



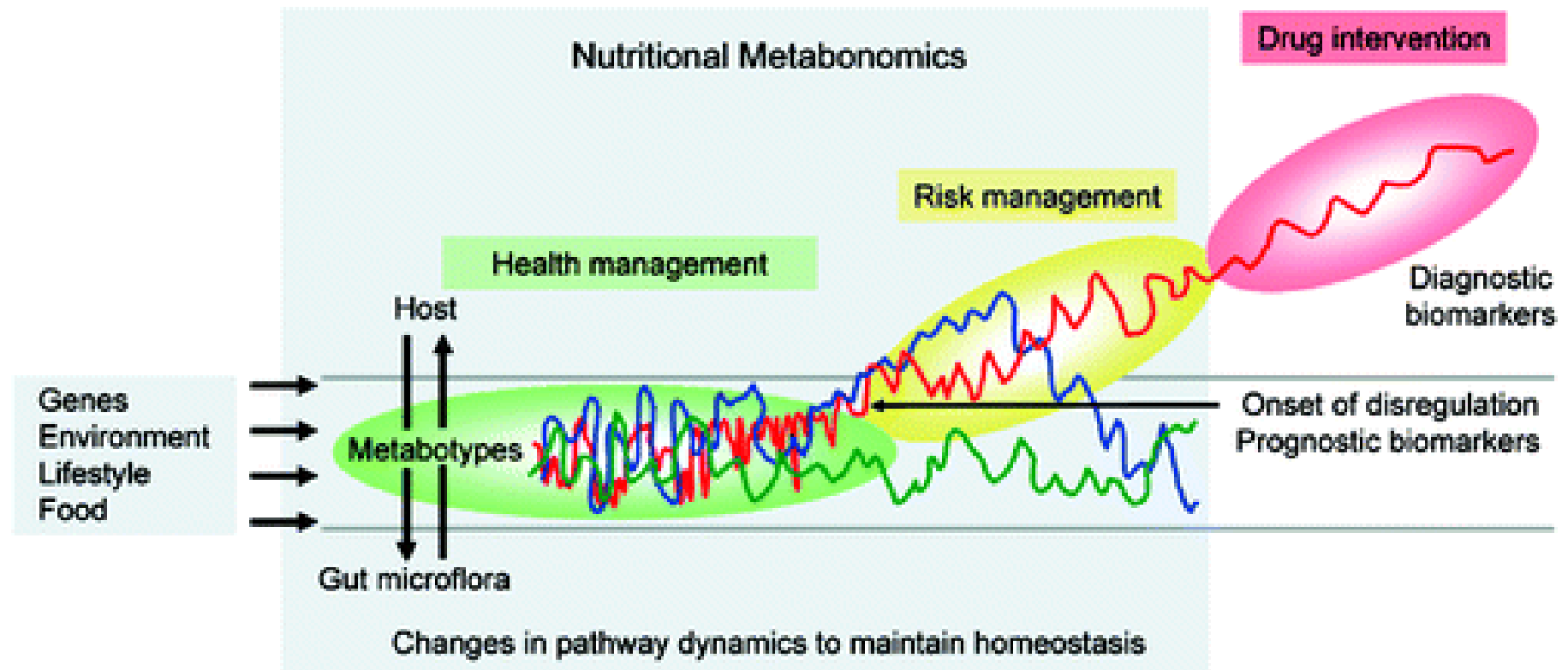
Nutritional metabolomics and clinical applications

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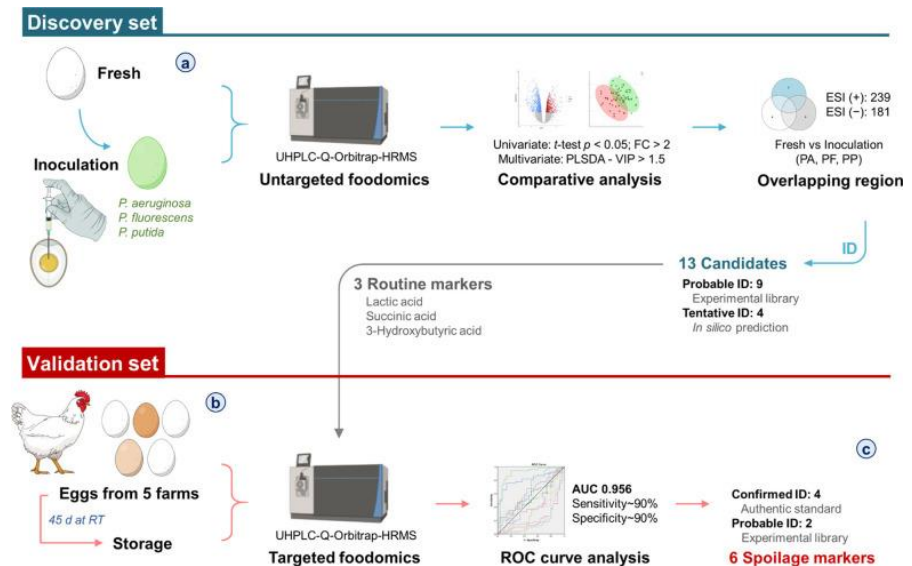
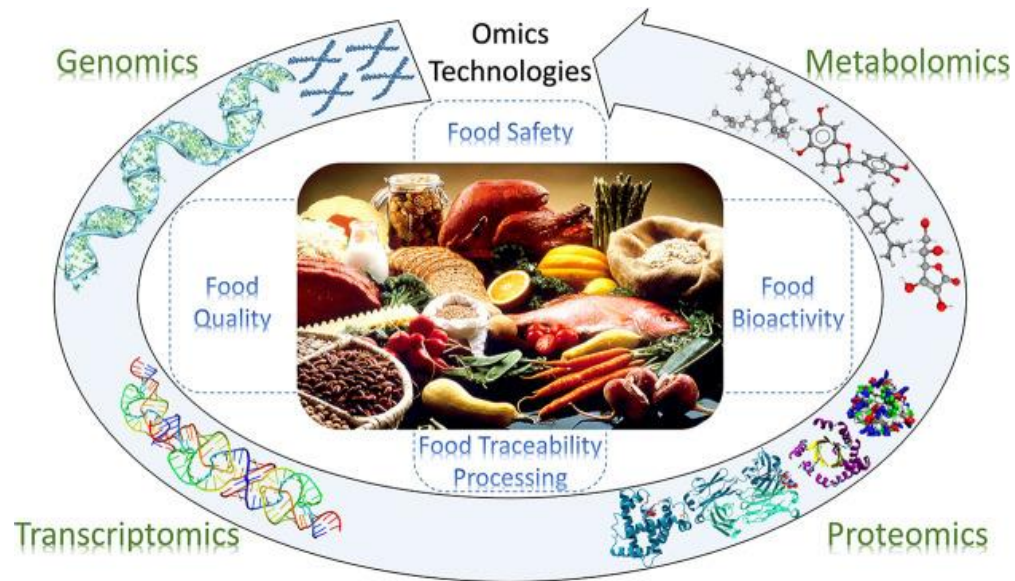
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Study the molecular relationships of metabolic phenotype with dietary habits and nutrient intake



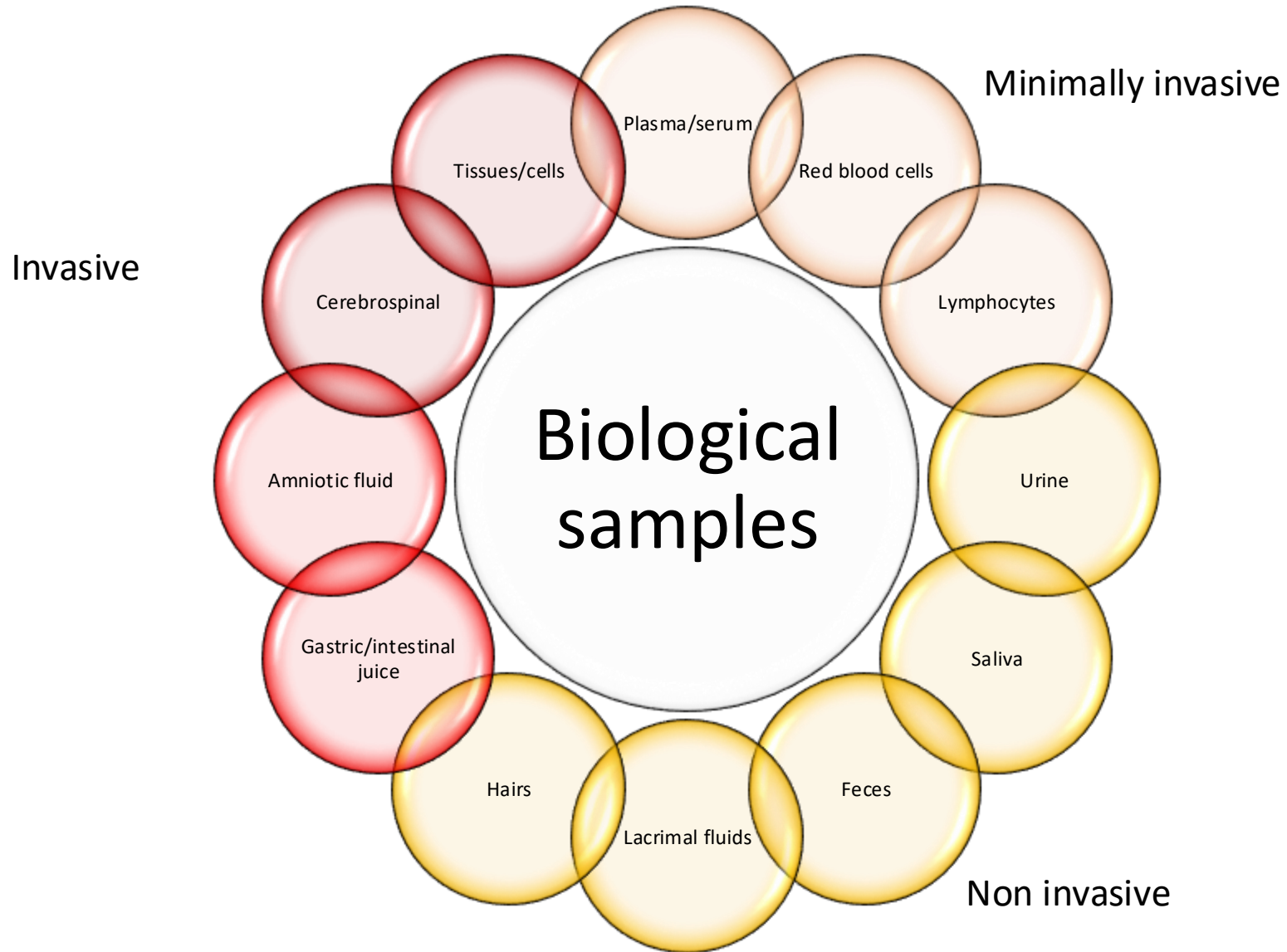
Foodomics

“a discipline that studies the food and nutrition domains through the application and integration of advanced omics technologies to improve consumer’s well-being, health, and confidence” (Cifuentes A. J Chromatogr A. 2009 Oct 23;1216(43):7109.)



Valdés A et al. Anal Chem. 2022 Jan 11;94(1):366-381.

