FACULTY OF
VETERINARY MEDICINE
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HIGH-RESOLUTION MASS SPECTROMETRY-BASED METABOLOMICS AND LIPIDOMICS TO UNRAVEL THE GUT MICROBIOME - DIET AXIS IN HEALTH AND DISEASE

Prof. Lynn Vanhaecke

GHENT
UNIVERSITY

6 April 2022

QUEEN'S
UNIVERSITY
BELFAST

INTRODUCTION

LIMET = LABORATORY OF INTEGRATIVE METABOLOMICS

Background:

- Faculty of Veterinary Medicine, Department of Translational Physiology, Infectiology and Public Health, Laboratory of Integrative Metabolomics
- 4 post-doctoral researchers, 13 PhD students and 4 technicians

Research domains:

- Metabolomics and lipidomics in health and disease (incl. wildlife reproduction)
- Nutrimetabolomics and DNA-adductomics in the food-health axis
- Metabolomics in food analysis, environmental and biotechnological applications

L I M E T
Laboratory of Integrative Metabolomics

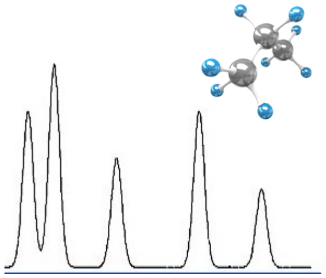
MATRICES & EQUIPMENT @ LIMET

Investigated biological matrices:

- **Tissue/cell lines**
- Urine
- Blood
- **Feces**
- **Saliva**
- Tomato
- Wheat
- Rice
- Seafood
- Insects
- Water
- Yeast

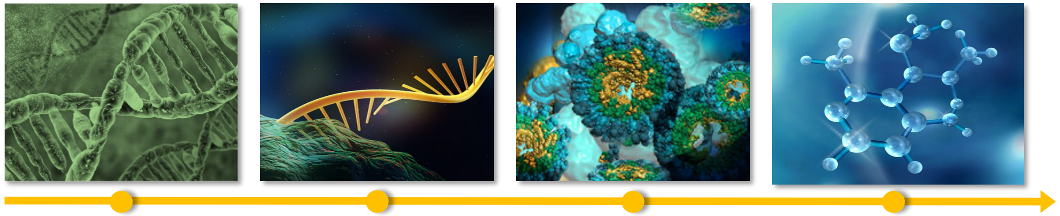
Equipment:

- UHPLC – Exploris^{TM} 120 **Orbitrap HRMS**
- UHPLC – Q- Exactive^{TM} **Orbitrap HRMS**
- UHPLC - QqQ MS
- HPLC - Ion Trap MS
- PDA, Fluorescence, ELSD
- **REI-Q-ToF-MS (2), iKnife and OPO laser**



⇨ **Special focus on holistic metabolomics/lipidomics analyses**

METABOLOMICS:



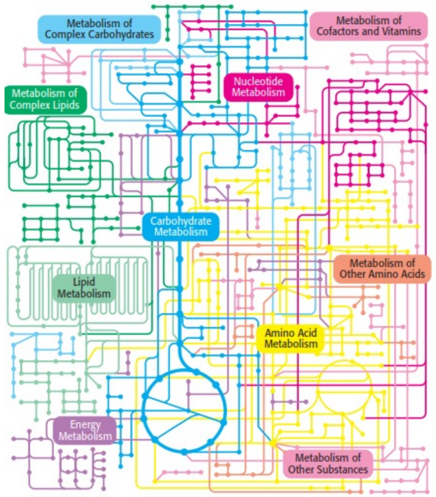
DNA
Genomics

RNA
Transcriptomics

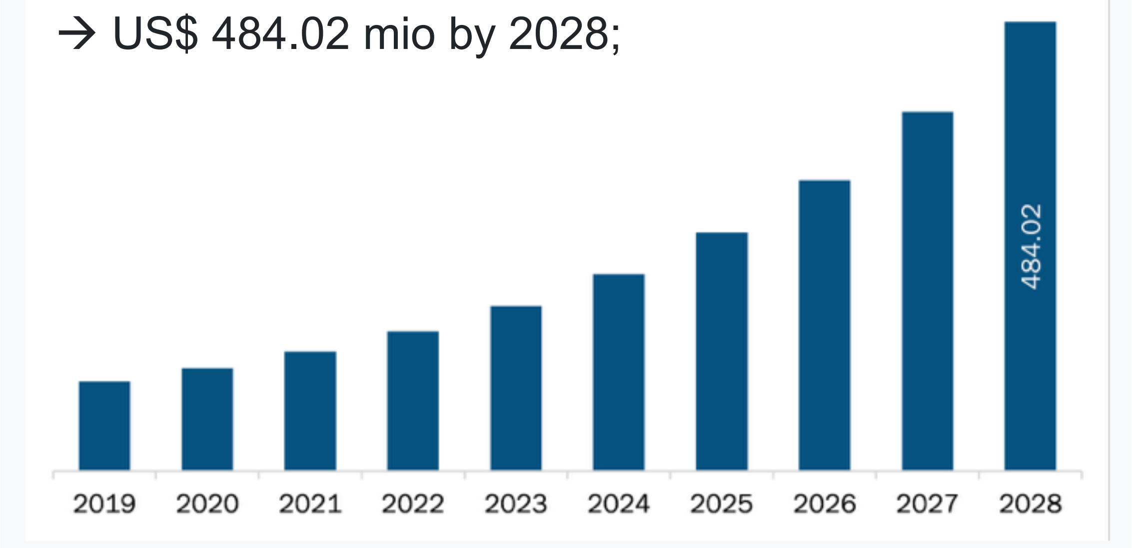
Proteins
Proteomics

Metabolites
Metabolomics

Biological phenotype



Market Europe: **CAGR = 20.8%**
US$ 128.72 mio in 2021
→ US$ 484.02 mio by 2028;



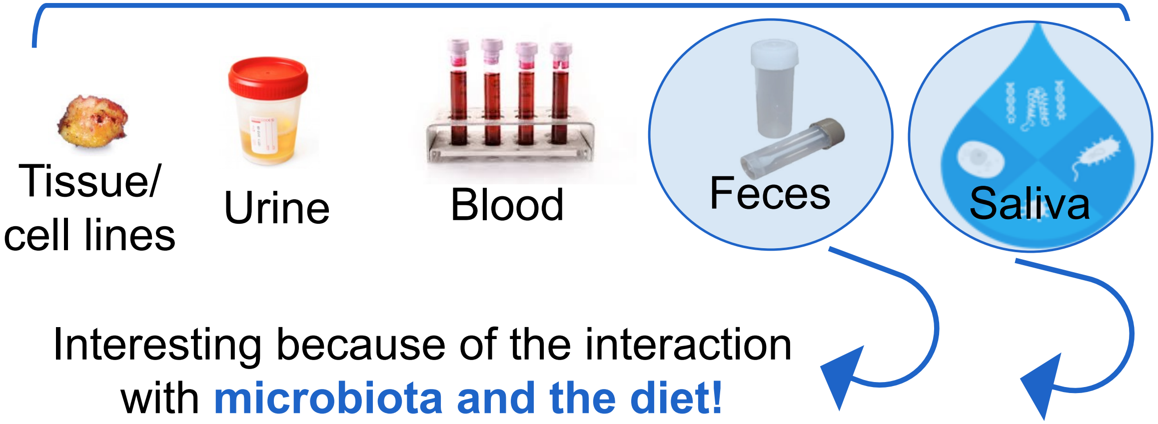
Main applications (pharma & biotech):
- Biomarker discovery
- Drug discovery
- Toxicology
- …

METABOLOMICS AND LIPIDOMICS METHODS

HOW DO WE PERFORM METABOLOMICS?

- **Metabolic health**
- **Homeostatic loss**
  - Allergy
  - Diabetes type 2
  - Metabolic syndrome
  - Obesity
  - IBD, IBS, Hirschsprung
  - Gastrointestinal cancers
  - …

Within my lab and in both a clinical and nutritional context, **various matrices** are targeted for metabolomics analyses:

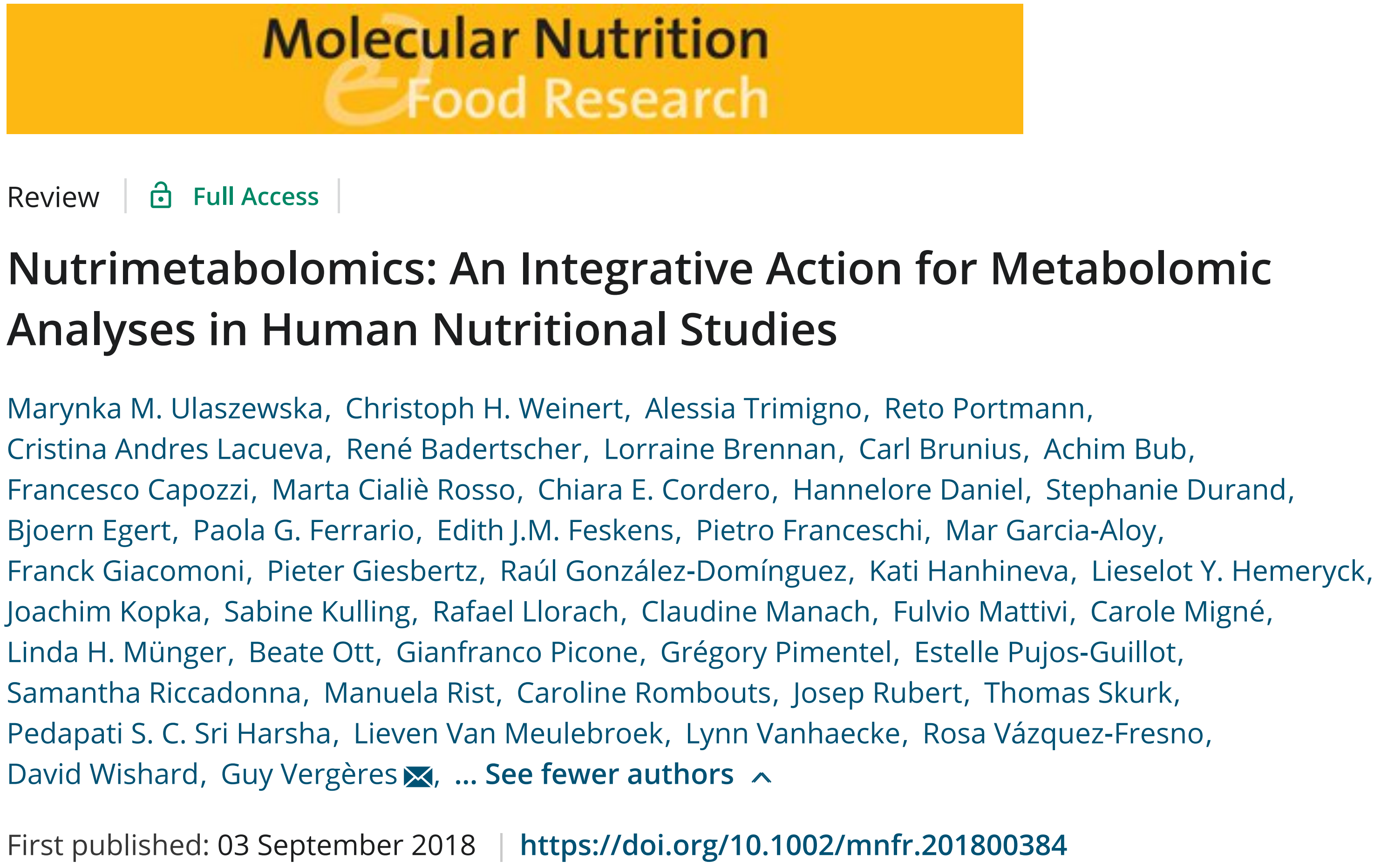


Interesting because of the interaction with **microbiota and the diet!**

Frequently investigated in light of many conditions that are linked to **GIT imbalance & direct link to diet-microbiome**

⇨ **Analytical methods have been developed for the various matrices for both polar metabolites and lipids**

NUTRIMETABOLOMICS: STATE-OF-THE-ART



Molecular Nutrition & Food Research

Review | Full Access |

Nutrimetabolomics: An Integrative Action for Metabolomic Analyses in Human Nutritional Studies

Marynka M. Ulaszewska, Christoph H. Weinert, Alessia Trimigno, Reto Portmann, Cristina Andres Lacueva, René Badertscher, Lorraine Brennan, Carl Brunius, Achim Bub, Francesco Capozzi, Marta Cialiè Rosso, Chiara E. Cordero, Hannelore Daniel, Stephanie Durand, Bjoern Egert, Paola G. Ferrario, Edith J.M. Feskens, Pietro Franceschi, Mar Garcia-Aloy, Franck Giacomoni, Pieter Giesbertz, Raúl González-Domínguez, Kati Hanhineva, Lieselot Y. Hemeryck, Joachim Kopka, Sabine Kulling, Rafael Llorach, Claudine Manach, Fulvio Mattivi, Carole Migné, Linda H. Münger, Beate Ott, Gianfranco Picone, Grégory Pimentel, Estelle Pujos-Guillot, Samantha Riccadonna, Manuela Rist, Caroline Rombouts, Josep Rubert, Thomas Skurk, Pedapati S. C. Sri Harsha, Lieven Van Meulebroek, Lynn Vanhaecke, Rosa Vázquez-Fresno, David Wishard, Guy Vergères ✉, ... **See fewer authors** ^

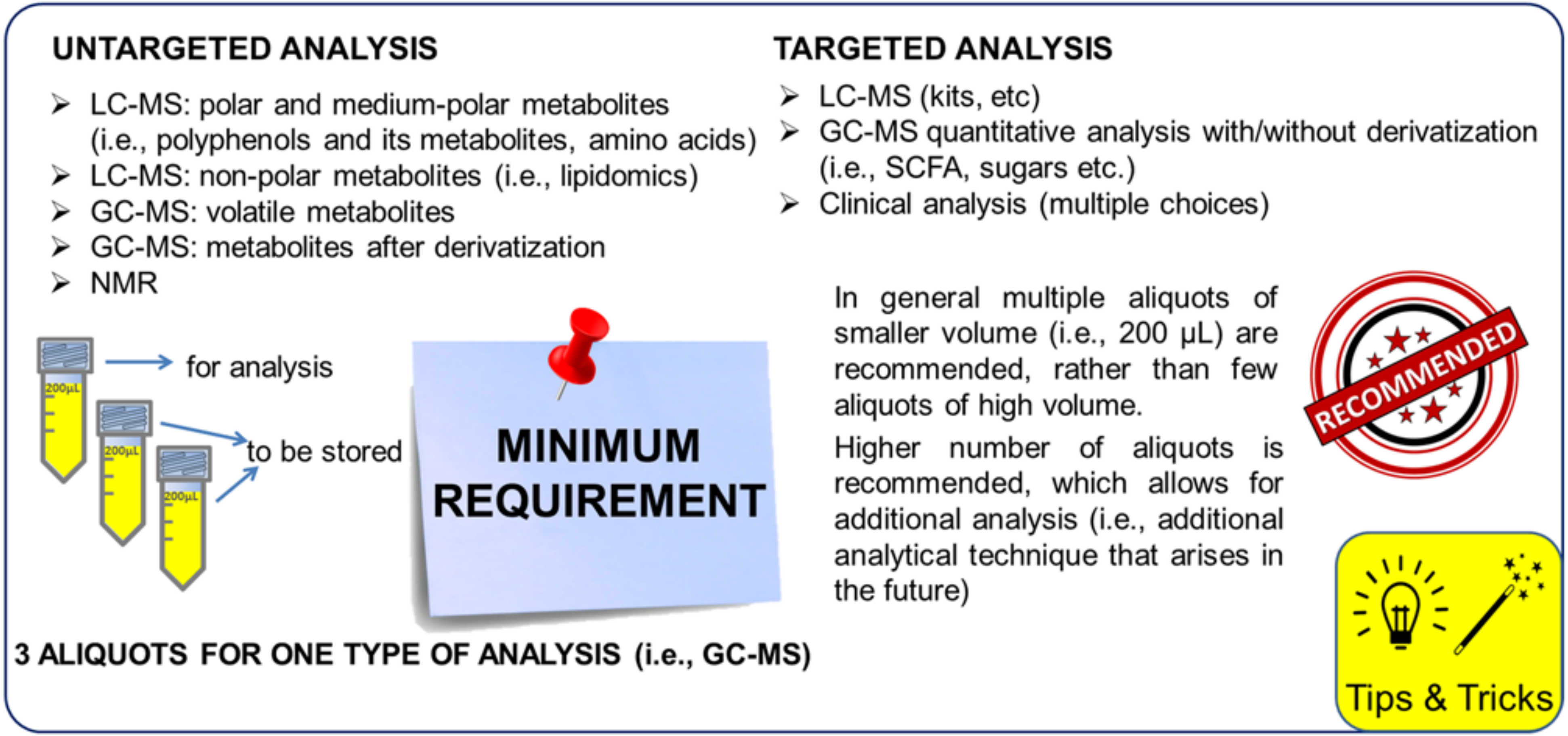
First published: 03 September 2018 | **https://doi.org/10.1002/mnfr.201800384**

JPI
a healthy diet
for a healthy life

FOODBALL
The Food Biomarker Alliance

NuGO

POSSIBLE ANALYSIS TYPES



UNTARGETED ANALYSIS

- LC-MS: polar and medium-polar metabolites (i.e., polyphenols and its metabolites, amino acids)
- LC-MS: non-polar metabolites (i.e., lipidomics)
- GC-MS: volatile metabolites
- GC-MS: metabolites after derivatization
- NMR

TARGETED ANALYSIS

- LC-MS (kits, etc)
- GC-MS quantitative analysis with/without derivatization (i.e., SCFA, sugars etc.)
- Clinical analysis (multiple choices)

MINIMUM REQUIREMENT

3 ALIQUOTS FOR ONE TYPE OF ANALYSIS (i.e., GC-MS)

In general multiple aliquots of smaller volume (i.e., 200 µL) are recommended, rather than few aliquots of high volume.

Higher number of aliquots is recommended, which allows for additional analysis (i.e., additional analytical technique that arises in the future)

Molecular Nutrition & Food Research

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Nutrimetabolomics: An Integrative Action for Metabolomic Analyses in Human Nutritional Studies

MASS SPECTROMETRIC ANALYSIS: DIFFERENT APPROACHES

Targeted analysis

Predefined list

Accurate mass

Retention time

Isotope profile

Suspect analysis

Specific Database

Accurate mass

Retention time prediction

Isotope profile

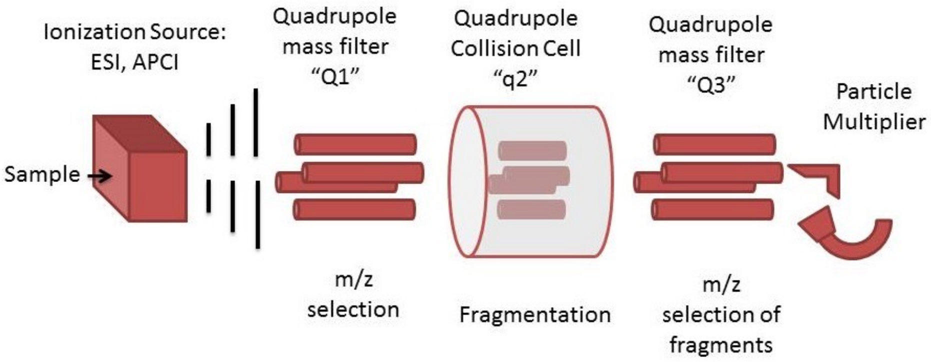
Untargeted analysis

No database

Top-down approach

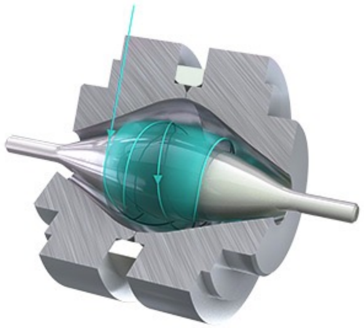
HIGH-END ANALYTICAL MS INSTRUMENTATION

- Analytical tools



QqQ technology

Advanced
instrumentation



Orbitrap

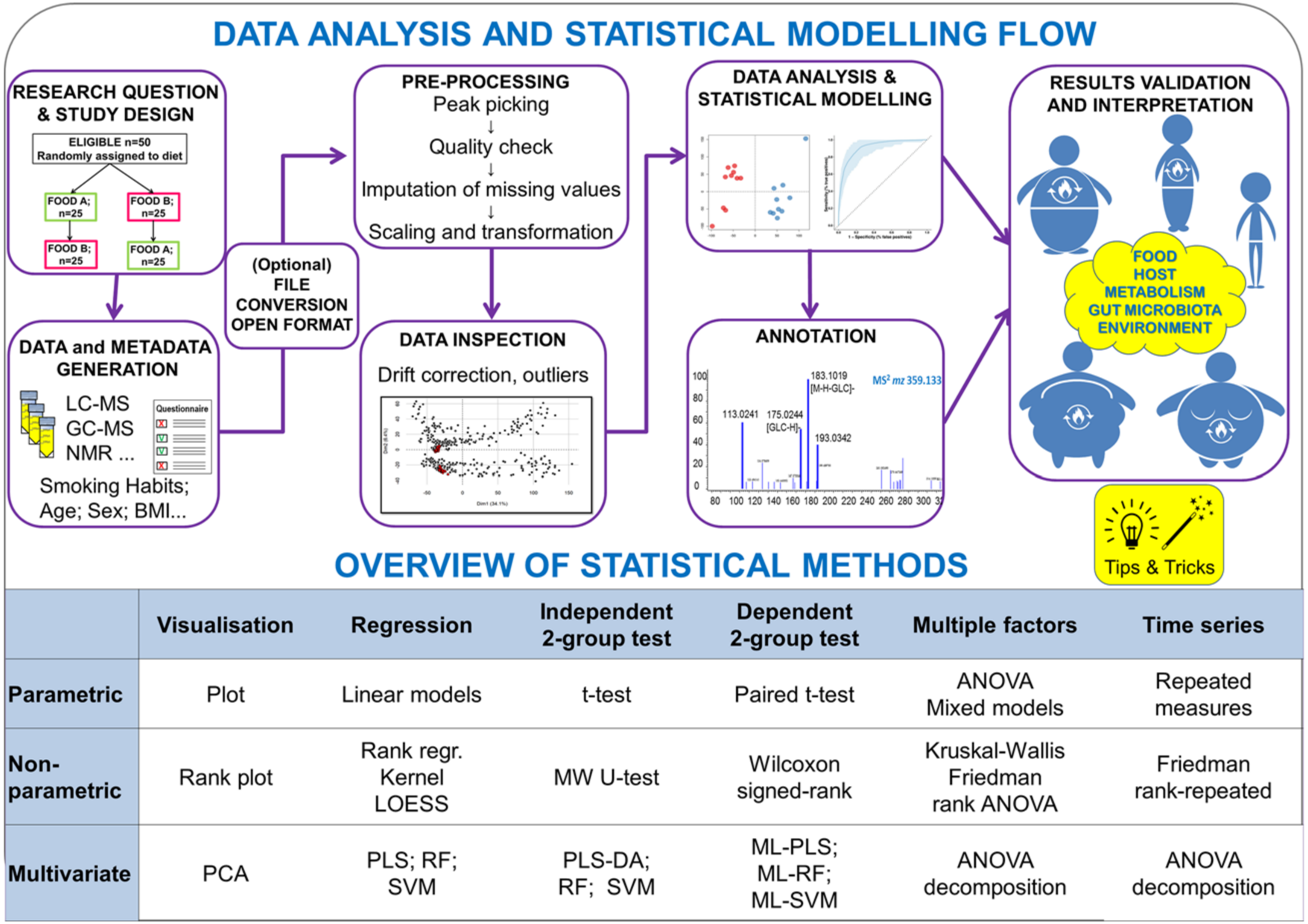
Alternatively:
time-of-flight

HRMS technology

Analysis of a predefined number of target compounds: quantitative

Accurate mass screening of a virtually unlimited number of compounds: (semi-)quantitative

GENERAL MODEL FLOW FOR DATA ANALYSIS



DATA ANALYSIS AND STATISTICAL MODELLING FLOW

RESEARCH QUESTION & STUDY DESIGN

DATA and METADATA GENERATION

LC-MS
GC-MS
NMR ...

