Methylmercury and long chain polyunsaturated fatty acids are associated with immune dysregulation in young adults from the Seychelles child development study.

Emeir M. McSorley, Edwin van Wijngaarden, Alison J. Yeates, Toni Spence, Maria S. Mulhern, Donald Harrington, Sally W. Thurston, Tanzy Love, Todd A. Jusko, Philip J. Allsopp, Marie C. Conway, Philip W. Davidson, Gary J. Myers, Gene E. Watson, Conrad F. Shamlaye, J.J. Strain

The Nutrition Innovation Centre for Food and Health (NICHE), School of Biomedical Sciences, Ulster University, Coleraine, Northern Ireland, United Kingdom
The School of Medicine and Dentistry, University of Rochester, NY, United States
The Child Development Centre, Ministry of Health, Mahé, Seychelles

Abstract

Background: Exposure to the environmental toxicant mercury (Hg) has been associated with immune dysregulation, including autoimmune disease, but few human studies have examined methylmercury (MeHg) exposure from fish consumption.

Objectives: We examined associations between MeHg exposure and biological markers of autoimmunity and inflammation while adjusting for long chain polyunsaturated fatty acids (LCPUFA).

Method: At age 19 years, hair total Hg (Y19Hg), LCPUFA status, a panel of 13 antinuclear antibodies (ANA), total serum immunoglobulins (Ig) IgG, IgA, and IgM and serum markers of inflammation (IL-1, IL-2, IL-6, IL-10, Creative protein (CRP), IFN-γ, TNF-α) were measured in the Seychelles Child Development Study (SCDS) Main Cohort (n =497). Multivariable regression models investigated the association between Y19Hg and biomarkers, adjusting for prenatal total hair Hg (MatHg) and other relevant covariates, and with and without adjustment for LCPUFA.

Results: With each 1 ppm increase in Y19Hg (mean 10.23 (SD 6.02) ppm) we observed a 4% increased odds in a positive Combined ANA following adjustment for the n6:n3 LCPUFA ratio (β = 0.036, 95% CI: 0.001, 0.073). IgM was negatively associated with Y19Hg (β=−0.016, 95%CI: 0.016,−0.002) in models adjusted for n-3, n-6 LCPUFA and when separately adjusted for the n-6:n-3 LCPUFA ratio. No associations were observed with MatHg. Total n-3 LCPUFA status was associated with reduced odds of a positive anti-ribonuclear protein (RNP) A. The n-3 LCPUFA were negatively associated with IL-6, IL-10, CRP, IFN-γ, TNF-α and positively with...
TNF-α:IL-10. There were positive associations between the n-6:n-3 ratio and IL-6, IL-10, CRP, IFN-γ, TNF-α and a negative association with TNF-α:IL-10.

**Discussion:** The Y19Hg exposure was associated with higher ANA and lower IgM albeit only following adjustment for the n-3 LCPUFA or the n-6:n-3 LCPUFA ratio. The clinical significance of these findings is unclear, but warrant follow up at an older age to determine any relationship to the onset of autoimmune disease.

**Keywords**
Autoimmunity; Methylmercury; Autoantibody; Cytokine; Immunoglobulin

---

**1. Introduction**

Exposure to the ubiquitous environmental toxicant mercury (Hg) has been associated with immune dysregulation including autoimmune disease (Blossom and Gilbert, 2018). It is proposed that Hg exposure, in combination with genetic predisposition, may result in autoimmune disease development or exacerbation (Silbergeld et al., 2005), albeit nearly all this evidence is derived from experimental animal studies with inorganic Hg exposure and evidence from human studies is lacking (Crowe et al., 2017; Bjørklund et al., 2017). Humans are primarily exposed to organic Hg following consumption of fish, which bio-accumulate organic methylmercury (MeHg) from their environment. If Hg is associated with autoimmune disease in people, it would be a major public health concern as fish are an important source of protein in many populations globally.

Immunotoxic effects of Hg have been observed in murine models where exposure to Hg (either organic or inorganic) results in the expression of autoimmune markers including anti-nuclear antibodies (ANA), anti-nucleolar antibodies (ANoA) and anti-chromatin (ACA); (Crowe et al., 2017; Pollard et al., 2019). In humans, several studies investigating occupational Hg exposure in artisanal gold mining communities have reported elevated titres of ANA and ANoA along with elevated concentrations of inflammatory markers (IL-1β, TNF-α and IFN-γ) (Silva et al., 2004; Alves et al., 2006; Nyland et al., 2011; Motts et al., 2014). Others, however, have observed no association between Hg and biomarkers of immune dysfunction (Barregård et al., 1997; Ellingsen et al., 2000; Alves et al., 2006; Sánchez Rodríguez et al., 2015). Analysis of the U.S. National Health and Nutrition Examination Survey (NHANES) data has identified associations, in women, between higher blood Hg concentrations and ANA positivity (Somers et al., 2015) as well as between Hg and higher concentrations of thyroid autoantibodies (Gallagher and Meliker, 2012). In a high fish consuming cohort from the Amazonian region, MeHg exposure was associated with higher IL-6, IFN-γ, IL-4 and IL-17 cytokine concentrations (Nyland et al., 2011), but other studies have observed no association (Monastero et al., 2017). The majority of research to-date has investigated concurrent Hg exposure with one study reporting an inverse association between prenatal MeHg exposure at 28 weeks gestation and immune markers (McSorley et al., 2018).

Associations between markers of autoimmunity and MeHg exposure in populations with high fish consumption have not been widely investigated. An examination of prenatal and
postnatal MeHg exposure and total serum IgG and IgM concentrations in a fish-eating cohort from the Faroe Islands reported significant associations with postnatal MeHg exposure at age 7 years and both IgG and IgM concentrations (Osuna et al., 2014). Conflicting with this finding, no association was observed between concurrent MeHg and markers of autoimmunity within a seafood consuming population from Long Island, New York (Monastero et al., 2017). Overall, the interpretation of existing research is hampered by differences in sources of MeHg exposure, varying sample size and the presence in some studies of malaria which affects immunity (Sánchez Rodríguez et al., 2015). Thus, large population based studies are required to fully elucidate any potential impact of Hg exposure, particularly that of MeHg from fish consumption, in the development of autoimmune disease (Pollard et al., 2010). Adding to the complexity, fish are a rich source of the long chain polyunsaturated fatty acids (LCPUFA), predominately n-3 LCPUFA, which have anti-inflammatory properties and are associated with a reduction of circulating inflammatory markers (Calder, 2015). Therefore, when investigating immunotoxic effects of MeHg, research should also consider the potential beneficial effects of LCPUFA on immune function.

The fish-eating cohort of 19 year olds from the Seychelles Child Development Study (SCDS) have an average consumption of 7 fish meals per week and a MeHg exposure approximately 10 times the levels in the United States (van Wijngaarden et al., 2017). Using this cohort, we investigated whether MeHg exposure from fish consumption (prenatal and concurrent exposure) was associated with markers of autoimmunity and inflammation. It was hypothesised that both prenatal and concurrent MeHg exposure would be associated with markers of autoimmunity and inflammation and that n-3 LCPUFA would mitigate these associations.

2. Methods

2.1. Study design

The SCDS enrolled 779 pregnant women during 1989–1990 as the ‘Main cohort’ to investigate associations between prenatal MeHg exposure and child neurodevelopment. At the 6-month time point, data for 39 mother-child pairs were excluded owing to mother’s illness during pregnancy, insufficient maternal hair to recapitulate prenatal MeHg exposure, twin births or children born with conditions known to affect neurodevelopment (e.g. prematurity, severe perinatal illness, closed head trauma with loss of consciousness, encephalitis or meningitis). Subsequent exclusions through age 19 years for epilepsy, head trauma or meningitis resulted in the removal of 56 additional participants leaving a total of 684 participants at 19 years of age for analysis.

