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Prenatal methyl mercury exposure in relation to neurodevelopment and behavior at 19 years of age in the Seychelles Child Development Study

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Abstract

Background—Fish are important sources of protein and contain a variety of nutrients, such as n-3 longchain polyunsaturated fatty acids (PUFA), essential for normal brain development. Nevertheless, all fish also contain methyl mercury (MeHg), a known neurotoxicant in adequate dosage. Our studies of the Seychelles Child Development Study (SCDS) Main Cohort enrolled in 1989–1990 (n=779) have found no consistent pattern of adverse MeHg effects at exposures achieved by daily fish consumption. Rather, we have observed evidence of improved performance on some cognitive endpoints as prenatal MeHg exposure increases in the range studied. These observations cannot be related to MeHg and may reflect the role of unmeasured covariates such as essential nutrients present in fish. To determine if these associations persist into young adulthood, we examined the relationship between prenatal MeHg exposure, recent PUFA exposure and subjects' neurodevelopment and behavior at 19 years of age.

Methods—We examined 533 participants using the following test battery: the Profile of Mood States- Bipolar (POMS-Bi); Finger Tapping; Kaufman Brief Intelligence Test (K-BIT); measures of Fine Motor Control and Complex Perceptual Motor Control; and Visual Spatial Contrast Sensitivity. We collected the following covariates: maternal IQ, family life course stressors, socioeconomic status, and subjects' recent postnatal MeHg, sex, and computer use. Primary analyses (based on N=392–475) examined covariate-adjusted associations in multiple linear regression models with prenatal MeHg as the primary exposure measure. Secondary analyses additionally adjusted for total n-6 and fish-related n-3 PUFA measured in the subjects serum at the 19-year examination.

Results—Study participants had a mean prenatal MeHg exposure of 6.9 ppm, and a mean recent postnatal exposure of 10.3 ppm. There were no adverse associations between prenatal MeHg and

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any of the measured endpoints. For recent postnatal MeHg exposure, however, adverse associations were observed for Finger Tapping (non-dominant hand) among women and for the K-BIT matrices for both sexes, with or without adjustment for PUFA.

Conclusion—Our findings continue to provide no evidence for an adverse effect of prenatal MeHg exposure on development in a cohort that consumes fish daily. Observations for postnatal MeHg exposure will need to be confirmed using more comprehensive exposure measures.

Keywords

methylmercury; prenatal exposure; child development

Introduction

Fish are important sources of protein, and contain a variety of nutrients important for normal brain development, in particular n-3 long chain polyunsaturated fatty acids (PUFA) (Uauy and Dangour 2006). Nevertheless, all fish also contain methyl mercury (MeHg), a known neurotoxicant. The toxicity of MeHg to the fetal nervous system at high levels of exposure was demonstrated when children were born with severe neurological deficits in populations consuming seafood or seed grain heavily contaminated with methyl mercury (WHO 1990). However, the extent to which or even if low-level exposure from fish consumption affects child development is uncertain. A number of epidemiological studies of neurobehavioral development in children have been conducted to investigate whether any risks are associated with MeHg exposure that would result from daily ocean fish consumption during pregnancy. Some studies have reported adverse associations with prenatal MeHg exposure (Crump et al., 1998, Grandjean et al., 1997, Jedrychowski et al., 2006, Oken et al., 2005, Sagiv et al., 2012, Suzuki et al., 2010) whereas others have not (Daniels et al., 2004, Llop et al., 2012). Heterogeneity of findings across studies may be due to differences in population characteristics, types of seafood consumption, concomitant exposure to other toxicants, development endpoints evaluated, and statistical approaches to exposure-response modeling, or other factors.