Smoking Habits; Age; Sex; BMI...

(Optional) FILE CONVERSION OPEN FORMAT

PRE-PROCESSING

Peak picking
↓
Quality check
↓
Imputation of missing values
↓
Scaling and transformation

DATA INSPECTION

Drift correction, outliers

DATA ANALYSIS & STATISTICAL MODELLING

ANNOTATION

RESULTS VALIDATION AND INTERPRETATION

FOOD
HOST
METABOLISM
GUT MICROBIOTA
ENVIRONMENT

Tips & Tricks

OVERVIEW OF STATISTICAL METHODS

	Visualisation	Regression	Independent 2-group test	Dependent 2-group test	Multiple factors	Time series
Parametric	Plot	Linear models	t-test	Paired t-test	ANOVA Mixed models	Repeated measures
Non-parametric	Rank plot	Rank regr. Kernel LOESS	MW U-test	Wilcoxon signed-rank	Kruskal-Wallis Friedman rank ANOVA	Friedman rank-repeated
Multivariate	PCA	PLS; RF; SVM	PLS-DA; RF; SVM	ML-PLS; ML-RF; ML-SVM	ANOVA decomposition	ANOVA decomposition

Molecular Nutrition & Food Research

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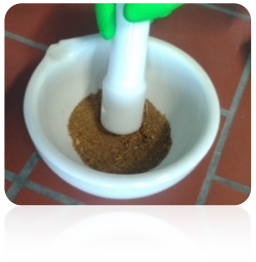
Nutrimetabolomics: An Integrative Action for Metabolomic Analyses in Human Nutritional Studies

HOW DO WE PERFORM METABOLOMICS: EX. FECES?



Extraction methods:

- Specifically developed for **faeces**: both for lipids and polar metabolites
- Using low amounts of starting material (100-200 mg dry weight)
- Applying solid- or liquid-liquid extraction

Detection methods:

- Using UHPLC for **chromatographic** separation
- Using Q-Exactive^{TM} Orbitrap MS for **detection** (Exploris 120 since mid 2021)
- High-resolution full-scan events for **discovery** phase (70,000 FWHM)
- MS/MS events for **identification** purposes (17,500 FWHM)

MS more sensitive than NMR
(nanomolar versus μmolar range)

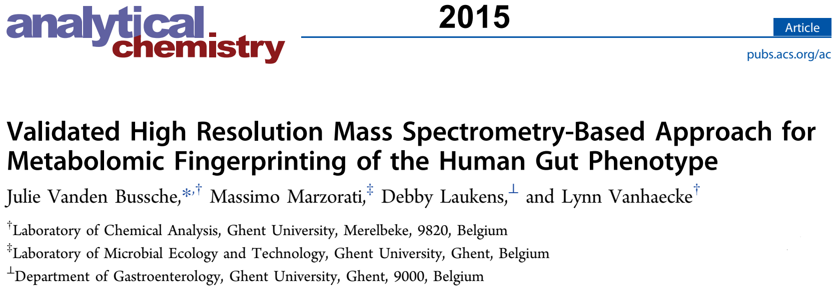
Method application:

- Methods applied in a truly **untargeted** (holistic) fashion
- But also a large set of analytical standards available for **targeted** interpretation

HOW DO WE PERFORM METABOLOMICS: EX. FECES?

Polar metabolomics – chemical targets:

- Amino acids
- Amines
- Other N-compounds
- Polyols
- Bile acids
- Carbohydrates
- Short chain fatty acids
- Hydroxy acids
- Multicarboxylic acids
- Monocarboxylic acids



2015

analytical chemistry

Article

pubs.acs.org/ac

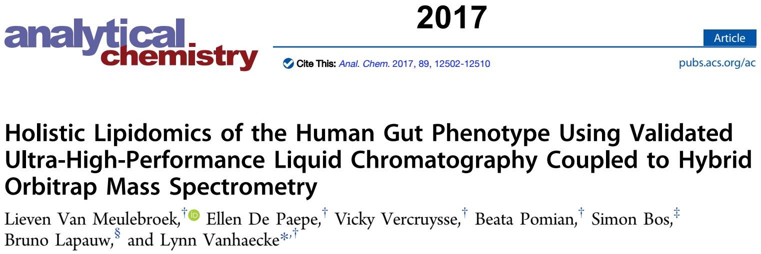
Validated High Resolution Mass Spectrometry-Based Approach for Metabolomic Fingerprinting of the Human Gut Phenotype

Julie Vanden Bussche,[*,†] Massimo Marzorati,[‡] Debby Laukens,[⊥] and Lynn Vanhaecke[†]

[†]Laboratory of Chemical Analysis, Ghent University, Merelbeke, 9820, Belgium
[‡]Laboratory of Microbial Ecology and Technology, Ghent University, Ghent, Belgium
[⊥]Department of Gastroenterology, Ghent University, Ghent, 9000, Belgium

Lipidomics – chemical targets:

- Fatty acyls
- Phospholipids
- Prenols
- Sterols
- Glycerolipids
- Glycerophospholipids
- Polyketides
- Sphingolipids



2017

analytical chemistry

Article

Cite This: Anal. Chem. 2017, 89, 12502−12510

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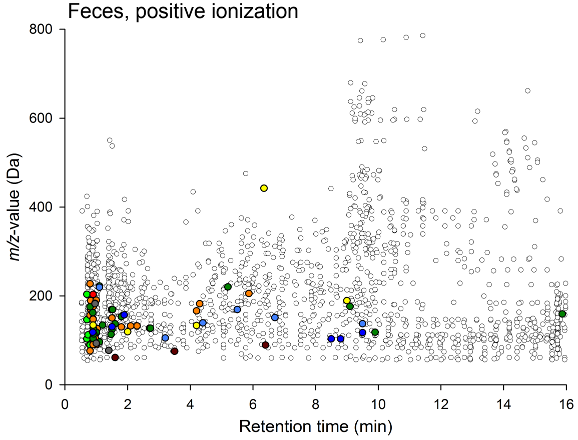
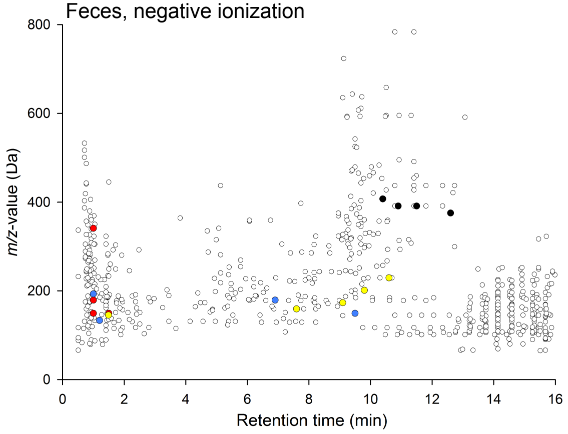
Holistic Lipidomics of the Human Gut Phenotype Using Validated Ultra-High-Performance Liquid Chromatography Coupled to Hybrid Orbitrap Mass Spectrometry

Lieven Van Meulebroek,[†] Ellen De Paepe,[†] Vicky Vercruysse,[†] Beata Pomian,[†] Simon Bos,[‡] Bruno Lapauw,[§] and Lynn Vanhaecke[*,†]



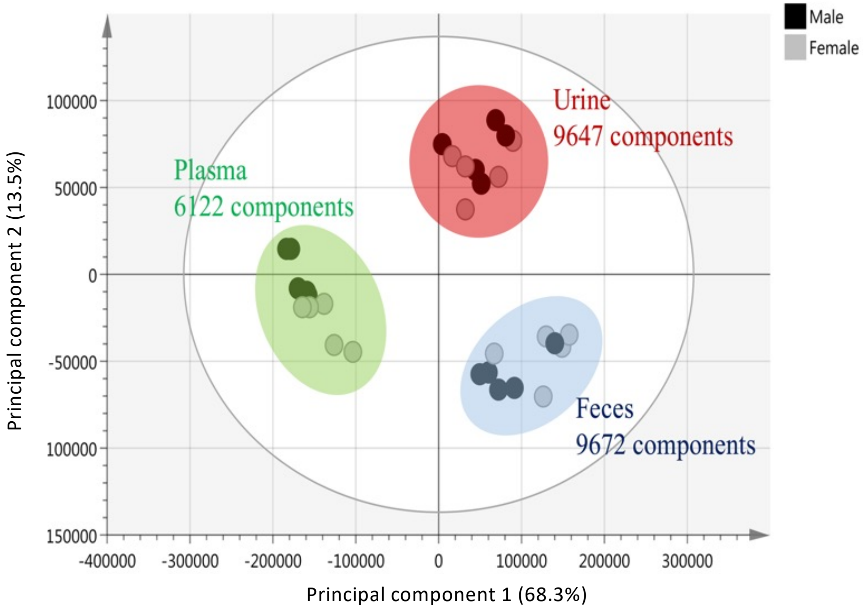
Feces, positive ionization

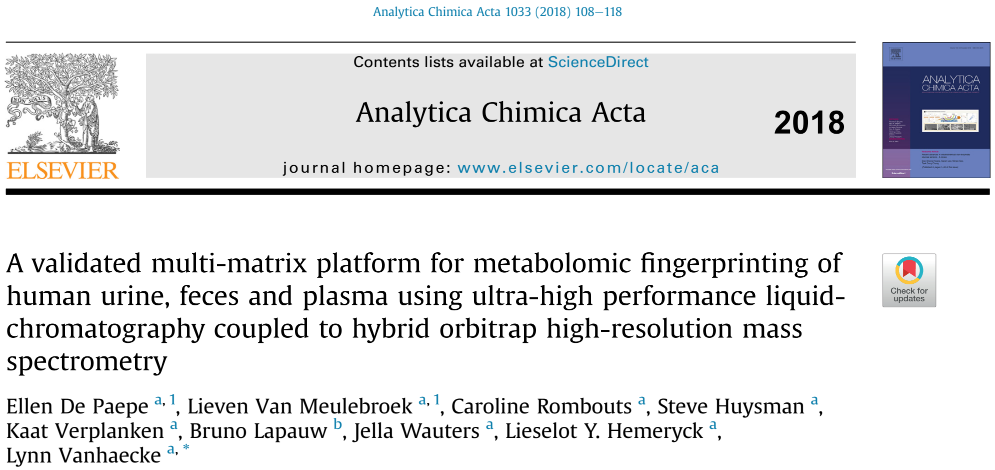


Feces, negative ionization

Dr. Lieven Van Meulebroek

OPTIMAL BIOFLUID (COMBI) FOR METABOLOMICS





Analytica Chimica Acta 1033 (2018) 108–118

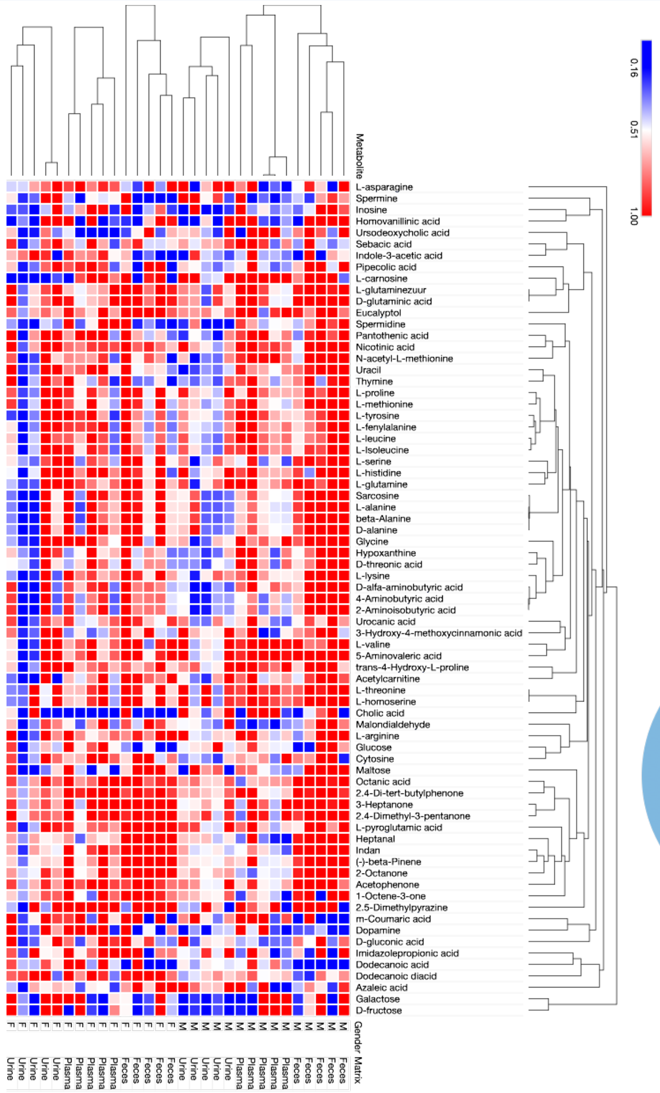
Contents lists available at ScienceDirect

Analytica Chimica Acta **2018**

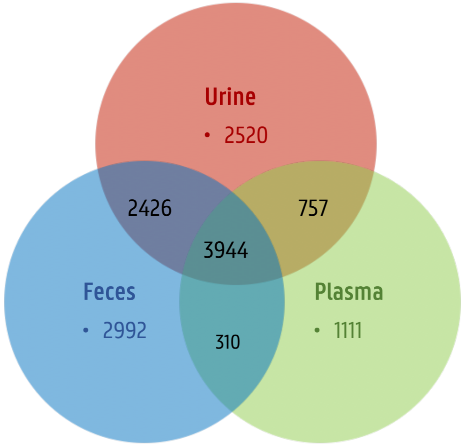
journal homepage: www.elsevier.com/locate/aca

A validated multi-matrix platform for metabolomic fingerprinting of human urine, feces and plasma using ultra-high performance liquid-chromatography coupled to hybrid orbitrap high-resolution mass spectrometry

Ellen De Paepe [a, 1], Lieven Van Meulebroek [a, 1], Caroline Rombouts [a], Steve Huysman [a], Kaat Verplanken [a], Bruno Lapauw [b], Jella Wauters [a], Lieselot Y. Hemeryck [a], Lynn Vanhaecke [a, *]



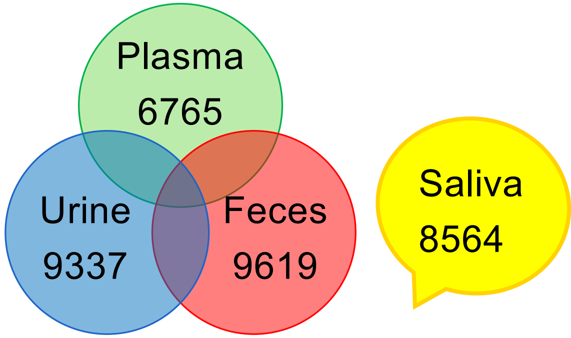
Ellen De Paepe



UNIQUE SALIVARY METABOLOMICS METHOD

Untargeted validation: 8564 metabolites (CV < 30%)

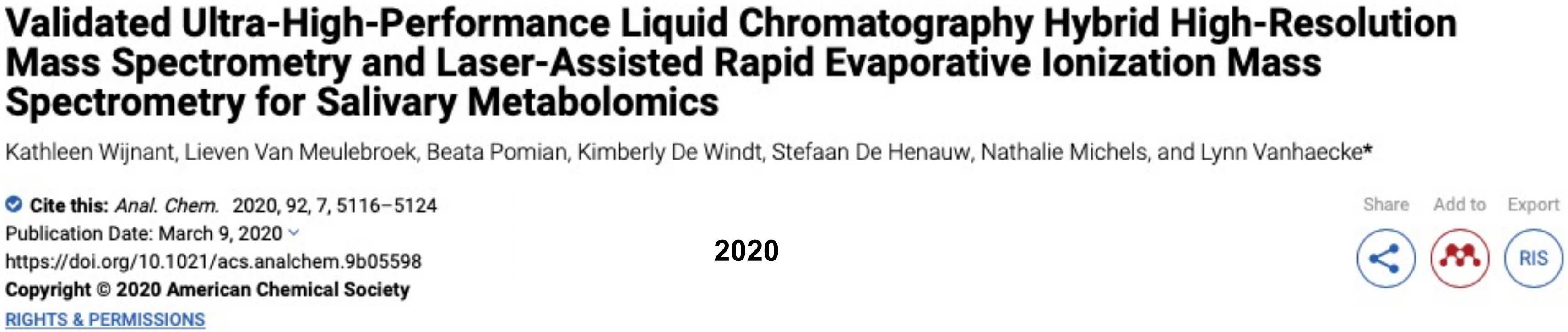
- ✓ Instrumental precision: 86.6%
- ✓ Inter-assay precision: 83.0%
- ✓ Intra-assay precision: 76.8%
- ✓ Linearity (9-point serial dilution: 1 to 0.01): $R^2 \geq 0.90$ = 82.7%



Endogenous metabolites

- Alcohols
- Phenols
- Benzenes
- Keto acids
- Monocarboxylic acids
- Dicarboxylic acids
- Hybrid peptides
- Imidazoles
- Pyridines
- Fatty acids
- Quaternary ammonium salts
- Ethers
- Carbohydrates
- Ketones
- Amino acids
- Amines
- Hydroxy acids
- Steroids

Kathleen Wijnant



Validated Ultra-High-Performance Liquid Chromatography Hybrid High-Resolution Mass Spectrometry and Laser-Assisted Rapid Evaporative Ionization Mass Spectrometry for Salivary Metabolomics

Kathleen Wijnant, Lieven Van Meulebroek, Beata Pomian, Kimberly De Windt, Stefaan De Henauw, Nathalie Michels, and Lynn Vanhaecke*

Cite this: *Anal. Chem.* 2020, 92, 7, 5116−5124
Publication Date: March 9, 2020
https://doi.org/10.1021/acs.analchem.9b05598
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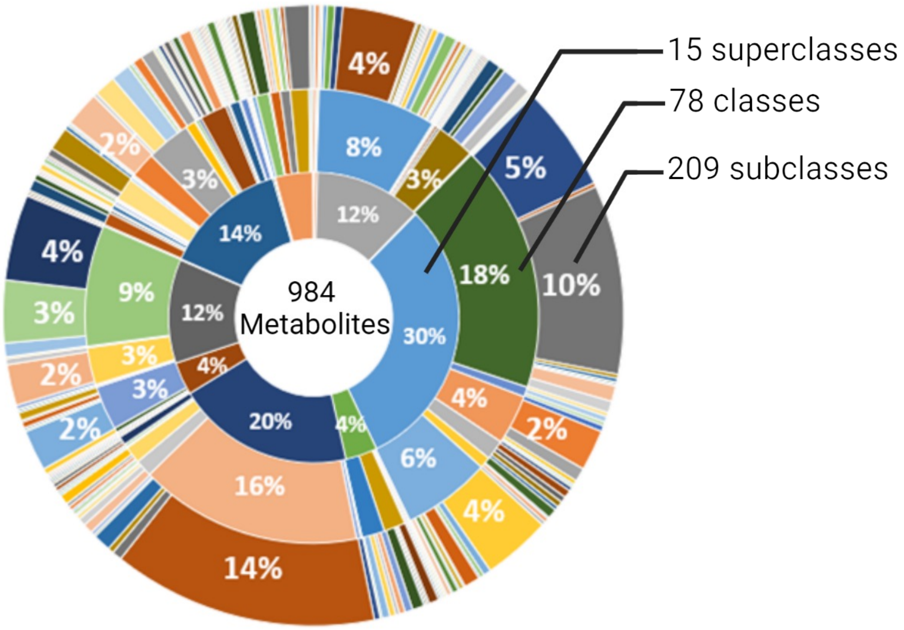
2020

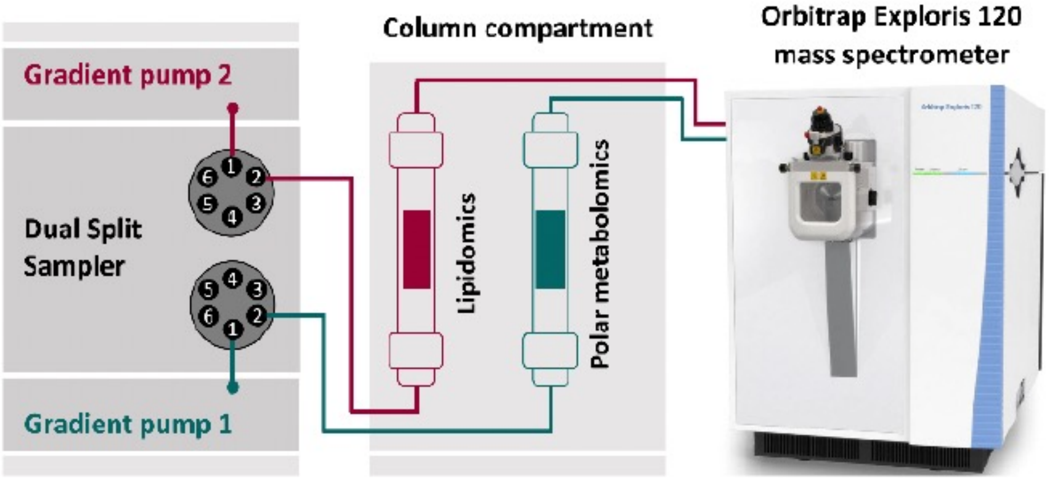
RECENT ANALYTICAL DEVELOPMENTS

Metabolite ontology search: in-house standard database comprising ca. 750 polar to medium polar and ca. 250 lipid metabolites

