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Mercury Exposure and Antinuclear Antibodies among Females of Reproductive Age in the United States: NHANES

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Abstract

Background: Immune dysregulation associated with mercury has been suggested, though data in the general population are lacking. Chronic exposure to low levels of methylmercury (organic) and inorganic mercury is common, such as through fish consumption and dental amalgams.

Objective: To examine associations between mercury biomarkers and antinuclear antibody (ANA) positivity and titer strength.

Methods: Among females 16-49 years (n=1352) from the National Health and Nutrition Examination Survey (NHANES) 1999-2004, we examined cross-sectional associations between mercury and ANAs (indirect immunofluorescence; cutoff $\geq 1:80$). Three biomarkers of mercury exposure were utilized: hair (available 1999-2000) and total blood (1999-2004) predominantly represented methylmercury, and urinary (1999-2002) inorganic. Survey statistics were used. Multivariable modeling adjusted for several covariates, including age and omega-3 fatty acids.

Results: 16% of females were ANA-positive; 96% of ANA-positives had a nuclear staining pattern of speckled. Mercury geometric means (standard deviations) were: 0.22 (0.03) ppm hair, 0.92 (0.05) $\mu\text{g/L}$ blood, and 0.62 (0.04) $\mu\text{g/L}$ urinary. Hair and blood, but not urinary, mercury were associated with ANA positivity (sample sizes 452, 1352, and 804, respectively), adjusting for confounders: hair odds ratio (OR)=4.10 (95% CI: 1.66, 10.13); blood OR=2.32 (95% CI: 1.07, 5.03) comparing highest versus lowest quantiles. Magnitudes of association were strongest for high-titer ($\geq 1:1280$) ANA: hair OR=11.41 (95% CI: 1.60, 81.23); blood OR=5.93 (95% CI: 1.57, 22.47).

Conclusions: Methylmercury, at low levels generally considered safe, was associated with subclinical autoimmunity among reproductive-age females. Autoantibodies may predate clinical disease by years, thus methylmercury exposure may be relevant to future autoimmune disease risk.

Introduction

Autoimmune disorders, while individually rare, are collectively estimated to afflict 7.6–9.4% of Americans (Cooper et al. 2009) and are among the 10 leading causes of death among women (Thomas et al. 2010; Walsh and Rau 2000). Almost all autoimmune diseases have a strong preponderance among females, with female to male ratios of up to 9:1 and onset often occurring during mid-adulthood (Cooper and Stroehla 2003; Somers et al. 2007; 2014).

Autoimmunity, which can include autoantibody formation, represents a breakdown of tolerance against self-antigens (Lleo et al. 2010). Self-reactive lymphocytes may occur in healthy individuals, and in the absence of related pathology, autoimmunity represents pre- or sub-clinical immune dysregulation. Thus, the term autoimmunity should be distinguished from autoimmune disease, as it does not denote clinical or symptomatic disease. Data are sparse regarding the prognostic significance of preclinical autoimmunity or the “conversion” rate to particular disorders, though autoantibodies may precede autoimmune diagnoses by several years (Arbuckle et al. 2003), and nearly all autoimmune diseases are characterized by circulating autoantibodies (Scofield 2004). Antinuclear antibodies (ANAs) are highly sensitive for a variety of autoimmune conditions, including systemic lupus erythematosus (SLE), scleroderma, and Sjögren’s syndrome. Estimates of ANA prevalence in individuals without autoimmune disease vary widely (1-24%) (Fritzler et al. 1985; Rosenberg et al. 1999) due to differing methodologies and population characteristics. ANA prevalence of approximately 13% has been reported in key studies using a 1:80 titer cutoff (Satoh et al. 2012; Tan et al. 1997) based on immunofluorescence assay, the method recommended by the American College of Rheumatology as the gold standard for ANA testing (Meroni and Schur 2010).

Mercury is a ubiquitous and persistent toxicant with pleiotropic effects, and currently ranked as a top three priority pollutant by the US Agency for Toxic Substances and Disease Registry (ATSDR 2011). Seafood consumption, particularly of large species, is a common source of organic mercury (methylmercury) exposure (Mergler et al. 2007). It is estimated that in the US, each year approximately 8% of mothers and 0.6 million newborns have mercury concentrations exceeding levels considered by regulatory agencies to be safe (Trasande et al. 2005).

Immunotoxic effects, including autoantibody production, have been clearly demonstrated in murine models in response to both organic and inorganic mercury (Pollard et al. 2010). In humans, occupational mercury exposure (predominantly inorganic and elemental species) among miners has been associated with increased risk of autoimmunity (Gardner et al. 2010; Silva et al. 2004), and an increased risk of SLE has been reported among dental professionals (Cooper et al. 2004). However, immune effects associated with low levels of exposure to each type of mercury in the general population are not well characterized (Mergler et al. 2007).