A total of 530 serum samples from the participants at 19 years were collected and stored at −80°. Data for 497 participants’ immune markers were used in the present study owing to inadequate serum volume for analysis in 29 samples and 4 specimens of immune markers could not be matched with study ID numbers. The study protocol was reviewed and approved by the Seychelles Ethics Board and the Research Subjects Review Board at the University of Rochester.
2.2. Immunology testing

Stored serum samples were thawed and analyzed at Ulster University. Inflammatory cytokines were measured using the electrochemiluminescence based Meso Scale Discovery (MSD) multiplex assay (Meso Scale Diagnostics, LLC.) and included interleukin (IL)-1β, IL-2, IL-6, IL-10, interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α). All cytokines are reported in pg/ml. Inter and intra assay cytokine CVs were IL-1β (28.61%;<28%), IL-2 (30.67%;<24%), IL-6 (25.81%;<23%), IL-10 (23.1%;<33%), IFN-γ (21.87%;<32%) and TNF-α (12.11%;<20%). CRP (mg/L), a marker of acute inflammation, was measured by an ultra-sensitive diagnostic kit (Werfen Ltd, England) using the iLab 650 Clinical Chemistry Analyzer and had an inter-assay CV of 16.37% and an intra assay CV of<12%. Cytokine measurements below the lower limited of detection (LLOD), as determined by the standard curve for each cytokine individually, was assigned a value of LOD/√2 for statistical analysis. Serum immunoglobulin-A (IgA), IgG and IgM (g/L) were measured by ELISA (Thermo Fisher, UK) at the Immunology Laboratory, Royal Victoria Hospital Belfast.

ANA status was screened for using the BioPlex ANA fully automated multiplex system (BioRad, UK) which has good concordance with comparative methods (Sohn & Khan, 2014). Screening of the samples by indirect immunofluorescence characterised the presence or absence of specific antinuclear antibodies (ANA). All samples were analyzed for the quantitative detection of anti-dsDNA and the semi-quantification in antibody of anti-ribosomal P, anti-chromatin, anti-Ro52, anti-Ro60, anti-La, anti-Sm, anti Sm/RNP, anti-RNP A, anti-RNP 68, anti-Scl-70, anti-Jo-1 and anti-centromere B. The ANA screen is reported as negative if the results for all 13 autoantibodies are negative. Conversely, if any of the 13 autoantibodies is positive, we report a positive ANA screen and the Antibody Index (AI) of individual antibodies. The AI is an arbitrary unit defined by the manufacturer when no official standards are available. Anti-dsDNA antibody is calibrated against World Health Organization Wo/80 standard and expressed in terms of IU/mL with values ≥ 4 IU/mL reported as positive. All other antibody results are semi-quantitative, expressed in terms of AI, and values ≥1.0 AI are taken as positive.

2.3. Methylmercury exposure

Prenatal MeHg exposure was determined using maternal hair samples collected either during pregnancy, at delivery, or at the 6-month enrolment (Myers et al., 1995). MatHg was measured as total Hg from these hair samples by cold vapour atomic absorption spectroscopy where the closest centimetre to the scalp represents the most recent exposure of one month. Concurrent postnatal exposure at 19 years of age (Y19Hg) was measured using the same approach in a 1 cm length of each participant’s hair taken at time of testing. All Hg results are presented as MeHg are total Hg (THg) based on the assumption that ~80% of THg in hair is MeHg within the Seychelles population (Cernichiaria et al., 1995).

2.4. Fatty acid analysis

Plasma phospholipids were measured at 19 years as outlined previously (van Wijngaarden et al., 2013). In brief, total lipids were extracted from plasma samples, using a modified method of Folch et al., (1957). A solid phase extraction using an NH2 cartridge system
conditioned with chloroform and followed by a series of solvent elution’s was used to isolate phospholipids. Absolute amounts of LCPUFA were determined using gas chromatography mass spectrometry (GCMS) as described previously (Bonham et al., 2008) and included linoleic acid (LA, C18:2 n-6), α-linolenic acid (ALA, C18:3 n-3), arachidonic acid (AA, C20:4 n-6), eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). Results were presented as mg/mL to indicate physiologic quantities. Total n-6 (mg/ml) was calculated by the addition of LA and AA concentrations, and ALA, EPA and DHA were summed to calculate total n-3 (mg/ml). The n-6:n-3 ratio was calculated.

2.5. Statistical analysis

Descriptive statistics summarised the distributions of MeHg, serum cytokines, immunoglobulins, autoantibodies and covariates. Cytokine and immunoglobulin measurements were log transformed for the linear regression models as these measures were extremely right skewed. Clinically elevated concentrations of individual ANA markers were uncommon, perhaps because of the relatively young age of the 19-year study participants. For 11 of the 13 ANA markers, >95% of the ANA values were below the LLLOD. Therefore, these ANA variables were not analyzed as individual markers. Instead, we designed a combined dichotomous ANA variable called ‘Combined ANA’ that was calculated based on being within or above reference range for one or more of the 13 measured ANA markers. Some 56% of the subjects met this criterion. In addition, because of their larger number of measurable values, Anti-dsDNA and Anti-RNP A were analyzed as both dichotomous (< LOD or LOD+) and categorical (< LOD, LOD-ref, > ref) individual markers.

Associations between serum cytokines, immunoglobulins, autoantibodies and pre- and postnatal MeHg exposure were examined through linear (cytokines and immunoglobulins) and logistic (autoantibodies) regression models. Covariates, known or suspected to be associated with (subclinical) autoimmunity or inflammation, included in the analysis were maternal age, child sex, socioeconomic status (SES) and obesity. Maternal socioeconomic status was measured using the Hollingshead Social Status Index modified for the use of employment codes relevant to the Republic of Seychelles (Davidson et al., 1998; Kobrosly et al., 2011). Models that included MatHg adjusted for maternal age at birth, maternal socioeconomic status (SES) and child sex. Models that included postnatal Y19Hg adjusted for child sex and waist circumference (WC) at 19 years as a proxy for abdominal obesity. Consistent with previous cross-sectional analyses of postnatal exposure in the SCDS (van Wijngaarden et al., 2013), postnatal Hg models also adjusted for prenatal MeHg exposure measured in maternal hair. We additionally controlled for 19-year LCPUFA status in separate models to evaluate the possibility that adverse associations may be missed or underestimated due to uncontrolled confounding by the immunomodulatory effect of LCPUFA. In separate models we also included interactions of child sex with both MatHg and Y19Hg to assess whether males or females are more susceptible to the effects of MeHg exposure. Following analysis, we found no evidence for interactions; therefore, we report results from the corresponding models without this interaction.

All statistical analyses were performed using R, Version 3.5.1. Statistical significance in all analyses was determined using a two-sided approach α = 0.05. Regression model...
assumptions were checked using standard methods (Weisberg, 2005). If violated, we consider transforming the outcome or fitting nonlinear additive models (Hastie and Tibshirani, 1990). Results were evaluated for extreme outliers and unduly influential points. All values presented in tables are log transformed.

