Diabetes Associated with Environmental Exposure to Arsenic: The Phenotype and Mechanisms

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Outline

1. Arsenic in the Environment: Sources of Exposure
2. Metabolism of Inorganic Arsenic
3. Adverse Effects of iAs Exposure: Diabetes
4. Research at UNC Chapel Hill – ViCTER:
   a) Mechanistic studies using adipocytes
   b) Population studies in Mexico
   c) Animal studies
   d) Mechanistic studies using pancreatic islets
   e) Metabolomic and epigenetic profiling
5. Conclusions and Future Directions
6. Acknowledgements
Arsenic in the Environment: Sources of Exposure
Natural Sources of Arsenic:  
20ᵗʰ most common element in Earth crust

- Metal ores, minerals, and geological formations
- Surface and underground water reservoirs
- Biosphere: microorganisms, animals, and plants
Anthropogenic Sources:

- Industrial emissions: metal smelting, coal burning, semiconductor production
- Herbicides and pesticides
- Wood preservatives (CCA-treated wood)
- Feed additives (Roxarson, poultry and swine production)
- Biosafe antimicrobial plastics
- Drugs, medications (Trisenox)
Inorganic Arsenic (iAs) in Drinking Water Reservoirs

Over 40 million in Southeast Asia alone are exposed to As in drinking water; hundreds of thousands suffer of chronic arsenicosis
~14 million US residents drink water with arsenic levels >10 $\mu$g/L

43 million drink water from unregulated private wells

Max. arsenic level = 800 ppb
1,436 wells contaminated above 10 ppb
Hundreds exceeded 50 ppb

North Carolina

75,000 people

Arsenic concentration (μg/L)
- EPA standard or below
- Above EPA standard

Environment International

Arsenic in North Carolina: Public Health Implications
Alison P. Sanders, Kyle P. Messier, Mina Shehee, Kenneth Rudo, Marc L. Serre, Rebecca C. Fry
Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, 1213 Michael Hooker Research Building, Chapel Hill, NC 27599, United States
Medical Evaluation and Response Assessment Unit, North Carolina Department of Health and Human Services, 1912 Mail Service Center, Raleigh, NC 27699, United States
Metabolism of Inorganic Arsenic
Metabolism = Detoxification of iAs (?)

**Prokaryotes:**

Enzymatic reduction of iAs\textsuperscript{V} to iAs\textsuperscript{III} and extrusion of iAs\textsuperscript{III} from the cell (*E. coli*; PO\textsubscript{4}\textsuperscript{3+} transporters, ArsC, Grx, GSH, ArsA/B, ATP)

**Eukaryotes:**

Enzymatic reduction of iAs\textsuperscript{V} to iAs\textsuperscript{III} and extrusion of iAs\textsuperscript{III} from the cell (*S. cerevisiae*; PO\textsubscript{4}\textsuperscript{3+} transporters, Acr2p, Grx, GSH, Acr3p)

Formation and accumulation of iAs\textsuperscript{III}(GS)\textsubscript{3} in vacuoles (*S. cerevisiae*; Ycf1p, ATP)

Enzymatic methylation of iAs\textsuperscript{III} to yield MAs, DMAs, and TMAs (*Mammals*; AdoMet-dependent As\textsuperscript{+3}-methyltransferase (AS3MT), As\textsuperscript{V}-reductases, GST, GSH)

None of the above (*Guinea pig, Marmoset, Chimpanzee*)
detoxification through protein binding ????)
Metabolism of Inorganic Arsenic (iAs)

Oxidative Methylation

\[ \text{iAs}^{\text{III}} \xrightarrow{\text{AdoMet}} \text{MAS}^{\text{V}} \xrightarrow{2e} \text{MAS}^{\text{III}} \xrightarrow{\text{AdoMet}} \text{DMAS}^{\text{V}} \xrightarrow{2e} \text{DMAS}^{\text{III}} \xrightarrow{\text{AdoMet}} \text{TMAS}^{\text{V,O}} \xrightarrow{2e} \text{TMAS}^{\text{III}} \]

Reductive Methylation

\[ \text{iAs}^{\text{III}} \xleftarrow{\text{GSH}} \text{iAs}^{\text{III}}(\text{GS})_3 \xleftarrow{\text{AdoMet}} \text{MAS}^{\text{III}}(\text{GS})_2 \xleftarrow{\text{GSH}} \text{DMAS}^{\text{III}}(\text{GS}) \xleftarrow{\text{GSH}} \text{DMAS}^{\text{V}} \]
The reduction of As\textsuperscript{V} to As\textsuperscript{III} in the methylation pathway can be viewed as a mechanism for activation of iAs as a toxin and a carcinogen!

