Low-level gestational exposure to mercury and maternal fish consumption: Associations with neurobehavior in early infancy

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Abstract

Background: Studies examining the effects of low-level gestational methylmercury exposure from fish consumption on infant neurobehavioral outcomes in the offspring are limited and inconclusive. Our objective was to examine the effects of low-level gestational exposure to methylmercury on neurobehavioral outcomes in early infancy.

Methods: We assessed neurobehavior of 344 infants at 5-weeks using the NICU Network Neurobehavioral Scale (NNNS). Gestational mercury exposure was measured as whole blood total mercury (WBTHg) in maternal and cord blood. We collected fish consumption information and estimated polyunsaturated fatty acid (PUFA) intake. We examined the association between gestational mercury exposure and NNNS scales using regression, adjusting for covariates.

Results: Geometric mean of maternal and cord WBTHg were 0.64 and 0.72 μg/L, respectively. Most mothers (84%) reported eating fish during pregnancy. Infants with higher prenatal mercury exposure showed increased asymmetric reflexes among girls (p = 0.04 for maternal WBTHg and p = 0.03 for cord WBTHg), less need for special handling during the assessment (p = 0.03 for cord WBTHg) and a trend of better attention (p = 0.054 for both maternal WBTHg and cord WBTHg). Similarly, infants born to mothers with higher fish consumption or estimated PUFA intake also had increased asymmetric reflexes and less need for special handling. In models simultaneously adjusted for WBTHg and fish consumption (or PUFA intake), the previously observed WBTHg effects were attenuated; and higher fish consumption (or PUFA intake) was significantly associated with less need for special handling.

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Conclusions: In a cohort with low level mercury exposure and reporting low fish consumption, we found minimal evidence of mercury associated detrimental effects on neurobehavioral outcomes during early infancy. Higher prenatal mercury exposure was associated with more frequent asymmetric reflexes in girls. In contrast, infants with higher prenatal mercury exposure and those whose mothers consumed more fish had better attention and needed less special handling, which likely reflect the beneficial nutritional effects of fish consumption.

Keywords

NNNS; Methylmercury; Fish consumption; Gestational exposure; Neurobehavior; Infancy

1. Introduction

Methylmercury (MeHg) is a widespread environmental pollutant to which humans are exposed primarily through the consumption of contaminated seafood. Neurotoxicity, the most sensitive endpoint used to assess the toxic effects of MeHg exposure, has been well recognized since the outbreak of MeHg poisonings in Minamata Bay, Japan and Iraq (Eto, 1997). Because the brain is the main target tissue for MeHg, and given that MeHg effectively crosses both the placenta and the blood-brain barrier (Stern and Smith, 2003), the developing central nervous system of the fetus and young child is especially vulnerable. During the Minamata outbreak, extreme fetal abnormalities and signs of neurotoxicity were even seen in infants born to women with minimal or no MeHg poisoning symptoms (Harada, 1995).

Effects of gestational exposure to MeHg on neurologic outcomes have been evaluated in epidemiological studies conducted typically among populations with a diet consisting of high levels of fish or sea mammal consumption including several large scale cohort studies. The Faroe Island study, using cord whole blood total mercury (geometric mean = 22.9 μg/L) as a biomarker of gestational exposure, reported MeHg associated deficits in language, memory, attention, motor skills, and visual spatial domains assessed at 7 and 14-years of age (Debes et al., 2006; Grandjean et al., 1997). At age 22 years, MeHg associated deficits in cognitive function were still observed in this cohort (Debes et al., 2016). However, the Seychelles study, in which MeHg exposure was measured as maternal hair mercury (mean = 6.8 ppm, equivalent to a blood mercury concentration of 27.2 μg/L), evaluated children during a follow up from 6 months to 17 years on similar neurocognitive and neurobehavioral functions (using different assessment tools) and found no evidence of adverse effects of gestational MeHg exposure (Davidson et al., 2011; Davidson et al., 1998; Davidson et al., 1995; Myers et al., 2003; Myers et al., 1995). More recently, in a cohort of Inuit infants from the Canadian Arctic, who were exposed to mercury at levels similar to those in Faroes and Seychelles studies, cord blood mercury (mean = 22.5 μg/L) was associated with poorer performance on working memory and school-age assessment of intelligence (IQ) (Boucher et al., 2014; Jacobson et al., 2015). It is important to note that within the Faroe Island and the Canadian Inuit cohorts, the sources of mercury exposure were both marine mammals and fish, whereas the Seychelles cohort was exposed to mercury primarily from consumption of fish. These different sources of exposure could contribute to the disparate outcomes reported.

