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Fish Consumption, Low-Level Mercury, Lipids, and Inflammatory Markers in Children

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Abstract

There is considerable evidence that consuming fish has numerous health benefits, including a reduced risk of cardiovascular disease. However, fish is also the primary source of human exposure to mercury (Hg). In a cross-sectional study of 9–11 year old children (N = 100), we measured fish consumption, blood lipids, total blood Hg, diurnal salivary cortisol (4 samples collected throughout the day), and performed a proteomic analysis of serum proteins using spectral count shotgun proteomics. Children that consumed fish had a significantly more atheroprotective lipid profile but higher levels of blood Hg relative to children that did not consume fish. Although the levels of blood Hg were very low in these children ($M = 0.77 \mu\text{g/L}$; all but 1 participant had levels below $3.27 \mu\text{g/L}$), increasing blood Hg was significantly associated with blunted diurnal cortisol levels. Blood Hg was also significantly associated with acute-phase proteins suggesting systemic inflammation, and several of these proteins were found to significantly reduce the association between Hg and diminished cortisol when included in the model. This study of a pediatric population is the first to document an association between blood Hg, systemic inflammation, and endocrine disruption in humans, in a pediatric sample. Without a better understanding of the long-term consequences of an atheroprotective lipid profile relative to blunted diurnal cortisol and systemic inflammation, a determination of the risk-benefit ratio for fish consumption by children is not possible.

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Keywords

Children; mercury; fish consumption; inflammation; neuroendocrine

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1. Introduction

There is considerable evidence that consuming fish is associated with a reduced risk of cardiovascular disease (CVD; Mozaffarian and Rimm 2006). This is likely derived from fish being a source of long chain omega-3 polyunsaturated fatty acids, which have been shown to decrease CVD risk (Dolecek and Grandits 1991) and promote an atheroprotective lipid profile (Singer and Wirth 2004). These benefits to cardiovascular health have led to calls for greater fish consumption (Kris-Etherton et al. 2003; Organization 2010). However, there are also risks associated with exposure to environmental toxicants as a consequence of fish consumption. For example, the primary source of mercury (Hg) exposure in humans is through consumption of contaminated fish (US EPA 2006). Ionic Hg is a highly reactive heavy metal that is quickly converted by microorganisms in the environment into organic methylmercury (MeHg), which bioaccumulates in the food chain and is most highly concentrated in larger, predatory fish. As such, fish consumption recommendations are typically accompanied by warnings regarding how much and what kind of fish should be consumed, particularly for pregnant women and children (US FDA 2004). Nonetheless, pervasive chronic low-level Hg exposure is widespread. A recent study of New York City residents estimate that almost one quarter of NYC residents and nearly half of Asian New Yorkers have levels of blood Hg above 5.8 µg/L (New York City Department of Health and Hygiene 2007), the Environmental Protection Agency's established level for potential health risks (US EPA 2011). Given that nonessential metals can have adverse effects at levels well below the current thresholds for identifying 'elevated' levels (Bellinger et al. 1992; Gump et al. 2011; Freire et al. 2010), it is important for us to understand the pathophysiological changes that may occur during chronic low-level metal exposure.

Chronic low-level Hg exposure appears to disrupt a number of physiological systems. For example, inorganic Hg (iHg) increases the release of pro-inflammatory cytokines from human immune cells *in vitro* (Gardner et al. 2009; Kempuraj et al. 2010). A cross-sectional study demonstrated elevated pro-inflammatory cytokines in miners exposed to elemental and iHg relative to levels in miners without occupational Hg exposure (Gardner et al. 2010). Unlike ethyl Hg, both iHg and MeHg were found to increase pro-inflammatory cytokine release *in vitro* in human peripheral blood mononuclear cells (Gardner et al. 2010). In addition, several types of Hg has been shown to be associated with endocrine dysfunction, as shown in catfish with reduced cortisol levels (Kirubakaran and Joy 1991). Similarly, the cortisol levels in yellow perch and northern pike following capture stress were reduced in populations exposed to a set of environmental pollutants including MeHg (Hontela et al. 1992). While human adult populations have not revealed a significant association between elemental Hg exposure and endocrine functioning (Langworth et al. 1990; Erfurth et al. 1990), research in this field is limited. As children are presumed to be more sensitive than adults to the effects of environmental toxicants (Faustman et al. 2000), such effects might be evident in children in the absence of any observable effects in adults.

The aim of this study was to elucidate the potential risks and benefits of fish consumption by children. Dietary and family histories, anthropomorphic measurements, blood, and saliva were collected from 100 children of ages 9–11. We determined the potential benefits of fish consumption to children's lipid profile and the potential adverse consequences of low-level Hg exposure in children, including changes to acute-phase proteins and disruption of adrenocortical function.

2. Patients and Methods

2.1. Study Population

Participants ($N = 100$) were recruited as part of an ongoing study designed to address the effects of nonessential metals on cardiovascular responses to acute stress. Using a direct mailing list, we mailed invitations to homes in Oswego County, NY, containing a child within our target age group of 9–11 year olds. This recruitment method elicits participation from a sample that closely resembles an eligible population and is cost effective (Hinshaw et al. 2007). Further inclusion criteria included: 1) reporting no use on the day of testing of medication that might affect cardiovascular functioning (e.g., Ritalin), and 2) having no significant developmental disorders that might affect task performance (a component of our broader study). A blood draw for measuring nonessential toxic metal levels was followed within 2 weeks by a laboratory visit. Children were paid \$100 for their participation in the current study.

2.2 Fish Consumption

Parents were asked to report their child's fish consumption using the food frequency questionnaire developed for the Oswego Children's Study (Lonky et al. 1996). This dietary record included an item regarding fish (specifically, 'Fish (including tuna)'). Responses were made using checkboxes for consumption frequency on a 9-point scale from none to 4+ servings/day. An additional survey requested data on lifetime consumption of 26 varieties of sport fish using an 8-point scale that ranged from never to 5/week. Because of the distribution of maternal reports of their child's fish consumption (53 reported no fish consumption on the first survey and 73 reported no sport fish consumption), we created a single dichotomous variable for fish consumption that included no fish consumption in either category ($N = 45$) vs. some fish consumption in at least one category ($N = 50$). This variable was coded as 0 (no fish) or 1 (fish consumed) for use in regression models.