(Thomas and Creed, unpublished data)
Examples of As species identified in biological samples, including foods (K. Francesconi et al.; Chris Le et al)

Marine Biota

Trimethylarsonioacetate (Arsenobetaine, AB)

Arsenite [As(III)]

Arsenocholine (AC)

Arsenate [As(V)]

Arsenosugar 2 (phosphate sugar)

Dimethylarsinate (DMA)

Tetramethylarsonium ion (TETRA)

Arsenosugar 4 (sulfate sugar)

As-fatty acids
Adverse Effects of iAs Exposure:
Diabetes Mellitus
Typical Symptoms of Chronic Arsenicosis

- Hypo/hyperpigmentation
- “Blackfoot disease”
- Keratosis
- Skin cancer
Diseases Associated with Chronic Exposures to Inorganic Arsenic (iAs)

Cancers:
Skin, urinary bladder, lung, and liver
Prostate, kidney, buccal cavity, pharynx, bone, large intestine and rectum

Non-cancerous Diseases:
Peripheral vascular diseases – Blackfoot disease
Cardiovascular and cerebrovascular diseases (hypertension)
Diabetes mellitus – Type II (noninsulin dependent)
Diseases of peripheral and central nervous systems
Respiratory system dysfunctions
Prevalence of Diabetes and Obesity in the U.S.

Could the environmental chemicals contribute to this epidemic?
Conclusion:

1. “The existing human data are “limited” to “sufficient” in support of an association between As and diabetes in populations with high exposure levels (≥ 150 ppb)

2. “The evidence is currently “insufficient” to conclude that As is associated with diabetes in lower exposure areas.....”

3. The extent of the existing literature was insufficient to consider obesity as an outcome

Maull et al., EHP, 2012
Arsenic-Induced Diabetes: Historically Characterized as Type 2 Diabetes Mellitus

- Adult onset disease
- Lack of diabetic keto-acidosis
- Fasting hyperglycemia
- Impaired glucose tolerance (by OGTT)
- Increased levels of glycated hemoglobin (HbA1c)
- Glucosuria
- Little or no data on insulin resistance, β-cell function, or insulin secretion!
Research at UNC Chapel Hill
Virtual Consortium for Translational/Transdisciplinary Environmental Research (ViCTER)
ViCTER: Environmental As & Diabetes Mellitus

**MODES OF ACTION**
- Insulin Signaling
- Insulin Production
- Diet & Obesity
- Glucose Tolerance
- Insulin Resistance
- Insulin Signaling
- iAs Metabolites in Tissues

**Exposed to iAs in Drinking Water**
- C57BL/6 Mice & As3mt-KO C57BL/6 Mice

**Exposed to iAs or Methyl-As in Culture**
- Adipocytes
- Myotubes & β-cells, islets

**Diet, Obesity & Physical Activity**
- Residents of Chihuahua (Mexico)

**Original Endpoints**
- Hypertension
- Skin Lesions
- Glucose Tolerance
- Insulin Resistance

**Expanded Endpoints**
- Genetic Polymorphism
- DNA Methylation

**New Endpoints**
- Urothelial Cells

**METABOLICOMICS**
- Urine Arsenome
- Urine & Blood Metabolome
- Urine Arsenome

**DISEASE PHENOTYPE**
- Diet, Obesity & Physical Activity
- Adipocytes
- Myotubes & β-cells, islets

**Exposed to iAs in Drinking Water**
-Residents of Chihuahua (Mexico)

- iAs Metabolites in Exfoliated Urothelial Cells

- Urine Arsenome

**DISEASE PHENOTYPE**
- Diet, Obesity & Physical Activity
- Adipocytes
- Myotubes & β-cells, islets

- Residents of Chihuahua (Mexico)

