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Mercury exposure and a shift toward oxidative stress in avid seafood consumers

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Abstract

Mechanisms of mercury (Hg) toxicity at low doses from seafood consumption, the most common exposure route, are not well understood. We tested the hypothesis that seafood Hg exposure is related to a shift in redox status, indicated by a decrease in the ratio of reduced to oxidized glutathione (GSH:GSSG) in blood, or increase in redox potential (E_h). We also examined whether key seafood nutrients (selenium (Se), omega-3 fatty acids) confound or modify this shift. We measured blood concentrations of total Hg, Se, GSH, GSSG, and the Omega-3 Index (% omega-3s of total fatty acids in red blood cell membranes) in seafood consumers in Long Island, NY. We examined relationships between Hg, GSH:GSSG ratio and E_h. Elevated blood Hg (> 5.8 μg L^{-1}) was associated with lower GSH:GSSG ($\beta = -116.73$, $p = 0.01$), with no evidence of confounding by Se or Omega-3 Index. However, in models stratified by Omega-3 Index levels, Hg-GSH:GSSG associations were weakened among those with high Omega-3 Index levels (> 6% of fatty acids, $\beta = -63.46$, $p = 0.28$), and heightened among those with low Omega-3 Index ($\beta = -182.53$, $p < 0.01$). We observed comparable patterns for E_h in relation to Hg. These results support the hypothesis that Hg exposure from seafood is linked to a shift in redox status toward oxidative stress, modified by omega-3 fatty acids in this population. Further work should examine the role of different seafood nutrients and Hg-induced shifts in redox status in the diverse health effects associated with elevated Hg exposure.

Keywords

Mercury; Glutathione; Seafood safety; Seafood nutrients; Omega-3 fatty acids; Selenium

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016Zj.envres.2015.12.023.

1. Introduction

Mercury (Hg) is a well-known contaminant that most people are exposed to in the organic form of methylmercury (MeHg) through seafood consumption (UNEP, 2013). MeHg exposure from fish consumption can result in diverse negative health effects, including neurobehavioral (Carta et al., 2003; Yokoo et al., 2003), neurodevelopmental (Oken et al., 2005), immunological (Nyland et al., 2011), and cardio vascular (Guallar et al., 2002; Stern, 2005) outcomes. However, the mechanism(s) of action underlying MeHg toxicity are not well understood, and multiple processes are thought to play a role (ATSDR, 2013; Clarkson, 2002). Potential mechanisms of toxicity include the inhibition of protein synthesis and cell division, and interactions with cellular defenses (Reviewed in Clarkson, 2002).

One mechanism of MeHg toxicity may occur through the glutathione antioxidant system. Glutathione is a major antioxidant in humans and many other organisms and plays a direct role in neurobehavioral, cardiovascular and immunological pathologies (Townsend et al., 2003; Valko et al., 2007; Wu et al., 2004), many of which are also known effects of MeHg toxicity. Therefore, MeHg's association with glutathione, and the oxidative stress that may result, may play a direct role in these diverse manifestations of Hg toxicity (Guallar et al., 2002; Motts et al., 2014; Sarafian and Verity, 1991). Currently, our understanding of MeHg's associations with the glutathione system, specifically the relative balance of reduced glutathione (GSH) and oxidized glutathione (glutathione disulfide, GSSG), is limited. One hypothesis is that MeHg lowers GSH levels (Franco et al., 2006, 2007; Grotto et al., 2010) by binding to sulfhydryl groups, reducing the availability of GSH, and GSH relative to GSSG, and increasing the accumulation of reactive oxygen species (Ercal et al., 2001; Wu et al., 2004). Alternatively, MeHg may induce oxidative stress by inhibiting the activity of antioxidant enzymes (e.g., glutathione peroxidase (GPX), glutathione reductase (GR), glutathione -S -transferase (GST), and catalase). Both human (Franco et al., 2007, 2009) and animal studies (Berntssen et al., 2003; Franco et al., 2009; Hoffman and Heinz, 1998; Larose et al., 2008) have found MeHg exposure associated with decreases in glutathione enzyme activities. Third, in response to MeHg exposure and binding to GSH, an organism may ramp up GSH production, by increasing the activity of GR, catalase, GPX and GST (Franco et al., 2006; Larose et al., 2008; Pinheiro et al., 2008; Woods and Ellis, 1995). Finally, genetic polymorphisms in GST (Goodrich et al., 2011; Gundacker et al., 2007, 2009; Lee et al., 2010) may influence Hg metabolism, including elimination from the body. Taken together, these studies indicate that MeHg exposure can result in either an increase or decrease in GSH levels and glutathione enzyme activities (Kaur et al., 2011), suggesting complex, nonlinear responses with in the glutathione system. Nevertheless, we lack basic information on whether MeHg exposure from seafood is linked to an overall shift in glutathione status toward oxidative stress, regard less of the pathway through which it may occur.