Compared to most previously published studies, the level of mercury exposure among US women, with a geometric mean of 0.78 µg/L in whole blood (CDC, 2009), is much lower than in populations that rely more on fish for their diets (for example, the Faroe Island cohort, had a cord whole blood total mercury geometric mean = 22.9 µg/L) (Grandjean et al., 1997). Studies conducted at this level of exposure are limited and findings have been inconsistent. Higher maternal mercury exposure was associated with poorer child cognitive performance (Oken et al., 2008; Oken et al., 2005) or delayed neurodevelopment (Jedrychowski et al., 2006), while higher fish consumption during pregnancy was associated with better child neurocognitive development (Daniels et al., 2004; Oken et al., 2008; Oken et al., 2005; Valent et al., 2013). There were also studies that reported no association between higher maternal mercury exposure and adverse child neurodevelopment outcomes (Daniels et al., 2004; Valent et al., 2013).

While fish is a main source of MeHg exposure for the general population, it is also a major dietary source of omega-3 poly-unsaturated fatty acids (PUFA): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA, an important structural component of the central nervous system, is believed to have important roles in both visual and neural functions (Innis, 2005). Randomized controlled trials have shown that maternal fish oil supplementation during pregnancy may have beneficial effects on children's eye and hand coordination, and on mental and cognitive development (Campoy et al., 2011; Dunstan et al., 2008; Helland et al., 2003). Longitudinal studies have also associated higher maternal or cord DHA concentrations with better attention, superior memory function, and more optimal visual, cognitive, and motor development in childhood (Boucher et al., 2011; Colombo et al., 2004; Jacobson et al., 2008).

Due to concerns about MeHg exposure, fishing advisories and fish intake guidelines have been developed and distributed (USEPA and USFDA, 2004), which may substantially change dietary behavior regarding fish consumption among pregnant women (Oken et al., 2003). Given the well-established neurotoxicity of MeHg and beneficial effects of fish nutrition (PUFA) on neurodevelopment, it is important to elucidate the neurobehavioral effects of low-level prenatal exposure to MeHg that occurs when fish is consumed. However, studies conducted among low fish consumption populations (and hence low level of MeHg exposure) are quite limited. Moreover, studies assessing neurobehavioral outcomes during early infancy are very limited. A Japanese cohort study reported a negative relationship between higher maternal hair total mercury and the quality of muscle tone and movement assessed using the Neonatal Behavioral Assessment Scale (NBAS)·(Suzuki et al., 2010) Another study conducted in China found that among male newborns, higher cord blood total mercury as well as higher maternal hair total mercury were associated with lower scores on a behavioral cluster of items including, habituation to stimuli during sleep, and orienting responses to visual and auditory stimuli (Gao et al., 2007). Thus, the purpose of the current study was to examine the effects of gestational MeHg exposure on neurobehavioral outcomes during early infancy among a cohort of mothers with exposure levels that are representative of US women. In addition, we aimed to examine the potential confounding effects of maternal fish consumption on the relationship between MeHg and neurobehavior.

2. Methods

2.1. Study participants

The current study utilized participants in the Health Outcomes and Measures of the Environment (HOME) Study, an on-going prospective pregnancy and birth cohort study, conducted in Greater Cincinnati, Ohio. The purpose of the HOME Study is to examine the associations between both prenatal and postnatal exposures to common environmental toxicants and health and developmental outcomes through infancy and childhood. Enrollment was conducted between February 2003 and January 2006. Women, at least 18 years of age, and residing in homes built before 1978 (a criterion for a nested study of lead exposure), were contacted at 16 ± 3 weeks of pregnancy. Of the 468 women enrolled, 389 remained in the study to deliver live-born singleton infants. Details of enrollment have been described elsewhere (Yolton et al., 2009). Institutional Review Boards of all involved research institutions, hospitals, and laboratories approved the study protocol. Written informed consent was obtained from each participant.

2.2. Gestational mercury exposure and fish consumption

We measured whole blood total mercury (WBTHg), a commonly used biomarker for MeHg exposure, in maternal samples collected at around 16 and 26 weeks gestation and at delivery, and in infant cord whole blood. The whole blood samples were collected in EDTA vacutainer tubes, and stored at −80 °C until shipment to the Centers for Disease Control and Prevention (CDC) for analysis. Total mercury was quantified using a quadrupole ICP-MS technology based method (CDC, 2003). The limit of detection (LOD) for total mercury was 0.2 μg/L. For results reported as below LOD (16%, 20%, and 14% for 16-week, 26-week and delivery samples, respectively), we imputed a value of LOD divided by square root of 2 (Hornung and Reed, 1990).