Tentative definition: “a discipline that studies all food components (foodome) to assess food quality, integrity/safety and nutritional profile”



Plasma/serum: provides insights on instantaneous metabolism at the time of sampling (homeostatic snapshot)

Urine: provides a “summary” of recent metabolic regulations to achieve homeostatic control

Red blood cells: provide proxy insights on storage of nutrients/micronutrients (ex: folic acid status)

Feces: provide insights on food digestive process and gut microbiota food/host interactions

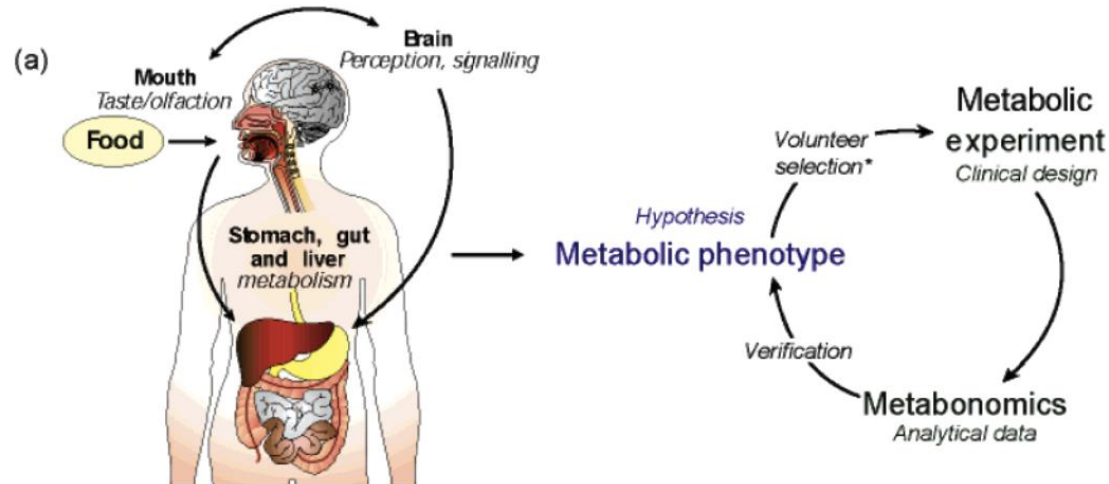
Cerebrospinal fluid: provides proxy insights on brain metabolism

Amniotic fluid: provides insights on mother-infant placental exchanges and infant metabolism



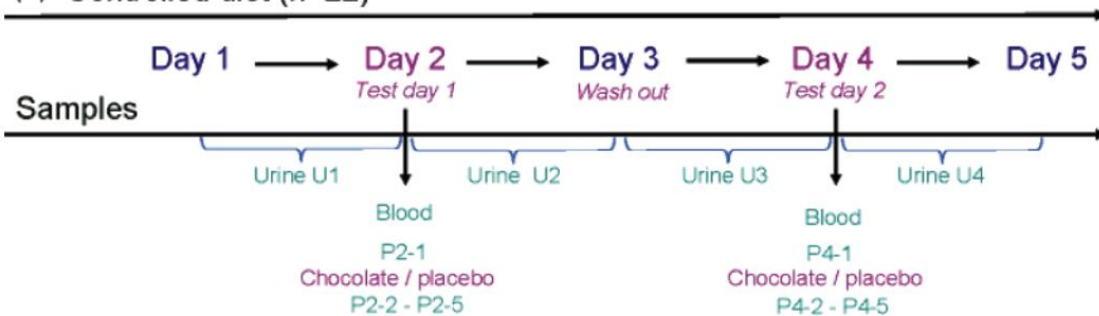
Study of food metabolome imprinting

Chocolate case study



* Based on dietary food consumption questionnaire

(b) Controlled diet (n=22)



Study duration: 5 days

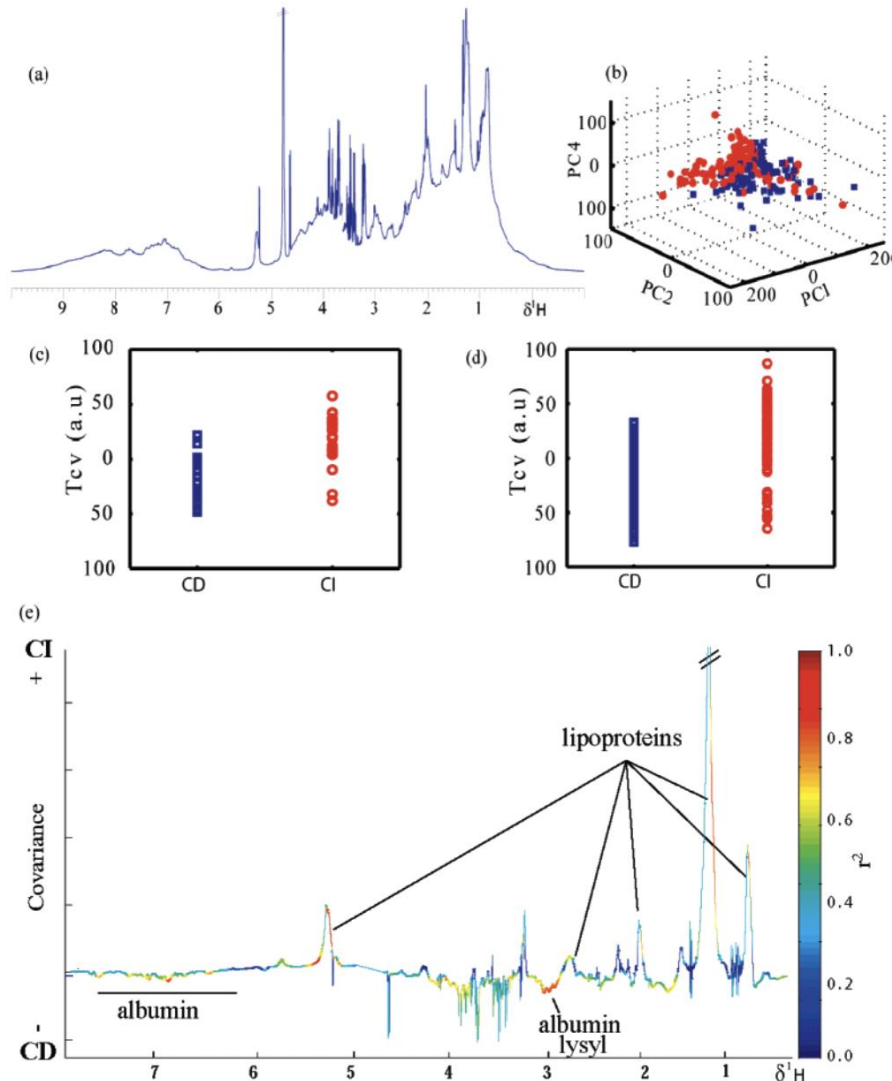
Study design:

- controlled diet 24 hours before the study and throughout the study)
- one test day with chocolate consumption and one test day with bread taken as a placebo
- Washout period of 1 day between each test day.
- On Day 1, a portion of a commercially available chocolate (50 g) was given to half the participants of each group to be eaten in the afternoon, and the other half received an equivalent amount of bread.
- On day 4: cross over
- Blood sampling: at 0 (P2-1 and P4-1), 5 (P2-2 and P4-2), 15 (P2-3 and P4-3), 30 (P2-4 and P4-4) and 60 (P2-5 and P4-5) min after chocolate/bread intake.
- Urine: 24 hours urine collection (U1-U4)

¹H NMR (600MHz) metabolic profiling of blood plasma and urine
Multivariate statistics

Study of food metabolome imprinting

Chocolate case study



Principal Component Analysis of ^1H NMR plasma

- PC1, PC2, and PC4, 55%, 12%, and 6% of the total variance

O-PLS-DA cross-validated scores (Tcv) plot, $Q^2 = 0.15$

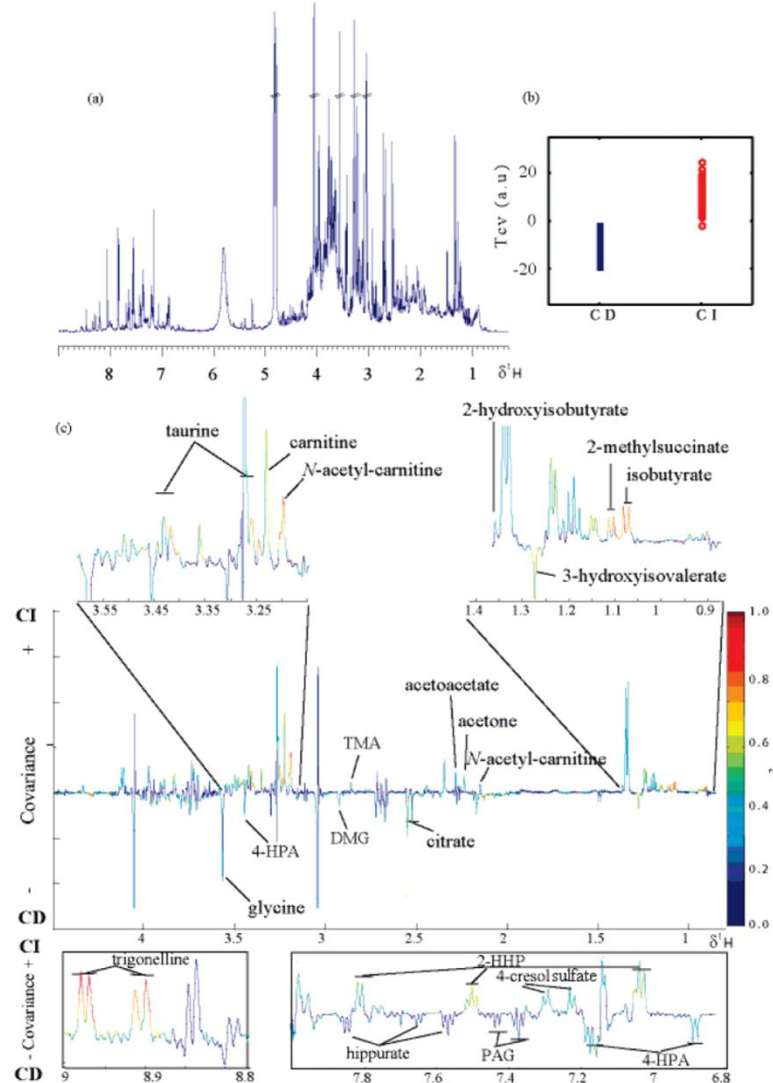
- 7-fold cross-validation of samples before chocolate/placebo intake (P2-1 and P4-1)
- Chocolate eating habit to define CD (chocolate desiring) and CI (chocolate indifferent)

Coefficients plot derived from all the plasma samples:

- back-scaling transformation which allows each variable to be plotted with a color code which relates to the significance of class discrimination as calculated from the correlation matrix.
- positive peaks are from metabolites that are higher in the "chocolate indifferent" class.

Study of food metabolome imprinting

Chocolate case study



O-PLS-DA cross-validated scores plot, $Q^2 = 0.39$ (7-fold cross-validation) of all samples

O-PLS-DA loadings plot from the model backscaled and plotted as a function of chemical shifts. The back-scaled coefficients plot is color coded to the weights of the selected latent variable.

Positive peaks are from metabolites that are higher in the “chocolate indifferent” class.