2.3 Serum Cholesterol

Fasting blood samples were collected in the morning. Serum was collected in a 4 mL Griener Vacuette® Serum Gel Evacuated Tube (Greiner Bio One North America, Inc., Monroe NC) and immediately shipped to the Oswego Hospital Laboratory (Oswego, NY). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were all analyzed on the Siemens Advia 1800 chemistry analyzer (Siemens Healthcare Diagnostics Inc., Deerfield, IL) per their protocol. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation: $LDL-C = (TC - HDL-C) - (TG \times 0.20)$. As a measure of atherogenic dyslipidemia, we used the TG/HDL-C ratio (Grundy et al. 2005).

2.4. Child's Blood Hg and Lead (Pb) Levels

Whole blood specimens (2 mL) were collected into Royal Blue Top Vacutainer® (BD Franklin Lakes, NJ, USA) tubes that had been pre-certified by the analyzing laboratory for low-level measurements of total Hg and Pb (a potential confound, see below). Blood specimens were refrigerated pending shipment to the Trace Elements section of the Laboratory of Inorganic and Nuclear Chemistry at the New York State Department of Health's Wadsworth Center, Albany, NY. The analysis for Pb and Hg in whole blood was carried out using a Perkin Elmer Sciex Model ELAN DRC Plus inductively coupled plasma-mass spectrometer (Shelton, CT, USA) equipped with a dynamic reaction cell (DRC-ICP-MS) (Palmer et al. 2006). For this study, the instrument was operated in the 'standard' rather than 'DNC' mode. The method detection limits (MDL) were 0.24 µg/L (Hg) and 0.34 µg/dL (Pb). Given the lower levels of blood Hg that were expected, the superior detection limits of

ICP-MS were appropriate for this study and have been used in several biomonitoring studies (McKelvey et al. 2007). Four levels of internal quality control materials were analyzed before, during and after each run. The method has been validated against NIST SRM 966 (Toxic Metals in Bovine Blood), as well as a new standard reference material SRM 955c (Toxic Metals in Caprine Blood) that has been certified for Pb and Hg (Murphy et al. 2009). Laboratory values below the MDL were used in data analyses with the assumption that such values constitute the best available estimate of the true value and are preferable to assigning a zero or an arbitrary constant such as ½ the MDL (Fitzgerald et al. 2004; Stewart et al. 2008).

2.5. Proteomics Analysis of Serum

For those samples with sufficient serum ($N = 95$), proteomic analysis was performed as described earlier (Birdsall et al. 2010), with some changes. Briefly, blood was collected using Vacutainers (BD Vacutainer K2, REF 367856), centrifuged to remove any cellular debris, and stored in Protein LoBind microcentrifuge tubes (Brinkmann Instruments) at -80°C until further analysis. One mL of serum samples were packed in dry ice and shipped overnight to METACyt Biochemical Analysis Center (MBAC) located in the department of Chemistry at Indiana University, Bloomington, IN where seven highly abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, anti-trypsin, and fibrinogen) were depleted using Agilent Multi Affinity Removal System column. The remaining proteins underwent buffer exchange into 50 mM ammonium bicarbonate and were pre-concentrated to ca. 0.5 $\mu\text{g}/\mu\text{L}$ using a 5 kDa MWCO spin concentrators.

After thermal denaturation, reduction, and alkylation, samples were treated with trypsin and LC-MS/MS analyses of the digests were performed in triplicate using a Dionex 3000 Ultimate nano-LC system (Dionex, Sunnyvale, CA) interfaced to LTQ Orbitrap hybrid mass spectrometer (Thermo Scientific, San Jose, CA). Prior to separation, a 1 μg -aliquot of protein equivalent was loaded onto a PepMap300 C18 cartridge (5 μm , 300 $\text{\AA}$, Dionex) and eluted through the column (150 mm $\times$ 100 μm i.d, 200 $\text{\AA}$ pores) packed with C18 (Michrom Bioresources, Auburn, CA). Peptides originating from tryptic digests were separated using a reversed-phase gradient from 10–55% of 99.9% acetonitrile with 0.1% formic acid over 3 hrs, at 350 nL/min flow rate. The mass spectrometer was operated in an automated data-dependent mode that was switching between MS scan and CID-MS. In this mode, eluted LC products undergo an initial full-spectrum MS scan from m/z 300 to 2000 in the Orbitrap at 15,000 mass resolutions, and subsequently CID-MS (at 35% normalized collision energy) was performed in the ion trap. The precursor ion was isolated using the data-dependent acquisition mode with a 2 m/z isolation width to select automatically and sequentially five most intense ions (starting with the most intense) from the survey scan. The total cycle (6 scans) was continuously repeated for the entire LC-MS run under data-dependent conditions with dynamic exclusion set to 60 sec. Performing MS scanning in the Orbitrap offers high mass accuracy and accurate charge state assignment of the selected precursor ions.

Mascot version 2.1.3 was used to search against *Homo sapiens* in the Swiss-Prot database using appropriate parameters (Birdsall et al. 2010). The quantitative analysis of proteins was carried out using the spectral count method (Liu et al. 2004; Lundgren et al. 2010). Using this method, 109 proteins were quantified and identified. However, proteins with very low abundance had progressively worse reliability across triplicate runs. Therefore, we reduced the set of reliably identified proteins to the 58 most abundant proteins. We tested distribution of spectral counts using the Kolmogorov-Smirnov test in Prism 4.03 (GraphPad Software Inc., La Jolla, CA) and log-transformed those demonstrating significant skewness.

2.6. Salivary Cortisol

After the blood draw, participants were sent home with a saliva collection kit containing 4 Sorbette saliva collection tubes (Salimetrics, State College, PA) and instructions regarding collection procedures and collection times (upon awakening, 30 min after awakening, 12 noon, and 6 pm). Participants were instructed on and practiced the procedures during the visit for the blood draw. Parents were also informed of the procedures and times to improve adherence. Participants were instructed not to collect samples within 60 minutes of eating a major meal and to rinse their mouth thoroughly 10 minutes before collection as dairy products and high acidic or high sugar foods can compromise the assay. After sample collection, participants were asked to store the collection tubes in their home freezer until their scheduled laboratory visit. Saliva was recovered from the collection tube via centrifugation and was stored at -80°C until used.