The ratio of reduced GSH to oxidized GSSG (GSH: GSSG) provides a measure of overall redox state, with lower GSH: GSSG levels indicating a greater degree of oxidative stress (Jones, 2001). Therefore, GSH: GSSG ratios integrate overall changes in GSH, GSSG, and indirectly, individual glutathione enzyme activities. Our major hypothesis is that MeHg exposure from seafood is associated with lower GSH; GSSG ratios, indicating an overall, net shift toward oxidative stress. Studies have found relationships between increasing MeHg

exposure and lower GSH: GSSG in birds (Hoffman et al., 2011; Hoffman and Heinz, 1998; Hoffman et al., 1998). However, MeHg-redox relationships in human populations, particularly those that are exposed to relatively low doses of MeHg from seafood are unknown.

Hg-induced oxidative stress from seafood consumption is important to understand, not only because seafood is the primary exposure route for most humans, but also because co-exposure to key seafood nutrients may counteract or weaken Hg-induced oxidative stress. In particular, selenium (Se) is a known component of the antioxidant enzyme GPX. This function may represent one of the ways in which Se can occasionally protect against Hg toxicity (Ralston and Raymond, 2010), in addition to the formation of equimolar Hg-Se compounds (Khan and Wang, 2009; Ralston et al., 2008). Seafood is relatively high in Se compared to most other food items (Svensson et al., 1992; USDA, 2012), and may be a major source of Se for seafood consumers. We hypothesize that Se intake is associated with a relatively reduced redox state, potentially counteracting Hg's role in promoting a relatively oxidized state; Se may act as a confounder or an effect measure modifier in this role. Other sea food nutrients, specifically omega −3 fatty acids, are known to help mitigate potential Hg toxicity on cardio vascular (Guallar et al., 2002; Mozaffarian and Rimm, 2006), neurobehavioral, and neurodevelopmental end points (Oken and Bellinger, 2008; Oken et al., 2005). Although omega −3 fatty acids are not known as antioxidants, emerging evidence suggests they can have a protective effect against oxidative stress, and may play a role as an effect measure modifier (Arnal et al., 2010; Avramovic et al., 2012), possibly by lessening GSH depletion (Wu et al., 2004). However, omega −3 fatty acids are also thought to be susceptible to lipid peroxidation (Nenseter and Drevon, 1996; Song et al., 2000), thus may potentially lead to oxidative stress. Therefore, we include omega −3 fatty acids in the analysis of Hg-induced oxidative stress and consider it for confounding or effect measure modification. Here, we examine whether seafood Hg exposure is associated with a shift in redox status toward oxidative stress in seafood consumers, measured by the balance between GSH and GSSG blood concentrations and redox potential, E_h (mV). Our secondary objective is to explore whether seafood nutrients might confound or modify this association.

2. Materials and methods

2.1. Study population and enrollment procedures

We conducted a cross-sectional study on Hg and nutrient exposure from seafood consumption in which we measured concentrations of Hg, nutrients, reduced glutathione, and oxidized glutathione in blood samples from adult, avid seafood consumers. Stony Brook University's Institutional Review Board approved the study for human subjects research (IRB # 2010–1179). Recruitment of study participants, determination of study eligibility, Hg and nutrient blood collection methods and analyses, and demographic characteristics of study participants are detailed elsewhere (Karimi et al., 2014a, 2014b). Briefly, we enrolled 290 adult, avid seafood consumers, defined as those who regularly eat sea food and are predicted to be at risk for elevated Hg exposure due to regular fish consumption (e.g., weekly or daily, depending on seafood type), from Long Island, NY. Advertisements and recruitment activities occurred at local sites including fishing piers, seafood markets, gyms,

newspapers, and on university websites. Individuals were informed that they were being recruited for a study to investigate benefits and risks of seafood consumption. To determine eligibility, participants completed a screening survey that assessed potential Hg exposure based on the frequency and types of fish consumed, and seafood Hg concentrations from the Seafood Hg Database (Karimi et al., 2012). Individuals estimated to exceed the USEPA reference dose (RfD) of 0.1 μg kg^{-1} day^{-1} were eligible. We defined avid seafood consumers in this manner to ensure adequate study power for examining the effects of low-level Hg exposure (in our sample, ~50% of individuals had blood Hg concentrations indicating exposure below the reference dose). Eligible participants who enrolled in the study completed a clinical appointment at the Clinical Research Core at Stony Brook University Medical Center. We obtained informed consent from all enrolled participants. At the appointment, trained nurses obtained written informed consent and collected questionnaires, anthropometric data, and blood specimens.

2.2. Blood analyses

We collected fasting whole blood samples for total Hg, total Se, reduced and oxidized glutathione analysis, and blood plasma samples for fatty acid an alysis from 285 participants at a single time point for each participant. We did not obtain blood samples for the remaining 5 participants due to complications with blood draw. We chose biomarkers of Hg and sea food nutrients that reflect relatively long term exposures (monthly or longer). Both total Hg and total Se in whole blood are commonly used as biomarkers of dietary exposure, including studies of populations that frequently consume sea food (Ayotte et al., 2011; Barany et al., 2003; Choi et al., 2008; Gundacker et al., 2006).

