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Negative confounding by essential fatty acids in methylmercury neurotoxicity associations

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ABSTRACT

Background: Methylmercury, a worldwide contaminant of fish and seafood, can cause adverse effects on the developing nervous system. However, long-chain n-3 polyunsaturated fatty acids in seafood provide beneficial effects on brain development. Negative confounding will likely result in underestimation of both mercury toxicity and nutrient benefits unless mutual adjustment is included in the analysis.
Methods: We examined these associations in 176 Faroese children, in whom prenatal methylmercury exposure was assessed from mercury concentrations in cord blood and maternal hair. The relative concentrations of fatty acids were determined in cord serum phospholipids. Neuropsychological performance in verbal, motor, attention, spatial, and memory functions was assessed at 7 years of age. Multiple regression and structural equation models (SEMs) were carried out to determine the confounder-adjusted associations with methylmercury exposure.
Results: A short delay recall (in percent change) in the California Verbal Learning Test (CVLT) was associated with a doubling of cord blood methylmercury (−18.9, 95% confidence interval [CI] = −36.3, −1.51). The association became stronger after the inclusion of fatty acid concentrations in the analysis (−22.0, 95% confidence interval [CI] = −39.4, −4.62). In structural equation models, poorer memory function (corresponding to a lower score in the learning trials and short delay recall in CVLT) was associated with a doubling of prenatal exposure to methylmercury after the inclusion of fatty acid concentrations in the analysis (−1.94, 95% CI = −3.39, −0.49).
Conclusions: Associations between prenatal exposure to methylmercury and neurobehavioral deficits in memory function at school age were strengthened after fatty acid adjustment, thus suggesting that n-3 fatty acids need to be included in analysis of similar studies to avoid underestimation of the associations with methylmercury exposure.

1. Introduction

Methylmercury (MeHg), an organic form of mercury, is primarily generated from inorganic form in micro-organisms in the aquatic environment as part of the natural global biogeochemical cycling of mercury (NRC, 2000). MeHg bioaccumulates up the aquatic food chain so that the highest concentrations occur in large, long-lived predatory species in freshwater and marine food chains. This worldwide contaminant is a well-established neurotoxicant that can have serious adverse effects on the developing nervous system. Thus, infants exposed to high prenatal methylmercury exposure in Minamata, Japan were born with serious neurological damage, even if their exposed mothers were virtually unaffected (Igata, 1993; Harada, 1995). Recent epidemiological studies have documented subtle mercury-associated neuropsychological dysfunctions in the domains of language, attention, and memory, and to a lesser extent, in visuospatial and motor functions (Grandjean et al., 1997; NRC, 2000; Debes et al., 2006).

Fish and seafood, however, contain n-3 polyunsaturated fatty acids (PUFA), mainly docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) (Hearn et al., 1987; Raper et al., 1992) that are essential for normal brain development and therefore

Abbreviations: AA, arachidonic acid; CVLT, California Verbal Language Test; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatrienoic acid; MeHg, methylmercury; PCB, polychlorinated biphenyls; PUFA, polyunsaturated fatty acid; SEM, structural equation model.

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may attenuate the apparent methylmercury toxicity. DHA is a component of membrane structural lipids that are enriched in non-myelin membranes of the nervous system and certain phospholipid components of the retina. EPA is a precursor of the n-3 eicosanoids, which have a range of beneficial effects (Kinsella et al., 1990; Connor, 2000; Mahaffey, 2004). The demand for PUFA is the greatest from the beginning of the third trimester of gestation through about 18 months after birth when the human brain grows the fastest; insufficient supplies of PUFA and other nutrients may result in deficits in brain development (Innis, 1991).

This creates a situation of negative confounding, where MeHg and nutrients affect the outcome in opposite directions, thus resulting in substantial underestimation of the effects of mercury toxicity and of fish benefits unless there is mutual adjustment of both (Choi et al., 2008a; Stewart et al., 2012). Among the small number of studies that examined the effects of both nutrients and MeHg at the same time as predictors of developmental outcomes, several found that the effects of both predictors were strengthened when both were included in the modeling of the outcomes (Oken et al., 2005, 2008; Budtz-Jørgensen et al., 2007; Strain et al., 2008; Boucher et al., 2011). A recent review suggested that further studies are needed to examine the association of MeHg and nutrients from fish consumption with neurological development (Dzirony et al., 2012). Without mutual adjustment of both the mercury exposure and nutrients from fish, inherent bias will result from the underestimation of both toxicants and nutrients. The bias will be greater for parameters that are measured with a larger imprecision (Budtz-Jørgensen et al., 2007). Hence there is a great need to clarify the relative risks and benefits of fish and seafood consumption.