We collected maternal fish consumption information during pregnancy with questionnaires at 16 weeks gestation and 5 weeks postpartum. Using a list of fish selected *a priori* based on their popularity and estimated mercury content (salmon, tuna, shellfish, lake trout, mackerel, swordfish, tilefish, shark), we asked women to identify the types of fish they ate during pregnancy and frequency of consumption for each type. We calculated total fish consumption by summing all fish types. We also estimated total intake of poly-unsaturated fatty acids (PUFA) from fish consumption, based on EPA and DHA (two most abundant omega-3 PUFA in fish) content data reported in the USDA National Nutrient Database (USDA).

2.3. Infant neurobehavior assessment

We assessed infant neurobehavior at around 5-weeks of age during a home visit, using the NICU Network Neurobehavioral Scale (NNNS), administered by trained examiners who were blinded to prenatal exposures. The NNNS is a neurobehavioral evaluation that integrates several infant assessment tools with the heaviest influence from the Neonatal Behavioral Assessment Scale (NBAS) (Lester et al., 2004). The NNNS has primarily been used in the assessment of neurobehavior in infants exposed to drugs of abuse, but the potential value of this measure for the detection of effects of gestational

exposure to environmental toxicants has also been recognized (Tronick and Lester, 2013; Yolton et al., 2009; Yolton et al., 2013). The predictive validity of the NNNS has been demonstrated in several studies, which reported NNNS scales or profiles predicted childhood neurodevelopmental outcomes, including behavior problems, motor development and IQ (Liu et al., 2010; Stephens et al., 2010; Sucharew et al., 2012). The NNNS includes evaluation of neurologic and behavioral qualities of the infant as well as observation of both overt and subtle signs of stress during the exam. Processing of NNNS raw data produces scores on thirteen scales: habituation, attention, arousal, regulation, need for special handling, quality of movement, excitability, lethargy, non-optimal reflexes, asymmetry, hypertonia, hypotonia and stress abstinence. A higher score on a particular scale indicates more of the quality of that scale, regardless of whether it is positive or negative in nature. For example, a higher score on the attention scale suggests more attention was exhibited during the exam (a positive quality); while a higher score on the asymmetry scale indicates more asymmetric reflexes observed during the exam (a negative quality) (Lester et al., 2004).

2.4.   Covariates

In the prenatal questionnaires, we collected information on maternal demographic and social economic status, such as age, race/ethnicity, education, household income, marital status, and employment. We also asked the mothers about any alcohol or illicit drug use, tobacco smoking, and diet during pregnancy. We measured blood lead, serum cotinine, and serum PCBs in the maternal samples collected during pregnancy and at delivery. For PCBs, we calculated total PCBs as the sum of the 35 PCB congeners measured (Patterson et al., 2009); PCB levels were lipid adjusted. We conducted a review of the mother and infant medical charts to collect pregnancy information, labor and delivery, and newborn characteristics. The mothers also completed the Beck Depression Inventory (BDI-II) (Beck et al., 1996) prenatally and at 5-weeks postpartum. Mothers who had a total score > 13 in either BDI-II were considered as exhibiting some depressive symptoms.

2.5.   Statistical analyses

We began with univariate analyses to examine data distributions including descriptive statistics. We log-transformed (with a natural base) WBTHg concentrations for further analyses due to a positively skewed distribution. We examined maternal mean WBTHg and cord WBTHg separately as primary exposure variables. Maternal mean WBTHg was calculated as the mean of WBTHg measures at 16-week, 26-week and delivery before log-transformation. In bivariate analyses, we assessed associations among the NNNS scales, the exposure variables, and potential covariates. Consistent with previous analyses of the NNNS in this cohort, we excluded the habituation and hypertonia scales from the analyses as the number of infants with useable scores on these scales (37 and 12 of 344, respectively) was too small for meaningful interpretation. Therefore, 11 NNNS subscales were examined. The hypotonia scale was analyzed dichotomously due to limited variability in the scores. Linear regression, logistic regression, and Poisson regression models, as well as ANOVA and correlation analysis, were used as appropriate. We conducted multivariable analyses examining the association of prenatal mercury exposure with NNNS scales where we observed an effect with p < 0.1 in bivariate analyses. We explored the potential mediating

