# LC-HR-MS/MS BASED UNTARGETED METABOLOMICS FOR URINARY BIOMARKER DISCOVERY OF CHRONIC HIGH AND LOW FLAVONOID FRUIT AND VEGETABLES INTAKE IN HUMANS

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## INTRODUCTION:

Human intervention trials are providing evidence for protective effects of various (poly)phenol-rich foods against chronic diseases like cardiovascular disease, neurodegeneration, osteoporosis and cancer. However, the overall impact of polyphenols on human metabolome is not fully understood.

## OBJECTIVE:

To determine the **impact of diets** enriched in fruit and vegetables (F&V), 2, 4 and 6 portion increases for consequative 6 week intervals, on urine metabolite profiles in people at risk of cardiovascular disease (CVD) (The FLAVURS study, University of Reading).


## STUDY DESIGN:

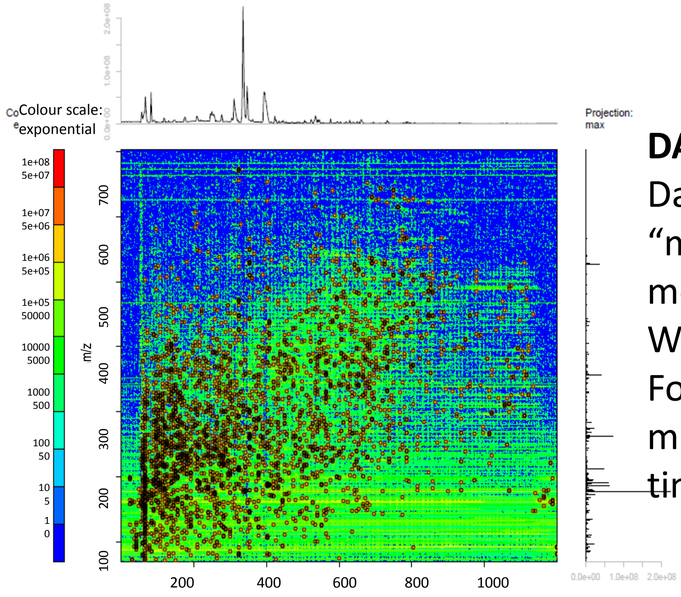
- Randomised, controlled, dose-dependent, parallel study
- Sequentially increasing doses of high flavonoid (HF) or low flavonoid (LF) F&V, compared to control habitual diet (CT).

| Rt | Annotation; Elemental Composition, MW, adjusted p value |
|---|---|
| **1.10** | Proline Betaine; MMW: 143.0946, p 0.002 ↑Diet A; |
| **3.80** | Hydroxy Hippuric Acid (isomer); MMW: 195.0531, p 0.02 ↑Diet A; |
| **4.40** | Hydroxy Hippuric Acid (isomer); MMW: 195.0531, p 0.002 ↑Diet A, |
| **4.82** | Vanilloylglycine, MMW: 225.0637; p.0.03 ↑ Diet A, B; |
| **5.70** | Hippuric Acid, MMW: 179.0582; p 0.002 ↑Diet A |
| **5.89** | Phenylacetylglutamine, MMW: 264.1110, p 0.04 ↑Diet A; |
| **6.15** | Tyrosine , MMW: 181.0731, p 0.04; ↑Diet B |
| **6.26** | Dihydroxyphenyl-γ-valerolactone-O-sulphate MMW:288.0306 p 0.0003 ↑Diet A; |
| **6.56** | Dihydroxyphenyl-γ-valerolactone-O-methyl-O-GLC, p 0.01 ↑ Diet A; |
| **7.27** | Unknown cysteine derivative, p 0.02 ↓DietA |
| **7.35** | Hydroxy Hippuric Acid (isomer), MMW: 195.0531, p 0.01 ↑ Diet A; |
| **7.76** | Hydroxy-tridecenoic acid GLC, MMW: 404.2046, p 0.001 ↑ Diet A; |
| **9.95** | Unknown C19H28O4-GLC; MMW: 496.2308, p 0.03 ↑ DietA |
| **12.30** | Unknown menthol-glucuronide derivative; MMW: 316.1885, p 0.02 ↓ Diet A; |

*Rest of biomarkers is still under construction ....*

## METHODS:

- LTQ FT Orbitrap MS with Dionex HPLC system.
- Chromatographic separation: Kinetex C18 column (150 mm × 2.1 mm I.D., 3.5 um).
- Positive and negative ionization mode, full scan (Res.30000), 3 consecutive MS/MS scan events (res.7500)

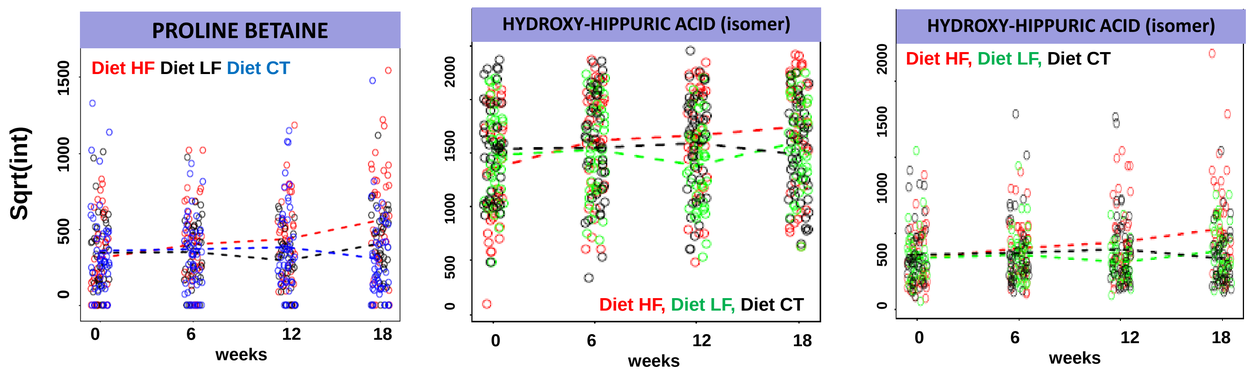



## DATA ANALYSIS:

Data processing - XCMS using the "matchedFilter" peak picking method with Spectra Filter Window Mower function.

For each mass feature two linear mixed models were fitted, diet-time interaction and time alone.

Both models were adjusted for baseline. p values for all features were corrected for multiple testing according to the two-stage Benjamini and Hochberg step-up false discovery rate (FDR).




**CONCLUSIONS:** Chronic, 16 week, intake of high/low-flavonoid F&Vs modulated the metabolic profiles of urine in people at risk of CVD. Biomarkers were principally end-products of microbial polyphenol metabolism like hippuric acid and hydroxy-hippuric acid isomers. Moreover phenylacetyl glutamine (correlated with bifidobacteria activity), tyrosine (related to amino acids), hydroxyphenyl-γ-valerolactone-O-methyl-glucuronides&sulfates, vanilloylglycine (catechin metabolites) and proline betaine were found to be significantly higher in diet HF compared to CT, what is in good agreement with previous studies and our microbiological analysis (data not shown). LC-HR-MS/MS based untargeted metabolomics succeeded in identifying novel biomarkers of chronic F&V intake. Further studies are needed to correlate metabolite profiles with the primary clinical outcome of the study, a significant improvement in vascular function and inflammatory status following enrichment with F&V.

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