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Metal exposure and oxidative stress markers in pregnant Navajo Birth Cohort Study participants

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Abstract

Contamination of soil and water by waste from abandoned uranium mines has led to chronic exposures to metal mixtures in Native American communities. Our previous work demonstrated that community exposures to mine waste increase the likelihood of developing cardiovascular disease, as well as the likelihood of developing multiple chronic diseases including diabetes, hypertension and kidney disease. Exposure to various environmental metals is associated with elevated oxidative stress, which is considered a contributor to these and other chronic disease states. The purpose of the current research was to assess potential associations between exposure to uranium and arsenic and evidence for increased oxidative stress as measured by urinary F_2-isoprostanes in pregnant women enrolled in the Navajo Birth Cohort Study. The current study also included an analysis of zinc as a potential mediator of oxidative stress in the study population. Urinary arsenic and uranium, serum zinc and urinary F_2-isoprostanes were measured for each study participant at enrollment. Study participants were pregnant women with median age of 26.8; 18.9 % were enrolled in the 1st trimester, 44.7% were enrolled in the 2nd trimester, and 36.4%

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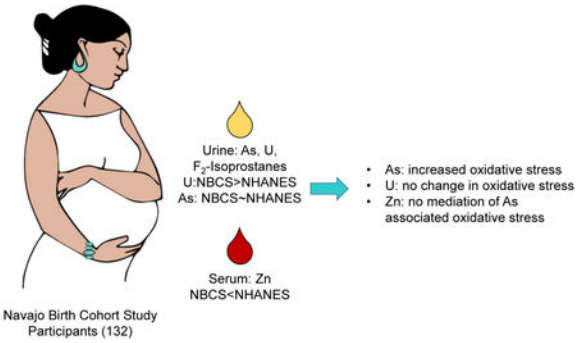
were enrolled in the 3rd trimester. Median urinary metal levels were 5.5 and 0.016 μg/g creatinine for arsenic and uranium, respectively. Multivariable regression analysis indicated a significant association between arsenic exposure and the lipid peroxidation product 8-iso-prostaglandin $F_{2\alpha}$, controlling for zinc and trimester. No associations were detected with uranium despite evidence that levels were in the Navajo Birth Cohort samples were 2.3 times the median reported for women in the National Health and Nutrition Examination Survey (2011-12). Zinc was not found to have any causal mediation of the effects of the other metals on oxidative stress. The current work is consistent with other studies that have detected an association between arsenic and elevated oxidative stress. In contrast to arsenic, uranium did not appear to increase oxidative stress response in this study population. These findings are relevant to assessing the potential human impact of chronic exposure to mixed metal waste from abandoned uranium mines.

Graphical abstract



Keywords

Arsenic; uranium; zinc; oxidative stress; isoprostanes; Navajo Birth Cohort Study; AI/AN

INTRODUCTION

There are more than 4,000 abandoned uranium mines (AUMs[9]) in the Western United States [1] with more than 500 located on the Navajo Nation [2, 3]. These abandoned mine sites are often located in close proximity to Native American communities leading to community concerns about health impacts due to mine waste exposures. People may be exposed to AUM waste containing uranium, arsenic, and other co-occurring metals via air, soil or ground water [4-6]. On the Navajo Nation for example, more than 30% of the population lacks access to regulated public drinking water and must rely on unregulated water supplies or other sources for drinking water. A previous analysis of more than 500 unregulated water sources indicated that 15.1% and 12.8% of these unregulated sources exceeded national drinking water maximum contaminant levels (MCLs) for arsenic and uranium, respectively [7], providing potential exposure sources for arsenic and/or uranium. Navajo community members have expressed particular concern about uranium exposures [9]. Exposure to metals found in abandoned mine waste is associated with chronic disease occurrence among the Navajo and other Native American tribes. A survey of over 1300 people living on the Navajo Nation discovered that Navajo community members with ongoing community-level

exposures to uranium mine waste have an increased likelihood of several chronic conditions such as cardiovascular disease, diabetes, and kidney disease [8, 9]. These findings suggest that chronic exposures to AUM waste affect human health.

Numerous chronic diseases, including cardiovascular and renal disease, are associated with elevated oxidative damage due to excess generation of reactive oxygen species [10-13]. Experimental studies in cells and animal models link both arsenic [14-18] and uranium [19-23] to generation of oxidative stress. In contrast, studies to investigate the association between arsenic exposure and biomarkers of oxidative stress in human populations have not yielded consistent findings [24-34] due in part to different study populations and detection methods for oxidative stress. The lack of studies on uranium exposure and direct biomarkers of oxidative stress is a gap in knowledge that limits our ability to understand the relationships between different metal exposures, oxidative stress and associated diseases in humans.

An ongoing cohort study on the Navajo Nation, the Navajo Birth Cohort Study (NBCS) [35], is examining the effects of uranium and other metal/metalloid exposures on birth outcomes and development. Currently there is limited information about the association between metal exposure and oxidative stress during pregnancy. In the NBCS, biomonitoring results indicate urine uranium concentrations exceed those in the US population, while serum zinc, a metal with antioxidant properties, is frequently below World Health Organization (WHO) recommended sufficiency concentrations [3]. This zinc deficiency has been observed in pregnant Navajo women for more than 35 years [36], but also occurs in men in the population. Therefore, samples from this study provide an opportunity to understand the effect of exposures to known metal inducers of oxidative damage on measures of oxidative stress, and test the hypothesis that zinc may have a potential modifying role in those responses.

