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Assessment of *in vivo* Bioaccessibility of Arsenic in Dietary Rice by a Mass Balance Approach

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Abstract

A pilot dietary experiment was conducted over ten days to evaluate whether a simple yet often under utilized approach of constructing mass balance of arsenic metabolites can be used to assess *in vivo* bioaccessibility of arsenic in cooked rice. Two volunteers were involved in this study. The quantity of drinking water, food and urine samples, together with arsenic concentration and speciation of these samples were monitored to construct a mass balance of arsenic intake and excretion. In the first five days, the two volunteers on a wheat diet had an average arsenic daily intake of $15.4 \pm 2.6 \mu\text{g}$ and $9.6 \pm 0.7 \mu\text{g}$, respectively. In the next five days, these volunteers switched to a rice diet, increasing the average arsenic daily intake to $36.4 \pm 2.8 \mu\text{g}$ and $34.1 \pm 7.7 \mu\text{g}$, respectively. Daily excretion of urinary arsenic, mostly as dimethylarsenic acid (DMA), doubled from $9.8 \pm 0.3 \mu\text{g}$ to $21.0 \pm 3.0 \mu\text{g}$, and from $6.5 \pm 0.8 \mu\text{g}$ to $11.6 \pm 4.5 \mu\text{g}$, respectively. The percentage of ingested arsenic excreted in urine remained constant at ~ 58% for one volunteer before and after the rice diet, and was ~ 69 % for another. Mass balance established during a controlled dietary experiment over 10 days is shown to be a useful approach to evaluate *in vivo* bioaccessibility and metabolism of arsenic uptake from diet and is applicable to study with more subjects.

Keywords

arsenic; rice; urine; diet; bioaccessibility

1. Introduction

Studies in the last two decades have greatly improved our understanding of exposure, metabolism and health effects of arsenic (As) (Ahsan et al. 2007; Brown et al. 1989; Parvez et al. 2006). Drinking water exposure to As is clearly linked to cancer occurrence in highly exposed populations (Chen and Lin 1994). However, growing evidence points to risks to certain types of cancers and adverse health effects in population exposed to even low concentrations (<50 $\mu\text{g/L}$) of As from drinking water. An ecological study has identified a link between

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residual bladder cancer mortality and use of private wells in both white men and women in New England, New York and New Jersey (Ayotte et al. 2006). Such results suggested that greater exposure to carcinogens, especially As which is known to frequently exceed 5 µg/L in private well water from this region, increases the chance of developing bladder cancer (Ayotte et al. 2006). In a cohort of 11,746 people in Bangladesh, the risk of developing skin lesions was found to be 1.91 times greater for a group exposed to drinking water As concentration ranging from 8.1 µg/L to 40 µg/L than a group exposed to < 8.1 µg/L of As (Ahsan et al. 2006). Additionally, the intellectual function of children in Bangladesh is adversely affected by water As exposure. Ten year old children exposed to water As concentration between 5.6 µg/L to 50 µg/L showed declining intellectual function consistent with a dose-response relationship (Wassermann et al. 2004).

Recently, dietary exposure to As, especially that from rice, has drawn increasing attention (Kile et al. 2007; Meacher et al. 2002; Schoof et al. 1999; Williams et al. 2006). As exposure from a rice-dominated diet with daily consumption of 0.5 kg of rice can amount to 100 µg of As, assuming an As concentration of 200 µg/kg in rice. This exposure is equivalent to drinking 1 L of water containing 100 µg/L of As per day. The major reason for concern is because inorganic forms of As, e.g. arsenite (AsIII) and arsenate (AsV), were found to be the dominant species in many rice samples surveyed around the world (Ackerman et al. 2005; Williams et al. 2006). In these surveys, As concentrations ranged from < 100 µg/kg to as high as 1700 µg/kg (Abedin et al. 2002; Meharg and Rahman 2003; Williams et al. 2006; Xie and Huang 1998), but most samples contained less than 700 µg/kg of As. A market basket survey (Williams et al. 2007) showed that the mean and median arsenic grain levels for rice processed in the South Central U.S. were 300 and 270 µg/kg (n=107), respectively. Rice from California displayed a mean As concentration of 170 µg/kg and a median value of 160 µg/kg (n=27) (Williams et al. 2007). Thus, those for whom rice is a staple of the diet, intake of inorganic As can easily exceed the 10 µg daily level, calculated using the US EPA drinking water standard of 10 µg/L and average drinking water consumption rate of 1 L per day. Even for an average American, rice could still contribute significantly to the total dietary exposure of As (Williams et al. 2007).

An open question in evaluating health effects of dietary arsenic exposure is how much arsenic in the diet is bioavailable and thus is harmful for human body. Although enzymatic extraction, in which an artificial gastrointestinal fluid containing pepsin and bile extracts, has been used to extract arsenic from samples, providing an *in vitro* estimate of the bioaccessibility of arsenic (Ackerman et al. 2005; Koch et al. 2007; Laparra et al. 2003), it cannot replace *in vivo* studies. Using enzymatic extraction, a range of arsenic bioaccessibility from over 60 % to 108 %, was observed (Ackerman et al. 2005; Laparra et al. 2005). To the best of our knowledge, no study has assessed arsenic bioaccessibility of rice *in vivo*. Such a knowledge gap is perhaps in part due to the difficulty of conducting an *in vivo* experiment. Without such knowledge, the health effects of arsenic from dietary exposure such as rice cannot be simply estimated by equivalent dose-response established by epidemiological studies of inorganic arsenic exposure from drinking water. In addition, recent studies have found that the capacity to methylate arsenic differs by nutrient intake (Gamble et al. 2006; Heck et al. 2007). Thus it is probably not justified to directly adopt a dose-response relationship established from drinking water As exposure to that from rice.