As the biologic effects, sources, and patterns of exposure to organic and inorganic mercury are expected to differ, it is important to examine both species. Biomarkers of mercury exposure in humans include hair mercury, representing predominantly organic (methyl) mercury; total blood mercury, a combination of organic and inorganic mercury; and urinary mercury, a marker predominantly of inorganic/elemental mercury. In the US National Health and Nutrition Examination Survey (NHANES), hair mercury was measured in adult females (16-49 years), but not males.

Utilizing NHANES data, we explored the associations between three types of biomarkers of mercury exposure and the presence, strength, and patterns of antinuclear antibodies in a representative sample of reproductive-age females from the US population.

Methods

Study population

NHANES is conducted by the Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS) (CDC/NCHS 2015b). It uses a stratified, multistage probability cluster design, with oversampling of selected subpopulations, to obtain a representative sample of the civilian, non-institutionalized US population. NHANES protocols were approved by the NCHS Institutional Review Board, and informed consent was obtained. Our study utilizes data from three cycles (1999-2004) of continuous NHANES (CDC/NCHS 2015a). Participation rates were 76% for 1999-2000, 80% for 2001-2002, and 76% for 2003-2004 (CDC/NCHS 2013). The eligible population for our analysis included participants who completed physical examination with biospecimen collection for ANA and mercury assessment, thus females ages 16-49 years.

From a total of 5984 females 16-49 years in NHANES 1999-2004, 1932 were included in the one-third subsample with ANA assessment, of whom 1354 had non-missing ANA data. Hair mercury was available for one cycle (1999-2000), total blood mercury for three (1999-2004), and urinary mercury for two (1999-2002). For hair, blood, and urinary mercury, respectively, samples sizes were 452, 1352, and 804 (after excluding 16, 2 and 29 persons with missing data).

Antinuclear antibodies (ANAs)

As detailed elsewhere (CDC/NCHS 2012), standard methodology for ANA screening was used, involving indirect immunofluorescence with HEp-2 substrate for detection of IgG antibodies to cellular antigens. Titers to which fluorescence remained positive (serial dilution range 1:80-1:1280) and staining patterns were determined for positive specimens. ANA patterns refer to indirect immunofluorescence patterns (*eg*, speckled, nucleolar, homogeneous) reflecting the

anatomic distribution of intracellular antigens, thus different nuclear components. A variety of different antinuclear antibodies can give rise to a given pattern. Follow-up immunoprecipitation was employed for identification of specific antigens from a standard panel.

Mercury exposure assessment

Three types of biomarkers for mercury exposure were utilized: hair (organic), total blood (organic and inorganic), and urinary (predominantly inorganic/elemental). 1-cm hair segments were utilized (approximating exposure during preceding 2.5 months). Standard methodology for mercury measurement was used, as detailed elsewhere (CDC/NCHS 2005, 2007). In brief, cold vapor atomic fluorescence spectrometry following analyte extraction was used for hair. For blood and urine, flow-injection cold vapor atomic absorption spectrometry (PerkinElmer Flow Injection Mercury System-400, Waltham, MA) was used in NHANES 1999-2002, and inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer ELAN 6100, Waltham, MA) in 2003-2004. Limits of detection (LODs) for hair mercury varied by batch and ranged between 0.011-0.027 ppm (method detection limit); 6% of the females in our study had hair mercury levels below the LOD. LODs for total blood mercury varied according to cycle and batch, ranging between 0.14-0.2 $\mu\text{g/L}$. 7.4%, 6.5% and 7.5% of the females in our study had total blood mercury levels below the LOD, for the three cycles, respectively. We did not separately investigate the inorganic fraction of blood mercury, as a large proportion were $< \text{LOD}$ (97.4%, 95.1%, and 77.2% for the three cycles). LOD for urinary mercury was 0.14 $\mu\text{g/L}$, with 13.3% and 14.3% of the participants having urinary mercury levels $< \text{LOD}$ for the two cycles, respectively.

Other variables

Sociodemographic data were collected by self-administered questionnaires. Body mass index (BMI), calculated as weight/height (kg/m^2), was included due to the role of obesity in chronic inflammation. Serum cotinine (ng/mL), a marker of active and passive smoking, was measured by isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry; tobacco exposure has been linked to increased risk autoimmune diseases (Costenbader and Karlson 2006). C-reactive protein (CRP), a non-specific inflammatory marker, was quantified (mg/dL) by latex-enhanced nephelometry. Nutrient data were estimated based on a multiple pass, computer-assisted dietary interview of food and beverage consumption, with recall assessment of individual foods consumed in the previous 24-hours. Data on selenium (mcg), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), all found in seafood, were thus derived; omega-3 fatty acid intake was calculated as (EPA+DHA). Selenium potentially mitigates effects of mercury (Civin-Aralar and Furness 1991), and omega-3s have anti-inflammatory effects (Simopoulos 2002). Among participants who underwent the dietary interview, weekly seafood intake was estimated based on recall of fish/shellfish consumption in the previous 30 days. Serum polychlorinated biphenyls (PCBs) were measured by high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry (HRGC/ID-HRMS) (CDC 2006). A summary measure for coplanar (dioxin-like) polychlorinated biphenyls (cPCBs), which included congeners with suspected immunotoxicity (Wolff et al. 1997), was calculated as the sum of the products of the concentration of each serum lipid-adjusted congener (PCBs 81, 105, 118, 126, 156, 157, 167, 169) and its corresponding 2005 World Health Organization-defined toxic equivalency factor (TEF) (Van den Berg et al. 2006). An alternate PCB summary measure was comprised of the

