Nutritional Manipulation of One-Carbon Metabolism: Effects on Arsenic Methylation and Toxicity

Mary V. Gamble, PhD
Columbia University, Department of Environmental Health Sciences
mvg7@cumc.columbia.edu
I have no conflict of interest to declare.
Naturally occurring contamination of As in groundwater:

- South and East Asia
- China
- Taiwan
- Mexico
- Chile
- United States

- 140 million exposed worldwide
- > 70 countries exposed

Installation of wells began in 1960’s

Arsenic concentrations in well water in Bangladesh range from 0 --> 1600 µg/L

50 µg/L Bangladesh standard
10 µg/L WHO standard
Targets of Arsenic Toxicity

**Fetus**
- Infant mortality
- Reduced birth weight/gestational age
- Potential latent effects
  - Diabetes
  - Cancer
  - Bronchiectasis

**Adolescent**
- Nervous System
  - Neurological impairment
- Soft Organs
  - Non-alcoholic fatty liver disease

**Adult**
- Nervous System
  - Movement/Motor function
  - Neuropathy
- Immune System
  - Infections
- Respiratory System
  - Bronchiectasis
  - Lung cancer
- Cardiovascular System
  - Heart and vascular disease
  - Hypertension
- Endocrine System
  - Diabetes
- Soft Organs
  - Bladder cancer
  - Liver cancer
- Skin
  - Skin lesions
  - Skin cancer

Abuawad, Bozack, Saxena and Gamble
Toxicology 427, 2021
Proposed Mechanisms of As Toxicity

- enzyme inhibition
- altered DNA repair
- chromosomal instability
- oxidative stress
- endocrine disruption
- epigenetic modifications

\[
\text{SH} \quad \text{SH} + \text{OH} \rightarrow \text{SH} \quad \text{As}^{III} - \text{OH}
\]

\[
\text{S} \quad \text{As}^{III} - \text{OH}
\]
S-adenosylhomocysteine (SAH)

\[ \text{As}^{\text{III}} \]
\[ \text{OH} \]
\[ \text{HO - As}^{\text{III}} - \text{OH} \]

\[ \text{MMAs}^{\text{V}} \]
\[ \text{OH} \]
\[ \text{O = As}^{\text{III}} - \text{CH}_3 \]
\[ \text{OH} \]

S-adenosylmethionine (SAM)

\[ \text{MMAs}^{\text{III}} \]
\[ \text{OH} \]
\[ \text{As}^{\text{V}} - \text{CH}_3 \]
\[ \text{OH} \]

\[ \text{DMAs}^{\text{V}} \]
\[ \text{CH}_3 \]
\[ \text{O = As}^{\text{V}} - \text{CH}_3 \]
\[ \text{OH} \]

As shown in the diagram:
- \( \text{AS3MT} \) catalyzes the transfer of a methyl group from SAM to MMAs III.
- Folate is involved in the process.
- MMAs V is converted to DMAs V by another reaction, possibly catalyzed by a different enzyme.

The process likely involves additional cofactors and enzymes that are not explicitly shown in the diagram.
Mthfr gene ablation enhances susceptibility to arsenic prenatal toxicity

Bogdan J. Wlodarczyk a,b,1, Huiping Zhu 1, Richard H. Finnell 1,2

Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A&M University System Health Science Center, 2211 West Holcombe Blvd., Houston, TX 77030, USA

Toxicology Letters 215 (2012) 214–218

Arsenic urinary speciation in Mthfr deficient mice injected with sodium arsenate

Bogdan Wlodarczyk a,c,⁎, Ofer Spiegelstein a, Denise Hill a, X. Chris Le b, Richard H. Finnell a,c

Folic acid protects SWV/Fnn embryo fibroblasts against arsenic toxicity

Ying Ruan a, Mary H. Peterson a, Eric M. Wauson a, Janee Gelineau-Van Waes a,b,c,d, Richard H. Finnell a,b,c,d, Roseann L. Vorce a,c,⁎
AS3MT Knockout Mice Retain InAs
Estimated Relative Risks (95 % CIs) for Health Outcomes Associated with %MMAs in Urine

