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Two complementary metabolomics studies to identify biomarkers of banana intake

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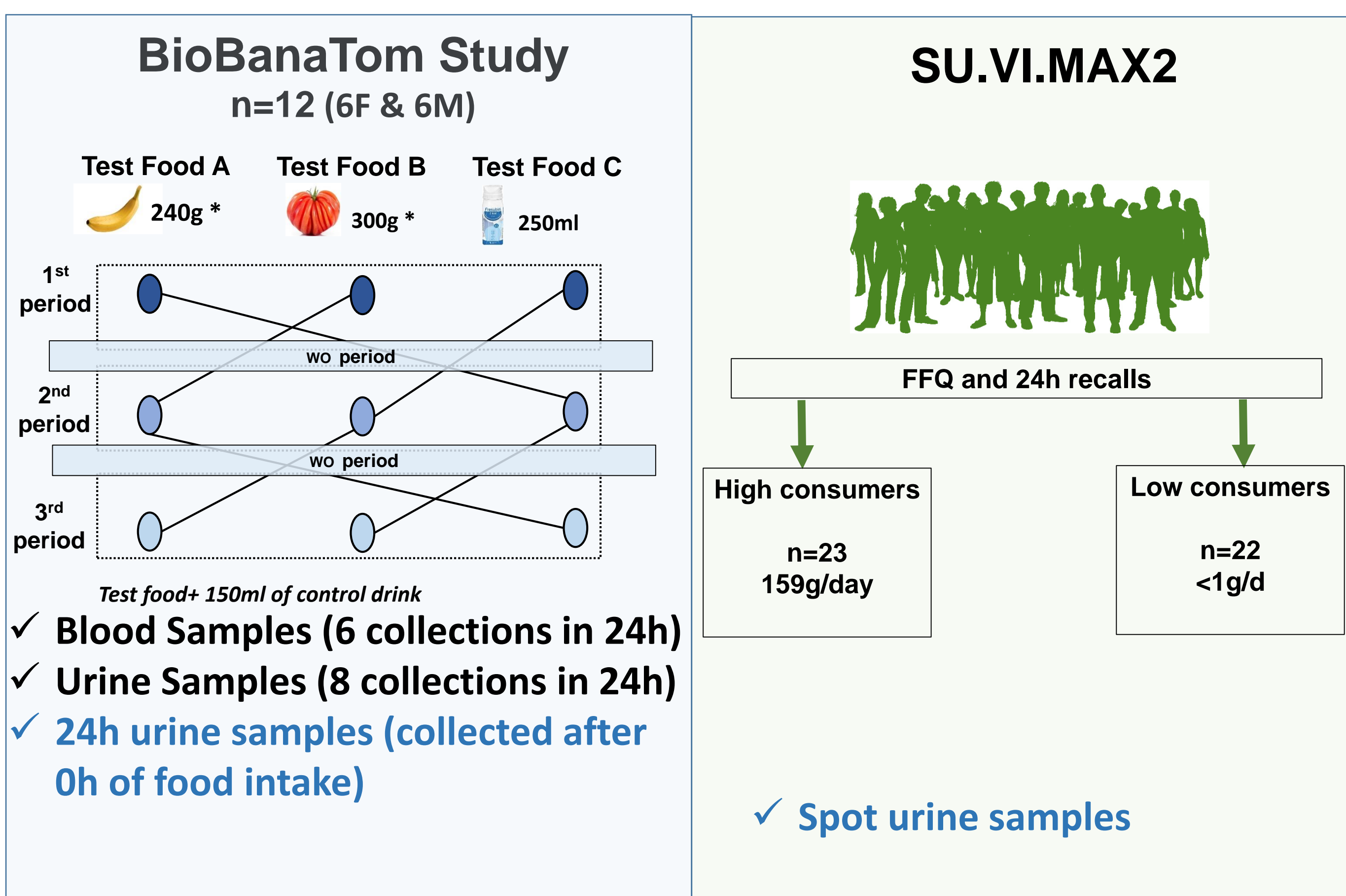
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INTRODUCTION