The objective of this study is to demonstrate the feasibility of evaluating the bioaccessibility of arsenic in rice *in vivo* through a simple mass balance approach during a short period of controlled diet. Although only two participants were involved, it is sufficient for method development purposes because many samples were collected over time. The experiment is designed to determine whether urinary As excretion responds to dietary changes with food containing different but still normal concentrations of arsenic. Rice was chosen to be the main

source of As dietary exposure. Other non-rice diet items were chosen for their low As content to allow comparison with those from rice. During the experiment, the diet of the volunteers was rice-free for the first five days. For the second five day, ~ 300 g (wet weight) of rice replaced the wheat staple in the diet each day. All drinking water, food and urine samples were measured for mass or volume and analyzed for arsenic concentration. Arsenic speciation in the rice and urine was also measured.

2. MATERIALS AND METHODS

2.1 Experimental design

Two adult volunteers participated in the dietary experiment over the course of 10 days. The first volunteer (V1) is a 38 year old Asian female with a body mass of 50 kg and a height of 1.65 m. The second volunteer (V2) is a 34 year old Asian female with a body mass of 55 kg and a height of 1.64 m. They fully understood the project and experimental details. The experimental protocol was approved by John Jay College Institutional Review Board (IRB) for human subject protection. Wheat and rice were the staple foods for the first and the second five days of experiment, respectively (Table 1). When switching from a wheat diet to a rice diet, V1 began the rice diet on the 5th day at dinner whereas V2 began the rice diet on the 6th day at lunch. The other food items largely consisted of vegetables and fruits were measured and displayed low As concentrations. The concentration of arsenic in drinking water was ≤ 1 $\mu\text{g/L}$. The purpose of this experiment was to establish urinary arsenic background in the first five days, and to monitor urinary arsenic changes caused by rice diet in the second five days.

The diet prior to the experiment was not controlled. Instead, an exposure contrast was designed to further evaluate the effect of un-controlled diet prior to the diet experiment. V1 had fish (grilled salmon) for lunch on the same day when the experiment began at dinner. V2 started the low As, non-rice diet five days before the experiment. The purpose for such contrast in the study design is to investigate how much time is needed to obtain a reliable background because there is no such a guideline available and the time frame that volunteers refrain from seafood ranged from 3 days to overnight in previous studies (Koch et al. 2007; Tam et al. 1979; Johnson and Farmer 1991). V1 and V2 represented two real-world situations and information obtained is essential for the future experimental design with a large cohort.

2.2 Food and drink samples

All food and drink items were purchased from supermarkets in New York City metro-area. Most food items were organic. New York City tap water was used for drinking and cooking by V1. Bottled water was used by V2.

The raw rice was labeled 'Grown in USA'. The rice was cooked without rinsing in a rice cooker by adding 1000 mL water to 300 g raw rice. Vegetables were first cleaned with tap water and then cooked. Fruits were served with skin removed. Wheat flour made bun, the carbohydrate staple for the first five days, ham, soymilk and drinking water were served as is. For each meal or snack, the weight of every food item consumed and the time it was served, and the volume of every drink consumed and the time it was served, were measured and recorded. Small aliquots of the food and drink samples served at each meal were frozen in zip-lock bags and in acid-cleaned HDPE bottles for arsenic measurement.

2.3 Urine sample collection

Every single urine excretion of the volunteers was collected in acid-cleaned plastic bottles, with the volume and time recorded. All samples were kept in a refrigerator at 4 °C or on ice in a cooler for at most 24 hours. A composite daily urine sample from the first morning urine to the last evening urine was made by mixing a volume of urine proportional to the volume

excreted at each time. This 24-hr composite thus represents the urine excreted during the entire day. All urine samples, including the first morning urine and the 24-hr composite urine sample, were stored in a -80°C freezer before analysis was completed within 2 months of sample collection.

2.4 Reagent and standards

The stock solution containing 1000 mg/L As of AsV, (Na_2HAsO_4 , ACS reagent, Sigma, St. Louis, MO, USA), AsIII (NaAsO_2 , 96.7% purity, Sigma), monosodium acid methane arsonate (MMA), (99.0% purity, Chem Service, West Chester, PA, USA) dimethylarsinic acid (DMA) (98% purity, Sigma) and arsenobetaine (AsB) (Argus Chemicals, Italy) were used in the experiments. A 1000 mg/L Ge (High-Purity Standards, Charleston, SC, USA) was used as internal standard (IS). Optima grade nitric acid, trace metal grade hydrochloric acid, ACS reagent grade ammonium carbonate, ammonium hydroxide and sodium chloride were obtained from Fisher (Pittsburg, PA, USA). Ultrapure water was prepared from a Millipore system (18.2 m Ω , Millipore, Bedford, MA, USA).

NIST standard reference materials (National Institute of Standards and Technology, Gaithersburg, MD, USA) used in this work included 1568a rice flour (certified value 290 ± 30 $\mu\text{g/kg}$; determined value 305 ± 19 $\mu\text{g/kg}$, $n=3$) and 2670a toxic elements in urine (certified value 220 ± 10 $\mu\text{g/kg}$, determined value 231 ± 8 $\mu\text{g/kg}$, $n=8$). NIST samples were analyzed frequently through the entire work to ensure the quality of analysis.