Study Design:
- **Cohort**
- **Nested case-control**
- **Case-control**
- **Cross-sectional**

| Health Outcome (Reference) | MMAs Comparison Groups |
|----------------------------|-------------------------|
| Bladder cancer (Argentina) (Steinmaus et al. 2006) | ≥16.7 vs <16.7% |
| Bladder cancer (U.S.) (Steinmaus et al. 2006) | ≥14.9 vs <14.9% |
| Bladder cancer (Pu et al. 2007) | >9.2 vs ≤3.0% |
| Bladder cancer (Chung et al. 2013) | ≥15.3 vs <8.3% |
| Bladder cancer (Melak et al. 2014) | ≥12.5 vs <12.5% |
| Breast cancer (López-Carrillo et al. 2014) | >13.3 vs ≤7.0% per 1% |
| Breast cancer (López-Carrillo et al. 2020) | >17.2 vs ≤11.8% per 1% |
| Lung cancer (Steinmaus et al. 2010) | ≥12.5 vs <12.5% |
| Lung cancer (Melak et al. 2014) | ≥16.5 vs <9.8% |
| Skin cancer (Yu et al. 2000) | ≥0.59 vs <0.35 |
| Skin cancer (Gilbert-Dimond et al. 2013) | ≥16.5 vs <9.8% |
| Skin lesions (Ahsan et al. 2007) | >17.6 vs ≤12.9% |
| Skin lesions (Lindberg et al. 2008) | >17.6 vs ≤12.9% |
| Skin lesions (Zhang et al. 2014) | ≥16.5 vs <13.4% |
| Atherosclerosis (Wu et al. 2006) | 17.1 vs ≤10.5% |
| Peripheral arterial disease (Newman et al. 2016) | >11.4 vs ≤11.4% |
| Peripheral arterial disease (Tseng et al. 2005) | ≥15.6 vs <8.1% |
| Hypertension (Huang et al. 2007) | NR |
| Hypertension (Li et al. 2013a) | >14 vs ≤12% |
| Hypertension (Li et al. 2013b) | >15.6 vs ≤11.5% per 5% |
| Hypertension (Li et al. 2015) | per 5% |
| Diabetes (Kuo et al. 2015) | per 5% |
| Diabetes (Grau-Perez et al. 2017) | per 1% |
| Diabetes (Zhang et al. 2020) | per 5% |
| Insulin resistance (Grau-Perez et al. 2017) | per 5% |
| Insulin resistance (Spratlen et al. 2018) | per 5% |
| Metabolic syndrome (Chen et al. 2012) | >11.3 vs ≤5.8% per 1% |
| Metabolic syndrome (Kazemifar et al. 2020) | >11.3 vs ≤5.8% per 1% |

Abuawad, Bozack, Saxena, Gamble
Toxicology 457, 2021
One-Carbon Metabolism

Bozack, Saxena and Gamble
Annu. Rev. Nutr. 38:401-29

Abuawad, Bozack, Saxena and Gamble
Toxicology 427, 2021
One-Carbon Metabolism

MAJOR CONSUMERS OF SAM

|                | 0% | 25% | 50% | 75% | 100% |
|----------------|----|-----|-----|-----|------|
| GAMT → creatine| 50%|     |     |     |      |
| PEMT           |     |     |     |     |      |
| Other methyltransferases | (10%) |      |      |      |      |
As concentrations in 6,000 wells
Prevalence of Hyperhomocysteinemia and Folate and B12 Deficiencies in Araihazar, Bangladesh (N = 1650)
Folic acid supplementation to folate deficient adults increases arsenic methylation.