Discovery of dietary intake biomarker

Citrus intake case study

Rationale

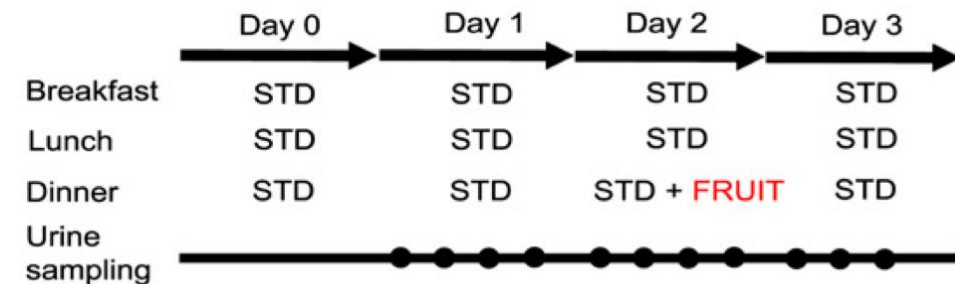
Dietary assessment by questionnaires lack accuracy
Food intake biomarkers are needed to objectively evaluate dietary intakes

Objective

Develop a metabolomics-based strategy for biomarker discovery and validation using large-scale epidemiologic cohorts

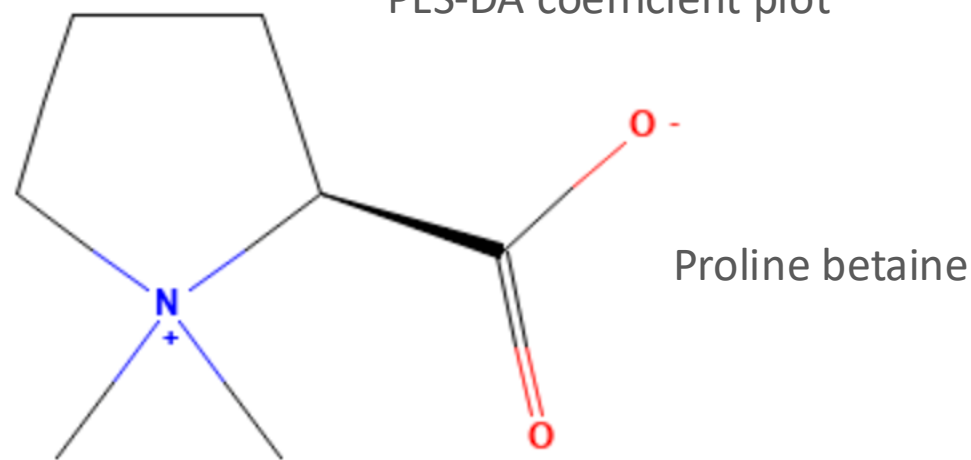
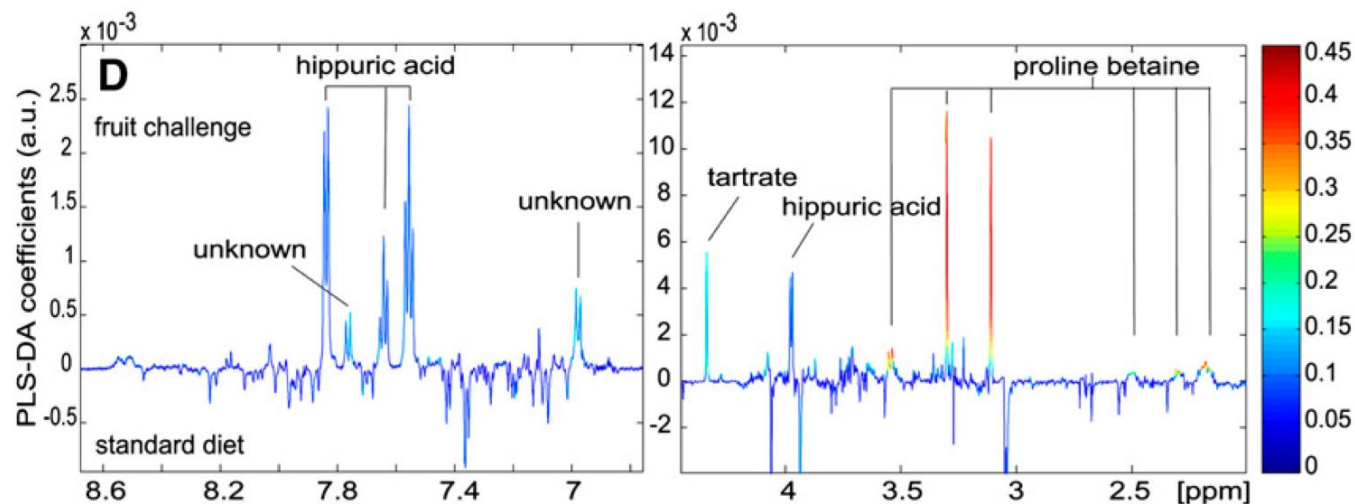
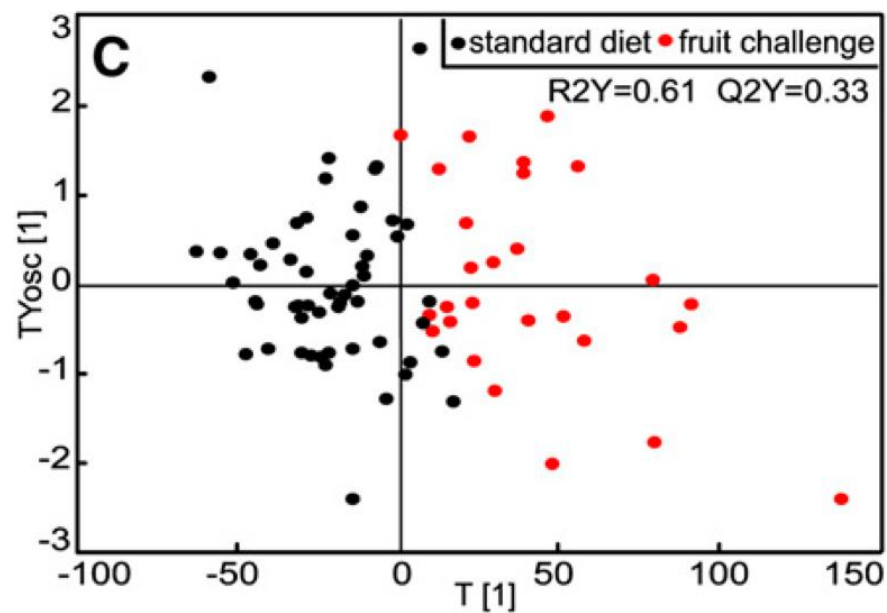
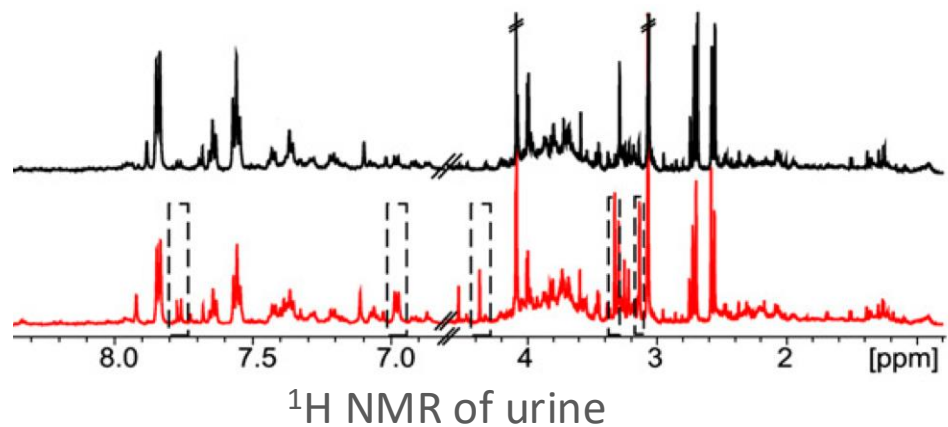
Design

8 individuals under standardized diet for 4 days
 ^1H NMR of urine
Citrus consumption determined by 24 h dietary recalls
Excretion kinetics of the biomarker measured on n=8 (orange juice consumption)



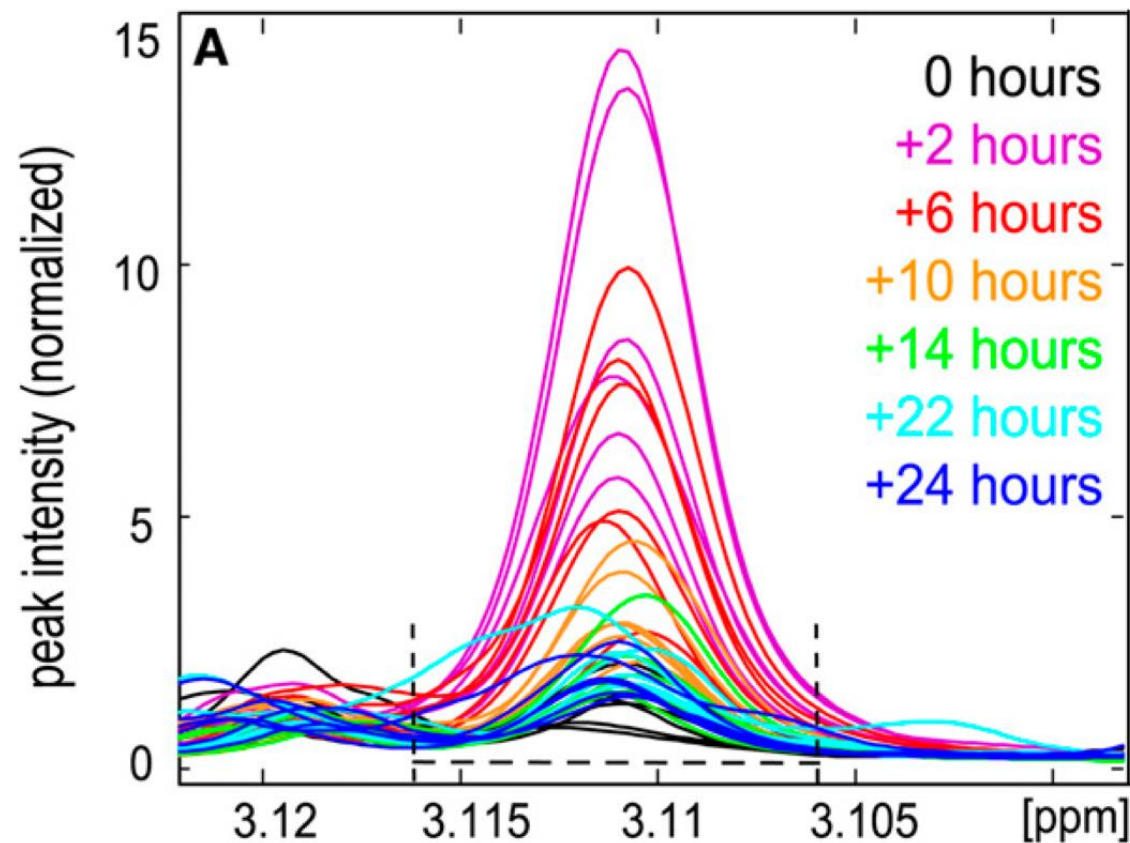
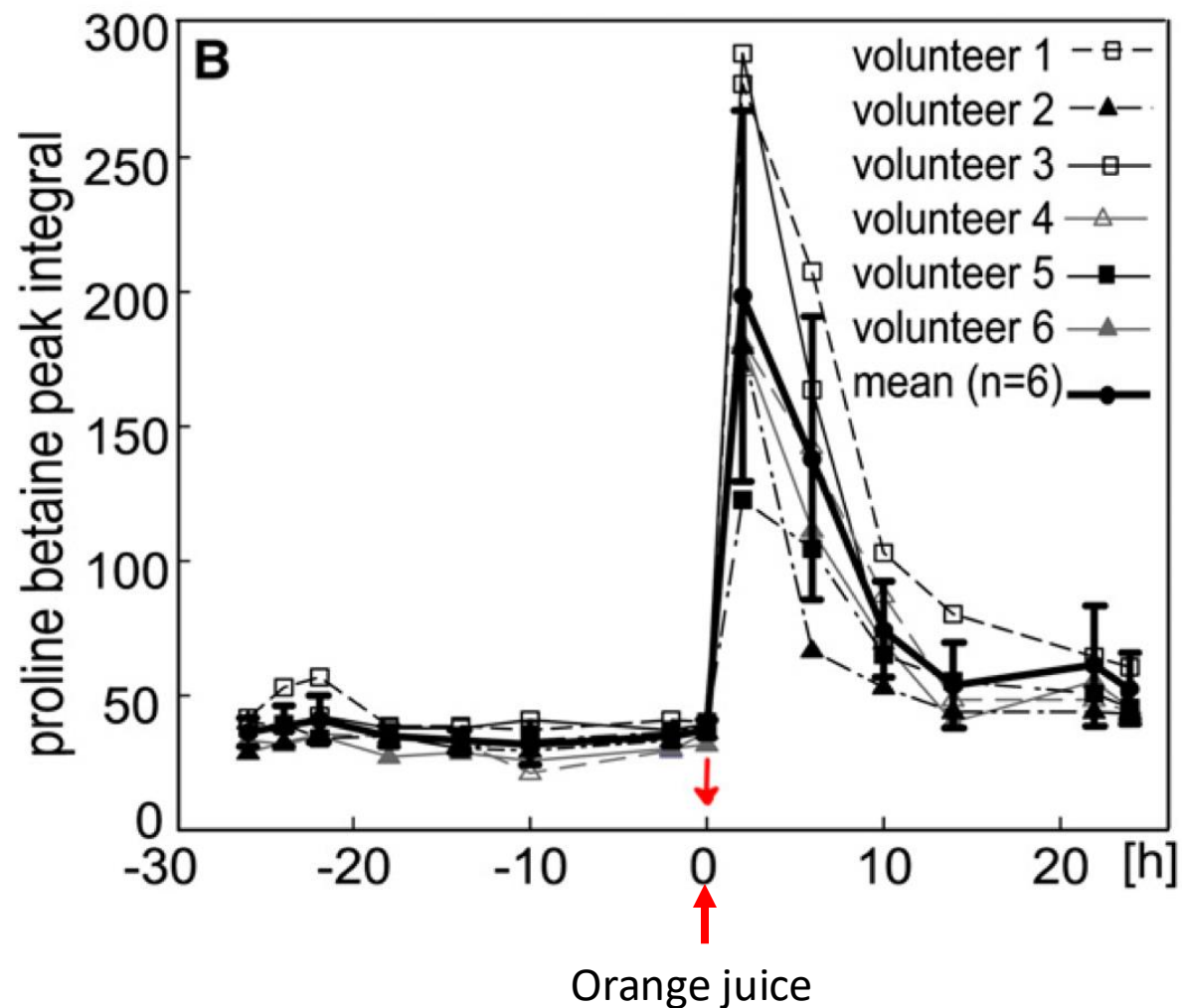
Discovery of dietary intake biomarker

