3rd International Electronic Conference on Metabolomics
15-30 November 2018
chaired by Prof. Peter Meikle, Dr. Thusitha W. Rupasinghe, Prof. Susan Sumner, Dr. Katja Dettmer-Wilde
sponsored by metabolites

Applying an untargeted metabolomics approach using two complementary platforms for the discovery and validation of banana intake biomarkers

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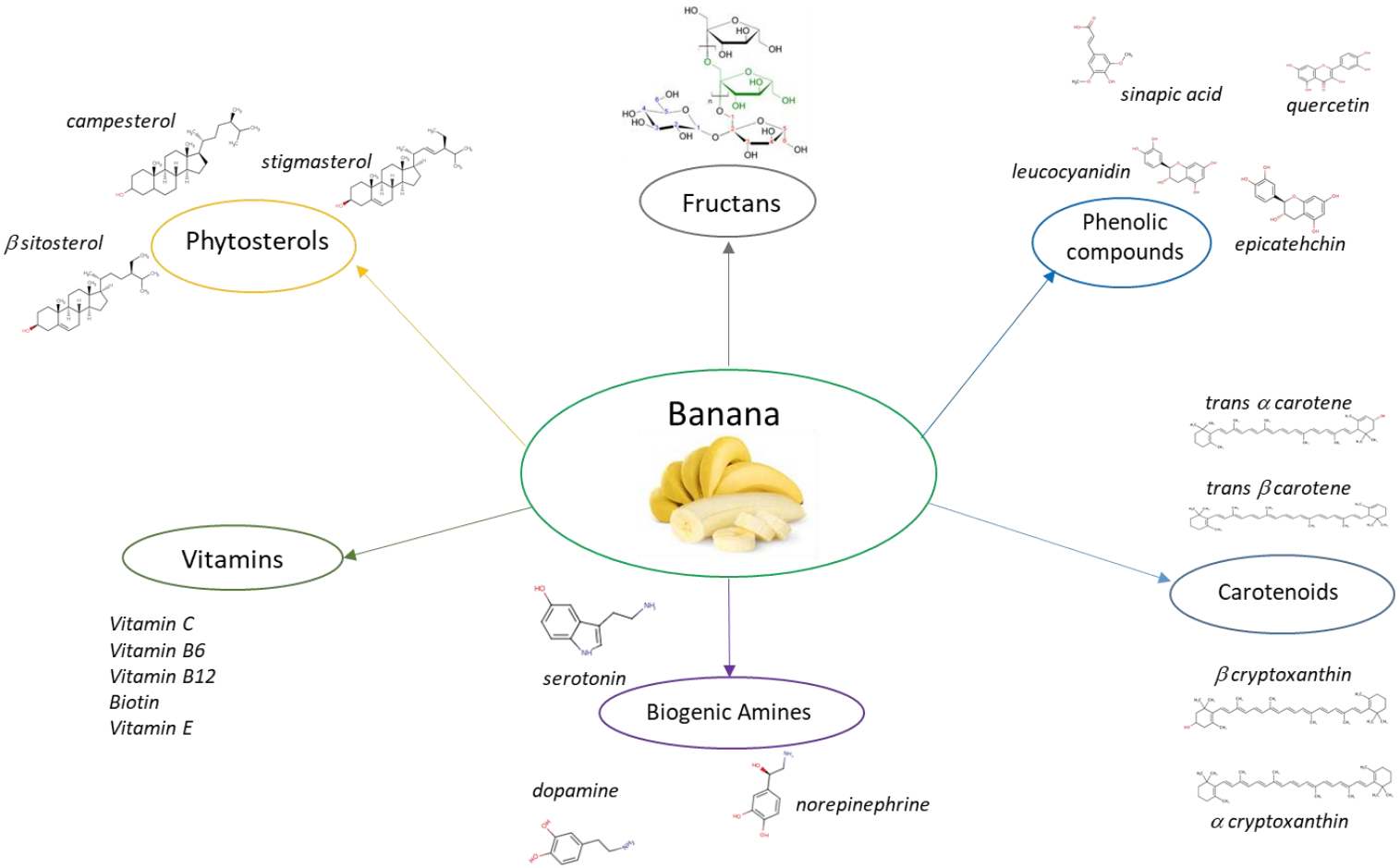
What do we know about banana?



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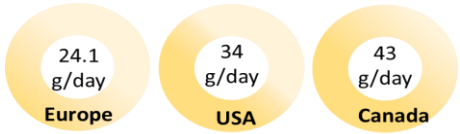
What do we know about banana?

✓ Highly consumed fruit in different countries.



✓ Intake of unripe banana ameliorates diarrhoea in children.

