Influence of maternal nutrition in pregnancy

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Metabolomics as a tool for the assessment of
A. the embryo growth and viability in *in vitro* fertilization and

B. The influence of maternal nutrition on
   1. Fetal and neonatal growth
   2. maternal blood and amniotic fluid composition
FETAL PROGRAMMING
alteration of gene expression during critical periods of development

- thrifty phenotype hypothesis
- fetal overnutrition hypothesis

- maternal undernutrition
- maternal overnutrition

- Fetal malnutrition

- fetal undernutrition
- Fetal overnutrition

SGA & LGA
Metabolic, endocrine, and cardiovascular diseases in adult life

Hales, C. N., & Barker, D. J.: *British medical bulletin*, 2001
Influence of maternal nutrition in pregnancy

1. Relation of maternal pre-pregnancy BMI with amniotic fluid amino acids
2. Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles
3. Dietary patterns reflecting 2\textsuperscript{nd} trimester amniotic metabolomic profile
Second trimester amniotic fluid amino acid levels in relation to maternal pre-pregnancy Body Mass Index

Maria Fotiou¹; Gianna Georganta¹; Foteini Tsakoumaki¹; Alexandra-Maria Michaelidou¹; Apostolos P. Athanasiadis²; George Menexes³; Maria Ganidou²; Georgios Theodoridis⁴; Costas G. Biliaderis¹; Basilis Tarlatzis²

(1) Aristotle University of Thessaloniki, School of Agriculture, Department of Food Science & Technology; (2) Aristotle University of Thessaloniki, 1st Department of Obstetrics & Gynecology; (3) Aristotle University of Thessaloniki, School of Agriculture, Department of Field Crops and Ecology; (4) Aristotle University of Thessaloniki, Department of Chemistry, Laboratory of Analytical Chemistry

Aim of the study

2nd trimester amniotic fluid amino acid composition

↓

maternal pre-pregnancy Body Mass Index (BMI)
Patients - Method

Subjects:
54 singleton pregnant women undergoing amniocentesis for prenatal diagnosis, between 17 and 24 weeks of gestation.

Somatometric parameters:
Data collection from questionnaires

According to the World Health Organization BMI criteria, women were divided into three groups:

- the normal group (pre-pregnancy BMI 18.5-24.9 kg/ m², n= 25),
- the overweight group (pre-pregnancy BMI 25-29.9 kg/ m², n= 19),
- the obese group (pre-pregnancy BMI >30 kg/ m², n=10)

Amniotic fluid analysis:
RP-HPLC / dabsyl chloride derivatives
## Correlation between amniotic fluid amino acids and maternal pre-pregnancy BMI

<table>
<thead>
<tr>
<th>maternal pre-pregnancy BMI (kg/m²)</th>
<th>ala</th>
<th>val</th>
<th>ile</th>
<th>leu</th>
<th>phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s $r$</td>
<td>0.253</td>
<td>0.422</td>
<td>0.306</td>
<td>0.363</td>
<td>0.329</td>
</tr>
<tr>
<td>$P$</td>
<td>0.065</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*BMI, body mass index*
Correlation between amniotic fluid amino acids and maternal pre-pregnancy BMI

Multivariate analysis of variance (MANOVA):

Obese group

↑

val
leu
ile

Normal group (p<0.05)

It has long been recognized that several amino acids, including val, leu, ile, are elevated in the blood of obese subjects relative to healthy individuals.


↑↑ Amino acid concentrations

↑↑ Placental transport systems and


that are required by fetal development
Gestational age and maternal pre-pregnancy body mass index: potential determinants of human amniotic fluid metabolic profile

Apostolos Athanasiadis¹; Panagiotis Zoumpoulakis²; Alexandra-Maria Michaelidou³; Charalambos Fotakis²; Maria Fotiou³; Foteini Tsakoumaki³; Gianna Georganta³; George Menexes⁴; Maria Ganidou¹; Georgios Theodoridis⁵; Costas G. Biliaderis³; Basilis Tarlatzis¹

