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Neurodevelopmental Impacts of Maternal Nutritional Status and Mercury Exposure from Fish Consumption during Pregnancy

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Abstract

Methylmercury (MeHg) is found in fish and can have hazardous effects, but it also contains minerals that promote healthy brain development and growth. The current study evaluated the hypothesis that specific fish elements or indicators of maternal nutritional status may illustrate relevant confounders when assessing the impacts of prenatal methylmercury exposure on child development. The study was conductedon the Andaman Islands, an archipelago in the Indian Ocean known for its extensive seafood industry. The study used a cohort structure. About 300 expectant mothers signed up for the study. A number of nutrients thought to have an impact in brain development were examined alongside prenatal MeHg exposure. Throughout their first 30 months of life, the children were regularly tested and monitored. There were 229 children whom the information was collected on both outcomes and covariates. Primary outcome measures were 9- and 30-month scores on the Bayley Scales of Infant Development-II (BSID-II). At the ages of 5, 9, and 25 months, infants were additionally given a composite of four supplementary tests of their cognitive and memorising abilities. On average, mothers in the cohort ate 537 g of fish each week (that's nine meals). MeHg levels in maternal hair averaged 5.9 ppt throughout pregnancy. The primary study detected a negative relationship among MeHg, maternal nutritional indicators, & children's BSID-II scores, as well as between MeHg & the mean PDI score at 30 months.At 30 months, we found a small but statistically significant connection between MeHg and the PDI. However, we found no correlations between the two. At 5 months of age, one experimental measure was positively connected with iodine status but not with prenatal MeHg exposure. These findings suggest that research examined associations among prenatal MeHg exposures & child developmental outputs may need to account for maternal diet as a confounding variable.

Keywords: Mercury, neurodevelopmental, exposures, mother, pregnancy

INTRODUCTION

Mercury (Hg) is just a climatic pollutant that can harm the central nervous system of humans, especially in developing children [1]. There is ample evidence that methylmercury (MeHg) can cause neurotoxicity in developing brains when exposed to it at high enough levels during pregnancy. It is still unknown what level of exposure to MeHg can occur from a mother eating a fish-heavy diet while pregnant, and whether or not this will have a negative impact on her child's neurodevelopment.

Neurodevelopmental and neuropathological problems have not been found consistently in our research inside the Andaman Islands, where most people are exposed by eating fish.MeHg levels in maternal hair have been recorded at around 6 ppm in Andaman Islands, which is an unsafe level for prenatal exposure. In the Andaman Islands, we observed that for a variety of outcome indicators, greater maternal hair MeHg values were linked to improved child development. We hypothesised

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that the positive benefits of nutritional intake from fish could be connected to this seemingly abnormal outcome. Incorporating fish into one's diet is a great way to promote healthy brain function [3-5]. Docosahexaenoic acid (DHA), which is an omega-3 fatty acid and a subtype of eicosapentaenoic acid (LCPUFA).DHA has been linked to better brain growth and function [6-8]. Arachidonic acid (AA), a V-6 LCPUFA, shares many of DHA's structural and functional activities in the human CNS [9]. Even in developed countries like Canada, women have reported low DHA levels in their pregnancies [10]. Choline and small amounts of things like iodine or iron (Fe), which have been associated to motor or cognitive development, are also abundant in fish [11, 12]. Around the world, fish is a vital part of people's diets. According to the FAO, fish is the primary food source for up to one billion people worldwide, and that many of these people have few viable food options beyond fish [4].

Multiple cohort and cross-sectional studies [13–24] have examined the effects of maternal fish diet on prenatal MeHg exposure at low and moderate doses on neurodevelopment.