2.5 Sample treatment and analysis

Nitric acid digestion for total As assay: A homogenized wet food sample was transferred to a Teflon beaker and weighed. After the sample was freeze dried, it was weighed again. Two mL of concentrated Optima HNO_3 was added to allow reaction at room temperature for about 10 min. The sample was then digested on a hot plate (130°C) with gentle heating in a fume hood until all solids dissolved. The heating continued until almost all acid evaporated. The beaker was cooled, and 20 ml of 2% HNO_3 was added to bring the digested sample into solution.

Water-methanol extraction for As speciation of rice samples: About 0.5 g of powdered raw rice sample was placed into a Teflon vial, to which 2 mL of 1:1 water:methanol solution was added. The vial was capped and sonicated for 2 hours using a Branson 5510 Sonicator. After sonication, the extraction continued overnight at 4°C in the dark before filtration through Whatman nylon filters (0.2 μm pore size) immediately before speciation analysis.

Urine sample pretreatment: Urine samples were thawed and filtered through Whatman nylon filters (0.2 μm pore size) before analysis. For total As assay, individual urine samples were diluted 10 times using 2% HNO_3 containing 10 $\mu\text{g/L}$ Ge internal standard. NIST SRM 2670a, toxic element in urine, was diluted 20 times due to its high As concentration. For speciation, filtered samples were used directly.

Total As was determined using a Thermo X-series Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) (Waltham, MA, USA) operating in the automated Collision Cell Technique (CCT) mode, which effectively eliminates $^{75}\text{ArCl}$ interference. A Thermo high pressure liquid chromatograph (HPLC) coupled with the Thermo X-series ICP-MS operated at normal mode was used for As speciation analysis. The separation was performed with a Hamilton PRP X-100 anion exchange column ($2.1 \times 250\text{mm}$, particle size 5 μm) using ammonium carbonate as mobile phase. Gradient elution employed 10 mM (A) and 20 mM (B) buffer solutions of pH 8.9 ammonium carbonate. The step-gradient program was 0–5 min buffer A, 5–15 min buffer B, 15–25 min buffer A with a flow rate of 1.0 mL/min. This method

neither differentiates MMA (III) from MMA(V), nor DMA(III) from DMA(V). Therefore, only total MMA and total DMA were quantified.

3. RESULTS AND DISCUSSION

3.1 Dietary Arsenic Intake

The arsenic concentration was found to be significantly higher in rice than in all the other food items involved in this study (Table 1). The plain cooked rice samples has 52 ± 2 $\mu\text{g/kg}$ of As on wet weight basis, equivalent to 140 ± 5 $\mu\text{g/kg}$ on dry weight basis. This compares well with the 148 ± 4 $\mu\text{g/kg}$ of total arsenic measured by digestion of raw powdered rice. Among the eleven food items other than rice, seven displayed total arsenic concentrations < 6 $\mu\text{g/kg}$ on wet weight basis, and four displayed total arsenic concentrations between 12 to 23 $\mu\text{g/kg}$ on wet weight basis. No detectable arsenic was found in bottled drinking water. New York City tap water contained 1 ± 0.1 $\mu\text{g/L}$ As ($n=3$). Soy milk contained 2 ± 0.2 $\mu\text{g/L}$ As ($n=3$). The speciation analysis of raw rice powder found that As III was the major species accounting for 76 %, or 101 $\mu\text{g/kg}$, of the 148 $\mu\text{g/kg}$ of total arsenic. DMA accounted for 19 %, or 25 $\mu\text{g/kg}$, and other forms (MMA and AsV) accounted for 5 %. The total extractable As by water-methanol method was 90%. Thermal treatment, and by extension, cooking, should not cause the changes in the chemical forms in which arsenic is found in the raw material (Devesa et al. 2008). The average daily consumption of a specific food item, and the day when it is consumed by V1 and V2, are also summarized (Table 1).

Rice was found to be a major contributor to dietary arsenic exposure for both participants. Total daily arsenic intake of both volunteers (Table 2) was calculated by summing arsenic intake from each food and drink consumed daily. During the rice-free diet, the total daily arsenic intake was 15.4 ± 2.6 μg ($n=4$) for V1, and 9.6 ± 0.7 μg ($n=5$) for V2. In the second five-day period, when rice accounted for 39% of the daily food consumption for both V1 and V2, the total daily arsenic intake increased to 36.9 ± 2.9 μg ($n=4$) for V1 and 34.1 ± 7.8 μg ($n=5$) for V2, in which 27.8 ± 3.3 μg ($n=4$) for V1 and 28.7 ± 7.9 μg ($n=5$) for V2 were from rice. Because 76% of arsenic in rice is AsIII, the average daily AsIII consumed from rice was therefore approximately 21.1 ± 2.5 μg ($n=4$) for V1, and 21.8 ± 6.0 μg for V2. Because rice was such an overwhelming source of As in diet, As speciation analysis was made for it but not for other diet items.