Salivary cortisol was measured using an enzyme immunoassay kit (Salimetrics) per the manufacturer's instructions. Briefly, samples were thawed completely at room temperature and centrifuged at $1,500 \times g$ to remove precipitated mucins. Standards (in duplicate), samples (in triplicate), and controls (in triplicate) were added to a 96-well microplate, a 1:1600 dilution of conjugated antibody was added to each well, the plate was placed on a shaker for 5 minutes, and then incubated at room temperature for 55 minutes. The plate was washed four times using a Bio-Tek ELx50 plate washer (Winooski, VT), 3, 3', 5, 5'-tetramethylbenzidine solution was added, mixed on a shaker for 5 minutes, and then incubated at room temperature in the dark for 25 minutes. Stop solution was added and the absorbance at 450 nm for each well was measured using a Bio-Tek PowerWave XS plate reader. The absorbance of each sample, standard, and control was used to calculate the percent cortisol bound and the concentrations of the samples and controls were determined from the standards using a 4-parameter sigmoid minus curve fit calculated using KC Junior software (Bio-Tek). Pooled saliva was used as an internal control for all analyses. The intra- and inter-assay coefficients of variance were 4.7% and 6.8%, respectively.

Following standards in the field (e.g., (Cohen et al. 2006), measures of diurnal cortisol levels included the following: 1) log-transformed values (to approximately normalize the distribution) at each time point, 2) diurnal slope and intercept by separately fitting a linear regression line for each participant that predicted the log-transformed cortisol values from the actual collection times reported, 3) peak cortisol (maximum for the 4 samples for each participant), 4) mean cortisol (average across 4 samples), and 5) 'area under the curve with respect to ground' (AUCg). AUCg is calculated as the average log-transformed cortisol for two consecutive samples divided by the length of time between these samples, and these values are then summed across multiple time intervals representing the multiple collection times (Pruessner et al. 2003). Two participants did not return 2 or more saliva samples and were treated as missing all data for this measure. Four participants had 1 missing sample and linear extrapolation based on the remaining 3 samples was used to assign a cortisol level for this missing sample.

2.7. Potential Confounds

Potential confounders were chosen based on the National Institutes of Health guidelines for the inclusion of covariates in multivariate models, which recommends *a priori* selection of a limited set of variables shown in prior literature to relate to the outcome (Ewout and Harrell 2009). This approach avoids over-fitting a model that occurs when 'cherry picking' covariates from a larger set of potential confounds (Babyak 2004). The following covariates were included: gender, age, race, BMI percentile standing (age and gender adjusted using a SAS program developed by the Centers for Disease Control and Prevention (Prevention), family history of high cholesterol (yes vs. no for parents or grandparents), and

socioeconomic status (SES). SES was a single measure derived from normalizing (using z-scores) and averaging the parents' education (using an 8 level item), occupation (using a 9 level occupational classification developed by Hollingshead (Hollingshead 1975), and income (using a 9-level item). Because of the potential effects of Pb exposure on the adrenocortical system (Gump et al. 2008) this metal was measured as a potential confound and included as a covariate in all analyses of Hg associations.

2.8. Data Analyses

All statistical analyses were performed using SAS software (SAS, Cary, NC). Hg was log transformed because the distribution of blood Hg levels was not normally distributed and showed a significant positive skew (skewness = 6.77, standard error of skewness = 0.24, $z = 28.04$, $p < 0.05$). Log Hg was analyzed in regression models (using SAS PROC REG). In addition to this approach, we analyzed nontransformed Hg by creating quartiles that contained a roughly equal number of participants and corresponded to the following blood levels: < 0.24 $\mu\text{g/L}$ (1st quartile; $N = 25$), $0.25 - 0.45$ $\mu\text{g/L}$ (2nd quartile; $N = 25$), $0.46 - 0.79$ $\mu\text{g/L}$ (3rd quartile; $N = 24$), and $0.80 - 11.82$ $\mu\text{g/L}$ (4th quartile; $N = 26$). For the analysis of quartiles, SAS PROC GLM was used with a linear contrast to test the effects of increasing blood Hg levels. Partial correlations (using SAS PROC CORR) were used to analyze associations between serum proteins, Hg, and cortisol. In all analytic models, the seven covariates outlined above were entered first. Sample size varied slightly across analyses due to missing data (as outlined for each measure above). Analysis of salivary cortisol involved separate analytic models for each outcome (e.g., awaking and noon cortisol were analyzed in separate models).

For the proteomics data analysis, we increased our protection from Type I error by focusing on groups of proteins generated from a principal components factor analysis (PCA) with varimax rotation, a common approach to reducing the complexity of intercorrelated proteomics data (Lancashire et al. 2005; Meyrick et al. 2008). Because inflammatory processes are integrally related to cortisol (Miller et al. 2002), we tested proteins as potential mediators of the associations between Hg and diurnal cortisol as well as diurnal cortisol as a potential mediator for the association between Hg and proteins. Meditational modeling was performed using the Sobel test with bootstrapping (Preacher and Hayes 2004).

3. Results and Discussion

3.1. Characteristics of Participants

Table 1 presents characteristics of children in our sample. Our sample included 9, 10, and 11 year-olds ($Ns = 47, 50$, and 3 , respectively), a roughly equal number of males and females (57 and 43 , respectively), and was predominantly white (87%). The parents of these children had, on average, 'some college' (scale score = 5.4), a family income between $\$45,000$ and $\$65,000$, and an occupational status between 'Clerical and Sales' (Hollingshead score = 5) and 'Technician or Semiprofessional' (Hollingshead score = 6). The mean BMI percentile for our sample was 75.1% and 55.0% of the children had a family history of high cholesterol (at least 1 parent and grandparent with known high cholesterol). Based on parental reports, 50 children (52.6%) consume either commercial fish (e.g., tuna) or sport fish at least once/week. Mean blood Pb in our study population was 1.00 $\mu\text{g/dL}$ (90^{th} percentile = 1.53 $\mu\text{g/dL}$). Mean blood Hg was 0.77 $\mu\text{g/L}$ and with the exception of one participant at 11.82 $\mu\text{g/L}$, all participants had Hg levels below 3.27 $\mu\text{g/L}$. The 90^{th} percentile for Hg was 1.55 $\mu\text{g/L}$. The average for our study population is well below the Environmental Protection Agency's established level 5.8 $\mu\text{g/L}$ for potential health risks (US EPA 2011).