LITERATURE REVIEW

Vejrup et al. (2018) [25] analysed the link between parental seafood consumption, prenatal mercury exposure, and a child's communication and language abilities by the age of five.Data from Norwegian Child and Mother Cohort Study were used in the study, which included data from 38,581 mother-child couples. A subsample of 2239 pregnant women had their mercury levels in their blood analysed during week 17. Maternal mercury exposure throughout pregnancy was estimated using a validated FFQ completed around the time of the midpoint of the pregnancy. The mothers of five-year-olds were polled using ASQ, SLAS & the 20 Declarations on Language-Related Difficulties to gauge their children's linguistic and communicative development (language 20). We used linear regression analysis to control for the mother's demographic characteristics, lifestyle choices, and socioeconomic status. The typical maternal diet included 217 grammes of seafood per week, and the median maternal blood mercury level was 1.03 micrograms per litre.No correlations were found between blood mercury levels and tests of language or communication ability.

Strain et al. (2015) [26] examined how factors including maternal PUFA status and prenatal MeHg exposure were linked to 20-month-old children's growth and development. The Seychelles Child Development Study Nutrient Cohort 2 is currently recruiting participants. This is still being investigated. Individuals there consume significantly more fish on a daily basis than those in other regions. At 20 months of age, the BSID-II, the MacArthur Bates CDI, & the Infant Behavior Questionnaire-Revised were administered to the children.Their mothers took part in the study while they were still in the womb.A total of 1265 mom-and-kid pairings had full information recorded for them. Prenatal exposure to MeHg was not found to have any adverse effects on any neurodevelopmental measures. The BSID-II PDI showed significant associations between MeHg and polyunsaturated fatty acids. Only the offspring of moms with a high n-6/n-3 ratio had a lowered risk of PDI as MeHg increased. Improvements in PDI were observed among children born to moms with greater MeHg levels who also had higher n-3 PUFA intakes. High serum docosahexaenoic acid (DHA) has been linked to better CDI total gesticulations (language development) with or without MeHg correction. However, it was significantly linked to worse Mental Development Index (MDI).Results on the three CDI outcomes were negatively correlated with increasing n-6:n-3 ratios.

Sagiv et al. (2012) [27] investigated the link between prenatal mercury exposure & fish consumption & ADHD-related behaviour. Our study looked at data from such a community, prospective birth cohort that was recruited throughout New Bedford, Massachusetts, from 1993 to

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1998. It focused on 8-year-old children whose mothers' hair was tested for mercury before and after birth (n = 421) or whose mothers said they ate fish while pregnant (n = 515). We used a teacher rating scale and neuropsychological testing to look at behaviours like not paying attention, acting on impulse, and being too active. The majority of mothers consumed more than two fish servings each week, and the average quantity of mercury found in their hair is 0.45 g/g (0.03-5.14 g/g), according to study.Exposure to mercury was linked to both attention problems and hyperactivity/impulsivity in a multivariate regression analysis; for some outcomes, the link became significant only at a mercury concentration of 1 g/g or above. Low-level prenatal mercury exposure has been linked to an increased risk of ADHD behaviours; however, fish consumption during pregnancy reduces this risk.

Bloomingdale et al. (2010) [28] investigated the consumption of fish by pregnant women. Twenty-two pregnant women in the Boston area who ate fewer than two servings of fish per week participated in five focus groups.The transcripts were examined via immersion-crystallization. Many women were aware that fish may contain mercury, a neurotoxin, and were advised to reduce their fish consumption. Fewer women were aware that fish contains DHA or the purpose of DHA. None of the women had been encouraged to eat fish, and most had no idea which species have the highest DHA content or the lowest mercury levels.

METHODOLOGY

Setting

The people of Andaman Islands consume a wide range of cuisine, including a lot of fruit and seafood. Healthcare, education& social services are all free, accessible & on par with those in industrialised nations. Other neurotoxic pollutants including lead, pesticides, and polychlorinated biphenyls (PCBs) do not constitute a serious threat to the general populace. Karang (Carangoidesgymnostethus), Shoemaker (Sigamussutor), Tuna (Thunnusalbacares), Mackerel (Rastrelligerkanagurta), and Barracuda are the most popular fish eaten in the Andaman Islands. Both MeHg& nutrient contents fluctuate substantially among and across species of all ocean fish [29].