effect of birthweight and infant high-risk status (defined as preterm, low birth weight, or experiencing a NICU stay) on mercury-NNNS relationship. In our sample, WBTHg is not significantly associated with birthweight nor infant high-risk status, suggesting no mediating effect from these two variables (Baron and Kenny, 1986). In all initial full models, we considered the same set of covariates selected *a priori* or based on their bivariate association ($p < 0.2$) with both the NNNS outcomes and mercury exposure: infant age at exam, infant sex, maternal race, marital status, education, and household income, maternal depressive symptoms, alcohol use, BMI at 16 weeks gestation, blood lead, serum cotinine, urinary diethyl phosphates (metabolites of organophosphates), lipid adjusted serum total PCB. Although we had found associations between NNNS outcomes and BPA and phthalates in this cohort (Yolton et al., 2011), maternal BPA and phthalate levels were not associated with WBTHg or the specific NNNS scales which we examined in the multivariable models, and thus they were not included as covariates. We used a step-wise backward elimination approach to obtain the most parsimonious final models. We decided, *a priori*, to retain infant sex and age at exam, regardless of its statistical significance, in all models. At each step, we removed a covariate that was not statistically significant ($p > 0.05$) and whose removal did not result in > 10% change in the beta estimate of the exposure variable (maternal mean WBTHg or cord WBTHg). The final list of covariates included infant sex and age at exam, household income, maternal marital status, maternal alcohol use during pregnancy, and maternal serum cotinine during pregnancy. After final models were obtained for WBTHg concentrations, we examined models further adjusted for reported fish consumption and estimated PUFA intake, in separate models. We tested for mercury by sex and mercury by PCB interactions in the final models. A secondary analysis examined reported fish consumption and estimated PUFA intake as the exposure variable. Level of significance was set at 0.05 or as otherwise noted. We did not adjust for multiple comparisons because each analysis of NNNS subscale outcomes was independent of all others. All statistical analyses were conducted using SAS® version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Maternal and infant characteristics

Of the 389 live-born singleton infants in the HOME study, 355 completed the 5-week infant neurobehavior assessment; of these, 344 also had available maternal or cord WBTHg data and thus were included in the current study. As presented in Table 1, the mothers were mostly non-Hispanic White with middle class income, married, employed, and had at least some college education. The infants were mostly term with normal birth weight, suggesting little medical risk. The mean age of the infants at the time of the NNNS exam was 34 (±5) days.

3.2. Gestational mercury exposure and reported fish consumption

WBTHg (Table 2) measured in maternal samples had a geometric mean of 0.65, 0.56, and 0.60 μg/L, for samples collected at 16-week, 26-week and delivery, respectively, with maternal mean WBTHg over gestation of 0.64 μg/L, slightly lower than cord WBTHg (0.72 μg/L). The level of exposure in this cohort was representative of the US population (NHANES 2003–2004: 0.78 μg/L for females).(CDC, 2009) Only 3 (0.9%) women had

WBTHg above 5.8 μg/L, the EPA level of concern for women of childbearing age. Maternal WBTHg at the different time points during pregnancy were highly correlated (Pearson's r = 0.74 to 0.80), and they were also highly correlated with the infant cord WBTHg (Pearson's r = 0.73 to 0.82).

In the prenatal questionnaire (covering the period of estimated conception date to about 20-weeks' gestation), 84% of women reported eating fish; 63% of women consumed fish 1–3 times a month or less than monthly. The fish consumed were mostly from stores or restaurants, with very few women (7%) reporting eating fish caught from local rivers or lakes, which could have high content of mercury or other environmental pollutants. Tuna, shellfish, and salmon were the most commonly consumed fish, reported by 60%, 59%, and 42% of the women, respectively. Reported fish consumption from the 5-week postpartum survey (covering the period of 20-weeks' gestation to delivery) was very similar. Combining all types of fish, the median number of fish-containing meals consumed during pregnancy per woman was 13 (interquartile range: 6–17). WBTHg concentrations significantly correlated with reported fish consumption (Pearson's r = 0.46 to 0.56) and estimated PUFA intake from fish consumption (Pearson's r = 0.44 to 0.55).