Based on evidence from experimental models that arsenic and uranium exposures and zinc deficiency increase oxidative stress [14-23, 37-40], the goal of the current study was to investigate the association between metal exposure and oxidative stress biomarkers, and determine whether serum zinc moderates the effects of these exposures. The lipid peroxidation product 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) was used as a urinary oxidative stress biomarker. Because 8-iso-$PGF_{2\alpha}$ is also generated through an enzymatic pathway by prostaglandin-endoperoxide synthases, the ratio of 8-iso-$PGF_{2\alpha}$ to prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$) has been established to distinguish enzymatic versus chemical lipid peroxidation as a biomarker of oxidative stress and was included in this study [41, 42]. We measured urinary arsenic and uranium, serum zinc and urinary F_2-isoprostanes for 132 participants from the parent NBCS study. This group was then stratified by serum zinc concentrations (low vs. high) while preserving the range of uranium and arsenic found in the population to allow assessment of the impact of zinc on metal-associated oxidative stress. Metal values in the study population were compared to national values obtained from the National Health and Nutrition Examination Survey (NHANES) [43]. The reported findings provide insights into oxidative stress and exposure to metals associated with AUMs.

MATERIALS AND METHODS

Study Overview

The study site, the Navajo Nation, is located in the Four Corners area of the southwestern United States. In 2013 the NBCS began recruiting pregnant women between 14 and 45 years of age who had lived on the Navajo Nation for at least 5 years, were willing to deliver at a participating hospital, and have their child followed up for one year postnatally. At the time of enrollment, a blood and urine sample was collected from the participant. Socioeconomic, demographic, and lifestyle information was also collected in the home shortly after enrollment. A subset of the maternal enrollment urine samples was selected for oxidative stress analysis, which forms the basis of this investigation.

Study Population

The NBCS was initiated to address Navajo community concerns about how chronic environmental exposure to uranium mine waste affects human health. The research team led by the University of New Mexico (UNM) Health Sciences Center Community Environmental Health Program included partnerships with Navajo Nation Department of Health, Navajo Area Indian Health Service, Southwest Research Information Center and the US Centers for Disease Control and Prevention Agency for Toxic Substances and Disease Registry (CDC/ATSDR) National Center for Environmental Health (NCEH). Trained Indian Health Service staff recruited pregnant women during prenatal visits at one of six participating Indian Health Service or Public Law 638 hospitals on the Navajo Nation. The enrollment protocol prioritized recruitment of women during their 1st trimester but an open enrollment process was used, allowing women to enroll at any time during their pregnancy. For the present study, urine samples from NBCS participants were stratified by serum zinc concentration above and below the WHO level of sufficiency of 70 μg/dL. Additionally, we limited the sample population to individuals who also had their enrollment urine sample analyzed for uranium, total arsenic, arsenous (III) acid (arsenite, AsIII), and dimethylarsinic acid (DMA). Then we randomly selected 66 urine samples from each zinc group for inclusion in the present study out of 204 women enrolled in the NBCS at that time. Written informed consent was obtained from all study participants and the study protocol approved by the University of New Mexico Institutional Review Board (HRPO 11-310) and the Navajo Nation Human Research Review Board (NNR 11.323).

Sample Collection and Preparation

Trained hospital staff collected biospecimen samples using pre-screened metal-free collection cups, transfer pipettes, and Nalgene cryo-vials provided by CDC NCEH Division of Laboratory Sciences (DLS). During a prenatal hospital appointment trained hospital staff collected spot urine samples in sterile 50 mL urine collection cup. After collection, a laboratory staff member transferred a 1.8 mL aliquot of urine to separate 2.0 mL Nalgene cryo-vials for multi-element metals, total arsenic, speciated arsenic, and creatinine analysis. Hospital laboratory staff also collected peripheral blood via venipuncture and then allowed the blood to clot at room temperature for 30 to 40 minutes. Once clotted laboratory staff centrifuged the blood tube at 2,400 revolutions per minute for 15 minutes to separate the serum and then transferred 1.8 mL aliquot of serum to a 2.0 mL Nalgene cryo-vial. After

processing urine and serum samples, hospital staff placed all cryo-vials in a −80°C freezer for storage and transferred on dry-ice to freezer storage facilities at UNM. Chain of Custody forms were completed, reviewed, and validated at each stage of collection, storage and analysis.

Metal Biomonitoring Analysis

UNM staff shipped samples on dry ice to CDC DLS for analysis. NCEH laboratory staff prepared urine samples for uranium and other metals analyses using NCEH Method 3018.3; samples analyzed for total arsenic were prepared using Method 3018A.2 [44]; and samples analyzed for speciated arsenic were prepared using Method 3000.11 [45]. Chemical concentrations in urine and serum were measured using Inductively Coupled Plasma – Dynamic Reaction Cell – Mass Spectrometry (ICP-DRC-MS) [46]. Arsenic species concentrations were determined in separate aliquots using High Performance Liquid Chromatography (HPLC) and an anion exchange column to separate species prior to ICP-DRC-MS [47]. Urinary creatinine was determined using Roche/Hitachi Modular P Chemistry Analyzer [48].

Measurement of Urinary Isoprostanes by HPLC-Tandem Mass Spectrometry

Analysis of urinary F2-isoprostanes and prostaglandin F2α in urine was performed by the Linus Pauling Institute Oxidative and Nitrative Stress Core Laboratory by HPLC-tandem mass spectrometry as described previously [49]. Creatinine was quantified in each sample by the same laboratory. Briefly, urine was thawed at room temperature, mixed by inversion, and centrifuged (200x *g*, 5 min). Aliquots of the supernatant were mixed with methanol and internal standards followed by addition of 0.02 M bis-tris-HCl. Samples were pH adjusted to 6.0 −/+ 0.05. Strata X-AW cartridges (100 mg/3 mL, Phenomenex, Torrance, CA) were each pre-conditioned with methanol, then water. Diluted urine samples were loaded, the cartridges rinsed with sucsessively with methanol/water, acetonitrile and cartridges dried under a gentle vacuum (~5 mm Hg, 30 sec). Cartridges were then eluted 3-times with 1 mL methanol, and the eluants from each cartridge pooled and collected into glass tubes. Samples were dried under nitrogen gas, reconstituted in 200 μL methanol containing 0.1% formic acid (v:v), and injected onto the LC-MS-MS. Analytes were detected and quantified using SRM: F2-isoprostanes/prostaglandin F2α, *m/z* 353 to 193; 8-iso-$PGF_{2\alpha}$-d_4 internal standard, *m/z* 357 to 197. Samples were analyzed by HPLC-tandem mass spectrometry against standard curves for authentic standards [49].