Identification of proline betaine as putative biomarker



Discovery of dietary intake biomarker

Kinetic variation of proline betaine signal



Discovery of dietary intake biomarker

Validation of proline betaine as citrus biomarker

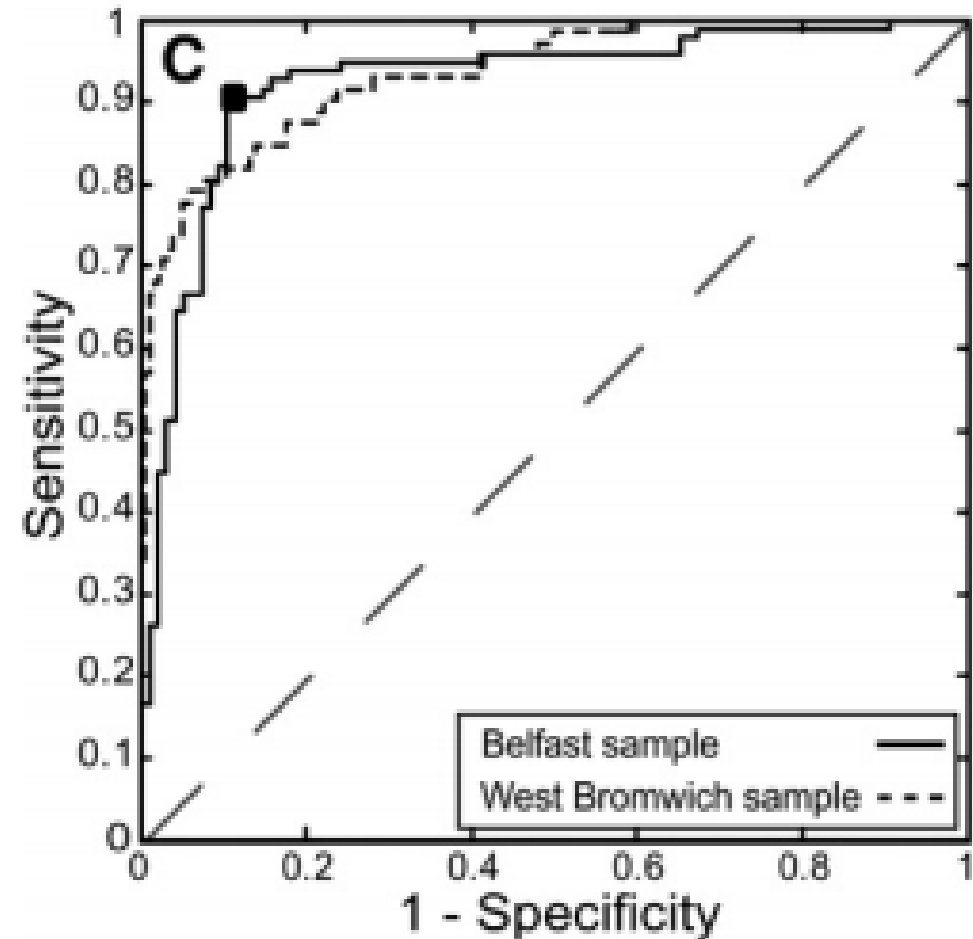
Epidemiological cohort with training set
(n=220) and validation set (n=279)
Analysis of the 24-h dietary recall data for
citrus intake classification
Prediction of citrus intake from proline betaine
signal in NMR spectrum of urine

Training set:

- Sensitivity (true positive rate): 86.3%
- Specificity (false positive rate): 90.6%

Validation set:

- Sensitivity: 80.6%
- Specificity: 92.3%



Nutrikinetic study of fermented soybean paste (Cheonggukjang) isoflavones according to the Sasang typology

Sasang constitutional medicine (traditional Korean classification system)

- 4 constitutional types based on the biopsychosocial characteristics, emphasis of the balance between Yin (the feminine passive principle, persistence, wetness, cold, darkness) and Yang (the masculine active principle, dryness, heat, and light):
 - Soyangin (SY, “lesser yang”)
 - Soeumin (SE, “lesser yin”)
 - Taeumin (TE, “greater yin”)
 - Taeyangin (TY, “greater yang”)
- 48 healthy Korean men (TE, n=15; SY, n=15; SE, n=18)
- Intake of 50g Cheonggukjang per 60Kg of body weight
- Kinetic profile of blood isoflavones by LC-MS/MS



Cheonggukjang

Quick fermented soybean paste used in Korean foods

Nutrikinetic study of fermented soybean paste (Cheonggukjang) isoflavones according to the Sasang typology

- Outcomes:
 - Time to maximum concentration (T_{max})
 - Elimination half-life ($t_{1/2}$)
 - Absorption rate as AUC
 - Maximum peak area (C_{max})
- T_{max} and $t_{1/2}$ of nine metabolites higher in the SE group than in the other groups
- Absorption rates of intact isoflavone 5.3 and 9.4 times higher in the TE group than in the SY and SE groups, respectively
- Highest AUC values for phase I and II metabolites observed in the TE group
- Isoflavone bioavailability, following Cheonggukjang ingestion, is high in individuals with the TE constitution, and relatively lower in those with the SE and SY constitutions

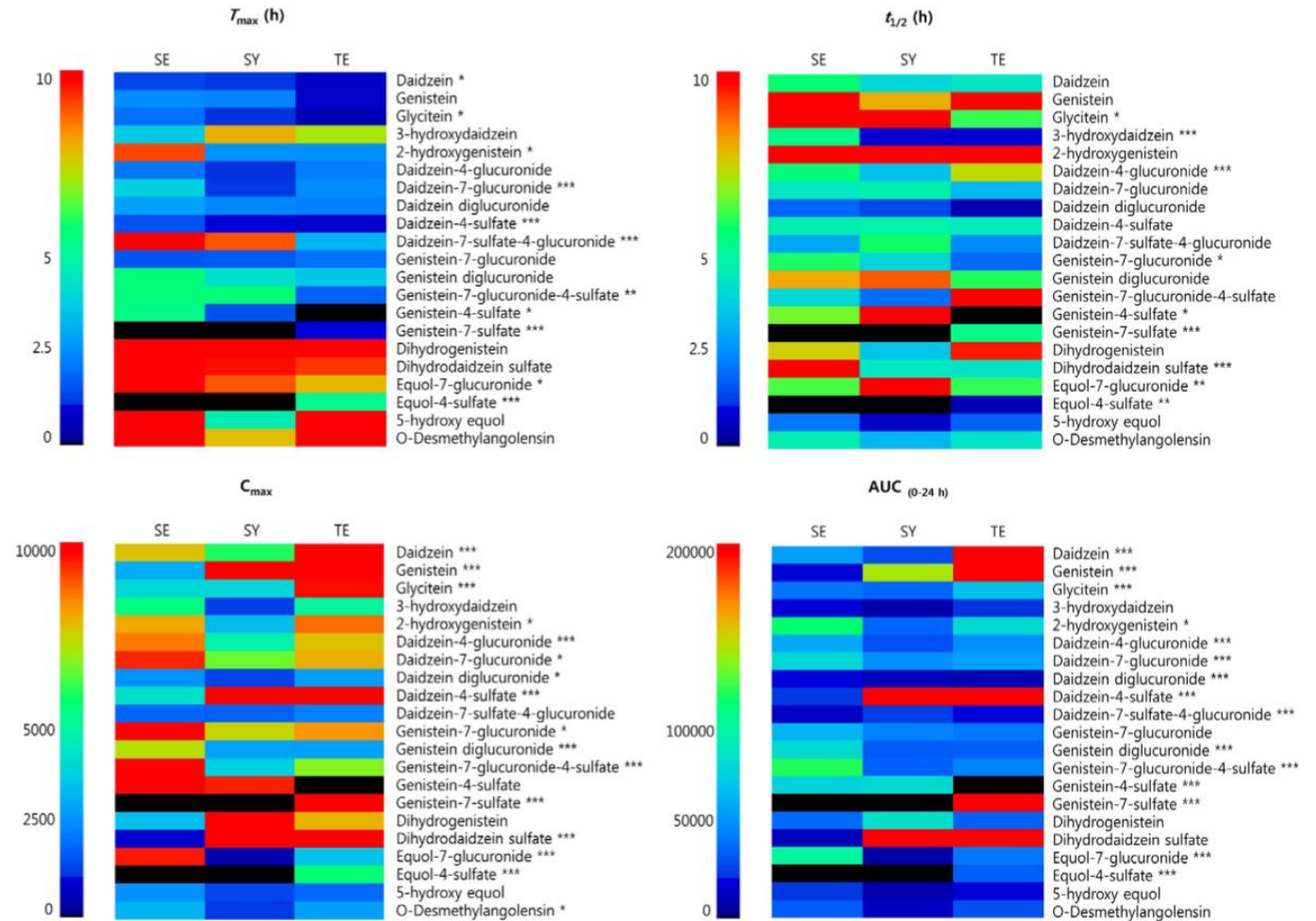


Fig. 1. Overview of T_{max} (h), $t_{1/2}$ (h), C_{max} , and AUC_(0-24 h) for 21 isoflavone metabolites detected in the plasma following ingestion of *Cheonggukjang* according to sasang typology. Error bars represent the mean $\pm$ SEM (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). SE, *Soeumin*, SY, *Soyangin*, TE, *Taeumin*, AUC_(0-24 h): area under the curve of the metabolite peak area versus time; T_{max} , time to maximum concentration; $t_{1/2}$, elimination half-life; C_{max} , maximum peak area.

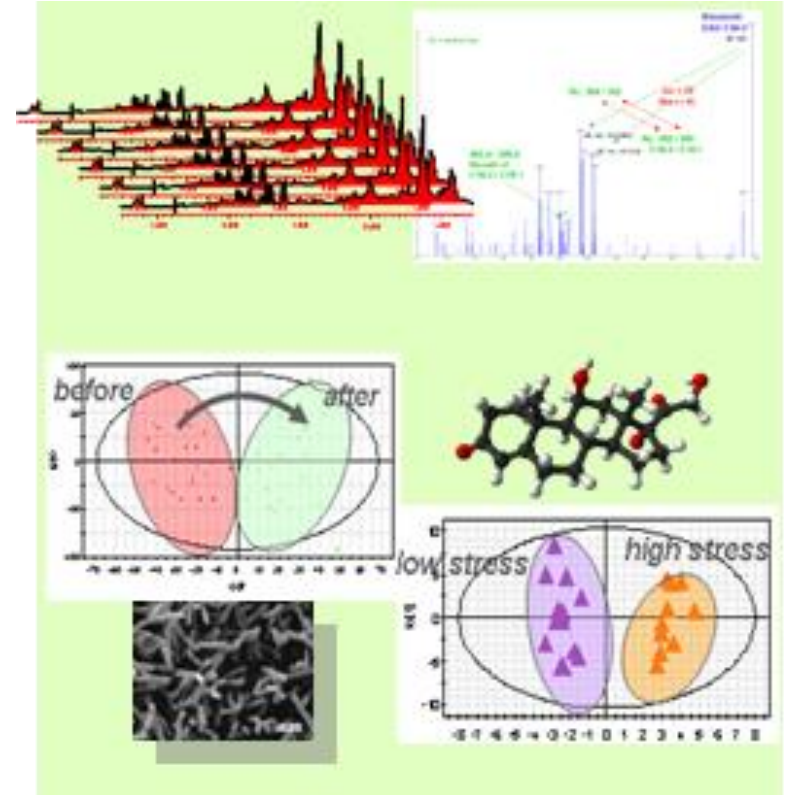
Metabolic effects of specific dietary intake

Case study: dark chocolate

Monitoring of 30 **free living subjects** classified according to their “everyday” stress using validated questionnaires.