3.3. Association between gestational mercury exposure and infant neurobehavior

3.3.1. Bivariate models—In bivariate models of WBTHg and NNNS scales (Table 3), higher cord WBTHg was significantly associated with less need for special handling (β = –0.040, SE = 0.019, p = 0.03) and higher asymmetry scores (β = 0.120, SE = 0.056, p = 0.03). There were also several trends that fell short of statistical significance: higher maternal mean WBTHg with better attention (β = 0.184, SE = 0.096, p = 0.06) and with less need for special handling (β = –0.035, SE = 0.021, p = 0.09); and higher cord WBTHg with better attention (β = 0.150, SE = 0.081, p = 0.07).

3.3.2. Multivariable model with mercury concentrations—We further investigated the three NNNS scales with bivariate associations at p < 0.10 (attention, handling, and asymmetry) in multivariable models (Table 4). After adjustment for covariates, we continued to observe that infants with higher maternal mean WBTHg had a non-significant trend of better attention (β = 0.197, SE = 0.102, p = 0.054), and less need for special handling (β = –0.038, SE = 0.022, p = 0.08). We examined asymmetry in sex-stratified models as there was an indication of mercury by sex interaction (p-value of interaction term = 0.07). Higher maternal mean WBTHg was associated with higher asymmetry scores among girls (β = 0.171, SE = 0.083, p = 0.04), but not boys (β = –0.09, SE = 0.104, p = 0.38). Models with cord WBTHg showed results similar to the maternal WBTHg models. Higher cord WBTHg was associated with less need for special handling (β = –0.042, SE = 0.019, p = 0.03), higher asymmetry scores among girls (β = 0.159, SE = 0.072, p = 0.03) and a non-significant trend of better attention (β = 0.165, SE = 0.085, p = 0.054) (Table 4). Further examination of individual NNNS items that attributed to the asymmetry score revealed that 95% of the asymmetry responses were due to weaker responses on one side. In addition, the asymmetry responses were most often related to upper extremity reflexes (rooting and arm recoil) and upright responses (incurvation).

We also explored possible effect modification from PCB exposure and found the PCB by mercury interaction was significant only with respect to the handling scale (p-value of interaction term = 0.03 and 0.07 for maternal mean WBTHg and cord WBTHg, respectively). Stratified analyses by PCB exposure group (high *vs.* low using the geometric mean as cutoff) showed a statistically significant association of less need for special handling with mercury in the higher PCB group only (β = −0.10, SE = 0.03, p = 0.002 for maternal mean WBTHg; β = −0.08, SE = 0.03, p = 0.008 for cord WBTHg).

3.3.3. Multivariable model with mercury concentrations and fish consumption—In the WBTHg models further adjusted for fish consumption (number of meals), neither maternal mean WBTHg nor cord WBTHg was significantly associated with NNNS scales, while higher fish consumption was associated with less need for special handling (Table 4).

Similarly, when we adjusted for estimated PUFA intake based on specific fish consumption in the final WBTHg models, we no longer observed a significant association between the mercury concentrations and NNNS scales (Table 4). Instead, we found higher estimated PUFA intake was associated with less need for special handling in both the maternal WBTHg model and the cord WBTHg model. Higher estimated PUFA intake was also associated with increased asymmetry score in female infants.

3.3.4. Multivariable model with fish consumption alone—In secondary analyses, we examined fish consumption and PUFA intake (separately) in relation to the NNNS outcomes, adjusting for the same covariates used in the models with WBTHg concentrations. We found that greater fish consumption was associated with less need for special handling (β = −0.0027, SE = 0.0009, p = 0.002) and among girls, higher asymmetry scores (β = 0.007, SE = 0.003, p = 0.02). Similarly, higher PUFA intake was associated with less need for special handling (β = −0.0028, SE = 0.0008, p = 0.001), and among girls, higher asymmetry scores (β = 0.008, SE = 0.002, p = 0.002).

4. Discussion

In the current study of a cohort with low fish consumption and mercury exposure representative of US women, we found minimal evidence of mercury associated detrimental effects on neurobehavioral outcomes during early infancy, as evaluated with the NNNS. The only potentially detrimental effect we observed was increased asymmetry among girls with higher mercury exposure as measured by maternal WBTHg and cord WBTHg. However, this association between mercury and asymmetrical reflexes in girls was no longer significant when fish consumption was included in the model. We also observed better attention and less need for special handling with increases in WBTHg. These associations were attenuated when we further adjusted for fish consumption, and the association of fish consumption with the handling outcome was significant. In addition, when examining fish consumption or PUFA intake as exposure variable, we found both higher fish consumption and higher estimated PUFA intake were associated with less need for special handling. The current study adds to the limited literature that evaluates effects of low-level gestational mercury exposure on early infant neurobehavioral outcomes.