Randomized, placebo-controlled Clinical Trial (RCT)
N = 200 folate deficient adults
400 µg folic acid (FA) per day x 12 weeks
Effects of Folic Acid Supplementation on Arsenic Metabolites in Urine

Reference: Gamble et al., Am J Clin Nutr 2006; 84:1093-1101
Hypothesis:

Increasing arsenic methylation lowers blood arsenic concentrations
Influence of Folic Acid Supplementation on Total Blood Arsenic Concentrations

Gamble et al, Am J Clin Nutr 2007;86:1202-9
Does Folic Acid Supplementation Influence As Metabolites in Blood? …Blood MMAs(III+V)

Gamble et al, Am J Clin Nutr 2007;86:1202-9
Hypothesis: NONE
# Urinary Creatinine: a Predictor of Arsenic Methylation

Results from 6 out of 6 Independent Studies

| Study Description                                      | %InAs   | %MMA    | %DMA    |
|--------------------------------------------------------|---------|---------|---------|
| Cross-sectional study (N=300)¹                          | -0.32   | -0.09   | 0.30    |
|                                                        | (<0.0001)| (N.S.)  | (<0.0001)|
| NIAT (N=194 folate deficient adults)²                  | -0.494  | -0.069  | 0.404   |
|                                                        | (<0.0001)| (N.S.)  | (<0.0001)|
| Children (N=165 6 year olds)³                           | -0.391  | 0.021   | 0.22    |
|                                                        | (<0.0001)| (N.S.)  | (<0.01) |
| Pregnant women (N=101)⁴                                 | -0.3529 | -0.1221 | 0.3205  |
|                                                        | (0.0003)| (N.S.)  | (0.001) |
| Nested case control (N=274 controls)                    | -0.446  | -0.0816 | 0.261   |
|                                                        | (<0.0001)| (N.S.)  | (<0.0001)|
| Torreon, Mexico (N=191 adults)⁵                         | -0.431  | -0.264  | 0.434   |
|                                                        | (<0.0001)| (0.0002)| (<0.0001)|

¹Gamble, Liu, Ahsan, et al. EHP 2005; 113(12):1683-8
²Gamble, Liu, Ahsan, et al. AJCN 2006; 84(5):1093-1101
³unpublished data from Bangladesh
⁴Hall, Gamble, Slavkovich et al. EHP 2007; 115(10):1503-9
⁵unpublished data provided by Drs. Uttam Chowdhury, and H. Vasken Aposhian, Dept of Molecular and Cellular Biology, Univ. AZ, and Dr. Gonzalo Garcia-Vargas, Universidad Juarez del Estado de Durango Lasalle in Sept. 2007

Kile et al. EHP 2009, 117(3):455-60
Basu et al. EHP 2011, 119(9):1308-13
Creatine/Creatinine Metabolism

Dietary Cr

SAM → SAH

GAA → Cr

GAA + ornithine → Arg + Gly

ATP, ADP

Cr, PCr

Urinary Crn excretion

B. Peters

Peters et al., J Nutr 2015;145:2245–52
The Folic Acid and Creatine RCT

Folic Acid and Creatine as Therapeutic Approaches to Lower Blood Arsenic (FACT)
Hypotheses:

- FA supplementation lowers bAs in mixed folate deficient/replete participants
  (by chance, 20% were folate deficient)

- 800 FA µg/d lowers bAs > 400 µg/d
## FACT Study Design (N=610)

| Treatment                        | N     | First Phase | Second Phase |
|----------------------------------|-------|-------------|--------------|
| Placebo                          | 102   |             | Placebo (N=102) |
| 400 µg FA                        | 153   |             | 400 FA (N=77)   |
|                                  |       |             | Placebo (N=76)   |
| 800 µg FA                        | 151   |             | 800 FA (N=77)   |
|                                  |       |             | Placebo (N=74)   |
| 3 g creatine                     | 101   |             |               |
| 3 g creatine + 400 µg FA         | 103   |             | Placebo (N=103) |

Week: 0---1---6---12---13---18---24---24
The “New Toy Effect”