For nearly three decades, the Seychelles Child Development Study (SCDS) in a series of investigations has examined the potential adverse effects of prenatal MeHg exposure from fish consumption on neurodevelopmental outcomes. In our Main Cohort, findings at 66 months of age of slightly better performance on the Preschool Language Scale as MeHg increased within the range studied suggested that nutrients in fish might be influencing the results (Davidson et al., 1998). Indeed, there continues to be evidence in this cohort, most recently at 17 years of age (Davidson et al., 2011), of improved performance on some endpoints (e.g. Woodcock-Johnson-II math calculation scores, numbers of trials on the Intra-Extradimensional Shift Set of the CANTAB, fewer reports of substance use and incidents of and referrals for problematic behaviors in school) as prenatal MeHg exposure increased. There is no biological reason to believe that MeHg at any dosage could improve cognitive performance, but MeHg could be a possible surrogate biomarker for beneficial nutrients present in fish. To determine if these patterns persist, we examined the association between prenatal MeHg exposure and subjects' cognitive test performance at 19 years of age.

Methods

Study Population

The SCDS is a longitudinal observational study which is being conducted in the Republic of Seychelles. The population of about 87,000 inhabitants lives mainly on the main island of

Mahé They have multiethnic roots of mostly mixed African, European and East Asian origins. Seychellois health care, education and social services are free, readily available and focus on prevention to foster child health and development. One hundred percent of children are enrolled in pre-school at age 3.5 years and all children complete compulsory education through age 16 years. Comprehensive preventive maternal and child health care in the Seychelles yields measures of infant mortality, childhood infectious diseases, maternal antenatal care and pediatric care comparable to those found in the US (Govinden et al., 2004).

The primary goal of the SCDS was to measure developmental outcomes in children whose mothers consumed a diet high in fish during pregnancy and to determine if there were associations with the prenatal exposure to MeHg. Fish consumed in Seychelles contain levels of MeHg similar to those found in ocean fish in the US. Karang (*Carangoides gymnostethus*), Barracuda (*Sphyraena jello*), Snapper (*Lutjanidae*), Mackerel (*Rastrelliger kanagurta*) and Shoemaker (*Sigamus sutor*) are the five most commonly consumed fish (Robson et al., 2004). Marine mammals, which can contain polychlorinated biphenyls (PCBs), other toxins, and much higher MeHg concentrations than fish, are not consumed in the Seychelles. The islands are 1,000 miles from the nearest continent and there are no meaningful local sources of industrial pollution in Seychelles. Environmental assessments suggested that exposure levels to other pollutants and contaminants in Seychelles, such as lead, PCBs, and pesticides are low (Shamlaye et al., 2004).

In 1989–1990, a longitudinal study of a cohort of 779 mother-child pairs (Main Cohort) in the Seychelles Islands was initiated. Children were enrolled at 6 months (+/- 2 weeks) postpartum and after exclusions 740 of the children were eligible for further study (Myers et al., 1995). Exclusion criteria included inadequate maternal hair to recapitulate prenatal MeHg exposure, twins, and illnesses or injuries known to adversely affect neurodevelopment including prematurity, severe perinatal illness, closed head trauma with loss of consciousness, encephalitis, and meningitis. Cohort children have subsequently been evaluated at 19, 29, 66, and 107 months of age, and around 10.5, 17 and 19 years of age. Some children were not tested at a specific follow up evaluation because they were residing abroad, refused to participate, or we were unable to locate them (Davidson et al., 2011, Davidson et al., 1998, Davidson et al., 1995, Myers et al., 2003, Myers et al., 1995).

Neurodevelopmental and Behavioral Assessment

Children's cognitive functioning was assessed using the Kaufman Brief Intelligence Test. We also administered several additional tests to measure specific developmental functions hypothesized to be affected by either MeHg or nutrients in fish, including the Profile of Mood States-Bipolar; Finger Tapping; a measure of Fine Motor Control; visual spatial contrast sensitivity; and measures of visual/motor function. Training procedures and reliability checks followed standard SCDS protocols. Participants and evaluators were blinded to pre- and postnatal MeHg concentrations and evaluators were specifically trained on the administered tests (Davidson et al., 1995).

