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Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7,8-dihydro-2'-deoxyguanosine in urine

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¹*Abbreviations used:* 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; B, blood; BMI, body mass index; DMA, dimethylarsinic acid; GW, gestational week; ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh; ICPMS, inductively coupled plasma mass spectrometry; LC–MS/MS, liquid chromatography tandem mass spectrometry; MMA, methylarsonic acid; P-Ft, plasma ferritin; ROS, reactive oxygen species; U, urinary.

Abstract

Exposure to arsenic (As), cadmium (Cd), and lead (Pb) may generate oxidative stress, which can be assessed by 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in urine, a sensitive marker of oxidatively damaged DNA. We have evaluated oxidative stress induced by chronic mixed exposure to As, Cd, and Pb, as well as the influence of As metabolism and nutritional status, i.e., levels of ferritin (Ft), selenium (Se), zinc (Zn), and manganese (Mn) and body weight. 8-OxodG was measured in urine from 212 women in early pregnancy from Matlab, in rural Bangladesh, using LC–MS/MS. Cd and Pb were analyzed in urine and erythrocytes, and Se, Mn, and Zn were analyzed in erythrocytes, all by ICPMS. As and As metabolites were analyzed in urine by HPLC–ICPMS. Ferritin was analyzed in plasma by radioimmunoassay. The median concentration of 8-oxodG was 8.3 nmol/L (adjusted for specific gravity), range 1.2–43, corresponding to a median of 4.7 µg/g creatinine, range 1.8–32. 8-OxodG was positively associated with urinary Cd ($\beta = 0.32$, $p < 0.001$), urinary As ($\beta = 0.0007$, $p = 0.001$), the fraction of the monomethylated arsenic metabolite in urine ($\beta = 0.0026$, $p = 0.004$), and plasma Ft ($\beta = 0.20$, $p < 0.001$). A joint effect was seen for urinary Cd and As, but whether this effect was additive or multiplicative was difficult to discern. *Keywords:* 8-OHdG; Arsenic; Cadmium; Ferritin; Oxidative stress; Pregnancy; Lead; Free radicals

Arsenic (As), cadmium (Cd), and lead (Pb) are among the most commonly encountered toxic metals for

humans after environmental or dietary exposure. Inorganic As exposure often occurs via drinking water, but also via certain foods, whereas Cd and Pb exposure mainly comes from food. The consequences of long-term exposures may be extensive, especially for fetuses and young children, who often are particularly susceptible to toxic insult [1]. As, Cd, and Pb are known to generate reactive oxygen species (ROS)¹ [2]. An excessive rise in ROS production due to insufficient antioxidant defense or exposure to exogenous pro-oxidants may lead to pathological effects at the cellular and organ levels. Indeed, As- and Cd-induced ROS have been suggested as a mechanism for carcinogenesis [3,4]. Mixed exposure to ROS-generating substances may produce additive or multiplicative effects on oxidative stress levels [5].

One of the major ROS-induced DNA base-modified products, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), is a sensitive marker of oxidatively damaged DNA. Associations with increased urinary 8-oxodG concentrations have been seen mainly for As [6–12]. Arsenic is metabolized via methylation. A high fraction of the monomethylated As metabolite methylarsonic acid (MMA) in urine, which is a susceptibility factor for As-induced toxicity, including carcinogenicity, has been linked to high concentrations of 8-oxodG in urine [7,13,11]. An association between Cd in urine and 8-oxodG was reported by Ketelslegers et al. [14], but not by De Coster et al. [15]. There seems to be no information linking Pb exposure with urinary 8-oxodG.

Enzymes involved in antioxidative defense or DNA repair may change the effects of these elements. Selenium (Se), zinc (Zn), and manganese (Mn) are necessary for the function of a number of antioxidant/DNA repair enzymes, e.g., Se as a component in glutathione peroxidase that protects against oxidative stress, Zn as a component in enzymes maintaining DNA integrity, and Mn as a cofactor in the antioxidant enzyme Mn superoxide dismutase.

We report on a population-based cohort study, evaluating the impact of concurrent As, Cd, and Pb exposure on 8-oxodG concentrations in early pregnancy, as well as the effects of As metabolism efficiency. This study was carried out in rural Bangladesh, where the exposure to As in drinking water from tube wells is a major public health problem and where we have previously shown that pregnant women were exposed to a wide range of As concentrations in the water [16]. People in the study area are also exposed to Cd, mainly through elevated levels in rice, the main staple food [17,18], and to some extent also to Pb, also via rice, at least partly (Bergkvist C et al., unpublished data in “Assessment of early life lead exposure in rural Bangladesh”, 2010). Because the toxicity of both As and Cd may be influenced by nutritional status, and malnutrition is prevalent in the area [18,19], we have also evaluated the effects of As, Cd, and Pb considering nutritional status, as measured by body weight and levels of Se, Zn, Mn, and Fe (using plasma ferritin as a biomarker of iron status).

