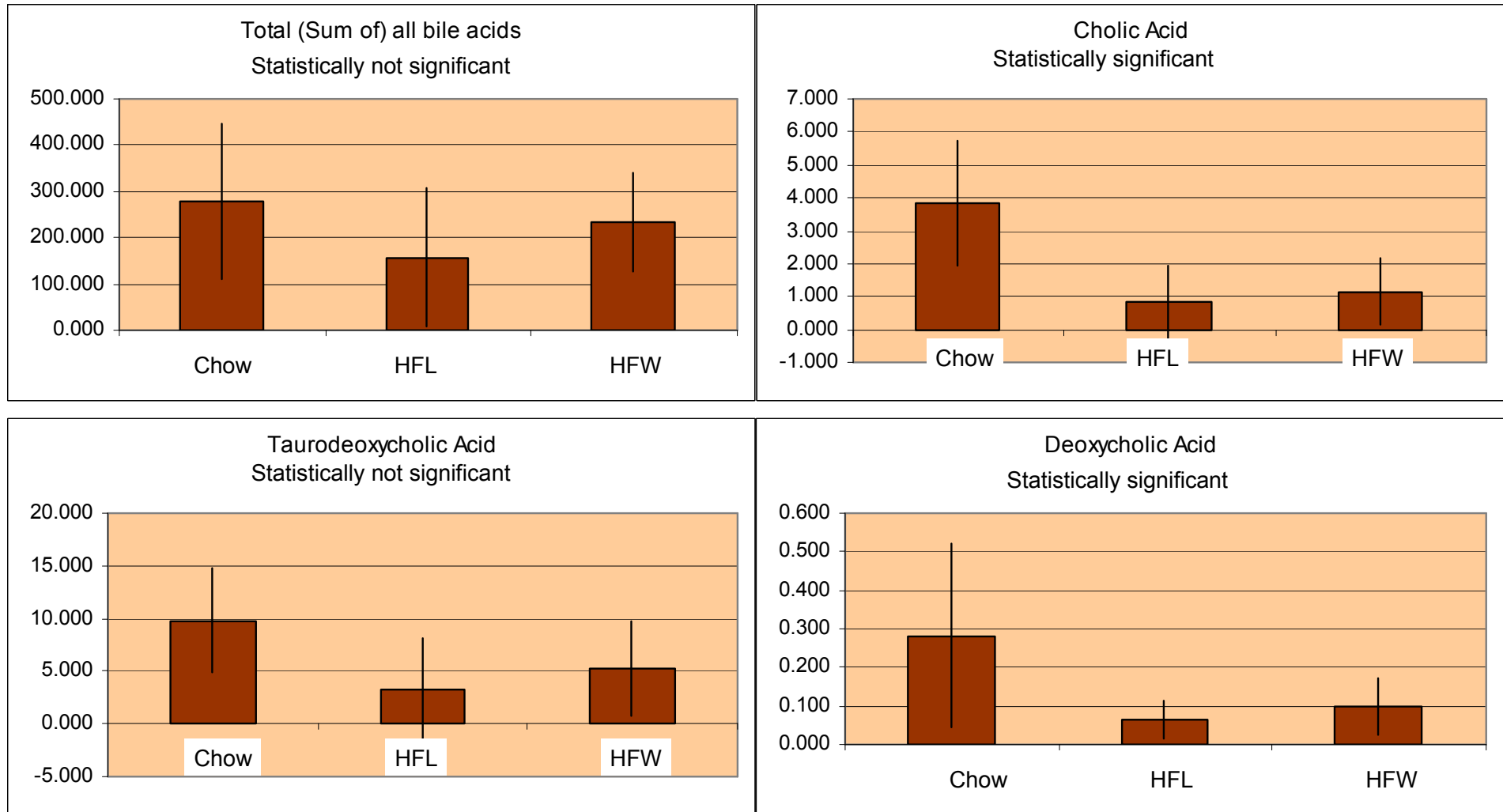
Application 1:

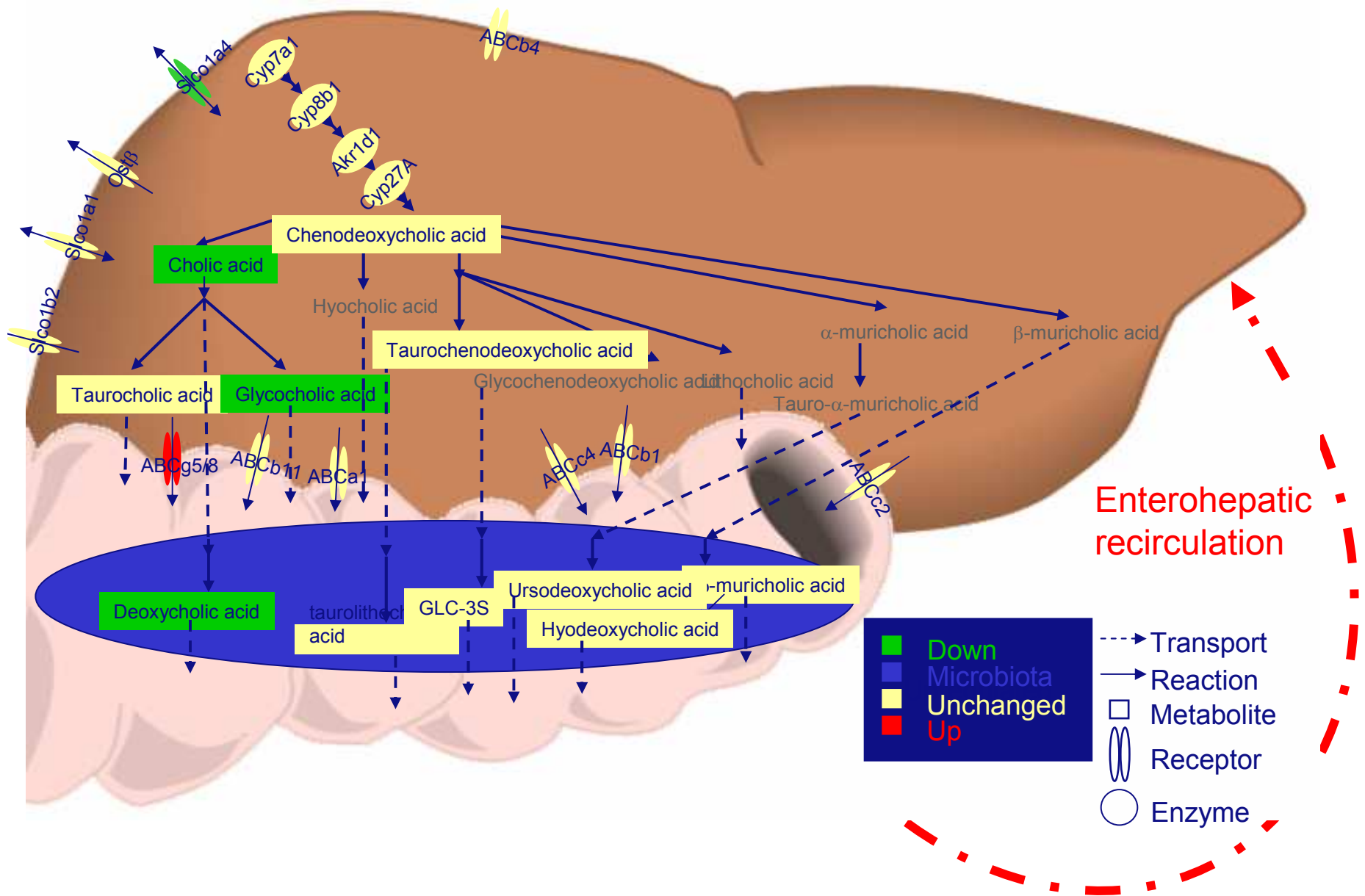
- Influence of lifestyle on health
- Mice study
- 3 diets:
 - Chow diet
 - High fat diet (lard)
 - High fat diet (palm oil)
- After 16 weeks mice were sacrificed
- Various metabolomics platforms were applied,
- Bile acid method was used to analyse liver extracts
- 17 liver extracts were analysed