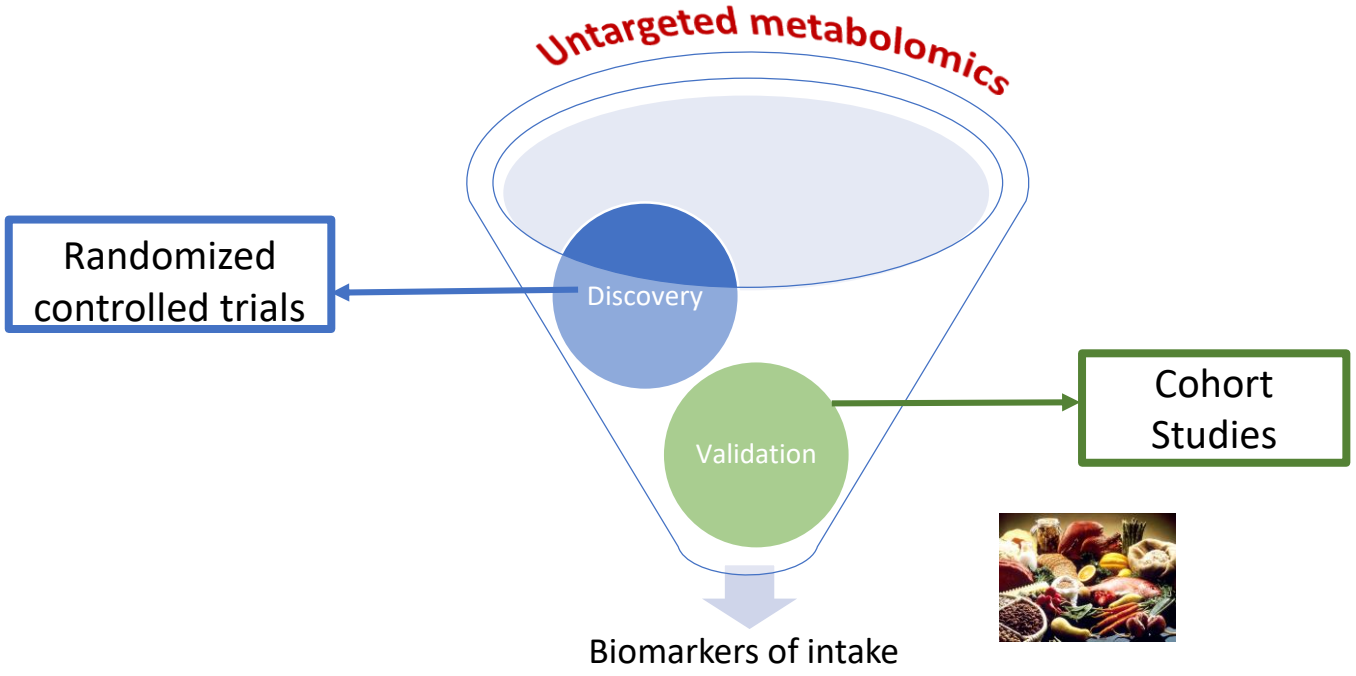
Green Banana Reduces Clinical Severity of Childhood Shigellosis
A Double-Blind, Randomized, Controlled Clinical Trial

Golam H. Rabbani, MD, PhD, FACG, Shamsir Ahmed, MBBS, Md. Iqbal Hossain, MBBS, PhD, Rafiqul Islam, MBBS, MPH, Farzana Marni, MSc, Mastura Akhtar, MSc, and Nashiha Majid, MSc

✓ **Biomarkers of banana intake** following a meal intervention **have not yet been reported.**

Why do we need biomarkers?

- ✓ Strengthening the information obtained from paper based dietary assessment tools (FFQ, 24HR) is needed.
- ✓ The use of biomarkers of intake to determine dietary exposure offers more objective information.



Cheung,W et al 2017 A metabolomic study of biomarkers of meat and fish intake doi:10.3945/ajcn.116.146639
Kristensen M, et al 2017 A High Rate of Non-Compliance Confounds the Study of Whole Grains and Weight Maintenance in a Randomised Intervention Tria.l doi:10.3390/nu9010055.

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Main Objective

- ✓ Identify and validate novel urinary biomarkers of intake of banana using an untargeted metabolomics approach.
- ✓ Untargeted metabolomics approach in **two different platforms** (UPLC-QTOF-MS and GC×GC-MS) to analyse urine samples of **two different study designs.**

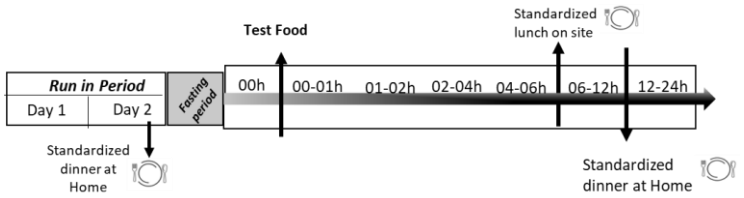
Discovery — RCT, cross-over

n=12
M=6 W=6
Age:18-40 years
BMI: 19.01-25.9kg/m²
Nonsmokers

Banana 240g + 150ml of control drink
250ml of control drink

Wash out period 3 days

250ml of control drink
Banana 240g + 150ml of control drink



- ✓ 24h urine in 7 time intervals
- ✓ 24h urine pool

Validation — The KarMen Study *Bub A et al., 2016 doi: 10.2196/resprot.5792*

n=301
Healthy men and women
Age: >18 years
Nonsmokers

Declared intake of banana 24HR questionnaires

- n=26 "High-consumers" (176±59 g banana)
- n=26 "Low-consumers" (87.7±12g banana)
- n=26 "Non-consumers"