The Profile of Mood States: Bipolar Version (POMS-Bi) is a self-reported questionnaire suitable for adolescents and was designed to assess moods and feelings in normal respondents, including both positive and negative affect (Lorr et al., 1982). POMS-Bi requires the participant to rate his or her mood against 72 adjectives or phrases by rating it as much like this, slightly like this, slightly unlike this, and much unlike this. Positive affect is defined by questions addressing the following factors: composed, agreeable, elated, confident, energetic, and clear-headed. Negative affect is based on the factors anxious, hostile, depressed, unsure, tired, and confused. Both the positive and negative affect scales are scored from 0 to 108. Higher scores on the negative affect scale and lower scores on the

positive affect scale would be considered adverse. The POMS-Bi was given in either English or Creole, and was not administered to participants who could not read.

The Finger Tapping test determines the average number of taps in five consecutive 10-second periods, and is an assessment of subtle motor and cognitive impairment. Test scores were obtained for the dominant and non-dominant hand. Higher scores indicate better performance on this test.

The Kaufman Brief Intelligence Test (K-BIT) assesses both verbal and non-verbal intelligence. The verbal scale assesses knowledge of words and their meanings, whereas the non-verbal scale assesses the ability to solve new problems by perceiving relationships and completing analogies. The K-BIT is comprised of three subtests: Verbal Knowledge, Riddles (verbal scale) and Matrices (non-verbal scale). All three are required to derive an IQ score. However, riddles are an uncommon means of communication in the Seychelles; therefore, we did not administer this subtest. Greater scores on this test are considered beneficial.

The non-commercially available system for assessing Fine Motor Control involved a task which has been described in detail elsewhere (Davidson et al., 2006). In short, subjects were asked to make positioning responses in accordance with a computer display by gripping a handle attached to a rod, which acts as a lever whose fulcrum lies at the elbow. Rotation of the forearm in the vertical plane changes the angular position of a rotary variable displacement transducer (RVDT) located at the fulcrum. On each of 20 trials spaced 20 seconds apart, a narrow band appears on the computer's display screen at 0°, then moves to a horizontal displacement of $\pm 25^\circ$. The subject's task is to rotate the lever so that a large dot, representing the angular position of the forearm, falls within the band and rests there for 8 seconds. At the end of the 8-second period, the band moves to a new position. Our primary outcome measure was reaction time (RT, in milliseconds), i.e., the latency to begin movement. Greater RT is considered an adverse outcome.

Visual Contrast Sensitivity (VCS; Vector Vision, 1991) was measured using a back-lighted display that provides constant luminance independent of ambient lighting (Davidson et al., 2006). This grating-based chart displays six rows and nine columns of circular targets varying in both contrast and in spatial frequency. Each participant was asked to indicate, as they proceeded from left (most discriminable contrast) to right (less discriminable), the orientation of the display (left tilt, right tilt, or vertical) by the position of their hand. Each eye was tested separately, and a log contrast sensitivity score was computed based on the accuracy of the subjects' assessment of the orientation of the display.

Visual/Motor Function was assessed using a custom computer "aircraft piloting" game (Davidson et al., 2006). The game measured a participant's ability to simultaneously engage in three tasks (i.e., multitask). The first task required a participant to use a joystick to keep a drifting visual cue (dot) centered on a "target" at the middle of a display screen. The second task was to pull the trigger of the joystick to increase a decreasing "fuel" meter. The third task was to push another button, on top of the joystick, to decrease an increasing "temperature" gauge. The game lasted 10 minutes and was divided into three measurement periods "acquisition" (T1), "peak" (T2), and "maintenance" (T3). The first period would capture variability in performances as the participant acquired and learned the tasks. The second period would capture the best or "peak" performance. The third period would capture fatigue or boredom as the participant maintained his/her performance. The average horizontal distance from the target was calculated for each period. Higher T2 values (distance from target) indicate worse task performance. Endpoints for statistical analyses included the logarithm of the average distance on T2 (peak performance), the difference in

average distance between T2 and T1 (acquisition), and the difference in average distance between T3 and T2 (fatigue/boredom). Positive values for T2-T1 (change from acquisition period) and T3-T2 indicate worsening performance compared to the earlier period. Negative values indicate improving performance compared to the earlier period.