(1) Aristotle University of Thessaloniki, 1st Department of Obstetrics & Gynecology; (2) Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation; (3) Aristotle University of Thessaloniki, School of Agriculture, Department of Food Science & Technology; (4) Aristotle University of Thessaloniki, School of Agriculture, Department of Field Crops and Ecology; (5) Aristotle University of Thessaloniki, Department of Chemistry, Laboratory of Analytical Chemistry

Aim of the study

Second trimester amniotic fluid profile

gestational age

maternal pre-pregnancy body mass index (BMI)

using
a conventional analytical research protocol and
a holistic NMR metabolomics approach
Method

Subjects:
44 singleton pregnant women, undergoing amniocentesis for prenatal diagnosis between 17 and 24 weeks of gestation.

Somatometric parameters:
Data collection from questionnaires

According to the World Health Organization BMI criteria, women were divided into two groups:

- the normal group (pre-pregnancy BMI 18.5–24.9 kg/m², n= 25),
- the overweight group (pre-pregnancy BMI 25–29.9 kg/m², n= 19)

Amniotic fluid analysis:
RP-HPLC / dabsyl chloride derivatives

Varian-600MHz NMR spectrometer with a 1H {13C – 15N} 5mm PFG Autox ID probe - National Hellenic Research Foundation
Effect of maternal pre-pregnancy BMI on amniotic fluid by the conventional analytical research protocol (HPLC)
Maternal nutritional status, as it is reflected in pre-pregnancy BMI and gestational week of 2nd trimester may have an effect on amniotic fluid composition.

Figure 1: OPLS-DA, Par, A=3+2, N=19, R^2= 0.92, Q^2= 0.33

- Group B: BMI=NL, Test Week >20.
- Group C: BMI=OW Test Week < 20.
- Group D: BMI=NL, Test Week < 18.
Influence of maternal nutrition in pregnancy

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2. Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles
3. Dietary patterns reflecting 2\textsuperscript{nd} trimester amniotic metabolomic profile
Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles

Aim of the study

- second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations
- gestational age
- neonatal growth
- explore the possible associations between BMI and:
  - amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations
  - birth weight centiles

Fotiou M: JMNFM, 2014
Second trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles

Method

Subjects:
52 pregnant women, undergoing amniocentesis for prenatal diagnosis in the second trimester of pregnancy.

Amniotic fluid analysis

automatic multichannel analyzer (Abbott Diagnostics—ARCHITECT c8000)

Department of Biochemistry-Microbiology-Virology, Papageorgiou General Hospital of Thessaloniki, Thessaloniki, Greece

Neonatal somatometric parameters:
Information from individuals’ medical records.

Fotiou M: JMNFM, 2014
2\textsuperscript{nd} trimester amniotic fluid glucose, uric acid, phosphate, potassium, and sodium concentrations in relation to maternal pre-pregnancy BMI and birth weight centiles

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>glucose</th>
<th>uric acid</th>
<th>phosphate</th>
<th>potassium</th>
<th>sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>maternal pre-pregnancy BMI (kg/m(^2))</td>
<td>Pearson’s ( r )</td>
<td>0.274</td>
<td>0.460</td>
<td>0.159</td>
<td>0.161</td>
</tr>
<tr>
<td></td>
<td>( p )</td>
<td>0.052</td>
<td>&lt;0.01</td>
<td>0.261</td>
<td>0.254</td>
</tr>
</tbody>
</table>

BMI, body mass index

Fotiou M: JMNFM, 2014
Correlations between urea, uric acid and potassium in maternal serum and amniotic fluid

<table>
<thead>
<tr>
<th>Biochemical markers</th>
<th>$r'$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.233</td>
<td>0.263</td>
</tr>
<tr>
<td>Urea</td>
<td>0.568</td>
<td>0.002</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.426</td>
<td>0.027</td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.060</td>
<td>0.769</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.016</td>
<td>0.941</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.635</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.227</td>
<td>0.254</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.318</td>
<td>0.106</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.266</td>
<td>0.180</td>
</tr>
</tbody>
</table>

Urea and potassium levels demonstrate strong positive correlations between maternal serum and amniotic fluid,

Fotiou M: Fetus as Patient, 2014
Correlation between biochemical markers in maternal serum and amniotic fluid in the 2nd trimester of pregnancy