3.2. Fish consumption is associated with an atheroprotective lipid profile and elevated Hg

This cross-sectional study of 9–11 year old children demonstrated some associations that are well established in adults. In these children, fish consumption was associated with a significantly more atheroprotective lipid profile. In unadjusted models (no covariates included), children consuming fish had significantly higher HDL-C ($F(1, 93) = 4.44, p < 0.05$), less dyslipidemia ($F(1, 93) = 5.89, p < 0.05$). However, they also had greater blood Pb ($F(1, 93) = 7.20, p < 0.01$) and Hg levels ($F(1, 93) = 4.30, p < 0.05$). As shown in Table 2, these associations remained after inclusion of covariates. In these multivariate models, children consuming fish had significantly higher HDL-C ($F(1, 85) = 4.17, p < 0.05$; full model $R^2 = 0.16$), a better ratio of TC:HDL-C ($F(1, 85) = 7.12, p < 0.01$; full model $R^2 = 0.23$), lower triglycerides ($F(1, 85) = 6.76, p < 0.05$; full model $R^2 = 0.23$), less dyslipidemia ($F(1, 85) = 10.03, p < 0.01$; full model $R^2 = 0.28$), greater blood Pb ($F(1, 87) = 8.05, p < 0.01$; full model $R^2 = 0.25$), and greater blood Hg ($F(1, 87) = 14.48, p < 0.05$; full model $R^2 = 0.23$). While studies have demonstrated the benefits of fish consumption for lipid profiles in adults (Bulliyya 2002; Gunnarsdottir et al. 2008), particularly by increasing HDL-C (Smith et al. 2009), we are not aware of this being shown before in children. Fish consumption in children was also associated with significantly greater blood Hg, which is not surprising given that fish consumption represents the primary exposure route for Hg in humans. Therefore, the data indicates that while fish consumption in children leads to a more atheroprotective lipid profile, there is also a significant increase in nonessential toxic heavy metal exposure.

3.3. Increasing blood Hg is associated with blunted diurnal cortisol

Some research suggests Hg-induced endocrine disruption in animals (Hontela et al. 1992) and humans (Langworth et al. 1990; Erfurth et al. 1990); however, we are not aware of research on the potential effects of Hg on cortisol levels. To determine if Hg exposure disrupts endocrine function in children, saliva was collected at four points in the day and cortisol levels were measured. We observed an association between increasing blood Hg and blunted diurnal cortisol. In unadjusted models, increasing blood Hg in children was associated with lower levels of cortisol as reflected by numerous significant associations (see Table 3), including: awakening cortisol ($\beta = -0.27, p < 0.05$; full model $R^2 = 0.16$), awakening + 30 min cortisol ($\beta = -0.24, p < 0.05$; full model $R^2 = 0.10$), 6 pm cortisol ($\beta = -0.29, p < 0.01$; full model $R^2 = 0.17$), mean cortisol ($\beta = -0.37, p < 0.001$; full model $R^2 = 0.16$), peak cortisol ($\beta = -0.31, p < 0.01$; full model $R^2 = 0.16$), and AUCg ($\beta = -0.31, p < 0.01$; full model $R^2 = 0.16$). Neither fish group nor blood HDL-C was significantly associated with diurnal cortisol in multivariate models (p values > 0.10). As such, the addition of these variables as covariates did not alter the association between Hg and diurnal cortisol. When testing for linear contrast (Table 3), increasing Hg quartiles were associated with significantly less noon cortisol ($F(1, 76) = 4.53, p < 0.05$; full model $R^2 = 0.14$), 6 pm cortisol ($F(1, 76) = 12.74, p < 0.001$; full model $R^2 = 0.21$), mean cortisol ($F(1, 76) = 12.32, p < 0.001$; full model $R^2 = 0.16$), peak cortisol ($F(1, 76) = 8.29, p < 0.01$; full model $R^2 = 0.21$), and AUCg ($F(1, 76) = 7.27, p < 0.01$; full model $R^2 = 0.15$). Figure 1 shows the mean diurnal cortisol for the top and bottom blood Hg quartile. These findings clearly show that blood Hg levels are associated with blunted diurnal cortisol level.

In contrast to the limited research on Hg-induced endocrine disruption in humans, the effects of psychological stress on cortisol have been extensively studied and may offer a model on how chronic Hg exposure could blunt cortisol levels. The onset of a chronic stress produces high cortisol output; however, as time passes and the chronic stressor remains, the body is unable to mount a normal adrenocortical response and cortisol output declines to below normal (Miller et al. 2007; Dallman 1993). It is possible that Hg is acting as an ‘environmental stressor’ (Yunus et al. 2000) that blunts diurnal adrenocortical functioning.

If so, this would be an important finding as a persistent lack of cortisol (whether it be induced by environmental or psychosocial stressors) may result in the development of stress-related physical and psychological disorders (Chikanza et al. 1992; Yehuda et al. 1993; Dreger et al. 2010).

3.4. Blood Hg levels are associated with serum inflammatory markers

We used a powerful exploratory tool, proteomics, to see if serum protein levels are altered due to Hg exposure. Shotgun spectral count proteomics provides a method to simultaneously identify and quantify numerous proteins in a heterogeneous sample. We used PCA (with rotation) to identify intercorrelated proteins that were grouped into 'Factors'. A Scree plot from PCA suggested eight distinct factors, which was confirmed with a Minimum Average Partial (MAP) test (O'Connor 2000). Factor 1 (Eigenvalue = 16.96) explained the greatest variance relative to the other factors (all other Eigenvalues < 4.14). The remaining seven Factors revealed by the PCA contained fewer proteins loading at 0.60 or greater (3 or less for all factors) and were not readily interpretable. Fourteen proteins (Table 4) loaded strongly at 0.60 or greater on Factor 1 and of these, eight are clearly identified as acute-phase proteins (Gabay and Kushner 1999). In addition, the remaining proteins (e.g., alpha-1B-glycoprotein, gelsolin, and apolipoprotein A-II) may be implicated in the inflammatory process (Hashim et al. 2001; Bucki et al. 2005; Thompson et al. 2008). Many of the proteins had a positive association with Hg and a negative association with cortisol, except for apolipoprotein A-II, which had a marginally significant negative association with Hg and a significant positive association with cortisol (Table 4). Figure 2 illustrates the significant association between Hg and the combined Factor 1 score ($r = 0.36$, $p < 0.001$).