Statistical Methods

Summary statistics including median (interquartile range) for continuous variables and frequency (%) for categorical variables were used to describe the demographics, environmental characteristics, chemical exposures of urinary uranium, urinary total arsenic, DMA, AsIII, and serum zinc of the participants in the NBCS, overall and by zinc groups (> 70 vs < 70 μg/dL for high vs low categorical membership).

The urine chemical measurements were corrected for urine creatinine, and values below limit of detection (LOD) were replaced by the LOD value divided by 2. Wilcoxon rank-sum tests were used to compare continuous variables between zinc groups. Chi-squared tests and

Fisher Exact tests for small expected numbers were performed to compare categorical variables between zinc groups. Pearson correlation coefficients along with the corresponding 95% confidence intervals (CIs) and scatterplots were used to summarize the correlation between log-transformed chemicals.

We compared the chemical exposures in the NBCS study to the national levels measured in women surveyed in the NHANES. The chemical exposure data were extracted from the NHANES year 2011-2012 database for women to represent the national population. Summary statistics of NHANES were calculated following the analysis guideline to account for the complex design features of NHANES including stratification, cluster sampling, and weighting [50].

Geometric mean along with 95% CIs were summarized for the chemical exposures in NBCS and the NHANES study. Statistical significance was determined using two-sample Welch's t-test comparing the mean (standard error) of the log transformed analytes, which took into account the NHANES design features.

The oxidative stress biomarkers as the primary outcomes include 8-iso-$PGF_{2\alpha}$, $PGF_{2\alpha}$, and the ratio of 8-iso-$PGF_{2\alpha}$ to $PGF_{2\alpha}$ to distinguish enzymatic versus chemical lipid peroxidation [41, 42]. Descriptive statistics were summarized for those variables by trimester at enrollment (1^{st}, 2^{nd}, 3^{rd}). The measurements were corrected for urine creatinine and variables with skewed distributions were log transformed.

Univariable linear regression analyses were used to examine the association between each demographic variable and chemical exposure and the oxidative stress outcome (prostaglandin ratio or 8-iso-$PGF_{2\alpha}$). Multiple linear regression models for the oxidative stress biomarkers were used to evaluate the impact of chemical exposures while adjusting for other potential confounding covariates. Covariates evaluated in the analysis included: age at interview, pre-pregnancy body mass index (BMI), trimester at enrollment, educational attainment (above or below high school), household income (above or below $20,000), employment status (currently employed or not), alcohol in the past year (yes or no), tobacco smoking (never, current or former smoker), vitamin intake (yes or no), and use of wood or coal for home heating (yes or no). Linear models with a backwards variable selection method based on the Akaike information criterion (AIC) measure were used for assessing the effects of variables along with their interactions on the oxidative stress biomarker variables. We also performed multiple linear regression stratified by zinc groups to describe the different effects of urinary total arsenic on 8-iso-$PGF_{2\alpha}$ moderated by high vs low zinc.

To assess the potential for zinc to mediate the effect of each metal (uranium, total arsenic, AsIII and DMA) on oxidative stress biomarkers (prostaglandin ratio and 8-iso- $PGF_{2\alpha}$), we used a quasi-Bayesian Monte Carlo causal mediation analysis [51, 52]. Covariates including trimester, education, household income, and tobacco were evaluated and adjusted for in the mediation analyses. We also then stratified this analysis by trimester to assess any confounding effects of changes in zinc across trimesters.

No adjustments for the multiple comparison tests were considered due to the discovery nature of this study. All analyses were conducted using the SAS 9.4 and R 3.4.1.

RESULTS

Selected Study Population Demographic Characteristics

Selected demographic characteristics for the subjects in this study are shown in Table 1. Study participants were drawn from the NBCS and all 132 participants included in this study were pregnant women between the ages of 16 to 42 with a median age of 26.8 years old. Analysis of survey information indicated that 38.6% of women had education beyond high school, 57.6% were unemployed and 43.2% had an annual household income below $20,000 at the time of study enrollment. The majority of women (59.8%) were taking vitamin supplements and were overweight or obese based on pre-pregnancy BMI (52.2%). Current cigarette smoking is negligible among the NBCS pregnant women (<1%) which is consistent with low tobacco usage overall in the Navajo population as reported previously by Redwood et al. [53]. However, other exposures such as wood or coal heating or use of ceremonial tobacco were noted as potential contributors to oxidative stress. The survey questions used to generate Table 1 are provided in Supplemental Table 1.

Metal Biomonitoring Characteristics of the Study Population

Urinary total arsenic, AsIII, DMA, uranium and serum zinc, were measured by ICP-DRC-MS as described in Materials and Methods (Table 2). Values for the same metals in women surveyed for the NHANES [54] are shown in Table 2. Of the 132 individuals in the sample population, urinary metals were detected among 132 (100%) for urinary total arsenic, 105 (79.5%) for urinary DMA, 88 (66.7%) for urinary AsIII, and 128 for urinary uranium (97.0%) (Table 2). Serum zinc was detected in 132 (100%) individuals from the sample population (Table 2). Total arsenic concentrations had a weak to moderate correlation with AsIII levels within the population (Supplemental Figure 1A), however, as expected, total arsenic correlated strongly with the metabolite DMA (Supplemental Figure 1B).