sum of the lipid-adjusted values for the four most prevalent PCB congeners [$\Sigma(118, 138, 153, 180)$] (Laden et al. 2010); three of these are non-coplanar and without defined TEFs to take into account. To address the potential for drug-induced autoimmunity, we assessed utilization within the past month of four prescription medications that have been implicated in this phenomenon (procainamide, hydralazine, carbamazepine and minocycline) (Schoonen et al. 2010).

Statistical analysis

To account for the complex, stratified, multistage cluster sampling design, analyses were conducted using the survey packages of *Stata* (v.12) and *R* (v.2.11.1) to obtain appropriate estimates and standard errors. ANAs were measured in a one-third subsample, and we constructed and applied weights to our subsample according to NCHS analytic guidelines (Johnson et al. 2013). Values below the LOD for laboratory assays were handled as the LOD divided by the square root of 2. Hair and blood mercury were log-transformed due to their skewed nature, or handled as quantiles based on distributions in the study population. Two-sample t-tests for survey data and the Rao-Scott chi-square test were used for continuous and categorical data, respectively. *P* values <0.05 were considered significant. Crude models included mercury as the independent variable; separate models were performed for each source of mercury (hair, blood, urine). Multivariable logistic regression was utilized to estimate adjusted odds ratios (ORs) for ANA positivity in association with mercury exposure. Model A included age, race/ethnicity, education, serum cotinine, and selenium; an indicator for NHANES cycle was included in models combining data across cycles, to account for potential methodological differences between cycles. Models B and C were further adjusted for omega-3 fatty acids and seafood intake, respectively, which have been suggested to negatively confound mercury health effects (Budtz-Jørgensen et al. 2007; Guallar et al. 2002). Multivariable urinary mercury models

adjusted for urinary creatinine to account for dilution of spot urine specimens. We performed sensitivity analyses adding BMI and CRP in Models A-C as potential confounders for all mercury types. Separate sensitivity analyses were performed including the coplanar and prevalent PCB measures. For urinary mercury, we also conducted models excluding persons with impaired renal function [glomerular filtration rate (GFR) <60 mL/min/1.73m²], to account for potential reverse causation whereby chronic kidney disease may increase urinary mercury excretion. Piecewise continuous models were constructed, and linearity with the log-odds of ANA examined by predicted probability plots with natural cubic splines with four degrees of freedom (three for hair). Multinomial logistic regression was utilized to examine ANA titer strength as the outcome, with negative ANA ($<1:80$) as the base outcome, and low/moderate-titer (1:80-1:640) and high-titer ($\geq 1:1280$) as the other outcome levels.

Results

Participant characteristics

Characteristics of the study population, according to ANA positivity, are summarized in Table 1. Sociodemographics were largely similar for ANA-positive and negative persons, though in the first cycle there was a larger proportion of non-Hispanic whites and Mexican-Americans among ANA-positives ($p=0.08$). In the combined 3-cycle population, education level differed between ANA positive and negative groups, with a higher proportion of ANA-positive adults having less than a high school education ($p=0.04$). Among ANA-positive compared to negative participants, hair and total blood mercury levels in Cycle 1 were higher ($p=0.03$ and $p=0.06$, respectively), and blood mercury in the 3-cycle population was non-significantly higher.

ANAs and mercury

The weighted proportion of participants with ANA positivity was 12% for Cycle 1 and 16% for Cycles 1-3. Among ANA-positives, the speckled pattern was predominant (>94%); Table 1. The geometric means (standard deviations) for mercury corresponding to all participants included in Table 1 for whom data were available were as follows: hair 0.22 (0.03) ppm; total blood 0.92 (0.05) µg/L; urinary 0.62 (0.04) µg/L. Correlations between the sources of mercury in Cycle 1 were as follows: hair and total blood ($r=0.69$; $p<0.01$); hair and urinary ($r=0.34$; $p<0.01$); total blood and urinary ($r=0.41$; $p<0.01$). Among the females in this study, 12.8% had a total blood mercury level above 3.5 µg/L, the reference dose (RfD) extrapolated from the US Environmental Protection Agency cord blood mercury RfD of 5.8 µg/L (Mahaffey et al. 2003; Stern and Smith 2003).

Based on multivariable logistic regression (Table 2), we detected positive and statistically significant associations (confidence intervals > 1) between both hair and total blood mercury and ANA positivity, but not urinary mercury. From the multivariable models incorporating omega-3 fatty acids (Model B), the adjusted OR for ANA positivity comparing females in the highest versus lowest tertile of hair mercury was 4.10 (95% CI: 1.66, 10.13), and for the highest versus lowest quartile of blood mercury was 2.32 (95% CI: 1.07, 5.03).