We collected blood specimens for Hg and Se quantification in trace element blood collection tubes (BD Medical, Mississauga, ON, Canada) before any other aliquot in order to avoid background contamination. Metals analyses are described in detail elsewhere (Karimi et al., 2014a, 2014b). Metal specimens were stored at 4°C and sent to RTI International's Trace In organics Laboratory (Research Triangle Park, NC) for Hg and Se analysis. Hg and Se concentrations were analyzed using ICP-MS (Thermo X -Series II). A 1000 μg m L^{-1} Au solution (High Purity Standards) was added to each sample to stabilize the Hg. Samples were microwave digested with HNO_3 and H_2O_2 (J.T. Baker, Ultrex Grade), and diluted with deionized water prior to analysis. Quality control procedures included the digestion and analysis of standard reference materials (NIST SRM955c caprine blood, NIST SRM 966 bovine blood, and UTAK human blood). Over the course of the ICP-MS analysis different concentrations of NIST reference materials, at the low and high end of the appropriate analyte range, were analyzed every ten samples or fewer, in order to provide a measurement of instrumental performance. Analytical recoveries of quality check samples ranged between 95 −106 % for Se and 64–106% for Hg. The overall inter-assay coefficients of variation (CV) between analytical batches were 5.1% for Se and 2.7% for Hg, showing good instrument stability with in each day. The instrument performance over the long term was also stable, with average recovery values around 100%. We also prepared and analyzed sample blanks and method blank samples to assess Hg and Se background due to the sample collection method and the digestion method, respectively. Hg and Se concentrations for blanks were negligible, confirming no background contamination. Detection limits ranged

from 0.10 to 0.70 μg Hg L blood^{-1} and 2–8 μg Se L blood^{-1} among sample batches. Samples that were below the detection limit (n =2 for Hg) were assigned a value of one -half the detection limit for that batch.

We collected blood specimens for fatty acid analysis in 3 mL Vacutainer tubes with K_2EDTA (BD Medical, Mississauga, ON, Canada). Blood was centrifuged within 4 5 min of collection, and an aliquot of plasma was removed and stored at - 80°C. Fatty acid analyses of the phospholipid fraction of plasma aliquots were conducted by Lipid Analytical Lab, (University of Guelph,ON, Canada). We specifically collected plasma because omega-3 fatty acid concentrations in the phospholipid fraction of plasma can be used to estimate the Omega-3 Index, the percent of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of total fatty acids in red blood cell membranes (Harris et al., 2013; Harris and von Schacky, 2004). The Omega-3 Index is a clinical measure associated with cardiovascular outcomes (Harris and von Schacky, 2004) that are also potential targets of Hg toxicity (Guallar et al.,2002; Stern, 2005). Fatty acid concentrations based on the phospholipid fraction reflects fatty acid in take over the last year (Hodge et al., 2007).

Methods for determining fatty acid composition are described in detail elsewhere (Karimi et al., 2014a). Briefly, lipids were extracted from the plasma samples after addition of an internal standard (Folch et al., 1957) and the phospholipid fraction separated from the neutral lipids by thin-layer chromatography (Dewailly et al., 2001; Stark and Holub, 2004). The fatty acid methyl esters were prepared (Morrison and Smith, 1964) and analyzed on a Varian 3400 gas-liquid chromatograph (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. For one serum sample analyzed 10 times, our assay showed an inter-assay CV of 0.7% for omega-3 fatty acids. The mean intra-assay CV for 10 samples measured in duplicate was 2.4% for omega-3 fatty acids.

We collected whole blood for glutathione analysis in a plastic 3-m L Vacutainer blood collection tube containing K_2EDTA (BD Medical, Mississauga, ON, Canada). Immediately following collection, blood specimens were placed on ice. GSH and GSSG blood concentrations were analyzed using a GSH/GSSG assay kit according to instructions provided (Product # GT40, Oxford Biomedical Research, Inc., Rochester Hills, MI, USA). GSH and GSSG concentrations in blood indicate current, overall redox status, and therefore integrate fluctuations in GSH and GSSG that occur through multiple chemical processes over varying reaction rates (Jones, 2001; Owen and Butterfield, 2010). The GSH: GSSG ratio reflects cellular health, or redox state, and chronic oxidative stress of individuals (Jones, 2001; Lu, 1999). In human studies, blood is most often used to measure this GSH: GSSG ratio, is thought to reflect GSH: GSSG in other tissues, and is widely used as an indicator of whole body status and disease risk (Rossi et al., 2002). GSH and GSSG concentrations were measured using a SpectraMaxM5 spectrophotometer (Sunnyvale, CA) in kinetic mode recording the absorbance at 412 nm every 10 minutes. In general, intra-assay CVs (3.24% GSH, 5.71% GSSG) were lower than inter-assay CVs (6.07% GSH, 20.39% GSSG). The higher inter-assay CV for GSSG was due to a high CV observed in one-third of the samples.