<H1>Material and methods

<H2>Study area and population

This population-based cross-sectional study was nested into a large food and micronutrient supplementation trial (Maternal and Infant Nutrition Interventions of Matlab, or MINIMat) among pregnant women in Matlab, a rural area 53 km southeast of Dhaka, Bangladesh. In this area, the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is running a large-scale health and demographic surveillance system that is updated monthly

with the help of community health workers visiting all families in the area. All women living within the coverage area of ICDDR,B who became pregnant between November 2001 and October 2003 were invited to participate in the MINIMat. From the 2119 women who were enrolled during January 2002 to December 2002, 500 women were randomly chosen. In total, 440 of the 500 women donated a urine specimen and 408 provided a blood sample in early pregnancy (described in [20]). Of the 408 blood samples, 220 were randomly selected for measurements of 8-oxodG in the present study. Urine for 8-oxodG, Cd, and As analyses was sampled in gestational week (GW) 8 (range 1–15 weeks) and blood/plasma for Cd, Fe, Mn, Pb, Se, and Zn in GW 14 (range 9–20 weeks). Pb was also analyzed in urine sampled at the same time as the blood.

Information on age, body weight, and BMI in GW 8, as well as parity and socioeconomic status, was available. Socioeconomic status was defined in terms of an asset score, relevant for rural settings. The asset score was generated through principal component analysis [21], standardized in relation to a standard normal distribution with a mean of 0 and a standard deviation of 1. None of the women were smokers.

<H2>*Arsenic analysis*

Exposure to inorganic arsenic was assessed by concentrations of As metabolites in urine (U-As) measured as the sum of inorganic arsenic (iAs) and the main methylated metabolites MMA and dimethylarsinic acid (DMA). Speciation of As metabolites in urine was performed using high-pressure liquid chromatography online with hydride generation and inductively coupled plasma mass spectrometry (ICPMS; Agilent 1100 Series System; Agilent 7500ce; Agilent Technologies, Santa Clara, CA, USA) employing adequate quality control [22,23]. To compensate for variations in dilution of urine, concentrations were adjusted to the mean specific gravity of 1.012 g/ml, measured by a hand refractometer (Atago, Japan).

<H2>*Multielement analyses*

Cd and Pb were analyzed in both blood and urine. For Cd, blood is a biomarker for both short-term exposure (half-life is a couple of months) and long-term retention (half-life is up to several decades), whereas Cd in urine is a biomarker for long-term retention only. Pb in blood has at least two compartments and reflects a combination of the exposure during the past months and several years back in time [24]. Because of the relatively young population, the Cd and Pb in blood probably mainly reflect short-term exposure. Pb in urine is closely associated with the concentration in blood.

Metals in urine, Cd (U-Cd) and Pb, were measured using ICPMS (Agilent 7500ce; Agilent Technologies) with a collision/reaction cell system, autosampler (Cetac ASX-510), integrated sample introduction system, and a MicroMist nebulizer in quartz [17,18]. Concentrations were adjusted to the mean specific gravity (1.012 g/ml). Fewer individuals (around 170–175 for each element) were analyzed for elements in erythrocytes, because of an insufficient amount of blood. Erythrocyte element concentrations were measured by ICPMS after digestion using a microwave-assisted high-temperature/high-pressure acid digestion system (Milestone UltraCLAVE II; EMLS, Leutkirch, Germany) as described in more detail elsewhere [18]. Reference material analyses for Cd are described in Kippler et al. [17]. For Pb quality control purposes (Bergkvist C. et al., unpublished data, 2010), two commercial

control materials were analyzed for blood (Serorm trace elements whole blood L-1, Ref. 201505, Lot MR4206, and Serorm trace elements whole blood L-2, Ref. 201605, Lot 0503109) and for urine, Serorm trace elements urine, Ref. 201205, Lot NO2525 was used. The mean lead concentrations and standard deviations (mean \pm SD) in the control materials were 24.8 ± 0.8 $\mu\text{g/L}$ ($n = 22$; analytical value 27.6 ± 1.4 $\mu\text{g/L}$; Lot MR4206), 366 ± 6 $\mu\text{g/L}$ ($n = 22$; analytical value 393 ± 21 $\mu\text{g/L}$; Lot 0503109), and 80 ± 1.6 $\mu\text{g/L}$ ($n = 16$; analytical value 91.1 ± 7.0 $\mu\text{g/L}$; Lot NO2525). In general, the obtained results showed a good agreement with the analytical values.

Plasma ferritin (P-Ft) was measured using a radioimmunoassay as described previously [17,19].

<H2>8-OxodG analysis

Concentrations of 8-oxodG in urine were measured using liquid chromatography tandem mass spectrometry (LC–MS/MS), with prior purification using solid-phase extraction [7,25]. Oasis HLB columns, 1 cc, 30 mg (Waters, Milford, MA, USA), were used for the solid-phase extraction. Analysis of the samples was performed using LC–MS/MS with electrospray ionization (API 3000; Applied Biosystems, Foster City, CA, USA) coupled to a liquid chromatography system from Perkin–Elmer (Norwalk, CT, USA). All samples were above the limit of detection (0.5 nmol/L). All data acquisition and processing were performed using the Analyst 1.4.2 software (Applied Biosystems). The samples were prepared and analyzed in duplicates in two different analytical sample sets on different days and the interassay coefficient of variance for these was 7%. Also, two internal controls were included in each run (unadjusted mean concentrations 4.2 and 13.1 nmol/L) and the coefficients of variance for these controls were 11.5 and 8.9%, respectively. Concentrations were adjusted to the mean specific gravity (1.012 g/ml). To make comparisons of levels of 8-oxodG in this population with 8-oxodG levels in other studies, the data were also corrected by urinary creatinine, where the ratio of 8-oxodG ($\mu\text{g/L}$) to creatinine content (g/L) was evaluated as a complement to the adjustments for mean specific gravity. Creatinine adjusted values are used only for descriptive data comparing levels of 8-oxodG with other studies; we have done no other statistical analyses using creatinine adjusted values. The creatinine levels were analyzed using an enzymatic method described by Mazzachi et al. [26].