We performed sensitivity analyses including BMI and CRP as covariates in the logistic regression models for all mercury types, and there were no substantive changes in results. Likewise, in separate sensitivity analyses incorporating each PCB summary measure, the mercury associations with ANA positivity remained similar (Supplemental Material, Table S1). Of the 8 study participants (0.78 weighted percent) reporting utilization of a prescription drug associated with drug-induced autoimmunity (4 carbamazepine, 4 minocycline), none were ANA

positive. In the urinary mercury sensitivity analyses excluding participants with GFR $<60\text{mL}/\text{min}/1.73\text{m}^2$, there were no substantive changes in results.

Spline regression models showed a non-linear dose-response relationship for log-transformed hair and total mercury (Figure 1). To examine mercury as a continuous variable, we fit adjusted piecewise logistic regression models, with cut-points based on visual inspection of the spline graphs. The dose-response relationship for both hair and total blood mercury increased in a statistically significant fashion within the lower ranges of mercury exposure (through -1 ppm log hair mercury and $0\ \mu\text{g}/\text{L}$ log total blood mercury), and then plateaued.

We also evaluated strength of ANA titer as an outcome. For both hair and total blood mercury, compared to the lowest mercury quantile, the upper quantiles contained a substantially higher proportion of individuals with high-titer ANA ($\geq 1:1280$); Figure 2. Consistent with the logistic regression models, results from multinomial logistic regression (Supplemental Material, Table S2) demonstrated a significant association between hair and total blood, but not urinary mercury, and ANA positivity (data for urinary mercury not shown). Further, magnitudes of association were strongest for high-titer ANA ($\geq 1:1280$), where adjusted ORs were >10 for hair mercury and >4 for total blood mercury.

Discussion

In this population-based study, we found that mercury exposure is associated with increased risk of high-titer ANA positivity among reproductive-age females in the general US population. Specifically, this association appears to be driven by organic (methyl) mercury, the predominant species in hair and total blood. Notably, a dose-response relationship was observed for low methylmercury exposure levels (<0.37 ppm hair mercury; $<1\ \mu\text{g}/\text{L}$ total blood mercury), in the

range generally considered safe for women of childbearing potential by regulatory agencies (Mergler et al. 2007). The predominant nuclear staining pattern of speckled found in our population is a marker of autoimmunity with a wide variety of clinical associations, including SLE, mixed connective tissue disease, Sjögren's syndrome, and idiopathic inflammatory myopathies (Klippel et al. 2008). The methylmercury association was robust across models, whereas other suspected risk factors in the multivariable models, including age and smoking, were not found to be associated with ANA risk.

Our findings are compatible with murine data demonstrating development of autoimmunity in response to methylmercury exposure in genetically susceptible strains (Haggqvist et al. 2005; Hultman and Hansson-Georgiadis 1999). Results from human studies have been inconsistent regarding the relationship between organic mercury and autoimmunity. An ecologic study of two Brazilian riverine communities with high fish consumption found suggestion of higher ANA prevalence in the community with higher average hair mercury levels (8 ppm vs 6.4 ppm) (Silva et al. 2004). A further study in a riverine Brazilian community failed to detect an association (Alves et al. 2006); the mean hair mercury level in this study was 34.5 ppm, thus it is possible that a dose-effect between methylmercury and ANA positivity could have been obscured if, as our data suggest, the response plateaus at a low exposure threshold. A study of females ages 12-85 years from one cycle of NHANES (2003-2004) failed to detect a significant association between total blood mercury and ANA positivity, though the non-linear nature of association that we observed was not addressed in their analyses (Gallagher et al. 2013). Further, they did not report the titer for defining ANA positivity, and in their smaller sample (632 compared to 1352 in our blood mercury analyses) statistical power may have been inadequate to detect an association.

Our study focused on females, ages 16-49 years. It is well recognized that females have a higher risk of autoimmune diseases (Cooper et al. 2009; Somers et al. 2007; 2012; 2014), and that risk among females may also correlate with reproductive stage. Moreover, estrogenic hormones may promote autoimmunity (Somers and Richardson 2014). Mercury metabolism may also contrast between sexes, and differences in mercury excretion and distribution have been observed between sexes in mouse models (Hirayama and Yasutake 1986; Hirayama et al. 1987), as well as immunotoxic effects at lower internal doses in females (Nielsen and Hultman 2002).

Oxidative stress has been shown to contribute to the induction of autoimmune phenotypes in animal models, such as through epigenetic mechanisms converting normal “helper” T cells to autoreactive lymphocytes sufficient to cause lupus in the absence of added antigen (Somers and Richardson 2014). Mercury induces oxidative stress through sulphhydryl reactivity and depletion of cellular antioxidants (Ercal et al. 2001). In human T cells treated with methylmercury, reductions in intracellular glutathione (GSH) concentration, glutathione S-transferase activity and mitochondrial transmembrane potential have been observed, followed by generation of reactive oxygen species; intracellular GSH depletion has further been linked to susceptibility of T cells to undergo methylmercury-induced apoptosis (Shenker et al. 1999).