2.3. Statistical analyses

We used GSH: GSSG values (based on molar concentrations), and the redox potential of GSH: GSSG (E_h), as indicators of redox status as in previous studies (Hall et al., 2013; Hoffman and Heinz, 1998; Hoffman et al., 1998; Xie et al., 2009). Lower GSH:GSSG ratios indicate a relatively oxidized state. We calculated E_h from GSH and GSSG in (Eq. (1), Jones, 2001)

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

E_h specifically accounts for the proportion of 2 GSH oxidized per GSSG. Therefore, compared to GSH: GSSG ratio, E_h may better reflect the potential for the GSH: GSSG pool to accept or donate electrons, where higher E_h values indicate a greater potential for oxidation to occur.

We quantified relationships between blood Hg concentrations and redox status measures using linear regression analysis. In a secondary analysis, we also examined relationships between blood nutrient concentrations and redox status using linear regression. Consistent with previous reports, blood Hg and Omega-3 Index followed a non-normal distribution (Choi et al., 2009; Soon et al., 2014), but were not log transformed as they were not used as dependent variables in our analyses. Sensitivity analyses examining log transformation of Hg and Omega-3 Index produced similar findings.

In multiple regression analyses, the following variables were initially selected as potential confounders: age (continuous), sex (male (reference), female), income (< US $ 25,000 yr^{-1} (reference), $ 25,000 −$ 70,000, $ 70,000 −$ 110,000, $ 110,000 −$ 200,000, > $ 200,000), level of education (up to some college (reference), college graduate), ethnicity (white/Caucasian (reference), other), body mass index (continuous), and estimated daily vitamin E intake (continuous, mg d^{-1} of α-Tocopherol equivalent). Age, gender, and income altered the beta coefficient of the main independent variable in multiple regressions by at least 10%, and had a p value < 0.1, and were therefore retained in the analysis. We also included Se (continuous) and the Omega-3 Index (continuous) in the model to evaluate confounding, and used interaction terms with Hg to evaluate effect measure modification. We also examined the role of Omega-3 Index and Se on Hg and redox state by stratifying the data using median cut-offs for Omega-3 Index and Se. Hg was treated as a continuous variable and as a categorical variable (Hg below or above 5.8 μg/L, the blood concentration corresponding to the RfD) in separate regression models. Linear spline models were built with either 4, 5 or 6 knots to further investigate the association between Hg and redox markers. Thirteen individuals with missing values in any of the variables used for the models were excluded. Additionally, four individuals with plasma omega-3 concentrations below 0.45 mg dL^{-1}, more than 10 times lower than any other value, were considered outliers and excluded from the analysis. For each regression model we report the adjusted R^2 (ad j. R^2), defined as the proportion of the variation in the dependent variable accounted for by the explanatory variables, the β coefficient and its 95% confidence interval (CI), and the regression p value for the variables of interest. All analyses were done in SAS 9.3 (Cary, NC).

We also report molar concentrations of blood Hg and Se and calculated the molar Se:Hg ratio for each sample to estimate the potential Se protection against Hg toxicity (Khan and Wang, 2009; Ralston et al., 2008) in this population.

3. Results

3.1. Population characteristics

Descriptors of the study population (N = 268) can be found in Table 1. Mean blood Hg, Se, and Omega-3 Index values were higher than those measured in reference populations of a cross-section of US adults (Centers for Disease Control and Prevention, 2013; Harris et al., 2013; Laclaustra et al., 2010). Females (N = 156, 58%, mean blood Hg = 6.77 μg L^{-1}) comprised a higher proportion of the population than males (N = 112, 42%, mean blood Hg = 9.02 μg L^{-1}). As reported previously, fish consumption rates for our full study population (mean = 14.4 kg y r^{-1}) were higher than those estimated for the US population (6.8 kg yr^{-1}) (Karimi et al., 2014b; National Marine Fisheries Service, 2012), and salmon, canned white tuna and shrimp were the most commonly consumed seafood items although individuals tended to consume a wide variety of seafood (Karimi et al., 2014a). Hg levels were not strongly associated with Se (Spearman r = 0.11, p = 0.08), consistent with our previous finding that blood Hg, but not blood Se, is positively related to total seafood consumption frequency in this population (K arimi et al., 2014a). The molar Se:Hg ratio exceeded 10 in all individuals.