Sanchez et al., Sci Total Environ, 2016
Folate Status

Plasma Folate

RBC Folate

Peters et al, EHP, 123(12):1294-1301, 2015
FACT: 800 µg Folic Acid vs. Placebo
Change in Total Blood Arsenic at Week 12

p = 0.03
800 µg FA/PBO vs. PBO, p = 0.02
800 µg FA/FA vs. PBO, p = 0.04
800 µg FA/FA vs. 800 µg FA/PBO, p = 0.72

Bozack et al, EHP 123(12):1294-1301, 2015
Percent Change in total Blood Arsenic
Folic Acid (800 ug/d) vs. Placebo

Bozack et al, EHP 123(12):1294-1301, 2015
Hypotheses:

• FA supplementation increases arsenic methylation

• 800 FA µg/d increases As methylation more than 400 µg/d
Change in Urinary Arsenic Metabolites Over Time (Weeks 0 to 12)

%InAs

%MMAs

%DMA

Weeks 0 to 12

Bozack et al, Am J Clin Nutr 2019; 109:380-391
Change in Urinary Arsenic Metabolites Over Time (Weeks 0 to 12)

*\( p < 0.05 \) for Treatment vs. Placebo by Wilcoxon Rank Sum Test

Bozack et al, Am J Clin Nutr 2019; 109:380-391
Hypotheses:

- Creatine supplementation downregulates creatine biosynthesis
- Creatine supplementation increases arsenic methylation
Creatine Hypothesis

Liver

Creatine Supplementation

Kidney

GAA → GAMT → Arg + Gly + ornithine → AGAT → SAH → SAM → GAA → creatine

Peters et al., J Nutr 2015;145:2245–52
Creatine Decreased %MMAs

| TABLE 3  | Treatment group differences in mean within-person change since baseline in As metabolite proportions |
|----------|---------------------------------------------------------------------------------------------|
|          | Mean change at wk 1 (95% CI) (wk 1 – wk 0)                                                                 |
|          | Mean change at wk 6 and 12 (95% CI) (wk ≥6 – wk 0)                                              |
| ln(%)ln(As) | Placebo | 0.01 (–0.08, 0.09) | 0.05 (0.0, 0.10) |
|           | 400FA    | −0.01 (–0.12, 0.10) | −0.09 (–0.17, −0.01)* |
|           | 800FA    | −0.10 (–0.22, 0.02) | −0.14 (–0.21, −0.06)** |
| Creatine + 400FA | −0.01 (–0.12, 0.10) | −0.11 (–0.18, −0.03)** |
| Creatine | −0.06 (–0.17, 0.06) | −0.02 (–0.10, 0.06) |
| %MMAs | Placebo | −0.62 (–1.13, −0.12) | 0.15 (–0.37, 0.68) |
|         | 400FA | −0.85 (–1.57, −0.14)* | −1.80 (–2.53, −1.07)** |
|         | 800FA | −0.86 (–1.62, −0.09)* | −2.60 (–3.35, −1.85)** |
| Creatine + 400FA | −0.66 (–1.43, 0.11) | −1.85 (–2.61, −1.09)** |
| %DMAs | Placebo | 0.19 (–1.30, 1.67) | −1.17 (–2.18, −0.17) |
|         | 400FA | 1.02 (–0.85, 2.88) | 3.25 (1.81, 4.68)** |
|         | 800FA | 2.27 (0.26, 4.28) | 4.57 (3.20, 5.95)** |
| Creatine + 400FA | 0.85 (–1.04, 2.74) | 3.11 (1.67, 4.55)** |
| Creatine | 2.11 (0.01, 4.21) | 1.43 (–0.21, 3.06) |

1) Treatment group differences in mean changes were derived from relevant group-by-time interaction parameters of the linear models with repeated measures where time was a variable with 3 categories (wk 0, wk 1, wk 6 and 12). 400FA, 400 μg FA per day treatment group; 800FA, 800 μg FA per day treatment group; creatine, 3 g creatine per day treatment group; creatine + 400FA, 3 g creatine and 400 μg FA per day treatment group; DMAs, dimethyl-arsenical species; FA, folic acid; InAs, inorganic arsenic; MMAs, monomethyl-arsenical species.