<H2>Statistical analysis

To analyze the associations between elements or Ft and 8-oxodG, a univariate analysis was run for each element/Ft, with natural-log-transformed 8-oxodG as the dependent variable. 8-OxodG, P-Ft, and U-Cd were natural log transformed to normalize its distribution. For As and its metabolites, Cd, and Pb, two multivariate analyses were performed: first, an analysis with adjustments for influential covariates (potentially influential covariates were GW, age, parity, and asset score) and second, a multivariate analysis with adjustments for influential covariates as well as for markers of nutritional status (B-Zn, B-Se, B-Mn, P-Ft, and the most influential of body weight or body mass index (BMI)). Covariates were included if they demonstrated a p value of <0.2 in the univariate analyses with 8-oxodG (natural log transformed) as the dependent variable. Finally, we simultaneously evaluated the impact of the elements/Ft that were significantly associated with 8-oxodG.

Additionally, when evaluating the effect of U-As on 8-oxodG concentrations, we stratified for %MMA (below/above median) to evaluate the U-As effect among different metabolism efficiency strata.

We evaluated the joint effect of the elements two by two (all elements were evaluated in combinations, the combination of Cd and As is given here as an example) by dichotomizing each element into two groups (above and below the median concentration). Then, the dichotomized groups were combined for two elements into a variable with four groups (e.g., group 1, individuals that were below the median for Cd and below the median for As; group 2, below the median for Cd and above the median for As; group 3, above the median for Cd and below the median for As; group 4, above the median for Cd and above the median for As) and ANOVA was performed. P-Ft was dichotomized according to the levels for deficiency (<20 µg/L) instead of the median value.

All statistical analyses were performed using SPSS (version 15; SPSS, Chicago, IL, USA).

<H1>Results

<H2>Study participant characteristics

Descriptive data of the women as well as their As metabolite values and biomarker concentrations (in erythrocytes, plasma, and urine) are shown in Table 1. In total, 212 women had valid 8-oxodG concentrations; there was not enough urine left in the sample from one woman, and samples from seven women were excluded because their duplicate samples demonstrated >20% difference in mean values of 8-oxodG, despite reanalysis. The median 8-oxodG concentration, adjusted to the average specific gravity of 1.012 g/ml, was 8.3 nmol/L (range 1.2–43 nmol/L), corresponding to 4.7 µg/g creatinine (range 1.8–32 µg/g creatinine).

<H2>Metal exposure, nutrition, and 8-oxodG

U-Cd was strongly associated with 8-oxodG concentrations (Fig. 1, Table 2). There was no effect of B-Cd on 8-oxodG concentrations (Table 2), despite the fact that U-Cd and B-Cd were strongly correlated ($r_s = 0.50$). Also U-As (Fig. 2) and %MMA were associated with 8-oxodG concentrations (Table 2), although the effect of %MMA disappeared when adjustments were made for nutritional factors. No significant associations between 8-oxodG and Pb in blood or in urine were seen (Table 2).

When evaluating the impact of U-As on 8-oxodG concentrations among different metabolism efficiency strata, the strongest effect on 8-oxodG was found for individuals within the high %MMA stratum (median %MMA 13%, range 10–22%, median U-As 170 µg/L, range 22–1216 µg/L), in which the association between U-As and 8-oxodG was significant, whereas it was nonsignificant in the low %MMA stratum (median %MMA 7.9%, range 0–10%; median U-As 65 µg/L, range 10–538 µg/L). A difference in the impact of U-As on 8-oxodG for the high and low %MMA groups was also seen within the same U-As range: among individuals with U-As ≤ 538 µg/L (the range for low %MMA), the group with higher %MMA demonstrated $\beta = 0.001$, $p = 0.051$, whereas the group with lower %MMA demonstrated $\beta = 0.0001$ and $p = 0.73$.

Among the potential covariates, age ($\beta = 0.01$, $p = 0.11$) and GW ($\beta = -0.027$, $p = 0.13$) had p values below 0.2 in the univariate analysis and were included in the multivariate analysis. Among the nutritional factors measured, body weight ($p = 0.07$) had a stronger association with 8-oxodG than BMI ($p = 0.31$). P-Ft was positively associated with 8-oxodG (Table 2). B-Zn was positively, though weakly, associated with 8-oxodG concentration ($p = 0.080$), whereas no clear effects of B-Se or B-Mn were seen.

When simultaneously evaluating the elements/Ft that were associated with 8-oxodG (U-Cd, U-As, and P-Ft), a statistically significant effect was still seen for all elements/Ft. After adjustments were made for covariates and nutritional factors, U-Cd ($\beta = 0.32$, $p < 0.001$) and P-Ft ($\beta = 0.22$, $p < 0.001$) were still strongly associated with 8-oxodG, whereas the effect of U-As became weaker ($\beta = 0.0004$, $p = 0.056$).