It is unclear why we did not find evidence linking inorganic mercury to ANAs, whereas this species has been more thoroughly linked to autoimmunity in animal models (Vas and Monestier 2008) and industrially-exposed human populations (Cooper et al. 2004; Gardner et al. 2010; Silva et al. 2004). However, the higher doses in such studies limit their generalizability. Indeed, median urinary mercury levels were over 3.7 µg/L (compared to our median of 0.64 µg/L) in a pair of studies in a Brazilian gold-mining population (Gardner et al. 2010; Silva et al. 2004).

Another distinction is that these studies used an ANA titer of $\geq 1:10$ as detectable and a restricted

dilution range (to 1:320); a more robust approach would be to employ higher titration levels to better assess strength of ANA positivity. Mechanisms of and degree of immunotoxicity may differ according to level of inorganic mercury. For instance, mercuric chloride at high concentrations (40 μ M) has been associated with non-apoptotic cell death, rapid cellular permeabilization (Pollard et al. 1997), and modification of the nucleolar antigen fibrillarin from a 34 kDa non-disulfide bonded to a 32 kDa disulfide bonded form. It is conceivable that structurally altered fibrillarin would be more immunogenic than in its native form by unveiling of cryptic epitope(s), and together with cellular necrosis and permeabilization, could be more readily accessible to the immune system. At lower concentrations, fibrillarin migrated at both 32 kDa and the predominant 34 kDa forms, and greater cellular viability was maintained (Pollard et al. 1997). In contrast to inorganic species, organic mercury is lipophilic and more readily crosses cellular membranes, but may demethylate intracellularly to inorganic mercury (Clarkson and Magos 2006), which may ultimately be more immunotoxic. It is plausible that sub-cytotoxic levels of organic mercury, such as those in our study, over long periods might yield higher intracellular doses of inorganic mercury and more efficient access to the nuclear environment than would occur with direct exposure to similarly low levels of inorganic mercury.

In our study, we found that speckled patterns predominated (96% of ANA-positives). A variety of speckled antinuclear antibody patterns can be seen by indirect immunofluorescence. Antigen specificities include U1-SnRNP (small nuclear ribonucleoproteins), Sm (Smith), U2-snRNP, U4/U6-snRNP, SSA/Ro, SSB/La, and other less common antigens (Bradwell et al. 2003). In contrast, the nucleolar pattern has primarily been reported in association with inorganic and methylmercury, with specificity of autoantibody formation to fibrillarin/U3RNP demonstrated in murine models in response to mercuric chloride (Hultman et al. 1989; Reuter et al. 1989). A

proposed mechanism is that inorganic mercury cross-links with free sulfhydryls on two cysteines of fibrillar, resulting in physiochemical protein modification (Pollard et al. 1997). While anti-fibrillar antibody formation is best recognized in response to inorganic mercury, anti-chromatin and anti-histone antibody formation have also been demonstrated (Hultman et al. 1996). For all three types of autoantibodies, the response varies according to mouse strain, underscoring the relevance of genetic susceptibility. Hultman *et al.* demonstrated that antibodies to fibrillar and chromatin tended to persist several months following cessation of mercuric chloride treatment, whereas anti-histone antibodies resolved more rapidly (Hultman et al. 1996). We found only 14 cases with the nucleolar pattern, none of which demonstrated anti-fibrillar antigenicity upon immunoprecipitation. Only three cases had a nuclear homogeneous pattern, which would be compatible with histone or chromatin antigens. The nucleolar pattern, and particularly anti-fibrillar antibodies, in humans are associated with scleroderma, especially among blacks and males (Arnett et al. 1996). The rarity of scleroderma (prevalence ~27.6/100,000 adults) (Mayes et al. 2003) and its associated autoantibodies make it unlikely that our study would have adequate power to detect an association with these specific autoantibodies. However, it is difficult to explain why the speckled pattern was prominent in our study, but not in the animal literature or human occupational studies. Whether organic mercury preferentially targets different nuclear antigens than inorganic, or if an alternate biologic pathway is relevant to low compared to high doses of either species, remains to be elucidated.

Fish consumption is an exposure route in common both to methylmercury and essential nutrients that may have beneficial impact on the immune system. Both organic and inorganic mercury are suggested to increase the production of prostaglandin E2 and phospholipase A2 (Mazerik et al. 2007) leading to release of arachidonic acid. Omega-3 fatty acids are an alternative substrate to

arachidonic acid for cyclooxygenase and lipoxygenase enzymes, and induce a series of anti-inflammatory eicosanoids (Simopoulos 2002). As such, we incorporated omega-3 fatty acids into our modeling given the potential for negative confounding (Choi et al. 2008). There was indeed a modest increase in the magnitudes of association for hair mercury when adjusting for omega-3 intake. PCBs are persistent toxicants with suggested immune effects (Gallagher et al. 2013; Heilmann et al. 2010) and fish consumption as an exposure route. The hair and total blood mercury associations were not appreciably altered with inclusion of PCBs in the models.