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 for group-by-time interaction parameters of the linear models with repeated measures.
FACT Study Design (N=610)

| Treatment                        | N   | First Phase | Second Phase |
|----------------------------------|-----|-------------|--------------|
| Placebo                          | 102 |             |              |
| 400 µg FA                        | 153 |             |              |
| 800 µg FA                        | 151 |             |              |
| 3 g creatine                     | 101 |             |              |
| 3 g creatine + 400 µg FA         | 103 |             |              |

Week: 0---1---------6---------12---13---------18---------24

400 FA (N=77)
Placebo (N=76)
800 FA (N=77)
Placebo (N=74)
Placebo (N=101)
Placebo (N=103)
Hypotheses:

• Total blood As may rebound after cessation of FA supplementation

• Arsenic methylation capacity may decrease after cessation of FA supplementation
FACT: 800 µg Folic Acid vs. Placebo
Change in Total Blood Arsenic

p = 0.03

800 µg FA/PBO vs. PBO, p = 0.02
800 µg FA/FA vs. PBO, p = 0.04
800 µg FA/FA vs. 800 µg FA/PBO, p = 0.72

Peters et al., EHP, 2015 Dec;123(12):1294-1301
Change in As Methylation after Cessation of 800 µg Folate Supplementation

- p = 0.03
- p = 0.09
Percent Change in Blood As Metabolites from Baseline to Week 12

PMI = MMAs/InAs  SMI = DMA/MMAs

Percent change in geometric mean of blood arsenic metabolite concentrations and methylation indices from baseline by treatment group.

| Blood As Metabolite | Placebo | 400FA | 800FA | Creatine | Creatine+400FA |
|---------------------|---------|-------|-------|----------|---------------|
|                     | % change (95% CI) | % change (95% CI) | % change (95% CI) | % change (95% CI) | % change (95% CI) |
| InAs                | -9.2 (-15.8, -2.1) | -13.8 (-19.5, -7.8) | 0.31 | -24.7 (-30.8, -18.0) | 0.001 | -9.7 (-16.8, -2.0) | 0.93 | -17.6 (-24.8, -9.8) | 0.11 |
| MMAs                | -16.2 (-22.8, -9.0) | -26.8 (-31.9, -21.3) | 0.016 | -36.2 (-41.6, -30.3) | < 0.001 | -21.2 (-27.1, -14.8) | 0.29 | -31.1 (-37.7, -23.7) | 0.003 |
| DMAs                | -13.8 (-20.3, -6.8) | -6.7 (-13.0, 0.15) | 0.14 | -19.8 (-26.2, -12.9) | 0.21 | -13.6 (-20.2, -6.5) | 0.97 | -17.8 (-25.2, -9.6) | 0.45 |
| Indices             |         |       |       |          |    |
| PMI                 | -7.5 (-11.9, -2.9) | -15.2 (-18.1, -12.0) | 0.005 | -21.0 (-24.6, -17.3) | 0.004 | -12.1 (-16.9, -7.1) | 0.18 | -16.2 (-19.6, -12.7) | 0.002 |
| SMI                 | 2.8 (-0.8, 6.6) | 27.5 (23.3, 31.8) | < 0.001 | 25.6 (21.5, 29.9) | < 0.001 | 9.5 (5.2, 14.0) | 0.023 | 19.3 (5.6, 23.0) | < 0.001 |

P-values were from Wald test on coefficients of group-by-time interaction terms in the linear models with repeated measures for differences in changes from baseline between each treatment and placebo group.
Limited studies on Nutrition x As Methylation in Children
Children are more efficient at methylating arsenic than adults,\textsuperscript{1-4} and they have better folate nutritional status than adults.