<H2>Effects of combined exposures

The analyses of combinations of elements/Ft in groups of lower and higher concentrations (low/high defined as relative to median concentrations of all study individuals) showed that the effects of neither U-Cd nor U-As seemed to be the result of the correlation with each other ($R_s = 0.22$; Fig. 3, $p < 0.001$). At these exposure levels, it was difficult to discern whether the effect was additive or multiplicative (Fig. 3, the median concentrations of 8-oxodG for each exposure group are given in the figure legend). The median 8-oxodG in the group with high U-As and high U-Cd was 10.2 nmol/L. If assuming an additive model, the group with higher U-Cd and higher U-As would have an expected 8-oxodG concentration of 10.2 nmol/L ($6.5 + (7.7 - 6.5) + (9.6 - 6.5) = 10.8$). If assuming a multiplicative model, the group with higher U-Cd and higher U-As would have an 8-oxodG concentration of 10.2 nmol/L ($6.5 \times (7.7/6.5) \times (9.6/6.5) = 11.4$).

The influence of age was further investigated, because both age (weakly) and Cd (strongly) were associated with 8-oxodG concentration and Cd and age were correlated as well. Age was divided into four groups (<20, 20–25, 25–30, and >30 years). Univariate analyses were performed between U-Cd and 8-oxodG, age and 8-oxodG, and age and U-Cd, within each age stratum. The association between U-Cd and 8-oxodG was strongly significant in all age groups (p values ranged between <0.001 and 0.008 and β ranged between 0.26 and 0.32), whereas neither the association between age and 8-oxodG nor that between age and U-Cd was significant in any of the age groups.

In the analyses of other element/Ft combinations than the ones mentioned above (all combinations were tested), no statistically significant results were found.

<H1>Discussion

Chronic exposure to As and, in particular, Cd strongly increased urinary 8-oxodG concentrations in a dose-dependent manner in the rural Bangladeshi women studied. We observed no effect of Pb exposure; however, the exposure to Pb was fairly modest. Consistent with previous reports, P-Ft (a biomarker of Fe status) was positively associated with increased concentration of 8-oxodG. Inefficient methylation of As, as measured by a high fraction of MMA in urine, seemed to be a susceptibility factor for As-induced 8-oxodG.

One of the major strengths of this study is that samples were obtained reflecting wide ranges of Cd and As exposure, as well as the variation in nutritional status; about one-third of the women had a BMI below 18.5 kg/m². Furthermore, this female population is homogeneous for several factors that may potentially influence 8-oxodG concentrations, i.e., there were no smokers [27], no alcohol consumption, and minor exposure to pollution from automobile exhaust and industries. The biomarkers studied in blood and urine are considered relevant biomarkers for exposure to As, Cd, and Pb, as well as for Se, Mn, Fe, and Zn status. There was a difference in sampling time of urine analyzed for Cd, As, and 8-oxodG (week 8) and sampling of blood and urine analyzed for the other elements

(week 14), although the time periods overlapped. This does not represent a problem because the concentrations of Cd and Pb in blood cells have a half-life of a couple of months, longer in subjects who have a long-term exposure. Hence, the blood levels should not be markedly affected by a difference in sampling time of 5–6 weeks in subjects with exposure via drinking water and food, which is likely to be fairly stable.

U-Cd showed the strongest association with 8-oxodG, with a clear dose–effect relationship. B-Cd and U-Cd were strongly positively correlated, but no similar association between B-Cd and 8-oxodG was found. The probable reason for this is that oxidatively damaged DNA is an effect of long-term Cd retention, as reflected by U-Cd, rather than short-term exposure, which is mainly reflected by B-Cd. The induction of oxidative stress by Cd can be interpreted by its inhibitory effects on antioxidant enzymes such as catalase and superoxide dismutase or depletion of glutathione (reviewed in Beyersmann and Hartwig [2] and Joseph [28]).

Despite the wide range of As exposure, with U-As concentrations up to 1200 µg/L, and the known pro-oxidative effects of As, the association of 8-oxodG with U-As was weaker than that with the moderate Cd exposure. 8-OxodG may not be as sensitive a biomarker for As-induced oxidative stress as it is for Cd-induced oxidative stress. We have previously demonstrated that the efficiency of As metabolism via methylation influences the urinary concentrations of 8-oxodG in Andean Argentinean women [7]. The positive association between 8-oxodG and %MMA in urine in both studies provides further supporting evidence that a low capacity for arsenic methylation to DMA via one-carbon metabolism is associated with an increased risk of oxidative damage, which in turn may lead to malignant transformation [29–31] and other toxic effects, e.g., preeclampsia and negative pregnancy outcomes [32]. The diminished effect of %MMA after adjustments for nutritional factors is probably due to a reduced number of individuals with data on essential elements, excluding a large number of individuals with higher fraction of MMA. The percentage of MMA formed is largely influenced by genetic factors, in particular *AS3MT* polymorphisms [23,33–35]. The results for the combination of Cd and As exposure were not conclusive for determination of an additive or multiplicative effect on 8-oxodG levels.

It is rather unexpected that Pb, which can generate free radicals by itself and inhibit DNA damage repair [2], was not associated with urinary 8-oxodG. However, the Pb concentrations were fairly low in this population, and 8-oxodG may not be a sensitive marker of Pb-related oxidative stress.