Untargeted metabolomics allows the discovery of biomarkers of intake of different foods that provide more reliable information about the dietary habits and compliance of volunteers in observational studies and clinical studies than that offered by FFQ or 24hr questionnaires. Banana is ranked amongst the most eaten fruits in the world however, there are no biomarkers of intake reported in the literature yet and little is known about the effect of banana intake on human health. Therefore, in order to elucidate novel biomarkers for acute intake of banana we applied an untargeted metabolomics approach on 24h urine samples of 12 volunteers that participated in an acute intervention study with banana, tomato and a control drink. In parallel, to identify long-term biomarkers of banana intake we applied the same methodology to morning spot urine samples of 45 high and low consumers of banana that participated in the SU.VI.MAX2 cohort study.

METHODOLOGY

STUDIES DESIGN



UNTARGETED METABOLOMICS WORKFLOW

1. UPLC-QTOFMS (QTOF MS Impact II Bruker)

-Column BEH shield RP10 100x41x1.7
-25 min gradient
-ESI(+) and (-)

2. Data pre-processing

-Ion Extraction
-Annotation of ions (CAMERA)



3. Multivariate and Univariate Analysis

-PCA, PLSDA, OSC-PLSDA
-Student T test and Wilcoxon (BH)

SIMCA



4. Identification of Features

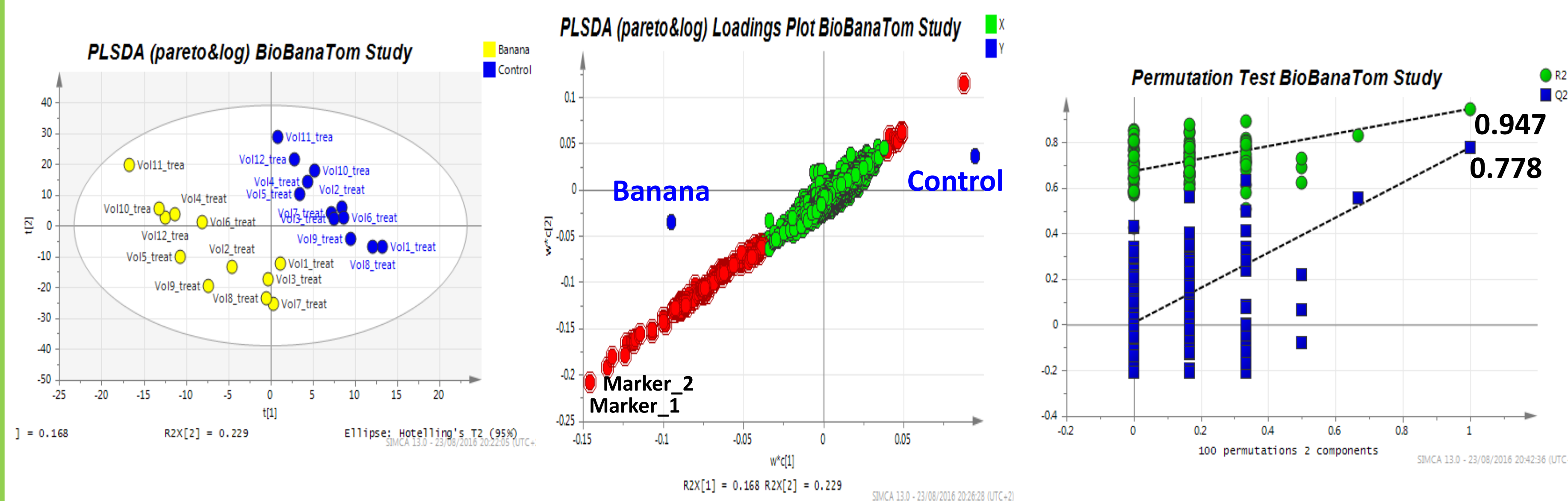
-LTQ-Orbitrap MS
-Database query.
-Literature search.