3. Results

Year 19 demographic characteristics for participants are displayed in Table 1. Immune markers for a total of 497 mother-child pairs were analyzed, consisting of 268 females and 229 males. Mean (SD) MatHg and Y19Hg was 6.84 (4.55) and 10.23 (6.02) ppm, respectively. An average (SD) of 7.22 (3.66) fish meals per week were consumed by participants who had a mean (SD) n-6:n-3 LCPUFA ratio of 3.81 (1.97).

3.1. MeHg, LCPUFA, and anti-nuclear antibodies

Regression analyses for covariate adjusted associations between MeHg exposure and immunologic markers are presented in Table 2. The Y19Hg was significantly associated with a higher Combined ANA (β = 0.036, 95%; CI: 0.001, 0.073) where for each 1 ppm increase in Y19Hg we observed a 4% increased odds for a positive Combined ANA following adjustment for the n-6:n-3 LCPUFA ratio. MatHg was not significantly associated with a change in odds for a positive Combined ANA. Separately, a significant association was observed between the anti-RNP A and n-3 LCPUFA (Table 3). No significant association was found between n-6 LCPUFA and ANA or any immune marker.

3.2. MeHg, LCPUFA, and immunoglobulins (Ig)

Y19Hg was negatively associated with IgM in the models adjusted for n-3 LCPUFA (β = −0.009, 95%CI: 0.016, −0.002) and when separately adjusted for the n6:n3 LCPUFA ratio (β = −0.009, 95%CI: 0.016, −0.001) (Table 2). MatHg was not associated with any Ig. None of the MeHg metrics were associated with IgA. No significant associations were found with n-3 LCPUFA, n-6 LCPUFA and the n6:n3 LCPUFA ratio and IgG.

3.3. MeHg, LCPUFA, and cytokines and CRP

Significant positive associations were observed between Y19Hg and CRP in the models that adjusted for n-3 and n-6 LCPUFA (β = 0.031, 95%CI:0.007, 0.054) and the n-3:n-6 ratio (β = 0.031, 95%CI:0.007, 0.054) (Table 2). Y19Hg was significantly associated with IL-10 in the model that adjusted for n-3 and n-6 LCPUFA (β = 0.016, 95%CI:0.002, 0.030) and in the model which adjusted for the n-3:n-6 ratio (β = 0.016, 95%CI:0.002, 0.031). A statistically significant association was observed between Y19Hg and the TNFa:IL-10 ratio in the model adjusted for n-3 and n-6 LCPUFA (β=−0.016, 95%CI: 0.027,—0.005) and in the model which adjusted for the n-3:n-6 LCPUFA ratio (β = −0.016, 95%CI: 0.027, −0.005). MatHg was not associated with any of the measured cytokines or CRP.

There were significant negative associations between n-3 LCPUFA and CRP, IFN-γ, TNF-α, IL-6 and IL-10 (Table 3). A positive association was observed between n-3 LCPUFA and the TNFa:IL-10 ratio. Statistically significant positive associations were found between the n-6:n-3 ratio and CRP, IFN-γ, TNF-α, IL-6 and IL-10 and a significant negative association...
with the TNFα:IL-10 ratio. No significant associations were found between n-6 PUFA and any of the cytokines.

3.4. Covariates

Females had significantly greater levels of Combined ANA, anti-dsDNA, CRP and IgM in all models. Female had significantly lower levels of TNF-α in all models. Higher levels of maternal SES was significantly associated with increased IgA concentrations without adjustment for LCPUFA ($\beta = 0.003, p = 0.006$). Larger values of Year 19 WC was significantly associated with positive IL-6 concentrations ($\beta = 0.022, p<0.0001$) and positive CRP concentrations ($\beta = 0.047, p<0.0001$) in all models.

4. Discussion

Evidence from animal models suggests a potential link between Hg exposure and the pathogenesis of autoimmune disease. Less, however, is known in human populations especially with respect to MeHg exposure through fish consumption. In this high fish-eating population from the SCDS, we found that current MeHg exposure at 19-year of age was associated with a novel measure of ANA (Combined ANA) but only following adjustment for the n-6:n-3 LCPUFA ratio. Furthermore, Y19Hg was associated with lower IgM, higher CRP, higher IL10 and a lower TNFα:IL10 ratio. Prenatal Hg exposure was not associated with any specific marker of ANA, cytokines or Ig. Although current MeHg exposure at 19 years was associated with higher odds of having a higher ANA combined score the clinical significance of these findings is unclear and further research is warranted to determine if these associations precede autoimmune disease development. Total n-3 LCPUFA was associated with lower anti-RNP A, ANA and overall a more anti-inflammatory profile supporting the well-known benefits of n-3 LCPUFA in regulating the immune system (Calder, 2015).

In this cohort, hair Hg concentrations were on average 10 times those reported from the USA (Davidson et al., 1998; Myers et al., 2003; van Wijngaarden et al., 2017). At 19 years, this cohort reported an average consumption of 7 fish meals per week which correlated with Hg supporting the evidence that fish consumption is a significant predictor of MeHg exposure in humans (Schober et al., 2003; Clark et al., 2007; Bjermo et al., 2013; Somers et al., 2015). The higher exposure of Hg in the 19 year olds may, in part, explain why the associations between MeHg and ANA were only evident at this time point following adjustment for the n6:n3 LCPUFA ratio. It is also plausible that the measure of MeHg exposure at 19 years reflects better than MatHg exposure what is happening systemically in the blood sample taken at the same timepoint.

Previous research by our group has emphasised the importance of n-3 LCPUFA, obtained primarily in the diet through fish consumption, in mitigating any potential effects of MeHg (Strain et al., 2008, 2015). The National Health and Nutrition Examination Survey (NHANES) investigated young females with hair Hg of 0.22 ppm and blood Hg of 0.944 μg/L and, similar to our results, reported associations between ANA positivity and a high titre of ANA positively in models which also adjusted for n-3 LCPUFA (Somers et al., 2015). Associations between Hg exposure and ANA have been reported in gold mining
communities (Alves et al., 2006, Gardner et al., 2010) and to a lesser extent in a high fish-eating riverine community in Amazonian Brazil (Silva et al., 2004, Nyland et al., 2011). Within these studies, malaria infections are suggested to add to the strength of the association between Hg exposure and ANA. Furthermore, exposure to Hg is associated with dysregulation of inflammatory cytokines and cellular oxidative stress proteins which authors suggest contributes to the immunotoxicity of Hg (Motts et al., 2014). Contrary to these findings, analysis in an American Sioux Tribe, who regularly consume fish from a river known to be ubiquitously contaminated with Hg showed that some 30% of individuals were positive for ANA, however there was no overall association between blood Hg values and ANA (Ong et al., 2014). Within their analysis these workers did not adjust for n-3 LCPUFA which may have negatively confounded any associations with Hg (Budtz-Jørgensen et al., 2007). Similarity, in a gold mining population in the Andes, Columbia, no difference in ANA status was observed between those exposed to Hg compared to those not exposed (Sánchez-Rodríguez et al., 2015). Whilst they did adjust for estimated Hg intake from fish consumption it would have been interesting to see if adjustment for biological status of n-3 LCPUFA would have revealed an association given that there was a higher consumption of fish in those exposed to Hg compared to the non-exposed group. A pilot study of a fish-eating cohort from Long Island, USA also found no association between Hg and the expression of genes known to be involved in autoimmunity; however, they reported, owing to small sample size, that they did not control for n-3 LCPUFA in the analysis. Taken together, those studies that have investigated MeHg exposure from fish consumption, and have not adjusted for n-3 LCPUFA in the statistical models, have found no associations with markers of autoimmunity whereas those that have controlled for n-3 LCPUFA have reported associations, albeit the magnitude of effect appears to be related to the source of Hg (gold mining or from fish consumption) as well as other confounders including infections. The Seychelles population is not affected by malaria and has a primary route of MeHg exposure through fish consumption; therefore, the findings reported here support the existing literature that the benefits of LCPUFA from fish consumption outweigh any adverse effect of MeHg on health outcomes including autoimmunity.