- 2 weeks trial (free living human subjects classified in low and high anxiety traits using psychological questionnaires)
- Urine and plasma were sampled at beginning, mid-time and end of the trial
- Daily consumption of 40 g dark chocolate



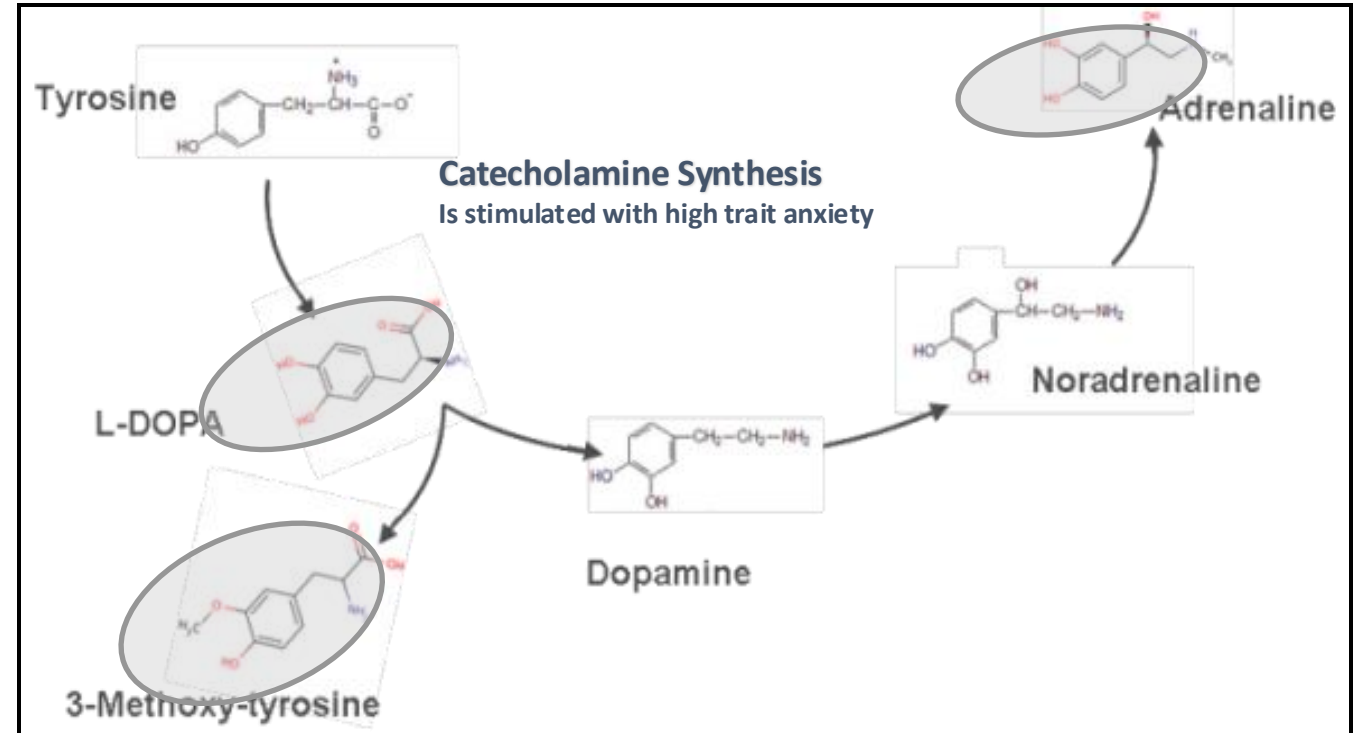
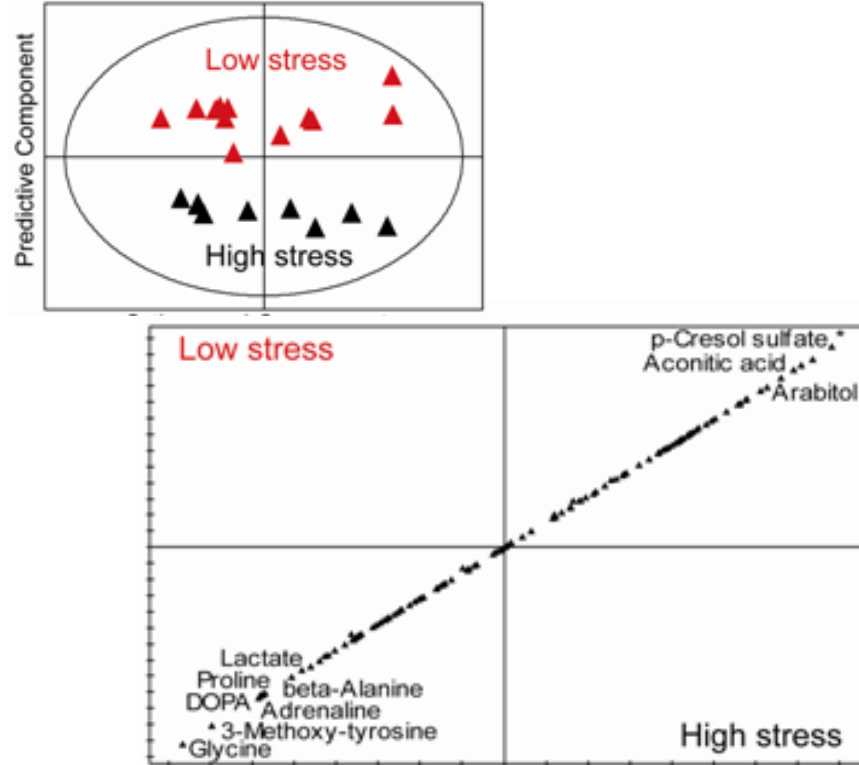
research articles **Journal of proteome** research

Metabolic Effects of Dark Chocolate Consumption on Energy, Gut Microbiota, and Stress-Related Metabolism in Free-Living Subjects

Francois-Pierre J. Martin,^{1,†} Serge Rezzl,^{1,†} Emma Peré-Trepat,[†] Beate Kamlage,[‡] Sebastiano Collino,[†] Edgar Leibold,[§] Jürgen Kastler,[‡] Dietrich Rein,[¶] Laurent B. Fay,[†] and Sunil Kochhar^{*,†}

Nestlé Research Center, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland, Metanomics GmbH, Tegeler Weg 33, 10589 Berlin, Germany, BASF SE, 67056 Ludwigshafen, Germany, and Metanomics Health GmbH, Tegeler Weg 33, 10589 Berlin, Germany

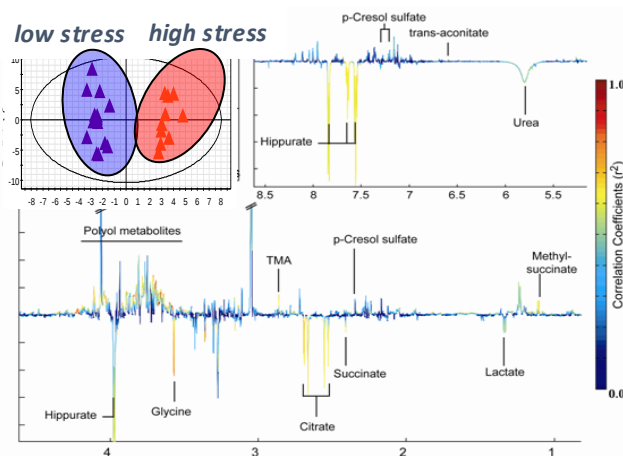
Background stress is associated with specific metabolic signatures



Anxiety trait was associated with:

- Hormonal metabolism: adrenaline, DOPA, 3-methoxy-tyrosine
- Energy metabolism: lactate, citrate, succinate, trans-aconitate, urea... Enhanced rates of aerobic glycolysis: increased mitochondrial respiration *via* Krebs Cycle
- Gluconeogenesis inducing increased lactatemia

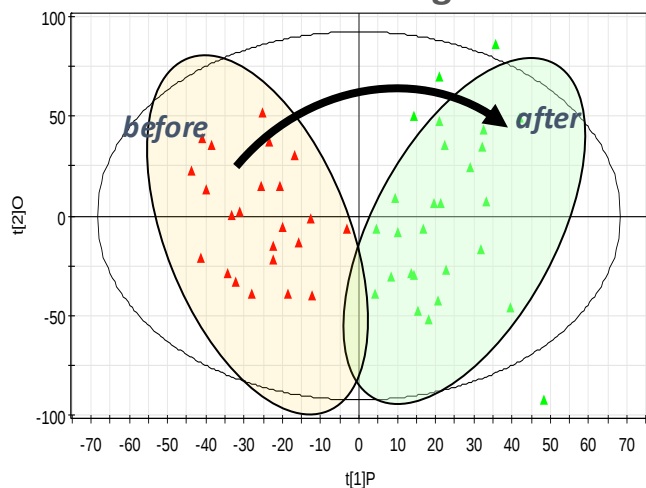
Metabolic signature of stress in urine



Regular intake of dark chocolate associates with:

- Reduction of the excretion of the stress hormone cortisol and catecholamines
- Partial normalization of stress-specific signature energy homeostasis and gut microbial activities (hippurate and p-cresol sulfate).