Exposure Measures

Prenatal MeHg in this cohort was measured in maternal hair collected during pregnancy, at delivery, or at the 6-month enrollment (Myers et al., 1995). Prenatal exposure to MeHg was determined by measuring total maternal hair Hg during pregnancy in the sample that best recapitulated growth during pregnancy using cold vapor atomic absorption spectroscopy with quality control procedures. Over 80% of Hg in hair is known to be organic. We assumed a hair growth rate of 1.1 cm per month and a delay of 20 days between current blood concentrations and appearance of Hg in the first centimeter of scalp hair (Cernichiari et al., 1995). Recent postnatal MeHg exposure at 19 years of age was measured using the same approach in a 1 cm length of each participant's hair closest to the scalp taken at the time of testing.

PUFA concentrations, including n-3 and n-6, were measured at the time of developmental assessment as total plasma phospholipids, a known effective biomarker of PUFA status (Hodge et al., 2007). Total plasma phospholipids have been shown to be indicative of dietary fatty acid intake, appear to track well over time (assuming dietary patterns remain relatively stable over time), and have been recommended as valid measures to describe exposure-disease relationships in prospective studies (Lindberg et al., 2013). Total lipids were extracted from plasma samples, using a modified method of Folch and colleagues (Folch et al., 1957). A solid phase extraction using an NH₂ cartridge system conditioned with chloroform and followed by a series of solvent elutions was used to isolate phospholipids. Absolute amounts of PUFA were determined using GC-MS as described previously (Bonham et al., 2008) and included linoleic acid (LA, n-6 PUFA), arachidonic acid (AA, n-6 PUFA), α -linolenic acid (ALA, n-3 PUFA), eicosapentaenoic acid (EPA, n-3 PUFA), docosapentaenoic acid (DPA, n-3 PUFA) and docosahexaenoic acid (DHA, n-3 PUFA). The sum of DHA and EPA, the main n-3 PUFA found in fish, and total n-6 concentrations were included in statistical models.

Covariates

In addition to recent postnatal MeHg exposure and PUFA status, we considered the following covariates that were *a priori* expected to be associated with one or more of our endpoints. For the analysis of the POMS-Bi we included child sex and life course family stress. For the analysis of Finger Tapping and K-BIT we included child sex, socioeconomic status, and maternal IQ, and for the analysis of Fine and Visual/Motor control and VCS we included child sex and computer use.

Our measure of life course family stress was defined as the sum of events up to the time of developmental testing at 19 years of age. At each evaluation we solicited by maternal self-response information about significant life events. These included caregiver separation or divorces (107 months, 17 years), "major family episode" (66 months), death of someone close to the subject (107 months, 17 and 19 years), a severe illness in the immediate family (107 months, 17 years) and household disasters such as fires, floods, or robberies (107 months, and 17 and 19 years). At each follow up period, information was asked on new events since the last time the child was tested in order to not doublecount traumatic events. Socioeconomic status was measured as a continuous variable at 9 years of age by the Hollingshead Four-Factor Socioeconomic Status scale modified for use in the Seychelles (Davidson et al., 1998). Maternal intelligence was determined by the Matrices subtest of the

K-BIT given to mothers at the 10-year evaluation and used as a continuous variable. Computer use was defined as prior experience with computers or computer games and was ascertained on the subject questionnaire. Computer use was categorized as rare (less than once per month), some (once a week or few times per month), or often (every day or most days).

Statistical Analysis

Descriptive analyses of endpoints, primary predictors and covariates were conducted on non-excluded subjects with data on at least one outcome (n=533). There were no clear differences in prenatal MeHg exposure, maternal K-BIT and socioeconomic status between participants with data on at least one outcome as compared to those without outcome data, although males appeared to be slightly more represented in the latter group (60% vs, 46%, respectively). The mean, median and standard deviation for continuous measures, and proportions for nominal and ordinal variables were computed. Primary regression analyses were based on 392 to 475 young adults who had complete information on cognitive endpoints and covariates, with the sample size varying depending upon the specific outcome measure and relevant covariates.