RESULTS

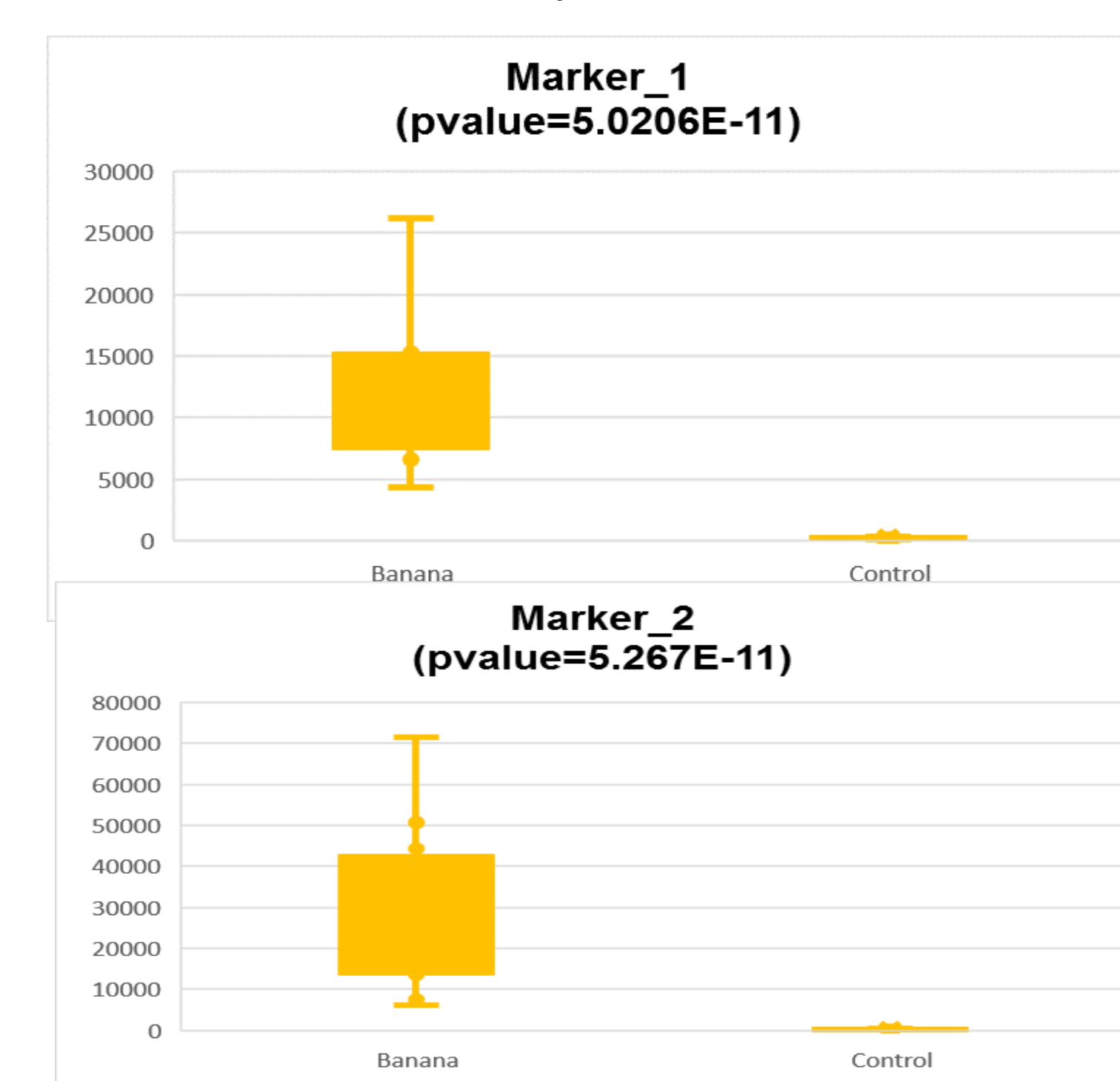