The focus of this paper is MeHg exposure through fish consumption. Nevertheless, it is important to recognise that research to date, in murine models, has shown a stronger association between inorganic Hg exposure and the development of autoimmune type responses (Crowe et al., 2018). Differences in intracellular diffusion and biodistribution of the two forms of Hg may explain why inorganic Hg is more strongly associated with the development of autoimmunity (Bjørklund et al., 2017; Pollard et al., 2019). MeHg is proposed to have a delayed and less inflammatory response without the development of immune complexes whereas inorganic Hg is associated with renal damage (Crowe et al., 2017).

The exact mechanisms involved in Hg-associated autoimmunity remains elusive with some suggestions that Hg may contribute to the stimulation and survival of autoreactive immune cells due to its ability to disrupt self-antigen presentation, functional B-cell signalling, effective class switching and the deletion of autoreactive immune cell clones (Crowe et al., 2017; Khan and Wang, 2018; Pollard et al, 2018). Hg may also contribute to the stimulation of an autoimmune response due to its ability to simulate the innate and adaptive

\[\text{Environ Res. Author manuscript; available in PMC 2021 April 01.}\]
response (Pollard et al., 2018). One potential mechanism by which Hg exposure could lead to autoimmunity is through Hg induced tissue damage resulting in the release of damage associated molecular patterns (DAMPs) and/or modified DAMPs with the subsequent activation of a local innate immune response alongside the activation of autoreactive B & T cells in the lymph node to illicit an autoimmune adaptive response (Pollard et al., 2018). Hg may also contribute to autoreactive B cell clone survival as it has been shown to disrupt B cell receptor signalling in immature B cells by targeting the tyrosine kinase protein, Lyn (Gill et al., 2017). Furthermore, disruption of BCR signalling mechanisms in immature B cells may disrupt negative selection of self-reactive clones and Ig class switching resulting in the loss of self-tolerance and the production of autoreactive B cells (Gill et al., 2017).

In the current study, higher Y19Hg was significantly associated with lower IgM which may suggest an alteration in class switching. IgM is the first antibody to respond to an antigen or self-antigen and is involved in enhanced antibody response and activation of the complement cascade resulting in the inflammatory response. IgM plays a regulatory role in subsequent immune response development, thereby accelerating the production of high-affinity IgG. Lower IgM has been associated with decreased T helper activity, increased isotype-specific suppressor T cell activity, and intrinsic B cell defects (Louis and Gupta, 2014). The association between lower IgM and higher Hg in this study could be indicative of a dysfunctional B cell activity frequently reported with exposure to Hg. Reduced concentrations of IgM have been associated with clinical disorders, including autoimmune diseases such as celiac disease and systemic lupus erythematosus (Manson et al., 2005; Yel et al., 2009). Class switching can be influenced by the cytokines available in the inflammatory milieu of the B-cell and the noted increased IL-10 in the Y19Hg cohort could potentially contribute to increased class switching from IgM to other Ig subtypes (Tangye et al. 2002). Y19Hg was found to be significantly associated with higher CRP, IL-10 and a lower TNFα:IL-10. In normal healthy adults, such as this cohort, liver production of the acute phase protein CRP forms part of the innate immune response, for example Hg induced DAMPs (Pollard et al., 2018), which would be accompanied by a regulatory anti-inflammatory response via IL-10 induction and thereby suppressing TNF-α. IL-10 is associated with non-cell mediated immunity where IL-10 functions as a potent B cell stimulator that enhances activation, proliferation, and differentiation of B cells and may have a role in autoimmune disease through opposing the cellular mediated inflammatory Th17 response (Jörg et al., 2016). There is some evidence indicating that the anti-inflammatory actions of IL-10 are defective in autoimmune conditions with a noted increased IL-10 concentrations alongside reduced IL-10 receptor expression (Tournoy et al., 2000; Wang et al., 2017). In autoimmunity, elevated IL-10 has the potential to result in the persistent activation of autoreactive B cells and therefore exacerbate autoimmune disease where the normal immunoregulatory function of IL-10 is defective (Peng et al., 2013).

A recent examination of the Nutrition Cohort 2 (NC2) from the SCDS found that increasing MeHg was associated with decreasing Th1:Th2 (McSorley et al., 2018). Hg modulation of cytokine and antibody responses may affect an individual’s susceptibility to autoimmune type disease and also significantly alter host-pathogen interactions increasing susceptibility to infectious disease (Gardner et al., 2010). Interestingly, at 19 years the n-3 LCPUFA were found to be associated with decreased CRP, INF-γ, TNFα, IL6, IL10 and with a

Environ Res. Author manuscript; available in PMC 2021 April 01.
higher TNFα:IL-10 ratio suggesting a regulatory effect on the immune system. Therefore, it is speculated where individuals are exposed to MeHg from fish consumption, the co-consumption of n-3 LCPUFA will prevent chronic inflammation and associated disease. A large observation study in Italy also reported that lower n-3 PUFA was associated with higher CRP and that higher n-3 PUFA was associated with lower IL-6, TNF-α and CRP (Ferrucci et al., 2006). These results are supported by previous studies that have shown n-3 LCPUFA to have anti-inflammatory properties associated with reduced biomarkers of inflammation (Rangel-Huerta et al., 2012; Pischon et al., 2003; Ferrucci et al., 2006; Calder, 2015). Furthermore, interventions with n-3 LCPUFA have been shown to reduce disease activity and disease progression in a number of inflammatory conditions including autoimmune disease (Miles and Calder, 2012, Calder, 2013).

A strength of this research is the sizeable cohort who are high consumers of fish, have a wide range of hair MeHg (0.54–52.08 ppm) and a good status of n-3 LCPUFA as indicated by the low n6:n3 ratio. This longitudinal cohort has a low dropout rate and is well characterised across numerous timepoints including prenatally. The methods used in this study to analyse MeHg exposure, PUFA status and markers of autoimmunity are considered to be highly sensitive in order to give the most precise results. Limitations of this study include its cross-sectional analysis and like all observational epidemiological studies no cause and effect can be determined. The analyses of this study focused on MeHg exposure and within the Seychelles it is believed that some 80% of hair THg is MeHg (Davidson et al., 2004); however, this may vary among other populations (Ou et al., 2014). Future studies should consider speciation of hair Hg or the use of Hg isotope ratios in hair in addition to total Hg concentrations to better assess exposure from fish derived MeHg (Sherman et al., 2015). Genetic differences within individuals with respect to susceptibility to mercury-induced immune dysfunction (Gardner et al., 2010) may explain differences in ANA concentrations within this and other cohorts. It is also important to remember that although ANA are used in the diagnosis and management of autoimmune disease their identification is not always associated with clinical disease and interpretation must be cautious. It would be important to also consider additional ANAs which have been previously linked with Hg exposure such as anti-fibrillarin autoantibodies and anti-glomerular basement membrane (Yang et al., 2001). Furthermore, blood samples were stored for circa 10 years before analyses which could affect cytokine measurements (Zhou et al., 2010) and in part may explain the large number below the LOD and thereby reducing the percentage of positivity.