- iAs Metabolites in Exfoliated Urothelial Cells

- Urine Arsenome
Mechanistic studies using cultured adipocytes
Trivalent Arsenicals Inhibit Insulin-Stimulated Glucose Uptake by Adipocytes

Walton et al., TAAP, 2004
GLUT-4 Transporters in Plasma Membrane of Arsenic-Treated Adipocytes (4-hour exposure)

Control - Insulin

Control + Insulin

50 μM iAs^{III} + Insulin

2 μM MAs^{III} + Insulin

Paul et al., EHP, 2007
Inhibition of Insulin Signaling by Arsenicals

Paul et al., EHP, 2007
Human population studies in Mexico
Population Studies in Mexico

Study 1: Zimapan, Lagunera
- iAs in drinking water: 3 - 215 ppb As (mean = 77.3 ppb)
- 258 subjects

Study 2: Chihuahua
- iAs in drinking water: 0.1 to 420 ppb As
- ~1,150 subjects (to date)

The study areas within the region of Mexico with high levels of iAs in drinking water supplies
Study Design:

- **Questionnaires:** sources of drinking water, health, arsenic exposure, diet (FFQ), physical activity…

- **Examination:** skin lesions, fasting blood glucose, glucose tolerance, HbA1c, indicators of obesity (BMI), ….

- **Analyses:** skin lesions, BMI, iAs metabolites in urine, genetic polymorphism, plasma and urine metabolomes, DNA methylation pattern in blood…..

Skin disease among residents of Zimapán and Lagunera: (A) hypopigmentation, (B) hyperkeratosis, (C) Bowen’s disease, and (D) epidermoid cancer.
## Study 1:
Subjects Recruited in Zimapán and Lagunera

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Lagunera</th>
<th>Zimapán</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N (Mean)</td>
<td>% (SD)</td>
<td>N (Mean)</td>
</tr>
<tr>
<td>Population</td>
<td>258</td>
<td>100</td>
<td>111</td>
</tr>
<tr>
<td>Female</td>
<td>174</td>
<td>67.4</td>
<td>65</td>
</tr>
<tr>
<td>Age</td>
<td>(34)</td>
<td>(18.3)</td>
<td>(40)</td>
</tr>
<tr>
<td>Water consumption (L/day)</td>
<td>(1.9)</td>
<td>(0.9)</td>
<td>(2.2)</td>
</tr>
<tr>
<td>Use bottled water</td>
<td>144</td>
<td>55.8</td>
<td>24</td>
</tr>
<tr>
<td>Skin lesions</td>
<td>61</td>
<td>23.6</td>
<td>11</td>
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<tr>
<td>BMI&gt;30</td>
<td>88</td>
<td>34.1</td>
<td>40</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>97</td>
<td>37.6</td>
<td>29</td>
</tr>
<tr>
<td>Diabetic</td>
<td>23</td>
<td>8.9</td>
<td>15</td>
</tr>
</tbody>
</table>

Subjects by Age

**Zimapán:**
- 5-15 years (30%)
- 16-30 years (22%)
- 31-49 years (34%)
- 50-64 years (14%)

**Lagunera:**
- 7-15 years (16%)
- 16-30 years (15%)
- 31-49 years (35%)
- 50-64 years (27%)
- 65-88 years (7%)

Del Razo et al., EH, 2011
## iAs Exposure Increases Risk of Diabetes

Association of diabetes classified by FBG or 2HBG with exposure to iAs in Zimapán and Lagunera, Mexico (adjusted for age, sex, obesity and hypertension)

| iAs in water (ppb) | FBG | | | | | |
|-------------------|-----|-----|-----|-----|-----|
|                   | Cases | Non-cases | OR  | 95% CI | p   |
| <50               | 8    | 186   | 1.00 |       |     |
| 50-124.9          | 9    | 50    | 3.30 | 1.11  | 9.85 | 0.03 |
| ≥125              | 6    | 22    | 7.40 | 2.13  | 25.65| <0.01|