The level of mercury exposure in the current study resembled U.S. reference level for women (CDC, 2009). Based on a hair (ppm) to blood (μg/mL) ratio of 250 (FAO/WHO, 2003), the maternal WBTHg of 0.64 μg/L in the current study translated to a hair mercury concentration of 0.16 ppm. No doubt the level of mercury exposure in the current study is much lower than levels reported in populations with high fish consumption such as the Faroe Islands (geometric mean of cord WBTHg = 22.9 μg/L).(Grandjean et al., 1997) It is also lower than previous studies conducted among populations with moderate fish consumptions: for example, the project VIVA (maternal hair geometric mean was 0.45 ppm) (Oken et al., 2005), and a study in Italy (maternal hair geometric mean was 0.785 ppm). (Valent et al., 2013) On average, mothers in the current study consumed fish 13 times during pregnancy, an amount equivalent to 2 oz/week (assuming 6 oz serving size per meal). This was much lower than the 12 oz/week limit recommended by the fish consumption advisory (USEPA and USFDA, 2004). Fish consumed by the mothers in the current study were mostly tuna, shellfish, and salmon from stores or restaurants, which normally have low mercury content. Seafood reported in previous studies were of a much greater variety and included marine mammals and fish with high mercury content, such as whale and shark. In summary, the mothers in current study consumed a small amount of fish, usually of low mercury content, and had low mercury exposure, representative of the US general population.

Our finding of increased asymmetry among infants with higher mercury exposure was consistent with an association between maternal hair mercury and lower motor cluster scores in neonates reported from a Japanese cohort (Suzuki et al., 2010), even though the level of mercury exposure in our cohort was considerably lower. When we further examined the individual NNNS exam items, it appeared that the asymmetric responses were most often indicative of weaker reflexes on one side. It is also worth noting that the adverse effects of mercury on asymmetry were observed only among girls in our sample. Although the biological mechanism is still not known, similar sex-specific effects of mercury on motor skills have previously been reported in a Spanish cohort, in which higher cord mercury was associated with a trend of lower psychomotor scores among girls (Llop et al., 2012). This finding could be due to chance considering the multiple analyses within this study. However, given that mercury associated deficits in motor skills have also been reported in other studies of children at older ages but with higher levels of exposure (Debes et al., 2006; Grandjean et al., 1997), we plan to further investigate effects of low-level mercury exposure on later neurobehavioral functioning using data collected at an older age in this cohort.

Infants with higher gestational mercury exposure in our cohort study showed reduced special handling and a trend of improved attention, as did the infants with higher maternal fish consumption or PUFA intake during pregnancy. Our findings with regards to fish consumption were consistent with a few other studies conducted in a population with low-level mercury exposure, which reported higher fish consumption during pregnancy was associated with higher neurodevelopment performance among offspring in early childhood (Daniels et al., 2004; Oken et al., 2008; Oken et al., 2005; Valent et al., 2013). The better neurobehavioral performance observed in infants with higher mercury biomarkers in our cohort should not be interpreted as a beneficial effect of MeHg exposure, since MeHg is clearly neurotoxic. Instead, it likely reflects the beneficial effects of fish consumption primarily through the mechanisms of PUFA, as fish is the primary

source of PUFA in the human diet, and higher PUFA intake has been associated with improved neurodevelopment, including memory, attention and cognition (Boucher et al., 2011; Colombo et al., 2004; Helland et al., 2003; Innis, 2005). Several studies have shown simultaneous detrimental effects of MeHg and beneficial effects of PUFA from fish consumption on neurodevelopment, and better neurodevelopment outcomes could be observed as a result of beneficial effects of PUFA (Oken et al., 2008; Stokes-Riner et al., 2011; Strain et al., 2008; Strain et al., 2012). In our cohort, mercury exposure was very low and fish consumption was the main source of mercury exposure, so any detrimental effects of mercury exposure might have been outweighed by the beneficial effects of fish nutrition. When we attempted to adjust for the negative confounding of fish nutrition by putting mercury concentrations and fish consumption in the same model, higher fish consumption was associated with less need for special handling; while mercury concentration was no longer significantly associated. Models that simultaneously adjusted for mercury concentration and estimated PUFA intake showed similar results. Although beta estimates of mercury concentration did not change direction, their magnitude decreased remarkably after models were adjusted for fish consumption or PUFA intake. Our results did not fully reveal negative confounding of fish consumption, likely due to the relatively crude estimate of fish consumption or PUFA intake and the moderate correlation between mercury biomarkers and fish consumption in this cohort.