Effect of dark chocolate on the recovery of stress metabolic signature



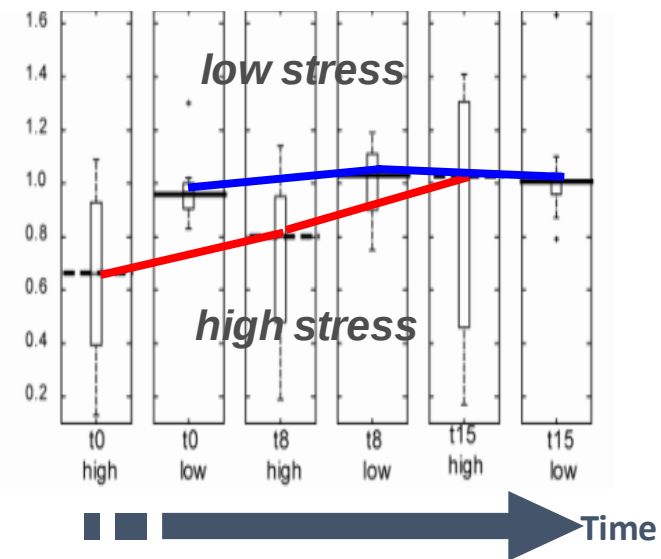
¹H NMR profiling of urine

Nutrients

Gut microbiota

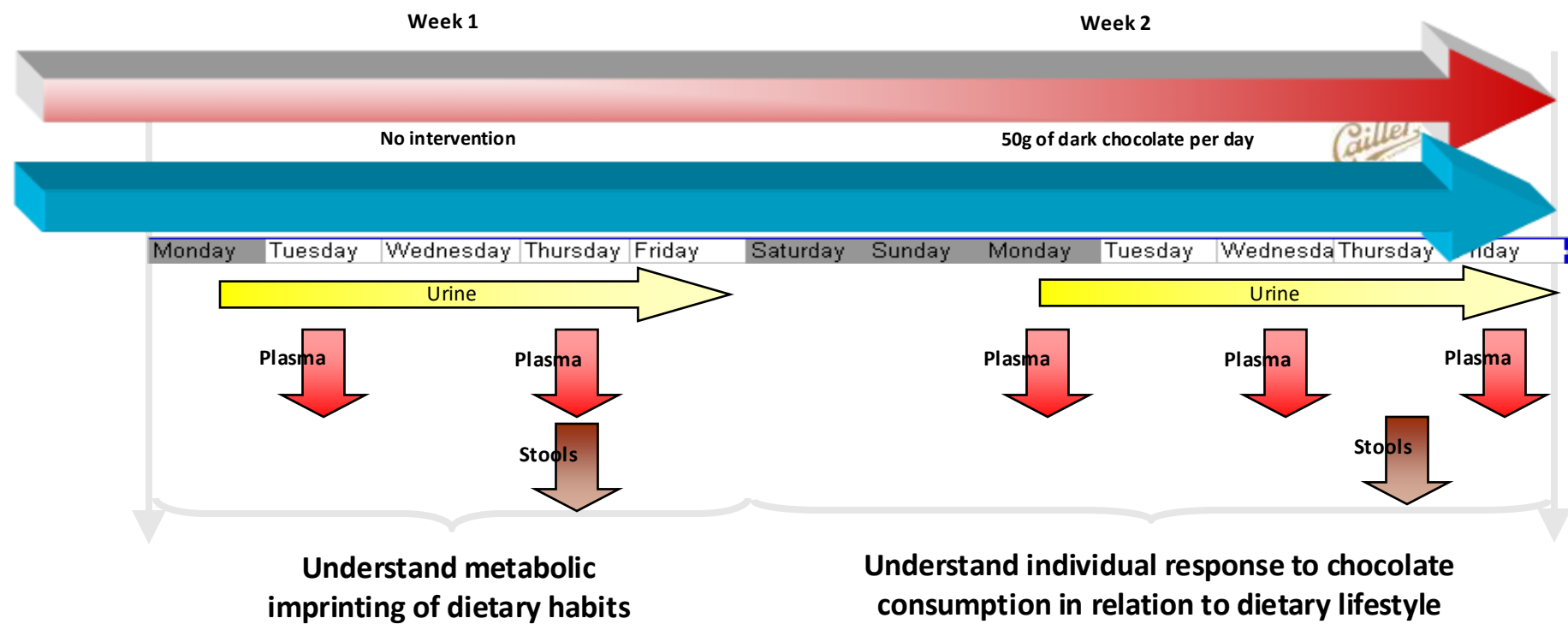
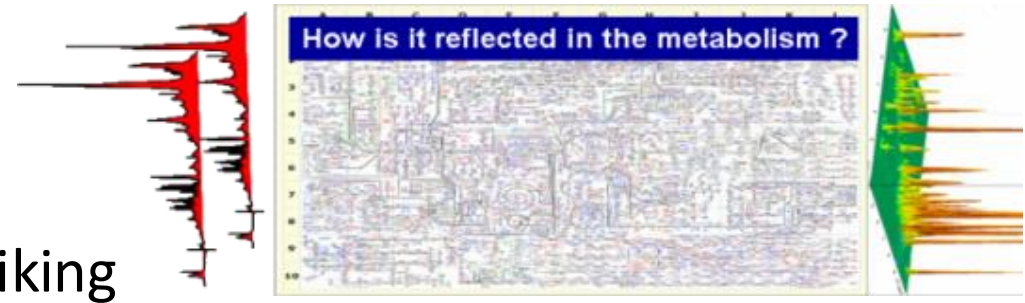
Co-metabolites
(ex: p-cresol sulfate)

Recovery of p-cresol sulfate urinary excretion



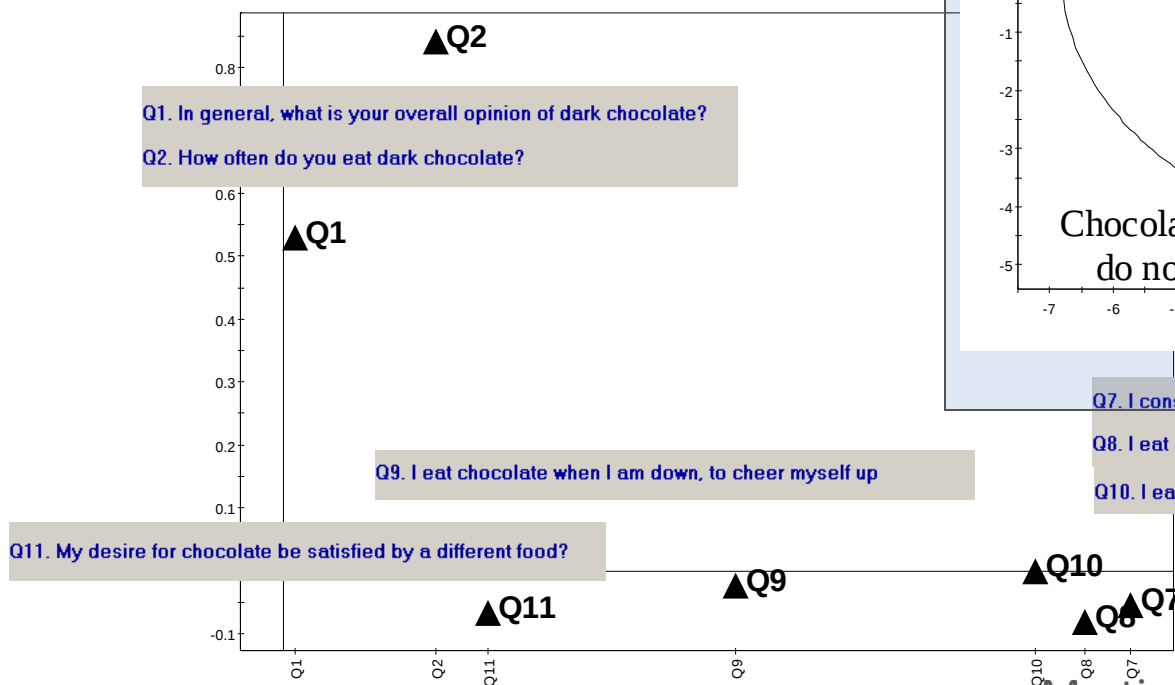
Chocolate dietary habits reflects specific metabolic status

50 subjects stratified according to chocolate liking



Assessment of chocolate dietary preference for subject selection

Emphasis was given to dark chocolate preference



Q1. In general, what is your overall opinion of dark chocolate?

Q2. How often do you eat dark chocolate?

Q9. I eat chocolate when I am down, to cheer myself up

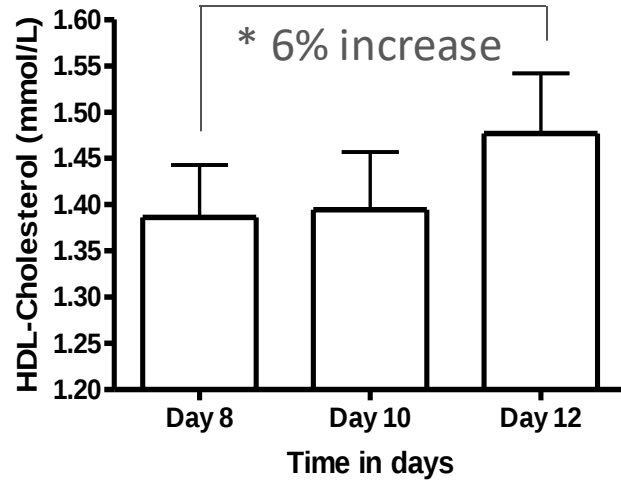
Q11. My desire for chocolate be satisfied by a different food?

Q7. I consider myself a chocolate lover

Q8. I eat chocolate often?

Q10. I eat chocolate even if I don't feel hungry?

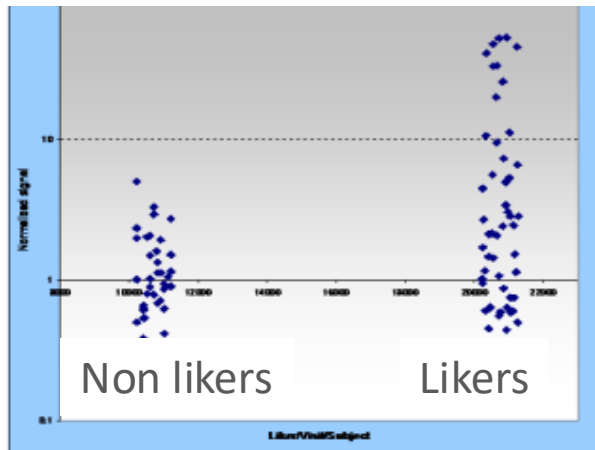
Dark chocolate improves plasma lipid profile and modulates polyphenol co-metabolism



Blood plasma metabolic profiles reveal:

- Beneficial modulation of blood plasma lipids (6% increased in HDL within 4 days of consumption)
- modulation of amino acid and phospholipid metabolism

LC-MS profiling of polyphenol metabolites



Urine metabolic profiles indicate:

- Adaptive response to chocolate consumption
- Potential metabolic programming
- Chocolate dietary habits have effect on gut microbiota

Aging cohort (n=396):

- Centenarians (n=143, mean age: 100.9y)
- Elderly centenarian's offspring (n=210, mean age: 70y)
- Elderly from non long-lived parents (n=73, mean age: 70y)
- Young (n=21, mean age: 30.6y)
- Down syndrome individuals (n=51)

Analysis of urine by ^1H NMR and of blood serum and by targeted MS (lipids, eicosanoids, amino acids...)

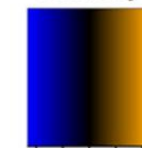
Analysis of fecal microbiota by HITChips
In a subgroup of subjects