| iAs in water (ppb) | 2HBG | | | | | |
|-------------------|------|-----|-----|-----|-----|
|                   | Cases | Non-cases | OR  | 95% CI | p   |
| <50               | 9    | 177   | 1.00 |       |     |
| 50-124.9          | 9    | 41    | 2.89 | 0.97  | 8.56 | 0.06 |
| ≥125              | 6    | 16    | 7.01 | 2.02  | 24.44| <0.01|
HbA1c is Only Weakly Associated with As Exposure
(β and CI for 10 ppb As in water or 1 ng As/mL urine)

| Del Razo et al., EH, 2011 | **HbA1c** | 95% CI |  
|---------------------------|-------------------|-----------|---|
| **Water iAs** | **β** | 0.019 | 0.002 | 0.037 | 0.031 |
| Urinary tAs | 0.004 | -0.015 | 0.023 | 0.660 |
| Urinary iAs$^{III}$ | -0.001 | -0.016 | 0.015 | 0.936 |
| Urinary MAs$^{III}$ | 0.008 | -0.011 | 0.028 | 0.393 |
| Urinary DMAs$^{III}$ | 0.005 | -0.007 | 0.016 | 0.398 |
| Urinary iAs$^{V}$ | 0.008 | -0.005 | 0.020 | 0.230 |
| Urinary MAs$^{V}$ | 0.001 | -0.017 | 0.020 | 0.879 |
| Urinary DMAs$^{V}$ | 0.004 | -0.012 | 0.019 | 0.647 |
| Urinary DMAs/MAs ratio | 0.022 | -0.013 | 0.056 | 0.215 |
| Urinary MAs/iAs ratio | 0.008 | -0.019 | 0.034 | 0.561 |
## Negative Associations of iAs Exposure with FPI & HOMA-IR (β and CI for 10 ppb As in water or 1 ng As/mL urine)

<table>
<thead>
<tr>
<th>Del Razo et al., EH, 2011</th>
<th><strong>FPI</strong></th>
<th><strong>HOMA-IR</strong></th>
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<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Water iAs</strong></td>
<td>-0.208</td>
<td>-0.272</td>
</tr>
<tr>
<td><strong>Urinary tAs</strong></td>
<td>-0.136</td>
<td>-0.207</td>
</tr>
<tr>
<td><strong>Urinary iAs^{III}</strong></td>
<td>-0.117</td>
<td>-0.176</td>
</tr>
<tr>
<td><strong>Urinary MAs^{III}</strong></td>
<td>-0.091</td>
<td>-0.166</td>
</tr>
<tr>
<td><strong>Urinary DMAs^{III}</strong></td>
<td>-0.085</td>
<td>-0.128</td>
</tr>
<tr>
<td><strong>Urinary iAs^{V}</strong></td>
<td>-0.027</td>
<td>-0.076</td>
</tr>
<tr>
<td><strong>Urinary MAs^{V}</strong></td>
<td>-0.082</td>
<td>-0.152</td>
</tr>
<tr>
<td><strong>Urinary DMAs^{V}</strong></td>
<td>-0.053</td>
<td>-0.110</td>
</tr>
<tr>
<td><strong>DMAs/MAs ratio</strong></td>
<td>-0.118</td>
<td>-0.252</td>
</tr>
<tr>
<td><strong>MAs/iAs ratio</strong></td>
<td>0.104</td>
<td>0.003</td>
</tr>
</tbody>
</table>
DMAs\\textsuperscript{III} in Urine Increases Risk of Diabetes

OR increases by \(\sim 5\%\) for every ng As/ml as DMAs\\textsuperscript{III} (\(p = 0.041\)).

After controlling for creatinine, OR increases by \(\sim 7\%\) for every ng As/ml as DMAs\\textsuperscript{III} (\(p \leq 0.016\)).
Associations with AS3MT(M287T) Polymorphism

Drobna et al., JESEE (in press)

FBG

DMSA^{III}

HbA1c

287M 287T 287M 287T

p < 0.001

p = 0.002

p = 0.007

p = 0.023
Interaction of M287T (●) and G4965C (▲) polymorphisms and exposure to iAs in water categorized at the 75th percentile (>52 ppb As in water)

Drobna et al., JESEE (in press)
Study 2:
Associations of Diabetes classified by FPG≥126 mg/dl with iAs Exposure and iAs Metabolism in the Chihuahua Study Population (n = 1,002) (adjusted for age, sex, obesity and hypertension)