In summary, MeHg exposure at 19 years was associated with higher ANA and lower IgM but only following adjustment for LCPUFA which may suggest immune dysregulation. Total n-3 LCPUFA was associated with lower markers of inflammation. This study has global relevance given the importance of fish consumption as a source of protein and nutrition and that the global consumption of fish has reached an all-time high (FAO, 2018). Nevertheless, the clinical significance of these findings is unclear and further research is warranted to determine if these associations precede autoimmune disease development.
Acknowledgments

We acknowledge with thanks the contribution of the nursing and laboratory teams in Seychelles and Mr David Callaghan (MSc Human Nutrition).

Funding sources

This research was supported by grants R01-ES008442, R03-ES027514, and P30-ES01247, from the United States National Institute of Environmental Health Sciences, National Institutes of Health) and inkind by the Government of the Republic of Seychelles. The study sponsors had no role in the design, collection, analysis, or interpretation of the data; in the writing of the report; or in the decision to submit the article for publication. The authors declare they have no conflicts of interest.

References

Alves MFA, Fraiji NA, Barbosa AC, De Lima DS, Souza JR, Dórea JG, Cordeiro GW, 2006. Fish consumption, mercury exposure and serum antinuclear antibody in Amazonians. Int. J. Environ. Health Res 16 (4), 255–262. [PubMed: 16854670]

Barregård L, Eneström S, Ljunghusen O, Wieslander J, Hultman P, 1997. A study of autoantibodies and circulating immune complexes in mercury-exposed chloralkali workers. Int. Arch. Occup. Environ. Health 70 (2), 101–106. [PubMed: 9253638]

Bjermo H, Sand S, Nilsén C, Lundh T, Enghardt Barbieri H, Pearson M, Lindroos AK, Jönsson BA, Barregård L, Dannerud PO, 2013. Lead, mercury, and cadmium in blood and their relation to diet among Swedish adults. Food Chem. Toxicol 57:161–169. 10.1016/j.fct.2013.03.024. Epub 2013 Mar 26. [PubMed: 23537601]

Bjørklund G, Dadar M, Mutter J, Aaseth J, 2017. The toxicology of mercury: Current research and emerging trends. Environ. Res 159, 545–554. 10.1016/j.envres.2017.08.051. Epub 2017 Sep 8. Review. [PubMed: 28889024]

Blossom SJ, Gilbert KM, 2018. Epigenetic underpinnings of developmental immunotoxicity and autoimmune disease. Curr. Opin. Toxicol 8. 10, 23–30. 10.1016/j.cotox.2017.11.013. Epub 2017 Dec 1. [PubMed: 30613805]

Bonham MP, Duffy EM, Wallace JM, Robson PJ, Myers GJ, Davidson PW, Clarkson TW, Shalmyay CF, Strain JJ, 2008. Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study). Prostaglandins Leukot. Essent. Fatty. Acids 78 (6), 343–350. 10.1016/j.plefa.2008.04.005. Epub 2008 Jun 26. [PubMed: 18585023]

Budtz-Jørgensen E, Grandjean P, Weihe P, 2007. Separation of risks and benefits of seafood intake. Environ. Health Perspect 115 (3), 323–327. Epub 2006 Dec 14. [PubMed: 17431478]

Calder PC, 2013. n-3 fatty acids, inflammation and immunity: new mechanisms to explain old actions. Proc. Nutr. Soc 72 (3), 326–336. 10.1017/S0029665513001031. Epub 2013 May 14. Review. [PubMed: 23668691]

Calder PC, 2015. Functional Roles of Fatty Acids and Their Effects on Human Health. JPEN J Parenter Enteral Nutr 39 (3), 18S–32S. 10.1177/0148607115595980.. [PubMed: 26177664]

Cernichiari E, Toribara TY, Liang L, Marsh DO, Berlin MW, Myers GJ, Cox C, Shalmyay CF, Choisy O, Davidson P, et al., 1995. The biological monitoring of mercury in the Seychelles study. Neurotoxicology (Little Rock) 16 (4), 613–628.

Clark NA, Teschke K, Rideout K, Copes R, 2007. Trace element levels in adults from the west coast of Canada and associations with age, gender, diet, activities, and levels of other trace elements. Chemosphere 70 (1), 155–164 Epub 2007 Aug 20. [PubMed: 17707880]

Crowe W, Allsopp PJ, Nyland JF, Magee PJ, Strain JJ, Doherty LC, Watson GE, Ball E, Riddell C, Armstrong DJ, Penta K, Todd JJ, Spence T, McSorley EM, 2018. Inflammatory response following in vitro exposure to methylmercury with and without n-3 long chain polyunsaturated fatty acids in peripheral blood mononuclear cells from systemic lupus erythematosus patients compared to healthy controls. Toxicol In Vitro 10 (52), 272–278. 10.1016/j.tiv.2018.05.008. [PubMed: 29778720]
Crowe W, Allsopp PJ, Watson GE, Magee PJ, Strain JJ, Armstrong DJ, Ball E, McSorley EM, 2017. Mercury as an environmental stimulus in the development of autoimmunity - A systematic review. Autoimmun Rev. 16 (1), 72–80. 10.1111/sms.12785. [PubMed: 27666813]

Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, Berlin M, Clarkson TW, 1998. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. J. Am. Med. Assoc 280 (8), 701–707.

Davidson PW, Myers GJ, Weiss B, 2004. Mercury exposure and child development outcomes. Pediatrics 113 (4 Suppl. 1), 1023–1029 submitted for publication. [PubMed: 15060195]

Ellingsen DG, Efskind J, Berg KJ, Gaarder PI, Thomassen Y, 2000. Renal and immunologic markers for chloralkali workers with low exposure to mercury vapor. Scand. J. Work Environ. Health 26 (5), 427–435. [PubMed: 11103842]

FAO, 2018. The State of World fisheries and aquaculture. Available from: http://www.fao.org/3/i9540EN/i9540en.pdf.

Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, Martin A, Andres-Lacueva C, Senin U, Guralnik J, 2006. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J. Clin. Endocrinol. Metab 91 (2), 439–446. [PubMed: 16234304]

Folch J, Lees M, Sloane Stanley GH, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem 226 (1), 497–509. [PubMed: 13428781]

Gallagher CM, Meliker JR, 2012. Mercury and thyroid autoantibodies in US women, NHANES 2007–2008. Environ. Int 40, 39–43. [PubMed: 22280926]

Gardner RM, Nyland JF, Silva IA, Ventura AM, de Souza JM, Silbergeld EK, 2010. Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in Amazonian Brazil: a cross-sectional study. Environ. Res 110 (4), 345–354. [PubMed: 20176347]

Gill R, McCabe MJ Jr., Rosenspire AJ, 2017. Low level exposure to inorganic mercury interferes with B cell receptor signaling in transitional type 1 B cells. Toxicol. Appl. Pharmacol, 330, 22–29. 10.1016/j.taap.2017.06.022. 2017. [PubMed: 28686464]

Hastie TJ, Tibshirani RJ, 1990. Generalized Additive Models. Monographs on Statistics and Applied Probability, first ed. 9780412343902 CAT# C4390.

