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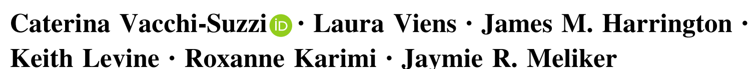
ORIGINAL PAPER

Low levels of lead and glutathione markers of redox status in human blood



Caterina Vacchi-Suzzi · Laura Viens · James M. Harrington · Keith Levine · Roxanne Karimi · Jaymie R. Meliker

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Abstract Exposure to lead (Pb) is implicated in a plethora of health threats in both adults and children. Increased exposure levels are associated with oxidative stress in the blood of workers exposed at occupational levels. However, it is not known whether lower Pb exposure levels are related to a shift toward a more oxidized state. To assess the association between blood lead level (BLL) and glutathione (GSH) redox biomarkers in a population of healthy adults, BLL and four GSH markers (GSH, GSSG, GSH/GSSG ratio and redox potential E_h) were measured in the blood of a cross-sectional cohort of 282 avid seafood-eating healthy adults living on Long Island (NY). Additionally, blood levels of two other metals known to affect GSH redox status, selenium (Se) and mercury (Hg), and omega-3 index were tested for effect modification. Regression models were further adjusted for demographic and smoking status. Increasing exposure to Pb, measured in blood, was not associated with GSSG, but was associated with lower levels of GSH/GSSG ratio and more positive GSH redox potential E_h, driven by its association with GSH. No effect modification was observed in analyses stratified by Hg, Se, omega-3 index, sex, age, or smoking. Blood Pb is associated with lower levels of GSH and the GSH/GSSG ratio in this cross-sectional study of healthy adults.

Keywords Lead · Blood lead levels · Pb · Glutathione · GSH · Oxidative stress

C. Vacchi-Suzzi (✉) · L. Viens · J. R. Meliker
Program in Public Health, Department of Family, Population, and Preventive Medicine, Stony Brook University, Stony Brook, NY 11794, USA
e-mail: caterina.vacchi-suzzi@stonybrookmedicine.edu

J. M. Harrington · K. Levine
Trace Inorganics Laboratory, RTI International, Durham, NC 27709, USA

R. Karimi
School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA

Introduction

Lead (Pb) is the 37th most prevalent element in the earth's crust, yet it has accumulated in our environment from anthropogenic uses, both historic, in gasoline and paint, and ongoing, in plumbing and in commercial and mining operations (Agency for Toxic Substances and Disease Registry (ATSDR) 2010). Current use exceeds 5 million tons annually for this nonessential, toxic metal. Humans are primarily exposed to Pb occupationally, or through contact with commercial products, Pb paint and plumbing in older homes, or in contaminated soil near highways or waste sites (Occupational Safety and Health Administration (OSHA) 2014). Exposure in the US population is primarily via ingestion and upon entering the bloodstream, 97–99% of absorbed Pb binds to erythrocyte proteins, while a smaller fraction (1–3%) is found in

blood serum (Sinicropi et al. 2010). Federal safety regulations require intervention when workers' blood lead levels (BLL) exceed 30 µg/dL (Occupational Safety and Health Administration (OSHA) 2014). Children are more sensitive to Pb exposure than adults (Centers for Disease Control and Prevention (CDC) 2014). Even low levels of Pb are associated with toxicity, and there is no known threshold for developmental effects in children (Centers for Disease Control and Prevention (CDC) 2014). The CDC action level for blood Pb in children has been progressively lowered from 60 µg/dL in the 1960s to the current reference level of 5 µg/dL, above which children are referred for case management (Centers for Disease Control and Prevention (CDC) 2014). The US Agency for Toxic Substances and Disease Registry (ATSDR) currently reports that neurological symptoms can be found with blood Pb levels at or below 10 µg/dL in children, and as low as 40 µg/dL in adults (Agency for Toxic Substances and Disease Registry (ATSDR) 2007; Canfield et al. 2003; Lanphear et al. 2000; Chiodo et al. 2004; Bellinger 2008; Chen et al. 2005).