ODDS RATIO

OR and 95% CI are shown for the <50th, 50th-75th, 75th-95th, and >95th percentiles; *p ≤0.038.
Arsenic Metabolites in Exfoliated Urothelial Cells (BECs) from the Chihuahua Cohort

Hydride generation-cryotrapping-ICP-MS analysis

Correlation between tAs in urine & BECs

As species in urine & in BECs

\[ y = 0.65x + 0.39 \]
\[ R^2 = 0.27 \]
\[ p < 0.0001 \]
Association Between As Metabolites in BEC and Diabetes (classified by FPG, 2HPG, diagnosis or medication)

Odds Ratios by IQR.
Age, gender and BMI were included in the logistic regression model. 
n = 374
Animal studies
Mouse Studies:
Adult male C57BL/6 mice exposed to arsenite (0, 25 or 50 ppm As) in drinking water and fed a regular, grain-bases laboratory diet (contains ~20-80 ppb As)

No significant differences in Hb1Ac levels

Paul et al., TAAP 2007
Mouse Studies, Role of Diet:
Adult male C57BL/6 mice exposed to arsenite (0, 25 or 50 ppm As) in drinking water and fed purified Low-Fat Diet or an obesogenic High-Fat Diet

Body Composition (by MRI) After 20 Weeks

Paul et al., EHP, 2011
Oral Glucose Tolerance Test (OGTT) after 20-week exposure

- No Arsenic
- 25 ppm Arsenic
- 50 ppm Arsenic

Blood Glucose (mg/dl) vs. Time (min.)

- High-Fat
- Low-Fat

Area under the OGTT Curve

Paul et al., EHP, 2011
Association Between iAs Exposure and Plasma Insulin (FPI) or HOMA-IR

Male C57BL/6 mice exposed for 20-weeks to 0, 25 or 50 ppm As in drinking water

Paul et al., EHP, 2011
Mechanistic studies using isolated pancreatic islets
Trivalent Arsenicals Inhibit Glucose Stimulated Insulin Secretion by Isolated Murine Pancreatic Islets

Douillet et al., TAAP 2013

48-hour exposure
Low As$^{III}$ Concentrations Have Little or No Effects on Insulin Expression/Synthesis in Pancreatic Islets

Effect of Secretagogue KCl

48-hour exposure

Insulin Content

Insulin mRNA

Douillet et al., TAAP 2013
Arsenic Species in Mouse Tissues Regulating Glucose Homeostasis after Exposure to Arsenite: 4-week exposure to 50 ppm As in drinking water As species in tissue analyzed by HG-CT-AAS

Currier et al., unpublished data
Hypothetical Mechanisms of the Inhibition of GSIS by As$^{\text{III}}$

1. Insulin (iAs$^{\text{III}}$ & MAs$^{\text{III}}$) binds to the glucose transporter GLUT2, facilitating glucose uptake.
2. Glucose is converted to glucose-6-P by glucokinase.
3. Glucose-6-P is further converted to pyruvate by PDH.
4. Pyruvate is converted to ATP by the pyruvate dehydrogenase complex (PDH).
5. ATP/ADP ratio affects the ATP-sensitive K$^+$ channel, leading to depolarization.
6. Depolarization activates the voltage-dependent Ca$^{2+}$ channel, increasing Ca$^{2+}$ influx.
7. Ca$^{2+}$ influx activates calpain-10, which may lead to the inhibition of GSIS.
Hypothetical Mechanism of the Inhibition of GSIS by Arsenite (Fu et al., EHP, 2011)

iAs\textsuperscript{III} provokes a Nrf2/ARE mediated adaptive oxidative stress response that increases antioxidant levels and dampens ROS signaling that is thought to be essential for regulation of GSIS.
Metabolome and Epigenome associated with iAs exposure & Diabetes
Plasma (A) and Urine (B) Metabolomes Associated with Diabetes in iAs-Exposed Chihuahua Subjects:

Partial least square discriminant analysis (OPLS-DA) scores plots for LC-TOFMS data only (n = 180)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fold change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrose 4-phosphate</td>
<td>0.39</td>
<td>5.82E-20</td>
</tr>
<tr>
<td>Myoinositol</td>
<td>1.63</td>
<td>9.36E-17</td>
</tr>
<tr>
<td>Succinyladenosine</td>
<td>1.98</td>
<td>2.34E-16</td>
</tr>
<tr>
<td>3-Methylthiopropionic acid</td>
<td>1.41</td>
<td>6.03E-16</td>
</tr>
<tr>
<td>Gluconate</td>
<td>1.55</td>
<td>1.06E-09</td>
</tr>
<tr>
<td>Galactonic acid</td>
<td>1.55</td>
<td>1.06E-09</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.18</td>
<td>1.73E-05</td>
</tr>
<tr>
<td>4-Methyl-2-oxovaleric acid</td>
<td>1.20</td>
<td>3.53E-05</td>
</tr>
<tr>
<td>5-Aminoisimidazole ribonucleotid</td>
<td>1.43</td>
<td>6.86E-05</td>
</tr>
<tr>
<td>Malic acid</td>
<td>1.29</td>
<td>3.48E-04</td>
</tr>
<tr>
<td>L-leucyl-L-proline</td>
<td>1.31</td>
<td>3.69E-03</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.91</td>
<td>8.35E-03</td>
</tr>
<tr>
<td>Phosphoenolpyruvic acid</td>
<td>0.93</td>
<td>1.06E-02</td>
</tr>
<tr>
<td>Ethenodeoxyadenosine</td>
<td>0.78</td>
<td>1.94E-02</td>
</tr>
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</table>

90 subjects with and 90 without diabetes, matched for iAs exposure, BMI, sex and age

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fold change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanidineacetic acid</td>
<td>3.42</td>
<td>8.08E-10</td>
</tr>
<tr>
<td>Valine</td>
<td>1.77</td>
<td>4.80E-04</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.07</td>
<td>6.17E-04</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>1.54</td>
<td>3.05E-03</td>
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<tr>
<td>Methylcysteine</td>
<td>0.81</td>
<td>6.47E-03</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.33</td>
<td>9.49E-03</td>
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<td>Tyrosine</td>
<td>1.47</td>
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<tr>
<td>Guanosine</td>
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<td>Oxoglutaric acid</td>
<td>1.47</td>
<td>2.58E-02</td>
</tr>
<tr>
<td>Indoxyl sulfate</td>
<td>0.65</td>
<td>3.25E-02</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.24</td>
<td>4.90E-02</td>
</tr>
</tbody>
</table>
1. Gluconate (gluconic acid): product of glucose oxidation

2. 5-Aminoimidazole ribonucleotide: an intermediate/precursor of purine & pyrimidine nucleotide biosyntheses and synthesis of TPP; also a substrate for a number of proteins, including scaffold attachment factor B2, multifunctional protein ADE2, pulmonary surfactant-associated protein B, tumor necrosis factor receptor superfamily member 2, etc.

3. Lanthionine is a sulfur-containing aminoacid formed by cystathionine gamma-lyase during condensation of 2 cysteines, yielding H₂S; found in urine of subjects with homocysteinuria

4. Cystine formed by oxidation/dimerization of cysteine; preferred substrate for GSH synthesis in some cell types (e.g., macrophages, astrocytes)

Pearson's correlation: P ≤ 0.05
*P-values adjusted for multiple comparisons by the Bonferroni method
Urinary Metabolites Associated with Diabetes and/or Exposure to iAs

Pearson's correlation: $P \leq 0.05$
*P-values adjusted for multiple comparisons by the Bonferroni method

1. **Methionine**: one of the bioactive substances in garlic. It has been suggested that it could protect partially oxidized and glycated LDL in plasma against further oxidative and glycation deterioration, which might benefit patients with diabetic-related vascular diseases

2. **Aspartic acid** (aspartate): a proteinogenic, non-essential amino acid, a major excitatory neurotransmitter, and a natural sweetener. Aspartic acid may also be a significant immunostimulant of the thymus.
Epigenome Associated with Arsenicosis in Zimapan Population (Mexico)