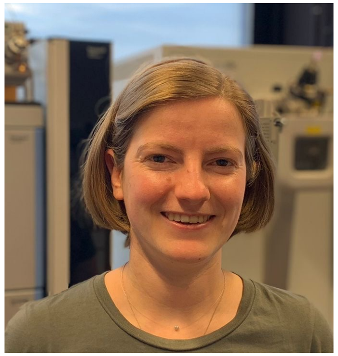
Dual extraction and UHPLC approach metabolomics & lipidomics combined: e.g. 100 mg freeze dried feces

Beata Pomian

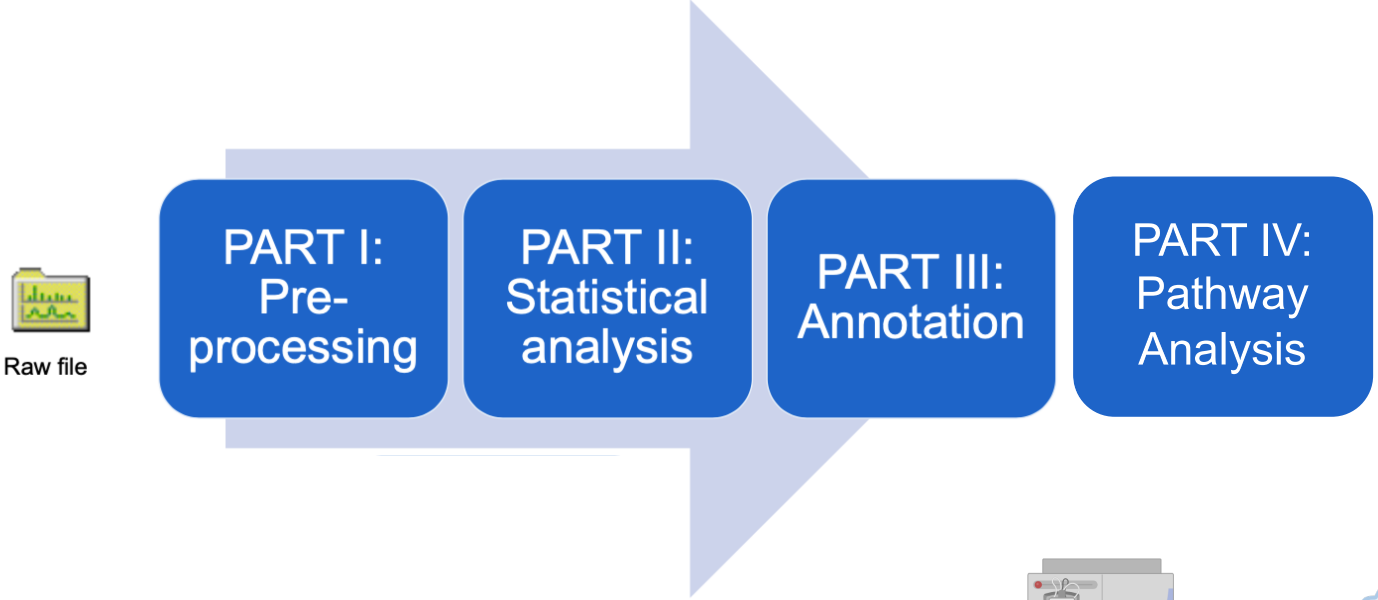




DATA ANALYSIS DEVELOPMENTS: AUTOMATION



Dr. Marilyn De Graeve



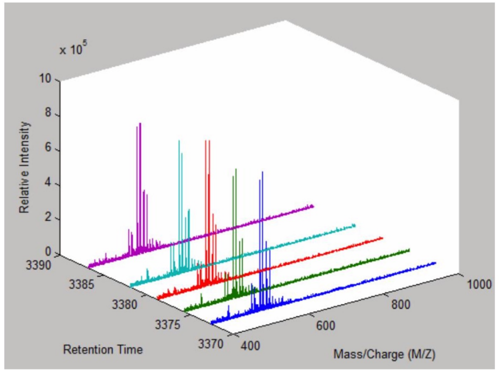
Raw file

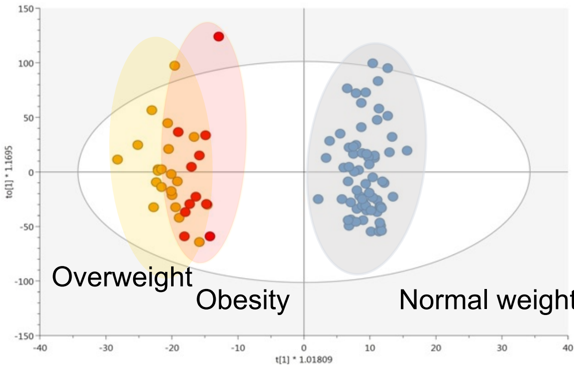
PART I: Pre-processing

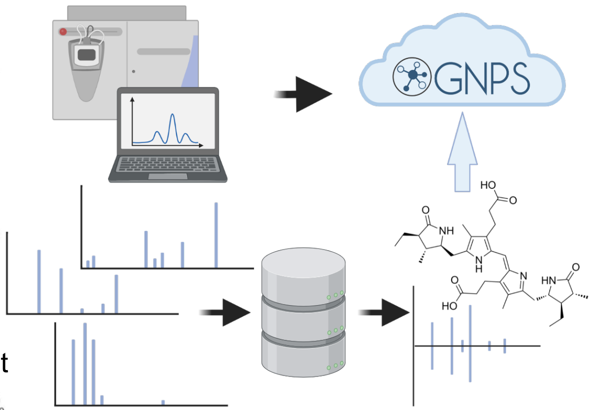
PART II: Statistical analysis

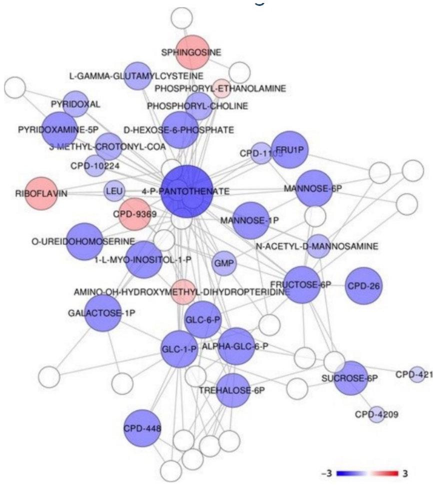
PART III: Annotation

PART IV: Pathway Analysis





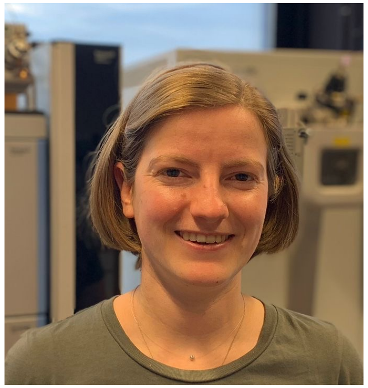




DATA ANALYSIS DEVELOPMENTS: INTEGRATION

- Supervised and unsupervised multi-omics analysis (DIABLO and MOFA)
- Spearman-based metabolome driver analysis: clinical data, microbial OTUs, diet, drugs, stress markers, etc.)
- Metabolite abundance predictions (o.a. gradient-boosted decision tree) of highly correlated drivers

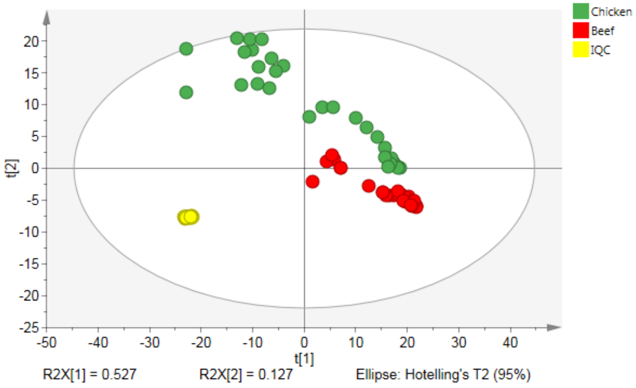
Pablo Vangeenderhuysen



Dr. Marilyn De Graeve

NUTRIMETABOLOMICS AND LIPIDOMICS TO UNRAVEL GUT MICROBIOME-DIET AXIS

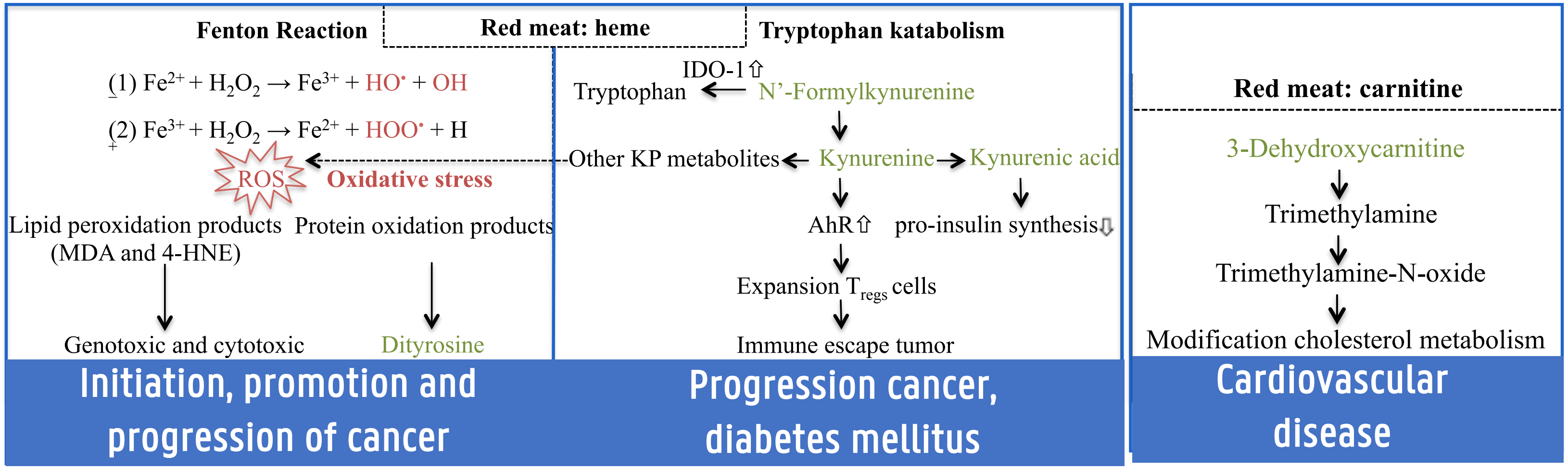
NUTRIMETABOLOMICS: RED VS. WHITE MEAT DIGESTION



Discovery of **5 discriminating metabolites** with potential involvement in red meat related diseases

- 3-dehydroxycarnitine
- dityrosine
- kynurenine
- N'-formylkynurenine
- kynurenic acid

Dr. Caroline Rombouts



Fenton Reaction

Red meat: heme

(1) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH$

(2) $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H$

ROS

Oxidative stress

Lipid peroxidation products (MDA and 4-HNE)

Protein oxidation products

Genotoxic and cytotoxic

Dityrosine

Initiation, promotion and progression of cancer

Tryptophan katabolism

IDO-1 ⇧

Tryptophan ← N'-Formylkynurenine

Other KP metabolites ← Kynurenine → Kynurenic acid

AhR ⇧

pro-insulin synthesis ⇩

Expansion T_{regs} cells

Immune escape tumor

Progression cancer, diabetes mellitus

Red meat: carnitine

3-Dehydroxycarnitine

Trimethylamine

Trimethylamine-N-oxide

Modification cholesterol metabolism

Cardiovascular disease

Rombouts et al., 2017 (Scientific Reports)

NUTRIMETABOLOMICS IN PIGS

WITHIN 2 CONTRASTING DIETS

Pig feeding trial: 2×2 factorial design

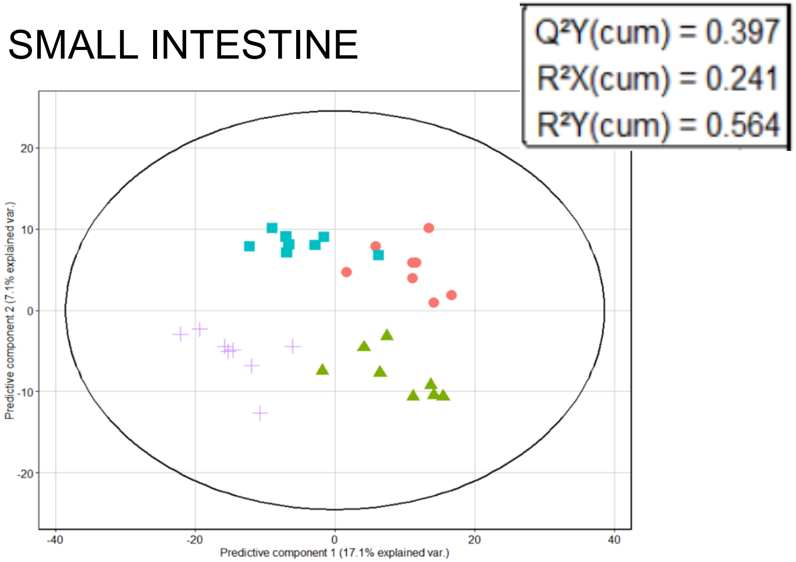
	red & processed	chicken
recommended		
Western		

● Recommended chicken

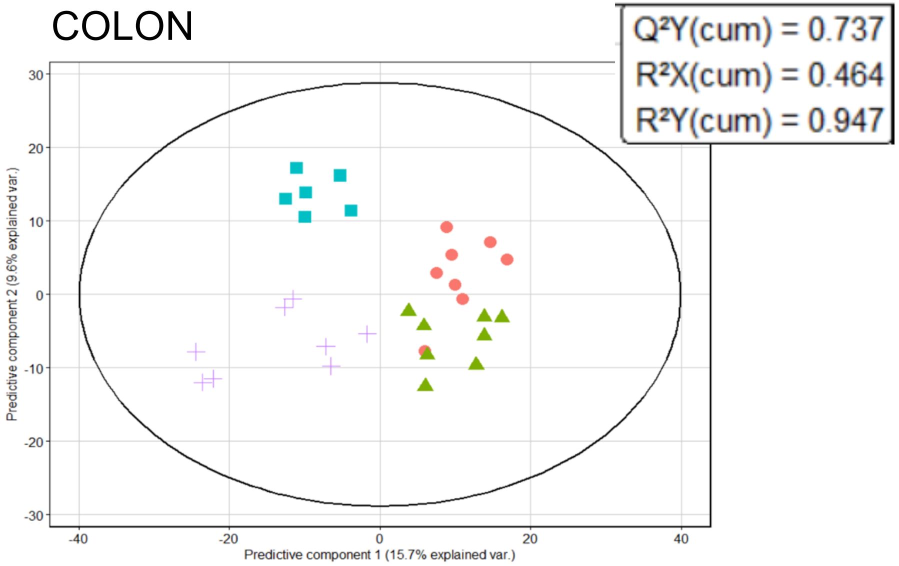
▲ Recommended red

■ Western chicken

+ Western red


SMALL INTESTINE

$Q^2Y(cum) = 0.397$
$R^2X(cum) = 0.241$
$R^2Y(cum) = 0.564$


COLON

$Q^2Y(cum) = 0.737$
$R^2X(cum) = 0.464$
$R^2Y(cum) = 0.947$

NUTRIMETABOLOMICS IN PIGS
WITHIN 2 CONTRASTING DIETS

Dr. Sophie Goethals

Number of differentiating metabolites (VIP >2; p-value < 0.05)

	Small intestine (1851)	Colon (1990)
Meat (chicken *vs.* red & processed)	**64** chicken: 26, red: 38	**99** chicken: 82, red: 17
Background diet (prudent *vs.* Western)	**299** prudent: 66, western: 233	**321** prudent: 128, western: 193

Interesting metabolites

short-chain acylcarnitines
carnitine, carnosine
3-dehydroxycarnitine

anserine
1- or 3-methylhistidine

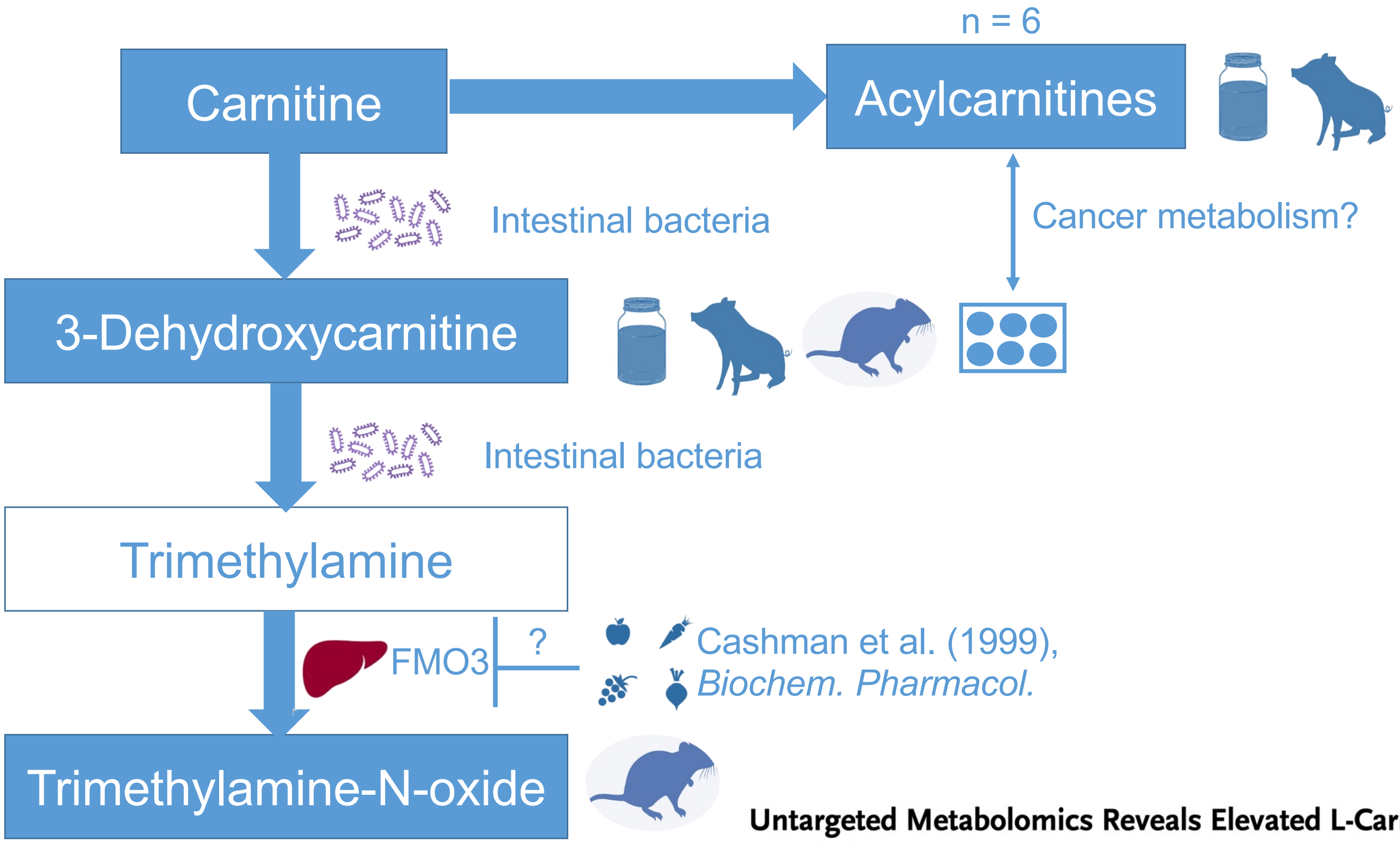
medium- and long-chain acylcarnitines
glycidyl esters
monoacylglycerols

Untargeted Metabolomics to Reveal Red versus White Meat–Associated Gut Metabolites in a Prudent and Western Dietary Context

*Sophie Goethals, Caroline Rombouts, Lieselot Y. Hemeryck, Lieven Van Meulebroek, Thomas Van Hecke, Els Vossen, John Van Camp, Stefaan De Smet, and Lynn Vanhaecke**

Mol. Nutr. Food Res. **2020**, 2000070

MICROBIAL-CARNITINE DERIVATES HIGHER IN TISSUE UPON RED MEAT DIGESTION



n = 6

Carnitine

Acylcarnitines

Intestinal bacteria

Cancer metabolism?

3-Dehydroxycarnitine

Intestinal bacteria

Trimethylamine

FMO3

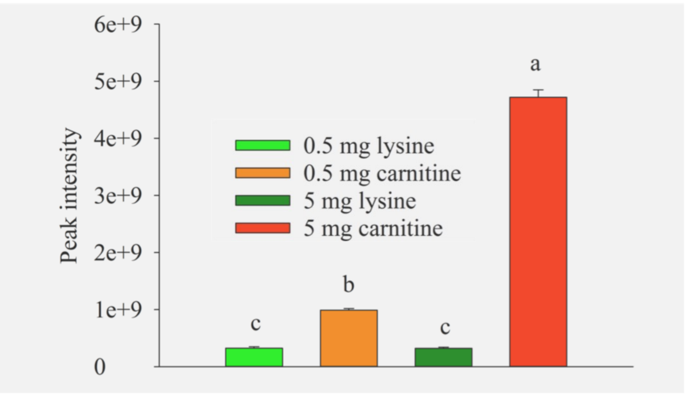
?

Cashman et al. (1999), *Biochem. Pharmacol.*

Trimethylamine-N-oxide

↑ in urine of women with CRC, Bae et al. (2014), *Cancer Res.*

In vitro colonic formation of 3-dehydroxycarnitine



Untargeted Metabolomics Reveals Elevated L-Carnitine Metabolism in Pig and Rat Colon Tissue Following Red Versus White Meat Intake

*Caroline Rombouts, Lieven Van Meulebroek, Margot De Spiegeleer, Sophie Goethals, Thomas Van Hecke, Stefaan De Smet, Winnok H. De Vos, and Lynn Vanhaecke**

Mol. Nutr. Food Res. **2021**, *65*, 2000463

CLINICAL METABOLOMICS: TYPE 2 DIABETES

DIABETES TYPE 2

Help mee om deze aandoening beter op te sporen, op te volgen en te behandelen.

Neem deel aan onze studie!

Waarom we uw hulp nodig hebben?

Diabetes type 2 is één van de meest uitdagende en voorkomende gezondheidsproblemen van de 21ste eeuw. In België heeft maar liefst 1 op 12 volwassenen deze ziekte! Bovendien hebben huidige geneesmiddelen meestal slechts een tijdelijke werking en worden vaak bijwerkingen vastgesteld. Diepgaand onderzoek is daarom cruciaal om de processen achter diabetes type 2 beter te begrijpen zodat men de ziekte beter kan opsporen, behandelen en opvolgen.

Hoe kan u ons helpen?

Binnen deze studie zal u gevraagd worden een stoelgangstaal ter beschikking te stellen. De aanwezige stoffen kunnen ons immers heel wat vertellen, onder meer over uw gezondheid. Daarnaast zal ook gevraag worden om een korte vragenlijst in te vullen.