- n=27
- Uric acid in AF and maternal serum were positively associated

Uric acid serum (mg/dl): 2.76 ± 0.63
Uric acid AF (mg/dl): 3.51 ± 0.61

$r=0.426$ $p=0.027$

Fotiou M: Fetus as Patient, 2014
Possible mechanism for the distribution of URIC ACID in maternal blood and amniotic fluid

These studies could provide useful information towards lifestyle and dietary interventions

- maternal uric acid
- fetal uric acid
- amniotic fluid uric acid


Influence of maternal nutrition in pregnancy

1. Relation of maternal pre-BMI with amniotic fluid amino acids
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3. Dietary patterns reflecting 2\textsuperscript{nd} trimester amniotic metabolomic profile
Nutrient intake

Amniotic fluid amino acid levels

Preterm Delivery
Estimated fetal weight
Neonatal weight
**Impact of maternal nutrition on amniotic fluid amino acid concentrations in humans**


<table>
<thead>
<tr>
<th>Amniotic fluid</th>
<th>Fed††</th>
<th>Fasted§</th>
<th>P‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td>77.2 ± 5.4†</td>
<td>98.9 ± 7.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Threonine</td>
<td>197.7 ± 28.2</td>
<td>173.7 ± 14.2</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>33.5 ± 3.4</td>
<td>42.9 ± 2.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Proline</td>
<td>175.2 ± 12.9</td>
<td>159.9 ± 9.7</td>
<td>NS</td>
</tr>
<tr>
<td>Citrulline</td>
<td>12.0 ± 0.5</td>
<td>8.1 ± 1.5</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Glycine</td>
<td>169.8 ± 18.2</td>
<td>151.9 ± 11.6</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>353.0 ± 21.5</td>
<td>264.8 ± 17.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>α-NH₂ butyrate</td>
<td>13.4 ± 2.7</td>
<td>52.7 ± 2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Valine</td>
<td>136.2 ± 9.4</td>
<td>255.3 ± 18.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystine</td>
<td>77.8 ± 8.0</td>
<td>72.8 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Methionine</td>
<td>19.2 ± 2.0</td>
<td>20.3 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>23.8 ± 3.1</td>
<td>68.3 ± 5.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leucine</td>
<td>51.7 ± 5.1</td>
<td>133.4 ± 11.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>43.7 ± 1.8</td>
<td>46.6 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>48.5 ± 1.9</td>
<td>52.2 ± 3.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

* The basic amino acids were not measured in the amniotic fluid samples.
†† Amniotic fluid and maternal plasma were obtained 3–6 hr after the previous meal in subjects maintained on a 2200–2400 kcal diet (n = 6).
§ Amniotic fluid and maternal plasma were obtained after 84–90 hr of fasting (n = 12).
‖ P = significance of differences between mean values in fed and fasted groups (unpaired t test).
† Mean ± se, μmoles/liter.
Impact of maternal nutrition on amniotic fluid amino acid concentrations in ewes