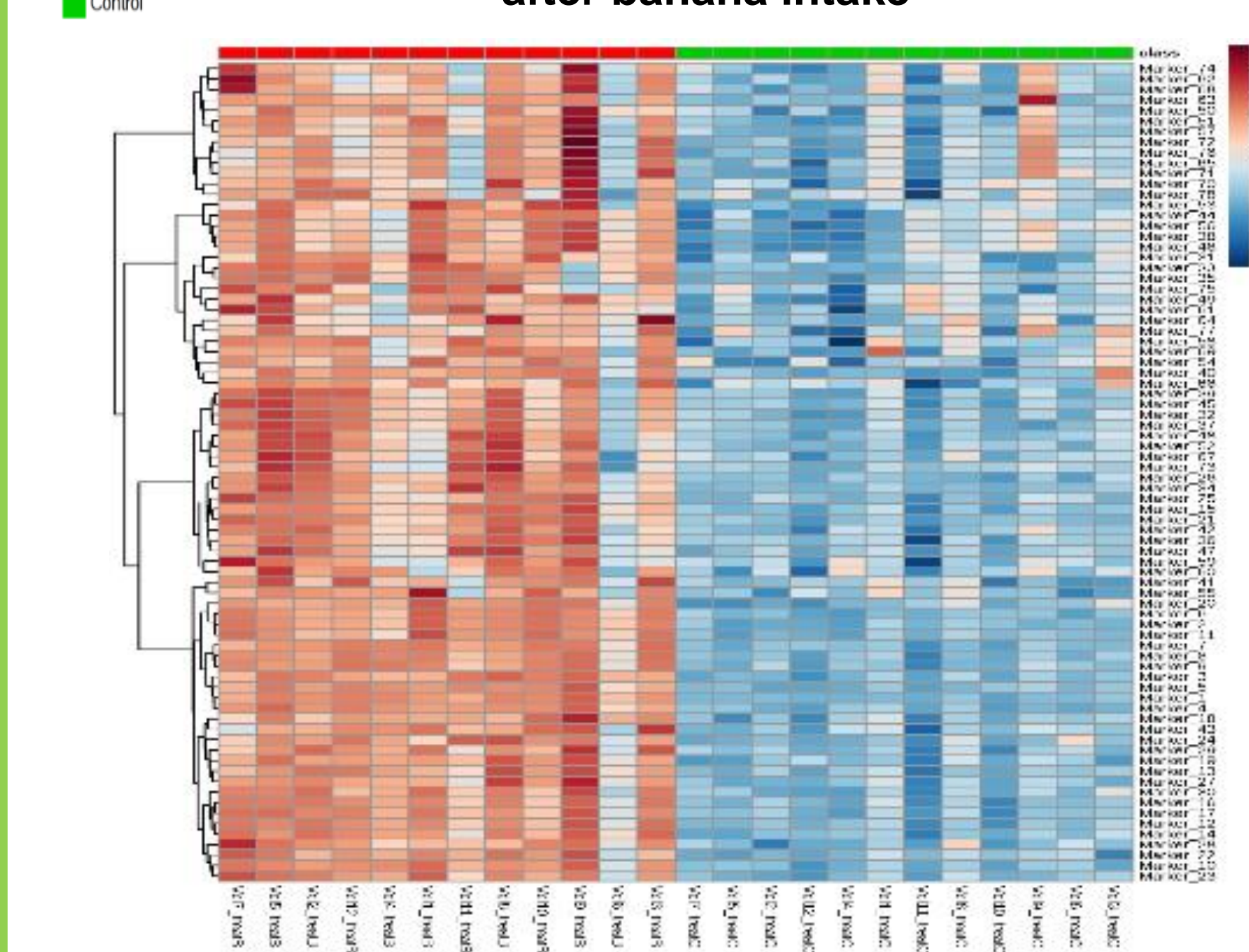
BioBanaTom Study