The current study has several strengths, including a prospective cohort design using serial biomarkers for mercury exposure assessment, and available data regarding fish consumption. Limitations of the study are primarily related to the estimation of fish consumption and PUFA intake. We assumed equal serving sizes for fish consumption since we did not have precise information on serving size. In addition, we did not have a biomarker of PUFA and had to estimate the PUFA intake based on reported fish consumption and PUFA content data provided by the national nutrition database (USDA). We likely obtained a good estimate of the amount of fish consumed and PUFA intake since there was limited variability in the type of fish consumed among the mothers in this cohort and we had frequency of consumption for each specific type of fish. Nevertheless, imprecision in the fish consumption or PUFA intake estimation limited our power to identify its confounding effect. In addition, the level of mercury exposure in this cohort is low and at this level of exposure, whole blood total mercury may not predominantly represent MeHg (Mahaffey et al., 2004) and therefore could introduce exposure misclassification. However, it is important to recall that the mercury concentrations among women in our sample are similar to those of US females. Although we have attempted to adjust for a wide range of potential confounders, it may still be possible that the better performance in infant neurobehavioral assessment in relation to higher mercury biomarker concentration or higher fish consumption seen in this cohort was a result of residual confounding from some other unmeasured factors. Lastly, as we did not adjust for multiple comparisons when examining the 11 NNNS subscales, it is possible that our statistically significant findings could be by chance alone.

5. Conclusions

In this cohort of mothers with blood mercury concentrations representative of US women, we found higher maternal mercury concentrations to be associated with increased attention

and less need for special handling. These effects were attenuated after adjustment for fish consumption; when examined alone higher fish consumption was also associated with less need for special handling. We also found higher maternal mercury concentrations to be associated with increased asymmetric reflexes among girls during neurobehavioral examination but this adverse association was no longer significant when fish consumption was included in the model. The adverse finding of increased asymmetric reflexes calls for additional studies of children with low-level mercury exposure during gestation to verify and follow-up at older ages to determine whether this association persists. Our finding relating better neurobehavioral performance in attention and handling with greater mercury exposure and concomitant higher fish consumption is consistent with the thesis that at low-level exposure the detrimental effects of mercury are outweighed by beneficial effects of fish consumption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Table 1

Maternal and infant characteristics (N = 344).

Maternal characteristics	
Maternal age at delivery (years)[a]	30 (5.8)
Race	
White, non-Hispanic	218 (63%)
Black, non-Hispanic	104 (30%)
Other	22 (7%)
Marital status	
Married	229 (67%)
Not married, living with someone	46 (13%)
Not married, living alone	69 (20%)
Household income ($)[b]	55K (27K–85K)
Employed	284 (82%)
Education	
<= High School or GED	74 (22%)
Some college or college graduate	195 (56%)
Graduate or professional school	75 (22%)
Moderate to severe depression (BDI > 13)	89 (26%)
Alcohol use during pregnancy	
Never drank alcohol during pregnancy	192 (56%)
Drank < 1 alcoholic drink per month	103 (30%)
Drank > =1 alcoholic drink per month	49 (14%)
Marijuana use during pregnancy	24 (7%)
Blood lead (μg/dL)[c]	0.827 (0.791–0.865)
Serum cotinine (ng/mL)[c]	0.099 (0.075–0.132)
Reported active smoking during pregnancy	39 (11%)
Serum total PCB (ng/g lipid)[c]	61.6 (57.8–65.5)
Infant characteristics	
Male	162 (47%)
Gestational age (weeks)[a]	39 (1.7)
Birth weight (g)[a]	3389 (614)
Age at 5-week NNNS exam (days)	34 (5)
At risk for neurodevelopmental deficits[d]	41 (12%)
Being breastfed at least 1 week	269 (78%)

Data reported as frequency (percent).

[a] Mean (SD).

[b] Median (25th, 75th percentile).

[c] Geometric mean (95% confidence interval).

[d]Infant defined as at risk for neurodevelopmental deficits if infant was preterm, low birth weight or had a NICU stay.

Table 2

Whole blood total mercury concentrations (μg/L).