Sample

Individual bile acids





F.P. Martin, et al., Mol Syst Biol. 2007;3:112.

Application 2



- Samples provided by Astra Zeneca in Sodertalje, Sweden
- Dog drug safety study 5 groups of animals
 - Group 2: low dose
 - Group 3: medium dose
 - Group 4: high dose
 - Group 5: control
 - Group 6: medium dose with recovery

Total number of study samples analysed: 104

In addition, several aliquots of a pooled QC sample

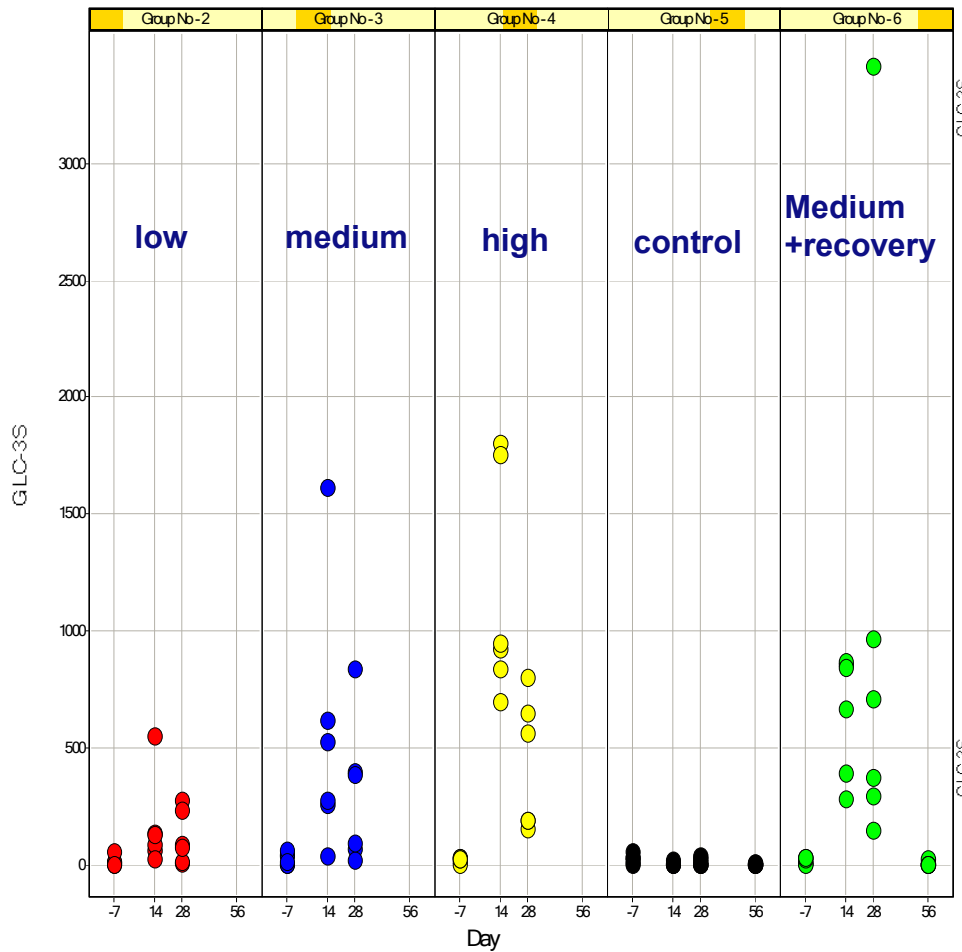
Calibration standards for bile acids

GLC-3S

Medium dose with recovery



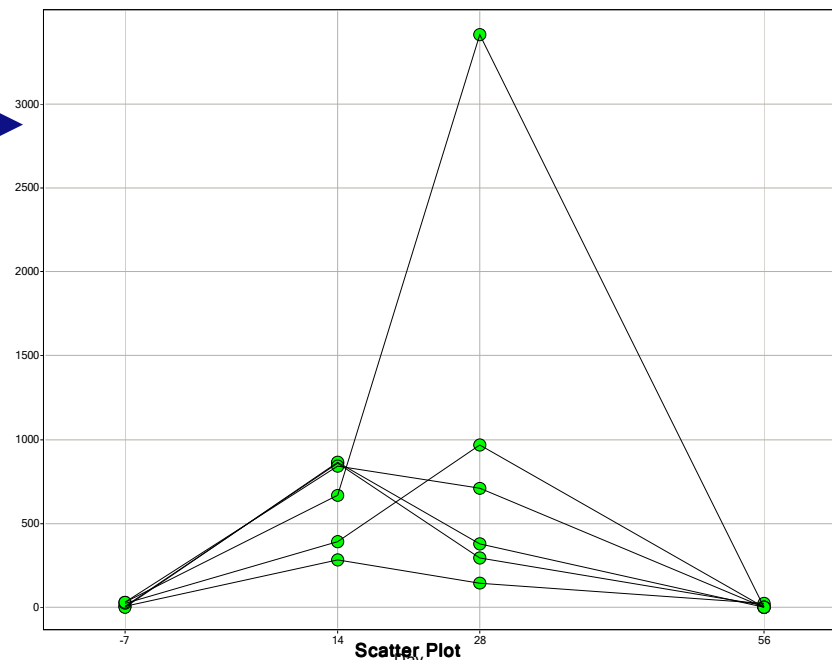
Scatter Plot



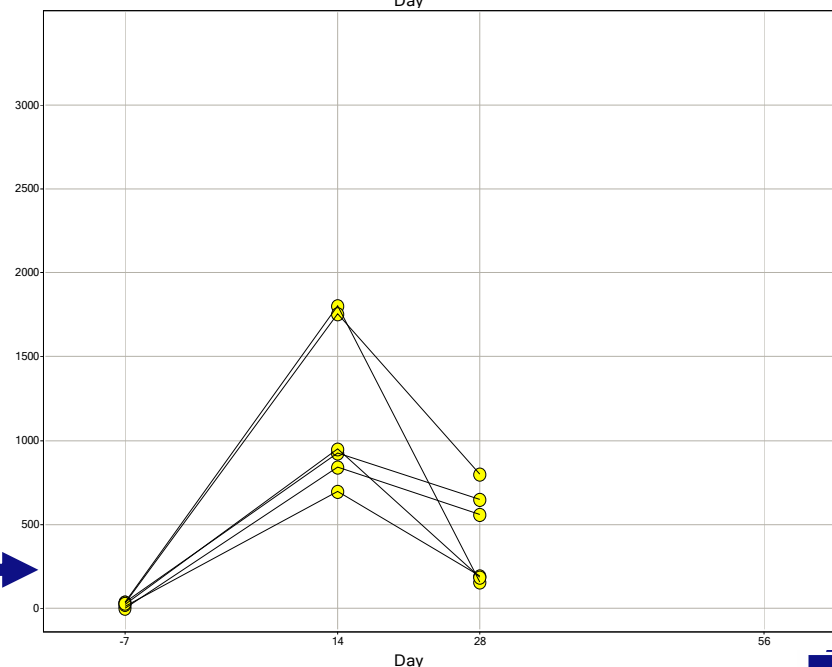
High dose



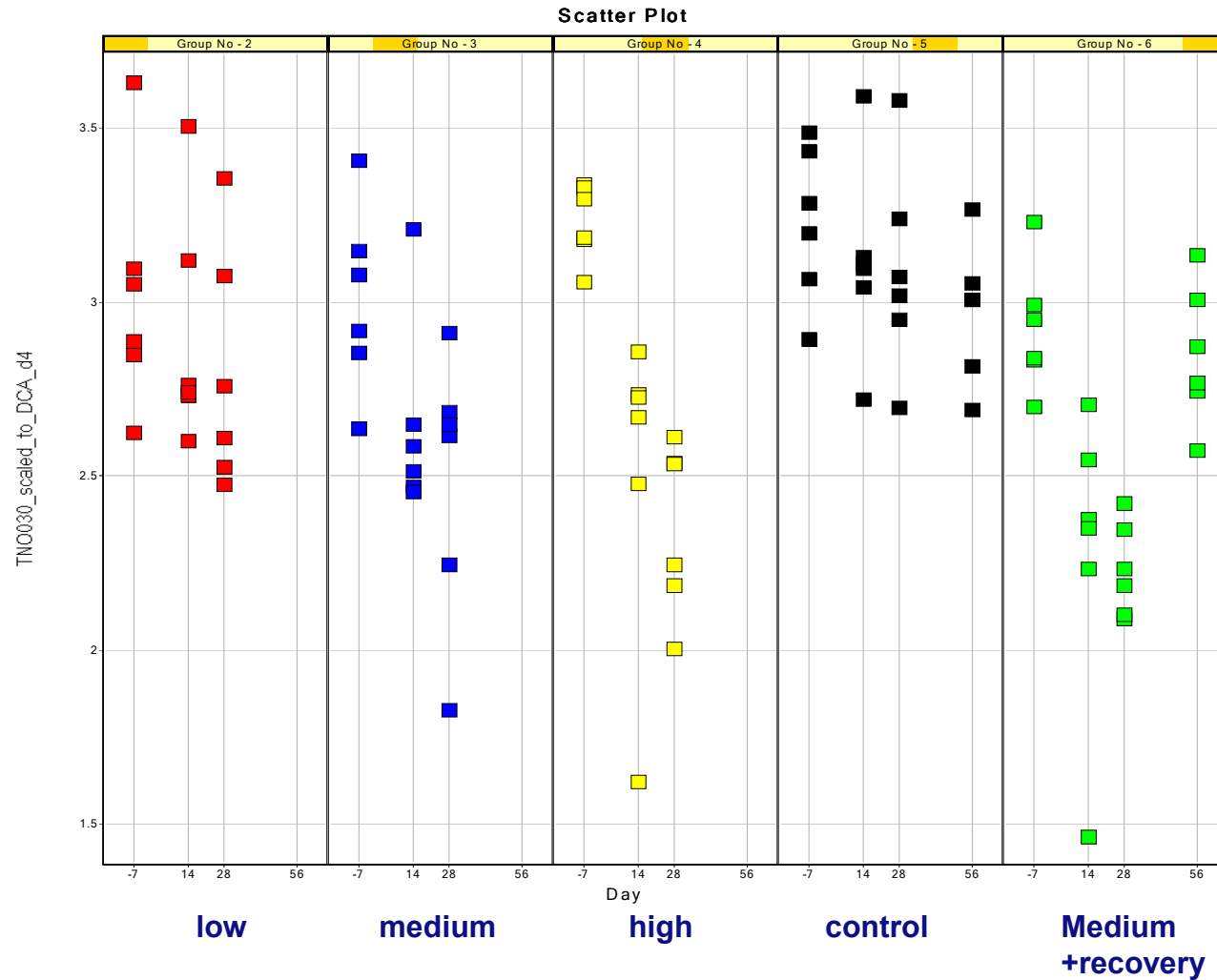
Scatter Plot



Scatter Plot

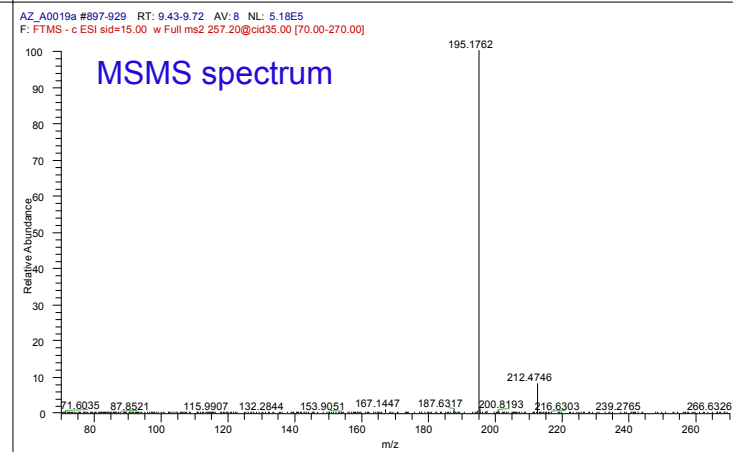
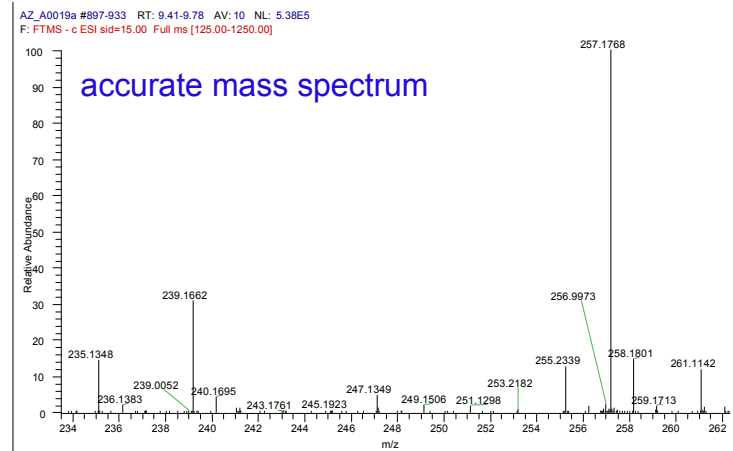
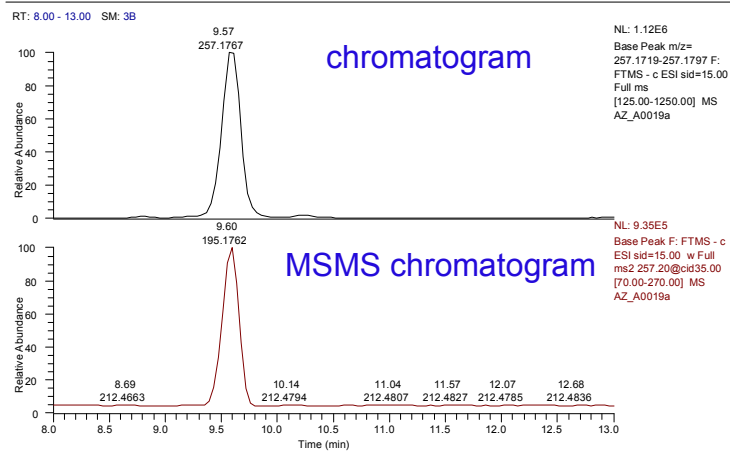


