**A 1H-NMR based metabolomics study of urine and plasma obtained from healthy human subjects**

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**OBJECTIVES**

The aim of this study was to assess the variability of metabolomic data in clinical studies, with severe lifestyle and dietary restrictions.

*Of particular interest were*

a) the variability in urine
b) the optimum time-point representing least variability
c) the detection of diurnal variation
d) the variability in plasma

e) the application of PCA as a preliminary screen to identify outliers and/or subjects who start to deviate from the protocol

**INTRODUCTION**

Metabolomics is well established as a means of disease and toxicity screening in experimental animals. Metabolomics will find increased application in the study of healthy and diseased humans. However, none of the large-scale human investigations is the greater variability in a human population.

Here, we describe an investigation on the plasma and urine metabolites of healthy male volunteers designed to evaluate the variability in metabolomic data under severe lifestyle and dietary restrictions imposed.

**METHODS AND MATERIALS**

Sample and Protocol:

- All subjects were from the A2 healthy volunteer panel.
- The volunteers were subjected to a standard diet and exercise regime in the CPU unit on study days 1 and 2.

1H-NMR spectroscopy:

- 3H NMR spectra of each urine sample were freeze-dried and reconstituted in 200mL D2O.
- Plasma NMR samples were analyzed at 20°C (35°C).

NMR spectra were acquired on a Bruker DRX500 MHz spectrometer using a 5 mm z-90° probe and 64 scans into 64K

- Data were imported into SIMCA-P 8.0 by Umetrics AB (www.umetrics.com) and analyzed by using the Hotelling T2 (Bruker Biospin Inc.)

- PCA scores plot of all urine samples (1H NMR, 0-12h, 12-24h, 24-48h, N=100) are shown in Figure 4a-b.

**CONCLUSIONS**

**Urine**

1. Observation of distinct inter-individual variability (representing normal genetic variation), but generally low intra-individual variability over 2 weeks.
2. The first void urines were more variable than later time-points (may reflect differences in the subject’s diets and life-style).
3. The urine spectra showed some diurnal variation (with prominent excitation in first void urines).
4. PCA didn’t reliably highlight volunteer 11’s urine sample (study day 2), containing high mannitol, as an outlier.

**Plasma**

1. Plasma are prone to inter-individual variability due to differences in glucose/lipid concentration.
2. However, it may be difficult to separate population ¹ from population ².
3. Generally, intra-individual variability was low over 2 weeks.
4. Blood sampling at the same time-point following dietary control may be advisable.

Urinary profiles are governed by dietary preferences and life-style effects.

*Hence, standardisation of diet, life-style and time of sample collection appear to be highly advisable!*

**REFERENCES**