Wie kan ons helpen?

We zijn op zoek naar zowel patiënten met diabetes type 2 als gezonde controlepersonen. We richten ons op zowel mannen als vrouwen binnen de leeftijdsgroep van 35 tot 65 jaar.

Wie kan u contacteren?

Prof. dr. Bruno Lapauw – arts Universitair Ziekenhuis Gent
Bruno.Lapauw@UZGent.be - 09 332 21 37

Dr. Lieven Van Meulebroek – onderzoeker Universiteit Gent
Lieven.Vanmeulenbroek@UGent.be - 09 264 73 16

De Diabolo-studie wordt uitgevoerd via een samenwerking tussen het Universitair Ziekenhuis Gent en de Universiteit Gent, met steun van de Vlaamse overheid (Vlaio).

Dr. Lieven Van Meulebroek

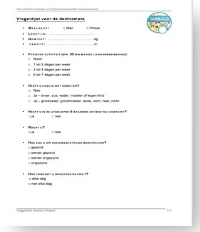
Type 2 diabetes (n = 41)

- Men and women
- BMI < 35
- 35 to 65 years
- HbA1c > 6%
- UZGent (prof. B. Lapauw)

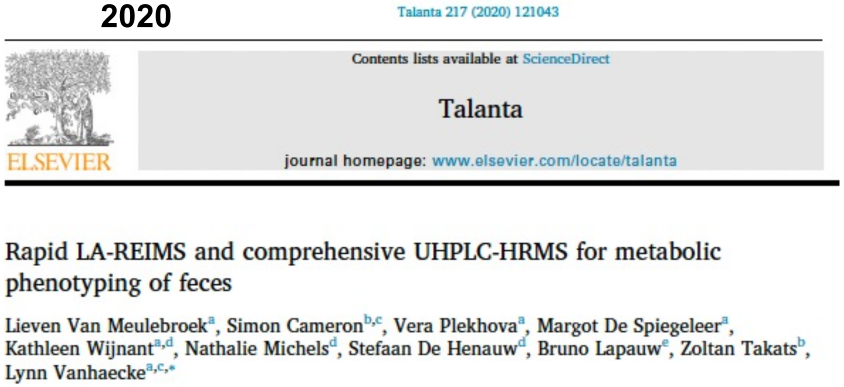
Normal glycaemic state (n=42)

- Men and women
- BMI < 35
- 35 to 65 years
- HbA1c < 5.5%
- Family

- 1 stool sample
- 1 blood sample
- 1 questionnaire



2020



Talanta 217 (2020) 121043

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid LA-REIMS and comprehensive UHPLC-HRMS for metabolic phenotyping of feces

Lieven Van Meulebroek[a], Simon Cameron[b,c], Vera Plekhova[a], Margot De Spiegeleer[a], Kathleen Wijnant[a,d], Nathalie Michels[d], Stefaan De Henauw[d], Bruno Lapauw[e], Zoltan Takats[b], Lynn Vanhaecke[a,c,*]

TYPE 2 DIABETES STOOL METABOLOMICS STUDY

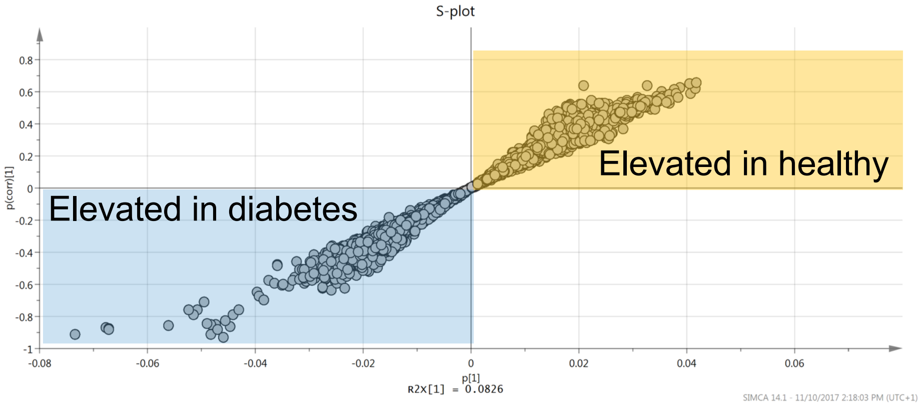
Lipidomics and polar metabolomics

- Use OPLS-DA model to define components with discriminating potential
- Start search for candidate biomarkers

Selection strategy

- VIP-score (> 1)
- S-plot (correlation 0.5, covariance 0.02)
- JK-confidence intervals (not across zero)

173 candidate markers



T2D STOOL BIOMARKER IDENTIFICATION

Polar metabolomics

Total of 70 markers

- 27 elevated in diabetes type 2
- 43 elevated in healthy control

Initial focus on diabetes markers

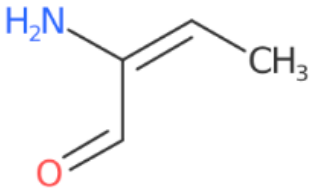
- Non-metformin markers (n = 2)
- Frequent link with metformin (n = 24)

> Three are directly linked to **metformin**
> Six are in source **fragments of metformin**
> Thirteen are **derived from metformin**

T2D STOOL BIOMARKER IDENTIFICATION

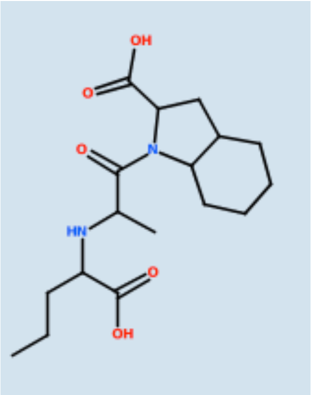
Polar metabolomics

Non-metformin markers

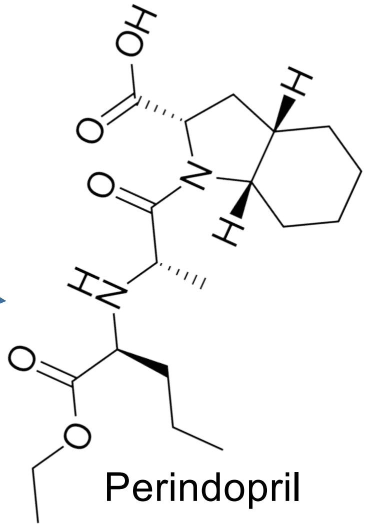


2-amino-2-butenal

Possible role in inflammation



Perindoprilat



Perindopril

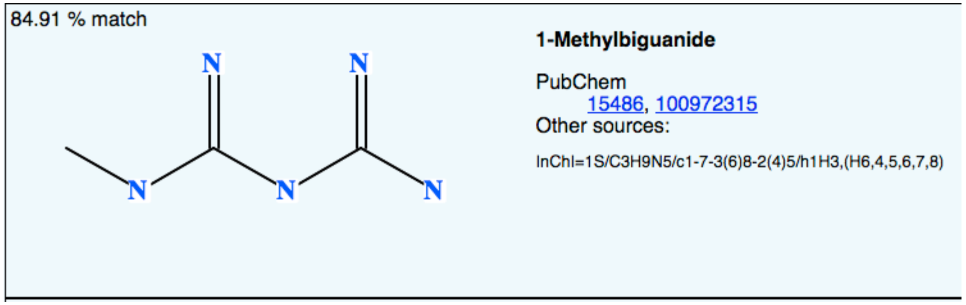
Lowers blood pressure and improves heart functioning

T2D STOOL BIOMARKER IDENTIFICATION

Polar metabolomics

Metformin-linked markers

- Metformin oxidation products

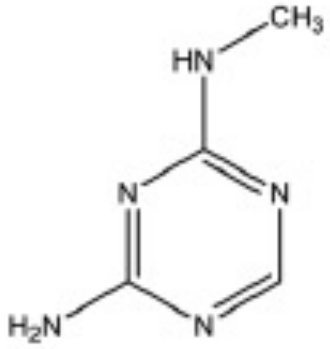


84.91 % match

1-Methylbiguanide

PubChem
15486, 100972315
Other sources:

InChI=1S/C3H9N5/c1-7-3(6)8-2(4)5/h1H3,(H6,4,5,6,7,8)



2,4-AMT

(2-amino-4-methylamino-1,3,5-triazine)

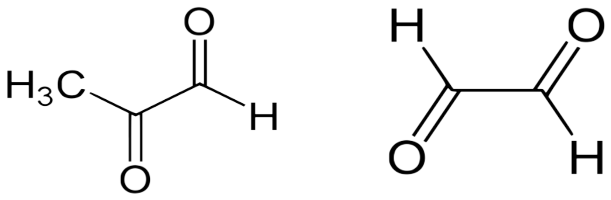
- Collin et al. 2004; $OH^{\bullet}$ -induced oxidation of metformin in aqueous solution.
- Research in context of **antioxidant properties** of metformin, other beneficial effects involved in the prevention of diabetic complications

T2D STOOL BIOMARKER IDENTIFICATION

Polar metabolomics

Metformin-linked markers

- Metformin reaction products with **dicarbonyls**
- Dicarbonyl compounds
    - Methylglyoxal and glyoxal



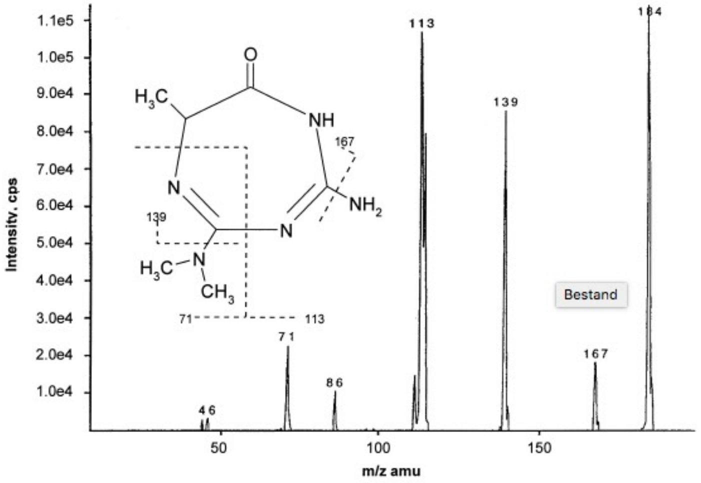
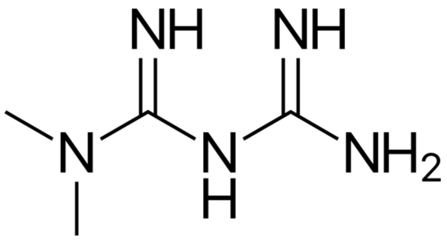


FIG. 2. Mass fragmentation spectrum of synthetic 2-amino-4-(dimethylamino)-7-methyl-5,7-dihydro-6*H*-[1,3,5]triazepin-6-one. 2-Amino-4-(dimethylamino)-7-methyl-5,7-dihydro-6*H*-[1,3,5]triazepin-6-one (triazepinone) was chemically synthesised and analysed by mass spectrometry as described in Materials and Methods.

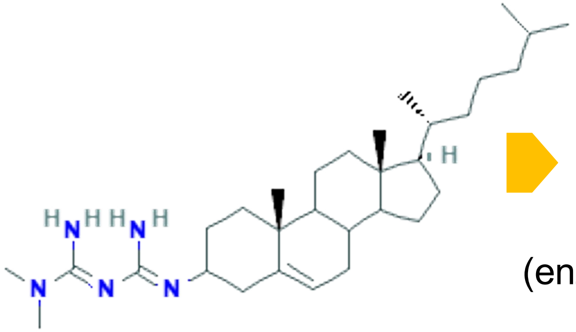
    - Reactive agents involved in formation of **advanced glycation end products (AGEs)**
- AGEs are associated with diabetic vascular complications (Ruggiero-Lopez et al. 1999)

T2D STOOL BIOMARKER IDENTIFICATION

Lipidomics



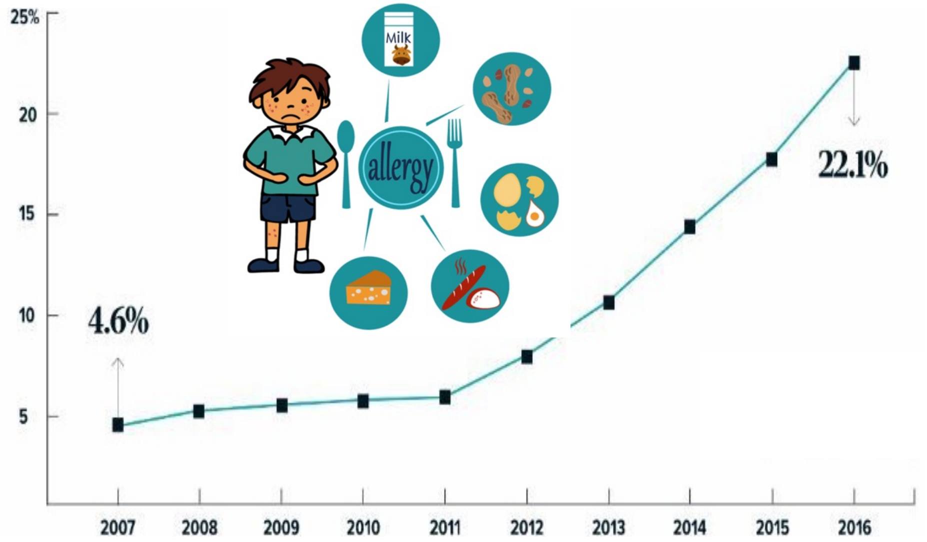
- m/z 498.4529 Da, RT 7.62 min
- C31H55N5
- 2-[N'-[(10R,13R,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta [a]phenanthren-3-yl] carbamimidoyl]-**1,1-dimethylguanidine**

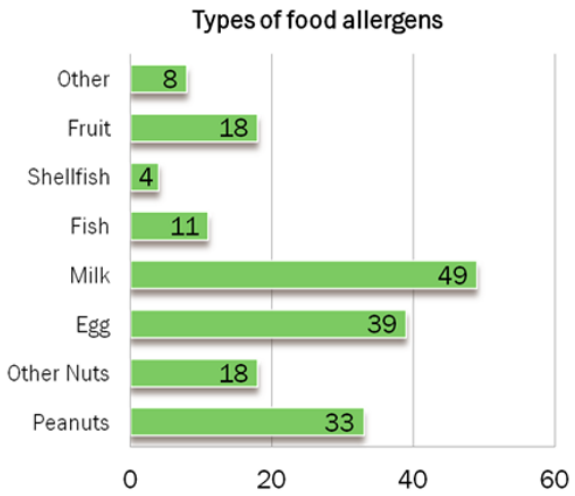


Cholesterol-lowering effects of metformin have been described

(enzymatic impact AMPK, genes and lower formation of LDL)

CLINICAL METABOMOLOMICS: COW'S MILK ALLERGY





Diagnosis

Oral food challenge
→ dangerous

Skin prick test
→ 25% false +

Serum IgE
→ 25% false +

Treatment

Elimination diet

Anaphylaxis
treatment

Ellen De Paepe

CLINICAL METABOMOLOMICS: FOOD ALLERGY

A Systematic Review of Metabolic Alterations Underlying IgE-Mediated Food Allergy in Children

Ellen De Paepe, Lynn Van Gijseghem, Margot De Spiegeleer, Eric Cox, Lynn Vanhaecke

First published: 14 October 2021 | https://doi.org/10.1002/mnfr.202100536

Microbial metabolism

SCFAs and bile acids

→ Altered microbial and host metabolic activity

Lipid metabolism

Sfingolipids

→ Hyperresponsive mast cell state

Lipid allergy mediators

→ PAF synthesis during anaphylaxis

Carbohydrate and energy metabolism

- Shift to **aerobic glycolysis**
- **Ketogenesis** during anaphylaxis

Amino acid metabolism

Tryptophan pathway

⇩Decreased IDO-1 activity

→ Th2-skewed immune environment

Nucleotide metabolism

Purine pathway

⇧Uric acid

→ Underlying inflammation

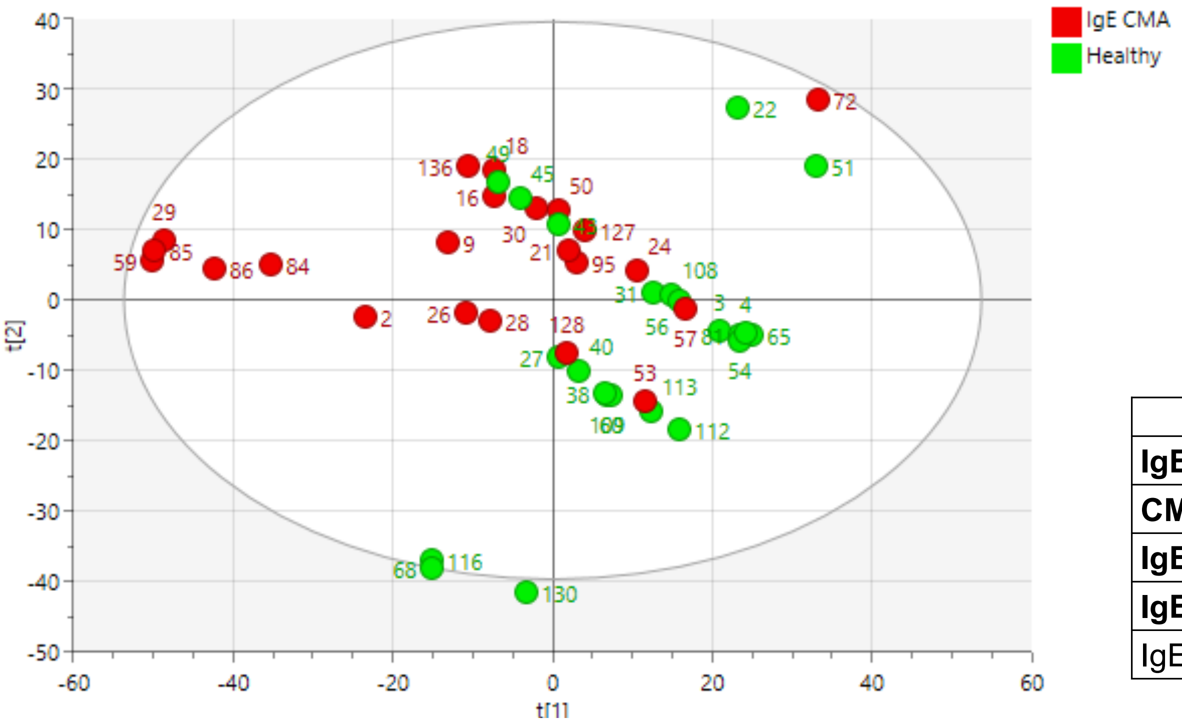
DISCRIMINATE HEALTHY AND IgE MEDIATED CMA

20 healthy

20 IgE mediated CMA

19 non-IgE mediated CMA

11 other IgE mediated FAs



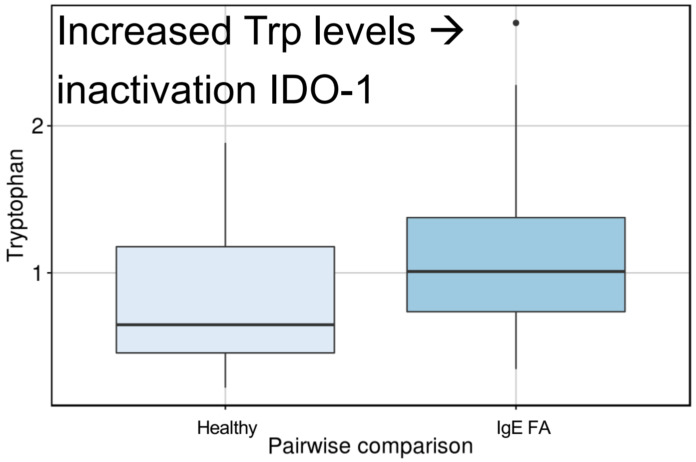
Polar metabolomics

feces

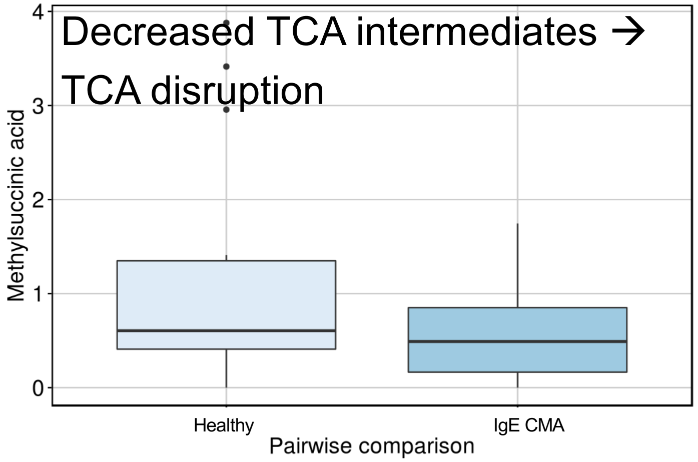
	R^{2}Y	Q^2	*p*-value
IgE CMA – Healthy	0.866	0.519	5.70e-05
CMA – Healthy	0.979	0.467	0.00029
IgE CMA – IgE other	0.996	0.626	0.0017
IgE CMA – non-IgE CMA	0.986	0.681	1.35e-05
IgE FA – Healthy	0.992	0.297	0.21