These findings suggest that increasing blood Hg levels are associated with inflammatory processes in children as indicated by significant associations with acute-phase proteins. This is important because these effects are being observed in children whose Hg levels are well below the levels presumed to result in negative health effects and pro-inflammatory cytokine activity and the acute-phase response have been implicated in the development atherosclerosis and diabetes (Ross 1999; Pickup and Crook 1998). Fish consumption was not associated with significantly different Factor 1 levels after controlling for our standard covariates (data not shown). Since these proteins (as well as diurnal cortisol) were not significantly associated with fish consumption, the observed proximal pathways (fish → Hg and Hg → proteins/cortisol) were presumably weakened somewhat by either consumption of fish with varying levels of Hg or alternative Hg exposure routes.

3.5. Acute phase proteins account for Hg-blunted cortisol association

Sobel tests for mediation were used to test whether the association between Hg and cortisol can be explained by the acute-phase serum proteins. Six of the proteins were found to significantly reduce the previously significant association between Hg and mean salivary cortisol (Table 4). This cluster of proteins (particularly hemopexin and complement factor C3) may represent cortisol-mediated promotion of the class I genes involved in the acute phase of inflammation (Poli 1998). In addition, the combined Factor 1 score was found to significantly reduce the previously significant Hg-cortisol association ($p < 0.05$).

The immune and neuroendocrine systems are integrally connected, suggesting an 'immunoneuroendocrine axis' (Beishuizen and Thijs 2004). As such, we were unable to statistically differentiate the two potential mediational models: 'Hg → inflammatory proteins → cortisol' and 'Hg → cortisol → inflammatory proteins.' It is possible that Hg induces proinflammatory cytokine activity (Gardner et al. 2009; Kempuraj et al. 2010) and thereby increases acute-phase proteins (a proximal effect) and eventually produces a blunted diurnal cortisol (a more distal effect resulting from chronic elevations in acute-phase

proteins). However, glucocorticoids (e.g., cortisol) also inhibit the inflammatory response and suppress the production and release of pro-inflammatory cytokines (Franchimont et al. 2002), which is seen as compensatory mechanism that restrains the inflammatory reaction and thereby prevents tissue damage (McEwen et al. 1997). Therefore, it is also possible that Hg-induced hypocortisolemia removes this restraint, resulting in greater systemic inflammation. The particular causal model linking Hg, cortisol, and acute-phase proteins remains to be determined.

3.6. Possible limitations

First, the cross-sectional design has known weaknesses. For example, it is possible that diurnal cortisol or acute-phase proteins affect the toxicokinetics of this nonessential environmental metal. Second, we measured total Hg in our samples and therefore cannot address the relationship between the various forms of Hg (elemental, ionic, organometallic) and elevated acute-phase proteins and adrenocortical dysfunction. While most of the Hg present in human originates from fish in the food supply in the form of MeHg (Ravichandran 200) we cannot exclude the possible contributions of other Hg species such as elemental Hg exposure through dental amalgams, especially in gum chewers (Hansen et al. 2004). Third, if we consider that most of the total blood Hg is found as MeHg, we must also take into account that the whole-body half-life of MeHg is estimated to be 70–80 days (Aberg et al. 1969; US EPA 1997) and that health behaviors and dietary characteristics likely vary as these children age. Therefore, it is difficult to assess the potential clinical implications for the current Hg-associated changes in cortisol and systemic inflammation. Without repeated measurement of Hg in their blood, we cannot determine if our current associations represent a short-term response to acute exposure or a chronic state associated with long-term exposure. This is an important distinction; if systemic inflammation is associated with an increased risk for CVD (Kaptoge et al. 2010), it likely involves chronic inflammation in order to contribute to a disease that develops over many years. Fourth, our measure of fish consumption relied on parental retrospective recall, and studies investigating the correlation between fish consumption recall reports and Hg biomarkers have been mixed (Lincoln et al. 2011; Hertz-Picciotto et al. 2010). However, our observed association between the fish consumption reports and lipids and blood Hg provides some validation for the parental recall measure used in this study. Finally, we did not measure proinflammatory cytokines that are presumed to produce the increase in acute-phase proteins. In the absence of direct measurement of proinflammatory cytokines, the hypothesized mediational pathway (Hg → proinflammatory cytokines → acute-phase proteins) remains to be tested.

4. Conclusions

This study confirmed the well-known association between fish consumption and atheroprotective lipid profiles as well as increased blood Hg. It also demonstrated a significant association between blood Hg levels, acute-phase proteins, and reduced diurnal cortisol in children. Although some research has suggested an association between proinflammatory markers and Hg, this is the first study that we are aware of that has demonstrated this association in children. Similarly, while the association between blunted cortisol and Hg has been shown in animals, this is the first demonstration of such an association in children. Most importantly, the associations demonstrated here were significant at Hg levels well below the potential health risk level of 5.8 µg/L that has been established by the Environmental Protection Agency (2011). The present finding of elevated acute-phase proteins and adrenocortical dysfunction at levels of Hg typical for many US children and produced by fish consumption suggests potentially broad public health implications.

Acknowledgments

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Abbreviations

CDC	Centers for Disease Control and Prevention
CVD	cardiovascular diseases
Hg	mercury
MDL	method detection limits
Pb	lead
SES	socioeconomic status

