P13: EXPLORING THE LINK BETWEEN THE CIRCULATORY AND EXCRETED METABOLOME DURING PREGNANCY – A PILOT STUDY

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Abstract

This exploratory research effort describes a metabolomic approach to elucidate the link between the circulatory and excreted metabolome during the 2nd trimester of pregnancy. NMR metabolomics was employed, in order to assess the maternal serum and urine fingerprint. The metabolites were integrated into a metabolic correlation network, to visualize the resulting correlation matrices in circulatory and excreted metabolomes. It would be of outmost importance to capitalize on metabolomics, in order to unravel the biochemical pathways towards further understanding of pregnancy metabolism.

Keywords: pregnancy, NMR metabolomics, serum, urine, metabolite correlations

1. INTRODUCTION

Metabolomics is considered to be a particularly promising field in scientific research, as it provides a high-throughput identification of a range of metabolites that are presented in biological matrices [1].

Several biofluids are employed in metabolomic research; however, the most commonly used are blood (serum and plasma) and urine [2]. Each of them has its own advantages and disadvantages. For instance, urine is less invasively collected and less costly compared to blood [3]. A major difference between urine and blood is the fact that urine contains a significantly higher proportion of non-metabolites - such as phytochemicals and xenobiotics - than blood [1].

The present exploratory research effort describes an NMR-metabolomic study of serum and urine obtained from healthy women during the 2nd trimester of pregnancy with the aim to assess the metabolic profile of these biospecimens. Furthermore, biomarkers measured in maternal serum are correlated to those present in maternal urine, an approach that, according to the recent literature [4], enables a more comprehensive metabolic picture of pregnancy.

2. EXPERIMENTAL

Twenty - three women in their 2nd trimester of pregnancy (18 - 22 weeks of gestation) participated in the current study. All participants provided one sample of serum and one of urine under non-fasting conditions, owing to medical restrictions in controlling or restricting maternal diet during pregnancy. Nuclear magnetic resonance (NMR) metabolomics was employed and assessed the maternal serum and urine fingerprint. NMR spectra were acquired using a 600MHz spectrometer and the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. Albumin was, also determined using an automatic analyzer (ARCHITECT c8000; Abbott Diagnostics). Correlation coefficients (Pearson's *r*) were calculated in order to test the association between biochemical markers in maternal serum and urine. These associations were then used to compute a correlation network. Statistical analysis was performed using the SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL). The significance level of all hypotheses tests was set at $p<0.05$.

This study was approved by the Bioethics Committee of the Faculty of Medicine of Aristotle University, Thessaloniki, Greece (A19479 – 26/2/08).

3. RESULTS AND DISCUSSION

The metabolic profile of maternal serum is presented in **Figure 1**. The metabolites were integrated into a metabolic correlation network to visualize the resulting correlation matrix in serum, as described in the red area of **Figure 2**. In this network, line width and color reflect strength and sign of the presented correlations, respectively. The recorded positively statistical significant correlation between HDL and LDL (p=0.002) and negative correlations between these lipoproteins and albumin (p=0.05 and p=0.049, respectively) are in accordance with the literature results [4]. Furthermore, albumin was found to be positively correlated with glutamine (p<0.001), as well as creatine (p=0.02). In turn, creatine was correlated with valine (p<0.001) and glutamine (p<0.001). A positive correlation was, also, recorded between the latter amino acid and alanine (p<0.001). It is worth mentioning that similar correlations and trends have also been reported in a very recent research paper by Pinto et al. (2015) [4].

As far as urine intermetabolite correlations are concerned **(Figure 2,** yellow area**)**, our data confirmed the recent recorded significant associations between urinary choline and betaine (p<0.001) [5], suggesting their common biochemical pathway. At this point, it should be highlighted that the enzymatic oxidation of choline delivers betaine, a methyl donor for the remethylation of homocysteine (Hcy) into methionine [5]. The essential amino acid methionine is of great importance during intrauterine life and may affect fetal growth and development, since *inter alia* the conjugated metabolic pathways of methionine and folate deliver methyl groups for use in critical processes, such as purine synthesis, as well as phospholipid and protein biosynthesis [6]. The statistically significant association recorded between the essential amino acids leucine and isoleucine (p<0.001) has also been underlined by Diaz et al. (2013) [7] and Pinto et al. (2015) [4], whose scientific interest has focused on the exploration of the excreted metabolome in healthy pregnancies. It should be emphasized that these two branched chain amino acids play a crucial role in metabolic regulation and fetal maturity [8].