P-Ft was clearly positively associated with 8-oxodG, consistent with the findings of previous studies [36,37]. P-Ft reflects the levels of ferritin in the liver that regulate the uptake of iron in the intestine [38]. Iron can cause oxidative stress by generating hydroxyl radicals via Fenton chemistry, which can oxidize lipids, proteins, and DNA.

The 8-oxodG concentrations can vary because of the analysis methods used; ELISA especially has been shown to overestimate 8-oxodG levels, whereas mass spectrometric techniques seem to be more robust [39,40]. The 8-oxodG concentrations determined were twice as high in this study compared to our previous study population from the Andes part of Argentina, employing the same LC–MS/MS method for analysis [7]. In the Andes, the population studied had a higher average exposure to As, lived at a high altitude (3800 m above sea level), and had a low consumption of fruit and vegetables, all factors that have been associated with higher oxidative stress [41–43]. However, the lower levels of 8-oxodG in the Andean population may depend on a more efficient metabolism of As in the population from Argentina [44], with lower %MMA in urine than in this study, or on genetic factors [23].

Furthermore, the women from Argentina were not pregnant, which probably reduces the oxidative stress levels. Pregnancy induces a state of oxidative stress, as a result of increased metabolic activity in the placental mitochondria [45]. There is a physiological increase in the production of ROS as early as the first trimester [46,47]. In this study, we could not decipher the effects of pregnancy, because all the women were pregnant.

The results from this study demonstrate that among these malnourished women with low antioxidant levels [19] there is a background of oxidative stress, partly from iron exposure. The combined chronic high-level exposure to several toxic metals in this population may result in a too-high body burden of oxidative stress with subsequent adverse toxic effects. Although it is evident that different environmental exposures result in increased levels of 8-oxodG [48], the relationship between urinary 8-oxodG and future health risks, such as cancer or adverse pregnancy outcomes, is not well characterized. A Danish prospective study on urinary 8-oxodG and lung cancer indicated a higher risk for cancer among nonsmokers with higher urinary 8-oxodG excretion. However, this was based on few individuals [49].

<H1>Conclusion

The strong positive association between U-Cd and urinary 8-oxodG suggests specific mechanisms for Cd-induced oxidatively damaged DNA. The influence of As exposure and Ft concentrations on 8-oxodG concentrations were verified in this study. The clinical effects of the increased body burden of oxidatively damaged DNA requires further investigation in this population of pregnant women.

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References

- [1] Rahman, A.; Vahter, M.; Ekstrom, E. C.; Rahman, M.; Golam Mustafa, A. H.; Wahed, M. A.; Yunus, M.; Persson, L. A. Association of arsenic exposure during pregnancy with fetal loss and infant death: a cohort study in Bangladesh. *Am. J. Epidemiol.* **165**:1389-1396; 2007.
- [2] Beyersmann, D.; Hartwig, A. Carcinogenic metal compounds: recent insight into molecular and cellular

mechanisms. *Arch. Toxicol.* **82**:493-512; 2008.

[3] International Agency for Research on Cancer. Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. In: IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 58. Lyon: Oxford Univ. Press; 1993. URL: <http://monographs.iarc.fr/ENG/Monographs/vol58/volume58.pdf>.

[4] International Agency for Research on Cancer. Some drinking-water disinfectants and contaminants, including arsenic. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 84. Lyon: Oxford Univ. Press; 2004.

[5] Wang, G.; Fowler, B. A. Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. *Toxicol. Appl. Pharmacol.* **233**:92-99; 2008.

[6] Chung, C. J.; Huang, C. J.; Pu, Y. S.; Su, C. T.; Huang, Y. K.; Chen, Y. T.; Hsueh, Y. M. Urinary 8-hydroxydeoxyguanosine and urothelial carcinoma risk in low arsenic exposure area. *Toxicol. Appl. Pharmacol.* **226**:14-21; 2008.

[7] Engström, K.; Vahter, M.; Lindh, C.; Teichert, F.; Singh, R.; Concha, G.; Nermell, B.; Farmer, P. B.; Strömberg, U.; Broberg, K. Low 8-oxo-7,8-dihydro-2'-deoxyguanosine levels and influence of genetic background in an Andean population exposed to high levels of arsenic. *Mutat. Res.* **683**:98-105; 2010.

[8] Fujino, Y.; Guo, X.; Liu, J.; Matthews, I. P.; Shirane, K.; Wu, K.; Kasai, H.; Miyatake, M.; Tanabe, K.; Kusuda, T.; Yoshimura, T. Chronic arsenic exposure and urinary 8-hydroxy-2'-deoxyguanosine in an arsenic-affected area in Inner Mongolia, China. *J. Expo. Anal. Environ. Epidemiol.* **15**:147-152; 2005.

[9] Kubota, R.; Kunito, T.; Agusa, T.; Fujihara, J.; Monirith, I.; Iwata, H.; Subramanian, A.; Tana, T. S.; Tanabe, S. Urinary 8-hydroxy-2'-deoxyguanosine in inhabitants chronically exposed to arsenic in groundwater in Cambodia. *J. Environ. Monit.* **8**:293-299; 2006.

[10] Wong, R. H.; Kuo, C. Y.; Hsu, M. L.; Wang, T. Y.; Chang, P. I.; Wu, T. H.; Huang, S. Increased levels of 8-hydroxy-2-deoxyguanosine attributable to carcinogenic metal exposure among schoolchildren. *Environ. Health Perspect.* **113**:1386-1390; 2005.