References

- Aberg B, Ekman L, Falk R, Greitz U, Persson G, Snihs JO. Metabolism of methyl mercury (203Hg) compounds in man. *Arch Environ Health*. 1969; 19:478–484. [PubMed: 5822994]
- Babyak MA. What you see may not be what you get: a brief, nontechnical introduction to overfitting in regression-type models. *Psychosom Med*. 2004; 66:411–421. [PubMed: 15184705]
- Beishuizen A, Thijs LG. The immunoneuroendocrine axis in critical illness: beneficial adaptation or neuroendocrine exhaustion? *Curr Opin Crit Care*. 2004; 10:461–467. [PubMed: 15616387]
- Bellinger DC, Stiles KM, Needleman HL. Low-level exposure, intelligence and academic achievement: A long-term follow-up study. *Pediatrics*. 1992; 90:855–861. [PubMed: 1437425]
- Birdsall RE, Kiley MP, Segu ZM, Palmer CD, Madera M, Gump BB, MacKenzie JA, Parsons PJ, Mechref Y, Novotny MV, Bendinskas KG. Effects of lead and mercury on the blood proteome of children. *J Proteome Res*. 2010; 9:4443–4453. [PubMed: 20681587]
- Bucki R, Georges PC, Espinassous Q, Funaki M, Pastore JJ, Chaby R, Janmey PA. Inactivation of endotoxin by human plasma gelsolin. *Biochemistry*. 2005; 44:9590–9597. [PubMed: 16008344]
- Bulliyya G. Influence of fish consumption on the distribution of serum cholesterol in lipoprotein fractions: comparative study among fish consuming and non fish consuming populations. *Asia Pacific Journal of Clinical Nutrition*. 2002; 11:104–111. [PubMed: 12074175]
- Centers for Disease Control and Prevention. [accessed December 20, 2008] A SAS Program for the CDC Growth. <<http://www.cdc.gov/nccdphp/dnpa/growthcharts/resources/sas.htm>>
- Chikanza IC, Petrou P, Kingsley G, Chrousos G, Panayi GS. Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. *Arthritis and rheumatism*. 1992; 35:1281–1288. [PubMed: 1445443]
- Cohen S, Schwartz JE, Epel ES, Kirschbaum C, Sidney S, Seeman T. Socioeconomic status, race and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Psychosom Med*. 2006; 68:41–50. [PubMed: 16449410]
- Dallman MF. Stress update Adaptation of the hypothalamic-pituitary-adrenal axis to chronic stress. *Trends Endocrinol Metab*. 1993; 4:62–69. [PubMed: 18407136]
- Dolecek, TA.; Grandits, G. *World Review of Nutrition and Diet*. Karger Press; Basel: 1991. Dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention (MRFIT) study; p. 205-216.
- Dreger LC, Kozyrskyj AL, HayGlass KT, Becker AB, MacNeil BJ. Lower cortisol levels in children with asthma exposed to recurrent maternal distress from birth. *J Allergy Clin Immunol*. 2010; 125:116–122. [PubMed: 19962747]

- Erfurth EM, Schutz A, Nilsson A, Barregard L, Skerfving S. Normal pituitary hormone response to thyrotrophin and gonadotrophin releasing hormones in subjects exposed to elemental mercury vapour. *Br J Ind Med*. 1990; 47:639–644. [PubMed: 2119795]
- Ewout, WF.; Harrell, E, Jr. Statistical models for prognostication. In: Max, MJB Lynn, Hrsg, editor. *Symptom research: Methods and Opportunities*. Bethesda: 2009.
- Faustman EM, Silbernagel SM, Fenske RA, Burbacher TM, Ponce RA. Mechanisms underlying children's susceptibility to environmental toxicants. *Environ Health Perspect*. 2000; 108(Suppl 1): 13–21. [PubMed: 10698720]
- Fitzgerald EF, Hwang SA, Langguth K, Cayo M, Yang BZ, Bush B, Worswick P, Lauzon T. Fish consumption and other environmental exposures and their associations with serum PCB concentrations among Mohawk women at Akwesasne. *Environ Res*. 2004; 94:160–170. [PubMed: 14757379]
- Food and Agriculture Organization of the United Nations and World Health Organization 2010. Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption: Executive Summary. http://ftp.fao.org/FI/DOCUMENT/risk_consumption/executive_summary.pdf
- Franchimont D, Kino T, Galon J, Meduri GU, Chrousos G. Glucocorticoids and inflammation revisited: the state of the art. NIH clinical staff conference. *Neuroimmunomodulation*. 2002; 10:247–260. [PubMed: 12759562]
- Freire C, Ramos R, Lopez-Espinosa MJ, Díez S, Vioque J, Ballester F, Fernández MF. Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. *Environmental research*. 2010; 110:96–104. [PubMed: 19909946]
- Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999; 340:448–454. [PubMed: 9971870]
- Gardner RM, Nyland JF, Silbergeld EK. Differential immunotoxic effects of inorganic and organic mercury species in vitro. *Toxicol Lett*. 2010; 198:182–190. [PubMed: 20600710]
- Gardner RM, Nyland JF, Evans SL, Wang SB, Doyle KM, Crainiceanu CM, Silbergeld EK. Mercury induces an unopposed inflammatory response in human peripheral blood mononuclear cells in vitro. *Environ Health Perspect*. 2009; 117:1932–1938. [PubMed: 20049214]
- Gardner RM, Nyland JF, Silva IA, Ventura AM, de Souza JM, Silbergeld EK. Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in Amazonian Brazil: a cross-sectional study. *Environ Res*. 2010; 110:345–354. [PubMed: 20176347]
- Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Executive summary. *Cardiol Rev*. 2005; 13:322–327. [PubMed: 16708441]
- Gump BB, MacKenzie JA, Bendinskas K, Morgan R, Dumas AK, Palmer CD, Parsons PJ. Low-level Pb and cardiovascular responses to acute stress in children: The role of cardiac autonomic regulation. *Neurotoxicology and Teratology*. 2011; 33:212–219. [PubMed: 20934510]
- Gump BB, Stewart P, Reihman J, Lonky E, Darvill T, Parsons PJ, Granger DA. Low-level Prenatal and Postnatal Blood Lead (Pb) Exposure and Adrenocortical Responses to Acute Stress in Children. *Environ Health Perspect*. 2008; 116:249–255. [PubMed: 18288326]
- Gunnarsdottir I, Tomasson H, Kiely M, Martínéz JA, Bandarra NM, Morais MG, Thorsdottir I. Inclusion of fish or fish oil in weight-loss diets for young adults: effects on blood lipids. *International Journal of Obesity*. 2008; 32:1105–1112. [PubMed: 18490931]
- Hansen G, Victor R, Engeldinger E, Schweitzer C. Evaluation of the mercury exposure of dental amalgam patients by the Mercury Triple Test. *Occup Environ Med*. 2004; 61:535–540. [PubMed: 15150394]
- Hashim OH, Ahmad F, Shuib AS. The application of Artocarpus integer seed lectin-M in the detection and isolation of selective human serum acute-phase proteins and immunoglobulins. *Immunol Invest*. 2001; 30:131–141. [PubMed: 11465670]
- Hertz-Picciotto I, Green PG, Delwiche L, Hansen R, Walker C, Pessah IN. Blood mercury concentrations in CHARGE Study children with and without autism. *Environ Health Perspect*. 2010; 118:161–166. [PubMed: 20056569]