3.2. Blood Hg levels were associated with a shift toward oxidative stress, modified by Omega-3 Index

We found that blood Hg concentration was associated with changes in redox status (GSH:GSSG ratio) and redox potential (E_h) indicating a shift toward oxidative stress with increasing Hg concentration. We found significant differences in GSH:GSSG ratio and E_h between participants with low blood Hg (≤ 5.8 μg L^{-1}) and elevated blood Hg (> 5.8 μg L^{-1}) levels (mean GSH:GSSG = 1324.1 and 1079.5 respectively, Student T test p = 0.025; E_h = -682.8 mV and - 676.5 mV respectively, p = 0.007). In regression models where Hg was treated categorically (Table 2), elevated Hg was significantly associated with decreased GSH:GSSG ratios (β=−116.73 and p = 0.01) and increased E_h values (β= 2.93 and p = 0.02). An investigation of non-linear relationships between Hg and redox markers using linear splines (3 knots an d 1 degree) revealed similar results (R^2 no higher than 0.03) to those presented in Table 2. When stratified by nutrients, these models indicated weaker, or nonsignificant associations in individuals with low blood Se concentrations, or high Omega-3 Index levels, respectively (Table 2). In contrast, we found stronger association s in individuals with low Omega-3 Index values (Table 2). Individuals in the high Se group also showed stronger association between Hg and GSH:GSSG ratio or E_h, although the R^2 was quite small (< 0.03). Results of stratified analyses were similar if we treated mercury continuously (Table S1). When we used interaction terms instead of a stratified analysis, there was a significant term for Hg*Omega-3 Index ($p \leq 0.01$), but not for Hg*Se (p = 0.53) for GSH:GSSG (similar results for E_h).

3.3. Blood Se levels were associated with a shift away from oxidative stress

When we examined relationships between seafood nutrients and redox state in secondary analyses, we found that higher blood Se concentration was associated with a relatively reduced state, indicated by increasing GSH:GSSG ratio and decreasing E_h in both unadjusted and adjusted models (Table 3). Se was also associated with relatively small decreases in GSH (Table 3). In contrast, while Omega-3 Index was associated with decreasing GSH concentrations, it was not significantly associated with GSH:GSSG ratio or redox potential (E_h) (Table 4).

4. Discussion

We report direct evidence that elevated Hg exposure from sea food is associated with a shift in redox status toward oxidative stress in this population of avid seafood consumers. In general, the direction of the association between Hg exposure and a relatively oxidized state persisted across model analyses, but the strength of the association varied depending on blood concentrations of omega −3 fatty acids. There are a number of implications from these findings. Redox status, measured by GSH:GSSG ratio and E_h, reflects general differences in physiology (e.g., due to aging), the presence of disease (e.g., rheumatoid arthritis, neurodegenerative diseases), or toxicity (e.g., due to drug or metals exposure) (Jones, 2001; Owen and Butterfield, 2010; Town send et al., 2003; Valko et al., 2007, 2005). Thus, redox status is a broad health indicator, and reflects the influence of the glutathione system across neurobehavioral, immunological and cardio vascular systems (Wu et al., 2004). These systems are also targets of elevated Hg exposure (Carta et al., 2003; Guallar et al., 2002; Nyland et al., 2011; Stern, 2005; Yokoo et al., 2003). Therefore, a Hg-induced shift in redox status may play a role in specific, known associations between Hg exposure and these systems, and in compromising an individual's overall ability to withstand oxidative challenge (Jones, 2001; Lu, 1999). Additional study is needed to examine causal molecular mechanisms, physiological and clinical consequences of the Hg-redox link, and the mechanistic role of nutrients.

4.1. Potential clinical implications

Clinical effects linked to the shifts in redox status that we observed are unknown. GSH and GSSG values are highly variable across studies (Rossi et al., 2002). Thus, oxidative stress is defined by relative decreases in GSH or GSH:GSSG ratio in case-control studies (Rossi et al., 2002). However, small decreases in these values may not necessarily indicate oxidative stress with clinical manifestations. The size of the decrease in GSH:GSSG we observed in participants with elevated Hg compared to low blood Hg (18%) is similar to that observed between mitochondrial disease patients and matched controls (32%) (Enns et al., 2014), and lower, but within the range observed between cases of diabetes or age-related macular degeneration and controls (18–54%) (Samiec et al., 1998). Similarly, another study observed a 34% decrease in GSH:GSSG in non-insulin dependent diabetic patients co m pared to controls (Bravi et al., 1997). Also, the shift in Eh was smaller for the elevated Hg group in our study (0.9% increase relative to the low Hg group), compared to mitochondrial disease patients (3.5% increase relative to controls) (Enns et al., 2014). Finally, the R^2 values that we observed in unadjusted models are similar to those found in other studies, but indicate

that while associations between Hg and redox status are significant, they explain a small percentage of the variation in redox status (GSH:GSSG or E_h: up to 7%, Table 2 and S1). Specifically, recent work identified similar R^2 values between arsenic and E_h (2–6%) at quite high levels of arsenic exposure in Bangladeshi adults (Hall et al., 2013), and between blood Hg and GSH (8%) (Grotto et al., 2010). The small, but significant relationships observed in this and other studies may be important for chronic exposures, and for potential subclinical effects.