Jörg S, Grohme DA, Erzler M, Binsfeld M, Haghikia A, Müller DN, Linker RA, Kleinewietfeld M, 2016. Environmental factors in autoimmune diseases and their role in multiple sclerosis. Cell. Mol. Life Sci 73 (24), 4611–4622 Epub 2016 Aug 4. Review. [PubMed: 27491297]

Khan MF, Wang G, 2018. Environmental agents, oxidative stress and autoimmunity. 2018 2. Curr. Opin. Toxicol 7, 22–27 10.1016/j.cotox.2017.10.012. Epub 2017 Oct 26. [PubMed: 29532040]

Kobrosly RW, van Wijngaarden E, Galea S, Cory-Slechta DA, Love T, Hong C, Shamlaye CF, Davidson PW, 2011. Socioeconomic position and cognitive function in the Seychelles: a life course analysis. Neuroepidemiology 36 (3), 162–168. 10.1159/000325779. [PubMed: 21508650]

Louis AG, Gupta S, 2014. Primary selective IgM deficiency: an ignored immunodeficiency. Clin. Rev. Allergy Immunol 4. 46 (2), 104–111. 10.1007/s12016-013-8375-x. submitted for publication. [PubMed: 23760686]

Manson J, Mauri C, Ehrenstein M, 2005. Natural serum IgM maintains immunological homeostasis and prevents autoimmunity. In: Springer Seminars in Immunopathology, vol. 26 pp. 425–432 4. [PubMed: 15611856]

McSorley EM, Yeates AJ, Mulhern MS, van Wijngaarden E, Grzesik K, Thurston SW, Spence T, Crowe W, Davidson PW, Zareba G, Myers GJ, Watson GE, Shamlaye CF, Strain JJ, 2018. Associations of maternal immune response with MeHg exposure at 28 weeks’ gestation in the Seychelles Child Development Study. Am. J. Reprod. Immunol 80 (5), e13046. 10.1111/aji.13046. Epub 2018 Sep 17. [PubMed: 30295973]

Miles EA, Calder PC, 2012. Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. Br. J. Nutr 107 (Suppl 2), S171–S184. 10.1017/S0007114512001560. Review. [PubMed: 22591891]

Monastero RN, Karimi R, Nyland JF, Harrington J, Levine K, Meliker JR, 2017. Mercury exposure, serum antinuclear antibodies, and serum cytokine levels in the Long Island Study of Seafood
Consumption: a cross-sectional study in NY, USA. Environ. Res 156, 334–340. [PubMed: 28390301]

Motts JA, Shirley DL, Silbergeld EK, Nyland JF, 2014. Novel biomarkers of mercury-induced autoimmune dysfunction: a cross-sectional study in Amazonian Brazil. Environ. Res 132, 12–18. 10.1016/j.envres.2014.03.024. Epub 2014 Apr 16. [PubMed: 24742722]

Myers GJ, Marsh DO, Davidson PW, Cox C, Shamlaye CF, Tanner M, Choi A, Cernichiari E, Choisy O, Clarkson TW, 1995. Main neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet: outcome at six months. Neurotoxicology. 16 (4), 653–664. [PubMed: 8714870]

Nyland JF, Fillion M, Barbosa F Jr., Shirley DL, Chine C, Lemire M, Mergler D, Silbergeld EK, 2011. Biomarkers of methylmercury exposure immunotoxicity among fish consumers in Amazonian Brazil. Environ. Health Perspect 119 (12), 1733. [PubMed: 21868305]

Osuna C, Grandjean P, Weihe P, El-Fawal H, 2014. Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicol. Sci 142 (1). 158–166. [PubMed: 25124724]

Pollard KM, Hultman P, Kono DH, 2010. Toxicology of autoimmune diseases. Chem. Res. Toxicol 15 (3), 455–466. 10.1021/tr9003787. Review.

Pollard KM, Christy JM, Cauvi DM, Kono DH, 2018. Environmental xenobiotic exposure and autoimmunity Curr. Opin. Toxicol 10, 15–22 [PubMed: 29503968]

Pollard KM, Cauvi DM, Toomey CB, Hultman P, Kono DH, 2019. Mercury-induced inflammation and autoimmunity. Biochim. Biophys. Acta Gen. Subj 10.1016/j.bbagen.2019.02.001. ([Epub ahead of print] Review).

Rangel-Huerta O, Aguiler a C, Mesa M, Gil A, 2012. Omega-3 long-chain polyunsaturated fatty acids supplementation on inflammatory biomakers: a systematic review of randomised clinical trials. Br. J. Nutr 107 (S2), S159–S170. [PubMed: 22591890]

Sánchez Rodríguez LH, Flórez-Vargas O, Rodríguez-Villamizar LA, Vargas Fiallo Y, Stashenko EE, Ramírez G, 2015. Lack of autoantibody induction by mercury exposure in artisanal gold mining settings in Colombia: findings and a review of the epidemiology literature. J. Immunotoxicol 12 (4), 368–375. [PubMed: 25477026]

Schober SE, Sinks TH, Jones RL, Bolger PM, McDowell M, Osterloh J, Garrett ES, Canady RA, Dillon CF, Sun Y, Joseph CB, Mahaffey KR, 2003. Blood mercury levels in US children and women of childbearing age, 1999–2000. J. Am. Med. Assoc 289 (13), 1667–1674.

Sherman LS, Blum JD, Basu N, Rajae M, Evers DC, Buck DG, Petrik J, DiGangi J, 2015. Assessment of mercury exposure among small-scale gold miners using mercury stable isotopes. Environ. Res 137, 226–234. 10.1016/j.envres.2014.12.021. Epub 2015 Jan 8. [PubMed: 25577187]

Sohn KY, Khan WI, 2014. ANA Testing. Available from: https://www.aacc.org/publications/cln/articles/2014/june/ana-testing, Access date: July 2019.
Silbergeld EK, Silva IA, Nyland JF, 2005. Mercury and autoimmunity: implications for occupational and environmental health. Toxicol. Appl. Pharmacol 207 (2 Suppl. 1), 282–292 submitted for publication. [PubMed: 16023690]

Silva IA, Nyland JF, Gorman A, Perisse A, Ventura AM, Santos EC, Souza JM, Burek CL, Rose NR, Silbergeld EK, 2004. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in Amazon populations in Brazil: a cross-sectional study. Environ. Health 2 (1), 11 3.

Somers EC, Ganser MA, Warren JS, Basu N, Wang L, Zick SM, Park SK. 2015. Mercury Exposure and Antinuclear Antibodies among Females of Reproductive Age in the United States: NHANES. Environ. Health Perspect 123 (8), 792–798. 10.1289/ehp.1408751. Epub 2015 Feb 10. [PubMed: 25665152]

Strain JJ, Davidson PW, Bonham MP, Duffy EM, Stokes-Riner A, Thurston SW, Wallace JM, Robson PJ, Shamlaye CF, Georgea LA, Sloane-Reeves J, Cernichiarie E, Canfield RL, Coox C, Huang LS, Marcieras J, Myers GJ, Clarkson TW, 2008. Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. Neurotoxicology (Little Rock) 29 (5), 776–782. 10.1016/j.neuro.