- Hinshaw LB, Jackson SA, Chen MY. Direct mailing was a successful recruitment strategy for a lung-cancer screening trial. *J Clin Epidemiol*. 2007; 60:853–857. [PubMed: 17606183]
- Hollingshead, AB. Four factor index of social status. New Haven, CT: 1975.
- Hontela A, Rasmussen JB, Audet C, Chevalier G. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. *Archives of Environmental Contaminants and Toxicology*. 1992; 22:278–283.
- Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J, Bladbjerg EM, Jespersen J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010; 375:132–140. [PubMed: 20031199]
- Kempuraj D, Asadi S, Zhang B, Manola A, Hogan J, Peterson E, Theoharides TC. Mercury induces inflammatory mediator release from human mast cells. *J Neuroinflammation*. 2010; 7:20. [PubMed: 20222982]
- Kirubakaran R, Joy KP. Changes in adrenocortical-pituitary activity in the catfish, *Clarias batrachus* (L.), after mercury treatment. *Ecotoxicology and environmental safety*. 1991; 22:36–44. [PubMed: 1914994]
- Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, thrombosis, and vascular biology*. 2003; 23:e20.
- Lancashire L, Schmid O, Shah H, Ball G. Classification of bacterial species from proteomic data using combinatorial approaches incorporating artificial neural networks, cluster analysis and principal components analysis. *Bioinformatics*. 2005; 21:2191–2199. [PubMed: 15746279]
- Langworth S, Rojdmarm S, Akesson A. Normal pituitary hormone response to thyrotrophin releasing hormone in dental personnel exposed to mercury. *Swed Dent J*. 1990; 14:101–103. [PubMed: 2115698]
- Lincoln RA, Shine JP, Chesney EJ, Vorhees DJ, Grandjean P, Senn DB. Fish consumption and mercury exposure among Louisiana recreational anglers. *Environ Health Perspect*. 2011; 119:245–251. [PubMed: 20980220]
- Liu H, Sadygov RG, Yates JR 3rd. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal Chem*. 2004; 76:4193–4201. [PubMed: 15253663]
- Lonky E, Reihman J, Darvill T, Mather J, Daly H. Neonatal Behavioral Assessment Scale performance in humans influenced by maternal consumption of environmentally contaminated Lake Ontario fish. *Journal of Great Lakes Research*. 1996; 22:198–212.
- Lundgren DH, Hwang SI, Wu L, Han DK. Role of spectral counting in quantitative proteomics. *Expert Rev Proteomics*. 2010; 7:39–53. [PubMed: 20121475]
- McEwen BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, Goldfarb RH, Kitson RP, Miller AH, Spencer RL, Weiss JM. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Brain Res Rev*. 1997; 23:79–133. [PubMed: 9063588]
- McKelvey W, Gwynn RC, Jeffery N, Kass D, Thorpe LE, Garg RK, Palmer CD, Parsons PJ. A biomonitoring study of lead, cadmium, and mercury in the blood of New York City adults. *Environmental Health Perspectives*. 2007; 115:1435. [PubMed: 17938732]
- Meyrick BO, Friedman DB, Billheimer DD, Cogan JD, Prince MA, Phillips JA 3rd, Loyd JE. Proteomics of transformed lymphocytes from a family with familial pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2008; 177:99–107. [PubMed: 17932379]
- Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*. 2007; 133:25–45. [PubMed: 17201569]
- Miller GE, Cohen S, Ritchey AK. Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. *Health Psychol*. 2002; 21:531–541. [PubMed: 12433005]
- Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA*. 2006; 296:1885–1899. [PubMed: 17047219]
- Murphy KE, Guthrie WF, Vetter TW, Turk GC, Palmer CD, Lewis ME, Geraghty CM, Parsons PJ. Comparison of clinical methods with isotope dilution inductively coupled plasma mass

- spectrometry for the new standard reference material 955c lead in caprine blood. *Journal of Analytical Atomic Spectrometry*. 2009; 24:1170–1178.
- New York City Department of Health and Hygiene. New York City Department of Health and Mental Hygiene (DOHMH) Health Advisory # 11: Blood Mercury Levels in NYC Adult Women are Higher than Women Nationally; Providers Should Encourage Healthy Fish Consumption. 2007.
- O'Connor BP. SPSS and SAS programs for determining the number of components using parallel analysis and velicer's MAP test. *Behav Res Methods Instrum Comput*. 2000; 32:396–402. [PubMed: 11029811]
- Palmer CD, Lewis ME Jr, Geraghty C, Barbosa F Jr, Parsons PJ. Determination of lead, cadmium and mercury in blood for assessment of environmental and occupational exposures: A comparison between inductively coupled plasma-mass spectrometry and atomic absorption spectrometry. *Spectrochimica Acta B-Atomic Spectrometry*. 2006; 61:980–990.
- Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia*. 1998; 41:1241–1248. [PubMed: 9794114]
- Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Methods Instrum Comput*. 2004; 36:717–731. [PubMed: 15641418]
- Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*. 2003; 28:916–931. [PubMed: 12892658]
- Ravichandran M. Interactions between mercury and dissolved organic matter - a review. *Chemosphere*. 2004; 55:319–331. [PubMed: 14987930]
- Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999; 340:115–126. [PubMed: 9887164]
- Singer P, Wirth M. Can n-3 PUFA reduce cardiac arrhythmias? Results of a clinical trial. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2004; 71:153–159.
- Smith KM, Barraj LM, Kantor M, Sahyoun NR. Relationship between fish intake, n-3 fatty acids, mercury and risk markers of CHD (National Health and Nutrition Examination Survey 1999–2002). *Public Health Nutrition*. 2009; 12:1261–1269. [PubMed: 18986590]
- Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal PCB exposure and intelligence (IQ) in 9-year-old children. *Environ Health Perspect*. 2008; 116:1416–1422. [PubMed: 18941588]
- Thompson PA, Berbee JF, Rensen PC, Kitchens RL. Apolipoprotein A-II augments monocyte responses to LPS by suppressing the inhibitory activity of LPS-binding protein. *Innate Immun*. 2008; 14:365–374. [PubMed: 19039060]
- US Environmental Protection Agency. Mercury study report to congress. ORD; 1997. EPA-452/R-97-003
- US Environmental Protection Agency. Mercury Study Report to Congress. 2006.
- US Environmental Protection Agency. Methylmercury (MeHg) (CASRN 22967-92-6). Integrated Risk Information System; 2011.
- US Food and Drug Administration. What you need to know about mercury in fish and shellfish. FDA/CFSAN Consumer Advisory; 2004. EPA-823-R-04-005
- Yehuda R, Resnick H, Kahana B, Giller EL. Long-lasting hormonal alterations to extreme stress in humans: normative or maladaptive? *Psychosomatic Medicine*. 1993; 55:287. [PubMed: 8346336]
- Yunus, M.; Singh, N.; De Kok, LJ. Environmental stress: indication, mitigation, and eco-conservation. 2000.