3.2 Urinary Arsenic Excretion

Total arsenic excreted from urine was much higher at the end of the rice diet experiment compared to the value for the rice-free diet for both participants (Table 3). The concentrations were 13 ± 1 $\mu\text{g/L}$ versus 6 $\mu\text{g/L}$ respectively for V1 and 8 ± 0 $\mu\text{g/L}$ versus 5 ± 0 $\mu\text{g/L}$ respectively for V2. V1 had a high initial arsenic excretion of 36.3 ± 1.5 μg due to fish consumption 6 hours before the initiation of low As diet. The amount decreased continuously and eventually lowered to 9.8 ± 0.3 μg on day 5 (Fig. 1). A baseline was most likely reached by day 5 because urinary AsB concentrations decreased from 24 $\mu\text{g/L}$ on day 1, to 8 $\mu\text{g/L}$ on days 2 and 3, and finally to 6 and 7 $\mu\text{g/L}$ on day 4 and day 5, respectively. Studies have also found that fish As could be eliminated in 4 days (Johnson and Farmer 1991). Our data suggested that a minimum 4-day, preferably one-week, dietary control is necessary to establish the background of urinary arsenic before the start of an *in vivo* experiment that requires ingestion of arsenic. Once the rice diet began, urinary As concentration increased similarly for V1 and V2. For both participants, about 30 % of daily urinary arsenic was found to be excreted as the first morning urine.

Except for when urinary AsB concentrations of V1 are high, DMA was found to be the dominant arsenic species in all the other urine samples (Table 3). The concentrations of AsIII,

AsV, and MMA were either non-detectable or very low in all urine samples (Fig. 2). It is worth noting that even though the average daily AsIII consumption from rice was $21.1 \pm 2.5 \mu\text{g}$ for V1 and $21.8 \pm 6.0 \mu\text{g}$ for V2, the average daily urinary AsIII excretion was only $1.4 \pm 0.4 \mu\text{g}$ for V1 and $0.6 \pm 0.5 \mu\text{g}$ for V2, respectively. The most profound effect of the introduction of the rice diet on urinary As speciation is reflected in the almost instantaneous doubling of urinary DMA concentration for both volunteers after the rice diet begun (Table 3). The average urinary DMA concentration increased from $4 \pm 1 \mu\text{g/L}$ ($n=5$) before the rice diet to $8 \pm 2 \mu\text{g/L}$ ($n=5$) after the rice diet for V1. The increase was from $2 \pm 0 \mu\text{g/L}$ ($n=2$) to $5 \pm 2 \mu\text{g/L}$ ($n=3$) for V2.

When the urinary arsenic excretion is compared to the As dietary exposure, the mass balance of arsenic show that, in a steady state condition, approximately two thirds of the dietary As is excreted in urine within 24 hours (Table 2). In addition, both V1 and V2 responded to the sudden increase of the arsenic burden from the introduction of the rice diet (Fig. 1). For V2, the average %As excreted in urine dropped from an average of $69 \pm 13 \%$ before the rice diet to 20% on the first day after rice consumption, and then slowly increased back to 68% on the fifth day of the rice diet. These results are consistent with findings showing that arsenic methylation efficiency of an individual is remarkably stable over time even though there was intra-individual variation (Concha et al. 2002).

3.3 Urinary arsenic profile in the last three days of rice diet

Because rice was consumed twice daily (lunch and dinner), participants were subjected to two large doses of As each day. From the 7th day to the 10th day of the experiment, arsenic consumption from lunch averaged $16.5 \pm 2.9 \mu\text{g}$ ($n=3$) whereas arsenic consumption from dinner averaged $21.2 \pm 2.1 \mu\text{g}$ ($n=2$) for V1. The daily As consumption from all other sources was less than $9.0 \mu\text{g}$ for V1.

The consistent pattern of urinary As excretion for V1 over the last three days (Fig. 3) of the rice diet shows that the body effectively eliminated most of the As intake from rice each day because by late morning before lunch, the urinary As concentration had reached a minimum concentration of $\sim 4 \mu\text{g/L}$. The first morning urine displayed the highest urinary As concentration as well as the maximum excreted urinary As mass. The increase in urinary As concentration and mass after lunch was much more subtle except for the last day when the dose was particularly high at $19.7 \mu\text{g}$. Further study is needed to confirm whether the most effective methylation and excretion of arsenic from human body happens at night during sleep.

3.4 Arsenic uptake and elimination

Previous studies of the uptake, biotransformation and elimination of arsenic in the human body have shown that arsenic is rapidly cleared through the blood stream and excreted mostly via urine. Small amounts of As are excreted in feces (Tam et al. 1979) and accumulated in hair, nails (Karagas et al. 2000) and skin (Koch et al. 2007). When a single dose of about $6 \mu\text{Ci}$ containing $\sim 0.01 \mu\text{g}$ arsenic was administered to six healthy adult male volunteers with age of 20–60 and weight of 64–84 kg, 58% of arsenic dose was excreted in the urine in the first days after dosing (Tam et al. 1979). Repeated ingestion of a low dose of $22 \mu\text{g}$ of AsV at a regular 8 hr interval, or a daily does of $66 \mu\text{g}$, for a 10 day period by one volunteer (55 kg) (Johnson and Farmer 1991), resulted in an average of 40–50% urinary excretion of the dose. Our results found that more than 60% of the As was excreted in urine when two daily dose of approximately $20 \mu\text{g}$ of rice As was ingested, and the data further confirm that excretion in urine is the major route for arsenic elimination by the body. In this study, the urinary arsenic excretion rate, or the percentage As excreted, can be used as a proxy for the bioaccessibility of arsenic in rice to the body. This is because urinary arsenic must have been initially absorbed by the digestive system, metabolized and circulated in the blood before being eliminated. The real bioavailability of arsenic in rice is likely to be higher than that indicated by the urinary

arsenic excretion rate due to arsenic accumulation and excretion through other pathways such as hair, nail and skin. Nevertheless, the *in vivo* data is more meaningful than those established by *in vitro* experiments.