Decrease with treatment: Unknown peak 12.1_714.315



Example of an unknown metabolite

257.1758, RT 11.5 min

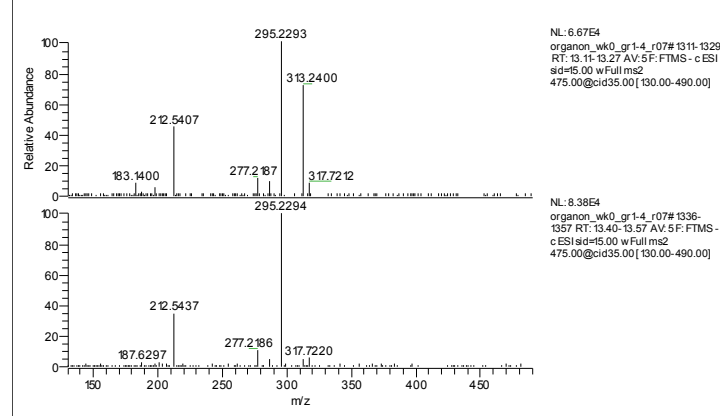
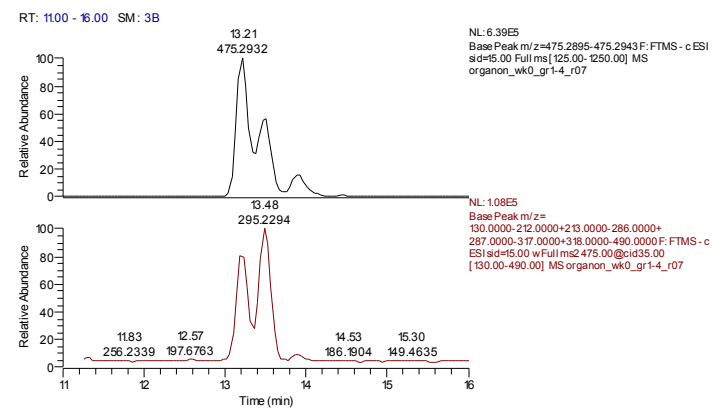
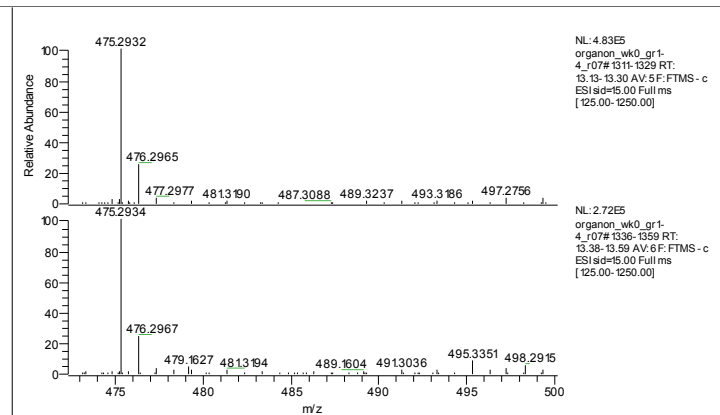
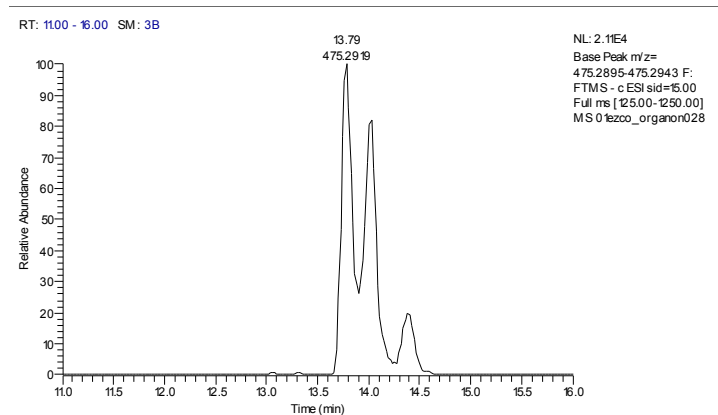


Unidentified metabolites 475.2932@138 & 475.2932@140 Drug efficacy markers RA model in rat

organon_wk0_gr1-4_r07

7/10/2008 3:03:56 AM

week 0, group 1-4, Rat 07



Clues

- Element composition : C₂₄H₄₄O₉
- Isotope pattern : supportive
- MS2 fragments
 - Loss of hexose C₆H₁₀O₅
 - Fragment C₁₈H₃₄O₄
- C₁₈:1 fatty acid + 2O + hexose (glucose)
- ????????
 - Web searches
 - No relevant hits for elem comp
 - No relevant hits for text search