- The total number of extracted ions was **4092** of which **103** ions showed a high contribution to the discrimination among groups on the PLSDA model (VIP>2).



- After univariate analysis using Student T test with Benjamini Hochberg correction, we detected 85 ions that were significantly different among our groups of treatment. Of these ions, **78** ions were found in higher intensity in the banana intake group.
- We detected **38 metabolite clusters** according to the RT of the 78 candidate biomarkers, will be now identified.
- Kinetic urine and plasma samples are available and will be further analyzed.

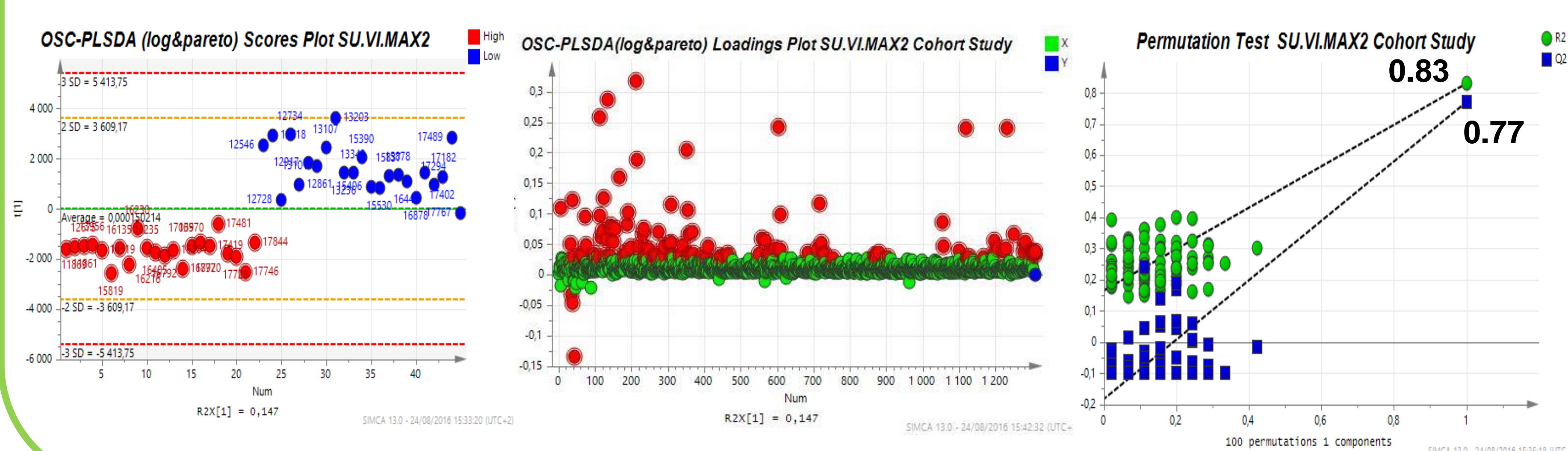
Heat map of 78 most significant metabolites after banana intake



Boxplots of the two most significant ions in univariate analysis; the p value of Student test with BH Correction.

SU.VI.MAX2

- In total **10,249** ions were extracted. Due the high number of ions detected given by the sensitivity of the instrument, we performed a pre selection of the significant ions among groups using a Wilcoxon Test (p value <0.05). We obtained **1308** significant ions on which we performed an OSC-PLSDA.
- With multivariate analysis (OSC-PLSDA), **111** ions were identified as discriminant markers (VIP>1) between high and low consumers of banana.
- We observed that only **3 metabolites** (VIP>1 and p-value <0.05) were in higher intensity in the high consumer group.
- Most the discriminant ions in multivariate and univariate analysis, had higher intensities in the low consumers group which suggests that these may be **markers of effect of the long term consumption of banana rather than biomarkers of intake**.



CONCLUSION

Through the application of an untargeted metabolomics approach in the 24h urine samples of the volunteers recruited in the BioBanaTom study, we were able to detect 78 discriminative ions for the acute intake of banana. The same approach applied to the SUVIMAX2 cohort study, revealed what mainly seem biomarkers of effect of the long term intake of banana. Currently, the identification of the markers selected in these two studies is in progress to later on determine their biological plausibility and validate their utility as biomarkers of banana intake.