DISCRIMINATE HEALTHY AND IgE MEDIATED CMA

Tryptophan metabolism


Increased Trp levels → inactivation IDO-1

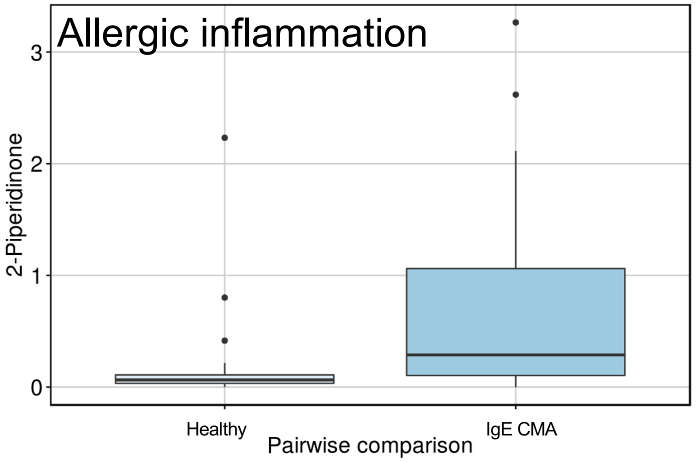
Energy metabolism


Decreased TCA intermediates → TCA disruption

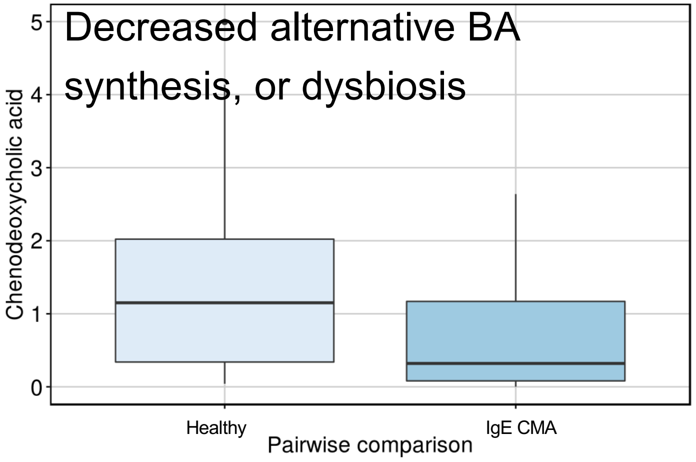
Other alterations

- Nucleotide metabolism

Inflammation

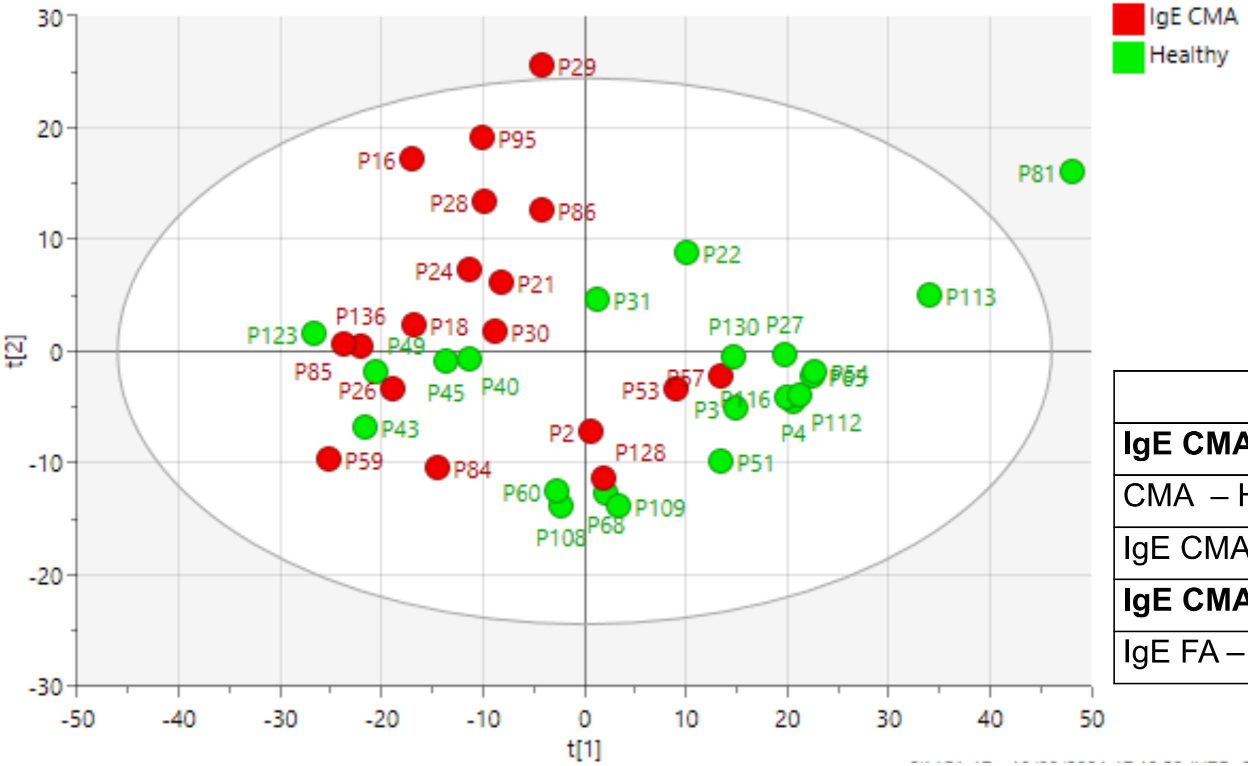

Allergic inflammation

Bile acids


Decreased alternative BA synthesis, or dysbiosis

DISCRIMINATE HEALTHY AND IgE MEDIATED CMA

20 healthy
20 IgE mediated CMA
19 non-IgE mediated CMA
11 other IgE mediated FAs

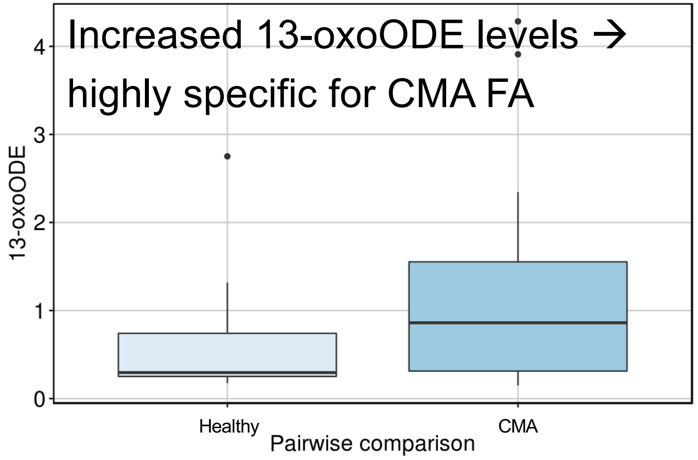


Polar metabolomics **urine**

	R^2Y	Q^2	*p*-value
IgE CMA – Healthy	0.981	0.462	0.027
CMA – Healthy	0.919	-0.0649	1
IgE CMA – IgE other	0.991	0.0285	0.999
IgE CMA – non-IgE CMA	0.983	0.539	0.011
IgE FA – Healthy	0.984	0.46	0.004

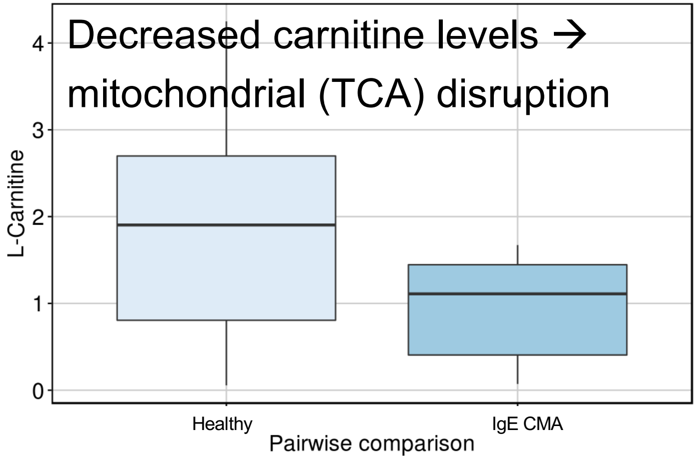
DISCRIMINATE HEALTHY AND IgE MEDIATED CMA

Inflammation



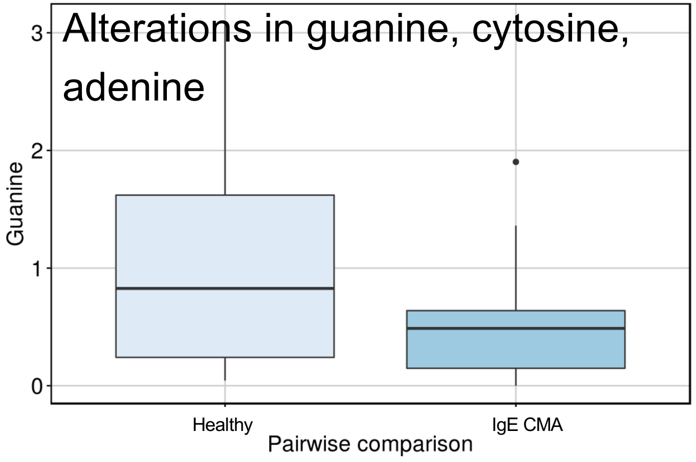
Increased 13-oxoODE levels → highly specific for CMA FA

Energy metabolism



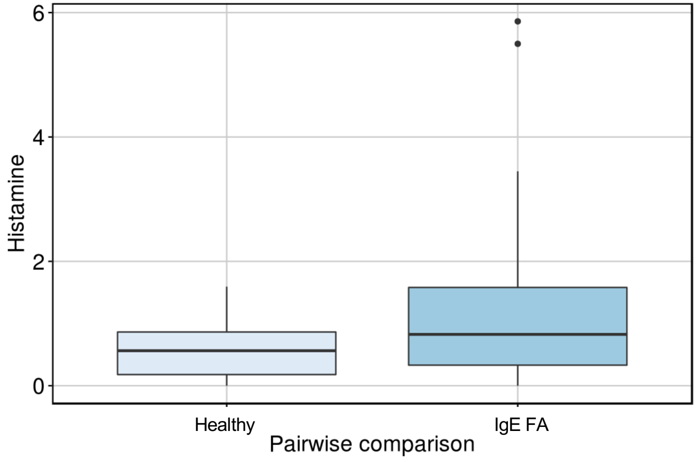
Decreased carnitine levels → mitochondrial (TCA) disruption

Purine metabolism



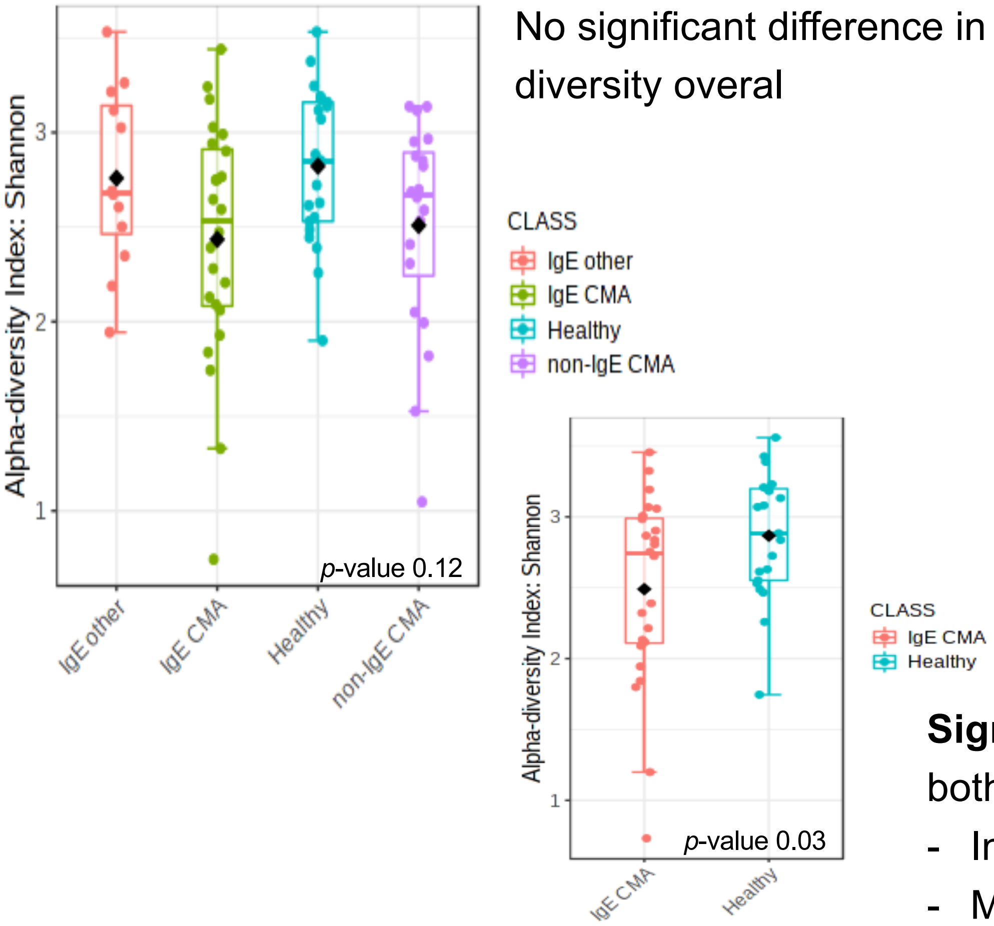
Alterations in guanine, cytosine, adenine

Histidine metabolism



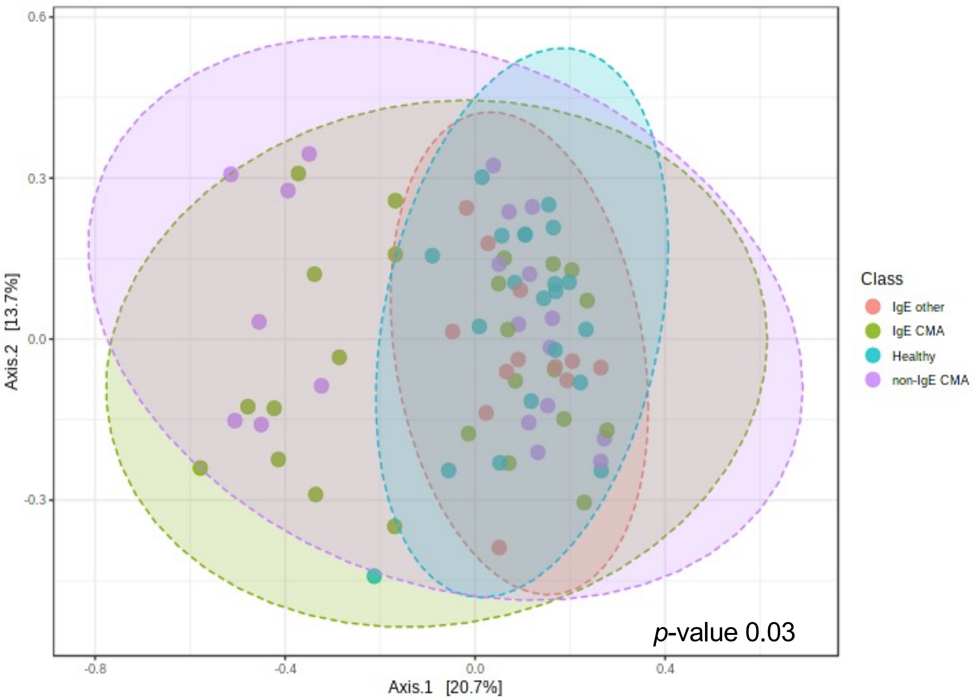
DISCRIMINATE HEALTHY AND IgE MEDIATED CMA

Alpha diversity



No significant difference in diversity overal

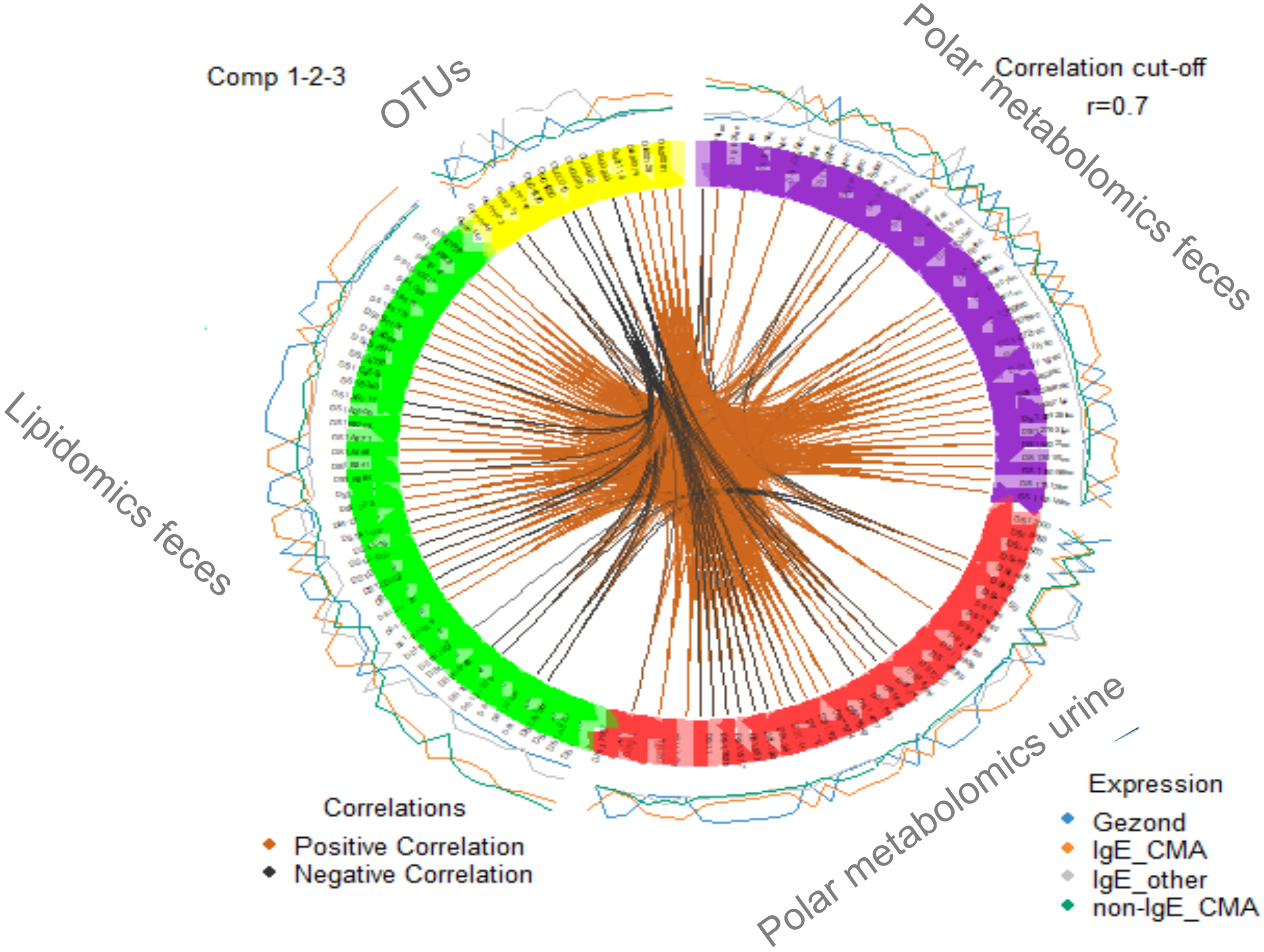
Beta diversity



Significant difference according to CMP intake for both **alpha and beta diversity:**

- Increased microbial richness if CMP consumption
- More similar/stabile core microbiome if CMP consumption

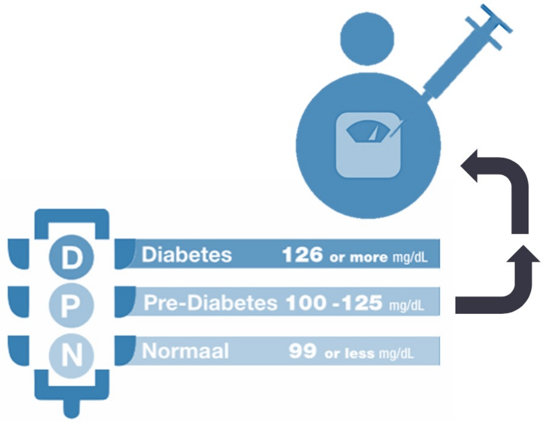
INTEGRATED METABOLOMICS AND MICROBIOMICS



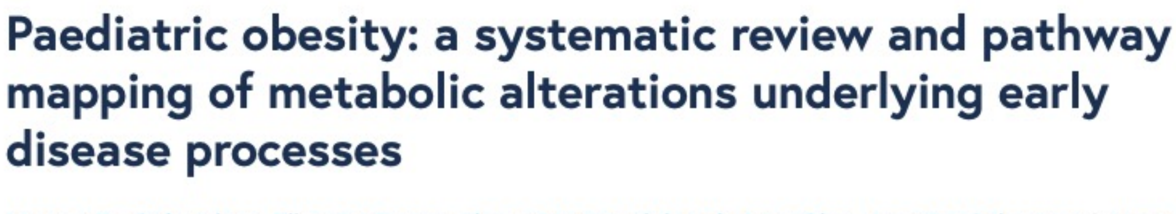
Pablo Vangeenderhuysen

CLINICAL METABOLOMICS: PEDIATRIC OBESITY

1. **Prediction and prevention of metabolic disorders**
2. **Unraveling the main drivers towards 3P medicine**



CLINICAL METABOLOMICS: PEDIATRIC OBESITY



Paediatric obesity: a systematic review and pathway mapping of metabolic alterations underlying early disease processes

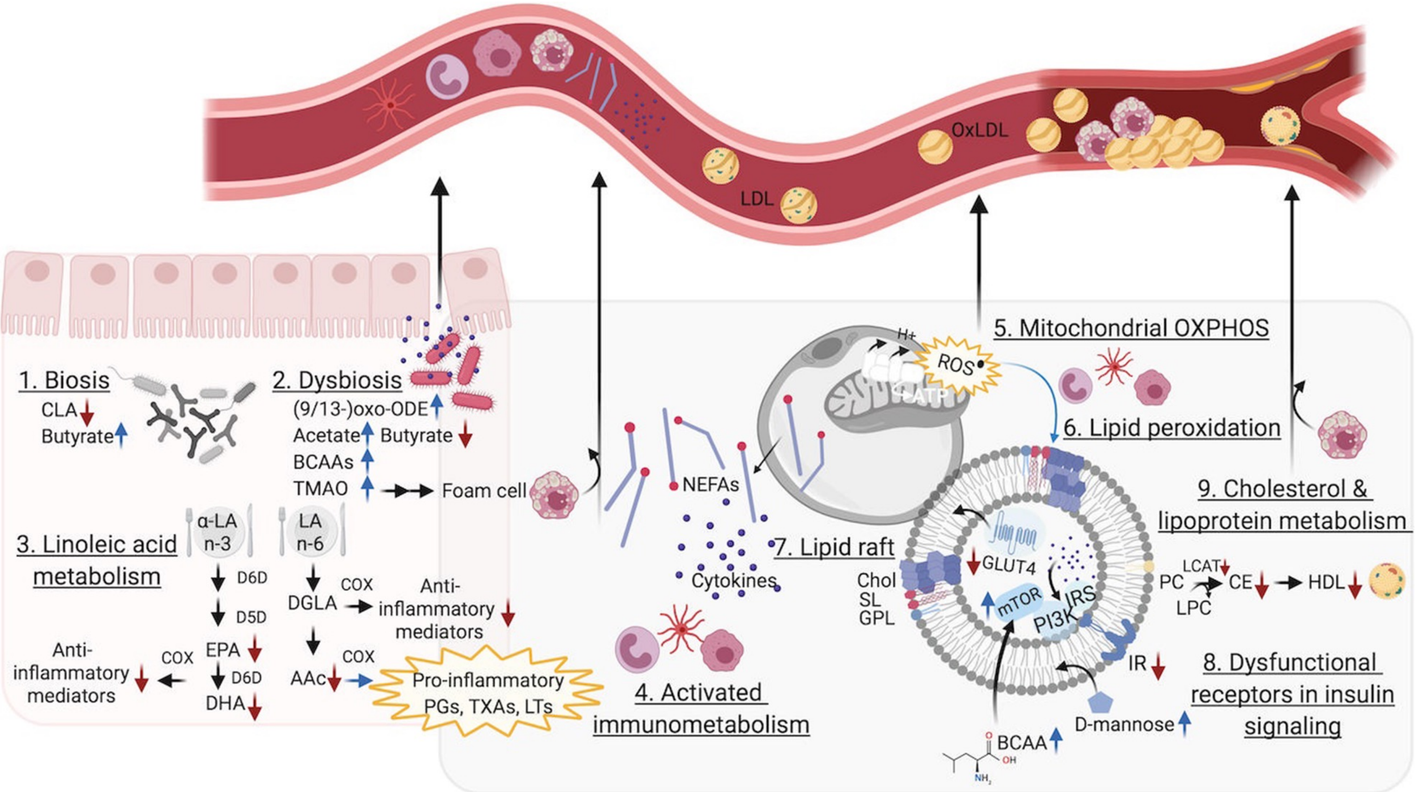
Margot De Spiegeleer, Ellen De Paepe, Lieven Van Meulebroek, Inge Gies, Jean De Schepper & Lynn Vanhaecke ✉

Molecular Medicine **27**, Article number: 145 (2021) | Cit

1320 Accesses | **11** Altmetric | Metrics

Margot De Spiegeleer

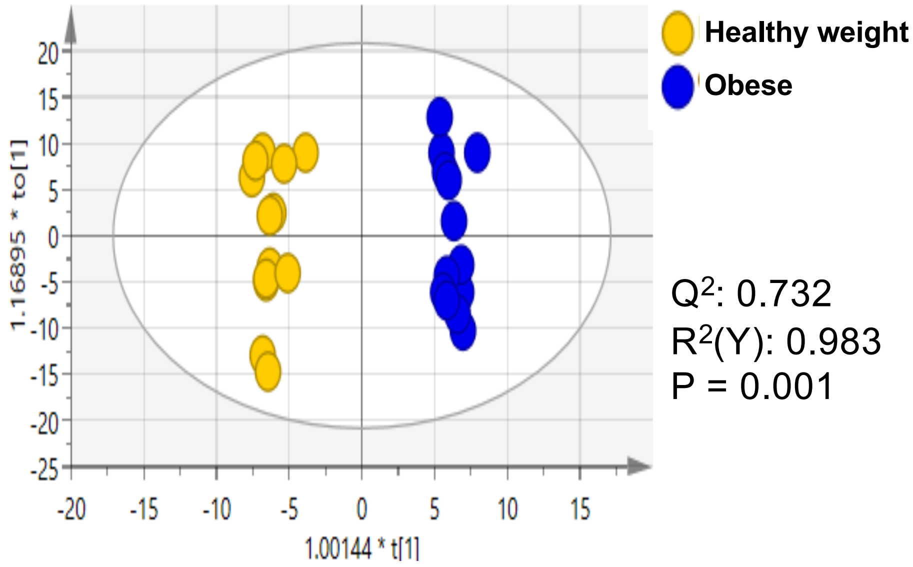
Overview of altered **lipid metabolism** and visualization of its complex interplay resulting in a **sustained oxidative environment and low-grade inflammation**.