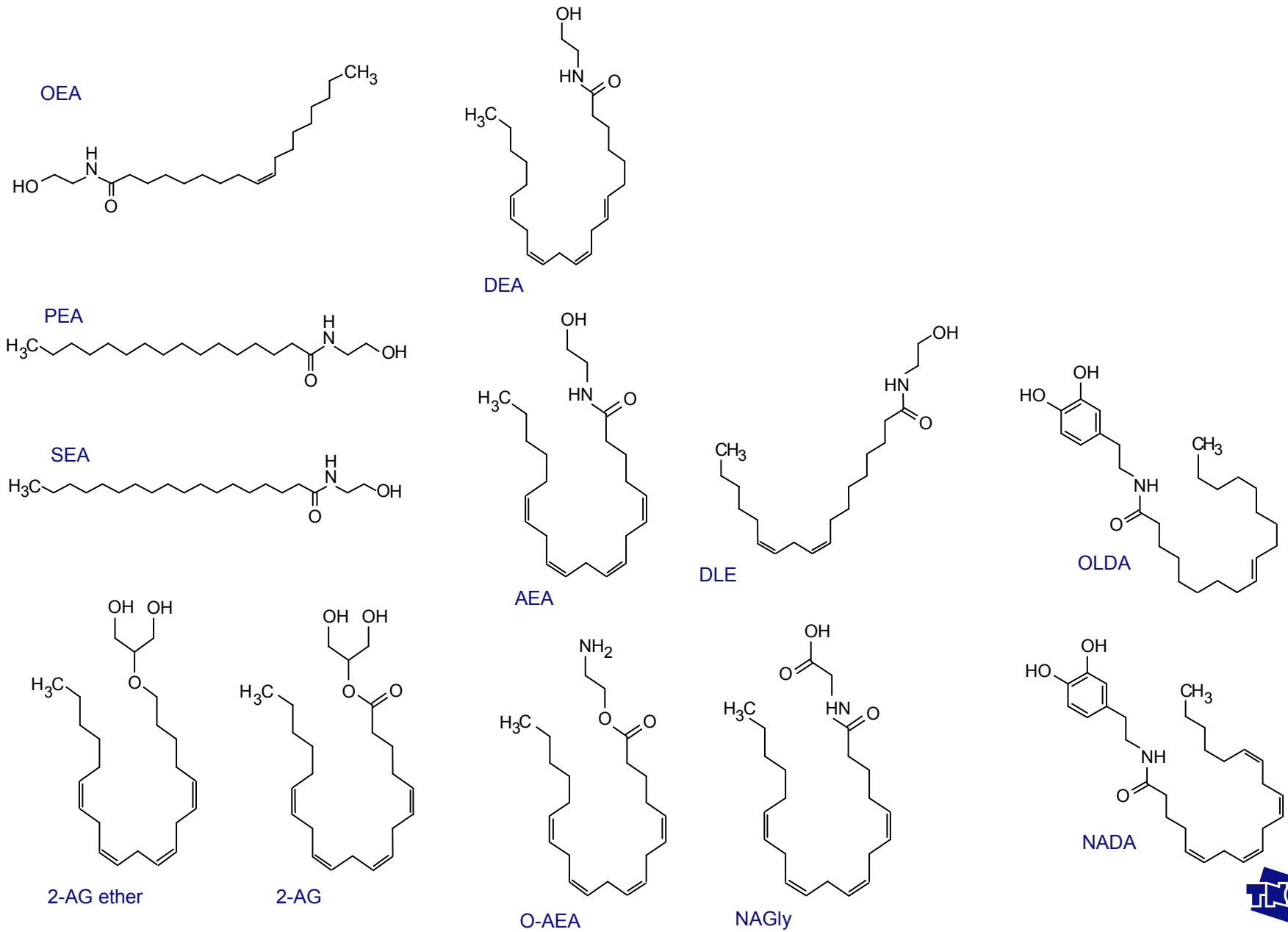
Biology driven Mx platform

- Literature , pathway analysis,
 - Inflammation
 - Oxidative stress
 -
 - Pain
 - Function
- List of relevant metabolites (n = 200, 300,?)
- Platform development
 - Current methods
 - Modification
 - Combination
 - New methods
 - (UP)LC-MS & MS²
 - FAST GC-MS