A priori multiple linear regression models tested the associations between endpoints and prenatal MeHg exposure. We included the sex by prenatal and sex by postnatal MeHg interactions, and if not significant, report results from models without the non-significant interactions. Within each model a two-tailed alpha level of 0.05 was used to determine the significance of interactions and independent variable effects. Regression assumptions were checked for each model. For models in which the assumption of normally distributed errors with constant variance was violated, the dependent variable was log transformed to stabilize the variance and produce more normally distributed errors. Variance inflation factors (VIFs) were used as a check for collinearity among variables. Statistical outliers (defined as observations with standardized residuals greater than 3 in absolute value) and influential points (defined as observations with a Cook's distance larger than 0.50) were identified for each model, and models which contained influential points or extreme outliers (standardized residuals greater than 5 in absolute value) were then run with and without these values. Where results differed substantially with the inclusion or exclusion of outliers or influential points the differences are noted.

In addition to the covariates mentioned above for each endpoint or group of endpoints, secondary models also adjusted for fish-related n-3 (i.e., DHA + EPA) and total n-6 concentrations which further reduced the sample size by up to an additional 28 individuals with missing PUFA data. To investigate the influence of excluding participants with missing covariate data on prenatal MeHg findings, we ran additional models adjusting for sex only. The interpretation regarding the magnitude and statistical significance of the association between prenatal MeHg exposure and any of our endpoints remained unaltered; therefore we report the findings of the fully-adjusted models that better predicted the endpoints.

Results

Characteristics of study participants are presented in Table 1. The average prenatal MeHg exposure was 6.9 ppm in maternal hair. Recent postnatal MeHg exposure in the children's hair was higher with an average of 10.3 ppm. Recent postnatal phospholipid PUFA concentrations were 0.04 mg/ml for DHA+EPA, and 0.15 mg/ml for total n-6. Prenatal MeHg concentrations and recent postnatal DHA+EPA and n-6 PUFA concentrations were similar for men and women, while recent postnatal MeHg concentrations were greater among men (12.5 ppm) than women (8.7 ppm). Correlations between PUFA and MeHg measures were low, ranging from 0.01 for the correlation between total n-6 and recent

postnatal MeHg concentrations to -0.11 for the correlation between DHA+EPA and prenatal MeHg levels. Men performed somewhat better on Finger Tapping (dominant and non-dominant hand), better on POMS-*Bi* (negative and positive affect) and K-BIT, and similar on VCS and Visual/Motor Function as compared to women. On average, participants had experienced 2.5 stressful family events up until the age of 19. About 75 percent of participants regularly used a computer.

Table 2 shows the regression coefficients for the associations between prenatal MeHg, covariates, and developmental endpoints. The set of covariates for POMS-Bi positive scale, Fine Motor Control RT, Visual/Motor Function T2-T1 and T3-T2, and VCS did not statistically significantly predict the endpoints and are not further discussed here. Prenatal MeHg exposure was not associated with any of our endpoints with or without adjustment for PUFA.

After adjusting for covariates, men had a lower score for POMS-Bi negative affect, and performed better on Finger Tapping (dominant hand) and peak performance period of the multi-tasking air-craft piloting game (T2) log average distance as compared with women. Recent postnatal MeHg was adversely associated with Finger Tapping (non-dominant hand) among women but not men, and also with K-BIT Matrices in the entire population. Maternal K-BIT and Hollingshead showed a positive association with participants' K-BIT, and those who used computers regularly performed better on the complex perceptual motor test (T2). Current fish consumption-related n-3 and total n-6 PUFA were not associated with any endpoints, and additional adjustment for these co-exposures did not alter findings (data not shown).