Pb exposure is well-documented to be associated with neurologic, renal, cardiovascular, and hematologic effects (Jomova and Valko 2011; Agency for Toxic Substances and Disease Registry (ATSDR) 2010). The three mechanisms responsible for lead toxicity include inhibition of the heme synthetic pathway, ionic mimicry (Ergurhan-Ilhan et al. 2008; Ahamed et al. 2005), and oxidative stress (Flora et al. 2012; Ercal et al. 2001; Patrick 2006). Oxidative stress is a well-characterized mechanism of lead-induced hypertension, infertility, liver, and kidney damage (Flora et al. 2012). Oxidative stress occurs when there is imbalance between the concentration of free radicals in the body (or individual cells) and the body's ability to detoxify free radicals' reactive intermediates. Therefore, an increase in free radicals or a decrease in antioxidants can give rise to oxidative stress: Enhanced generation of reactive oxygen species (ROS), or depletion of the antioxidant sulfhydryl-rich molecules, such as glutathione (GSH), can indicate oxidative stress.

GSH is a tripeptide and an important cellular antioxidant (Circu and Aw 2010; Ercal et al. 2001; Flora et al. 2012; Martinez-Haro et al. 2011). Once synthesized, GSH can be further metabolized through a variety of pathways (Kanehisa and Goto 2000; Kyoto Encyclopedia of Genes and Genomes 2000). The main outcome in the presence of oxidative stress is for GSH to be oxidized to the dimeric GSSG, a reaction catalyzed by the selenoenzyme glutathione peroxidase (GPX) in which GSH donates reducing equivalents from its thiol groups to stabilize reactive oxygen species, and then combines with another oxidized GSH molecule to create the oxidized glutathione dimer GSSG (Flora et al. 2012). The intracellular concentrations of GSH and GSSG in the blood are measurable biomarkers of the whole-body oxidative index in response to oxidative events (Schafer and Buettner 2001; Dalle-Donne et al. 2006). In a normal cell, at least 90% of cellular glutathione content is in the reduced form and with less than 10% in the oxidized form; a shift in these percentages (decreasing GSH and/or increasing GSSG) can indicate a state of oxidative stress (Gurer and Ercal 2000). The relative concentration of the reduced versus oxidized glutathione is typically quantified as the molar ratio between GSH and GSSG (GSH:GSSG).

There are a number of ways in which Pb exposure may lead to a shift in the balance of GSH/GSSG toward oxidative stress. Pb binds to proteins' sulfhydryl groups, depleting the reserves of reduced GSH (Sinicropi et al. 2010; Hunaiti and Soud 2000; Jomova and Valko 2011; Patrick 2006). Lead also strongly inhibits δ-aminolevulinic acid dehydratase (δ-ALAD), which may increase the accumulation of its substrate δ-aminolevulinic acid (δ-ALA). This accumulating δ-ALA autooxidizes, generating ROS (Gurer-Orhan et al. 2004), which is in turn detoxified via conversion of GSH to GSSG (Sugawara et al. 1991). Furthermore, Pb interferes with the resupply of reducing equivalents, by inhibiting the enzyme glutathione reductase, which catalyzes the reduction of GSSG back to GSH (Ercal et al. 2001; Malekirad et al. 2010). Other enzymes involved in the regeneration of the GSH pool or detoxification of ROS are inhibited by lead, including glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD), increasing the cells susceptibility to oxidative damage (Ercal et al. 2001; Sugawara et al. 1991). In animal studies, a decrease in GSH levels has been seen in brain, liver, and eye lens associated with lead toxicity (Ercal et al. 2001; Gurer and Ercal 2000; Martinez-Haro et al. 2011). Occupational exposure to Pb has been correlated with increased levels of malondialdehyde (MDA), an indicator of oxidative stress caused by lipid peroxidation, as well as decreased levels of

GSH or some interferences with its synthetic pathways (Garcon et al. 2004; Sciskalska et al. 2014; Devi et al. 2007).

Human studies of Pb in relation to glutathione-related biomarkers have been limited to occupationally exposed workers (Jomova and Valko 2011). While BLLs below 10 µg/dL have been found to correlate with levels of other oxidative stress markers, such as serum gamma-glutamyltransferase (GGT), vitamin C, carotenoids, and vitamin E (Lee et al. 2006), glutathione-based markers have only been correlated with BLL in workers with elevated mean BLLs (> 50 µg/dL) (Gurer-Orhan et al. 2004). It is therefore unknown whether Pb may affect glutathione balance at low BLLs (< 10 µg/dL) in nonoccupationally exposed populations of Western countries.

