Folate and Arsenic Metabolism: A double-blind placebo controlled folate supplementation trial in Bangladesh.

J. Richard Pilsner, MPH, MPhil

Dr. Mary V. Gamble’s Laboratory
Mailman School of Public Health
Columbia University

RO1 ES011601 & P42 ES10349
Arsenic metabolism by mono- and dimethylation.

**Arsenate**

\[
\begin{align*}
&\text{OH} \\
&\text{O} = \text{As}^V - \text{OH} \\
&\text{O}^-
\end{align*}
\]

**Arsenite**

\[
\begin{align*}
&\text{OH} \\
&\text{H} - \text{As}^{III} - \text{OH}
\end{align*}
\]

**Methylarsonic acid (MMA)**

**Dimethylarsonic acid (DMA)**

**Methylenedioxyarsenic acid (MDA)**

**GSH**

\[
\begin{align*}
\text{GSH} &\rightarrow \text{GSSG} \\
\text{GSSG} &\rightarrow \text{GSH}
\end{align*}
\]

**SAM**

\[
\begin{align*}
\text{SAM} &\rightarrow \text{SAH} \\
\text{SAH} &\rightarrow \text{SAM}
\end{align*}
\]
Overview of One-Carbon Metabolism

Folate

Methionine

Homocysteine

Transsulfuration Pathway

Cystathionine

Cysteine + Glutamate

γ-glutamylcysteine + Glycine

GSH → GSSG
As(V) → As(III)
MMA(V) → MMA(III)

Total urinary arsenic

InAs
MMA
DMA
SAH
SAM

5:10 Methylene-THF

10 formyl THF
purine synthesis

thymidylate
synthesis

5 Methyl THF

DHF
Serine
Glycine
Background: Arsenic Methylation & Folate

• Animal studies
  • Dietary folate deficiency
    • Toxicol. Lett. 2003: 145:167-74
  • Dietary methyl donor deficiency
    • Mutat Res 1997: 386:315-34

• Case-control studies in Taiwan
  • Lower DMA in urine increased the risk of skin and bladder cancers and peripheral vascular disease

Decrease total urinary arsenic excretion, particularly DMA

J Occup Environ Med 2003, 45:241-8
Cancer Epidemiol Biomarker Prev 1997: 589-96
Toxicol Appi Pharmacol 2005, 206: 299-308
Background: Arsenic Methylation in Bangladesh

- Prospective cohort
  - Increase %MMA in urine is a risk factor for skin lesions

- Cross-Sectional Study

<table>
<thead>
<tr>
<th></th>
<th>%lnAs</th>
<th>%MMA</th>
<th>%DMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (nM)</td>
<td>-0.12*</td>
<td>-0.12*</td>
<td>0.14*</td>
</tr>
<tr>
<td>Homocysteine (µM)</td>
<td>0.06</td>
<td>0.21#</td>
<td>-0.14**</td>
</tr>
</tbody>
</table>

Spearman correlation coefficients * p < 0.05, ** p < 0.001, # p < 0.001

Environ Health Perspect 2005, 113:1683-88
Placebo-controlled Folate Intervention Trial

Hypothesis

Folate supplementation to folate deficient Bangladeshi adults enhances the methylation of arsenic.
Study Design:
Placebo-controlled Folate Intervention Trial

• 200 folate-deficient Bangladeshi adults
  • (Plasma folate < 9 nM)

• 12 weeks: Folic Acid (400 µg/d) or placebo

• Urinary As metabolites analyzed:
  • 0, 1, & 12 weeks

• All participants received a supply of multivitamins upon completion of the study
Results: Nutritional Parameters Pre- and Post-Intervention

Folate

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=98)</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Placebo (n=98)</td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>

Folate (n=96)

a: p < 0.0001 within group; b: p < 0.0001 between groups; c: p < 0.001 between groups

Wilcoxon rank sum test for continuous variables

Homocysteine

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=98)</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Placebo (n=98)</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

Homocysteine (umol/L)

a: p < 0.0001 within group; b: p < 0.0001 between groups; c: p < 0.001 between groups
Results: Effects of Folate Supplementation on Arsenic Metabolites in Urine

Repeated measures linear regression

*p < 0.05; ** p < 0.001 (folate vs. placebo); # p ≤ 0.05 (placebo pre- vs. post intervention)
Distribution of %DMA in Urine after 12 Weeks Folate or Placebo Supplementation
• **Summary:**
  - Folate intervention study indicates a causal relationship between folate supplementation and arsenic methylation in a folate-deficient population in Bangladesh.

• **Implications:**
  - Enhancing arsenic methylation, as achieved by adequate folate status, could reduce arsenic-induced health outcomes.

• **Future research:**
  - In a nested case-control study, we will determine if folate deficiency is a risk factor for subsequent development of arsenic-induced skin lesions.
Special Thanks…

Mary V. Gamble
Vesna Ilievski
Merle Jalakas

Epidemiology
Habibul Ahsan
Yu Chen
Pamela Factor-Litvak

Bangladesh
Tarique Islam
Shafiul Alam
Mominul Islam
Field Staff

Joseph Graziano
Vesna Slavkovich
Marni Hall
Olga Balac
Michael Jerome

Environmental Health Sciences
Faruque Parvez

Biostatistics
Xinhua Liu
Diane Levy

Lamont
Lex van Geen