- ✓ 24h urine pool

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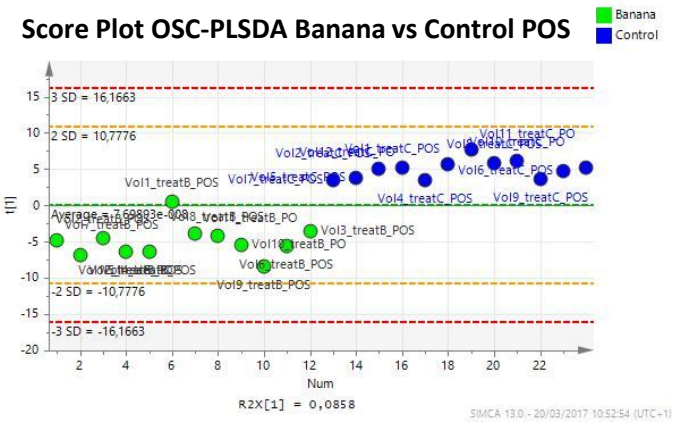
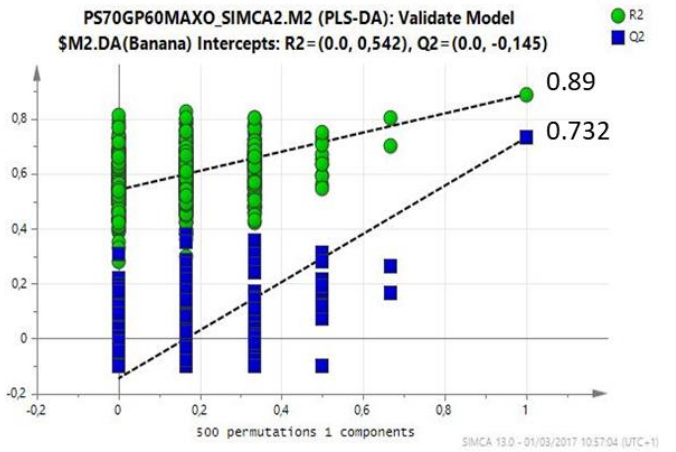
METABOHUB Infrastructure nationale en métabolomique et fluxomique

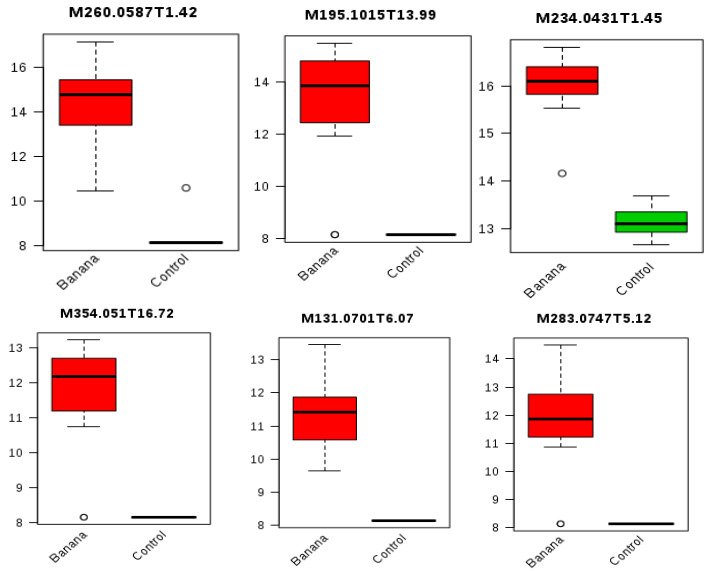
	UPLC-QTOF-MS	Data Preprocessing	Data Cleaning	Data Analysis
24h urine pools Meal intervention Study	✓ BEH shield RP18 100X41X1,7 ✓ 25 minute gradient ✓ Impact II Bruker ✓ ESI(+) and (-)	- Workflow4metabolomics **- XCMS** for spectral data analysis. **- CAMERA** for ion annotation.	ESI (+) **2,714** ESI (-) **1,289**	✓**OSC-PLSDA (VIP>2)** ✓**Student paired T test (p-FDR<0.05)**
24h urine pools Cohort Study	✓ BEH shield RP18 100X41X1,7 ✓ 25 minute gradient ✓ Impact II Bruker ✓ ESI(+)		ESI (+) **2,427**	✓**PLSDA** ✓**Student T test (p-FDR <0.05)** ✓**Logistic Regression with AIC**

Discovery
ESI (+)