Epigenetically modified (hypermethylated) iAs-induced networks:
(B) Tumor suppressor protein p53 (tp53)-associated network.
(C) The iAs-induced tumor suppressorome - a complex of 17 tumor suppressors known to be silenced in human cancers
Epigenetic Changes in Genes Linked to Diabetes: Promoter methylation status correlates with urinary As metabolites

Type-1 Diabetes

- Antigen-presenting cell
- Cytotoxic CD8+ T cell
- Macrophage

- IFNY
- TNFB
- TNFA

- MAP3K1
- MKK4/7
- TRAF2
- TRAF6
- TAK1
- JNK
- P38 MAPK
- STAT1
- STAT1
- NF-kB
- PDX1
- IRF1
- NOS2
- PRKCD
- INS
- NO

- VAMP2
- GAD1
- IFNGR1
- TNFR

- JAK-STAT signaling
- Apoptosis

- positive association
- negative association

Type-2 Diabetes

- obesity
- TNFA
- TNFRSF1B
- INS
- IR
- IRS
- SOCS6
- PIP2
- PIP3
- PTEN
- INPPL1

- P38 MAPK
- AKT
- glycogen synthesis
- protein synthesis
- lipogenesis

- cellular proliferation
- glucose transport

- VAMP2

- cytokine
- transcriptional regulator
- transmembrane receptor
- enzyme
- DMAs-associated gene
- iAs-associated gene
- positive association
- negative association
- transmembrane receptor

Bailey et al. JBMT 2013 (in press)
Conclusions

1. Exposures to moderate-to-high concentrations of iAs in drinking water increase the risk of developing diabetes.

2. iAs-induced diabetes is characterized by fasting hyperglycemia and impaired glucose tolerance but not necessarily by hyperinsulinemia or increased insulin resistance (HOMA-IR).

3. Human data and results of tissue culture studies suggest that iAs exposure impairs both insulin signaling and insulin production/secretion, with pancreatic β-cells being the primary target.

4. The methylation by AS3MT activates iAs as a diabetogen.

5. AS3MT(M287T) polymorphisms increases the risk of diabetes due to an increased production of DMAAs.
Future Prospective Study in Mexico

**Goals:**
1. To examine causality of the association between chronic iAs exposure and diabetes
2. To characterize phenotype of iAs-induced diabetes

**Longitudinal study in Chihuahua (Mexico):**
- Incidence of diabetes in the Chihuahua cohort over the next 5 years
- Conversion rates from impaired glucose tolerance to diabetes
- Characterization of the phenotype of iAs-induced diabetes

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### CURRENT PROJECT
- **Total subjects recruited:** 1003
- **Total subjects followed annually:** 450
- **Year:** 4, 5

### CONTINUATION PROJECT
- **Total subjects recruited:** 1200
- **Subjects recruited annually:** 350, 350, 300
- **Subjects followed annually:** 450, 450, 450
- **Years:** 1, 2, 3, 4, 5
- **Total subjects followed:** 1980

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**SUBJECTS FOLLOWED ANNUALLY**
- **Year 1:** 450
- **Year 2:** 450
- **Year 3:** 450
- **Year 4:** 450
- **Year 5:** 180

**SUBJECTS RECRUITED ANNUALLY**
- **Year 1:** 350
- **Year 2:** 350
- **Year 3:** 300
- **Year 4:** 450
- **Year 5:** 180

**TOTAL SUBJECTS RECRUITED**
- **CURRENT PROJECT:** 1003
- **CONTINUATION PROJECT:** 1200

**TOTAL SUBJECTS FOLLOWED**
- **CURRENT PROJECT:** 180
- **CONTINUATION PROJECT:** 1980
Future Research Directions & Topics

- Molecular mechanisms of the diabetogenic effects of iAs exposure, with focus on β-cells
- Exposure to iAs (and other arsenicals) in the diet
- Role of prenatal (in utero) exposure to iAs in the development of adult disease, including diabetes
- Epigenetic processes affected by chronic exposure to iAs (cross-generational effects, role of iAs metabolism)
- Effects of iAs exposure on urine and blood metabolome (implications for As-induced diabetes)
- Further optimization and development of analytical techniques for detection and quantification of iAs and its (toxic) metabolites in the environment, foods and biological matrices
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