The objective of this study was to assess the association between blood Pb levels in a non-occupationally exposed population with reduced and oxidized glutathione (measured singularly) and glutathione-derived biomarkers (i.e., reduced GSH/oxidized GSSG ratio and calculated redox potential E_h), as indicators of oxidative stress. We conducted this cross-sectional study in a cohort of healthy adult avid seafood consumers. Seafood is an important source of exposure for both Hg and Se as well as *n*-3 polyunsaturated omega 3 fatty acids, and we have previously shown associations between each of these and glutathione (Karimi et al. 2015). Therefore, a secondary objective was to investigate the impact of omega 3, Se, and Hg on the association between Pb and glutathione (Karimi et al. 2015).

Materials and methods

Study cohort

We conducted a study in which we measured concentrations of lead, selenium, mercury, GSH, and GSSG in blood samples from 282 healthy adults living on Long Island, NY. The cross-sectional study design is reported elsewhere (Karimi et al. 2014b) and involved collection of demographic characteristics, health outcomes, and blood samples in a population of avid seafood consumers. Stony Brook University's Institutional Review Board for research involving human subjects approved the study (IRB# 2010-1179).

Blood sample analysis

We collected fasting whole blood samples for total metal measurements and GSH and GSSG analyses at a single time point for each participant. Sample recruitment occurred from 2011 to 2012, and most participants provided a blood sample before 12 noon. Whole blood lead (Pb) levels (BLL) were used to estimate exposure to Pb. We collected fasting blood specimens for metal quantification in trace element blood collection tubes (BD Medical, Mississauga, ON, Canada) before any other aliquot to avoid background contamination. Metal analyses are described in detail elsewhere (Karimi et al. 2014a, b). Briefly, metal specimens were stored at 4 °C and sent to RTI International's Trace Inorganics Laboratory (Research Triangle Park, NC) for analysis. Total Pb, Se, and Hg concentrations were analyzed using ICP-MS (Thermo X-Series II). A 1000-µg/mL Au solution (High Purity Standards) was added to each sample to stabilize the mercury. Samples were microwave digested with HNO_3 and H_2O_2 (J.T. Baker, Ultrex Grade) and diluted with deionized water prior to analysis. Method performance was verified by digestion and analysis of method blank samples, to assess background elemental content resulting from the digestion method, and NIST SRM 966 (Toxic Metals in Bovine Blood). Calculated recovery of mercury and lead in this SRM was 140 and 83.9%, respectively (Se concentration was not certified in 966), demonstrating acceptable method performance.

For analysis of study samples, quality control procedures included the digestion and analysis of method blank samples to assess background metal concentrations in each analytical batch, as well as acid matrix-matched calibration verification samples to monitor instrument performance over a range of relevant concentrations. The average ± SD recovery of Pb, Se, and Hg from quality control samples were 95 ± 4, 101 ± 4 and 100 ± 4%, respectively. Our analytical limit of detection (LOD) ranged between 0.002 and 0.05 µg/dL for Pb, 2–8 µg/L for Se, and 0.1–0.7 µg/L for Hg across six batches. The limit of quantification (LOQ) was 0.5 µg/dL for Pb, while it ranged between 5 and 25 µg/L for Se and 1.5–5 µg/L for Hg across batches. There were two samples classified as < LOQ for Pb and 70 samples < LOQ and three samples < LOD for Hg. All other elements in all other samples were above the limit of

quantitation. Values < LOQ were retained in the analysis, and those < LOD were assigned to LOD/2.

We also measured the levels of omega-3 fatty acids in the plasma and used it to estimate the blood omega-3 index (von Schacky and Harris 2007), as previously described (Karimi et al. 2014a). Briefly, plasma lipids were first separated via thin layer chromatography, and then the fatty acids methyl esters fraction was measured on a Varian 3400 gas–liquid chromatography (Palo Alto, CA) with a 60-m DB-23 capillary column (0.32 mm internal diameter). Fatty acid standards (Nu Chek Prep, Elysian, MN) were used to ensure quantitative and qualitative accuracy and recovery. The inter- and intra-assay coefficients of variations for our omega-3 analytical methods were 0.7 and 2.4%, respectively.