74 ions had a VIP>2
47 ions have a higher intensity in the banana group

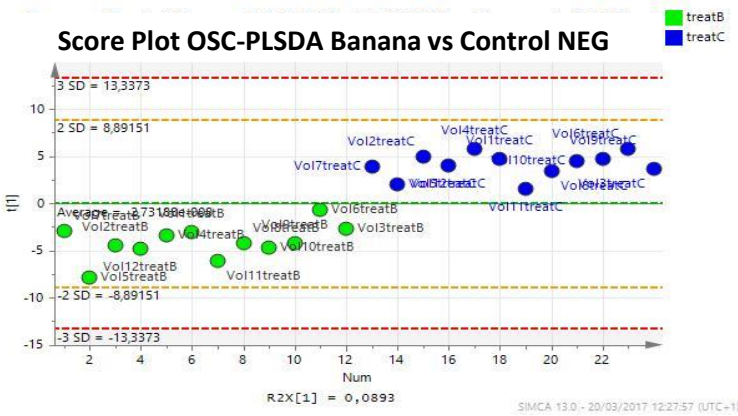


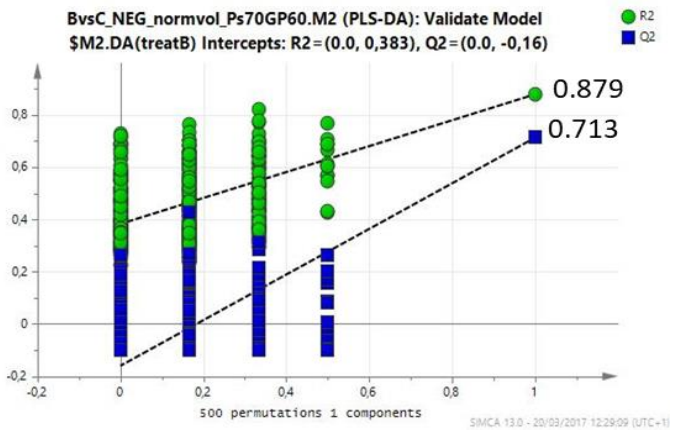
36 ions with p<0.05 BH

31 ions Higher in Banana

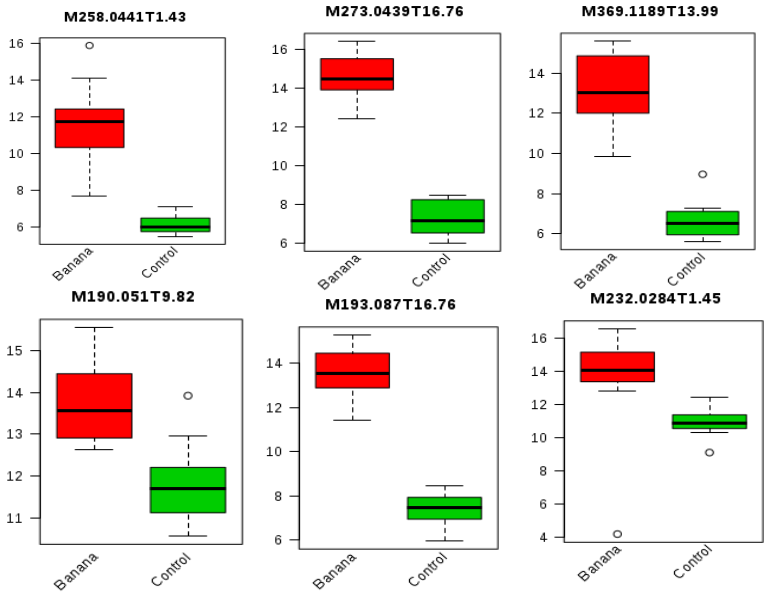
All significant ions in univariate have a VIP>2

Discovery ESI (-)





40 ions had a VIP>2
37 ions have a higher intensity in the banana group



22 ions with p<0.05 BH

22 ions Higher in Banana

All significant ions in univariate have a VIP>2

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Identification pipeline overview

Identification of significant parent ions MS Scan QTOF and Orbitrap

Determination of elemental formula

Query of databases and literature for biologically plausible compounds.
HMBD,FooDB, Phytohub, Knapsack, CHEBI, DFC

Metabolism predictions of banana compounds.
Meteor nexus

Fragmentation experiments

MSMS QTOF
(Impact II Bruker)
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MSMS Orbitrap
(LTQ orbitrap velos hybrid mass spectrometer)
FONDAZIONE EDMUND MACH CENTRO RICERCA E INNOVAZIONE

Marynka Ulaszewska-Tarantino, PhD

Query of Spectral libraries for specific compound or compound classes
Mzcloud, RESPECT, Mona, HMDB, FooDB, Metlin, Metfrag, CSI finder

Plausible candidates

- ✓ Acquisition of chemical standards
- ✓ Enzymatic conjugation of standards
- ✓ MSMS experiments for spectral matching

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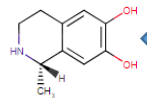
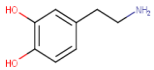
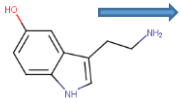
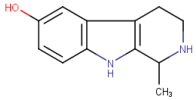
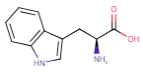
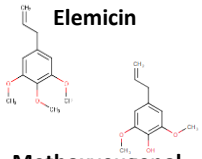

