Background

- One carbon (1C) metabolism is a system of biochemical pathways that supply methyl groups for DNA methylation and is catalyzed by many different enzymes where several diet-derived micronutrients serve as co-factors.
- Understanding how 1C metabolites change over the course of pregnancy has implications for maternal-fetal health. However, very few studies have longitudinally assessed metabolomics prior to and during pregnancy.
- Increased pre-pregnancy BMI (ppBMI) represents a key prenatal exposure that may increase the susceptibility for obesity, particularly in low- and middle-income countries undergoing a nutrition transition. Maternal obesity and other forms of malnutrition (micronutrient deficiency, high fat diet) are associated with altered 1C metabolism in rodent models and clinical studies.

Primary Hypothesis

- We aimed to investigate the relationship between 1C metabolite concentrations and body mass index (BMI) prior to and during pregnancy in a sub-cohort of Guatemalan women. We hypothesized that 1C metabolite concentrations would be altered by time and pre-pregnancy BMI (ppBMI).

Materials and Methods

Study Design: This was a cross-sectional, secondary study of a large randomized controlled trial called Women First (NCT01883193).

Subjects: This study included 113 Guatemalan women with normal weight (BMI 18.5-24.9; n=58), and overweight (BMI > 25; n=57) ppBMI. Whole blood was collected at 3 time points: (1) pre-conception, (2) 12 weeks gestation, and (3) 34 weeks gestation.

1C metabolomics: A targeted 1C metabolite assay was performed on two 3 mm punch biopsies from the umbilical cord blood spot (DBS) cards utilizing liquid chromatography/tandem mass spectrometry that covered 27 metabolites. Authentic isotopically-labelled standards were used as internal benchmarks for each metabolite. Several key 1C metabolites (including S-adenosylhomocysteine, S-adenosylhomocysteine, folate and homocysteine) were not detected due to the limitations of the DBS samples.

Statistics: Statistical analysis was performed using GraphPad Prism 8 and Microsoft Excel. An unpaired Student’s t-test was performed to test differences in 1C metabolite concentrations between groups. Two-way ANOVA was performed to evaluate differences in 1C metabolite concentrations by time and ppBMI and test for time x ppBMI interactions. Significance was set at p < 0.05.

1C Metabolism Pathway

Figure 1. One carbon metabolism pathway and interacting metabolic pathways.

Table 1. Maternal characteristics and anthropometric data were collected at enrollment, prior to pregnancy. Data are presented as mean ± SEM. A paired Student’s t-test was performed to test differences between women with NW or OW/Ob ppBMI. Significance was set at p ≤ 0.05. Socioeconomic status (SES) score was calculated as a score from 0-6, with one point each for electricity, improved water source, sanitation, man-made flooring, improved cooking fuels and household assets such as television, car or telephone. BMI= body mass index, NW= normal weight, OW/Ob= overweight/obese, SEM= standard error of the mean.

<table>
<thead>
<tr>
<th>Metabolite (molar)</th>
<th>Normal Weight</th>
<th>Overweight/Obese</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 weeks</td>
<td>34 weeks</td>
</tr>
<tr>
<td></td>
<td>M=SEM</td>
<td>M=SEM</td>
<td>M=SEM</td>
</tr>
<tr>
<td>Glutamine</td>
<td>192±13.3</td>
<td>166±6.6</td>
<td>165±5.5</td>
</tr>
<tr>
<td>1-Carnitine</td>
<td>15.9±7.0</td>
<td>17.8±8.5</td>
<td>17.3±7.1</td>
</tr>
<tr>
<td>Arginase</td>
<td>15.9±7.0</td>
<td>17.8±8.5</td>
<td>17.3±7.1</td>
</tr>
<tr>
<td>Prolinase</td>
<td>15.9±7.0</td>
<td>17.8±8.5</td>
<td>17.3±7.1</td>
</tr>
</tbody>
</table>

Table 2. 1C metabolite concentrations were measured at baseline, 12 weeks, and 34 weeks of gestation. Two-way analysis of variance was performed to test for an interaction of time and ppBMI and for the effects of time and ppBMI. A p-value of 0.05 was considered statistically significant. Significant results in **bold.** Data are shown as mean ± SEM. ADMA = Asymmetric Dimethylarginine, ADA = Adenine, ARG = Arginine, hARG = Homocitrulline, MeARG = (Mono)methylarginine, BET = Betaine, CHOL = Cholesterol, CIT = Citrulline, CRE = Creatine, CRN = Creatinine, total CYS = total Cysteine, GLU = Glutamine, total GSH = total Glutathione, HIS = Histidine, ILE = Isoleucine, LEU = Leucine, LYS = Lysine, MET = Methionine, ORN = Ornithine, PHE = Phenylalanine, PRA = Proline, SDMA = Symmetric Dimethylarginine, SER = Serine, TAU = Tauurine, THR = Threonine, TRP = Tryptophan, TYR = Tyrosine, VAL = Valine.

Conclusions

- Time had a much larger influence on 1C metabolite concentrations than ppBMI in NW and OW/Ob Guatemalan women during pregnancy.
- Maternal ppBMI appeared to only statistically influence homocitrulline and creative metabolite concentrations.
- Limitations of this study include the absence of dietary and insulin resistance data, which limits consideration of behavioral and metabolic variables related to maternal obesity that could influence the relationships between ppBMI and the metabolome. ppBMI was treated categorically, instead of as a continuous variable, which may have masked subtle BMI differences.
- Future studies will include longitudinal analysis and will examine the relationship between dietary micronutrients, DNA methylation, and maternal and infant health outcomes at birth and during the first six months of life.

Acknowledgements

Many thanks to the women who participated in the Women’s First trial, as well as all other international collaborators and on-site personnel. Funding for this project was provided by Bill & Melinda Gates Foundation OPP105367 and the NIH ODS U10 HD075474, DK109077, and U24 DK097209. Figure 1 was adapted from Eoin Quinlivan, PhD at the University of Florida. Finally, this MSA project would not have been possible without Sarah Borengasser, PhD and Stephanie Gilley, MD, PhD. I am deeply grateful for their guidance and mentorship.