Blood measurements of reduced and oxidized glutathione (GSH and GSSG), and in particular their ratio, are commonly used to estimate their respective concentrations in other tissues of the body and are widely accepted as an indicator of whole body status and disease risk (Rossi et al. 2002). As previously described (Karimi et al. 2015), whole blood for GSH and GSSG quantification was collected in a plastic 3-mL Vacutainer blood collection tube containing K_2EDTA (BD Medical, Mississauga, ON, Canada). Blood samples collected for GSSG quantification were quickly mixed on ice with a thiol-scavenging reagent to prevent analytical artifacts. GSH and GSSG concentrations were determined in duplicate using a GSH/GSSG spectrophotometric assay kit (part number # GT40, Oxford Biomedical Research, Inc., Rochester Hills, MI, USA), using a SpectraMax M5 spectrophotometer (Sunnyvale, CA) in kinetic mode recording the absorbance at 412 nm every 10 min. Intra-assay coefficients of variation (CVs) (3.2% GSH, 5.7% GSSG) were lower than inter-assay CVs (6.1% GSH, 13.9% GSSG). Each sample's CV was calculated by dividing the standard deviation by the mean of the technical duplicates. Large variability in the GSSG measurement (intra-assay CV ~ 14%) may cause a Type II error. Therefore, any measurement error for GSSG may contribute to shift associations toward the null, weakening rather than strengthening any existing association. As a sensitivity analysis, we excluded 62 samples (~ 22%) with a CV over 10% and re-ran the analyses obtaining very similar results (data not shown).

To estimate oxidation status, we calculated GSH/GSSG, and glutathione-derived redox potential (E_h). Decreasing GSH/GSSG values suggest a shift toward oxidized state. The E_h value, calculated adapting the Nernst equation as described by Jones et al. (Jones 2001), reflects the potential of the available GSH:GSSG to donate or accept electrons; a more positive E_h value indicates a shift toward an oxidized state. To calculate E_h, we used the measured GSH and GSSG levels in the Eq. (1).

$$E_h(\mathrm{mV}) = -264 + 30\log\left(\mathrm{GSSG}/\mathrm{GSH}^2\right) \quad (1)$$

Statistical analysis

We examined the relationship between blood Pb levels and markers of redox status (GSH/GSSG ratio and E_h) using linear regression analysis. Regression models were adjusted for seven potential confounders: age, gender, income level (categorized as (1) below \$70,000 or (2) above/equal to \$70,000), smoking status (never smoker or ever smoker), race (Caucasian vs. all other races), blood Se, and blood Hg. BMI did not increase the model's R^2 and was therefore not added as a covariate. For each regression analysis, we reported Pb's β coefficient and p value, the models' adjusted R^2, and ΔR^2, calculated as the difference in R^2 between a fully adjusted model with and without Pb. We also examined these associations in subsets of the data stratified by sex, age, smoking status, selenium and mercury, and ran interaction analyses to evaluate the potential relationship between Pb and each of those variables. Student t tests were used to compare mean differences. Adjusted redox potential E_h (mV), obtained by subtracting covariate values multiplied by the respective β coefficient, was used to generate a scatter plot, fit line and 95% CI of the regression BLL model, stratified by blood Se and Hg levels.

All statistical analyses and figures were done using SAS, version 9.3 (Cary, NC, USA).

Results

Cohort characteristics

Descriptive characteristics of the study population are shown in Table 1, along with the levels of BLL in study population subgroups. The mean BLL in the overall study population ($n = 282$) was