Salsolinol

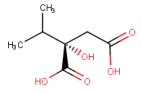

Dopamine

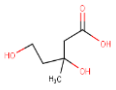

Serotonin

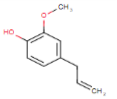

6-OH-1-methyl-1,2,3,4-tetrahydro-β carboline

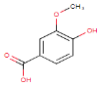

Tryptophan


Elemicin
Methoxyeugenol

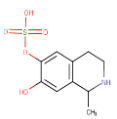

2 isopropylmalic acid

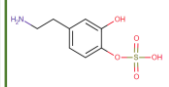

Mevalonic acid

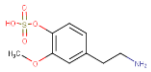

Eugenol

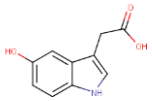

Vanillic acid

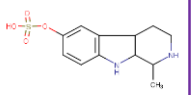
Human Metabolism

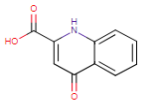

Salsolinol Sulfate

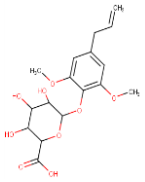

Dopamine Sulfate

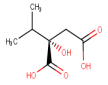

3-MT sulfate

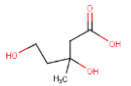

5-HIAA

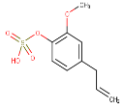

6-OH-1-methyl-1,2,3,4-tetrahydro-β-carboline Sulfate*

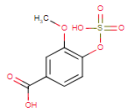

Kynurenic acid


Methoxyeugenol glucuronide


2-isopropylmalic acid


Mevalonic acid


Eugenol Sulfate


Vanillic acid sulfate

*putatively annotated

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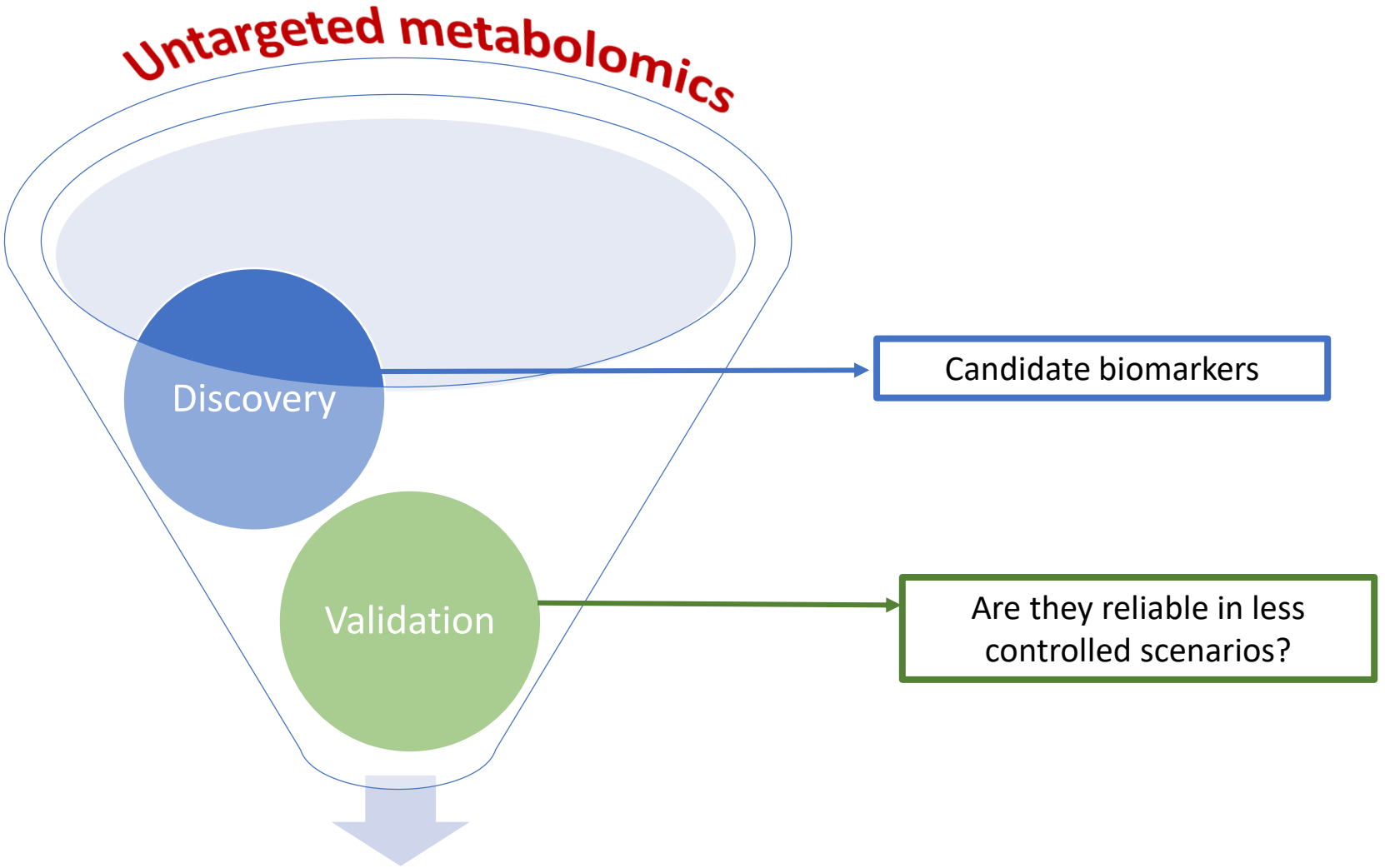

Untargeted metabolomics
Discovery
Validation
Candidate biomarkers
Are they reliable in less controlled scenarios?