Kwon et al: Biology of reproduction 2004

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control†</th>
<th>Restricted‡</th>
<th>Realimented§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>194 ± 8a</td>
<td>71 ± 2b</td>
<td>114 ± 11b</td>
</tr>
<tr>
<td>B-Ala</td>
<td>162 ± 11</td>
<td>149 ± 8</td>
<td>144 ± 13</td>
</tr>
<tr>
<td>Arg</td>
<td>186 ± 8b</td>
<td>39 ± 4c</td>
<td>95 ± 13b</td>
</tr>
<tr>
<td>Asn</td>
<td>37 ± 2a</td>
<td>17 ± 2b</td>
<td>32 ± 3a</td>
</tr>
<tr>
<td>Asp</td>
<td>22 ± 1a</td>
<td>15 ± 2b</td>
<td>17 ± 1b</td>
</tr>
<tr>
<td>Cit</td>
<td>178 ± 8a</td>
<td>22 ± 3c</td>
<td>43 ± 5b</td>
</tr>
<tr>
<td>Cys</td>
<td>127 ± 4a</td>
<td>50 ± 4c</td>
<td>87 ± 5b</td>
</tr>
<tr>
<td>Gln</td>
<td>386 ± 14a</td>
<td>86 ± 8c</td>
<td>121 ± 8b</td>
</tr>
<tr>
<td>Glu</td>
<td>69 ± 4a</td>
<td>41 ± 3b</td>
<td>50 ± 4b</td>
</tr>
<tr>
<td>Gly</td>
<td>326 ± 14a</td>
<td>184 ± 6c</td>
<td>240 ± 18b</td>
</tr>
<tr>
<td>His</td>
<td>26 ± 2a</td>
<td>12 ± 1c</td>
<td>18 ± 1b</td>
</tr>
<tr>
<td>Ile</td>
<td>29 ± 2a</td>
<td>10 ± 1c</td>
<td>14 ± 1b</td>
</tr>
<tr>
<td>Leu</td>
<td>50 ± 2a</td>
<td>17 ± 1c</td>
<td>31 ± 2b</td>
</tr>
<tr>
<td>Lys</td>
<td>55 ± 2a</td>
<td>19 ± 2c</td>
<td>28 ± 2b</td>
</tr>
<tr>
<td>Met</td>
<td>22 ± 2a</td>
<td>16 ± 1b</td>
<td>17 ± 1b</td>
</tr>
<tr>
<td>Orn</td>
<td>25 ± 1a</td>
<td>13 ± 1b</td>
<td>14 ± 1b</td>
</tr>
<tr>
<td>Phe</td>
<td>27 ± 1a</td>
<td>9 ± 1c</td>
<td>13 ± 1b</td>
</tr>
<tr>
<td>Pro</td>
<td>141 ± 5a</td>
<td>54 ± 3c</td>
<td>82 ± 5b</td>
</tr>
<tr>
<td>Ser</td>
<td>771 ± 43a</td>
<td>205 ± 4c</td>
<td>365 ± 31b</td>
</tr>
<tr>
<td>Taurine</td>
<td>127 ± 10</td>
<td>102 ± 8</td>
<td>103 ± 10</td>
</tr>
<tr>
<td>Thr</td>
<td>201 ± 13a</td>
<td>64 ± 4c</td>
<td>99 ± 6b</td>
</tr>
<tr>
<td>Trp</td>
<td>15 ± 1a</td>
<td>9 ± 1b</td>
<td>13 ± 1a</td>
</tr>
<tr>
<td>Tyr</td>
<td>44 ± 2a</td>
<td>13 ± 1c</td>
<td>20 ± 1b</td>
</tr>
<tr>
<td>Val</td>
<td>78 ± 4a</td>
<td>28 ± 2c</td>
<td>50 ± 4b</td>
</tr>
<tr>
<td>Total α-AA</td>
<td>2988 ± 86a</td>
<td>998 ± 22c</td>
<td>1620 ± 70b</td>
</tr>
</tbody>
</table>

* Data are means ± SEM. AA, amino acids.
† Ewes were fed 100% of NRC nutrient requirements between Days 28 and 135 of gestation (n = 8).
‡ Ewes were fed 50% of NRC nutrient requirements between Days 28 and 135 of gestation (n = 5).
§ Ewes were fed 50% of NRC nutrient requirements between Days 28 and 78 of gestation and realimented to 100% of NRC nutrient requirements between Days 78 and 135 of gestation (n = 8).

a,b,c Means with different superscripts are different (P < 0.05).
Impact of maternal nutrition on amniotic fluid amino acid concentrations in rats

Dietary and developmentally induced changes in AF amino acid concentrations during the last 4 d of gestation in rats whose dams were fed varying levels of carbohydrate

<table>
<thead>
<tr>
<th>Individual amino acids</th>
<th>Dietary glucose, 1 %</th>
<th>Main effect 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤12%</td>
<td>≥24%</td>
</tr>
<tr>
<td>Methionine</td>
<td>$72 \pm 12^a$</td>
<td>$135 \pm 20^b$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>$436 \pm 24^a$</td>
<td>$565 \pm 40^b$</td>
</tr>
</tbody>
</table>