Discussion

Our findings provide no evidence for an adverse association of prenatal exposure to MeHg from fish consumption on neurobehavioral development measured at 19 years of age. This extends our findings from the previous eight other assessment batteries of this cohort administered since age 6 months of age. Among over 100 primary endpoints evaluated, there were some associations that showed improved performance at greater prenatal MeHg exposure levels (Davidson et al., 1998). We have hypothesized that prenatal MeHg may act as a biomarker for one or more uncontrolled covariates, such as the presence of possible beneficial nutrients also present in fish and the Seychellois diet including n-3 and n-6 PUFA, selenium, vitamin E, and other nutrients essential for brain development and function. To evaluate whether nutrients influence the association between prenatal MeHg and developmental outcomes, we studied PUFA, iodine, and iron in a nutrition cohort (SCDNS). The psychomotor index (PDI) on the Bayley Scales of Infant Development (BSID) at 30 months of age showed an adverse association with prenatal MeHg after controlling for PUFA (Davidson et al., 2008). However, the adverse influences of prenatal MeHg exposure were no longer apparent at 5 years of age even after maternal PUFA status was taken into account (Strain et al., 2012).

Our SCDS results are consistent with those of other large studies that reported finding no association between low-dose prenatal MeHg exposure and children's neurodevelopment (Daniels et al., 2004; Llop et al., 2012). Llop and colleagues conducted a multicenter cohort study of almost 1,700 children in Spain. They reported no adverse associations of prenatal MeHg exposure, measured in cord blood, and the children's development when evaluated at 14 months of age using the Bayley Scales of Infant Development psychomotor and mental development indices (Llop et al., 2012). In that study, adjusting for maternal total fish intake during pregnancy did not change the results. Similarly, a comprehensive evaluation of effect modifiers did not identify susceptible subgroups, with the possible exception of an adverse

association in girls. However, our results differ from studies reporting adverse developmental influences of prenatal MeHg exposure (Crump et al., 1998, Grandjean et al., 1997, Jedrychowski et al., 2006, Oken et al., 2005, Sagiv et al., 2012, Suzuki et al., 2010). Studies vary in the source and degree of MeHg exposure, size of the cohort, study design, and numerous other factors which may partially explain the variability in findings across studies.

For example, confounding in epidemiological studies of fish consumption may be a factor in the disparate findings and can be due to several factors, including the type of fish or seafood consumed, the method of assessment of exposure and nutritional status, and the cognitive endpoints evaluated. For example, the correlation between measures of exposure to toxicants and nutrients may not be substantial, either due to consumption of fish in which toxicant and nutrient concentrations are not highly correlated, or because of exposure measurement error due to use of different biomarkers of prenatal MeHg exposure or maternal PUFA status, or use of self-reported fish intake. Consequently, the lack of correlation between toxicant and nutrient intake levels would result in either the true absence of confounding or a decreased ability to detect it. In addition, the possible adverse consequences of toxicants or beneficial influences of nutrients present in fish may vary by developmental outcome. That is, confounding is less likely to be present or to be detected if toxicants and nutrients do not affect the same or similar developmental endpoints. Thus, statements regarding the presence or absence of confounding cannot be made without careful consideration of toxicological mechanisms and potential methodological limitations.

The apparent discrepancy between our findings and those of the Faroe Islands and New Zealand studies has been the subject of considerable debate for more than a decade (National Research Council 2000), and has led to consideration of only a subset of epidemiological studies in government-based regulatory decision-making and consumer advice regarding fish intake during pregnancy (EPA/FDA 2004). Nevertheless, formal quantitative approaches such as meta-analysis and benchmark dose analysis have not supported the view that findings across studies are meaningfully different (Ryan 2008, van Wijngaarden et al., 2006). Specifically, benchmark dose estimates that may serve as the basis for regulatory decision-making have been largely comparable across Seychelles, Faroese and New Zealand studies (van Wijngaarden et al., 2006). Inconsistencies in findings across these and other studies may be due to variability between studies in genetic heritage, biomarkers of exposure, presence of concomitant exposures potentially affecting neurodevelopment, cognitive endpoints measured, and statistical analysis methods employed. Therefore, interpretation of the evidence regarding the neurodevelopment of low-dose exposures is increasingly complex. In an effort to explain the perceived heterogeneity of findings across studies addressing the potential developmental influences of prenatal MeHg exposure, future studies should focus on possible interactions of MeHg with nutrients found in fish and the diet (e.g., n-3 PUFA, selenium, vitamin E) and the role of differences in population characteristics (e.g. genetic heritage) in the toxicokinetics and toxicity of MeHg.