 - HR ICPMS
 - Multiplex immunoassays

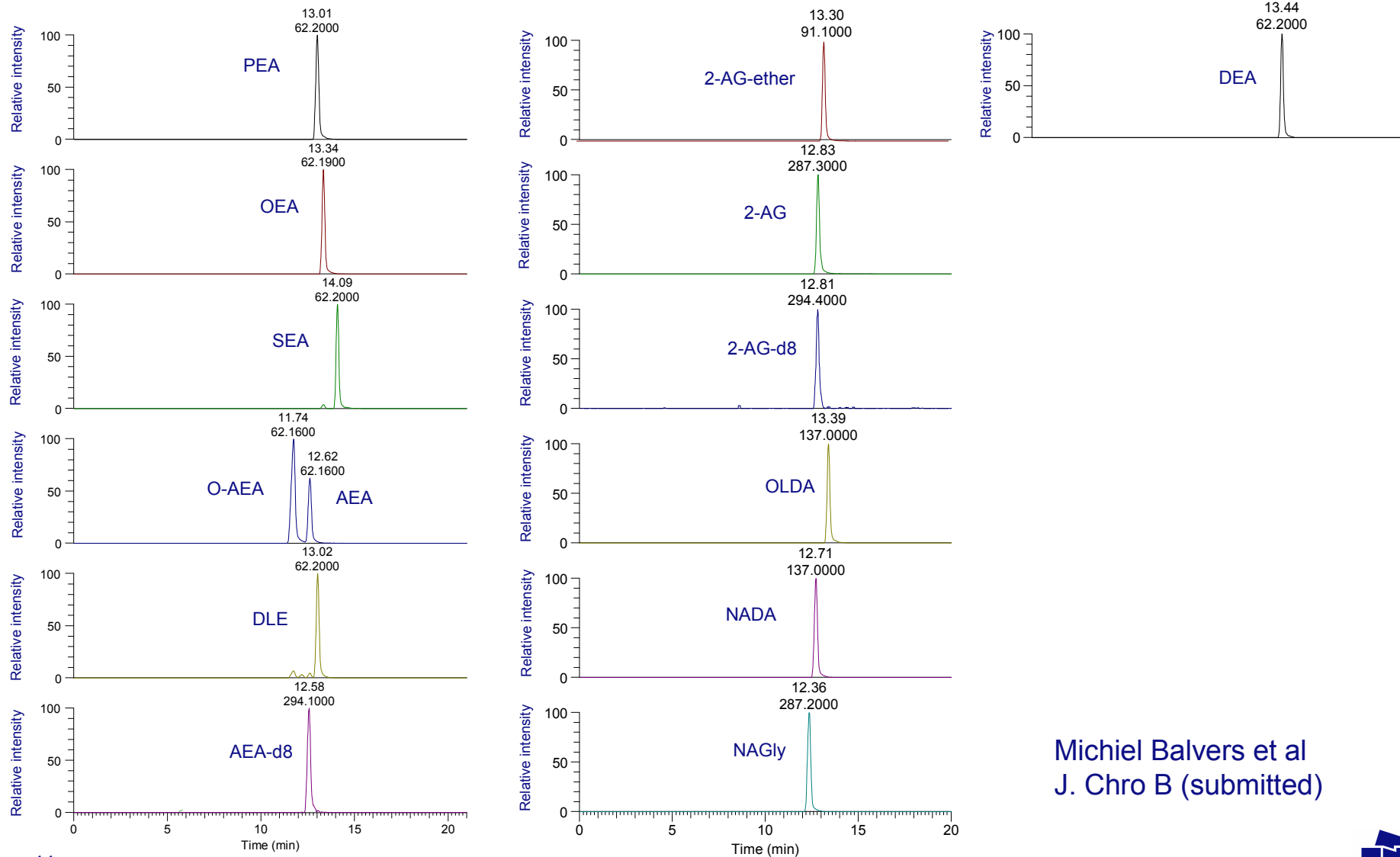
Endocannabinoids

- The endocannabinoid system plays an essential role in many physiological processes and pathological conditions,
 - inflammation,
 - cardiovascular diseases,
 - cancer,
 - neurological disorders,
 - obesity
 - metabolic syndrome
 - plasma