Overall study design

The study's goal is to determine whether or not maternal diet and MeHg exposure together influence a child's educational outcomes.Blood samples were collected when the mother signed up, when she was 28 weeks pregnant, and when she gave birth. This was done to see how well she was eating during her pregnancy and when she gave birth. A 4-day food journal and a food consumption survey were used to examine the mother's diet at 28 weeks of pregnancy. The BSID-II or another measure of newborn cognitive and memory were administered to children in the same group at ages 5, 9, and 25.Researchers didn't know how much MeHg was in the participants' bodies when they took developmental data and biological samples, and lab workers didn't know how much MeHg was in the participants' hair or what their nutritional status was when they looked at those things. Family members of children in the cohort did not know how much MeHg was in the mother's hair or how each child was doing in school.

Power

It was determined that a sample size equal to about one-third of the test's SD was needed to detect a difference of 5 points on the BSID-II alone. On the basis of our information of previous Andamanian cohorts, we assumed that nearly half of this group's mothers would have a hair MeHg level of more than 5 ppm. The sample size of 250 patients was calculated to have 80% power to

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detect a 5-point difference between the low- and high-MeHg exposure groups using a two-sided test with a significance threshold of 0.05.

Participants

In 2019, only those Andamanian women over the age of 16 who went to their health Clinic for the first time were asked to take part. Once 300 volunteers agreed, enrollment has been finished. Maternal age on such enrolment varied among 16 or 43 years, & gestational age among 14 as well as 24 weeks. Four of these women weren't pregnant, and thirteen of their pregnancies ended in stillbirths. There were 283 babies who lived. Outside of Andaman Islands, two women gave birth, preventing the collection of biological samples at birth. Four infants with serious congenital defects and a set of twins accounted for the exclusion of six newborns. At 5, 9, 25, & 30 months of age, mothers and their children were evaluated again. At the first study visit, when the kids were 5 months old, 265 of them took part. At later visits, the number of kids who took part changed. After the 30-month evaluation, there were 230 children to complete covariate information (114 boys and 116 girls) who could be used for the primary analysis. Subjects lacking complete data did not differ substantially from the remainder of the cohort on any measure.

Developmental assessment

The study examined 16 neurodevelopmental endpoints. Children's development was primarily assessed using the BSID-II, which was administered to them at the ages of 9 and 30 months. The BSID-II is a widely accepted method of gauging a baby's development, with MDI and PDI as its two primary outcomes.More studies looked at specific parts of how babies learn and think.The Mean Fixation Duration or the Overall Novelty Preferential% are the two results from the Fagan Infantest, which gauges a person's level of interest in novelty. A test of visual recognition memory utilising the Visual Expectation Paradigm [30] produces two outcomes: a global mean reaction time & a global proportion of anticipatory saccades. Five & nine months of age were used for administering the FTII and VEXP, respectively. Working memory, planning, inhibition, & attention were all tested using the A-not-B [31] or DSA at 25 months. For each evaluation, the percentage of incorrect reaches and the % of lose-stay errors are displayed.Three paediatric nurses with training in neurodevelopment gathered the neurodevelopmental data.

Reliabilities

During BSID-II testing, first author (PWD) has been available to finish reliabilities for 5% of a cohort. The average degree of agreement ranged from 92.3% to 98%. 10% of the remaining BSID-II tests were utilised to evaluate inter-tester agreement.The range of the mean agreement was 85.9% to 93%.Every week, these dependencies were checked to make it more likely that the skill levels needed to run the BSID would stay high.

Maternal nutrition

We concentrated on a select group of nutrients that were contained in fish, could be found in the mother's blood, and were specifically related to the growth of the offspring [5]. At 28 weeks of pregnancy and at delivery, we examined the total lipids, which included phospholipids, in samples of maternal serum. The primary LCPUFA for brain development and growth, DHA & AA, were the subject of this study [9].The same approach mentioned in a related study was used to examine other LCPUFA. The geometric mean of prenatal and postnatal measurements of DHA and AA was used in this study.This is because the third trimester is when the majority of LCPUFA transfer from the mother's blood to the foetus occurs.The geometric mean is found by taking the average of the logarithms of the two important numbers and then putting that number back on the original scale by