Validation

The KarMen Study

47 biomarkers of banana from meal study ESI (+)

↓

2427 ions ESI(+)

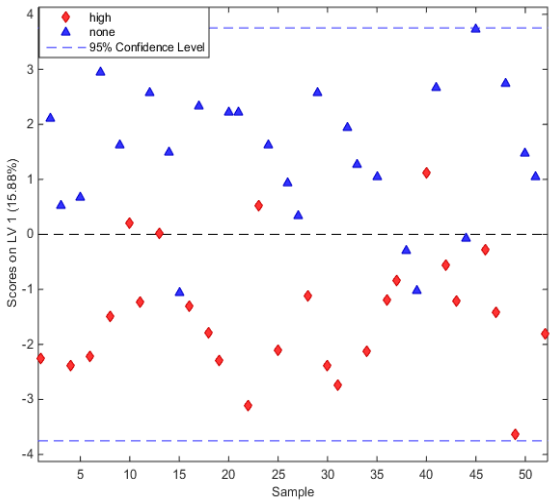
↓

22 ions *matched according to rt, mz and spectra*

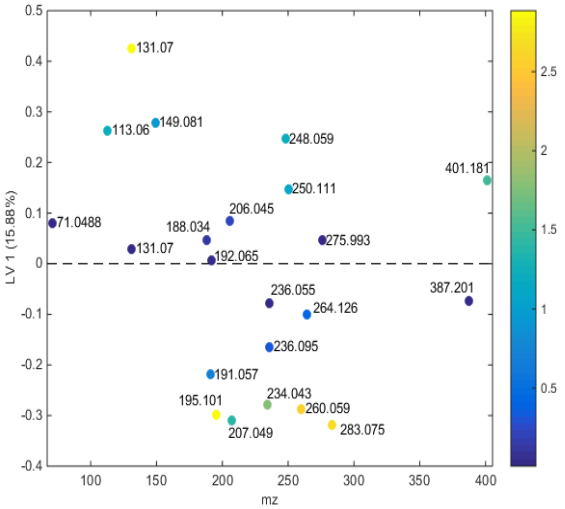
↓

PLSDA model

PLSDA Scores Plot High vs None

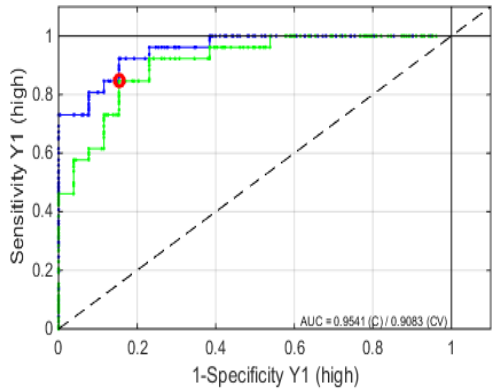


PLSDA Loadings Plot High vs None



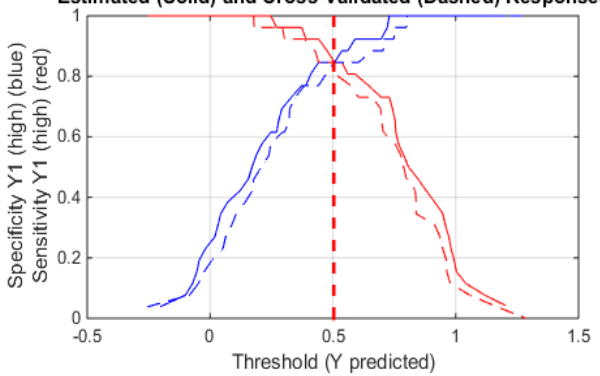
✓ **22 highly discriminant features** in the meal study are able to **predict the intake of banana** with a good sensitivity and specificity.

Estimated (blue) and Cross-Validated (green) ROC



AUC (CV)=0.90

Estimated (Solid) and Cross-Validated (Dashed) Responses



Sensitivity (CV) = 84%
Specificity (CV) = 80.7%

Is there a more parsimonious biomarker?