The current study was undertaken to assess the potential impact of negative confounding by n-3 PUFA on the methylmercury effects on children's neurobehavioral performance. Faroes Islands, the study location, is a Nordic fishing community with limited social differences, and the pollutant exposures primarily originate from traditional diets that include seafood and pilot whale meat (Grandjean et al., 1992). We made use of each individual's multiple exposure biomarkers which included prenatal mercury levels from maternal hair at parturition and cord blood samples. We also included the result of the child's hair and blood samples at 7 years of age. We used structural equation model (SEM) analysis to ascertain the association between the combination of MeHg exposure biomarkers on groups of outcome variables, thus avoiding the multiple comparison problems and adjusting for exposure imprecision and missing data which may not be adequately addressed by standard regression analysis (Budtz-Jørgensen et al., 2002).

2. Materials and methods

2.1. Study population

A cohort of 182 singleton term births was assembled during a 12-month period in 1994–1995 at the National Hospital in Torshavn in the Faroe Islands. The local marine diet includes also the consumption of pilot whale meat, a main source of methylmercury exposure (Steuerwald et al., 2000). Six children who had congenital neurologic disease or psychomotor retardation were excluded. A total of 176 were included in the study.

The protocol of the study was approved by the Faroese ethical review committee and by the Institutional Review Board in the US. Written informed consent was obtained from all parents.

2.2. Measurements of exposure

The mercury (Hg) concentration in whole blood from the umbilical cord was used as the primary indicator of prenatal exposure to MeHg (Grandjean et al., 1992, 1997). Cord blood samples were obtained at birth and Hg analysis was performed in duplicate by flow-injection cold-vapor atomic absorption spectrometry after digestion of the sample in a microwave oven. Hair samples were cut close to the root in the occipital area of the mother at parturition. Details of analytic methods and quality control procedures are described elsewhere (Grandjean et al., 1992, 1997).

Increased exposure to polychlorinated biphenyls (PCBs) may occur in the Faroes from the ingestion of blubber from pilot whales (Grandjean et al., 1997). The concentrations of major PCB congeners were therefore measured in maternal serum obtained at the mothers' last antenatal consultation at week 34. PCB congeners were quantified by a two-stage solid-phase extraction method, followed by gas chromatography analysis with electron capture detection (Steuerwald et al., 2000). To avoid problems with congeners not assessed and concentrations below the detection limit, a simplified total PCB concentration was calculated as the sum of congeners CB-138, CB-153, and CB-180 multiplied by 2 (Heilmann et al., 2006).

2.3. Measurements of seafood nutrients

Cord serum phospholipids were extracted and transmethylated before analysis on a gas chromatograph with a flame ionization detector (Bjerve et al., 1987). Results were reported as relative concentrations in weight percent of total phospholipid fatty acids for essential n-3 PUFA (DHA and EPA), arachidonic acid (AA, 20:4n-6) as an essential n-6 PUFA, and the three relevant elongation and desaturation products — eicosatrienoic acid (ETA, 20:3n-9), docosatetraenoic acid (DTA, 22:4n-6), and docosapentaenoic acid (DPA, 22:5n-6). The sum of DHA and EPA was highly correlated with the total n-3 fatty acid concentration ($r = 0.98$, $p < 0.001$) and was used as the nutrient adjustment.

Selenium in cord blood samples was determined by electrothermal atomic absorption with Zeeman background correction. Methods and procedures of the analysis have been documented (Grandjean et al., 1992). We found that selenium was only weakly correlated with the sum of DHA and EPA ($r = 0.27$, $p < 0.001$). Selenium in cord blood showed an average of 10-fold molar excess above methylmercury (Choi et al., 2008b).

2.4. Outcome measurements

We included neuropsychological tests that would be affected by the neuropathological abnormalities that have been described in congenital methylmercury poisoning (Harada, 1995; NRC, 2000) and functional deficits seen in children with early life exposure to neurotoxicants (Kjellström et al., 1989; White et al., 1994). These tests reflect different domains of brain functions. Details of the administration and results of the tests have been published (Grandjean et al., 1997; Choi et al., 2008b). In addition, based on a priori neurobehavioral knowledge and supported by exploratory factor analysis, the outcome variables were

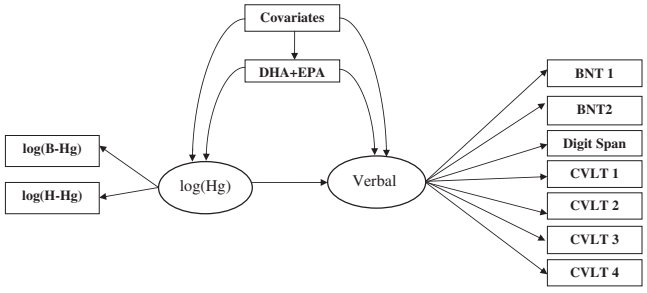

Fig. 1. Path diagram for a structural equation model (SEM) to determine the overall association of prenatal methylmercury exposure and verbal function, with adjustment for nutrients (expressed by the sum of DHA and EPA) and covariates. The estimated true mercury exposure (Hg) is modeled as a latent parameter based on mercury concentrations in cord blood (B-Hg), and maternal hair (H-Hg). The latent verbal parameter is expressed by a series of verbal neuropsychological test results (Boston Naming Tests — BNT1, and BNT2; Digit Span forward; and California Naming Tests — CVLT1, CVLT2, CVLT3, and CVLT4).