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multiplying it by itself. Among the 230 people with otherwise complete information, 6% of the cohort did not have DHA and AA status at 28 weeks and 20% did not have it at birth. Before figuring out geometric methods, missing LCPUFA value systems for certain points in time were filled in as follows. 30 ml of blood were drawn from non-fasting moms at 28 weeks of pregnancy again and one day since giving birth. Antecubitalvenipuncture blood samples were placed in vacuum-sealed serum tubes, placed on water ice for thirty min, and then spun at 1000 g for 15 minutes. Small amounts of samples have been kept at 80.8% Celsius until they could be analysed. Biochemical studies of nutrients have been done at the University of Kolkata and other affiliated institutes. As soon as the samples were no longer frozen, they were put through the mass spectrometry and looked at. Methylation was done on lipid extracts, and a ThermoFinnegan TRACE MS with such a 30 m FAMEWAX capillary section with an inside diameter of 0.25 mm & a film thickness of 0.25 mm was used to measure them. At 28 weeks of pregnancy, either a radiometric immunoassay or even a competitive immunoassay was used to measure the mother's levels of TSH as well as free thyroxine (T4).To figure out the mother's Fe status, the soluble transferrin receptor and ferritin were used to figure out the total amount of Fe in her body. Before starting to give mothers supplements, which is common in the Andaman Islands, we checked their Fe status when they signed up. Using a 4-day meal diary and a food usage questionnaire (FUQ), we were able to determine how much fish women consumed. The purpose of the FUQ was to determine how frequently respondents consumed fish and meals containing fish over the course of two weeks. The survey was created in Andaman Islands and put to the test by nurses in prenatal clinics there. This was done to supplement the food diary information. Each woman finished a four-day food diary (two consecutive weekdays & two weekends) at 28 weeks of pregnancy to evaluate how many grammes of fish & fish products she consumed daily [32].All of the people in the study were shown how to fill out meal diaries by nurses who had been received training by nutritionists from University of Kolkata. In the food diaries, people had to write down how much food and drink they ate and drank in household units (like spoons, cups, etc.), decimal digits, and multiples of industrially packaged goods,& fractions or multiples of non-packaged goods. The patients were shown three different sizes of small wooden cubes to help figure out how much meat and fish they ate in mixed foods like stews and curries. Within a week of the end of the study, nurses checked the diaries and talked to the people about any mistakes or missing information. About 90% of people in the Andaman Islands can read and write. Depending on what the parents wanted, the diaries have been written in English. After they were done, the project team members went over them with the mothers. At the University of Kolkata, nutritionists with a lot of experience looked at dietary data. They used WISP version 2, which was improved with composition data for items used in the Andaman Islands, as well as local recipe and serving size data. From the food diaries, the estimated amount of choline in the mother's diet (mg/day) was figured out &utilized as an indirect estimate of choline status.

Dosimetry

We only used the average amount of mercury inside the mother's hair while she was pregnant as a biomarker for MeHg exposure before birth. There is a connection between how much mercury is found in a daughter's hair and how much is throughout her baby's brain. This link is thought to show the type of mercury that can cross the blood-brain barrier [33]. Mercury was found in hair samples taken from the mother while she was giving birth. If hair grows 1.1 centimetres per month, people used the sample of hair that would have covered the most of the pregnancy. Magos reagents were employed to find out how much total or inorganic Hg there was. The selected hair sample was measured or dissolved in 2 ml of such a 45% (w/v) solution of NaOH or 1 ml of a 1% (w/v) way to solve of cysteine at 95.8 degrees Celsius. Stannous chloride turned inorganic Hg into a vapour, and

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atomic absorption spectroscopy was used to figure out what it was. All of the organic and inorganic mercury was turned into vapour with the help of a reagent made of cadmium chloride and stannous chloride. By adding the 0.9% NaCI solution, the volume was increased to 10 ml. A Laboratory Control MeHg was tested with a Mercury Monitor Model 11235. The length of a hair sample was then used to figure out the average Hg. The Mercury Analytical Laboratory at the University of Kolkata did regular quality checks both inside and outside the lab.