The median concentration for serum zinc was 67 μg/dL and median concentrations for urinary total arsenic, DMA, AsIII, and uranium were 5.5 μg/g, 4.3 μg/g, 0.41 μg/g, and 0.016 μg/g creatinine respectively. To provide context for the biomonitoring values, information for women was extracted from the NHANES 2011-12. Compared to the median for women included in the NHANES 2011-12 survey, urinary uranium was 2.3 times greater for NBCS samples (Table 2) with 88.6% and 15.9% of the sample population exceeding the NHANES 50^{th} and 95^{th} percentiles, respectively (Supplemental Table 2). Urine uranium concentrations for NBCS participants were greater than observed values from the NHANES study (p-value <0.001).

Urinary concentrations of total arsenic were lower for NBCS participants compared to NHANES values (p-value <0.001) (supplemental Table 2). Only 40.2% and 0.8% of NBCS participants had total arsenic urine concentrations exceeding the NHANES 50^{th} and 95^{th} percentiles, respectively. Serum concentrations of zinc were also lower in NBCS participants when compared to NHANES (p-value<0.001) (supplemental Table 2). No statistically significant difference was observed for DMA. Concentrations of urinary AsIII and serum zinc decreased from the 1^{st} to 3^{rd} trimester while concentrations of urinary uranium, total arsenic, and DMA remained stable (Table 3).

We observed no significant difference in urinary uranium or arsenic concentrations between the low versus high zinc groups suggesting that zinc status did not influence urinary metal levels (Table 2). The median concentration for the high zinc group was 78 μg/dL, which is similar to the NHANES values of 80 μg/dL and above the WHO sufficiency standard of 70 μg/dL. The median for the low zinc group was 48 μg/dL and well below the NHANES values and zinc sufficiency standard. The high and low zinc groups did not differ in the demographic characteristics with the exception of smoker classification. There were more Never Smokers and fewer Former Smokers in the low zinc group. Additionally, the high zinc group included more women who enrolled in the NBCS during the 1st trimester and low zinc group had more women enroll during the 3rd trimester (Table 1).

Characteristics of Oxidative Stress Biomarkers in the Study Population

Table 4 provides the summary statistics for oxidative stress biomarkers as measured by HPLC-Tandem Mass Spectrometry. The biomarker 8-iso-$PGF_{2\alpha}$ is a well-regarded marker for detection of chemical lipid peroxidation in human studies but does not take into account enzymatic contributions to total 8-iso-$PGF_{2\alpha}$. The use of a prostaglandin ratio (8-iso-$PGF_{2\alpha}$ to $PGF_{2\alpha}$) has been described to overcome this issue [41, 42]. Of the oxidative stress biomarkers measured in this study, $PGF_{2\alpha}$ and the prostaglandin ratio, but not 8-iso-$PGF_{2\alpha}$, were significantly different based on pregnancy trimester (Table 4). When only zinc is considered, comparison between the low and high zinc status subsets revealed no difference in the oxidative stress biomarkers 8-iso-$PGF_{2\alpha}$ or the prostaglandin ratio (Figure 1). The urinary 8-iso-$PGF_{2\alpha}$ levels measured in this study are comparable to previous reports in pregnant populations [55, 56]. Based on findings that the isoprostane metabolites dinorF1 and F2 values were subject to variability between different batch analyses (data not shown), only 8-iso-$PGF_{2\alpha}$, $PGF_{2\alpha}$ and the prostaglandin ratio were included for the regression models.

Ceremonial tobacco use was associated significantly with higher levels 8-iso-$PGF_{2\alpha}$ and prostaglandin ratio oxidative stress markers (Supplemental Tables 3 and 4). In addition, significantly lower 8-iso-$PGF_{2\alpha}$ levels were observed in populations with a household income above $20,000 and education above high school (Supplemental Table 3). The prostaglandin ratio was significantly affected by trimester stage (Supplemental Table 4).

Associations between metals and oxidative stress biomarkers

In a univariable analysis of the linear regression model there were no significant associations with environmental metals and 8-iso-$PGF_{2\alpha}$ (Table 5). Of the potential confounders, low income and ceremonial tobacco use had a significant association with urinary 8-iso-$PGF_{2\alpha}$ in the univariable analysis (Table 5). A multivariable analysis revealed significant main effects and interaction effects between total arsenic and low zinc on the 8-iso-$PGF_{2\alpha}$ outcome (Table 5).

The findings differed when using the prostaglandin ratio as the oxidative stress biomarker. In the univariable analysis there was a positive association with the arsenic metabolite DMA, but not other metals (Table 6) and a marginal positive association between total arsenic and the prostaglandin ratio was observed in the multivariable analysis. Notably, different sets of

confounding variables were identified for each of the oxidative stress biomarkers. For example, pregnancy trimester was identified as a confounding variable for the prostaglandin ratio but not for 8-iso-$PGF_{2\alpha}$ alone (Table 6). This can be accounted for by the lack of significant difference of 8-iso-$PGF_{2\alpha}$ by trimester whereas $PGF_{2\alpha}$ was significantly increased as pregnancy progressed from 1st to 3rd trimester (Table 4).

In order to better understand the arsenic and zinc interaction term from Table 5, we ran the stratified multivariable analysis by serum zinc group. We observed that the association between total arsenic and oxidative stress was modified by zinc group. Specifically, arsenic was positively associated with increased 8-iso-$PGF_{2\alpha}$ for the high serum zinc group, but not for the low serum zinc group (Table 7). In terms of the mediation effect of zinc on the association or metal/metalloids with oxidative stress, the causal mediation analysis (Materials and Methods) yielded no significant results of zinc mediation.