Figure 1: structures of the endocannabinoids and related compounds



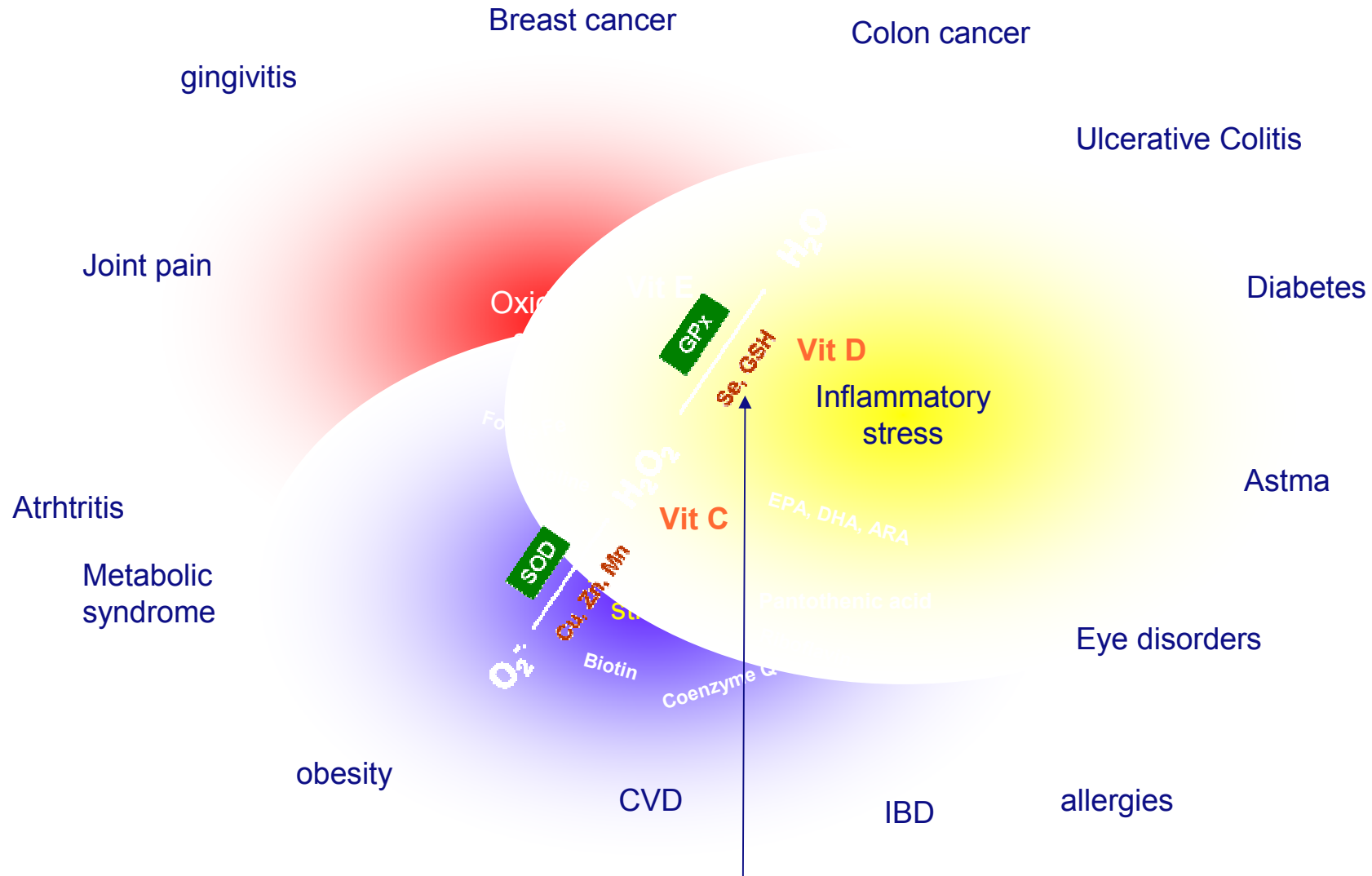
Fully validated method for tissue and plasma LC-MS/MS method on a triple Quad



Michiel Balvers et al
J. Chro B (submitted)



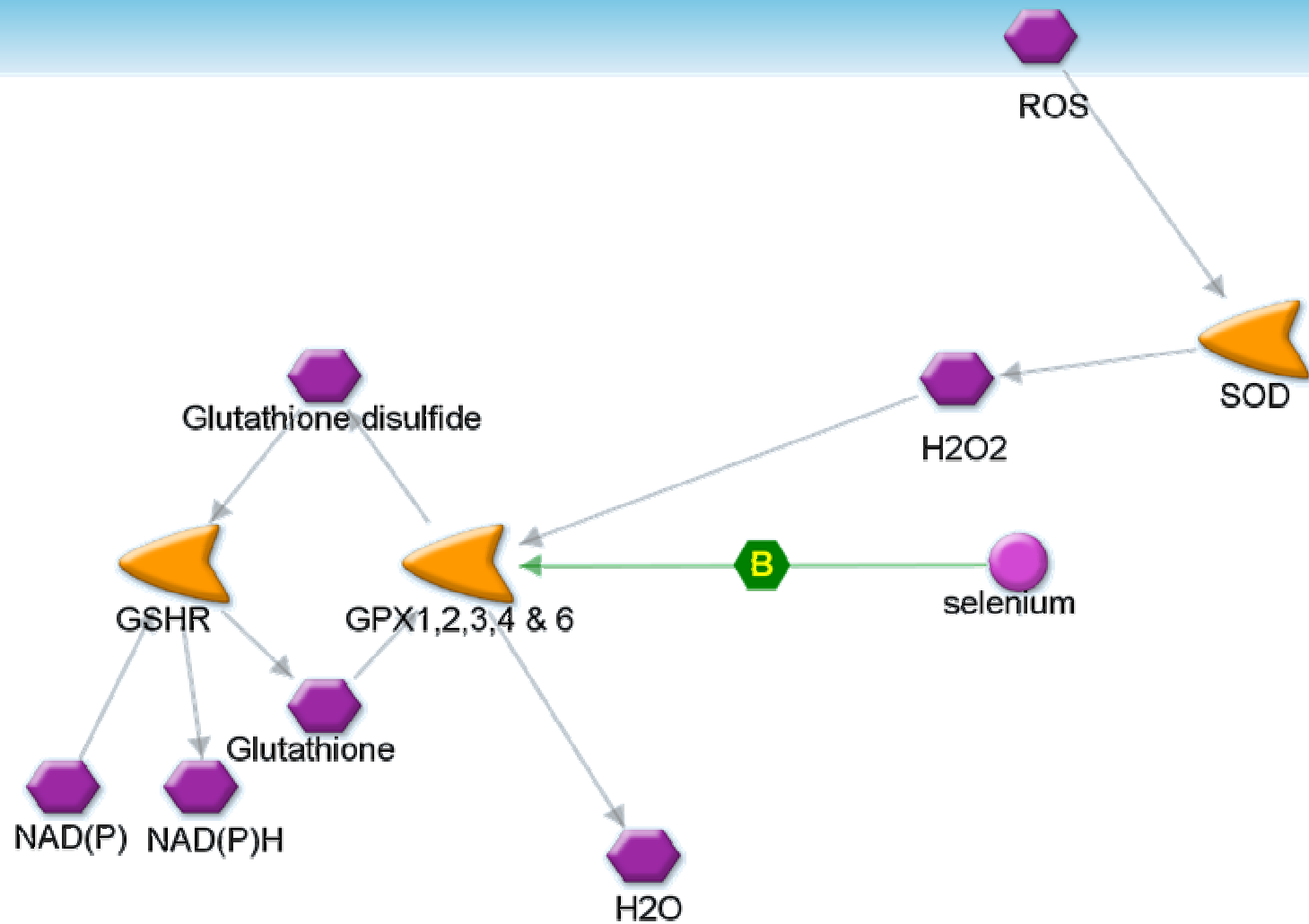
Many micronutrients act in maintaining the performance and elasticity of metabolism, oxidation and inflammation “overarching processes”