Limitations of this study include its cross-sectional nature, precluding the ability to determine the pattern and chronicity of mercury exposure, and persistence of ANA positivity or future risk of overt disease. However, the study of risk factors for preclinical disease is an important tool for dissecting the etiology of complex diseases with long latencies (Cooper 2009). Further, certain combinations of autoimmune diseases tend to co-occur within individuals and families (Cooper et al. 2009; Somers et al. 2006, 2009), to an extent inadequately explained by genetic background. The identification of shared environmental factors for immune dysregulation relevant to a variety of autoimmune phenotypes is an important goal (Dietert et al. 2010). The non-specific nature of the ANA patterns documented in this study supports the premise of organic mercury as a risk factor for multiple autoimmune conditions. Future research is also necessary to evaluate whether our study findings extend to other populations, including males and persons outside of the 16-49 year age range.

Conclusions

We provide evidence for the first time, to our knowledge, that low levels of methylmercury exposure are linked to subclinical autoimmunity, among females of reproductive age in the general population. As autoantibody development is a marker of immune dysregulation and may

predate clinical autoimmune diagnoses by several years, the prospect that organic mercury acts as an early but potentially modifiable trigger relevant to a spectrum of autoimmune conditions warrants more intense investigation.

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Table 1. Participant characteristics according to ANA positivity, among females 16-49 in the general US population (NHANES).

Characteristics	NHANES 1999-2000			NHANES 1999-2004		
	ANA positive ^a (n=56)	ANA negative ^a (n=396)	p-value	ANA positive ^a (n=213)	ANA negative ^a (n=1,139)	p-value
Age (years)	31.7 ± 2.8	32.7 ± 0.7	0.71	33.7 ± 0.02	33.6 ± 0.06	0.14
Race/ethnicity			0.08			0.25
White, non-Hispanic	15 (74.2)	135 (64.1)		85 (71.5)	458 (64.4)	
Black, non-Hispanic	12 (10.7)	73 (10.7)		48 (12.9)	256 (12.9)	
Mexican-American	26 (12.3)	136 (7.3)		63 (8.7)	306 (9.0)	
Other Hispanic	2 (2.6)	32 (9.9)		13 (5.1)	65 (6.5)	
Others	1 (0.2)	20 (8.1)		4 (1.8)	54 (7.1)	
Education			0.28			0.04
<High school	9 (18.1)	74 (14.8)		48 (17.2)	227 (14.7)	
High school grad/GED	15 (34.3)	61 (20.2)		38 (21.9)	198 (22.1)	
College some/grad	16 (43.3)	134 (52.7)		88 (55.8)	446 (55.1)	
Youth (16-19 yrs)	16 (4.3)	127 (12.3)		39 (5.1)	268 (8.2)	
Body mass index (kg/m²)	26.6 ± 1.1	27.3 ± 0.5	0.57	26.6 ± 0.1	27.5 ± 0.2	0.11
Smoking^b			0.77			0.32
Never	27 (60.7)	171 (57.1)		111 (61.2)	529 (55.6)	
Former	7 (17.4)	40 (15.0)		28 (15.1)	130 (15.3)	
Current	5 (21.9)	58 (28.0)		34 (23.8)	212 (29.2)	
Serum cotinine (ng/mL)^c	0.68 ± 0.43	0.75 ± 0.25	0.87	0.32 ± 0.11	0.62 ± 0.11	0.07
C-reactive protein (mg/dL)^c	0.13 ± 0.03	0.18 ± 0.02	0.30	0.14 ± 0.00	0.18 ± 0.02	0.25
<1 mg/dL	51 (84.5)	336 (89.3)	0.54	193 (92.1)	978 (87.4)	0.57
≥1 mg/dL	5 (15.6)	60 (10.7)		20 (7.9)	161 (12.6)	
Selenium, dietary (mcg)^c	87.9 ± 9.2	78.7 ± 4.0	0.30	85.5 ± 2.3	82.8 ± 1.6	0.52
Omega-3, dietary (gm)^c	0.02 ± 0.01	0.03 ± 0.003	0.57	0.02 ± 0.003	0.02 ± .001	0.57
Hair Hg (ppm)^c	0.27 ± 0.04	0.21 ± 0.03	0.03	NA	NA	NA
Total blood Hg (µg/L)^c	1.31 ± 0.17	1.01 ± 0.12	0.06	0.97 ± 0.00	0.91 ± 0.05	0.43
Urinary Hg (µg/L)^c	0.80 ± 0.16	0.65 ± 0.08	0.34	0.67 ± 0.03 ^d	0.62 ± 0.05 ^d	0.56
ANA titer			NA			NA
Negative (≤1:40)	NA	396 (100)		NA	1139 (100)	
1:80	0	NA		0	NA	
1:160	2 (1.2)	NA		3 (0.4)	NA	
1:320	16 (21.3)	NA		42 (17.6)	NA	
1:640	14 (18.1)	NA		64 (32.8)	NA	
≥1:1280	24 (59.5)	NA		104 (49.2)	NA	
ANA pattern^e			NA			NA
Nuclear (all)	52 (98.9)	NA		208 (99.7)	NA	
Speckled	50 (94.4)	NA		203 (96.3)	NA	
Nucleolar	4 (3.1)	NA		14 (5.1)	NA	
Homogeneous	1 (4.4)	NA		3 (2.3)	NA	