Table 1 Blood Pb (µg/dL) concentration by population subgroups

Variable	N	%	Mean ± SD	Median	Min.	Max.
All	282	100	1.77 ± 1.03	1.55	0.37	6.01
Age (years)*						
< 51	143	51	1.36 ± 0.76	1.09	0.37	4.43
≥ 51	138	49	2.20 ± 1.10	2.02	0.47	6.01
Sex						
Female	164	58	1.78 ± 1.00	1.58	0.37	6.01
Male	118	42	1.76 ± 1.07	1.50	0.55	5.72
Race/ethnicity						
Caucasian	224	79	1.83 ± 1.01	1.66	0.44	6.01
Other	58	21	1.54 ± 1.07	1.22	0.37	5.53
Income (USD per year)						
< 70,000	127	45	1.78 ± 1.14	1.55	0.37	6.01
≥ 70,000	146	55	1.75 ± 0.93	1.55	0.50	5.15
Cigarette smoking*						
Ever smokers	122	43	1.92 ± 1.14	1.59	0.59	6.01
Never smokers	158	57	1.65 ± 0.92	1.49	0.37	5.05
Blood Se (µg/L)						
Se < 318	141	50	1.66 ± 0.83	1.50	0.37	5.05
Se ≥ 318	141	50	1.88 ± 1.19	1.58	0.44	6.01
Blood Hg (µg/L)*						
Hg < 4.58	142	50	1.43 ± 0.86	1.14	0.44	5.53
Hg ≥ 4.58	140	50	2.12 ± 1.08	1.86	0.37	6.01
Blood omega 3 index (%)						
Index < 5.91	141	50	1.80 ± 0.97	1.59	0.44	6.01
Index ≥ 5.91	141	50	1.74 ± 1.09	1.45	0.37	5.72

* $p < 0.05$

1.77 ± 1.03 µg/dL. The Pb level was significantly higher in the older subgroup (> 51 years, $p < 0.01$), in ever smokers versus never smokers ($p = 0.04$), and among those with higher versus lower mercury levels ($p < 0.01$). Mean BLL did not differ significantly between males and females, Caucasian and other races, lower versus higher income, or low versus high selenium.

Levels of reduced GSH, oxidized GSSG, as well as their ratio (GSH/GSSG), and calculated glutathione redox potential E_h are reported in Table 2, along with the respective total blood concentration of total Hg and Se.

BLL and oxidative stress

As shown in Table 3, there were significant associations between lead and markers of oxidative stress GSH, GSH/GSSG ratio, and E_h in unadjusted and adjusted models, but not GSSG alone. As the Pb level increases, there is a shift to a more oxidized state, demonstrated by the β coefficient of − 28.6 for GSH, p 0.03, − 246.4 for GSH/GSSH, $p < 0.01$; and + 5.6 for E_h, $p < 0.01$ per 1 µg/dL increase in Pb in fully adjusted models. For these three markers, the associations remained significant in unadjusted and adjusted models. The overall R^2 value in the fully adjusted models for GSH and GSSG was strongly influenced by Se. Se and Pb equally contributed to the variability of GSH/GSSG ratio and E_h potential, with Pb accounting for 8% (out of overall R^2 19%) and 5% (out of overall R^2 11%) of GSH/GSSG and E_h models, respectively. The addition of omega-3 index and Hg to fully adjusted models, respectively, explained less than 3 and < 0.1% of the variability for all the glutathione markers, when both Pb and Se were included.

Table 4 shows the associations between lead and glutathione biomarkers when stratified by sex, age,

Table 2 Blood Hg, Se, and redox markers in the study population ($n = 282$)

Blood concentration	Mean ± SD	Median	Min.	Max.
Hg (μg/L)	7.64 ± 8.09	4.58	0.01	51.00
Se (μg/L)	294 ± 102	318	105	554
Omega 3 index	6.29 ± 1.75	5.91	3.49	13.91
GSH (μM)	1030 ± 190	1010	490	2020
GSSG (μM)	1.26 ± 1.14	0.99	0.25	9.59
GSH/GSSG	1200 ± 670	1000	100	3220
E_h (mV)	− 680 ± 20	− 680	− 730	− 600

Table 3 Association between Pb and glutathione biomarkers ($n = 282$), $p \leq 0.05$ is highlighted in bold

Blood marker	Unadjusted	Adjusted[a]	Adjusted[b]
GSH (mM)			
β	− 20.3	− 26.1	− 28.6
p	**0.05**	**0.04**	**0.03**
R^2	0.01	0.02	0.10
ΔR^2		0.01	0.01
GSSG (mM)			
β	1×10^{-2}	0.1	0.1
p	0.94	0.42	0.33
R^2	0.00	0.00	0.04
ΔR^2		0.00	0.00
GSH/GSSG			
β	− 161.9	− 186.6	− 246.4
p	**< 0.01**	**< 0.01**	**< 0.01**
R^2	0.06	0.06	0.19
ΔR^2		0.06	0.09
E_h (mV)			
β	3.7	4.6	6.1
p	**< 0.01**	**< 0.01**	**< 0.01**
R^2	0.04	0.04	0.12
ΔR^2		0.04	0.06