[11] Xu, Y.; Wang, Y.; Zheng, Q.; Li, X.; Li, B.; Jin, Y.; Sun, X.; Sun, G. Association of oxidative stress with arsenic methylation in chronic arsenic-exposed children and adults. *Toxicol. Appl. Pharmacol.* **232**:142-149; 2008.

[12] Yamauchi, H.; Aminaka, Y.; Yoshida, K.; Sun, G.; Pi, J.; Waalkes, M. P. Evaluation of DNA damage in patients with arsenic poisoning: urinary 8-hydroxydeoxyguanine. *Toxicol. Appl. Pharmacol.* **198**:291-296; 2004.

[13] Hu, C. W.; Pan, C. H.; Huang, Y. L.; Wu, M. T.; Chang, L. W.; Wang, C. J.; Chao, M. R. Effects of arsenic exposure among semiconductor workers: a cautionary note on urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Free Radic. Biol. Med.* **40**:1273-1278; 2006.

[14] Ketelslegers, H. B.; Gottschalk, R. W.; Koppen, G.; Schoeters, G.; Baeyens, W. F.; van Larebeke, N. A.; van Delft, J. H.; Kleinjans, J. C. Multiplex genotyping as a biomarker for susceptibility to carcinogenic exposure in the FLEHS biomonitoring study. *Cancer Epidemiol. Biomarkers Prev.* **17**:1902-1912; 2008.

[15] De Coster, S.; Koppen, G.; Bracke, M.; Schroyen, C.; Den Hond, E.; Nelen, V.; Van de Mieroop, E.; Bruckers, L.; Bilau, M.; Baeyens, W.; Schoeters, G.; van Larebeke, N. Pollutant effects on genotoxic parameters and tumor-associated protein levels in adults: a cross sectional study. *Environ. Health* **7**:26; 2008.

- [16] Vahter, M. E.; Li, L.; Nermell, B.; Rahman, A.; El Arifeen, S.; Rahman, M.; Persson, L. A.; Ekstrom, E. C. Arsenic exposure in pregnancy: a population-based study in Matlab, Bangladesh. *J. Health Popul. Nutr.* **24**:236-245; 2006.
- [17] Kippler, M.; Ekstrom, E. C.; Lonnerdal, B.; Goessler, W.; Akesson, A.; El Arifeen, S.; Persson, L. A.; Vahter, M. Influence of iron and zinc status on cadmium accumulation in Bangladeshi women. *Toxicol. Appl. Pharmacol.* **222**:221-226; 2007.
- [18] Kippler, M.; Lonnerdal, B.; Goessler, W.; Ekstrom, E. C.; Arifeen, S. E.; Vahter, M. Cadmium interacts with the transport of essential micronutrients in the mammary gland—a study in rural Bangladeshi women. *Toxicology* **257**:64-69; 2009.
- [19] Li, L.; Ekstrom, E. C.; Goessler, W.; Lonnerdal, B.; Nermell, B.; Yunus, M.; Rahman, A.; El Arifeen, S.; Persson, L. A.; Vahter, M. Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. *Environ. Health Perspect.* **116**:315-321; 2008.
- [20] Kippler, M.; Goessler, W.; Nermell, B.; Ekstrom, E. C.; Lonnerdal, B.; El Arifeen, S.; Vahter, M. Factors influencing intestinal cadmium uptake in pregnant Bangladeshi women—a prospective cohort study. *Environ. Res.* **109**:914-921; 2009.
- [21] Gwatkin, D. R.; Rustein, S.; Pande, J.; Wagstaff, R.P. Socio-economic Differences in Health, Nutrition, and Population in Bangladesh. Washington, DC: HNP/Poverty Thematic Group of the World Bank; 2000.
- [22] Lindberg, A. L.; Goessler, W.; Grandner, M.; Nermell, B.; Vahter, M. Evaluation of the three most commonly used analytical methods for determination of inorganic arsenic and its metabolites in urine. *Toxicol. Lett.* **168**:310-318; 2007.
- [23] Schlawicke Engstrom, K.; Broberg, K.; Concha, G.; Nermell, B.; Warholm, M.; Vahter, M. Genetic polymorphisms influencing arsenic metabolism: evidence from Argentina. *Environ. Health Perspect.* **115**:599-605; 2007.
- [24] Nordberg, G.; F. B.; Nordberg, M.; Friberg, L. Handbook on the Toxicology of Metals, 3rd ed. San Diego: Elsevier; 2007.
- [25] Teichert, F.; Verschoyle, R. D.; Greaves, P.; Thorpe, J. F.; Mellon, J. K.; Steward, W. P.; Farmer, P. B.; Gescher, A. J.; Singh, R. Determination of 8-oxo-2'-deoxyguanosine and creatinine in murine and human urine by liquid chromatography/tandem mass spectrometry: application to chemoprevention studies. *Rapid Commun. Mass Spectrom.* **23**:258-266; 2009.
- [26] Mazzachi, B. C.; Peake, M. J.; Ehrhardt, V. Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. *Clin. Lab.* **46**:53-55; 2000.
- [27] Lindberg, A. L.; Ekstrom, E. C.; Nermell, B.; Rahman, M.; Lonnerdal, B.; Persson, L. A.; Vahter, M. Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. *Environ. Res.* **106**:110-120; 2008.
- [28] Joseph, P. Mechanisms of cadmium carcinogenesis. *Toxicol. Appl. Pharmacol.* **238**:272-279; 2009.
- [29] Chen, Y. C.; Guo, Y. L.; Su, H. J.; Hsueh, Y. M.; Smith, T. J.; Ryan, L. M.; Lee, M. S.; Chao, S. C.; Lee, J. Y.; Christiani, D. C. Arsenic methylation and skin cancer risk in southwestern Taiwan. *J. Occup. Environ. Med.* **45**:241-

248; 2003.