DISCUSSION

The Navajo Nation was a site of extensive uranium mining for more than 40 years [2, 35] and even in the absence of current active mining, potential exposures persist through proximity to AUMs, and water and soil contamination [57]. Uranium and arsenic were the focus of this study based on the prevalence of these metals in AUM waste, elevated levels in many water sources on Navajo land [7] and biomonitoring evidence indicating elevated uranium exposures in individuals [9]. Both metals have been reported to induce oxidative stress in experimental models [14-23], although the specific mechanisms that might be pertinent to a human population at environmental levels are currently unknown. Numerous studies link exposures to uranium or arsenic or mixed metals to a multitude of adverse health effects such as renal, cardiovascular and immune diseases [8, 9, 58-63]. Although oxidative stress is viewed as a mechanism underlying many metal-associated adverse health effects, there is limited knowledge regarding the potential association between uranium exposure and oxidative stress in human populations.Understanding potential roles of environmental metal exposure and oxidative stress during pregnancy is important because oxidative stress has been implicated in various pregnancy disorders, most notably pre-eclampsia and preterm labor [64].

The median urinary uranium levels in this study population were more than double those reported in the general US population based on NHANES data, yet no significant association between uranium and the oxidative stress biomarkers was detected. This finding is consistent with experimental studies where concentrations of uranium at or above 100 μM were required to elicit a measureable oxidative stress response [19-23] and suggests that the level of uranium exposure in these NBCS participants is below the threshold for detectable increase in oxidative stress.

We identified a significant positive association between urinary total arsenic and the oxidative stress biomarker 8-iso $PGF_{2\alpha}$ in a multivariable analysis. This finding is in agreement with several other population studies investigating arsenic exposure and oxidative stress, but results vary across studies. Positive associations between arsenic exposure and the urinary oxidative stress markers of DNA damage (8-oxodG or 8-OHdG) [26, 30, 32-34] or

8-iso $PGF_{2\alpha}$ [34] have been reported. A study in children found a positive correlation between arsenic exposure and salivary 8-OHdG, but not urinary 8-OHdG [24] and a dose relationship study based on different arsenic exposures failed to identify a significant association between arsenic and protein carbonyl or 8-oxo-2'-deoxyguanosine levels [28]. Another study found the strongest association between 8-oxodG and percent monomethylarsonic acid (MMA) compared to a weak association with urinary inorganic arsenic [29]. Those authors proposed that the differences from other reported findings may have been due to differences in arsenic metabolism in their indigenous study population [29]. Despite differences in study populations in terms of age, ethnicity, gender, pregnancy status and other factors, as well as lack of uniformity in specific oxidative stress biomarkers selected overall, our findings in the NBCS participants and those reported in the literature provide evidence of arsenic-associated elevation of oxidative stress in humans.

In this study we hypothesized that zinc status would have an impact on metal-associated oxidative stress based on the antioxidant properties of zinc [65, 66]. However, we did not obtain definitive evidence that zinc mediated the arsenic effect with regard to the oxidative stress biomarker 8-iso-$PGF_{2\alpha}$. One possible explanation is the impact of pregnancy on zinc status. Decreased serum zinc is common in pregnancy with reported mean values of approximately 58-60 μg/dL at term [66-69]. During pregnancy, women with serum zinc values <56 μg/dL are considered zinc deficient [70] and the mean serum zinc value in the NBCS study participants was below this definition of zinc sufficiency. Multivariable analysis using the standard biomarker 8-iso-$PGF_{2\alpha}$ revealed a positive association between low serum zinc and elevated oxidative stress as has been reported previously in adults [66, 71].

The biomarker 8-iso-$PGF_{2\alpha}$ is considered the best fatty acid indicator of oxidative stress *in vivo* [10, 72] and is widely used in human studies. One caveat is that cyclooxygenases 1 & 2 can contribute to the formation of 8-iso-$PGF_{2\alpha}$ independent of oxidative stress stimuli. Recent studies in humans and animal models suggest that the prostaglandin ratio of 8-iso-$PGF_{2\alpha}$/$PGF_{2\alpha}$ is a better indicator of oxidative stress because it accounts for changes in biosynthesis pathways [41, 42]. In this study we compared 8-iso-$PGF_{2\alpha}$ or the prostaglandin ratio as the oxidative stress biomarker variables and noted differences based on the biomarker used. For example, in the linear regression models pregnancy trimester was significantly associated with oxidative stress when the prostaglandin ratio was used as the biomarker variable. This association was absent when 8-iso-$PGF_{2\alpha}$ alone was the biomarker variable. We found that $PGF_{2\alpha}$, but not 8-iso-$PGF_{2\alpha}$, levels increased as pregnancy advanced which is consistent with other findings that oxidative stress increases with pregnancy [55, 56]. This comparison provides an example of the potential value of incorporating the prostaglandin ratio into analyses of F_2-isoprostanes as biomarkers of oxidative stress in addition to the more standard use of 8-iso-$PGF_{2\alpha}$. Use of both markers would allow for comparison of findings with established literature and expand understanding of the benefits of the ratio especially under conditions or in populations were endogenous contributions to total production of 8-iso-$PGF_{2\alpha}$ may be an important factor.

Very few demographic characteristics were associated with either oxidative stress biomarker, but ceremonial tobacco use was significant in the regression models. Ceremonial tobacco use is associated with traditional practices and may reflect complex exposures from the local

plant sources or other aspects of cultural ceremonies incorporating this product. Although we do not know how or why intermittent ceremonial tobacco use might be associated with systemic oxidative stress, this example highlights the importance of taking into consideration culture and local practices in studies of indigenous populations.