Statistical analysis

Strategic Approach to Analysis

Using multiple regression, the relationship among the nutrition and diet of the mother, exposure to MeHg during pregnancy, and each of 16 neurodevelopmental outcomes has been looked at. Only models with such a total F-test p-value of 0.05 or less were looked at more closely and are shown in this section. p 0.05 was used to figure out how important the effects of each independent variable were in each model. Each test had two ends.

Primary and secondary analyses

In addition to examining the relationship among each outcome and the mean prenatal MeHg exposure, the primary analysis analysed 6 nutrition indicators: DHA, AA, TSH, Fe storage, maternal fish consumption & choline intake.In both the first & second models, no interactions were observed.There were also two secondary analyses that were done. The first looked at the covariable-adjusted connection between MeHg so each consequence without correcting for nutrition markers. It was a repeat of our earlier research. The second examined the relationship between each outcome & the 6 maternal nutrition and nutrition indicators, controlling for covariates but not for MeHg. Among 170 pregnant women whose LCPUFA levels were evaluated at 28 weeks of pregnancy and at birth, the average DHA level decreased from 0.20 mg/ml (S.D. = 0.07) to 0.17 mg/ml (S.D. = 0.07), whereas the average AA level decreased from 0.64 mg/ml (S.D. = 0.15) to 0.61 mg/ml (S.D. = 0.16).A single LCPUFA evaluation at a specific time point does not depict the average for the trimester because it is recognised that these declines occur during the third trimester. Based on the correlations between the two periods of observation, we were able to calculate an average value for the missing DHA & AA. Our working hypothesis was that the overall distribution of both LCPUFAs over time was bivariate normal on the logarithmic axis. The evidence suggests that this presumption was reasonable. The unknown LCPUFA concentration at the first time point was estimated to correspond to the predicted concentration based on the concentration at the second time point. In the next step of the evaluation, we calculated the geometric mean of the estimated & detected values.

Table 1: Nutrition and development statistics

Variable	N[a]	Mean	SD	Ranges
MeHg (measured in mothers' hair in ppm)	230	5.8	3.8	0.3-18.6
TSH @ 28 weeks (mlU/l)	230	1.3	0.7	0.2-3.8
Choline in mg/day (from diet diary)	230	224.3	82.7	27.4-538.9
Fish intake in g/day (from diet diary)	230	76.7	48.0	0-347.4
DHA (mg/ml)				
28 weeks gestation	217	0.20	0.07	0.08-0.5
Delivery	185	0.17	0.07	0.07-0.4
Geometric mean of 28 weeks and delivery[b]	230	0.18	0.06	0.08-0.4

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AA (mg/ml)				
28 weeks gestation	217	0.64	0.15	0.5-1.3
Delivery	185	0.61	0.16	0.4-1.3
Geometric mean of 28 weeks and delivery[b]	230	0.62	0.14	0.5-1.2
Fe (total body stores at enrollment in mg/kg bodyweight)	230	15.4	3.2	5.6-24.0
Bayley MDI 9 months	227	103.0	8.4	73-123
Bayley PDI 9 months	226	106.8	10.5	69-142
Bayley MDI 30 months	229	86.0	10.0	57-116
Bayley PDI 30 months	226	90.9	14.9	51-124
Fagan Fixation Duration 5 months (s)	216	2.4	0.8	0.9-5.4
Fagan % looking time 5 months	215	57.9	7.0	40.1-76.8
Fagan Fixation Duration 9 months (s)	221	1.7	0.5	0.9-3.0
Fagan % looking time 9 months	221	57.0	6.5	39.7-77.9
VEXP Mean Reaction Time 5 months (ms)	183	335.9	55.8	221.8-544.8
VEXP % Anticipatory Saccades 5 months	180	12.5	10.0	1.5-57.5
VEXP Mean Reaction Time 9 months (ms)	198	300.9	45.5	203.0-441.0
VEXP % Anticipatory Saccades 9 months	198	15.0	8.9	1.5-45.8
A not B % Correct (all trials)	219	69.0	9.5	41.5-97.5
A not B % Lose-Stay Errors	219	15.0	5.7	3.0-38.9
DSA % Correct (all trials)	186	61.7	8.0	25.5-96.5
DSA % Lose-Stay Errors	186	15.0	9.5	0-50.5

[a]Not all 229 subjects' complete data

[b]DHA and AA levels in maternal blood were measured at 28 weeks and at delivery.