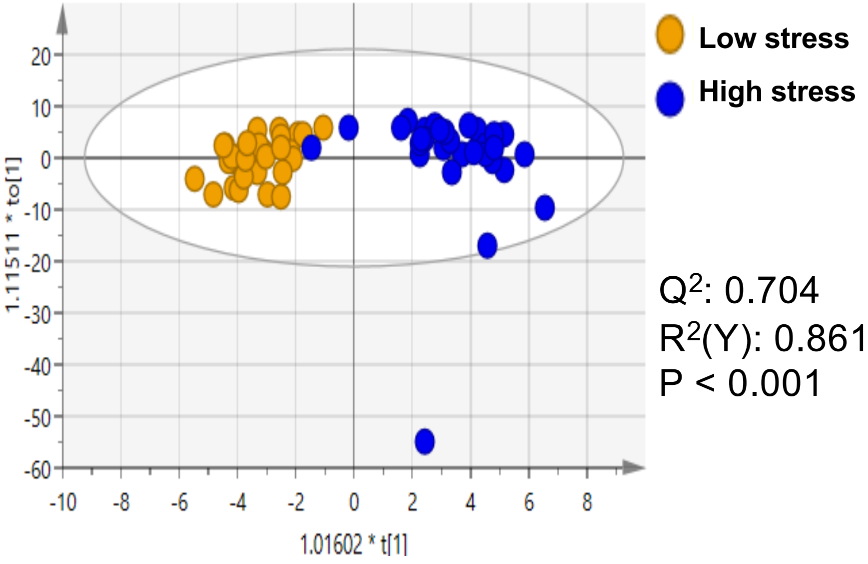
CLINICAL METABOLOMICS: OBESITY IN CHILDREN

Deeply phenotyped **OPERA** cohort (Prof. S. De Henauw)
137 children, 6-18y
70% normal weight (IOTF ≤ 0)
29% IOTF ≥ 1 (overweight or obese, including 9% obese: IOTF 2 or 3)

Kathleen Wijnant



Q^2: 0.732
$R^2(Y)$: 0.983
P = 0.001



Q^2: 0.704
$R^2(Y)$: 0.861
P < 0.001

Feature class	**Feature subgroups**
Microbiome data	OTUs
Antropometric data	BMI z-score
	IOTF
	Waist (cm)
	Waist-to-height
	Fat percentage (%)
Psychological data	Perceived Stress Scale (PSS)
	Child Depression Index 2 (CDI-2)
	Emotional Eating Scale (EE)
	Relative Cortisol Reactivity Score
	Resting heart rate variability RMSSDms (baseline timepoint S6) (ms)
	Hair cortisol
	Positive and Negative Affect Schedule (PANAS) Negative Effect score
Biological data	Age (yrs)
	Sex
	Puberty
Lifestyle	Screentime (daily)
	Physical activity (school transport)
	Physical activity (sports)
	Physical activity (hrs sports/week)
	Smoking
	Chronic Sleep Reduction Questionnaire (CSRQ)
Clinical data	Cardiometabolic markers
	Triglycerides (mg/dl)
	LDL (mg/dl)
	HDL (mg/dl)
	fasting insulin (mU/L)
	fasting glucose (mg/dL)
	HbA1c (mmol/mol)
	MeanHR (bpm)
	Inflammation markers
	IL6 (pg/ml)
	IL8 (pg/ml)
	IL10 (pg/ml)
	TNF-α (pg/ml)
	IFN-γ (pg/ml)
	CRP (mg/l)
	Appetite markers
	Leptin (ng/ml)
	Adiponectin (µg/ml)
	Ghrelin (not active) (pg/ml)
	GLP1 (pg/ml)
	Pancreatic polypeptide (PP) (pg/ml)
	PYY (pg/ml)
	Endocrine markers
	TSH (mU/L)
	FT4 (pmol/L)

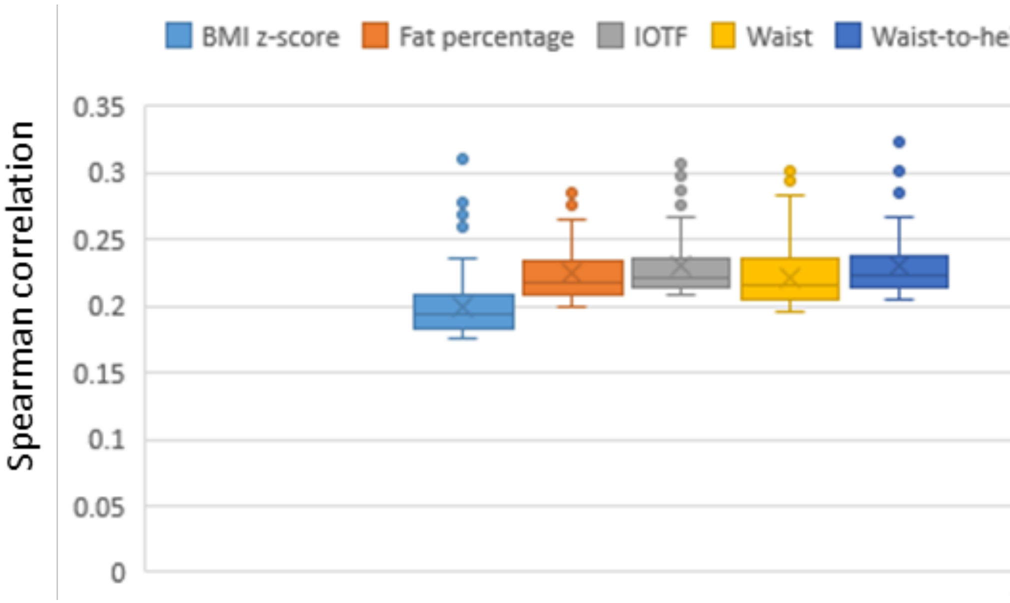
Diet (week intake frequencies)

- High fat snacks
- High fat food
- High sugar snacks
- High sugar food
- Fruit and vegetables
- Whole grain products
- Dairy products
- Animal products (meat, fish, eggs)
- Coffee
- Legumes
- Cooked vegetables
- Raw vegetables
- Milk
- Yoghurt
- Fish
- Red Meat
- White Meat
- Eggs
- Meat alternatives (soy,…)
- Cheese
- Butter
- Olive oil
- White bread
- Dark bread
- White pasta
- Whole grain pasta
- Grains / Cereals
- Nuts and seeds
- Dried fruits

Drug intake active components

- Isotretinoïne
- VitaminD
- Methylphenidate_hydrochloride
- Salbutamol
- Setraline
- Desloratadine
- Cetirizinedihydrochloride
- Insulin
- Melatonin
- Fumaraatdihydraat
- Budesonide
- Levocetirizinedihydrochloride
- Fluticasonpropionaat
- Levonorgestrel
- Ethinylestradiol
- Formoterol
- Rupatadine
- Magnesiumcitraat
- Valsartan
- Paracetamol
- Metformin
- Oxymetazoline_hydrochloride
- Prednisolonacetaat
- Framycetinesulfaat
- Nafazolinenitraat
- Clonidinehydrochloride

CLINICAL METABOLOMICS: OVERWEIGHT-OBESITY IN CHILDREN

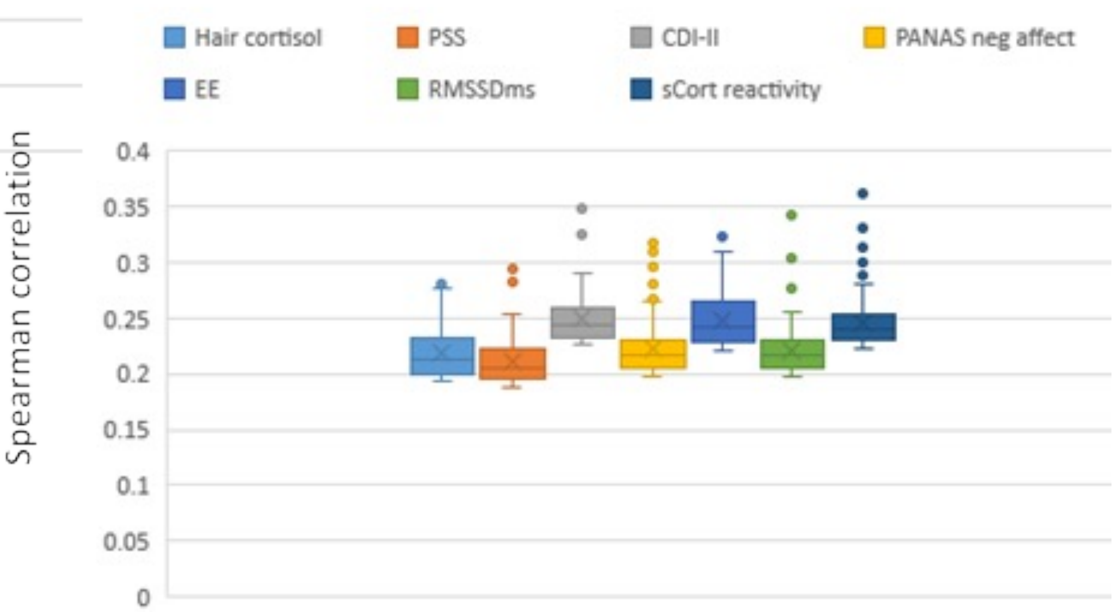


Top 100 significant metabolite – metadata correlations

Left: Antropometric

Below: Psychological markers

Metabolite annotation on-going!



METABEASE AND FAME COHORTS

Recruitment phase 1 finalized 03/2022

n = 240
Children TS 0 (6-11y)

Stool, urine and blood samples for metabolomics, microbiome and clinical analyses.

→ 2 follow-up points (Y0, Y1, Y2)

Margot De Spiegeleer

Recruitment started 03/2022

n = 600
Adolescents (12-16y)

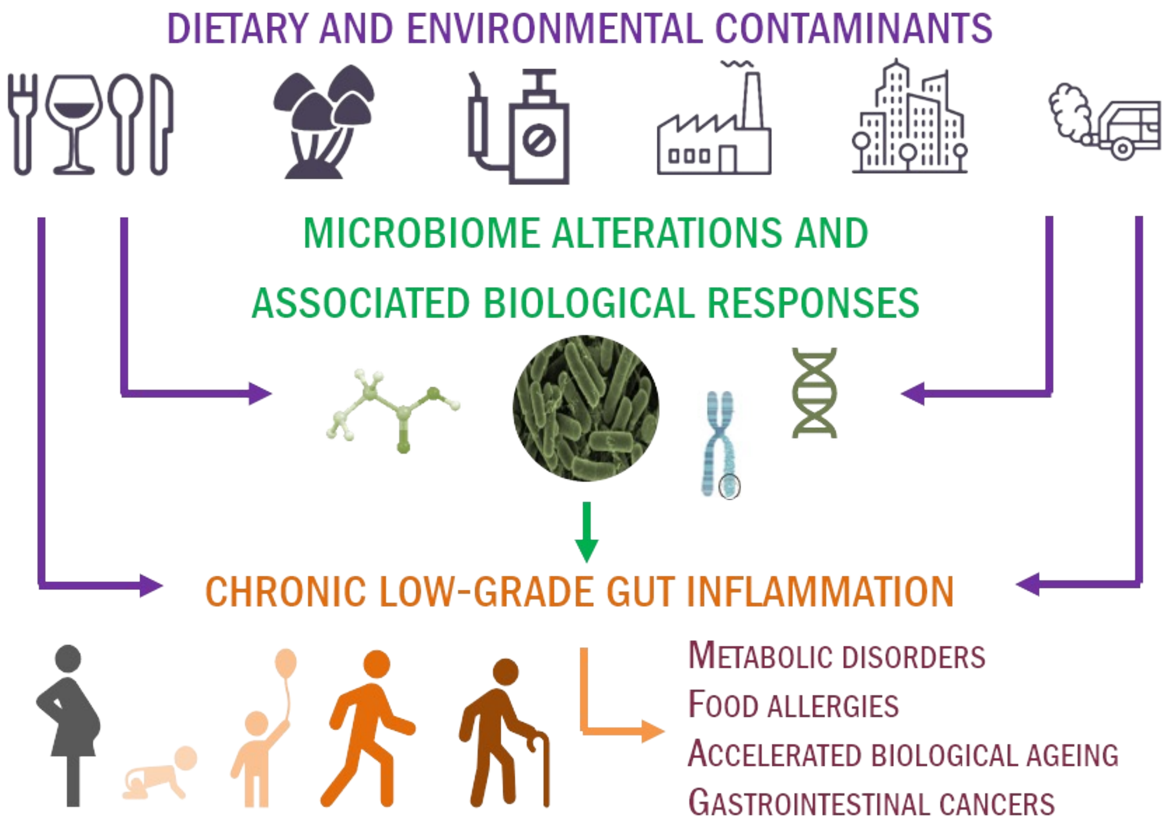
Stool, saliva and in patients blood samples for metabolomics, microbiome and clinical analyses.

Questionnaires on dietary intake and stress.

Jasmien Van Arnhem

FLEXIGUT - THE FLEMISH EXPOSOME PROJECT

**TOWARDS A COMPREHENSIVE UNDERSTANDING OF THE
LIFE-COURSE IMPACT OF DIETARY AND ENVIRONMENTAL EXPOSURE
ON CHRONIC LOW-GRADE GUT INFLAMMATION**



DIETARY AND ENVIRONMENTAL CONTAMINANTS

MICROBIOME ALTERATIONS AND
ASSOCIATED BIOLOGICAL RESPONSES

CHRONIC LOW-GRADE GUT INFLAMMATION

- METABOLIC DISORDERS
- FOOD ALLERGIES
- ACCELERATED BIOLOGICAL AGEING
- GASTROINTESTINAL CANCERS

Prof. S. De Saeger

UNIVERSITEIT
GENT

Prof. J. Raes

KU LEUVEN

VIB
science meets life

Prof. A. Covaci

Universiteit
Antwerpen

Prof. T. Nawroth

UHASSELT

RAPID HRMS-BASED FINGERPRINTING

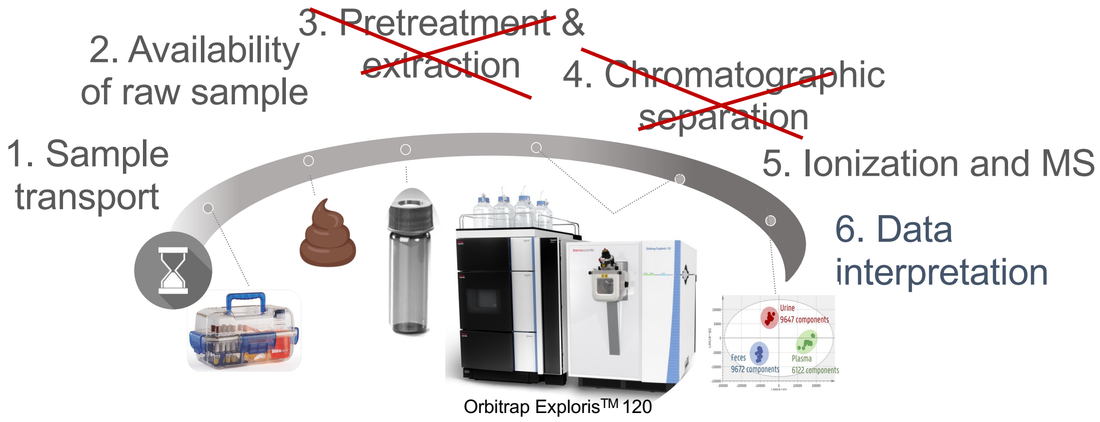
IN SITU OR **POINT OF CARE** METABOLOMICS: AIMS

< 50/day
> 200 EUR/sample

Conventional metabolomics workflow

> 500/day
< 50 EUR/sample

Point-Of-Care metabolomics workflow



1. Sample transport

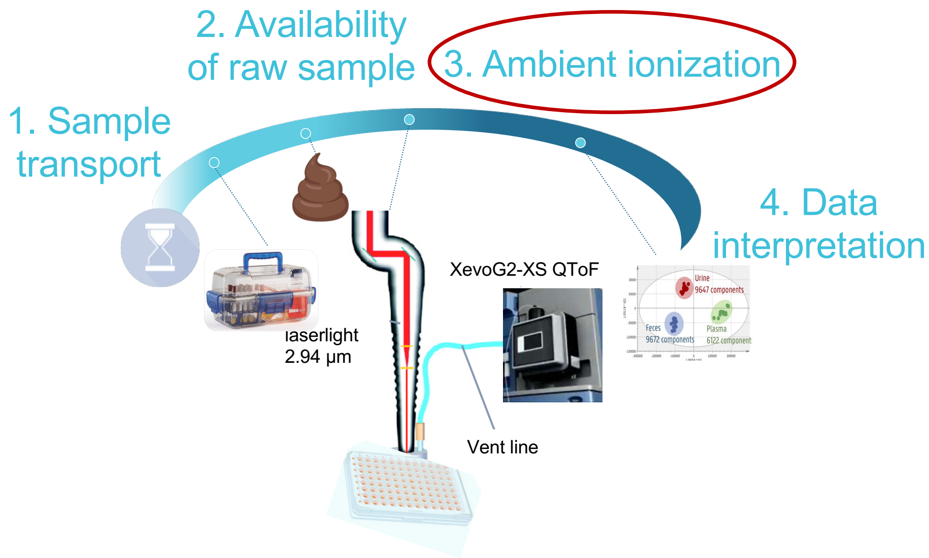
2. Availability of raw sample

~~3. Pretreatment & extraction~~

~~4. Chromatographic separation~~

5. Ionization and MS

6. Data interpretation



1. Sample transport

2. Availability of raw sample

3. Ambient ionization

4. Data interpretation

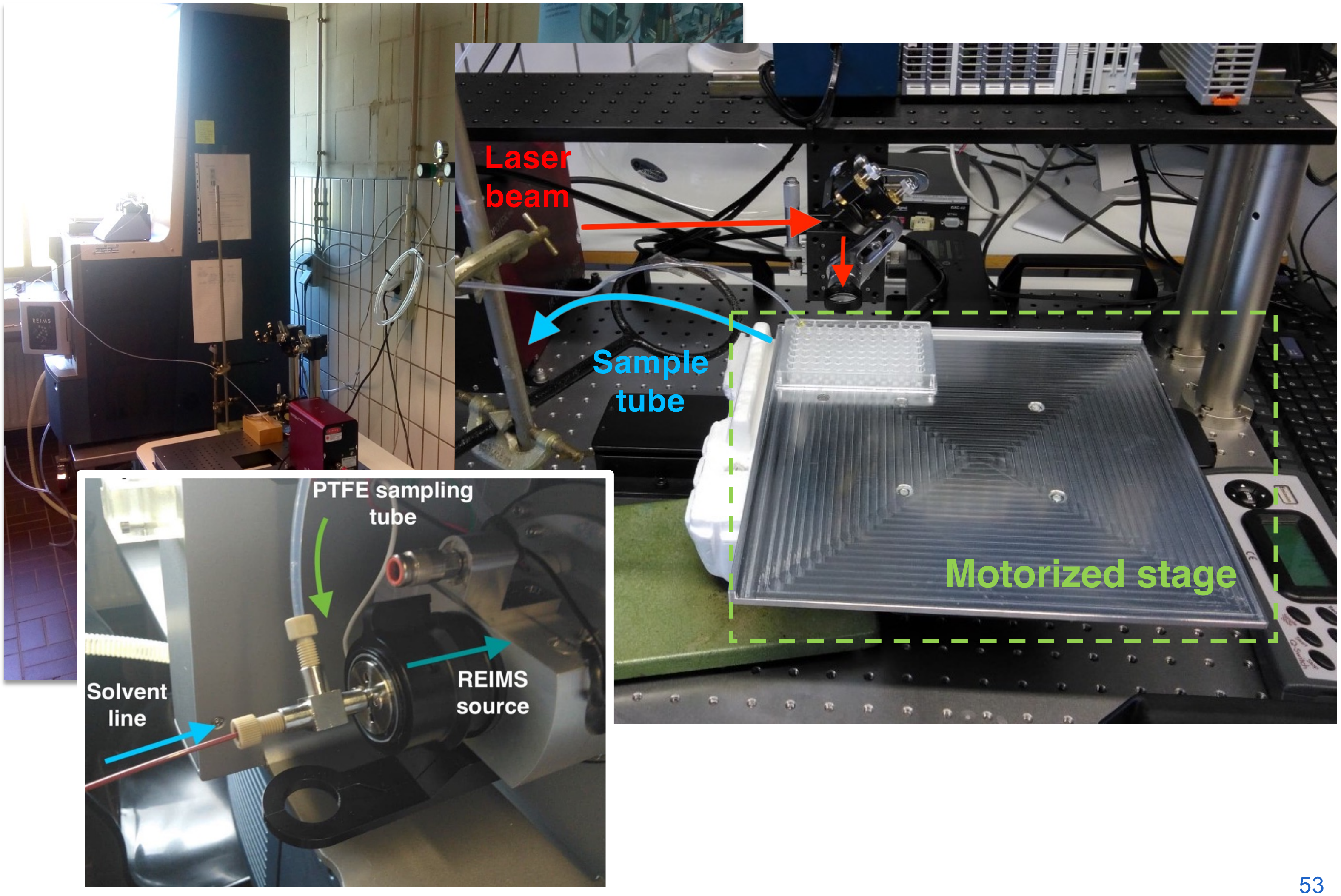
- Extensive sample preparation
- Approx. 30 min/sample
- High labor and material demands