Gestational age, 3 d

<table>
<thead>
<tr>
<th></th>
<th>18/19</th>
<th>20/21</th>
<th>Main effect 4</th>
</tr>
</thead>
</table>

\(^{1}\) Dietary glucose as a percentage of total carbohydrate intake; \(^{2}\) Main effect of dietary glucose on amino acid concentrations; \(^{3}\) Gestational age in days; \(^{4}\) Main effect of gestational age on amino acid concentrations.
Maternal dietary intake in our sample (n=240)

Macronutrient distribution

- **Proteins**: 39.8% (RDI 20-35%)
- **Carbohydrates**: 17.2% (RDI 10-35%)
- **Lipids**: 43% (RDI 45-65%)

Box plots for amniotic fluid amino acids concentrations (μmol/L) (n=78)
Amniotic fluid amino acid concentration in relation to protein intake at 18th gestational week

Spearman correlation coefficient (r) & Partial correlation coefficient (r') adjusted for BMI

Michaelidou AM, et al: AJOG (sup), 2008
Amniotic fluid amino acid concentration in relation to percentage of energy derived from proteins at 18th gestational week

- The percentage of energy derived from proteins was positively correlated with most AF amino acids.
- Stronger statistical significance were noticed during the 18th week of pregnancy.

Spearman correlation coefficient

Michaelidou AM, et al: AJOG (sup), 2008
Dietary patterns reflection in the metabolic profiles of second trimester maternal blood and amniotic fluid

Subjects:
Serum and amniotic fluid samples were obtained from 27 women that underwent amniocentesis for prenatal diagnosis - between the 18th and 24th gestational week.

Dietary assessment:
Dietary assessment was carried out by registered dietician using a food frequency questionnaire and 24-h recall.

Maternal serum and amniotic fluid analysis:
National Hellenic Research Foundation
Holistic metabolomics approach on Varian-600MHz NMR spectrometer and CPMG pulse sequence. MestReNova software.
Phase and baseline correction/ Bucketing of 0.001 ppm
Spectral alignment / The SIMCA-P 13.0 (PCA) & (OPLS-DA).
Cluster analysis identified 3 dietary patterns (clusters)

1. White bread
   - Rice - pasta
   - n = 5

2. Red meat
   - Meat products
   - Full-fat milk/yogurt
   - Eggs
   - Potatoes
   - Fruit juices
   - Confectionery
   - n = 16

3. Whole-meal bread
   - Fruits
   - Low-fat milk/yogurt
   - Nuts
   - Legumes
   - n = 6

C. Fotakis, XXIV ECPM, 2014
Dietary patterns reflection in the metabolic profiles of second trimester maternal blood and amniotic fluid

**Maternal Serum**

<table>
<thead>
<tr>
<th>Model</th>
<th>Type</th>
<th>A</th>
<th>N</th>
<th>R2X(cum)</th>
<th>R2Y(cum)</th>
<th>Q2(cum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M17</td>
<td>OPLS-DA</td>
<td>1+1+0</td>
<td>25</td>
<td>0.443</td>
<td>0.854</td>
<td>0.699</td>
</tr>
</tbody>
</table>

**Amniotic Fluid**

<table>
<thead>
<tr>
<th>Model</th>
<th>Type</th>
<th>A</th>
<th>N</th>
<th>R2X(cum)</th>
<th>R2Y(cum)</th>
<th>Q2(cum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M10</td>
<td>OPLS-DA</td>
<td>1+1+0</td>
<td>22</td>
<td>0.492</td>
<td>0.76</td>
<td>0.647</td>
</tr>
</tbody>
</table>

**Cluster 2**

- Higher concentration:
  - specific amino acids
  - carnitine
  - creatinine
  - ethanolamine
The present research effort suggests that the metabolic profile of second trimester amniotic fluid is influenced by maternal dietary pattern.
Conclusions

• The results of these studies show that there is an association between maternal nutritional status and amniotic metabolic profile.

• Further investigating in “metabolomics” is needed to unravel the biochemical pathways underlying fetal development and gain insight on the potential impact of maternal nutritional management on fetal growth regulation.

Thank you very much!!!