Recent postnatal MeHg exposure has been increasing in the Seychelles cohort children, from an average of 6.5 ppm at 5 years of age (Davidson et al., 1998) to 10.3 ppm in the current study. This was not expected as the Seychellois diet is becoming more diverse and less dependent on ocean fish. However, the increase at 19 years of age, particularly among men, could be explained by larger absolute quantities of overall dietary intake including seafood. While we did not find prenatal MeHg exposure to be associated with any of the outcomes in the current study, recent postnatal exposure at 19 years was associated with adverse effects on Finger Tapping among women only, and on K-BIT matrices in the overall population. In this cohort we previously reported some adverse associations with recent postnatal MeHg

exposure at 107 months (Connor's Teacher Rating Scale ADHD Index in both sexes; full scale IQ, the Grooved Pegboard with the non-dominant hand, and the Connor's Continuous Performance Task Risk Taking in females only) and at 17 years of age (for Woodcock-Johnson Test of Scholastic Achievement-II Passage Comprehension) (Davidson et al., 2011, Myers et al., 2009). While intriguing, the cross-sectional nature of postnatal exposure data and the numerous statistical comparisons made throughout the 19 years of follow up make these findings difficult to interpret. Nevertheless, given the repeated albeit limited occurrence of associations with recent postnatal exposure and increasing exposure levels as the cohort children get older, further research appears warranted to explore possible postnatal exposure effects using longitudinal assessments and metrics that better capture the accumulation of exposure during childhood and adolescence (Myers et al., 2009).

DHA and EPA, two n-3 PUFA present in fish, have substantial anti-inflammatory effects in several tissues (Stables and Gilroy 2011). DHA is highly concentrated in the central nervous system and essential for optimal brain development (Brenna 2002). DHA deficiency disrupts serotonin, norepinephrine, and dopamine transmission across cellular membranes. These neurotransmitters contribute to the mood and cognitive dysfunction aspects of depression (Su 2009). EPA can counter proinflammatory reactions which induce symptoms of physical sickness and may play a role related to somatic symptoms that affect a majority of individuals with major depression (Su 2009). Recent randomized and observational human studies have investigated the beneficial role of n-3 PUFA on mental illness, especially depression. Findings from randomized clinical trials indicate that increased intake of EPA and DHA may benefit those already suffering from depressive symptoms (Appleton et al., 2010, Martins 2009), but there is no consistent evidence for a role of these PUFA in the initial development of depression (Hoffmire et al., 2012). In our study inclusion of recent postnatal plasma phospholipid n-3 and n-6 concentrations in regression models did not contribute to the prediction of POMS scores or any other endpoint, so our results do not provide support for a beneficial influence of PUFA on the occurrence of depressive symptoms.

Strengths of the current study include levels of prenatal MeHg exposure that are at least ten times higher than those found in developed countries such as the U.S., large sample size, longitudinal design with repeated testing at multiple ages, and the use of developmental tests that increase in specificity as the children have aged and that have both clinical and environmental validity (Davidson et al., 2006). Key study limitations include the absence of information on maternal nutritional status or dietary behaviors during pregnancy and earlier biomarkers of nutritional status. Information on these factors would have permitted a direct assessment of prenatal nutrition's influence on subjects' development. Also, some of our developmental tests (i.e. POMS-Bi, K-BIT) may be influenced by cultural context, but we have no evidence to suggest that this introduced bias in our exposure-response analyses.

In conclusion, although potential confounding by prenatal exposure to nutrients cannot be excluded, the current findings suggest that prenatal exposure to MeHg from ocean fish consumption at the levels studied here is not adversely associated with neurocognitive and behavioral outcomes measured in late adolescence.