- Limited to no sample preparation
- Approx. 30 sec/sample
- Semi-automated

LA-REIMS: INSTALLATION & EQUIPMENT

Instrumentation

- Xevo G2-XS Q-ToF MS
- REIMS source
- Harvard11 Elite syringe pump
- Opolette 2940 nm mid-IR laser
- Free space optics
- Motorized positioning stage (optional)

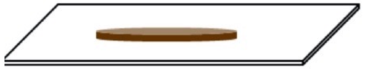


PROCEDURE

Sample pre-treatment

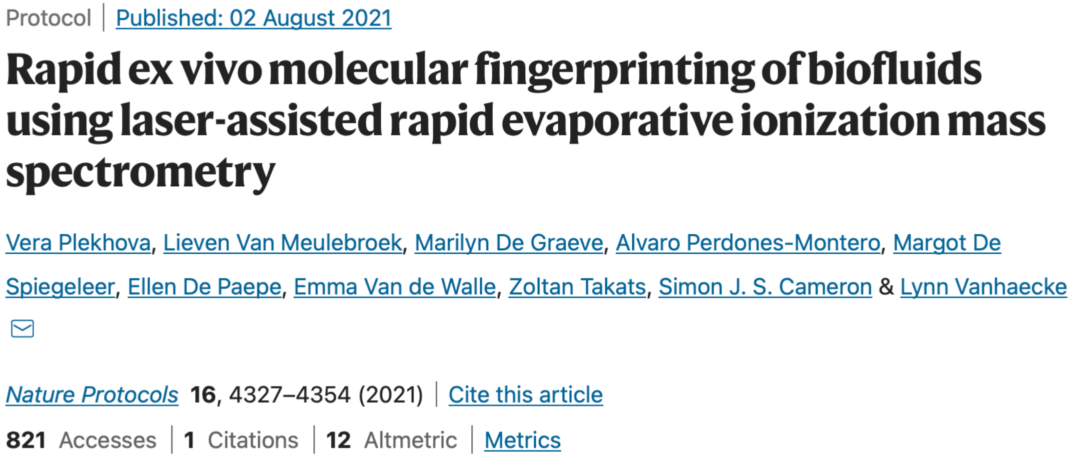
- Homogenisation, rehydration if lyophilized
- Centrifugation, filtration
- No pre-treatment
- No pre-treatment

15 – 100 µL



Single Sample

Vera Plekhova



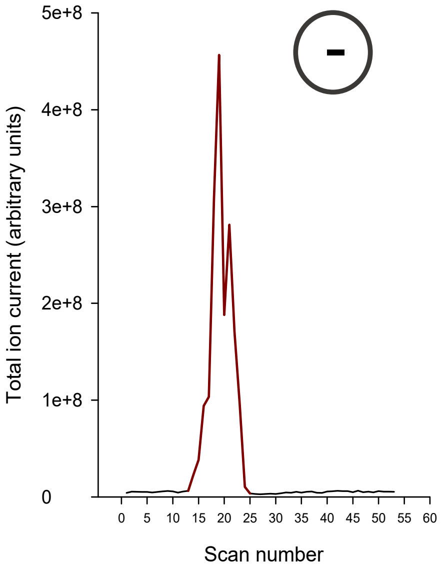
Protocol | Published: 02 August 2021

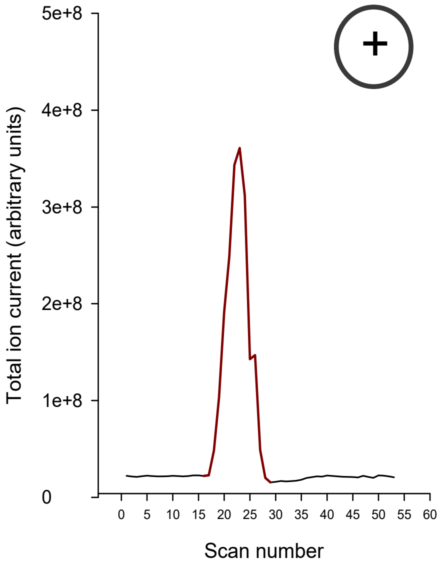
Rapid ex vivo molecular fingerprinting of biofluids using laser-assisted rapid evaporative ionization mass spectrometry

Vera Plekhova, Lieven Van Meulebroek, Marilyn De Graeve, Alvaro Perdones-Montero, Margot De Spiegeleer, Ellen De Paepe, Emma Van de Walle, Zoltan Takats, Simon J. S. Cameron & Lynn Vanhaecke

Nature Protocols **16**, 4327–4354 (2021) | Cite this article

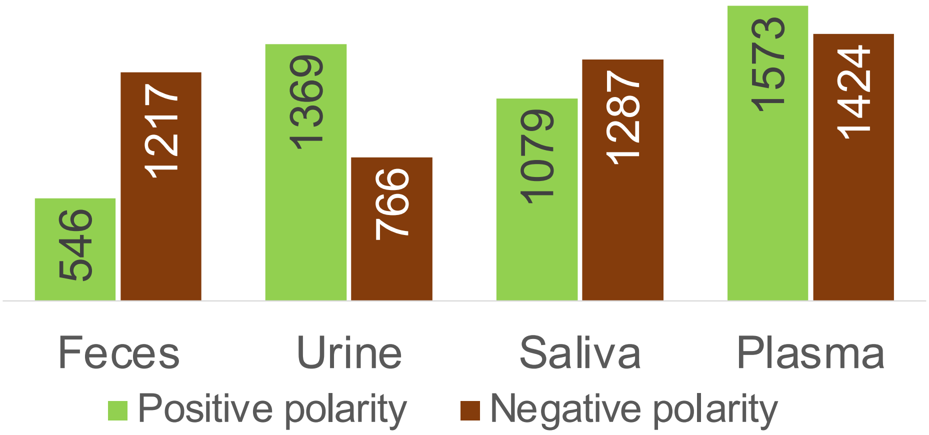
821 Accesses | **1** Citations | **12** Altmetric | Metrics



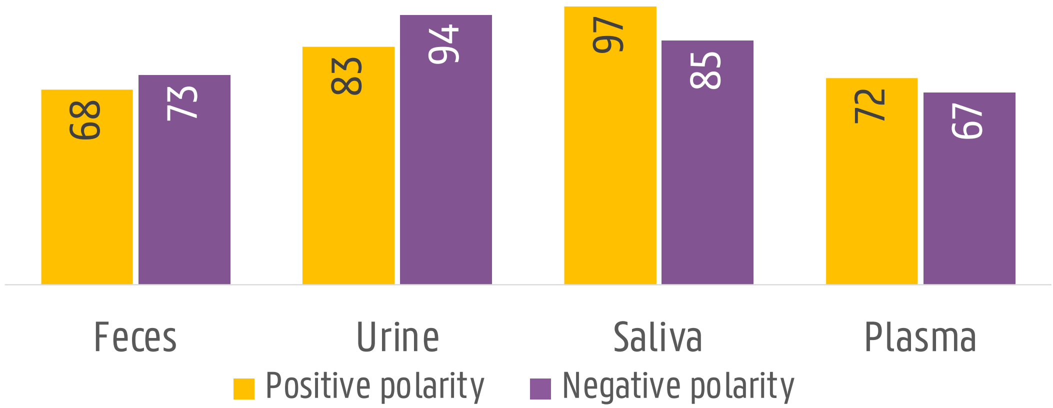


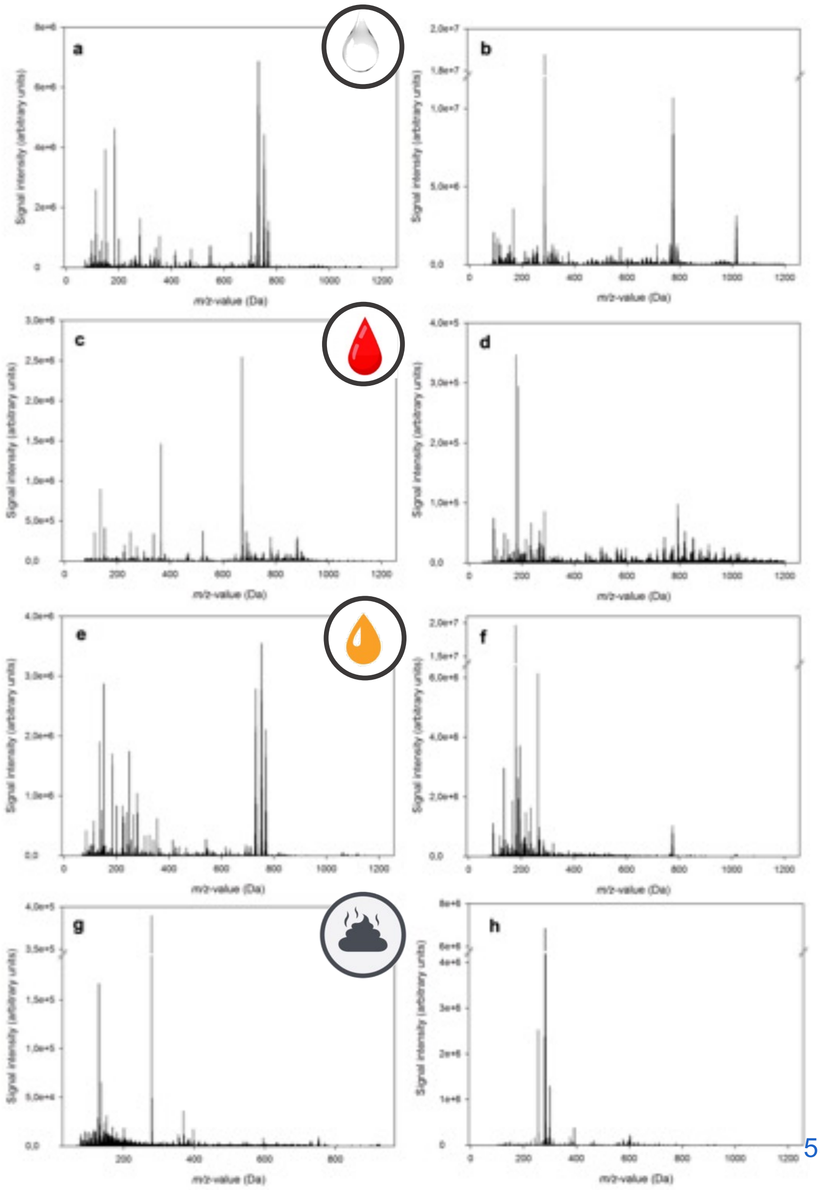
METHOD PERFORMANCE

Number of detected features

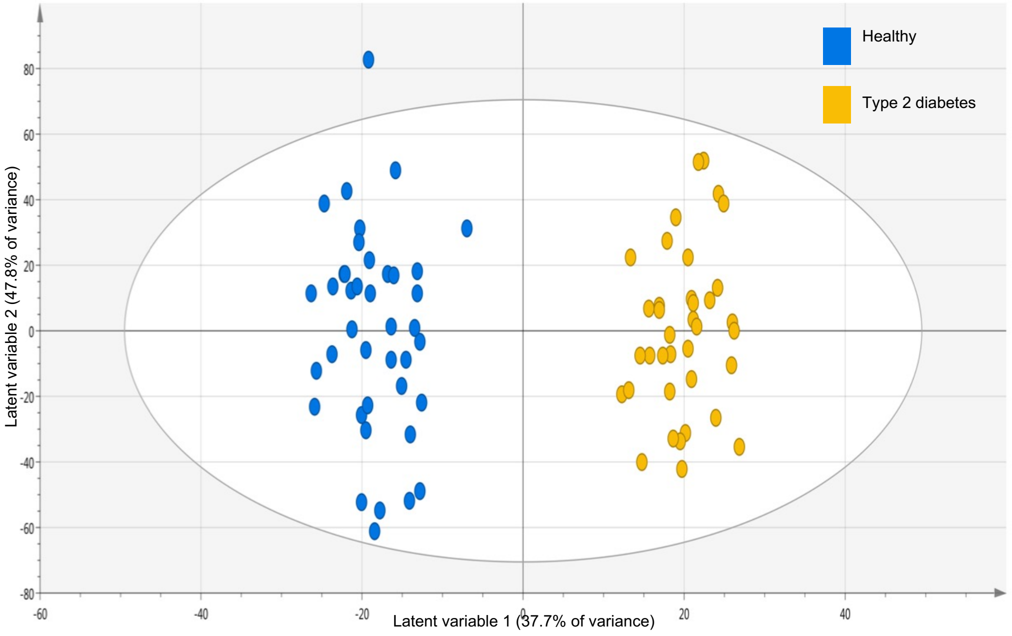


Percent of features with CV below 30% threshold





IN SITU FECAL METABOLOMICS IN T2D



$R^2(X)$ 0.420

$Q^2(Y)$ 0.662

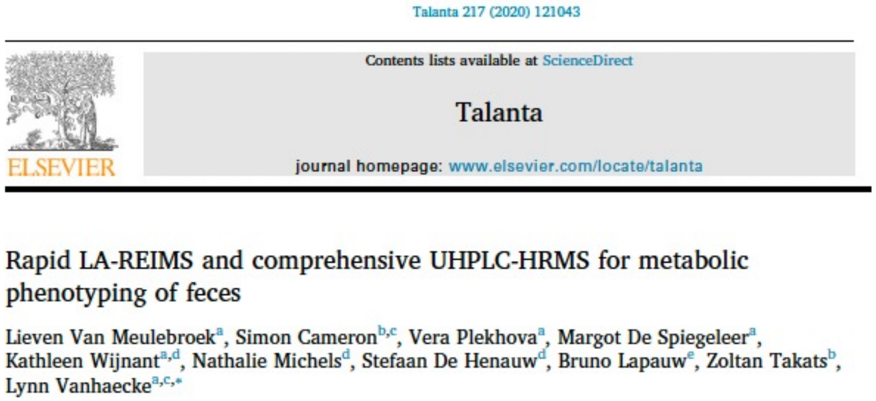
p-value of 2.3 e^{-13}

- Fingerprints containing 11,550 features
- 26 discriminating metabolites present
- Potential for POC application!

Proof of principle: LA-REIMS expedient platform to differentiate between
normal and high glycemic state!

Valid OPLS-DA model

2020



Talanta 217 (2020) 121043

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Rapid LA-REIMS and comprehensive UHPLC-HRMS for metabolic phenotyping of feces

Lieven Van Meulebroek[a], Simon Cameron[b,c], Vera Plekhova[a], Margot De Spiegeleer[a], Kathleen Wijnant[a,d], Nathalie Michels[d], Stefaan De Henauw[d], Bruno Lapauw[e], Zoltan Takats[b], Lynn Vanhaecke[a,c,*]

METASAMP® : NOVEL HIGH-THROUGHPUT DIRECT BIOFLUID SAMPLING AND METABOTYPING DEVICE

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(71) **Applicant: UNIVERSITEIT GENT** [BE/BE]; Sint-Pietersnieuwstraat 25, 9000 Gent (BE).

(72) **Inventors: VANHAECKE, Lynn**; Broek 17, 9860 Balegem (BE). **DE SPIEGELEER, Margot**; Ottergemsesteenweg 4, 9000 Gent (BE). **DE CLERCK, Karen**; Afsneekouter 48, 9051 Afsnee (BE). **GELTMEYER, Jozefien**; Rodonkstraat 21, 9030 Mariakerke (BE). **SINGH, Varoon**; A307, Alok Regency, Nilemore, Nalasopara (West), Palghar, Mumbay 401203 (IN).

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

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(74) **Agent: VAES, Peter** et al.; DenK, iP Leuvensesteenweg 203, 3190 Boortmeerbeek (BE).

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(54) **Title:** FIBROUS MAT FOR METABOLOME SAMPLING

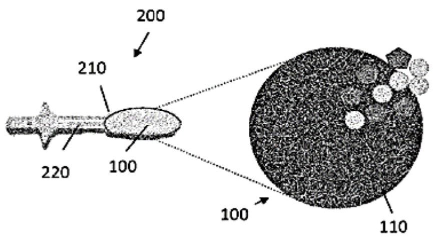
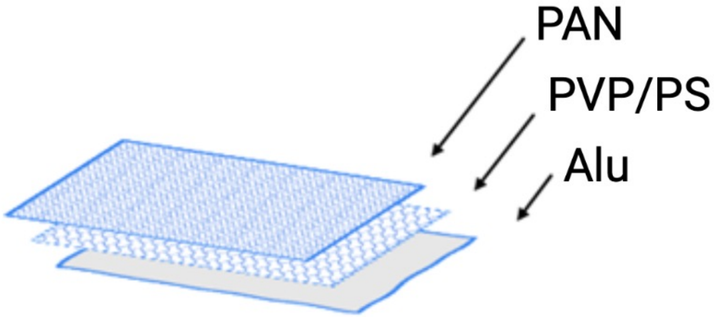
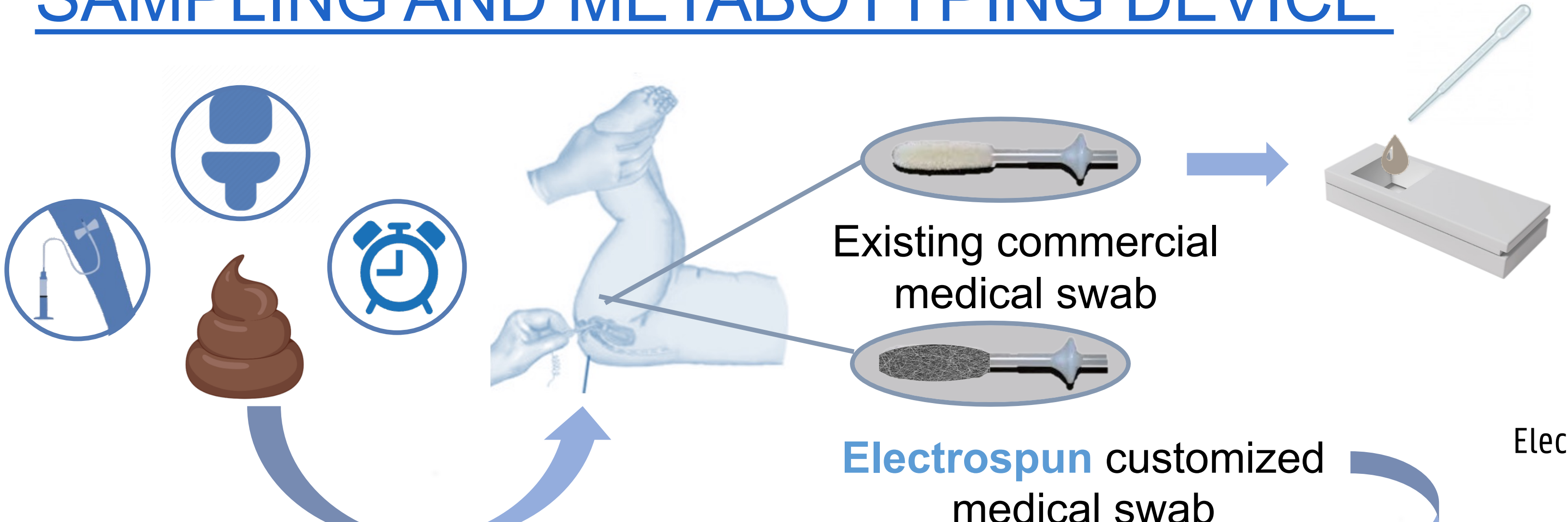


FIG. 1



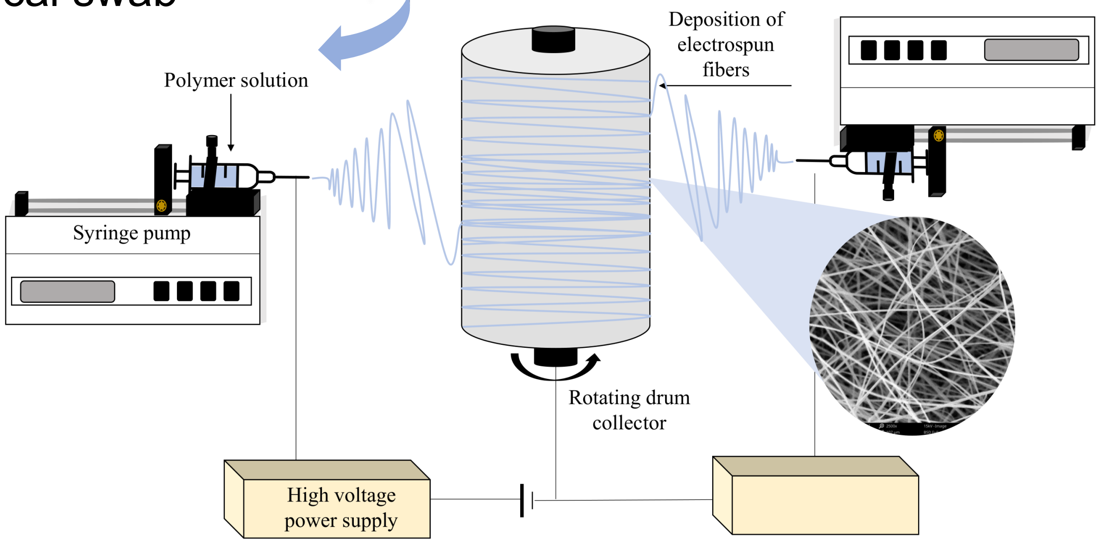
METASAMP® : NOVEL HIGH-THROUGHPUT DIRECT BIOFLUID SAMPLING AND METABOTYPING DEVICE