Validation

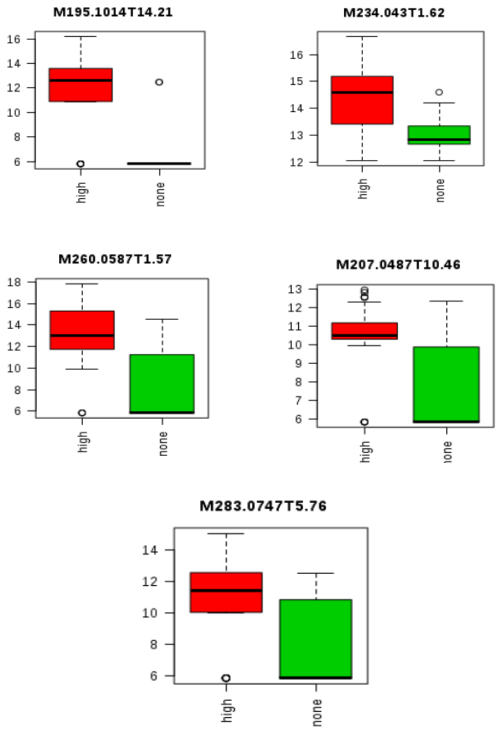
KarMen Study

Student T test

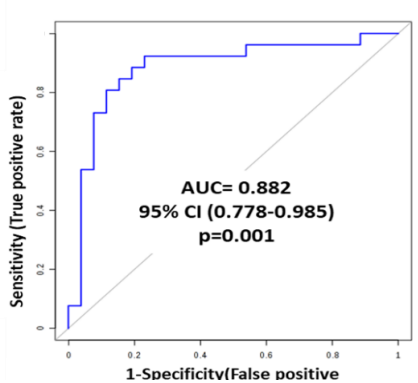
FDR-correction

Features with p-FDR value <0.05 were selected as **confirmed biomarkers of banana intake**

Logistic Regression with AIC to obtain a parsimonious biomarker of banana intake

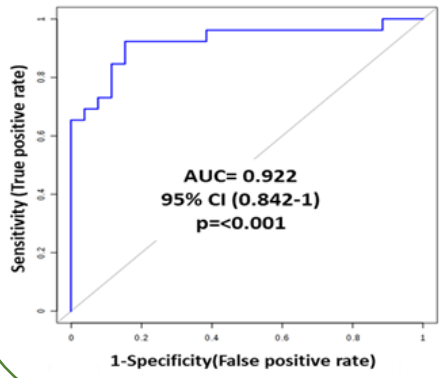


5 metabolites



Sensitivity (CV) = 84%
Specificity (CV) = 84.6%

m/z 195.1014+ m/z 283.0747



Sensitivity (CV) = 84.6%
Specificity (CV) = 92%

Parsimonious biomarker of banana intake!
Good sensitivity and higher specificity

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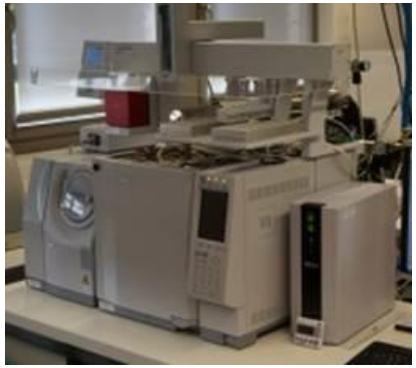
Untargeted GCxGC-MS analysis

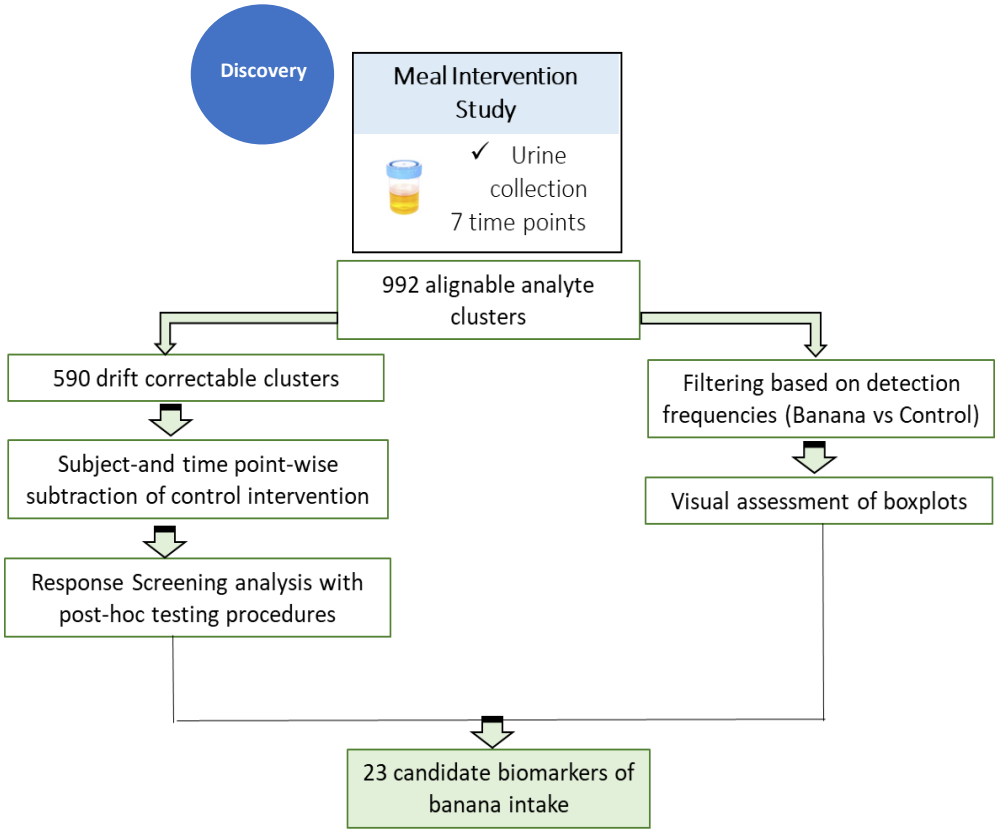


- ✓ To obtain a broader coverage of biomarkers of banana intake.
- ✓ Confirm the robustness of the biomarkers of banana intake identified using UPLC-QTOF-MS.