4.2. Comparison with previous studies

Our finding that Hg exposure is linked to a shift toward oxidative stress is generally consistent with previous studies. Specifically, animal studies have found links between increasing Hg exposure and decreases in hepatic GSH:GSSG ratio in avian populations (Hoffman et al., 2011; Hoffman and Heinz, 1998; Hoffman et al., 1998). However, results from studies on human populations exposed to dietary MeHg are less consistent. Two studies on populations consuming relatively contaminated freshwater fish in the Amazon have found links between MeHg exposure levels and glutathione responses, including decreased GPX activity and GSH levels (Grotto et al., 2010; Pinheiro et al., 2008). However, another study found no correlation between Hg exposure and glutathione measures (glutathione reductase (GR), GPX, total glutathione) in sport fishermen (Belanger et al., 2008). The Be-langer et al. study examined seasonal changes in these measures within individual subjects, and had a relatively small study population ($N = 31$) thus may have lacked sufficient power to detect Hg-glutathione relationships. In contrast with our study, these human population studies did not examine GSH:GSSG ratio, or the role of nutrients on glutathione measures. Additionally, our findings do not support the hypothesis that MeHg exposure lowers GSH levels (Franco et al., 2006, 2007; Grotto et al., 2010), and suggest that a H g-induced decrease in GSH:GSSG is more complex than GSH depletion alone. Instead, our results suggest a consistent inverse association between Hg and overall GSH:GSSG ratio.

4.3. Complex role of seafood nutrients

Our findings indicate that Se is associated with a more reduced state (h igher GSH:GSSG) (Table 3), while omega-3 fatty acids have no significant association with GSH:GSSG ratio (Table 4); neither factor confounded the association between Hg and glutathione markers. The interaction between Se and Hg was not significant, however, there was a significant interaction between Hg and Omega-3 Index, with associations between Hg and a more oxidized state heightened among those with lower Omega-3 Index. Thus, the role of seafood nutrients on redox state was not universally protective, and varied with nutrient concentration in the blood. In our study, Sew as in molar excess of Hg in the blood in all participants, which is thought to indicate Se protection against Hg toxicity (Khan and Wang, 2009; Ralston et al., 2008) and is consistent with Se:Hg ratios reported in other population studies of frequent seafood consumption (Choi et al., 2008). However, the lack of significant interaction with Se does not support the hypothesis that Se protects against mercury-associated changes in redox state in this population.

In contrast to the known antioxidant function of Se, the role of omega-3 fatty acids is less clear. Omega-3 fatty acids mitigate adverse cardiovascular effects of MeHg exposure (Guallar et al., 2002), and are thought to explain the net benefit of fish consumption on neurodevelopment (Oken and Bellinger, 2008; Oken et al., 2005, 2008). However, associations between omega −3 fatty acid sand glutathione are not hypothesized to explain these health benefits. Also, polyunsaturated fatty acids are susceptible to lipid peroxidation (Meydani et al., 1991; Song et al., 2000), thus have the potential to shift redox status toward a more oxidized state under certain conditions. This susceptibility is consistent with the inverse association between omega −3 fatty acids and GSH that we found (Table 4). Omega-3 Index levels in our study participants were similar to those in the general US population, and lower than what we expect for avid seafood consumers (Karimi et al., 2014a). The overall qualitative effect of omega −3 fatty acids on redox status, and on Hg-glutathione interactions, may differ in populations with higher Omega-3 Index levels.

4.4. Limitations

One challenge in interpreting results from our cross-sectional study with a single blood draw, and comparing results across studies, is that differences in exposure levels, timing between exposures and responses, and temporal relevance of different biomarkers may each influence the direction and magnitude of the Hg-glutathione association. Our study participants were recruited based on average fish consumption over the past year. Therefore, we chose Hg and nutrient biomarkers that reflect relatively long term dietary exposures. Total Hg in blood is known to reflect MeHg exposure from seafood (ATSDR, 1999; Mahaffey, 2005) over the past month or more, depending on the consistency of the diet (Mahaffey et al., 2004; Svensson et al., 1992). Similarly, total Se in whole blood represents longer term dietary exposure compared to plasma-Se (Thomson, 2004; World Health Organization (WHO), 1987). Whole blood Se also combines signals of long-term exposures from red blood cells, and short-term exposures from plasma (Thomson, 2004). However, total Se concentrations can be similar in whole blood and serum in individuals who eat fish (Barany et al., 2003).

In contrast, blood GSH:GSSG ratios fluctuate throughout the day, in response to exogenous factors within hours(Blanco et al., 2007). Despite this background variability in GSH:GSSG we still observed significant relationships between Hg exposure and a relatively oxidized state, which persisted across model analyses and included treating Hg as a continuous, log-transformed, or categorical variable; furthermore, analyses with linear splines also produced similar results. In addition, GSSG values are relatively low, with an IQR of 0.60–1.50(Table 1). Hence small differences in GSSG across individuals can result in proportionately large CVs. There was no association between mercury and the subset of samples with higher CVs; therefore this high variability in GSSG may cause a Type II, but not Type I error, also referred to as non-differential misclassification. The result of non-differential misclassification is a shifting of the association toward the null, and therefore the measurement error did not lead to the observed associations with GSH:GSSG. In terms of nutrients, little is known about the identities of, or biomarkers that reflect, Hg–Se compounds, such as bis (methyl mercuric) selenide, that are thought to lower the bio availability of Hg and Se(Khan and Wang, 2009; Knott et al., 2011), and may influence