Existing commercial medical swab

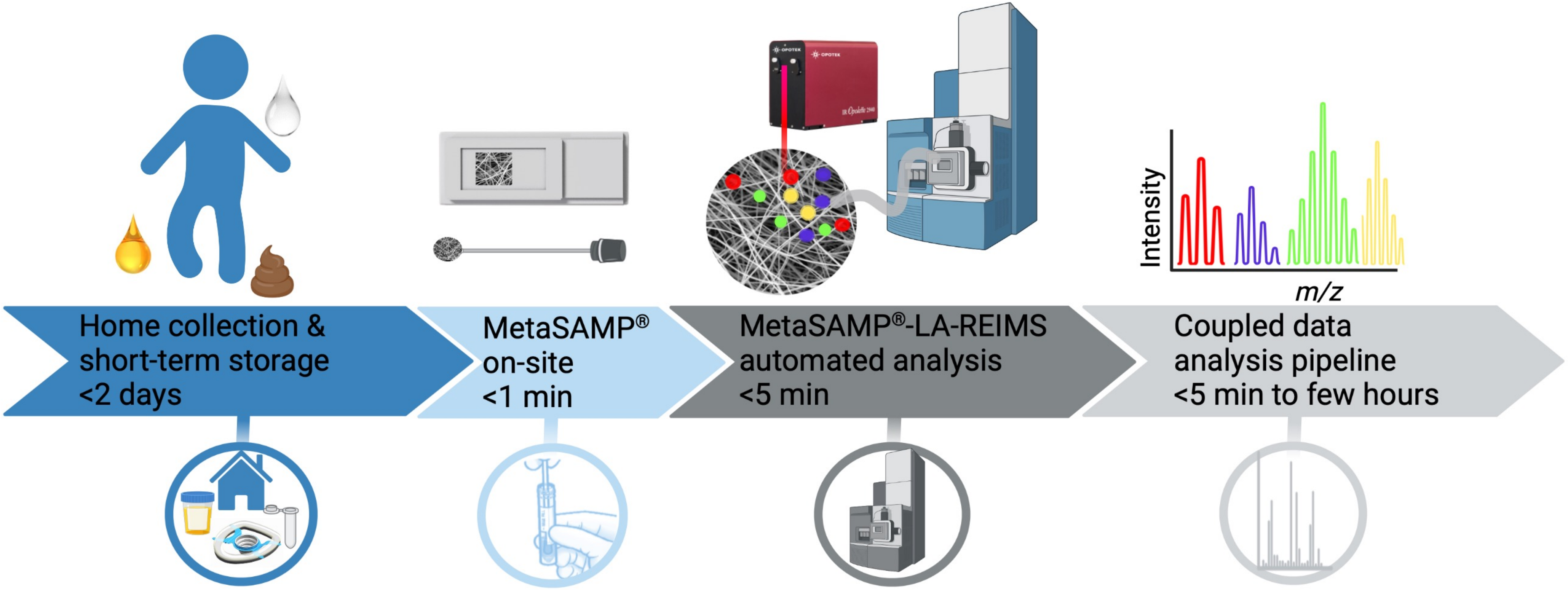
Electrospun customized medical swab

Electrospun nanofibrous membrane



- Direct sampling (no storage, pre-treatment, ...)
- Porous fibrous network
- High surface area
- Enhanced absorption of metabolites

METASAMP® : NOVEL HIGH-THROUGHPUT DIRECT BIOFLUID SAMPLING AND METABOTYPING DEVICE



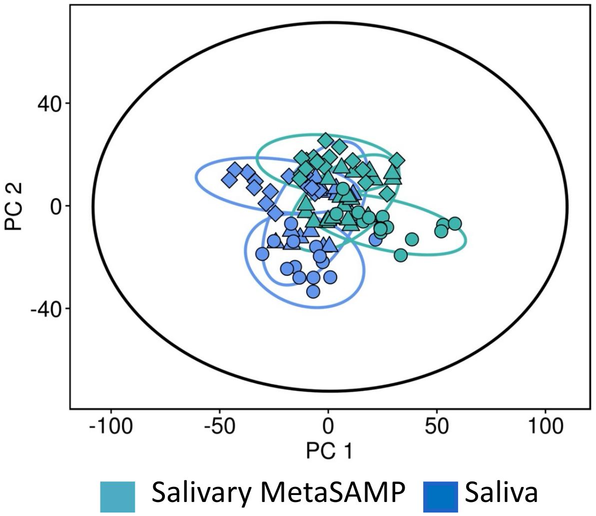
Home collection & short-term storage
<2 days

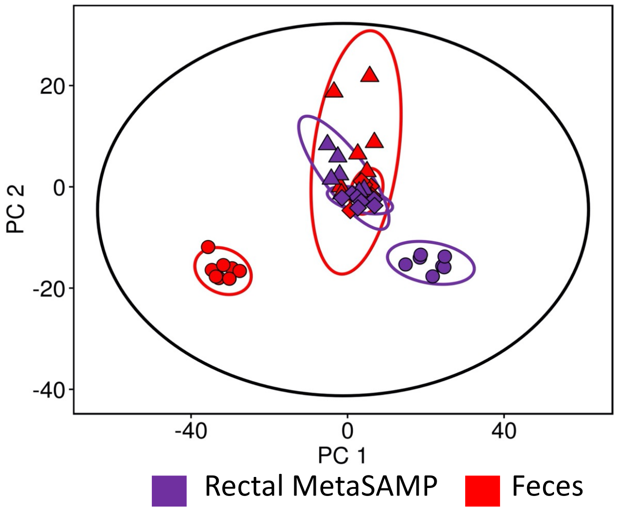
MetaSAMP®
on-site
<1 min

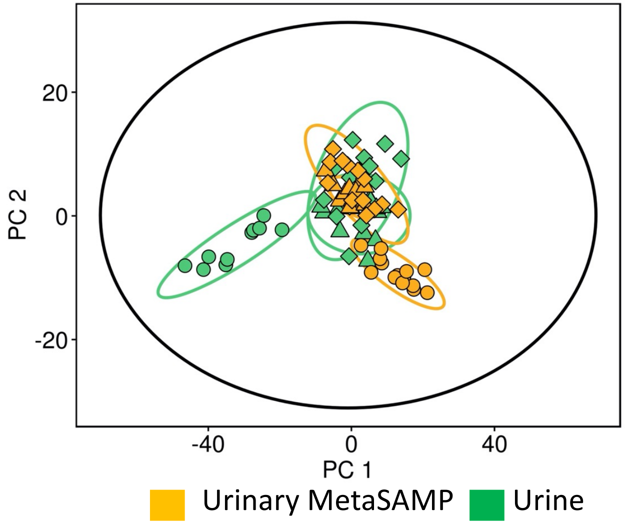
MetaSAMP®-LA-REIMS
automated analysis
<5 min

Coupled data
analysis pipeline
<5 min to few hours

METASAMP® : SUPERIOR STABILITY UPON SHIPPING



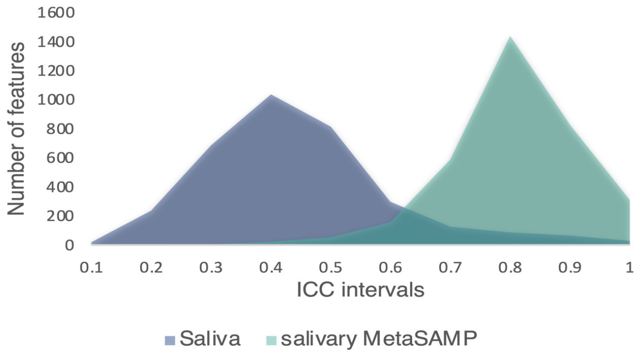


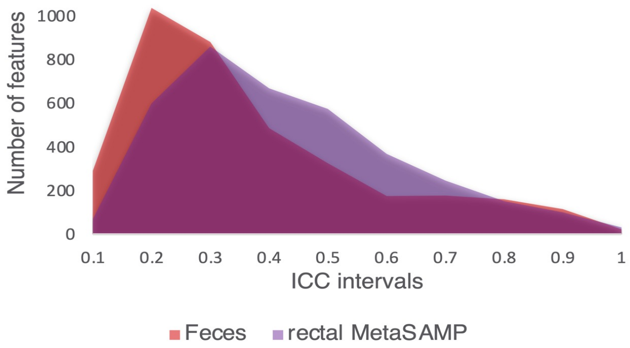


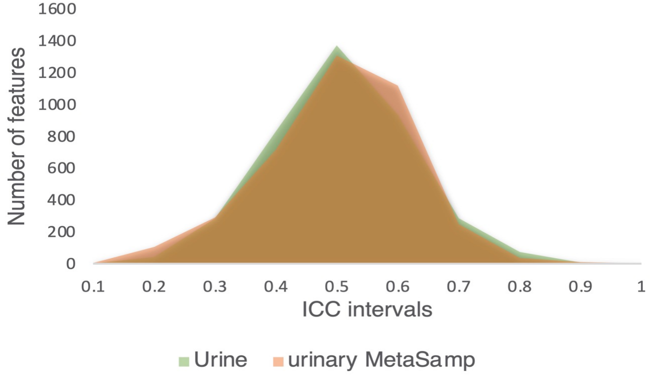
△ Day 0

◇ Day 2, 20°C

○ Day 2, 4°C

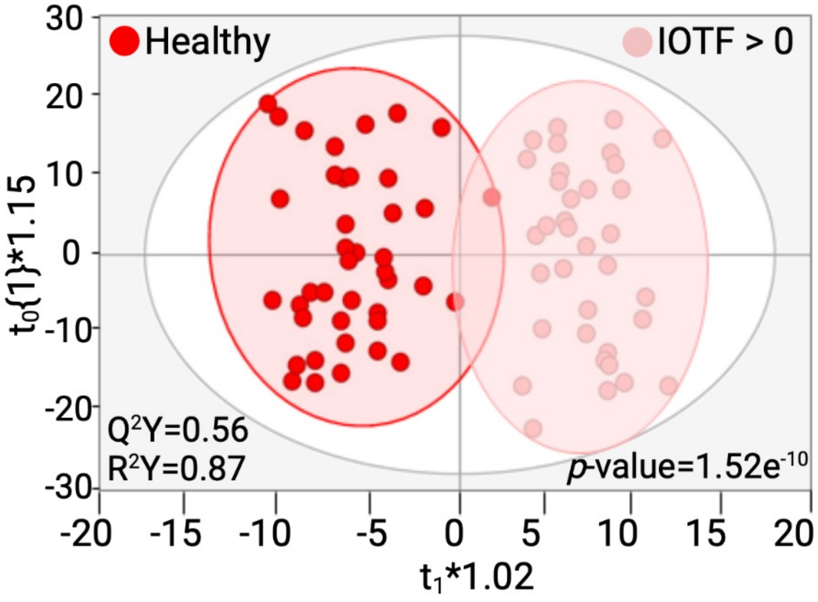




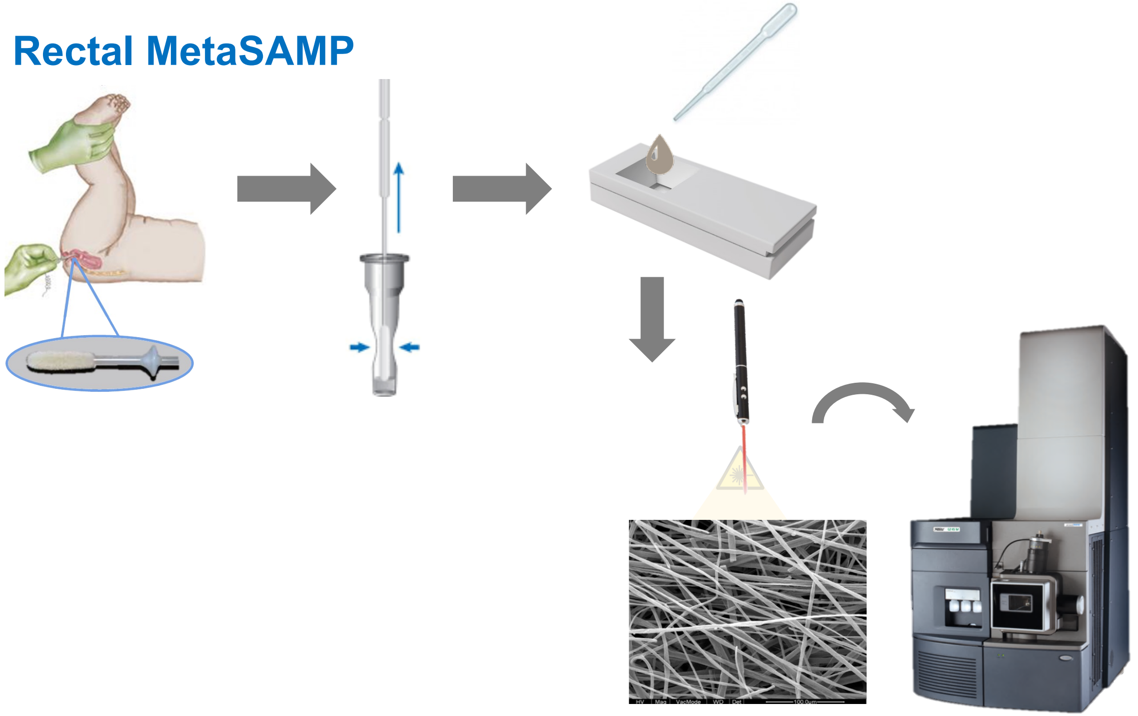


METASAMP® : SUPERIOR CLINICAL PREDICTIVE POWER

MetaBEASE
41 normal weight (IOTF ≤ 0)
35 IOTF ≥ 1 (overweight or obese)



Rectal MetaSAMP



OPLS-DA model parameters

IOTF ≥ 1 vs. healthy (IOTF ≤ 0)	Q^2Y	p-value
Feces	0.40	4.14e^{-6}
Rectal MetaSAMP	0.56	1.52e^{-10}

METASAMP® : SUPERIOR CLINICAL PREDICTIVE POWER

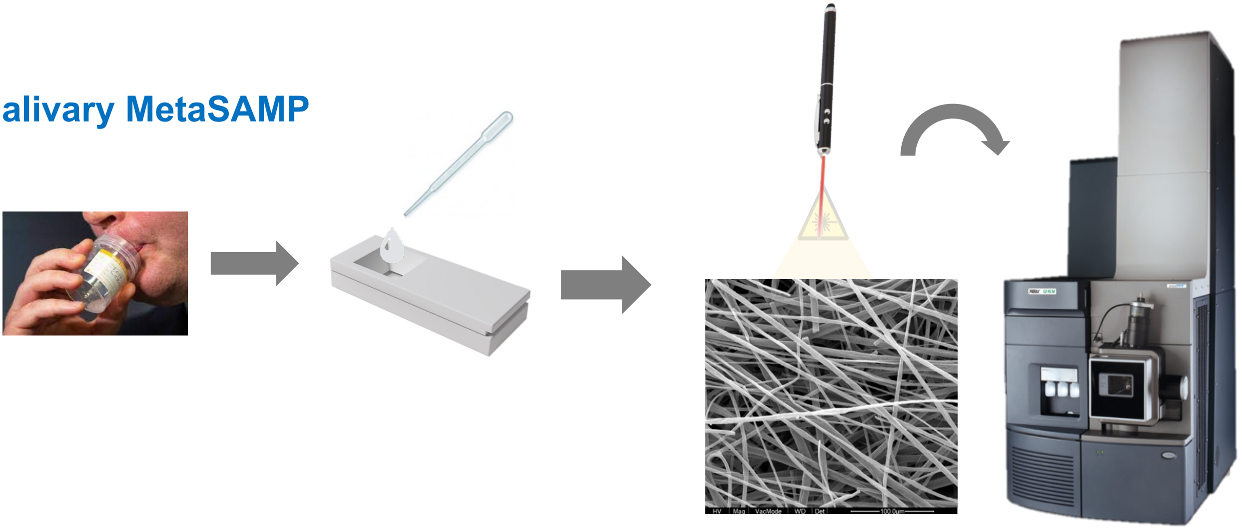
OPERA:

66 normal weight

20 overweight

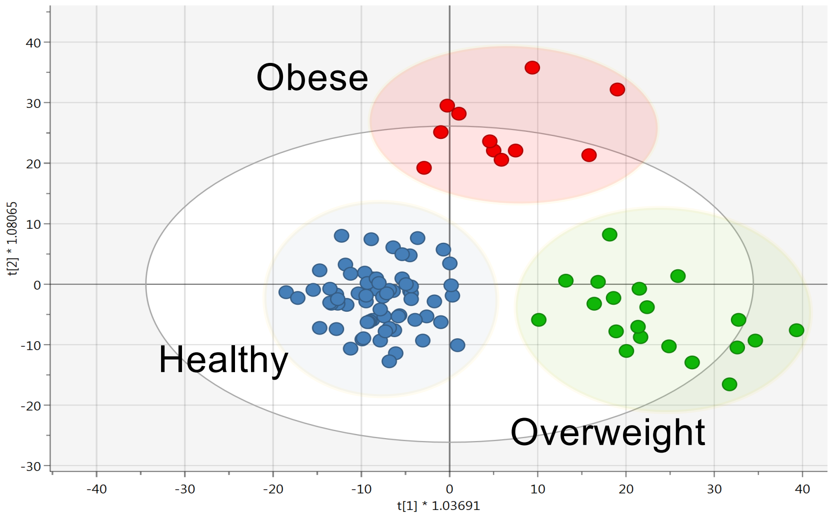
11 obese

Salivary MetaSAMP



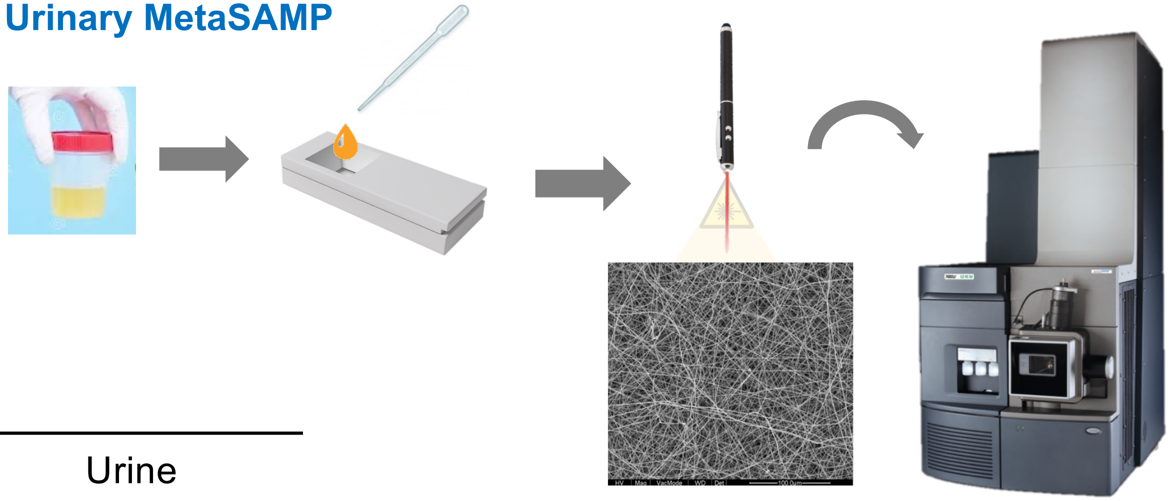
OPLS-DA model parameters

	Salivary MetaSAMP		Saliva	
	Q^2Y	p-value	Q^2Y	p-value
Obese vs. normal weight	0.545	$1.53e^{-10}$	0.442	$5.76e^{-9}$
Obese and overweight vs. normal weight	0.533	$4.00e^{-05}$	0.576	$4.06e^{-10}$
Obese vs. overweight	0.698	$4.63e^{-05}$	0.541	$2.71e^{-05}$



METASAMP® : SUPERIOR CLINICAL PREDICTIVE POWER

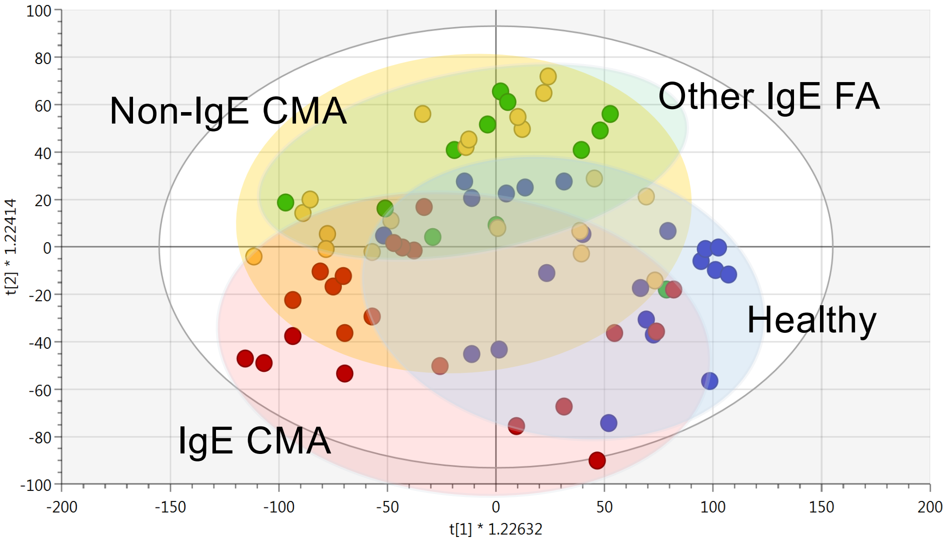
20 healthy
20 IgE mediated CMA
19 non-IgE mediated CMA
11 other IgE mediated FAs



Urinary MetaSAMP

OPLS-DA model parameters

	Urinary MetaSAMP		Urine	
	Q^2Y	p-value	Q^2Y	p-value
IgE CMA vs. healthy	0.642	$1.29e^{-11}$	0.523	$3.64e^{-7}$
IgE CMA vs. other IgE FA	0.535	$2.24e^{-06}$	0.536	$6.68e^{-6}$
IgE CMA vs. non-IgE CMA	0.550	0.00038	0.511	0.00010



TAKE HOME MESSAGE

TAKE HOME MESSAGE

- Nutri- or clinical (biofluid) metabolomics workflow: a complex process integrating branching into its structure in order to allow for the necessary **flexibility** associated with the complex analysis of **human biology** as well as with the **wealth of technological solutions**
- Combing data from dietary intake, gut microbiota, psychological parameters, drug intake, etc. (i.e. the **exposome**) with the **metabolome of biospecimens** will further aid to unravel the diet-gut microbiome-health axis and pave the way towards **3P medicine**.

→ **While physiology and biochemistry of (intermediate) metabolism were largely left behind when the genomics revolution arrived, metabolomics has revived the study of metabolism, as it in essence offers the most direct link to final health outcomes.**

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

LIMET
Laboratory of Integrative Metabolomics

FOOD2KNOW

Lynn Vanhaecke
Prof.

Department of Translational Physiology, Infectiology and Public health
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