Let`s have a closer look at selenium



The Selenium biological network in oxidative stress



Mixed mode metabolomics platform



Analytical technology

Statistics

Data push

Identification

Hypothesis generation

Unbiased

Biomarker discovery

Biology

Bioinformatics

Information pull

Knowledge

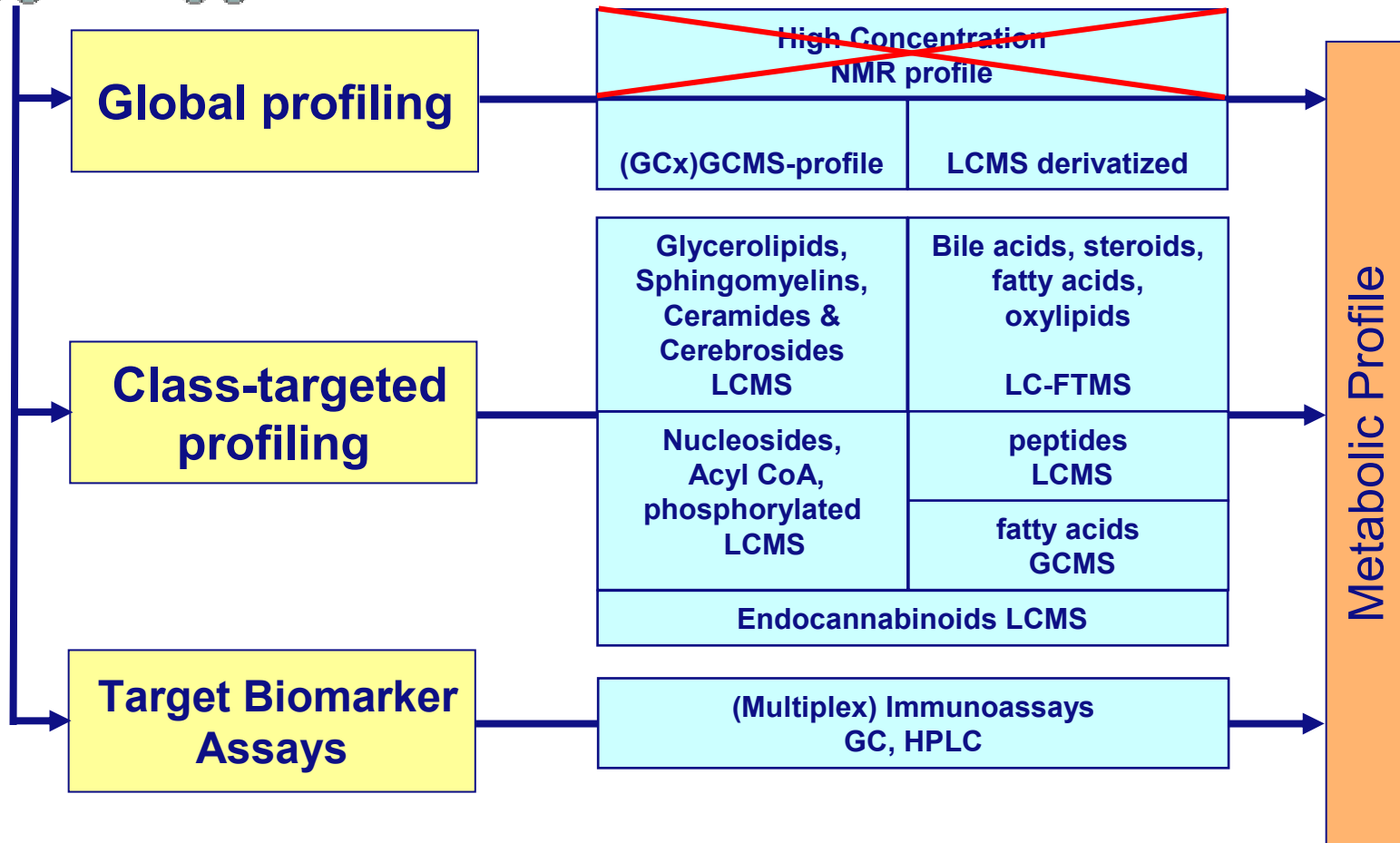
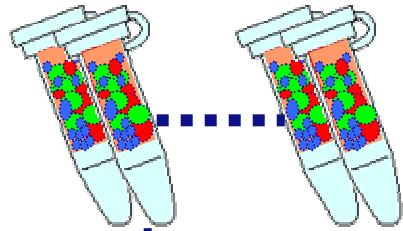
Hypothesis testing

Targeted

Mechanisms



Metabolomics platform



Acknowledgements

TNO

- Ivana Bobeldijk
- Maarten Hekman
- Suzan Wopereis
- Jildau Bouwman
- Frans van der Kloet
- Teake Kooistra
- Bas Muilwijk
- Marc Tienstra
- Renger Jellema
- Age Smilde
- Maud Koek
- Ben van Ommen
- Jan van der Greef

AZ

- Ina Schuppe-Koistinen
- Johan Lindberg (AZ)
- Pierre Donnes (AZ)
- Benita Forngren (AZ)