β values represent the difference in the predicted value of each glutathione marker for each one-unit (1 μg/dL) difference in Pb

[a]Adjusted for age, sex, income, smoking, race

[b]Adjusted for age, sex, income, smoking, race, omega 3 index, Se, and Hg. ΔR^2 represents the change in R^2 when Pb is added to the adjusted model

smoking, omega-3 index, Hg, and Se. The association remained significant for most of the groups, and there was no evidence of effect modification or interaction. For the lower Se group, the association somewhat diminished and became not significant, but there was no evidence of interaction. A visual depiction of the association between E_h and BLL, stratified by each of Hg and Se, is depicted in Fig. 1.

Discussion

To our knowledge, this is the first study to report on the association between blood glutathione redox markers (including GSH/GSSG ratio and E_h redox potential) and Pb in an adult low-exposure population. Even at low levels of exposure, we observed a significant trend toward a more oxidized state (lower GSH/GSSG ratio and more positive redox potential E_h) with rising BLL, in line with the body of literature, indicating oxidative stress as an important mechanism of lead toxicity in occupational settings (Ercal et al. 2001; Gurer and Ercal 2000; Flora et al. 2012; Martinez-Haro et al. 2011; Kasperczyk et al. 2013). Our study is unique in that we measured blood cells' levels of reduced and oxidized glutathione (GSH, GSSG) and calculated their ratio (GSH:GSSG ratio) and GSH redox potential (E_h). These indicators are commonly used to approximate cellular health, redox state, and chronic oxidative stress of the entire organism (Jones 2001; Owen and Butterfield 2010).

In this study, we did not observe a significant association between GSSG concentration and BLL. Others reporting on heavy metals and blood redox markers also failed to observe a significant correlation between exposure measures and blood GSSG levels (Hall et al. 2013). It could be speculated that, instead of being oxidized to GSSG, at low BLL blood GSH may be consumed via alternative metabolic pathways. For example, GSH can be covalently bound to a wide variety of moieties in response to xenobiotics, a process mediated by the enzyme glutathione

Table 4 Associations between BLL and GSH/GSSG ratio and blood redox potential E_h, stratified by covariates

Population group[a]	N	GSH/GSSG ratio				E_h (mV)			
		β	p_{reg}	R^2	p_{int}	β	p_{reg}	R^2	p_{int}
Sex					0.87				0.50
Women	164	− 196.7	**< 0.01**	0.17		3.9	**0.03**	0.11	
Men	118	− 272.5	**< 0.01**	0.22		7.2	**< 0.01**	0.14	
Age[b]					0.15				0.60
< 51 years	136	− 326.5	**< 0.01**	0.20		6.4	**< 0.01**	0.06	
≥ 51 years	135	− 195.9	**< 0.01**	0.18		5.4	**< 0.01**	0.16	
Smoking[c]					0.79				0.67
Ever smokers	122	− 273.7	**< 0.01**	0.24		6.8	**< 0.01**	0.17	
Never smokers	158	− 197.1	**< 0.01**	0.13		4.3	**0.04**	0.06	
Selenium					0.87				0.56
< 318 μg/L	141	− 141.5	0.07	< 0.01		4.0	0.07	0.02	
≥ 318 μg/L	141	− 240.6	**< 0.01**	0.14		5.5	**< 0.01**	0.10	
Mercury					0.08				0.42
< 4.58 μg/L	142	− 280.9	**< 0.01**	0.19		6.0	**< 0.01**	0.12	
≥ 4.58 μg/L	140	− 182.3	**< 0.01**	0.18		4.7	**0.01**	0.08	
Omega 3 index[b]					0.13				0.33
< 5.91	133	− 231.3	**< 0.01**	0.18		5.7	**0.01**	0.08	
≥ 5.91	138	− 225.4	**< 0.01**	0.19		5.3	**< 0.01**	0.14	