glutathione and health responses. In contrast with plasma and red blood cells, whole blood includes all Se compounds in circulation that may interact with Hg and glutathione (Fairweather-Tait et al., 2010), thus does not rely on assumptions about the molecular interactions between Hg, Se and glutathione components. There is a clear need to characterize these molecular interactions, particularly between the dominant chemical species of Hg and nutrients from seafood, and relate these interactions with measurements of exposure and toxicity. Such studies should explore the potential formation of MeHg–Se-glutathione complexes, that may be involved in MeHg excretion or detoxification, as has been observed with As, in organic Hg, and other metals and metalloids(Gailer, 2002; Manley et al., 2006). Finally, because GSH,GSSG and glutathione enzymes function together in redox reactions (Kelly et al., 1998), future research efforts should move beyond descriptive studies and examine the mechanistic roles of Hg and nutrients on redox status, glutathione enzyme activities, and lipid peroxidation.

5. Conclusions

Overall, our findings provide evidence for an association between elevated Hg exposure from seafood, the most common route of exposure, and a shift in redox status toward oxidative stress. We found this association in a population with relatively low blood Hg levels (IQR2.46–10.50 μg L^{-1}) and this association may be the basis for the diverse subclinical, and overt health effects that can result from Hg exposure. Additional research should focus on whether Hg-associated changes in GSH:GSSG ratio are related to Hg's known health effects and the role of seafood nutrients in modifying Hg toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

Hg	mercury
MeHg	methylmercury
Se	selenium
GSH	reduced glutathione

GSSG	oxidized glutathione
GPX	glutathione peroxidase
GST	glutathione-S-transferase
GR	glutathione reductase

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Table 1

Demographic characteristics and biomarker levels of the study population. Mean, standard deviation and interquartile range are reported for continuous variables. *N* and percent (%) of study population are reported for categorical variables. *N*=268.

Variable	N (%)	Mean ± SD	IQR
Age (years)		47.88 ± 18.24	30, 62
Males	112 (42%)	-	-
White/Caucasian	214 (80%)	-	-
Ever smokers[a]	114 (43%)	-	-
Eat fish 3 or more times a week[b]	140 (53%)	-	-
Household income (USD yr^{-1})			
< 25,000	45 (17%)	-	-
25,000–70,000	79 (30%)	-	-
70,000–110,000	68 (25%)	-	-
110,000–200,000	57 (21%)	-	-
> 200,000	19 (7%)	-	-
Graduate college education	181 (68%)	-	-
Body mass index	-	25.97 ± 5.13	22.48, 28.50
Hg (μg L^{-1})	-	7.71 ± 8.12	2.46, 10.50
Hg (nM)	-	47.56 ± 42.44	12.26, 52.35
Se (μg L^{-1})	-	293.54 ± 101.99	206.85, 365.50
Se (nM)	-	3728.54 ± 1287.65	2619.68, 4628.93
Se:Hg (nM ratio)	-	277.95 ± 560.94	65.79, 277.95
Omega-3 Index (%)	-	6.34 ± 1.76	5.09, 7.24
GSH (μM)	-	1022.29 ± ± 186.50	904.87, 1120.39
GSSG (μM)	-	1.23 ± 1.03	0.60, 1.50
GSH:GSSG	-	1206.35 ± 671.46	679.13, 1610.01
Eh (mV)	-	−679.69 ± 19.44	−694.25, −668.45

[a] *N* = 267.

[b] *N*=265.

Table 2

Association between elevated Hg (> 5.8 μg/L) and glutathione biomarkers, stratified by Omega-3 Index and Se (*p* values < 0.05 are in bold).