- A cross-sectional study of 6-y children in Bangladesh (N=165), plasma folate inversely correlated with %InAs and positively associated with %DMAs in urine (p=0.14)\textsuperscript{2}

- A cross-sectional study of 9-y children in Bangladesh (N=487), plasma folate was inversely correlated with %InAs and positively associated with %DMAs in urine (p<0.01)\textsuperscript{3}

- A cross-sectional study of adolescents in Bangladesh (N=679), in males, plasma folate was negatively correlated with %InAs and positively associated with %DMAs in urine, RBC folate was inversely associated with total blood arsenic concentrations in females.\textsuperscript{5}

- In a case-control study in Taiwan (N=178), a combination of high plasma folate and high vitamin B12 were associated with lower %InAs and higher %DMAs in urine and with reduced odds of developmental delay.\textsuperscript{6}

1. Chowdhury et al, 2003. PMID: 12635821
2. Hall et al, 2009. PMCID: PMC2685848
3. Skroder Loveborn et al, 2016. PMCID: PMC4922540
4. Sun et al, 2007. PMCID: PMC1852658
5. Saxena et al, 2021. PMCID: PMC7987757
6. Lin et al, 2019. PMID: 31473767
One-Carbon Metabolism in Early Life

Protein synthesis

Folic acid
DHF
THF

Thymidylate

5,10-methylene-THF
5-methyl-THF

Purine synthesis

Substrates
1. A5III
2. MMA
3. GAA
4. PE
5. Cytosine

Respective products
1. MMA
2. DMA
3. Creatine
4. PC
5. 5-methylcytosine

Glutathione
Glutathione
Cysteine

Methionine
SAM

AS3MT
GAMT
DNMT
PEMT

Betaine
BHMT
MTR

SAM

Homocysteine

Trans-sulfuration pathway

Choline
Cystathionine

Pyrimidine synthesis

Dietary folates

THF + formate

5,10-methenyl-THF

10-formyl-THF

Serine
Glycine

PLP
Conclusions

- Folate supplementation increases As methylation and lowers blood As in both folate deficient (400 µg) and folate sufficient (800 µg/d) individuals.
- Arsenic methylation patterns revert to baseline 12 weeks after cessation of FA supplementation.
- Risk factors for As induced skin lesions include folate deficiency and high homocysteine.
- Creatine supplementation lowers creatine synthesis (↓GAA, ↓Hcys) and lowers MMAs.
- Additional research is needed to fully understand the strong cross-sectional relations consistently observed between urinary creatinine and arsenic methylation.
10 countries with the most significant problems of As-contaminated drinking water: Bangladesh, Cambodia, China, India, Inner Mongolia, Myanmar, Pakistan, Taiwan, Thailand, and Vietnam do not have mandatory folate fortification programs; most voluntary programs are sparse or minimally enforced.
Acknowledgements

Bangladesh
Tarique Islam
Shafiul Alam
Milan & Unus
Field Staff
Study Participants

Gamble Laboratory
Ahlam Abuawad
Anne Bozack
Megan Hall
Kristin Harper
Caitlin Howe
Vesna Ilievski
Irene Morata-Martinez
Jessica Napolitano
Megan Niedzwiecki
Julie Oka
Brandi Peters
J. Richard Pilsner
Roheeni Saxena
Haotian Wu

CUMC Trace Metals Laboratory
Joe Graziano
Vesna Slavkovich
Olga Balac

Biostatistics
Xinhua Liu
Diane Levy
Jeff Goldsmith

Environmental Health Sciences
Faruque Parvez
Ana Navas-Acien

Lamont
Lex van Geen

Epidemiology
Megan Hall
Pamela Factor-Litvak
Habibul Ahsan

clinicaltrials.gov: NCT01050556
clinicaltrials.gov: NCT03384862

R01 DK123285
R01 ES030945
R01 CA133595
R01 ES011601
R01 ES017875
P30 ES009089
P42 ES10349
Thank you!
SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Arsenic and Children’s Health

Break 10:40 AM–10:50 AM ET