- [30] Chen, Y. C.; Su, H. J.; Guo, Y. L.; Hsueh, Y. M.; Smith, T. J.; Ryan, L. M.; Lee, M. S.; Christiani, D. C. Arsenic methylation and bladder cancer risk in Taiwan. *Cancer Causes Control* **14**:303-310; 2003.
- [31] Steinmaus, C.; Bates, M. N.; Yuan, Y.; Kalman, D.; Atallah, R.; Rey, O. A.; Biggs, M. L.; Hopenhayn, C.; Moore, L. E.; Hoang, B. K.; Smith, A. H. Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. *J. Occup. Environ. Med.* **48**:478-488; 2006.
- [32] Burton, G. J.; Jauniaux, E. Placental oxidative stress: from miscarriage to preeclampsia. *J. Soc. Gynecol. Invest.* **11**:342-352; 2004.
- [33] Lindberg, A. L.; Kumar, R.; Goessler, W.; Thirumaran, R.; Gurzau, E.; Koppova, K.; Rudnai, P.; Leonardi, G.; Fletcher, T.; Vahter, M. Metabolism of low-dose inorganic arsenic in a central European population: influence of sex and genetic polymorphisms. *Environ. Health Perspect.* **115**:1081-1086; 2007.
- [34] Meza, M. M.; Yu, L.; Rodriguez, Y. Y.; Guild, M.; Thompson, D.; Gandolfi, A. J.; Klimecki, W. T. Developmentally restricted genetic determinants of human arsenic metabolism: association between urinary methylated arsenic and CYT19 polymorphisms in children. *Environ. Health Perspect.* **113**:775-781; 2005.
- [35] Schlawicke Engstrom, K.; Nermell, B.; Concha, G.; Stromberg, U.; Vahter, M.; Broberg, K. Arsenic metabolism is influenced by polymorphisms in genes involved in one-carbon metabolism and reduction reactions. *Mutat. Res.* **667** 4-14; 2009.
- [36] Nakano, M.; Kawanishi, Y.; Kamohara, S.; Uchida, Y.; Shiota, M.; Inatomi, Y.; Komori, T.; Miyazawa, K.; Gondo, K.; Yamasawa, I. Oxidative DNA damage (8-hydroxydeoxyguanosine) and body iron status: a study on 2507 healthy people. *Free Radic. Biol. Med.* **35**:826-832; 2003.
- [37] Tuomainen, T. P.; Loft, S.; Nyssonen, K.; Punnonen, K.; Salonen, J. T.; Poulsen, H. E. Body iron is a contributor to oxidative damage of DNA. *Free Radic. Res.* **41**:324-328; 2007.
- [38] MacKenzie, E. L.; Iwasaki, K.; Tsuji, Y. Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxid. Redox Signaling* **10**:997-1030; 2008.
- [39] Cooke, M. S.; Singh, R.; Hall, G. K.; Mistry, V.; Duarte, T. L.; Farmer, P. B.; Evans, M. D. Evaluation of enzyme-linked immunosorbent assay and liquid chromatography-tandem mass spectrometry methodology for the analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine in saliva and urine. *Free Radic. Biol. Med.* **41**:1829-1836; 2006.
- [40] Cooke, M. S.; Barregard, L.; Mistry, V.; Potdar, N.; Rozalski, R.; Gackowski, D.; Siomek, A.; Foksinski, M.; Svoboda, P.; Kasai, H.; Konje, J. C.; Sallsten, G.; Evans, M. D.; Olinski, R. Interlaboratory comparison of methodologies for the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Biomarkers* **14**:103-110; 2009.
- [41] Dosek, A.; Ohno, H.; Acs, Z.; Taylor, A. W.; Radak, Z. High altitude and oxidative stress. *Respir. Physiol. Neurobiol.* **158**:128-131; 2007.
- [42] Magalhaes, J.; Ascensao, A.; Marques, F.; Soares, J. M.; Ferreira, R.; Neuparth, M. J.; Duarte, J. A. Effect of a high-altitude expedition to a Himalayan peak (Pumori, 7,161 m) on plasma and erythrocyte antioxidant profile. *Eur. J. Appl. Physiol.* **93**:726-732; 2005.
- [43] Thompson, H. J.; Heimendinger, J.; Gillette, C.; Sedlacek, S. M.; Haegele, A.; O'Neill, C.; Wolfe, P. In vivo