Strain JJ, Yeates AJ, van Wijngaarden E, Thurston SW, Mulhern MS, McSorley EM, Watson GE, Love TM, Smith TH, Yost K, Harrington D, Shamlaye CF, Henderson J, Myers GJ, Davidson PW, 2015. Prenatal exposure to methyl mercury from fish consumption and polyunsaturated fatty acids: associations with child development at 20 mo of age in an observational study in the Republic of Seychelles. Am. J. Clin. Nutr 101 (3), 530–537. 10.3945/ajcn.114.100503. Epub 2015 Jan 21. [PubMed: 25733638]

Tangye SG1, Ferguson A, Avery DT, Ma CS, Hodgkin PD, 2002. 10 15. Isotype switching by human B cells is division-associated and regulated by cytokines. J. Immunol 169 (8), 4298–4306. 10.4049/jimmunol.169.8.4298. [PubMed: 12370361]

Tournoy KG, Kips JC, Pauwels RA, 2000. Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness. Clin Exp Allergy 30 (30), 775–783. 10.1046/j.1365-2222.2000.00838.x. [PubMed: 10848896]

van Wijngaarden E, Thurston SW, Myers GJ, Harrington D, Cory-Slechta DA, Strain JJ, Watson GE, Zareba G, Love T, Henderson J, Shamlaye CF, Davidson PW, 2017. Methyl mercury exposure and neurodevelopmental outcomes in the Seychelles Child Development Study Main cohort at age 22 and 24 years. Neurotoxicol. Teratol 59, 35–42. 10.1016/j.ntt.2016.10.011. Epub 2016 Oct 28. [PubMed: 27989696]

van Wijngaarden E, Thurston SW, Myers GJ, Strain JJ, Weiss B, Zarcone T, Watson GE, Zareba G, McSorley EM, Mulhern MS, Yeates AJ, Henderson J, Gedeon J, Shamlaye CF, Davidson PW, 2013. Prenatal methyl mercury exposure in relation to neurodevelopment and behavior at 19 years of age in the Seychelles Child Development Study. Neurotoxicol. Teratol 39, 19–25. 10.1016/j.ntt.2013.06.003. Epub 2013 Jun 14. [PubMed: 23770126]

Wang T, Li Z, Li X, Chen L, Zhao H, Jiang C, Song L, 2017. Expression of CD19+CD24highCD38high B cells, IL-10 and IL-10R in peripheral blood from patients with systemic lupus erythematosus. Mol. Med. Rep. 11 (6), 6326–6333. 10.3892/mmr.2017.7381. Epub 2017 Aug 29. [PubMed: 28901380]

Weisberg S, 2005. Applied Linear Regression, third ed. John Wiley & Sons, Inc, Hoboken, NJ (Wiley Online Library Google Scholar).

Yang JM, Baserga SJ, Turley SJ, Pollard KM, 2001. Fibrillarin and other snRNPs proteins are targets of autoantibodies in xenobiotic-induced autoimmunity. Clin. Immunol. 10. 101 (1), 38–50. 10.1006 clinically.2001.0509. [PubMed: 11580225]

Yel L, Ramanuja S, Gupta S, 2009. Clinical and immunological features in IgM deficiency. Int. Arch. Allergy Immunol 150 (3), 291–298. 10.1159/000222682. Epub 2009 Jun 4. [PubMed: 19494527]

Zhou X, Fragala M, McElhaney J, Kuchel G, 2010. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. Curr. Clin. Nutr. Metab. Care 13 (5), 541. [PubMed: 20657280]
Table 1

Participant characteristics (n = 497).

|                      | n    | Mean | SD   | Range       |
|----------------------|------|------|------|-------------|
| Male:female          | 229:268 |
| Waist circumference (cm) | 490   | 75.86 | 10.48 | 52–118.20   |
| Maternal hair MeHg (ppm) | 497   | 6.84  | 4.55 | 0.54–26.73  |
| Y19 hair MeHg (ppm)  | 448   | 10.23 | 6.02 | 0.42–52.08  |
| Weighted average hair MeHg (ppm) | 368   | 7.46  | 2.82 | 2.28–20.32  |
| AntiCombined ANA (% > LOD) | 473   | 56    |      |             |
| IgA (g/L)            | 471   | 1.84  | 0.61 | 0.37–3.88   |
| IgG (g/L)            | 471   | 13.19 | 2.46 | 7.52–23.08  |
| IgM (g/L)            | 471   | 1.23  | 0.62 | 0.26–4.48   |
| Fish consumption (meals/week) | 217   | 7.22  | 3.66 | 0–29        |
| n-3 LCPUFA (mg/ml)   | 491   | 0.04  | 0.02 | 0.01–0.11   |
| n-6 LCPUFA (mg/ml)   | 491   | 0.15  | 0.04 | 0.02–0.55   |
| n-6:n-3 LCPUFA       | 491   | 3.81  | 1.97 | 0.96–17.73  |

MeHg, methylmercury; Y19, year 19; ppm, parts per million; ANA, antinuclear antibody; LCPUFA, long chain polyunsaturated fatty acids.
Main effect models reporting covariate-adjusted associations between methylmercury (MeHg) exposure and antinuclear antibodies (ANA) and immunoglobulins (Ig) and inflammatory markers with and without adjustment for long chain polyunsaturated fatty acids (LCPUFA).

|                        | MatHg          | Y19Hg          |
|------------------------|----------------|----------------|
| **ANA combined**        |                |                |
| Unadjusted             | 0.010 (−0.031, 0.051) p = 0.637 | 0.036 (0.001, 0.073) p = 0.051 |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | 0.017 (−0.028, 0.062) p = 0.472 | 0.036 (0.001, 0.073) p = 0.049 |
| Adjusted for n-3:n-6 LCPUFA | 0.019 (−0.025, 0.064) p = 0.395 |                |
| **Anti-dsDNA**          |                |                |
| Unadjusted             | −0.002 (−0.045, 0.039) p = 0.909 |                |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | 0.007 (−0.039, 0.053) p = 0.750 | 0.010 (−0.026, 0.046) p = 0.565 |
| Adjusted for n-3:n-6 LCPUFA | 0.005 (−0.041, 0.050) p = 0.826 | 0.010 (−0.026, 0.046) p = 0.570 |
| **Anti-RNP A**          |                |                |
| Unadjusted             | 0.002 (−0.030, 0.034) p = 0.908 |                |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | −0.004 (−0.040, 0.031) p = 0.810 | −0.010 (−0.037, 0.017) p = 0.467 |
| Adjusted for n-3:n-6 LCPUFA | −0.002 (−0.037, 0.033) p = 0.905 | −0.010 (−0.037, 0.017) p = 0.472 |
| **IgG**                |                |                |
| Unadjusted             | −0.001 (−0.045, 0.046) p = 0.950 |                |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | −0.001 (−0.052, 0.048) p = 0.969 | −0.002 (−0.043, 0.036) p = 0.921 |
| Adjusted for n-3:n-6 LCPUFA | 0.009 (−0.041, 0.057) p = 0.733 | −0.001 (−0.041, 0.037) p = 0.960 |
| **IgM**                |                |                |
| Unadjusted             | 0.003 (−0.006, 0.012) p = 0.459 | −0.003 (−0.008, 0.002) p = 0.453 |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | 0.002 (−0.008, 0.011) p = 0.714 | −0.009 (−0.016, −0.002) p = 0.016 |
| Adjusted for n-3:n-6 LCPUFA | 0.003 (−0.007, 0.012) p = 0.557 | −0.009 (−0.016, −0.001) p = 0.018 |
| **IgA**                |                |                |
| Unadjusted             | 0.004 (−0.003, 0.011) p = 0.274 | −0.002 (−0.008, 0.004) p = 0.569 |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | 0.004 (−0.004, 0.011) p = 0.356 | −0.002 (−0.008, 0.004) p = 0.562 |
| Adjusted for n-3:n-6 LCPUFA | 0.004 (−0.004, 0.011) p = 0.342 | −0.002 (−0.008, 0.004) p = 0.562 |
| **Il-1 beta**          |                |                |
| Unadjusted             | −0.002 (−0.007, 0.002) p = 0.321 |                |
| Adjusted for n-3 LCPUFA, n-6 LCPUFA | −0.003 (−0.008, 0.002) p = 0.241 | 0.002 (−0.002, 0.006) p = 0.307 |
| Adjusted for n-3:n-6 LCPUFA | −0.003 (−0.008, 0.002) p = 0.283 | 0.002 (−0.002, 0.006) p = 0.303 |
|                | MatHg                        | Y19Hg                      |
|----------------|------------------------------|----------------------------|
| **IL-2**       | Unadjusted                   | -0.000 (−0.003, 0.003) p = 0.951 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | -0.000 (−0.004, 0.003) p = 0.865 |
|                | Adjusted for n-3:n-6 LCPUFA  | -0.000 (−0.004, 0.003) p = 0.938 |
| **IL-6**       | Unadjusted                   | 0.014 (−0.007, 0.006) p = 0.199 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | -0.000 (−0.023, 0.022) p = 0.970 |
|                | Adjusted for n-3:n-6 LCPUFA  | 0.008 (−0.015, 0.011) p = 0.485 |
| **IL-10**      | Unadjusted                   | 0.011 (−0.006, 0.028) p = 0.200 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | 0.001 (−0.018, 0.019) p = 0.937 |
|                | Adjusted for n-3:n-6 LCPUFA  | 0.006 (−0.012, 0.025) p = 0.514 |
| **INF-γ**      | Unadjusted                   | 0.001 (−0.018, 0.020) p = 0.902 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | -0.008 (−0.029, 0.013) p = 0.443 |
|                | Adjusted for n-3:n-6 LCPUFA  | -0.002 (−0.023, 0.020) p = 0.888 |
| **TNF-α**      | Unadjusted                   | -0.000 (−0.008, 0.008) p = 0.995 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | -0.005 (−0.014, 0.004) p = 0.279 |
|                | Adjusted for n-3:n-6 LCPUFA  | -0.003 (−0.012, 0.006) p = 0.555 |
| **CRP**        | Unadjusted                   | -0.009 (−0.038, 0.021) p = 0.564 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | -0.014 (−0.044, 0.017) p = 0.377 |
|                | Adjusted for n-3:n-6 LCPUFA  | -0.010 (−0.040, 0.020) p = 0.517 |
| **TNH-α: IL10 ratio** | Unadjusted                   | -0.011 (−0.024, 0.002) p = 0.091 |
|                | Adjusted for n-3 LCPUFA, n-6 LCPUFA | -0.006 (−0.020, 0.009) p = 0.436 |
|                | Adjusted for n-3:n-6 LCPUFA  | -0.009 (−0.023, 0.005) p = 0.223 |