Metabolites maintained
in centenarians and young

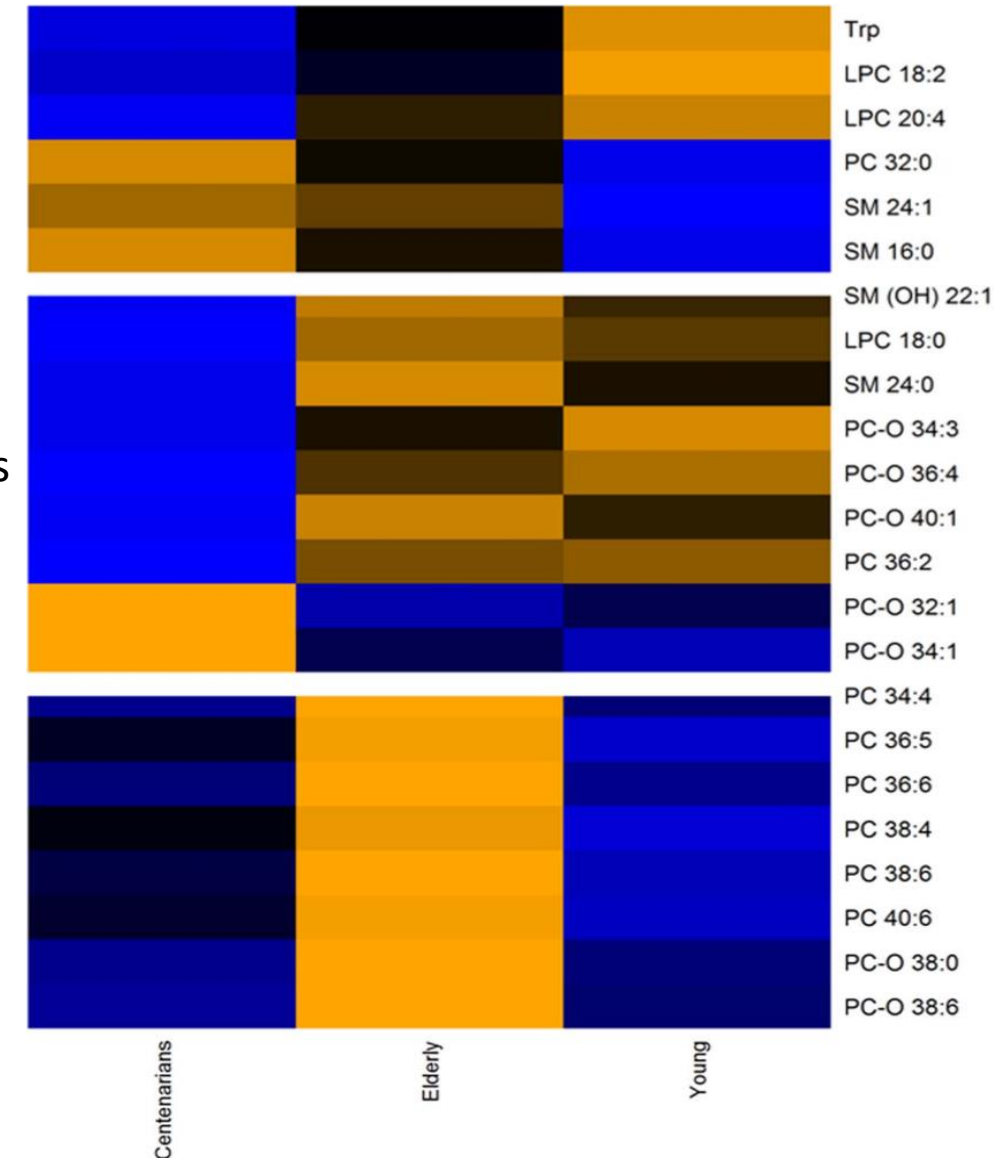
Changes
with age

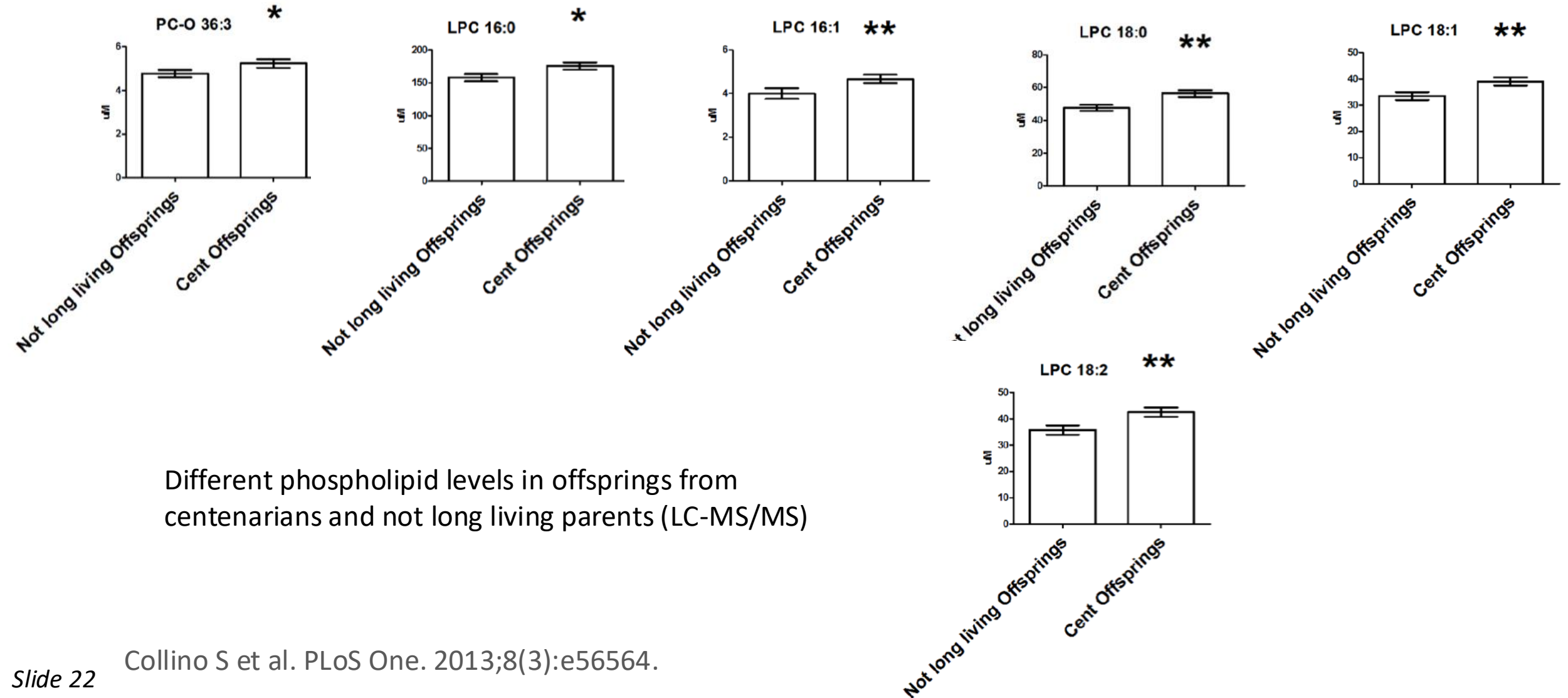
Changes
In centenarians

Color Key

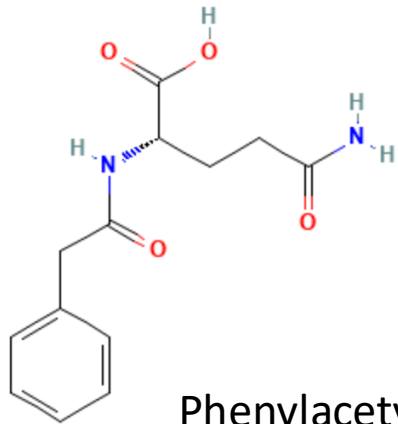
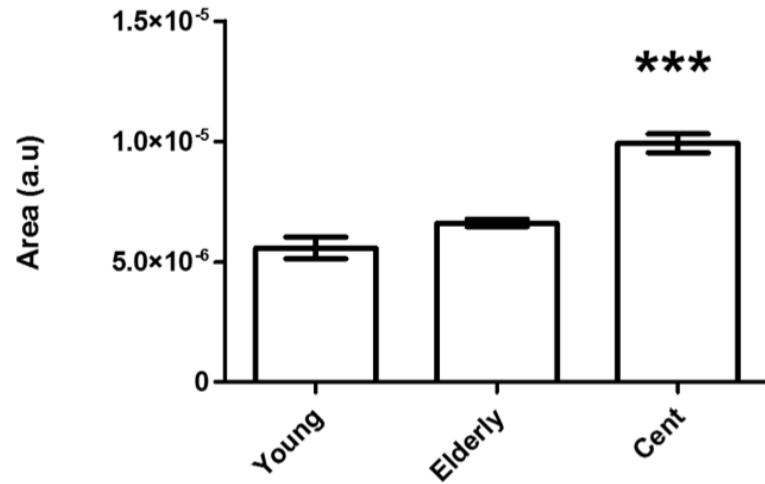


-1 0 1
Row Z-Score

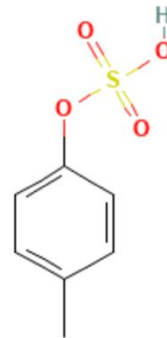
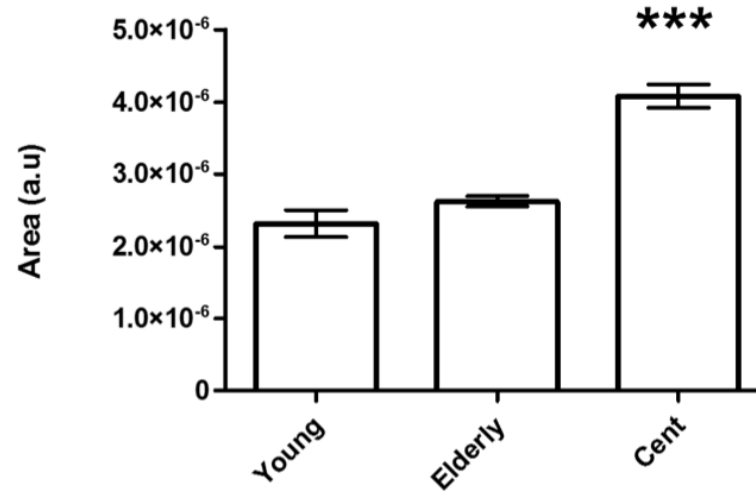




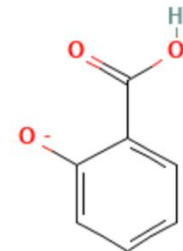
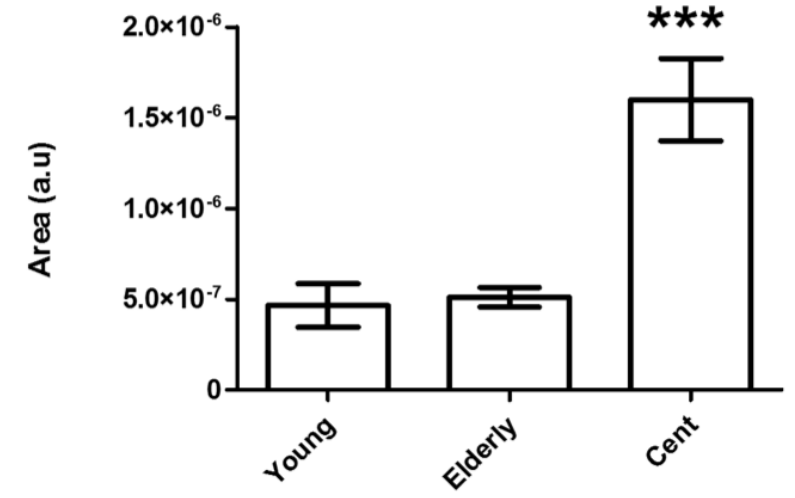
^1H NMR urine



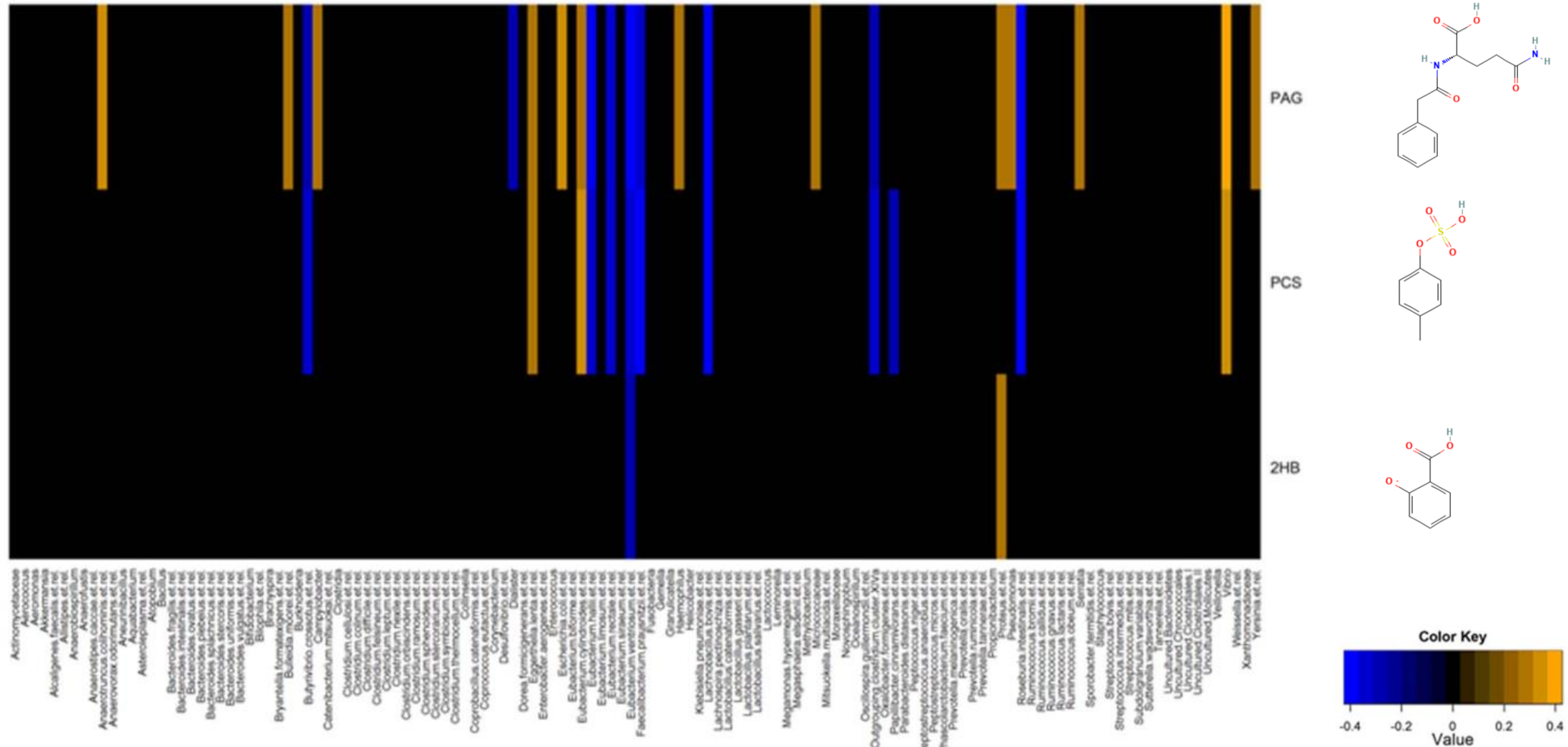
Phenylacetylglutamine



Para cresol sulfate



hydroxybenzoate



Rationale & Objective

Epicardial Adipose Tissue (EAT) is considered as a specific cardiovascular risk indicator, and a determinant of coronary artery disease