Christoph Weinert, PhD

Carina Mack, PhD

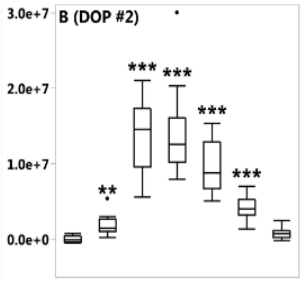
Björn Egert, PhD



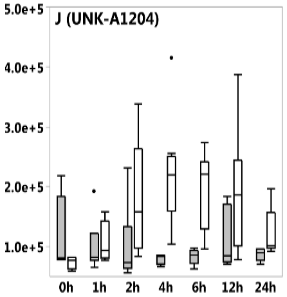
Discovery

Previously observed in UPLC-QTOF-MS

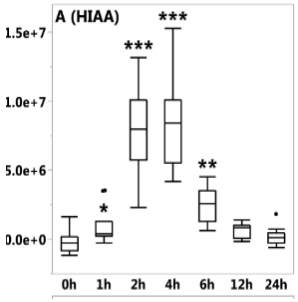
Dopamine



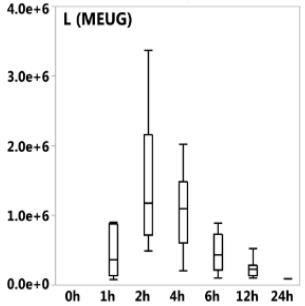
3-methoxytyramine



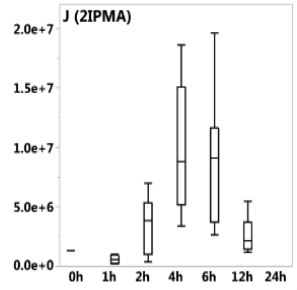
5-HIAA



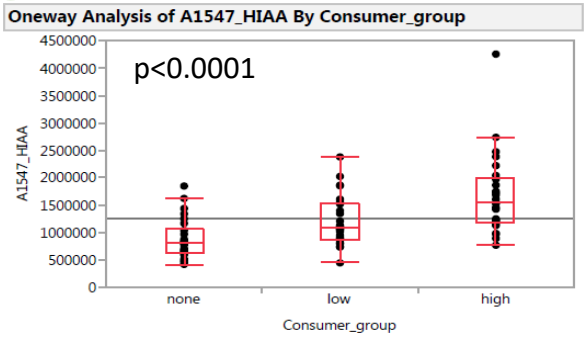
Methoxyeugenol

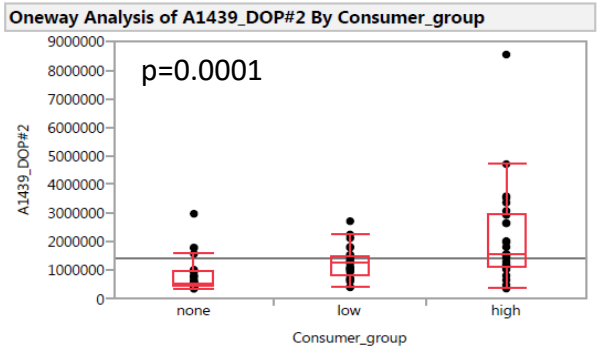


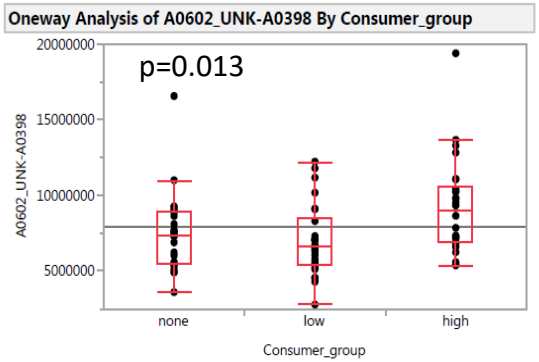
2-isopropylmalic acid



Validation







Conclusions

- Applying an untargeted metabolomics approach in two different platforms provided a broader coverage of metabolites and candidate biomarkers for banana intake.

- Dopamine and serotonin metabolites are among the most discriminant metabolites following banana intake.

- The combination of m/z 195.1014 and 283.0474 putatively annotated as methoxyeugenol and 6-OH-TβC sulfate offers a parsimonious biomarker of banana intake.

- Further validation in independent cohorts is needed using a quantitative method to further assess the utility of these biomarkers to predict the intake of banana.

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