3.5 Rapid Arsenic Elimination

Starting with the demonstration by Buchet et al. (Buchet et al. 1981) of rapid biotransformation of arsenic by the body and elimination through the urine, studies have all found evidence for fast kinetics of arsenic elimination (Johnson and Farmer 1991; Koch et al. 2007). In the Buchet et al. study, the maximum urinary excretion was observed after 4 to 12 hours of ingestion of 500 µg of arsenic. After 4 days, the amount of arsenic excreted in urine represented 46, 78 and 75% of the ingested dose of As III, MMA and DMA, respectively, and half of which was excreted approximately in 28 hrs for AsIII, < 4 hrs for MMA, and 11 hrs for DMA (Buchet et al. 1981). When a Caucasian male volunteer ingested a single dose of 1000 µg inorganic arsenic (presented as a mineral realgar As₄S₄, an ingredient of Niu Huang Jie Du Pian, a traditional Chinese medicine), the urinary As profile showed a peak of AsIII 4–8 hours after ingestion, followed by a larger peak of DMA and MMA 14 hours after ingestion (Koch et al. 2007). Approximately 40% of arsenic was estimated to have been excreted from the uptake of approximately 1 mg of bioaccessible arsenic measured by gastric fluid extraction. Our results are consistent with these studies in that increased urinary arsenic excretion signals were commonly observed within 24 hrs after rice ingestions (Fig. 2.) The faster elimination time of < 1 day may due to the lower dosage of As ingested than in the previous studies.

The elimination of fish-derived As is also rapid and occurs within days. A single oral dose of seafood arsenic leads to elevated urinary arsenic concentrations within 2–4 hours of intake followed by a rapid decline and eventually returned to baseline after four days (Johnson and Farmer 1991). The urinary As pattern exhibited by V1 followed the same trend, with a rapid decline of concentration to reach baseline on the fifth day of the experiment (Fig. 1). Speciation analysis verified that AsB was the major form to contribute a high urinary arsenic concentration.

3.6 Methylation of As from Rice Diet

Our results of methylation of As consumed in rice agreed with those from previous findings on methylation of As via various exposure routes. DMA has been consistently reported as the major metabolite in urine, even though there is considerable intra-individual variation in its proportion (Concha et al. 2002). DMA was 64.7% of urinary As two days after the initial intake when a volunteer ingested 66 µg of inorganic AsV daily (Johnson and Farmer 1991). In Tam's study, DMA accounted for 51% of urinary arsenic for the first 5 days following a single dose ingestion of ~0.01 µg of radioactive ⁷⁴As (Tam et al. 1979). Investigation of urinary arsenic metabolites in 233 residents, including children and adults, exposed to 20 (control), 90 or 160 µg/L inorganic arsenic via drinking water in Inner Mongolia, DMA was found to account for 85 % of urinary arsenic in control group (16 children and 20 adults), but decreased to 70% in the two exposed groups. Assuming daily consumption of 1L of drinking water, this control group is exposed to 20 µg of As, slightly less than the daily arsenic intake from rice in this study of ~ 26 µg. We find that DMA is 63 ± 5 % (n=5) of the urinary As for V1, and is 82 ± 8 % (n=3) of the urinary As for V2 with rice diet. In addition to the fact that our results are agreeable with %DMA in urine from population expose to low dose of As, it appears that V1 has a lower methylation rate than V2.

4. Implications and Limitations

The mass balance approach established by this pilot study has been demonstrated to be useful in providing critical information to establish bioaccessibility of As through *in vivo* experiment.

Whereas studies with a much larger population are needed to confirm the following preliminary findings, we show that arsenic in rice is rapidly eliminated in urine within 24 hours of ingestion, with urinary As elimination accounting for over 60% of the dietary dose. Individuals respond to the increased arsenic burden from rice ingestion quickly and maintain a relatively stable urinary excretion rate. Further research involving more participants can use this mass balance approach to obtain statistically significant data for risk assessment of dietary arsenic and to investigate individual variability. Factors that may affect bioavailability or methylation such as gender, age, body mass, ethnicity, and health history should be considered.