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Highlights

- Studies of prenatal exposure to MeHg and child development are inconclusive
- We found no link between prenatal MeHg exposure and development in young adults
- Study strengths include the prospective design and high maternal hair MeHg levels

Table 1

Summary statistics for covariates and neurodevelopmental endpoints among non-excluded subjects with at least one outcome (n=533): Seychelles Child Development Study Main Cohort at 19 years of follow up

Covariate or endpoint	N	Mean (SD) or %	Median
MeHg Exposure (ppm)			
Prenatal	533	6.89 (4.52)	5.98
Recent postnatal (19 yr)	481	10.29 (6.06)	9.06
Recent postnatal PUFA status (mg/ml)			
DHA + EPA	498	0.04 (0.01)	0.04
Total n-6	498	0.15 (0.04)	0.16
POMS			
Positive Affect	525	70.56 (14.73)	71
Negative Affect	525	38.39 (17.95)	38
Finger Tapping			
Dominant hand	530	46.25 (6.23)	46.4
Non-dominant hand	530	41.14 (5.78)	41
K-BIT			
Verbal	526	12.81 (2.48)	13
Matrices	524	102.14 (18.33)	100
Fine Motor Control (RT) (log)	533	4.28 (0.25)	4.26
Visual/Motor Function			
Average distance to center (T2) (log)	533	7.81 (0.30)	7.79
Average distance T2-T1	533	-126.85 (974.36)	-41.84
Average distance T3-T2	533	-22.44 (636.73)	-5.38
VCS (log)	529	1.72 (0.12)	1.72
Family stressors	533	2.53 (1.48)	2
Maternal K-BIT	477	80.40 (14.87)	78
Hollingshead	487	25.26 (10.98)	24
Computer use			
Rare	122	25.1	-
Some	212	43.5	-
Often	153	31.4	-

Table 2

Regression coefficients (95% confidence intervals) for prenatal MeHg exposure and covariates in relation to POMS, Finger Tapping, KBIT, and Visual/Motor Function: Seychelles Child Development Study Main Cohort at 19 years of follow up^a

	POMS-Bi Negative	Finger Tapping Dominant	Non-dominant	K-BIT K-BIT Matrices	K-BIT verbal	Visual/Motor Function Log Average Distance (T2)
Prenatal MeHg	-0.026 (-0.384, 0.332)	-0.028 (-0.166, 0.11)	0.031 (-0.089, 0.151)	0.047 (-0.362, 0.456)	0.014 (-0.043, 0.071)	0.001 (-0.005, 0.007)
Covariates						
Sex (male)	-7.628 (-11.01, -4.246)	4.81 (3.542, 6.078)	2.391 (0.238, 4.544)	-0.373 (-4.148, 3.402)	-0.24 (-0.773, 0.293)	-0.111 (-0.166, -0.056)
Recent postnatal MeHg	-0.064 (-0.343, 0.215)	-0.024 (-0.126, 0.078)		-0.388 (-0.693, -0.083)	0.004 (-0.039, 0.047)	0.002 (-0.002, 0.006)
Women			-0.23 (-0.387, -0.073)			
Men			0.047 (-0.063, 0.157)			
Family stressors	0.449 (-0.614, 1.512)					
Maternal K-BIT		-0.002 (-0.047, 0.043)	0.025 (-0.014, 0.064)	0.151 (0.017, 0.285)	0.012 (-0.008, 0.032)	
Hollingshead		0.051 (-0.01, 0.112)	0.038 (-0.015, 0.091)	0.211 (0.032, 0.39)	0.046 (0.02, 0.072)	
Computer Use						
Some						-0.048 (-0.113, 0.017)
Often						-0.15 (-0.221, -0.079)
Model Statistics						
N	475	396	396	392	394	441
R ² (p value)	0.05 (<0.001)	0.142 (<0.001)	0.206 (<0.001)	0.066 (<0.001)	0.058 (<0.001)	0.071 (<0.001)

* The overall regression models for the POMS-Bi Positive scale (model p-value=0.052), Fine Motor Control RT (model p-value=0.06), Visual/Motor Function (T2- T1, model p-value=0.11; T3-T2, model p-value=0.20) and VCS (model p-value=0.34) were not statistically significant, and are not reported in this table. Prenatal MeHg did not statistically significantly predict endpoints in these models.

^a p<0.05