Challenges in this study include the relative paucity of data in the Navajo population, limited published information on metals and oxidative stress in pregnant women, and the NHANES sample not being representative of many aspects of our study group including a sufficient numbers of Native Americans for comparison. While NHANES values are representative of the overall US population, the survey is not designed to assess pregnant women specifically. During the 2011-12 NHANES cycle for example, less than 5% of participating women had a clinically confirmed pregnancy. Pregnancy results in changes to urinary excretion of metals and decreases in serum zinc concentrations. For example, previous work has demonstrated that the prevalence of arsenic species changes throughout pregnancy due to greater methylation [73]. Additionally, previous work has indicated that pregnant women have lower serum zinc concentrations than the general population because increased copper absorption interferes with zinc absorption [74]. No information about changes in uranium excretion throughout pregnancy is available. Therefore, the representative NHANES values may not fully represent urinary or serum concentrations of pregnant women in the United States but it remains the only large sample data set using consistent, high quality, and comparable analytical methods to use for comparison of population biomonitoring data. This study was focused on metals (arsenic and uranium) known to exceed the MCL in local water sources. Future studies may include additional metals in relation to biomarkers of oxidative stress.

In conclusion, we find evidence for elevated urinary uranium in women enrolled in the NBCS when compared to NHANES values, but no corresponding increase in oxidative stress measures associated with uranium. In contrast, arsenic is associated with increased levels of urinary 8-iso-$PGF_{2\alpha}$ as has been reported in other populations. Despite established antioxidant properties of zinc, zinc was not found to have causal mediation of the effects of the other metals on oxidative stress. Direct comparison of the prostaglandin ratio versus 8-iso-$PGF_{2\alpha}$ alone as biomarkers of oxidative stress reveals the utility of the prostaglandin ratio for studies of populations with known underlying factors, such as pregnancy, that may affect oxidative stress measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations9

8-iso-PGF$_{2\alpha}$	8-iso prostaglandin F$_{2\alpha}$
AsIII	arsenite
AUM	abandoned uranium mine
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body Mass Index
CDC	Center for Disease Control and Prevention
CI	confidence interval
DLS	Division of Laboratory Sciences
DMA	dimethylarsinic acid
HPLC	High Performance Liquid Chromatography
ICP-DRC-MS	Inductively Coupled Plasma – Dynamic Reaction Cell – Mass Spectrometry
IQR	interquartile range
LOD	Limit of Detection
MCL	Maximum Contaminant Level
NBCS	Navajo Birth Cohort Study
NCEH	National Center for Environmental Health
NHANES	National Health and Nutrition Examination Survey
PGF$_{2\alpha}$	prostaglandin F$_{2\alpha}$

UNM	University of New Mexico
WHO	World Health Organization

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Highlights

- Urinary uranium, but not arsenic, is elevated in the study participants compared to NHANES.
- Arsenic is associated with increased levels of urinary 8-iso-prostaglandin $F_{2\alpha}$.
- Uranium is not associated with elevated urinary F_2-isoprostanes.
- Zinc was not found to have any causal mediation of the effects of the other metals on oxidative stress.

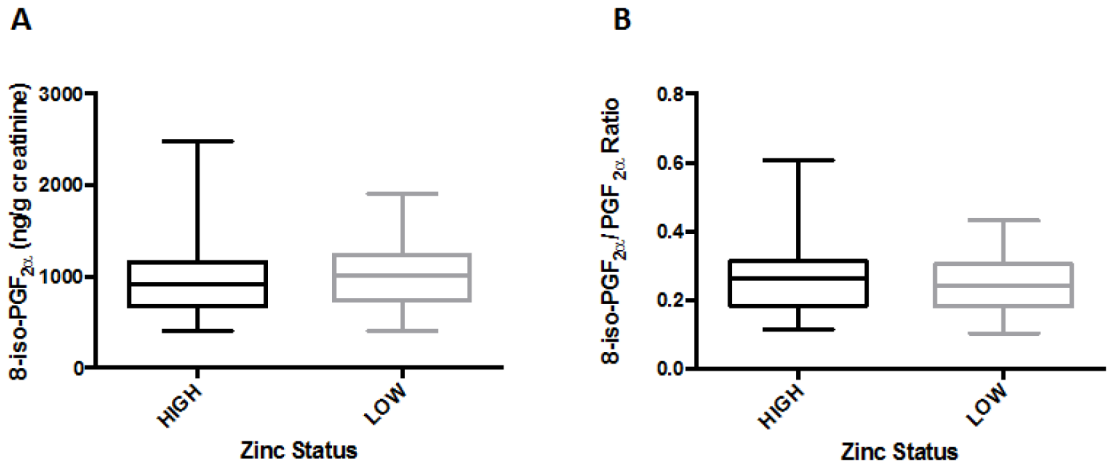

Figure 1. No difference in oxidative stress biomarkers by zinc status.

Participant results were separated by zinc status and analyzed for changes in the oxidative stress markers **A.** 8-iso-$PGF_{2\alpha}$ and **B.** Prostaglandin ratio (8-iso $PGF_{2\alpha}$/ $PGF_{2\alpha}$)

Table 1.