	All (N = 268)		Low Omega-3 index (< 6%, N = 133)		High Omega-3 index (> 6%, N = 135)		Low Se, (< 318 μg L^{-1}, N = 136)		High Se, (> 318 μg L^{-1}, N = 132)	
Hg ≥ 5.8 μg/L[a]	**Unadj.**	**Adj.[b]**	**Unadj.**	**Adj.[b]**	**Unadj.**	**Adj.[b]**	**Unadj.**	**Adj.[b]**	**Unadj.**	**Adj.[b]**
GSH										
Adj. R^2	0	0.05	0	0.01	0	0.04	0	0.03	0	0.02
β	−14.4	−0.4	−31.5	−8.0	20.0	13.6	2.5	15.9	−21.6	−37.9
95% CI	(−59.3, 30.5)	(−47.2, 46.4)	(−107.1, 44.1)	(−90.6, 74.6)	(−32.3, 72.4)	(−40.0, 67.1)	(−60.0, 65.1)	(−51.8, 83.7)	(−86.6, 43.3)	(−108.0, 32.2)
p Value	0.54	1.00	0.42	0.85	0.45	0.62	0.94	0.64	0.51	0.29
GSSG										
Adj. R^2	0.01	0.08	0.03	0.09	0	0.10	0.01	0.07	0.01	0
β	0.1	0.1	0.1	0.1	0.1	< 0.1	0.1	0.1	0.1	0.1
95% CI	(-5×10^{-3}, 0.2)	(< 0.1, 0.2)	(< 0.1, 0.3)	(-3×10^{-3}, 0.3)	(−0.1, 0.2)	(−0.1, 0.2)	(-2×10^{-2}, 0.2)	(-3×10^{-2}, 0.2)	(-3×10^{-2}, 0.2)	(-4×10^{-2}, 0.2)
p Value	0.07	**0.03**	**0.03**	0.05	0.42	0.43	0.10	0.12	0.13	0.15
GSH:GSSG										
Adj. R^2	0.02	0.10	0.07	0.18	0	0.09	0.02	0	0.04	0.02
β	−111.0	−116.7	−194.0	−182.5	−48.0	−63.5	−98.2	−97.1	−157.6	−151.8
95% CI	(−403.9, −85.5)	(−423.0, −96.2)	(−575.1, −111.5)	(−554.9, −78.7)	(−407.7, 51.6)	(−417.4, 40.9)	(−461.2, −64.2)	(−505.6, −65.0)	(−533.3, −49.6)	(−534.4, −1.0)
p Value	**0.01**	**0.01**	< **0.01**	< **0.01**	0.41	0.28	0.06	0.09	**0.01**	**0.03**
Eh										
Adj. R^2	0.02	0.05	0.06	0.08	0	0.08	0.03	0.03	0.02	0.02
β	6.38	6.5	10.8	9.0	2.7	3.1	6.9	6.5	7.3	7.7
95% CI	(1.8, 11.0)	(1.6, 11.4)	(3.8, 17.8)	(1.4, 16.6)	(−3.6, 9.1)	(−3.3, 9.4)	(0.8, 12.9)	(−0.1, 13.2)	(0.3, 14.2)	(0.1, 15.3)
p Value	< **0.01**	**0.02**	< **0.01**	**0.02**	0.85	0.34	**0.03**	0.05	**0.04**	0.05

Negative R^2 values were replaced with "0".

[a] Reference Hg < 5.8 μg L^{-1}

[b] Adjusted for age (continuous), sex (male (reference), female), income (< US$25,000 yr^{-1} (reference), $25,000–$70,000, $70,000–$110,000, $110,000–$200,000, > $200,000), and nutrients (continuous) (unstratified: Omega-3 Index (continuous) and Se (continuous); stratified: Omega-3 Index or Se).

Table 3

Associations between Se (continuous) and glutathione biomarkers (p values < 0.05 are in bold, N= 268).

	Unadj.	Adj.[a]
GSH		
Adj. R^2	0.05	0.05
β	−0.4	−0.4
95% CI	(−0.6, −0.2)	(−0.6, −0.2)
p-Value	<**0.01**	<**0.01**
GSSG		
Adj. R^2	0.07	0.08
β	-9×10^{-4}	-1×10^{-3}
95% CI	$(-1 \times 10^{-3}, -6 \times 10^{-4})$	$(-1 \times 10^{-3}, -6 \times 10^{-4})$
p-Value	<**0.01**	<**0.01**
GSH:GSSG		
Adj. R^2	0.07	0.10
β	1.8	2.0
95% CI	(1.0, 2.5)	(1.2, 2.8)
p-Value	<**0.01**	<**0.01**
E_h		
Adj. R^2	0.03	0.04
β	$-3x - 10^{-2}$	-4×10^{-2}
95% CI	$(-0.6, -1 \times 10^{-2})$	$(-0.1, -2 \times 10^{-2})$
p-Value	<**0.01**	<**0.01**

[a]Adjusted for age (continuous), sex (male(reference), female), income (< US$ 25,000 yr^{-1} (reference), $25,000–$70,000, $70,000–$110,000, $110,000–$200,000, > $200,000), Hg (continuous) and Omega-3 Index (continuous)

Table 4

Associations between Omega-3 Index (continuous) and glutathione biomarkers (p values < 0.05 are in bold, N=268).

	Unadj.	Adj.[a]
GSH		
Adj. R^2	0.02	0.06
β	−16.5	18.0
95% CI	(−29.1, −3.9)	(−31.4, −4.6)
p-Value	**0.01**	**0.01**
GSSG		
Adj. R^2	0	0.08
β	-6×10^{-3}	-9×10^{-3}
95% CI	$(-0.1, -4 \times 10^{-2})$	(−0.1, <0.1)
p-Value	0.62	0.47
GSH:GSSG		
Adj. R^2	0	0.08
β	−15.6	−8.4
95% CI	(−61.6, 30.4)	(−56.0, 39.1)
p-Value	0.51	0.73
Eh		
Adj. R^2	<0.01	0.03
β	0.7	0.6
95% CI	(−0.6, 2.0)	(−0.9, 2.0)
p-Value	0.30	0.44

[a]Adjusted for age (continuous), sex (male (reference), female), income (< US \$25,000 yr^{-1} (reference), \$25,000–\$70,000, \$70,000–\$110,000, \$110,000–\$200,000, > \$200,000), Hg (continuous) and Se (continuous).