Abbreviations: ANA, antinuclear antibody; Hg, mercury; NHANES, National Health and Nutrition Examination Survey; SD, standard deviation.

^aValues are means ± SD or n (%); means and percentages are weighted. ^bSmoking available for ages ≥20 years (n=308 for first cycle; n=1044 for 3-cycles). ^cGeometric mean. ^dUrinary mercury corresponds to NHANES 1999-2002 (unavailable for 2003-2004); n=804. ^eMore than one ANA pattern possible; 3.1% (Cycle 1) and 5.1% (Cycles 1-3) of participants had ≥1 pattern. Three major types of nuclear staining patterns are tabulated; others possible.

Table 2. Association between mercury exposure and ANA positivity among females 16-49 years in the general US population (NHANES).

Mercury exposure	ANA positive n (%) ^a	Crude Model ^b OR (95% CI)	Model A ^c OR (95% CI)	Model B ^d OR (95% CI)	Model C ^e OR (95% CI)
Hair mercury (ppm) ^f					
Tertile 1 (<0.11)	14 (8)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Tertile 2 (0.11-0.27)	22 (12)	1.58 (0.27, 9.33)	2.45 (0.47, 12.82)	2.70 (0.57, 12.80)	2.28 (0.40, 12.94)
Tertile 3 (0.271-5.96)	20 (14)	1.83 (0.54, 6.16)	4.01 (1.57, 10.28)	4.10 (1.66, 10.13)	3.75 (1.06, 13.28)
Total blood mercury (µg/L) ^g					
Quartile 1 (<0.4)	30 (10)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Quartile 2 (0.4-0.8)	71 (19)	2.18 (1.05, 4.52)	2.25 (1.08, 4.68)	2.25 (1.09, 4.66)	2.27 (1.06, 4.83)
Quartile 3 (0.9-1.5)	51 (16)	1.72 (0.82, 3.59)	2.04 (0.94, 4.46)	2.03 (0.95, 4.33)	2.14 (0.89, 5.12)
Quartile 4 (1.6-32.8)	61 (17)	1.84 (0.88, 3.87)	2.33 (1.05, 5.19)	2.32 (1.07, 5.03)	2.51 (1.04, 6.03)
Urinary mercury (µg/L) ^{h,i}					
Quartile 1 (<0.0029)	28 (12)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Quartile 2 (0.0029-0.0063)	30 (10)	0.73 (0.35, 1.53)	0.88 (0.42, 1.87)	0.88 (0.40, 1.94)	0.89 (0.42, 1.88)
Quartile 3 (0.0063-0.0135)	28 (17)	1.30 (0.55, 3.09)	1.36 (0.58, 3.20)	1.36 (0.58, 3.20)	1.40 (0.60, 3.25)
Quartile 4 (0.0137-0.8873)	33 (12)	0.90 (0.41, 1.96)	1.18 (0.49, 2.82)	1.18 (0.49, 2.83)	1.20 (0.50, 2.90)

Abbreviations: ANA, antinuclear antibody; Hg, mercury; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio.

^aWeighted percent. ^bCrude models included mercury as the independent variable; separate models were performed for each source of mercury (hair, blood, urine). ^cAdjusted for age, race/ethnicity, education, serum cotinine, selenium, and indicator for NHANES cycle for multi-cycle models. ^dModel A + further adjusted for omega-3 fatty acids. ^eModel A + further adjusted for total seafood intake. ^fNHANES 1999-2000, 1 cycle (n=452). ^gNHANES 1999-2004, 3 cycles (n=1352). ^hNHANES 1999-2002, 2 cycles (n=804). ⁱIncluded all participants with urinary Hg data, irrespective of availability of hair or blood data; all urinary Hg models (including crude) adjusted for urinary creatinine.