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REFERENCES

- Abedin MJ, Cresser MS, Meharg AA, Feldmann J, Cotter-Howells. Arsenic accumulation and metabolism in rice (*Oryza sativa* L.). *Environ. Sci. Technol* 2002;36:962–968. [PubMed: 11918027]
- Ackerman AH, Creed PA, Parks AN, Fricke MW, Schwegel CA, Creed JT, Heitkemper DT, Vela NP. Comparison of a chemical and enzymatic extraction of arsenic from rice and assessment of the arsenic absorption from contaminated water by cooked rice. *Environ. Sci. Technol* 2005;39:5241–5246. [PubMed: 16082952]
- Ahsan H, Chen Y, Kibriya MG, Slavkovich V, Parvez F, Jasmine F, Gamble MV, Graziano JH. Arsenic metabolism, genetic susceptibility, and risk of premalignant skin lesions in Bangladesh. *Cancer Epidemiol Biomarkers Prev* 2007;16:1270–1278. [PubMed: 17548696]
- Ahsan H, Chen Y, Parvez F, Zablotska L, Argos M, Hussain L, Momotaj H, Levy D, Cheng ZQ, Slavkovich V, et al. Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: Baseline results from the health effects of arsenic longitudinal study. *Am J. Epidemiol* 2006;163:1138–1148. [PubMed: 16624965]
- Ayotte J, Baris D, Cantor KP, Colt J, Tobinson GRJ, Lubin JH, Karagas MR, Robert NH, Fraumeni JFJ, Silverman DT. Bladder cancer mortality and private well use in New England: an ecological study. *J. Epidemiology and Community Health* 2006;60:168–172.
- Brown KG, Boyle KE, Chen CW, Gibb HJ. A dose-response analysis of skin cancer from inorganic arsenic in drinking water. *Risk Anal* 1989;9:519–528. [PubMed: 2608947]
- Buchet JP, Lauwerys R, Roels H. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. *Int. Arch. Occup. Environ. Health* 1981;48:71–79. [PubMed: 6894292]
- Chen, C-J.; Lin, L-J. Human carcinogenicity and atherogenicity induced by chronic exposure to inorganic arsenic. In: Nriagu, JO., editor. *Arsenic in the environment, Part II: Human health and ecosystems effects*. New York: John Wiley & Sons; 1994.
- Concha G, Vogler G, Nermell B, Vahter M. Intra-individual variation in the metabolism of inorganic arsenic. *Int. Arch. Occup. Environ. Health* 2002;75:576–580. [PubMed: 12373320]
- Devesa V, Velez D, Montoro R. Effect of thermal treatment on arsenic species contents in food. *Food Chem. Toxicol* 2008;46:1–8. [PubMed: 17928121]
- Gamble MV, Liu X-H, Ahsan H, Pilsner JR, Ilievski V, Slavkovich V, Parvez F, Chen Y, Levy D, Factor-Litavak P, et al. Folate and arsenic metabolism: a double-blind placebo-controlled folic acid-supplementation trial in Bangladesh. *Am J. Clin Nutr* 2006;84:1093–1101. [PubMed: 17093162]
- Heck JE, Gamble MV, Chen Y, Graziano JH, Slavkovich V, Parvez F, Baron JA, Howe GR, Ahsan H. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. *Am J. Clin Nutr* 2007;85:1367–1374. [PubMed: 17490975]
- Johnson LR, Farmer JG. Use of human metabolic studies and urinary arsenic speciation in assessing arsenic exposure. *Bull. Environ. Contam. Toxicol* 1991;46:53–61. [PubMed: 2001493]

- Karagas MR, Tosteson TD, Blum J, Klaue B, Weiss JE, Stannard V, Spate V, Morris JS. Measurement of low levels of arsenic exposure: A comparison of water and toenail concentrations. *Am J Epidemiol* 2000;152:84–90. [PubMed: 10901333]
- Kile ML, Houseman EA, Breton CV, Smith T, Quamruzzaman Q, Rahman M, Mahiuddin G, Christiani D. Dietary arsenic exposure in Bangladesh. *Environ. Health Perspect* 2007;115(6):889–893. [PubMed: 17589596]
- Koch I, Sylvester S, Lai VW-M, Owen A, Reimer KJ, Cullen WR. Bioaccessibility and excretion of arsenic in Niu Huang Jie Du Pian pills. *Toxicol. Appl. Pharmacol* 2007;222:357–364. [PubMed: 17239412]
- Laparra J, Velez D, Montoro R, Barbera R, Farre R. Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *J. Agric. Food Chem* 2003;51:6080–6085. [PubMed: 13129320]
- Laparra JM, Velez D, Barbera R, Farre R, Montoro R. Bioavailability of inorganic arsenic in cooked rice: practical aspects for human health risk assessments. *J. Agric. Food Chem* 2005;53:8829–8833. [PubMed: 16248591]
- Meacher DM, Menzel DB, Dillencourt MD, Bic LF, Schoof RA, Yost LJ, Eickhoff JC, Farr CH. Estimation of multimedia inorganic arsenic intake in the US population. *Hum. Ecol. Risk Assess* 2002;8:1697–1721.
- Meharg A, Rahman M. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. *Environ. Sci. Technol* 2003;37:229–234. [PubMed: 12564892]
- Parvez F, Chen Y, Argos M, Hussain A, Momotaj H, Dhar R, Van Geen A, Graziano JH, Ahsan H. Prevalence of arsenic exposure from drinking water and awareness of its health risks in a Bangladeshi population: Results from a large population-based study. *Environ. Health Perspect* 2006;114:355–359. [PubMed: 16507457]
- Schoof RA, Yost LJ, Eickhoff J, Crecelius EA, Cragin DW, Meacher DM, Menzel DB. A market basket survey of inorganic arsenic in food. *Food and Chemical Toxicology* 1999;37:839–846. [PubMed: 10506007]
- Tam GKH, Charbonneau SM, Bryce F, Pomroy C, Sandi E. Metabolism of inorganic arsenic (74As) in humans following oral ingestion. *Toxicol. Appl. Pharmacol* 1979;50:319–322. [PubMed: 505460]
- Wassermann GA, Liu XH, Parvez F, Ahsan H, Factor-Litavak P, Van Geen A, Slavkovich V, Lolacono NJ, Cheng ZQ, Hussain L, et al. Water arsenic exposure and children' intellectual function in Araihaazar, Bangladesh. *Environ. Health Perspect* 2004;112:1329–1333. [PubMed: 15345348]
- Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J, Meharg AA. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. *Environ. Sci. Technol* 2006;40:4903–4908. [PubMed: 16955884]
- Williams PN, Raab A, Feldmann J, Meharg AA. Market Basket survey shows elevated levels of As in South Central U.S. processed rice compared to California: Consequences for Human dietary exposure. *Environ. Sci. Technol* 2007;41:2178–2183. [PubMed: 17438760]
- Xie Z, Huang C. Control of arsenic toxicity in rice plants grown on an arsenic-polluted paddy soil. *Communications in Soil Science and Plant Analysis* 1998;29(15–16):2471–2477.