Data presented as odds ration (95% confidence interval) p value. All values are log transformed.

MatHg, prenatal methylmercury exposure; Y19Hg, year 19 concurrent MeHg exposure.

Unadjusted: controlled for sex of child, maternal SES, maternal age and MatHg.

Adjusted for n-3 LCPUFA, n-6 LCPUFA: controlled for sex of child, waist circumference (WC), MatHg and Y19Hg.

Adjusted for n-6:n-3 LCPUFA: controlled for sex of child, WC, MatHg and Y19Hg.

ANA; antinuclear antibody, dsDNA; double stranded DNA, RNP A; ribonuclear protein A, Ig; immunglobulin, IL; interleukin, CRP; C reactive protein, INF-γ; interferon gamma, TNF-α; tumour necrosis factor alpha.

1 ANA combined: within or above reference range for any of the 13 measured ANA.

2 Anti-dsDNA and anti-RNP A were analyzed as dichotomous (< LOD or LOD+).
Table 3

Associations between n-3 long chain polyunsaturated fatty acids (LCPUFA), n-6 LCPUFA and the n-6:n-3 LCPUFA ratio with immune markers controlling for maternal methylmercury (MatHg).

|                  | n-3 PUFA                | n-6 PUFA                | n6:n3 ratio              |
|------------------|-------------------------|-------------------------|--------------------------|
| ANA combined     | -1.148 (−15.030, 12.714) p = 0.871 | -2.380 (−7.166, 2.128) p = 0.311 | -0.021 (−0.125, 0.083) p = 0.690 |
| Anti dsDNA       | 9.343 (−4.888, 23.685) p = 0.198 | -1.019 (−5.966, 3.637) p = 0.676 | -0.059 (−0.175, 0.049) p = 0.299 |
| Anti RNP A       | -20.355 (−36.893, −4.336) p = 0.014 | -1.442 (−6.652, 3.393) p = 0.571 | 0.075 (−0.035, 0.185) p = 0.173 |
| CRP              | -13.805 (−23.155, −4.456) p = 0.004 | 0.451 (−2.627, 3.529) p = 0.774 | 0.091 (0.022, 0.161) p = 0.010 |
| INF-γ            | -14.654 (−21.098, −8.211) p = 0.000 | -1.559 (−3.680, 0.563) p = 0.149 | 0.054 (0.005, 0.103) p = 0.030 |
| IgA              | -0.221 (−2.586, 2.145) p = 0.855 | -0.058 (−0.824, 0.708) p = 0.882 | 0.003 (−0.015, 0.020) p = 0.753 |
| IgG              | -0.805 (−2.031, 0.420) p = 0.197 | -0.299 (−0.696, 0.097) p = 0.139 | 0.000 (−0.009, 0.009) p = 0.963 |
| IgM              | -1.215 (−4.156, 1.726) p = 0.417 | -0.423 (−1.376, 0.529) p = 0.383 | -0.007 (−0.029, 0.015) p = 0.533 |
| IL-1             | 0.236 (−1.387, 1.859) p = 0.775 | -0.377 (−0.912, 0.157) p = 0.166 | -0.005 (−0.017, 0.007) p = 0.419 |
| IL-10            | -18.461 (−24.125, −12.797) p < 0.001 | 0.560 (−1.305, 2.425) p = 0.556 | 0.110 (0.067, 0.153) p < 0.001 |
| IL-2             | 0.007 (−1.125, 1.138) p = 0.991 | -0.133 (−0.506, 0.239) p = 0.482 | -0.003 (−0.011, 0.006) p = 0.509 |
| IL-6             | -23.841 (−30.760, −16.921) p < 0.001 | -1.026 (−3.304, 1.252) p = 0.377 | 0.130 (0.077, 0.183) p < 0.001 |
| TNF-α            | -7.541 (−10.275, −4.808) p < 0.001 | 0.196 (−0.704, 1.096) p = 0.668 | 0.045 (0.024, 0.065) p < 0.001 |
| TNF-α: IL-10    | 10.920 (6.549, 15.291) p < 0.001 | -0.363 (−1.802, 1.076) p = 0.620 | -0.065 (< 0.09, −0.032) p < 0.001 |
| Anti dsDNA       | -7.354 (−18.227, 3.518) p = 0.184 | 0.523 (−2.990, 4.035) p = 0.770 | 0.041 (−0.040, 0.122) p = 0.322 |
| Anti RNA         | 295.607 (44.831, 546.383) p = 0.021 | 28.539 (−2.488, 109.565) p = 0.489 | −1.085 (−2.963, 0.793) p = 0.257 |

Models controlled for sex of child, maternal SES, maternal age and MatHg.

ANA; antinuclear antibody, dsDNA; double stranded DNA, RNP A; ribonuclear protein A, CRP; C reactive protein, INF-γ; interferon gamma, Ig; immuoglubulin, IL; interleukin, TNF-α; tumour necrosis factor-alpha.

All values are log transformed.

1 ANA combined: within or above reference range for any of the 13 measured ANA
2 Anti-dsDNA and anti-RNP A were analyzed as dichotomous (< LOD or LOD+)