Develop a systemic signature of EAT with MS-based lipidomics

Design

40 obese healthy females, age: 25–45 years, BMI: 28–40 kg/m²

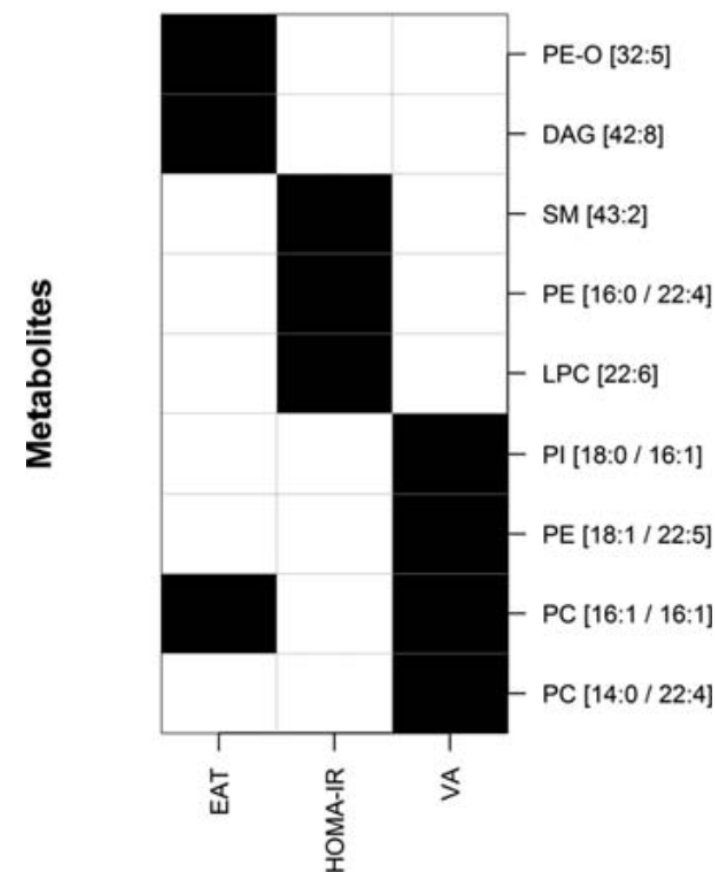
No demonstrated metabolic disease traits

Lipidomics analysis of blood plasma and in vivo quantitation of mediastinal fat depots by computerized tomography.

Design

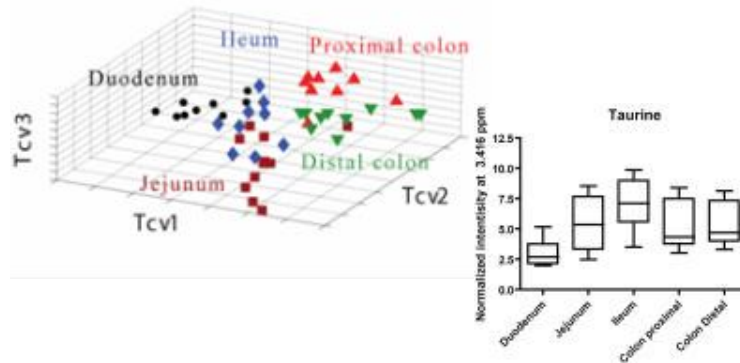
Plasma lipidomics appears as a promising diagnostic readout for patient stratification and monitoring

RF-highlighted metabolites



Exploring the host-gut microbiota transgenomic metabolic interactions

Gastrointestinal physiology

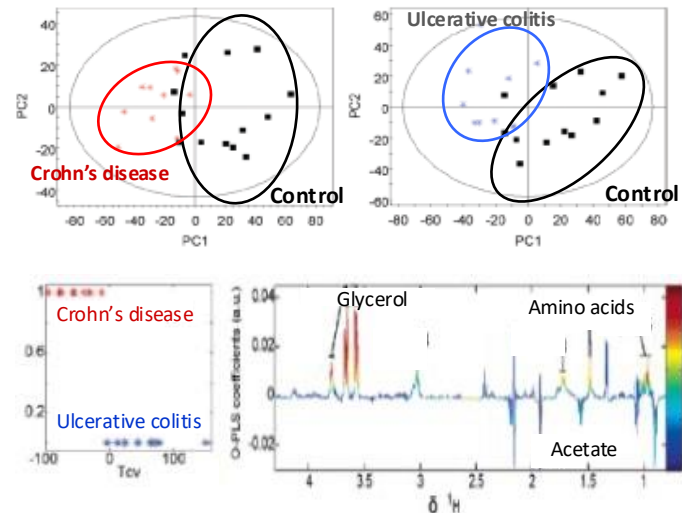


Colonization of germfree mice with probiotic induced region-specific changes:

- digestion,
- absorption of nutrients,
- energy metabolism,
- lipid synthesis and
- protective functions.

Martin et al., (2007) *J. Proteome. Res.* 6(4), 1471-1481

Gastrointestinal disorders

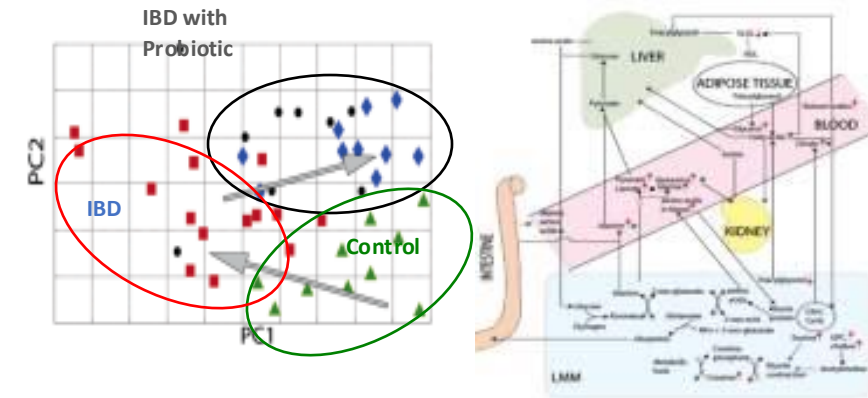


GI disorders were related to:

- microbial changes assessed due to reduced levels of butyrate, acetate, methylamine, and trimethylamine in feces.
- inflammation causing amino acid malabsorption & protein losing enteropathy.

Marchesi et al., (2007) *J. Proteome. Res.* 6(2), 546-551

Nutritional intervention efficacy



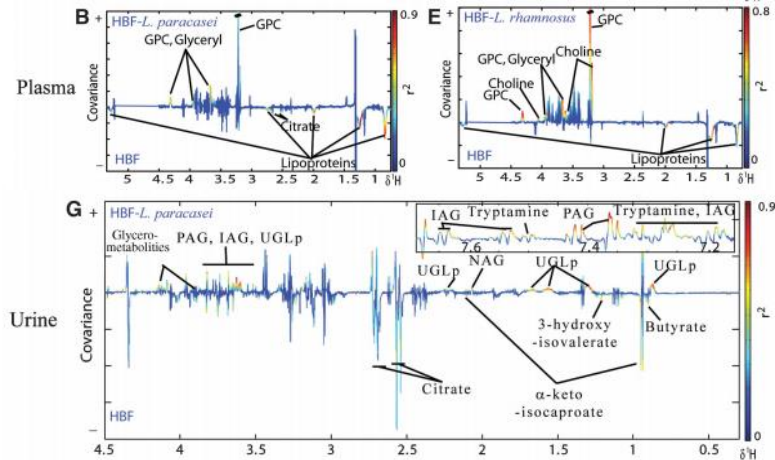
The probiotics resulted in normalizing

- gut microbiota activity,
- parasite-induced GI tract disturbances,
- and energy metabolism.

Martin et al., (2006) *J. Proteome. Res.* 5(9), 2185-2193

Nutritional modulation of the gut microbiota functional ecology

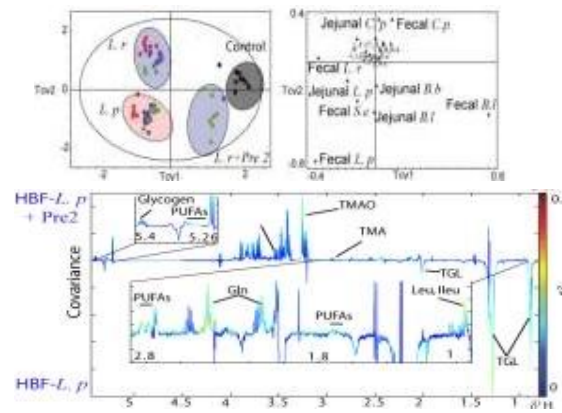
Probiotic effects on modulation of lipid metabolism



- Microbiome modification and altered hepatic lipid metabolism coupled with lowered plasma lipoprotein levels and stimulated glycolysis.
- Probiotic treatments altered several pathways including amino-acid metabolism, methylamines and SCFAs.

FP, Martin et al. Molecular Systems Biology (2008) 4:157

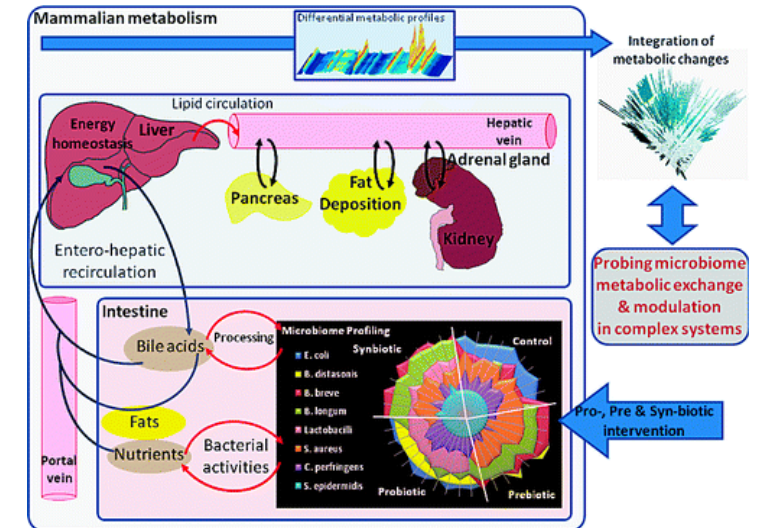
Prebiotic effects on energy homeostasis



- Development of Bifidobacteria & inhibition of pathogens
- Probiotic - Prebiotic interactions induced different faecal SCFAs, carbohydrate, amino acid and methylamine metabolism
- Low levels triglycerides, high levels of PUFAs and glucogenic amino acids in the liver

FP, Martin et al. Molecular Systems Biology (2008) 4:205

Synbiotic effects on caloric recovery and fat metabolism



- Synergistic effects between prebiotics, *L. rhamnosus* and *B. longum*
- Pre- and synbiotics resulted in different lipid mobilisation, recirculation, and deposition

FP, Martin et al. Journal of Proteome Research 2009, 8, 2090–2105