Fig. 1.

Changes in urinary As excretion over a ten-day period before and after the rice diet for two volunteers. The x-axis indicates the day during the experiment. The difference in total urinary arsenic between volunteer 2 (V2, solid circle) whose diet was controlled for 6 days prior to day 1 of the experiment and volunteer 1 (V1, solid square) whose diet included fish right before the start of the experiment was illustrated. V1 began rice diet on the fifth day at dinner time, and V2 started rice diet from day six at lunch time. High initial urinary arsenic of V1 is due to consumption of fish six hours before the experiment.

Fig. 2.

A representative chromatogram of urinary arsenic speciation. The sample shown is the first morning urine of V1 on day 10. The peak intensity is presented by the counts of ICP-MS signal, and the retention time is expressed in millisecond.

Fig. 3.

Urinary arsenic excretion in the last 3 days of the experiment with rice intake from lunch and dinner for V1. The solid squares are urinary arsenic concentration and the open circles are the mass of arsenic. “L” indicates lunch and “D” indicates dinner.

Table 1

Arsenic concentration in diet involved in this study and the average daily consumption of each food

Food category	Food name	Moisture content (%)	As Conc. ($\mu\text{g/kg}$ wet or $\mu\text{g/L}$ for drink, n=3)	V1	V2
Carbohydrate	Wheat bun (brand 1)	26	12 $\pm$ 1	413 $\pm$ 77g (day 1 to 5) 86 $\pm$ 0 g (day 6 to 10)	415 $\pm$ 33 g (day 1 to 5)
	Wheat bun (brand 2)	34	17 $\pm$ 2		194 $\pm$ 54g (day 6 to 10)
	Plain cooked rice ^a	63	52 $\pm$ 2	312 $\pm$ 115 g (day 5, 6, 8, 10)	
	Stirred fried rice ^b	63	52 $\pm$ 2	418 $\pm$ 165 g (day 6, 7, 9, 10)	
Vegetables	Fried bok choy	74	23 $\pm$ 5	290 $\pm$ 46 g (day 1,3,5,8)	
	Curry eggplant & cabbage	88	5 $\pm$ 1	377 $\pm$ 181 g (day 1,2,3,4,6,7)	
	Spicy broccoli	89	6 $\pm$ 0	290 $\pm$ 130g (day 1, 2, 4, 5, 6, 9)	
	Fried eggplant	86	13 $\pm$ 1	295 $\pm$ 100 g (day 2 to 5)	
Meat	Cabbage	82	2 $\pm$ 0		314 $\pm$ 108g (day 1 to 10)
	ham	75	5 $\pm$ 0		228 $\pm$ 51 g (day 1 to 10)
Fruit	banana	72	5 $\pm$ 2	137 $\pm$ 4 g (day 2, 4, 5, 7)	
	apple (Chile)	86	5 $\pm$ 0	150 $\pm$ 17 (day 1,3,5,6,8,10)	
	apple (Fuji)	86	3 $\pm$ 0		283 $\pm$ 107g (day 1 to 10)
Drink	soymilk		2 $\pm$ 0	442 $\pm$ 162 mL (day 1 to 10)	1140 $\pm$ 360 mL (day 1 to 10)
	Drinking water 1		N.D.		700 $\pm$ 355mL (day 1 to 10)
	Drinking water 2		1 $\pm$ 0	1140 $\pm$ 409mL (day 1 to 10)	

- 1) Total arsenic in NIST rice 1568a (certified value $290 \pm 30 \mu\text{g/kg}$) was $305 \pm 19 \mu\text{g/kg}$ (n= 3). As III: $78 \mu\text{g/kg}$; As V: $23 \mu\text{g/kg}$; MMA: $12 \mu\text{g/kg}$; DMA: $148 \mu\text{g/kg}$
 - 2) Total arsenic in raw rice sample purchased from local supermarket and labeled 'Grown in USA' was $148 \pm 4 \mu\text{g/kg}$ (n= 3). As III: $101 \mu\text{g/kg}$; As V: $2 \mu\text{g/kg}$; MMA: Non-detectable; DMA: $25 \mu\text{g/kg}$.
- ^aEquivalent to $140 \pm 5 \mu\text{g/kg}$ dry rice (n=3).
- ^bFried from plain cooked rice with one egg.