Selected demographic characteristics for NBCS participants

Characteristic		Total (n = 132)	Zinc Group		p
			High (n = 66)	Low (n = 66)	
Median Age [IQR]		26.80 [22.33 - 31.2]	26.35 [22.0 - 30.38]	27.15 [22.5 - 31.2]	0.664[a]
Trimester stage (%)	1st	25 (18.9)	25 (37.9)	0 (0.0)	<0.001[b]
	2nd	59 (44.7)	32 (48.5)	27 (40.9)	
	3rd	48 (36.4)	9 (13.6)	39 (59.1)	
Pre pregnancy BMI (%)	Normal[c]	28 (21.2)	13 (19.7)	15 (22.7)	0.536[b]
	Overweight[c]	37 (28.0)	13 (19.7)	24 (36.4)	
	Obese[c]	32 (24.2)	15 (22.7)	17 (25.8)	
	Information unavailable	35 (26.5)	25 (37.9)	10 (15.2)	
Vitamin usage (%)	No	39 (29.5)	22 (33.3)	17 (25.8)	0.241[b]
	Yes	79 (59.9)	34 (51.5)	45 (68.2)	
	No Response	14 (10.6)	10 (15.2)	4 (6.1)	
Education above high school (%)	No	65 (49.2)	28 (42.4)	37 (56.1)	0.385[b]
	Yes	51 (38.6)	27 (40.9)	24 (36.4)	
	No Response	16 (12.1)	11 (16.7)	5 (7.6)	
Annual household income <$20,000 (%)	No	41 (31.1)	23 (34.8)	18 (27.3)	0.182[b]
	Yes	57 (43.2)	23 (34.8)	34 (51.5)	
	No Response	34 (25.8)	20 (30.3)	14 (21.2)	
Currently unemployed (%)	No	42 (31.8)	24 (36.4)	18 (27.3)	0.17[b]
	Yes	76 (57.6)	32 (48.5)	44 (66.7)	
	No Response	14 (10.6)	10 (15.2)	4 (6.1)	
Alcohol usage in the past year (%)	No	84 (63.6)	42 (63.6)	42 (63.6)	0.506[b]
	Yes	34 (25.8)	14 (21.2)	20 (30.3)	
	No Response	14 (10.6)	10 (15.2)	4 (6.1)	
Cigarette usage (%)	Never Smoked	89 (67.4)	38 (57.6)	51 (77.3)	0.018[d]
	Current Smoker	1 (0.8)	0 (0.0)	1 (1.5)	
	Former Smoker	16 (12.1)	12 (18.2)	4 (6.1)	
	No Response	26 (19.7)	16 (24.2)	10 (15.2)	
Ceremonial tobacco usage (%)	No	73 (55.3)	38 (57.6)	35 (53.0)	0.278[b]
	Yes	45 (34.1)	18 (27.3)	27 (40.9)	
	No Response	14 (10.6)	10 (15.2)	4 (6.1)	
Wood used for home heating (%)	No	70 (53.0)	29 (43.9)	41 (62.1)	0.163[b]
	Yes	48 (36.4)	27 (40.9)	21 (31.8)	
	No Response	14 (10.6)	10 (15.2)	4 (6.1)	

Characteristic		Total (n = 132)	Zinc Group		p
			High (n = 66)	Low (n = 66)	
Coal used for home heating (%)	No	28 (21.2)	11 (16.7)	17 (25.8)	0.438[b]
	Yes	90 (68.2)	45 (68.2)	45 (68.2)	
	No Response	14 (10.6)	10 (15.2)	4 (6.1)	

Abbreviations: BMI, body mass index; IQR, interquartile range

[a] Wilcoxon rank sum test was used to calculate the P-value comparing low vs. high zinc groups.

[b] Chi-square tests were used to calculate the P-value comparing low vs. high zinc groups.

[c] BMI defined as Normal (18.5 - 24.9 kg/m2), Overweight (25 - 29.9 kg/m2), and Obese (>30 kg/m2)

[d] Fisher Exact tests were used to calculate the P-value comparing low vs. high zinc groups.

Table 2.

Summary of metal levels in the NBCS population

Metals	NHANES[a] Median [IQR]	NBCS Median [IQR]	% Below LOD	Zinc Group	
				High [IQR]	Low [IQR]
Uranium (μg/g creatinine)	0.007 [0.005 −0.013]	0.016 [0.0098 - 0.025]	3.0	0.013 [0.0094 - 0.021]	0.017 [0.011 - 0.027]
Total Arsenic (μg/g creatinine)	6.6 [4.2-15.0]	5.5 [4.2-8.2]	0.0	5.1 [4.1 – 7.9]	5.9 [4.8 - 8.6]
DMA (μg/g creatinine)	4.1 [2.7-6.7]	4.3 [2.9-5.8]	20.5	3.9 [2.6-5.2]	4.6 [3.2-6.2]
AsIII (μg/g creatinine)	<LOD [< LOD - <LOD]	0.41 [0.019-0.58]	33.3	0.43 [0.24 - 0.58]	0.37 [0.16-0.58]
Zinc (μg/dL)	80 [72 −88]	67 [48.0 - 78.0]	0.0	78 [74.0 - 85.0]	48 [44 -51]

Abbreviations: NBCS, Navajo Birth Cohort Study; NHANES, National Health and Nutrition Examination Survey; LOD, limit of detection; IQR, interquartile range; DMA, dimethylarsinic acid; AsIII, arsenite

[a]Summary statistics were based on 2011-2012 NHANES dataset restricted to women; Urine chemical measurements were corrected for creatinine.

Table 3.

Distribution of metals by trimester.