For each model, R^2, β, and p values (p_{reg}) are provided. P_{reg} values below 0.05 are highlighted in bold. β values represent the difference in the predicted value of each glutathione marker for a one-unit (1 μg/dL) increase of blood Pb. The interaction p value (p_{int}) for Pb with each covariate is also reported here

[a]All models were adjusted for income and race. Each stratified model was further adjusted for sex, age, smoking, Se and/or Hg, as appropriate

[b]$N = 271$

[c]$N = 280$

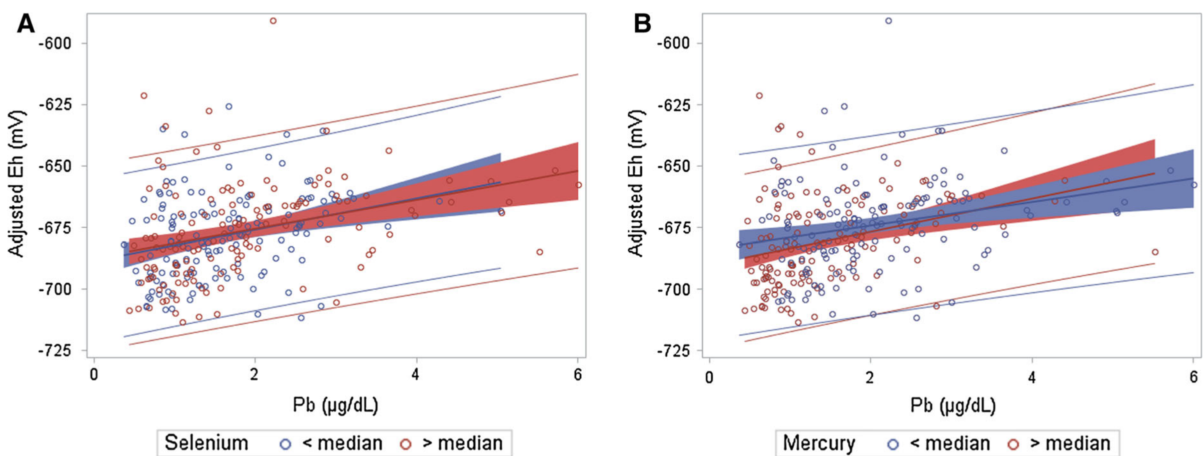

Fig. 1 Scatter plot, fit line, and 95% confidence intervals for adjusted redox potential E_h (mV) and BLL, stratified by blood selenium (Se) and mercury (Hg) levels. **a** Median Se 318 μg/L. **b** Median Hg 4.58 μg/L

S-transferase (GST). Alternatively, as Flora et al. summarize, Pb can bind directly to the sulfhydryl group of GSH itself. As a result, it is possible that the protocol we used to measure glutathione in our samples failed to detect the glutathione attached to either sulfhydryl moieties or Pb, which could potentially explain why we could see a decrease in GSH, but not an increase in GSSG, with higher BLLs. A third explanation is that Pb can bind and inactivate those enzymes necessary for GSH biosynthesis, resulting in a more depressed GSH state in the absence of a higher concentration of GSSG (Ahamed and Siddiqui 2007).

Importantly, the association between a shift toward a more oxidized state (i.e., a lower GSH/GSSG ratio or a more positive E_h potential) and blood Pb exposure was found to be significant at levels well below the current lower threshold of concern established in children (5 µg/dL).

Effect size and clinical relevance

While clinical consequences linked to shifts of GSH/GSSG ratio or E_h redox potential of the magnitude described here are currently unknown, the relative changes of markers in this study are of similar magnitude with those found in mitochondrial disease (Enns et al. 2014). According to the coefficient estimated in our fully adjusted regression model for E_h (+ 6.1 mV for each additional 1 µg Pb/dL blood, Table 3), a change of 5 µg Pb/dL blood is associated with an increased level of oxidation of + 30 mV, or 4.1% of the median E_h value (− 680 mV), which is comparable with the redox imbalance in E_h observed in mitochondrial disease patients (+ 3.4%) relative to a control group (Enns et al. 2014). It is, however, unknown whether such a decrease in GSH reducing potential would be associated with clinical consequences in otherwise healthy adults.

