Multi-Matrix Biofluid Metabolomics as a Promising Tool for Biomarker Discovery and Pathway Analysis

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1. Introduction

In recent years, metabolomics has surfaced as an innovative research strategy in human metabolism, whereby selection of the biological matrix and its inherent metabolome is of crucial importance. However, focusing on a single matrix may imply that relevant molecules of complementary physiological pathways, covered by other matrices, are missed. The combination of different matrices encloses the potential to reveal more significant results and interesting correlations in comparison to single-matrix analyses [1].

2. Approach

This study presents a unique multi-matrix platform for the concurrent polar metabolic fingerprinting of feces, plasma and urine, applying ultra-high performance liquid-chromatography coupled to hybrid quadrupole-Orbitrap high-resolution mass spectrometry. All three fingerprinting approaches were proven 'fit-for-purpose' through extensive validation in a targeted as well as an untargeted fashion [1, 2].

To demonstrate the potential of the platform, fecal, urine and plasma samples (collected within a single day) from ten healthy volunteers were subjected to metabolic fingerprinting to discriminate according to metabolic state. As no specific disease conditions or age differences were present among the participants, it was opted to determine the metabolic discrepancies between males and females.

3. Results and Discussion

For targeted and untargeted validation of all three matrices, linearity (coefficients of determination $R^2 \geq 0.99$ or 0.90, respectively), recovery (between 70 and 120%) and precision (coefficients of variance $\leq$ 15% or 30%, respectively) were assessed. The excellent validation results indicated that the analytical methods were 'fit-for-purpose' and suitable to accurately map the metabolome, along diverse chemical classes.

The effectivity of the platform was demonstrated by subjecting fecal, urine and plasma samples from ten healthy volunteers to metabolic profiling and fingerprinting, yielding respectively 9672, 9647 and 6122 components, with a substantial overlap of the plasma metabolome with the fecal (69.48%) and urinary metabolome (76.79%) (Figure 1). The fecal metabolome displayed the highest number of components, followed by urine and plasma. Subsequent OPLS-DA modelling suggested that plasma and feces are equally suited to assess gender-dependent metabolic differences. As such, due to its discriminative abilities, non-invasive nature of sample collection and excellent coverage, feces proved itself as an excellent alternative to plasma.

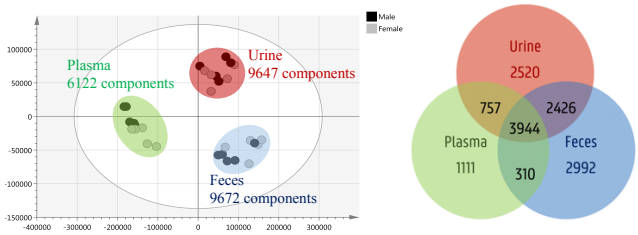

Figure 1. PCA-X score plot and Venn diagram of urine, fecal and plasma samples

To evaluate the added value of a multi-matrix over a single-matrix platform, the fingerprinting abilities of both platforms were compared, revealing an increased number of recovered metabolites and improved model predictivity for the multi-matrix approach. As such, it has the unprecedented potential to reveal more significant results, including the discovery of biomarkers and unraveling of mechanistic information. The latter was demonstrated using targeted profiling, which revealed noticeable differences (i.e. min. 30% difference) between male and female participants in the beta-alanine pathway (Figure 2). In fecal samples from men, higher concentrations of metabolites, associated with the beta-alanine pathway, were observed, while these metabolites were up-regulated in plasma samples from women, which may relate to the menstrual cycle. The increased plasma concentration was then reflected in higher levels of uracil and pantothenic acid in the urine of women [1].

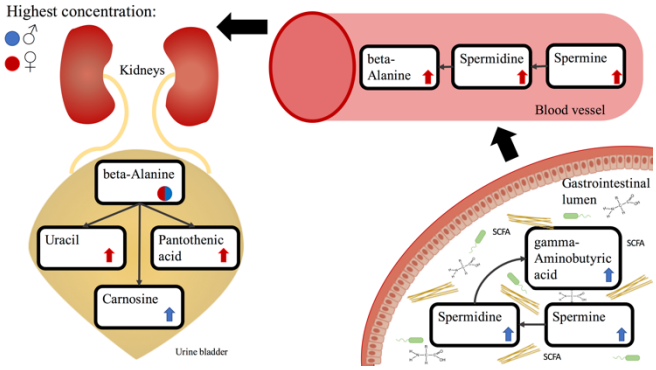

Figure 2. Noticeable changes in the beta-alanine pathway.

4. References

[1] De Paepe et al, "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". *Analytica Chimica Acta* DOI: 10.1016/j.aca.2018.06.065, 2018

[2] Vanden Bussche et al, "Validated High Resolution Mass Spectrometry-Based Approach for Metabolomic Fingerprinting of the Human Gut Phenotype". *Analytical Chemistry* 87:10927-10934, 2015