Table 2

Mass balance calculation of arsenic intake and excretion from urine

day	As intake from dietary (μg)			As excreted from urine (μg)	% As excreted from urine
	Rice	Other than rice	Total		
V1	1 0.0	18.4 $\pm$ 1.8	18.4 $\pm$ 1.8		
	2 0.0	14.1 $\pm$ 0.9	14.1 $\pm$ 0.9		
	3 0.0	16.3 $\pm$ 1.3	16.3 $\pm$ 1.3		
	4 0.0	12.6 $\pm$ 0.9	12.6 $\pm$ 0.9		
	5 15.7 $\pm$ 0.0 ^a	15.5 $\pm$ 0.6	31.3 $\pm$ 0.6	9.8 $\pm$ 0.3	63 ^b
	6 28.5 $\pm$ 0.6	7.1 $\pm$ 1.7	35.6 $\pm$ 1.8	17.4 $\pm$ 0.7	49
	7 26.1 $\pm$ 0.8	7.8 $\pm$ 0.4	33.9 $\pm$ 0.9	22.7 $\pm$ 1.7	67
	8 24.6 $\pm$ 1.0	12.7 $\pm$ 0.5	37.3 $\pm$ 1.1	18.1 $\pm$ 0.4	49
	9 32.1 $\pm$ 0.9	8.7 $\pm$ 1.9	40.8 $\pm$ 1.5	23.8 $\pm$ 0.4	58
	10 13.9 $\pm$ 1.2	7.8 $\pm$ 0.4	34.4 $\pm$ 1.3	23.1 $\pm$ 1.2	67
V2	1 0.0	9.0 $\pm$ 1.1	9.0 $\pm$ 1.1	7.0 $\pm$ 0.7	78
	2 0.0	9.7 $\pm$ 1.1	9.7 $\pm$ 1.1	6.6 $\pm$ 0.7	68
	3 0.0	9.7 $\pm$ 1.2	9.7 $\pm$ 1.2	6.6 $\pm$ 0.5	68
	4 0.0	10.6 $\pm$ 1.2	10.6 $\pm$ 1.2	5.1 $\pm$ 0.5	48
	5 0.0	8.9 $\pm$ 1.1	8.9 $\pm$ 1.1	7.2 $\pm$ 0.4	81
	6 22.8 $\pm$ 0.6	5.8 $\pm$ 0.4	28.6 $\pm$ 0.8	5.7 $\pm$ 0.8	20
	7 35.8 $\pm$ 1.0	3.3 $\pm$ 0.3	39.2 $\pm$ 1.0	11.6 $\pm$ 0.4	30
	8 37.8 $\pm$ 1.1	5.9 $\pm$ 0.5	43.7 $\pm$ 1.1	9.1 $\pm$ 0.6	21
	9 27.8 $\pm$ 0.8	6.9 $\pm$ 0.5	34.7 $\pm$ 0.9	15.1 $\pm$ 0.8	44
	10 19.6 $\pm$ 0.5	5.0 $\pm$ 0.4	24.6 $\pm$ 0.7	16.7 $\pm$ 0.8	68

^aV1 began rice diet on the fifth day at dinner time.^bOnly the fifth day's urine excretion of 9.8 mg of As was used as baseline for V1. This is because fish consumption a day before the experiment begin by V1 has resulted in AsB in urine at concentration of 24, 8, 8, 6 and 7 $\mu\text{g/L}$ for day 1, 2, 3, 4 and 5, respectively (Table 3).

Table 3

Urinary As concentration and speciation on a ten-day diet trial

Date	As species						Total ^e (μg/L, n=3)	Volume of urine (L)	Total As excreted from urine (μg)
	AsB (μg/L)	AsIII (μg/L)	DMA (μg/L)	MMA (μg/L)	AsV (μg/L)	SUM ^d (μg/L)			
Composite urine sample (V1)									
1	24	<i>a</i> ND	6	1	ND	31	19 ± 1	1.87	36.3±1.5
2	8	ND	3	<1	<1	12	12 ± 1	1.96	22.0±1.1
3	8	ND	3	<1	ND	11	10 ± 1	1.68	16.0±0.8
4	6	<1	3	<1	ND	9	6 ± 0	2.37	14.8±0.2
5	7	<1	4	<1	<i>b</i> NA	NA	6 ± 0	1.64	9.8±0.3
6	4	1	9	1	ND	14	9 ± 0	1.97	17.4±0.7
7	2	<1	7	1	ND	11	12 ± 1	1.90	22.7±1.6
8	2	1	5	1	ND	9	8 ± 0	2.17	18.1±0.4
9	2	1	9	2	ND	13	13 ± 0	1.79	23.8±0.4
10	2	1	11	2	ND	16	13 ± 1	1.76	23.1±1.2
First morning urine sample (V1)									
4	3	<1	2	<1	ND	6	7 ± 0	0.39	29±0.2
5	8	<1	9	2	ND	19	16 ± 0	0.21	33±0.1
9	2	2	18	4	2	29	38 ± 1	0.20	7.6±0.2
10	2	2	20	5	ND	30	25 ± 0	0.45	11.1±0.1
Composite urine sample (V2) ^c									
3	2	<1	2	ND	ND	5	5 ± 0	1.73	6.6±0.5
5	1	<1	2	ND	ND	4	5 ± 0	1.53	7.2±0.4
8	<1	<1	3	<1	<1	4	4 ± 0	2.57	9.1±0.6
9	<1	<1	7	<1	ND	8	7 ± 0	2.31	15.1±0.8
10	<1	<1	5	ND	ND	6	8 ± 0	2.11	16.7±0.8
First morning urine sample (V2)									

Date	As species					Total ^e (μg/L, n=3)	Volume of urine (L)	Total As excreted from urine (μg)
	AsB (μg/L)	AsIII (μg/L)	DMA (μg/L)	MMA (μg/L)	AsV (μg/L)			
4	1	<1	1	<1	ND	2 ± 0	0.70	1.3±0.1
5	2	<1	3	ND	<1	5 ± 0	0.41	2.1±0.1

^aNon-detectable

^bA electronic spike was observed at the same retention time, resulting no data for AsV. High concentration of total As observed in V1 from day 1 to day 4 is due to consumption of fish six hours before the experiment.

^cDue to the limit of time and cost, we only performed speciation analysis for V2 samples that could represent the change of the metabolites before and after rice diet, i.e. samples collected on day 3,5, and 8,9,10; and the speciation data listed here was from single measurement.

^dSum=[AsB]+[AsIII]+[MMA]+[DMA]+[AsV]

^eTotal is from direct measurement of [As] in a composite urine sample by ICP-MS operating at Collision Cell Technology mode.