Whole blood total mercury	n	range	Geomean (95% CI)	% < LOD	% > 5.8
Maternal(16w)	320	0.14–8.3	0.65 (0.59–0.71)	15.9	0.9
Maternal(26w)	287	0.14–6.7	0.56 (0.50–0.62)	19.5	0.3
Maternal(birth)	313	0.14–4.3	0.6 (0.55–0.66)	14.1	0.0
Maternal(mean)[a]	344	0.14–6.4	0.64 (0.59–0.75)	7.8	0.3
Cord	270	0.14–14.3	0.72 (0.64–0.81)	16.7	1.9

[a]Maternal (mean) = arithmetic mean of maternal 16 w, 26 w, and birth.

Table 3

Bivariate association between NNNS scales and mercury concentrations.

NNNS scales	Maternal mean WBTHg			Cord WBTHg		
	Beta	Std err	p value	Beta	Std err	p value
Attention	0.184	0.096	**0.06**	0.150	0.081	**0.07**
Arousal	0.025	0.047	0.59	−0.029	0.041	0.49
Self-regulation	0.068	0.054	0.21	0.052	0.045	0.25
Handling	−0.035	0.021	**0.09**	−0.040	0.019	**0.03**
Quality of movement	0.053	0.040	0.19	0.037	0.035	0.29
Excitability	−0.008	0.138	0.96	−0.066	0.120	0.58
Lethargy	−0.116	0.119	0.33	−0.040	0.101	0.69
Non-optimal reflex	0.032	0.111	0.77	0.115	0.099	0.24
Asymmetry	0.065	0.062	0.29	0.120	0.056	**0.03**
Hypotonicity	0.049	0.160	0.76	0.043	0.145	0.77
Total stress	−0.002	0.004	0.57	−0.0003	0.003	0.91

Bold numbers indicate significance at p < 0.1.

Table 4

Adjusted association between NNNS scales and mercury concentrations.

NNNS scales	Model[a]	Mercury concentration			Fish consumption (meals) or PUFA intake (g)		
		Beta	Std err	p value	Beta	Std err	p value
Attention	Maternal WBTHg	0.197	0.102	0.054			
Handling	Maternal WBTHg	−0.038	0.022	0.08			
Asymmetry (male)	Maternal WBTHg	−0.090	0.104	0.38			
Asymmetry (female)	Maternal WBTHg	0.171	0.083	**0.04**			
Attention	Cord WBTHg	0.165	0.085	0.054			
Handling	Cord WBTHg	−0.042	0.019	**0.03**			
Asymmetry (male)	Cord WBTHg	0.065	0.097	0.50			
Asymmetry (female)	Cord WBTHg	0.159	0.072	**0.03**			
Attention	Maternal WBTHg and fish	0.152	0.124	0.22	0.003	0.005	0.56
Handling	Maternal WBTHg and fish	−0.001	0.026	0.98	−0.003	0.001	**0.01**
Asymmetry (male)	Maternal WBTHg and fish	−0.129	0.124	0.30	0.003	0.005	0.55
Asymmetry (female)	Maternal WBTHg and fish	0.08	0.102	0.43	0.005	0.004	0.15
Attention	Cord WBTHg and fish	0.119	0.100	0.23	0.004	0.005	0.48
Handling	Cord WBTHg and fish	−0.021	0.022	0.35	−0.002	0.001	0.07
Asymmetry (male)	Cord WBTHg and fish	0.099	0.109	0.36	−0.002	0.006	0.73
Asymmetry (female)	Cord WBTHg and fish	0.073	0.087	0.40	0.008	0.004	0.09
Attention	Maternal WBTHg and PUFA	0.138	0.119	0.24	0.004	0.005	0.35
Handling	Maternal WBTHg and PUFA	−0.0003	0.025	0.99	−0.003	0.001	**0.002**
Asymmetry (male)	Maternal WBTHg and PUFA	−0.129	0.120	0.28	0.003	0.005	0.50
Asymmetry (female)	Maternal WBTHg and PUFA	0.054	0.097	0.58	0.007	0.003	**0.02**
Attention	Cord WBTHg and PUFA	0.108	0.098	0.27	0.005	0.005	0.31
Handling	Cord WBTHg and PUFA	−0.017	0.022	0.44	−0.003	0.001	**0.02**
Asymmetry (male)	Cord WBTHg and PUFA	0.087	0.110	0.43	0.001	0.005	0.92
Asymmetry (female)	Cord WBTHg and PUFA	0.052	0.083	0.53	0.010	0.004	**0.01**

Bold numbers indicate significance at p < 0.5.

[a] All models adjusted for child age at exam, child sex (in full sample only), income, maternal marital status, maternal alcohol use and maternal serum cotinine.