Highlights

- We measured fish consumption, lipids, mercury, and salivary cortisol in children.
- We performed an analysis of serum proteins using shotgun proteomics.
- Children that consumed fish had a more atheroprotective lipid profile.
- They also had increasing serum mercury and proteins suggesting systemic inflammation.
- Balancing the risks and benefits of fish consumption by children remains a challenge.

Figure 1.
Diurnal salivary cortisol (nmol/L) patterns for the top and bottom blood Hg quartiles.

Figure 2.
Inflammatory markers (Factor 1 score) in relation to Hg ($\mu\text{g/L}$, log), with an ordinary least squares line added.

Table 1 Sample Characteristics ($N = 100$)

Variable	M (SD)	%
Age	10.01 (0.62)	
Gender (% female)		43.0
Race (% white)		87.0
Childs' BMI (percentile ¹)	75.13 (23.19)	
Family Income ²	7.83 (1.82)	
Parent's Occupation (score)	5.92 (1.71)	
Parent's Education (score)	5.40 (1.11)	
Fish Consumption (% yes)		52.6
Blood Hg ($\mu\text{g/L}$)	0.77 (1.28)	
Blood Pb ($\mu\text{g/dL}$)	1.00 (0.47)	

¹ Percentiles are derived from national age and gender adjusted BMI tables provided by the CDC.

² On this income scale, a score of 8 corresponds to '45,000–65,000'. As outlined in the methods section, this income was subsequently adjusted for the number of people living in the household.

Table 2

Associations between fish consumption, lipids, and nonessential metals in blood.

	Fish Consumption ¹	
	No (N = 45)	Yes (N = 50)
Cholesterol (mg/dL)	159.20	158.02
LDL (mg/dL)	92.01	89.30
HDL (mg/dL)	51.08	56.91 *
Total: HDL Ratio	3.24	2.83 **
Triglycerides (mg/dL)	80.22	58.89 *
Dyslipidemia (Triglycerides: HDL Ratio)	1.75	1.09 **
Blood Hg (µg/L)	0.41	1.11 *
Blood Pb (µg/dL)	0.84	1.10 *

*
p < 0.05;**
p < 0.01

¹ Parents of 5 children did not complete the dietary questionnaire and were therefore not available for these analyses. All analyses are covariate adjusted for age, gender, race, BMI, SES, family history of high cholesterol, as well as Pb and Hg (for analysis of lipids).

Table 3

Associations between blood Hg and logged salivary cortisol measures ($N = 100$).

Salivary Cortisol Level	Logged Hg (β)	Hg Quartile				Linear Contrast
		1 st	2 nd	3 rd	4 th	
Awakening (nmol/L)	-0.27 *	0.41	0.36	0.28	0.31	#
+ 1/2 hour (nmol/L)	-0.26 *	0.28	0.21	0.22	0.19	#
12 pm (nmol/L)	-0.19 #	0.13	0.09	0.09	0.09	*
6 pm (nmol/L)	-0.31 **	0.10	0.05	0.05	0.04	***
Intercept (within-subject)	-0.08	-0.08	-0.09	-0.19	0.04	
Slope (within-subject)	-0.07	-0.0150	-0.0197	-0.0207	-0.0220	#
Average Cortisol (nmol/L)	-0.37 **	0.20	0.14	0.13	0.12	***
Peak Cortisol (nmol/L)	-0.28 **	0.53	0.36	0.31	0.33	**
Area under curve (AUC)	-0.32 **	-33558.92	-38480.30	-40934.89	-41506.07	**

# $p < 0.10$,

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$

Note. Each measure of salivary cortisol was analyzed in a separate model. All analyses include covariate control for gender, age, race, SES, BMI, family history of high cholesterol, and blood Pb levels. In addition, actual time of sample was included as covariate for analyses of each sample and mean sample time was included for analysis of composite cortisol measures. Log-transformed values were used in all analyses and the original units are reported here for each sample time, peak, and mean.

Table 4

Serum proteins correlations with Factor 1 (at 0.60 or greater) and corresponding associations with Hg and mean cortisol (standardized beta coefficients are reported from multiple regression equations controlling for covariates). Results of the Sobel test for simple mediation (Hg → Mean Cortisol → Protein) are also reported.

Proteins	Factor 1 Loading	Hg	Mean Cortisol	Sobel Test
Complement Factor B	0.89	0.40 ***	−0.34 **	p < 0.05
Ceruloplasmin	0.82	0.38 ***	−0.27 **	ns
Hemopexin	0.76	0.29 **	−0.29 **	p < 0.05
Alpha-1-antichymotrypsin	0.75	0.26 *	−0.30 **	p < 0.05
Apolipoprotein A-II	−0.72	−0.18 #	0.25 *	p < 0.05
Inter-alpha-trypsin inhibitor heavy chain H4	0.71	0.41 ***	−0.23 *	ns
Alpha-1B-glycoprotein	0.68	0.22 *	−0.23 *	ns
Gelsolin	0.66	0.28 **	−0.26 *	p < 0.05
Complement component C6	0.66	0.27 *	−0.16	ns
Carboxypeptidase N subunit 2	0.66	0.34 ***	−0.17	ns
Inter-alpha-trypsin inhibitor heavy chain H1	0.65	0.29 **	−0.27 *	ns
Complement C3	0.64	0.32 ***	−0.38 ***	p < 0.05
Plasma protease C1 inhibitor	0.61	0.30 ***	−0.02	ns
Alpha-2-antiplasmin	0.60	0.14	−0.08	ns
Factor 1 Score	--	0.38 ***	−0.36 ***	p < 0.05

# p < 0.10;

* p < 0.05;

** p < 0.01;

*** p < 0.001;

Notes: Partial correlations controlled for the standard set of covariates (including Pb).