- investigation of changes in biomarkers of oxidative stress induced by plant food rich diets. *J. Agric. Food Chem.* **53**:6126-6132; 2005.
- [44] Vahter, M.; Concha, G.; Nermell, B.; Nilsson, R.; Dulout, F.; Natarajan, A. T. A unique metabolism of inorganic arsenic in native Andean women. *Eur. J. Pharmacol.* **293**:455-462; 1995.
- [45] Potdar, N.; Singh, R.; Mistry, V.; Evans, M. D.; Farmer, P. B.; Konje, J. C.; Cooke, M. S. First-trimester increase in oxidative stress and risk of small-for-gestational-age fetus. *BJOG* **116**:637-642; 2009.
- [46] Jauniaux, E.; Watson, A. L.; Hempstock, J.; Bao, Y. P.; Skepper, J. N.; Burton, G. J. Onset of maternal arterial blood flow and placental oxidative stress: a possible factor in human early pregnancy failure. *Am. J. Pathol.* **157**:2111-2122; 2000.
- [47] Myatt, L.; Cui, X. Oxidative stress in the placenta. *Histochem. Cell Biol.* **122**:369-382; 2004.
- [48] Pilger, A.; Rudiger, H. W. 8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *Int. Arch. Occup. Environ. Health* **80**:1-15; 2006.
- [49] Loft, S.; Svoboda, P.; Kasai, H.; Tjonneland, A.; Vogel, U.; Moller, P.; Overvad, K.; Raaschou-Nielsen, O. Prospective study of 8-oxo-7,8-dihydro-2'-deoxyguanosine excretion and the risk of lung cancer. *Carcinogenesis* **27**:1245-1250; 2006.

Fig. 1. Scatterplot of the association between urinary cadmium (natural log transformed) and concentration of 8-oxodG (natural log transformed).

Fig. 2. Scatterplot of the association between total urinary arsenic and concentration of 8-oxodG (natural log transformed).

Fig. 3. Boxplots depicting 8-oxodG (natural log transformed) for the combination groups of U-Cd (urinary cadmium) and U-As (total urinary arsenic). The median untransformed 8-oxodG values for each combination group were the following (from the left): 6.5, 7.7, 9.6, and 10.2 nmol/L.

Table 1

Descriptive information on the women^a studied including concentrations of elements in urine (U), erythrocytes (B), and plasma (P)

Variable	N	Median	10th/90th percentile
U-Cd (µg/L) ^b	212	0.58	0.23–1.5
B-Cd (µg/kg)	175	1.2	0.64–2.4
U-Pb (µg/L) ^b	179	3.6	1.9–7.3
B-Pb (µg/kg)	175	79	48–150
U-As (µg/L) ^b	211	100	17–380
iAs (%)	211	14	7.3–23
MMA (%)	211	10	5.2–16
DMA (%)	211	75	62–85
Age (years)	212	27	19–35
Parity	212	1	0–3
Height (cm)	212	150	140–160
GW (week)	211	8.1	5.6–11
BMI (kg/m ²)	210	19	17–23
Weight (kg)	210	44	37–53
B-Se (µg/kg)	175	200	160–250
B-Zn (µg/kg)	175	9,700	7,800–120,000
P-Ft (µg/L)	168	28	11–66
B-Mn (µg/kg)	175	22	16–33
8-OxodG (µg/L) ^b	212	8.2	3.9–16

^aOnly individuals with valid 8-oxodG data are included.

^bAdjusted for specific weight (1.012).

Table 2

Association between concentrations of 8-oxodG in urine (dependent variable) and concentrations of cadmium (Cd), arsenic (As), and lead (Pb); influential covariates; and nutritional factors

Element/Ft/covariate	Univariate analyses ^a			Adjusted analyses ^b			Adjusted analyses with nutrition factors ^c		
	N	β	P value	R^2 ^d	N	β	P value	R^2 ^d	R^2 ^d
U-Cd ^e	212	0.32	<0.001	0.21	209	0.32	<0.001	0.20	0.23
B-Cd	175	-0.003	0.96	0	172	-0.02	0.71	0.001	0.002
U-Pb	179	0.013	0.35	0.005	176	0.019	0.19	0.010	0.004
B-Pb	175	0	0.91	0	172	0	0.94	0	0
U-As	211	<0.001	0.001	0.055	208	0.001	0.001	0.057	0.047
MMA	211	0.026	0.004	0.039	208	0.026	0.005	0.037	0.011
B-Mn	175	-0.005	0.43	0.004					
B-Se	175	0.002	0.17	0.011					
B-Zn	175	<0.001	0.08	0.018					
P-Ft ^e	168	0.20	<0.001	0.073					
Weight	210	0.010	0.07	0.016					
GW	211	-0.027	0.13	0.011					
Age	212	0.01	0.11	0.012					

^aUnivariate analyses: 8-oxodG (natural log transformed) = intercept + $\beta_1 \times \text{element/Ft/covariate}$.

^bAdjusted analyses: 8-oxodG (natural log transformed) = intercept + $\beta_1 \times \text{element} + \beta_2 \times \text{gestational week} + \beta_3 \times \text{age}$.

^cAdjusted analyses with nutrition factors: 8-oxodG (natural log transformed) = intercept + $\beta_1 \times \text{element} + \beta_2 \times \text{gestational week} + \beta_3 \times \text{age} + \beta_4 \times \text{B-Zn} + \beta_5 \times \text{B-Se} + \beta_6 \times \text{B-Mn} + \beta_7 \times \text{P-Ft}$. Because blood samples were available for fewer individuals, fewer individuals were included in the adjusted analyses with nutrition factors for the metals measured in urine.

^dUnadjusted R^2 values for the element/Ft.

^eU-Cd and P-Ft are natural log transformed for a better model fit.