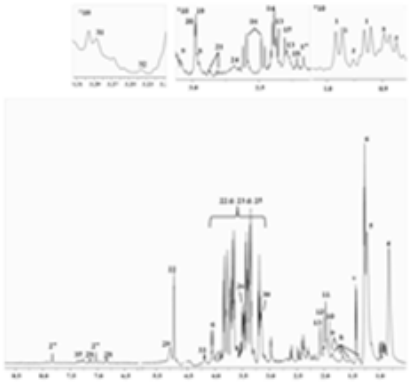

Figure 1. NMR spectra of a serum sample with annotation on the identified metabolites.
1:Valine, 2:Leucine, 3:Isoleucine, 4:Cholesterol-VLDL, 5:LDL2/VLDL2, 6:Lactic acid, 7:Alanine, 8:Lysine, 9:Arginine, 10:Acetic acid, 11:NAC1, 12:NAC2, 13:N-acetyl glutamine, 14:Glutamine, 15:Glutamic acid, 16:Citric acid, 17:Acetone, 18:Acetoacetate, 19:Creatine, 20:Creatinine, 21:Asparagine, 22:a-D-glucose, 23:b-d-glucose, 24:Methylamine, 25:Glycerol, 26:Glycine, 27:Histidine, 28:Tyrosine, 29:Unsaturated lipid, 30:Choline, 31:Betaine, 32:Phosphocholine, 33:Threonine, 34:Formic acid, 35:Phenylalanine.

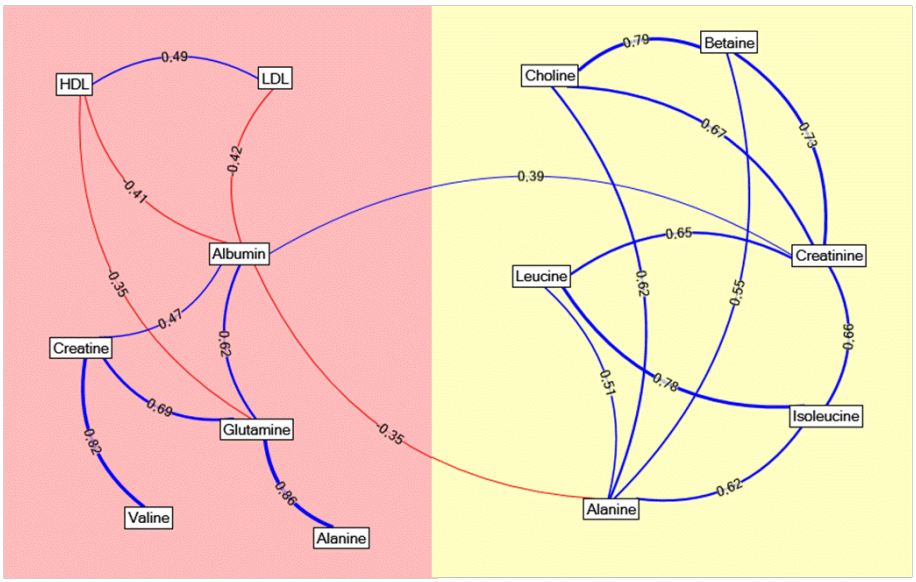

Figure 2. Correlation network of maternal serum (red area) and urine (yellow area) metabolites. Blue lines correspond to positive correlations, while red to negative. Line thickness reflects the magnitude of the correlation coefficients, also written numerically

Over the past decade, metabolomic analysis of maternal blood and urine has received much attention and may be considered a new dynamic field for assessing healthy pregnancy [9, 10, 11, 12, 13, 14]. Within this context, we explored the potential associations between the two metabolomes; the circulatory and excreted of our paired serum and urine samples. In particular, albumin levels in serum were related to urine creatinine concentrations (p=0.038), an observation that concurs with the findings of Pinto et al. (2015) [4]. However, the positive correlation between blood albumin and urine alanine (p=0.042) is not in conformity with the results of the aforementioned study [4]. Overall, these findings support and reflect the complexity of pregnancy metabolism. It would be of outmost importance to further capitalize on metabolomic profiling, in order to unravel the key metabolic pathways involved in human reproduction. Finally, we cannot rule out the possibility of residual confounding factors, such as maternal diet and its impact on metabolic process during pregnancy.

AKNOWLEDGMENT

This research has been co-financed by the European Union (European Social Fund—ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)—Research Funding Program: Thales. Investing in knowledge society through the European Social Fund.

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