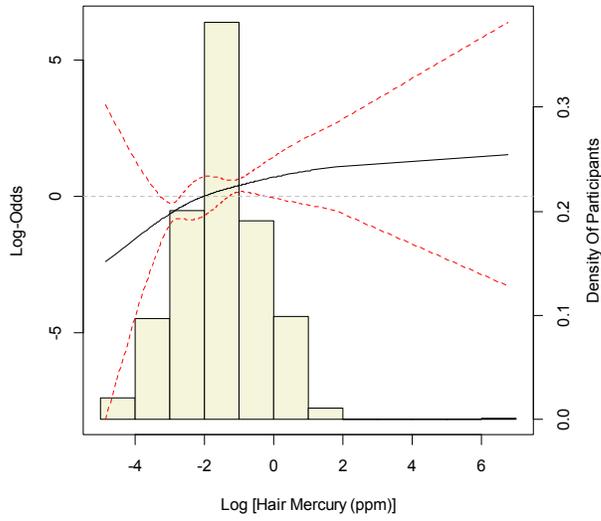
Figure Legends

Figure 1. Associations of ANA positivity with log-transformed hair and total blood mercury, adjusted for Model B covariates. Solid lines represent the smoothing trends estimated from the natural spline with three degrees of freedom (DF) for hair mercury, and four DF for total blood mercury (knots at 25th, 50th, and 75th percentiles). Red dotted lines represent 95% confidence intervals. Bars represent the weighted density distribution for mercury. The dose-response relationship for both hair and total blood mercury increased in a statistically significant fashion within the lower ranges of mercury exposure. (A) Hair mercury (1999-2000), n=452. (B) Total blood mercury (1999-2004), n=1352.

Figure 2. Weighted proportions of ANA positivity and titer categories according to mercury exposure quantiles. (A) Hair mercury (1999-2000), n=452. (B) Total blood mercury (1999-2004), n=1352. (C) Urinary mercury (1999-2002), n=804.

Figure 1

(A) Hair mercury (NHANES 1999-2000), n=452



(B) Total blood mercury (NHANES 1999-2004), n=1352

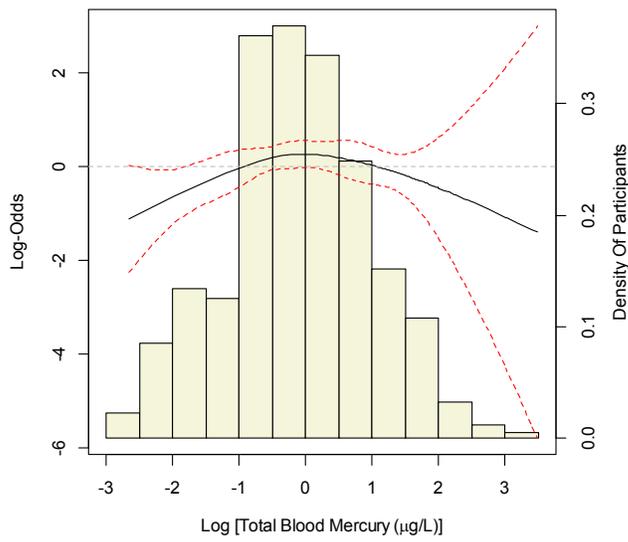
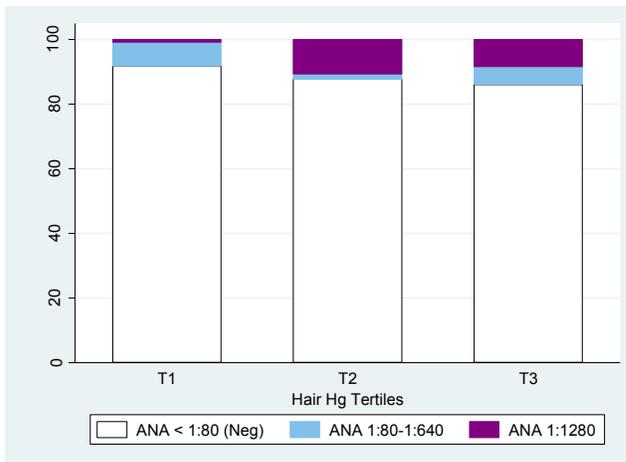
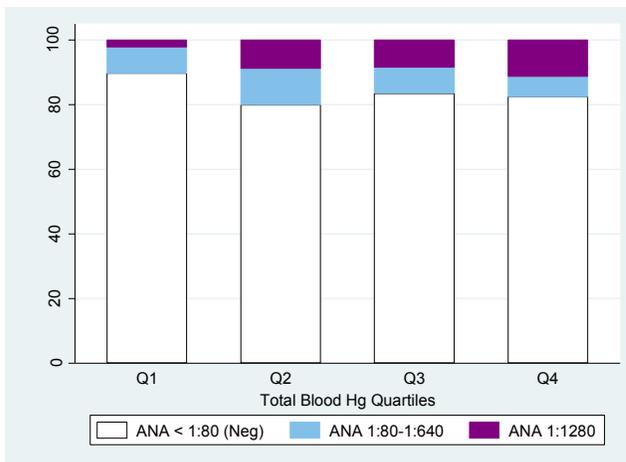


Figure 2.

(A) Hair mercury (NHANES 1999-2000), n=452



(B) Total blood mercury (NHANES 1999-2004), n=1352



(C) Urinary mercury (NHANES 1999-2002), n=804