Although not directly comparable, the relative change of GSH/GSSG ratio across a change of ~ 5 µg/dL Pb is of similar magnitude to those of a handful of redox status markers, including vitamin C, E, and carotenoids (Lee et al. 2006). According to data provided by Lee et al., comparing the ninth and second decile of blood Pb levels (5.3–7.1 and 1.1–1.5 µg/dL, respectively) in the US NHANES population, vitamin C and carotenoids dropped 29 and 7%, respectively. Serum gamma-glutamyl transferase (GGT), a proposed early marker of oxidative stress, was elevated by 11% in the higher versus the lower decile. As a comparison, our study found a decrease of 28% in GSH/GSSG ratio across a similar range of Pb values (our top BLL decile—3.1 to 6.0 µg/dL—versus those with BLL below 1 µg/dL) (data not shown).

Comparison with available literature

Our study found that a decrease in antioxidant equivalents (decreased GSH/GSSG ratio and E_h) and a shift toward a more oxidized status are part of the xenobiotic response even at very low BLL. Our results support the hypothesis that this Pb-induced shift in oxidative stress occurs through a decrease in GSH. Pb is known to directly or indirectly decrease the pool of GSH through various mechanisms (Kasperczyk et al. 2004; Hunaiti and Soud 2000; Kasperczyk et al. 2013; Pande and Flora 2002), and the intracellular pool of reduced GSH plays a pivotal role in counteracting oxidative stress.

In this study, a significant association between Pb and a decrease in redox ability was observed with GSH, GSH/GSSG ratio, and the redox potential E_h, while the correlation with GSSG concentrations alone was not significant. The R^2 was driven for the most part by Se in the case of GSH, but both Se and Pb contributed almost equally for GSH/GSSG ratio and E_h. This suggests that the response of GSH and GSSG individually to increased BLL is smaller than the GSH/GSSG ratio and the redox potential derived from them. GSH/GSSG ratio and E_h may be more sensitive to the oxidative effects of Pb than GSH and GSSG alone. This also suggests that the relationship between Pb and the GSH redox system is complex and potentially mediated by metabolic pathways not measured in this study.

Co-exposures and effect modifiers

Our study cohort was originally assembled to study co-exposure to fish nutrients (e.g., Se) and contaminants, such as Hg in a healthy adult population. For this reason, the intake of seafood and blood levels of omega-3 index, Se, and Hg were above the national average (Karimi et al. 2014a). It was therefore important to include these as covariates in our models and to evaluate their potential for effect modification. Se is necessary for the activity of the enzyme glutathione peroxidase (Reddy and Massaro 1983);

animal studies have demonstrated that selenium, among other antioxidants, may protect against metal toxicity, when administered before Pb (Othman and El Missiry 1998; Liu et al. 2013; Yuan and Tang 2001). However, its role in mitigating oxidative stress is not clear, especially in the absence of a specific Se deficit. Among other heavy metals, Hg is associated with a shift in glutathione blood markers, indicating increased oxidative stress (Karimi et al. 2015), and therefore was also included in the fully adjusted model. Adjusting for the covariates did not qualitatively alter the association between Pb and GSH:GSSH or E_h. Similarly, in stratified analyses, no effect modification was detected for age, sex, smoking status, omega 3 index, Se, or Hg, as indicated by nonsignificant interaction p values (Table 4, Fig. 1).

Strengths and limitations

Limitations of this study include the cross-sectional design, one-time measures of metals and glutathione, and limited information about smoking since the quantity or duration of cigarette use was not recorded. In addition, our measured value of GSSG is about ten times lower than that reported in the literature. Also, the translation of our findings into the general population may be biased by the fact that the cohort was selected based on avid seafood consumption, and this distinct dietary pattern may impact the redox balance. However, confounding due to seafood intake is unlikely as Pb was not strongly associated with Hg, Se, or other markers of seafood intake. The association between low Pb and glutathione markers is consistent with the literature available on heavily exposed populations. Future investigations should measure the activity of enzymes in the glutathione pathway, such as GPx and GPR in a more representative population, and perhaps longitudinally, to shed light on the source of the decreased redox potential.

Conclusions

There is a correlation between a more oxidized state (i.e., a lower GSH/GSSG ratio or a more positive E_h potential) and increasing BLL in this population. These results suggest that the relationship between Pb and the glutathione system is complex and not limited to the depletion of blood GSH pool, in our low exposure population.

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