**Global Metabolomics of the Placenta Over 24 Hours**

Walejko, J. (UF, Biochemistry & Molecular Biology); Chelliah, A.; Gregg, A. (UF, Obstetrics & Gynecology); Keller-Wood, M. (UF, Pharmacodynamics) and Edison, A. (UGA, Genetics & Biochemistry & Molecular Biology)

**Introduction**

The current data for optimization of placental specimen collection for metabolomics studies suggest

immediate processing of specimens.1 This poses a significant challenge during specimen collection and the lack of timely processing may result in loss of useful metabolic data. We aim to evaluate changes in metabolites of

placental tissue at various time points up to 24 hours post-delivery.

**Experimental**

Five gravid full-term, non-labored patients were identified at University of Florida (UF) Health Shands, Gainesville, Fl, and consented for participation in UF IRB approved study IRB20150007. Placentas were collected immediately following cesarean delivery, and stored at 4°C within 1 hr. Tissue specimens were frozen in liquid nitrogen at 4 time points post-delivery: 15 min, 30 min, 1 hr and 24 hrs. A total of 4 sections were taken from each placenta. Specimens were stored at -80°C until extraction. For extraction, 80-150 mg of tissue and 500 μL of 1:1 methanol-water solution were added to each sample. Samples were homogenized for 20 min and centrifuged at 3000 g for 15 min at 4°C. 450 μL of resulting supernatant was dried with nitrogen dryer. Extracts were kept at -80°C until preparation for NMR. During NMR preparation, extracts were brought up in 600 μL sodium phosphate buffer with 0.33 mM trimethylsilyl propanoic acid (TSP). The resulting solution was centrifuged at 3000 g for 15 min at 4°C and 590 μL of clean supernatant was transferred to 5-mm NMR tubes. Nuclear magnetic resonance spectroscopy was conducted on an Avance II 600 MHz spectrometer at the University of Florida Advanced Magnetic Resonance Imaging and Spectroscopy to gain global metabolic profiles of placental samples. Significance of metabolites was determined using a student's t-test of the area under the metabolite peak(s) of probabilistic quotient normalized spectra.

**\*p-value<0.05**

VLDL/LDL: Very low density lipoprotein/Low density lipoprotein

BCAA: Branched chain amino acids (leucine, isoleucine, valine)

**Table I: Percent change of placental metabolites following 15 minutes post-delivery**



**Results and Discussion**

A persistent profile of 19 identified metabolites was not significantly changed following 30 minutes post-delivery, and were still measurable across all time points independent of maternal subject variability (**Table I**). The profile of metabolites significantly increased at 24 hours, including amino acids, lactate and choline, while glycerophosphocholine (GPC), glucose, and taurine were significantly decreased (p<0.05). Acidic amino acids, creatine, acetate, histidine, trimethylamine N-oxide (TMAO), formate, and 3-hydroxybutyrate (3HB) did not significantly differ over 24 hours.

**Conclusions**

In our analysis, several metabolites persisted in placental tissue through all time points, up to 24 hours while lactate and glucose displayed variability reflecting anaerobic glycolysis. Our study challenges the current notion of immediate specimen processing, suggesting there may be valuable metabolic data present in tissue up to 24 hours post-delivery. To date, there is limited data on metabolic transport in the placenta, and the persistence of metabolites may be used to gain information on altered metabolism in this tissue.

**Acknowledgements**

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida. In addition, we would like to acknowledge NIH SECIM grant 1U24DK097209-01A1, NIH grants HD057871, and NIH/NCATS TL1 TR000066 and UL1 TR000064.

**References**

[1] Serkova N., *et al*., Placenta, **24**, 227-235 (2003).