Metal	Trimester	n	Median [IQR]	p^a
Uranium (μg/g creatinine)	1st	25	0.014 [0.010 - 0.021]	0.76
	2nd	59	0.016 [0.009 - 0.024]	
	3rd	48	0.016 [0.011 - 0.026]	
Total Arsenic (μg/g creatinine)	1st	25	4.992 [4.097 - 7.910]	0.55
	2nd	59	5.573 [4.293 - 8.287]	
	3rd	48	5.723 [4.565 - 8.632]	
DMA (μg/g creatinine)	1st	25	3.460 [2.629 - 5.806]	0.357
	2nd	59	4.572 [2.802 - 5.894]	
	3rd	48	4.307 [3.177 - 5.750]	
AsIII (μg/g creatinine)	1st	25	0.535 [0.379 - 0.892]	**0.0017**
	2nd	59	0.442 [0.257 - 0.574]	
	3rd	48	0.259 [0.137-0.482]	
Zinc (μg/dL)	1st	25	77 [74.0 - 82.9]	**1.78E-11**
	2nd	59	72 [49.65 - 82.0]	
	3rd	48	49.95 [44.075 - 52.851	

Abbreviations: IQR, interquartile range; DMA, dimethylarsinic acid; AsIII, arsenite

[a]P-values were calculated using one-way ANOVA F test of the log transformed chemical exposures among three trimesters.

Table 4:

Summary statistics for oxidative stress outcome variables.

		Overall	First Trimester	Second Trimester	Third Trimester	
	Unit[b]	Median [IQR]	Median [IQR]	Median [IQR]	Median [IQR]	*p*[c]
8-isoPGF$_{2a}$	ng/g creatinine	935 [708 - 1201]	918 [674 - 1162]	881 [699 - 1219]	1009 [850 - 1171]	0.4633
prostaglandin-F$_{2a}$	ng/g creatinine	3897 [2952 - 5373]	3225 [2571 - 4073]	3892 [2985 - 5058]	4929 [3572 - 6905]	**0.0013**
Prostaglandin Ratio[a]		0.25 [0.18 - 0.31]	0.30 [0.27 - 0.32]	0.25 [0.19 - 0.30]	0.20 [0.16 - 0.29]	**0.0036**

Abbreviations: IQR, interquartile range

[a]Ratio of 8-isoPGF$_{2a}$ to PGF$_{2a}$.

[b]Measurements were corrected for urine creatinine.

[c]Kruskal-Wallis tests were used to calculate the p-value comparing different trimesters.

Table 5.

Regression coefficients of metal exposure, income, and ceremonial tobacco use for oxidative stress biomarker 8-isoPGF2α.

	Univariable			Multivariable[d]		
Variable [a]	**Coefficient** [b]	**Standard Error** [b]	***p*** [c]	**Coefficient**	**Standard Error**	***p*** [c]
Uranium	−0.044	0.044	0.32	-	-	-
DMA	0.097	0.066	0.15	-	-	-
Total Arsenic	0.066	0.069	0.34	0.341	0.133	**0.012**
Low Zinc	0.086	0.066	0.20	0.679	0.327	**0.041**
Total As/Zinc Group Interaction	-	-	-	−0.380	0.181	**0.038**
Annual Household Income <$20,000	0.158	0.078	**0.044**	0.168	0.076	**0.030**
Ceremonial Tobacco Use	0.167	0.071	**0.021**	0.142	0.079	0.076

Abbreviations: DMA, dimethylarsinic acid

[a] Metals were log transformed. Zinc was a binary variable. Outcome variable (8-isoPGF$_{2\alpha}$) was log transformed.

[b] The estimates of the regression coefficients and the standard errors from the linear regression models.

[c] P-values were calcuated using t tests.

[d] Multivariable model with backward selection using AIC criteria modeling the relationship between metals and oxidative stress biomarker 8-isoPGF$_{2\alpha}$. R^2=0.15.

Table 6.

Regression coefficients of metal exposure, education, ceremonial tobacco use and trimester for the prostaglandin ratio.

	Univariable			Multivariable [d]		
Variable [a]	**Coefficient** [b]	**Standard Error** [b]	***p*** [c]	**Coefficient**	**Standard Error**	***p*** [c]
Uranium	0.008	0.010	0.44	−0.055	0.043	0.21
Total Arsenic	0.031	0.015	0.043	0.19	0.096	0.053
DMA	0.021	0.008	**0.01**	-	-	-
Uranium/Total Arsenic Interaction	-	-	-	0.036	0.023	0.11
Low Zinc Group	−0.015	0.015	0.320	-	-	-
Education above high school	−0.024	0.017	0.157	−0.03	0.015	**0.049**
Ceremonial Tobacco Use	0.035	0.016	**0.037**	0.035	0.016	**0.026**
2nd Trimester	−0.057	0.020	**0.004**	−0.052	0.021	**0.017**
3rd Trimester	−0.079	0.020	**0.00016**	−0.089	0.022	**0.00009**

Abbreviations: DMA, dimethylarsinic acid

[a] Metals were log transformed.

[b] The estimates of the regression coefficients and the standard errors from the linear regression models.

[c] P-values were calcuated using t tests.

[d] Final multivariable model with backward selection using AIC criteria modeling the relationship between chemicals and oxidative stress biomarker ratio. R^2=0.23.

Table 7.

Regression coefficients of total arsenic and the oxidative stress biomarker 8-isoPGF$_{2\alpha}$, stratified by zinc group.

	Low Zinc group			High Zinc group		
Variable [a]	**Coefficient** [b]	**Standard Error** [b]	***p*** [c]	**Coefficient**	**Standard Error**	***p*** [c]
Total Arsenic	−0.045	0.12	0.71	0.34	0.14	**0.018**
Annual Household Income <$20,000	0.097	0.10	0.35	0.24	0.11	**0.040**
Ceremonial Tobacco Use	0.099	0.10	0.34	0.20	0.13	0.13

[a] Metals were log transformed. Zinc was a binary variable. Outcome variable (8-isoPGF$_{2\alpha}$) was log transformed.

[b] The estimates of the regression coefficients and the standard errors from the linear regression models.

[c] P-values were calcuated using t tests.