Analysis of the 30-month PDI using tertiary data

The main study of a 30-month BSID-II PDI was clarified by various tertiary studies. The primary & secondary analyses for these studies were modified by: (1) switching from TSH to T4, a different biomarker for maternal iodine status (Model 1) & (2) omitting nutrient assessments from the mothers' diet diaries (choline & fish consumption); (3) displacing TSH with T4 but exclude choline or fish intake; and (4) displace TSH with T4 but exclude choline or fish intake; & (4) incorporating interactions of MeHg with environmental factors (Model 5). Under the assumption that DHA and AA were model covariates for all time periods, ten datasets were subjected to multiple imputation. To select samples from the posterior predictive distribution of a non-observed LCPUFA at each time point, we used MeHg, other nutritional & dietary condition indicator factors, other model covariables, or the BSID-II multidimensional & multistage imputation (MDI & PDI) at 9 & 30 months.For each imputed data set, we used a covariate in the regression model that was calculated as the geometric mean of each LCPUFA across the two time points.The 10 datasets with multiple imputations' regression estimates and standard errors were then combined for inference in the customary manner.

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RESULT

The average baby at birth weighed 3.24 kg, and the average amount of mercury in a mother's hair was 5.8 ppm (SD = 3.8, range = 0.3-18.6). The markers of mothers' nutritional and dietary status and their development are shown in Table 1 together with means (standard deviations), ranges, and developmental endpoints. The group had no clinical symptoms of nutritional deficits. Prenatal MeHg was weakly but significantly linked with mean DHA (r = 0.35, p = 0.0001) but not with mean AA (r = 0.07, p 0.27).Table 2 displays the associations between MeHg & each nutrition-related factor. Except for choline intake (r = 0.36, p= 0.0001) and prenatal MeHg (r = 0.07, p = 0.32), there was no association among maternal fish consumption and any of the dietary or nutritional status indicators.The average maternal fish consumption was 537 g per week, or nine fish-based foods per week.The averages of the developmental endpoints were within the normal range, and the differences between them were as expected. The average BSID-II MDI and PDI scores dropped by about one standard deviation among 9 & 30 months. The correlations among BSID scores at 9 months & 30 months on the MDI & PDI were insufficient at 0.12 & 0.27, respectively.In contrast, the correlations between the PDI & MDI at 9 months and 30 months were 0.35 and 0.57, respectively. This is consistent with our knowledge of standardised testing.

Table 2: Correlations of prenatal MeHg and maternal nutritional and dietary status

Nutritional and dietary status measure	Pearson r
AA (mean of 28 weeks and delivery in mg/ml)	0.074
DHA (mean of 28 weeks and delivery in mg/ml)	0.35
TSH @ 28 weeks (mlU/l)	-0.030
Choline in mg/day (from diet diary)	0.099
Fish intake in g/day (from diet diary)	0.069
Fe (total body stores at enrollment in mg/kg bodyweight)	-0.0069

The Primary & Secondary Regression Analyses' Results

Using a p-value of 0.05 as a cutoff, we found no correlation among prenatal MeHg exposure and any outcome.This was the case for all models that disregarded dietary intake & nutritional status indicators. The PDI at 30 months was one outcome for which there was a weak association between MeHg and PDI (p = 0.07). As with our previous analyses of the Andaman Island Child Development Study Main Cohort, these models were largely consistent with one another, in which we did not collect information on the mothers' dietary habits or nutritional status. There were no associations between food intake & nutritional status markers that did not account for MeHg and any outcome at any age. Statistical outliers appeared in many statistical analyses; they are discussed below. On the other hand, no regression analysis for the endpoints identified any influential points. Multiple collinearities were nonexistent as a result of the fact that all variance inflation factors were below than 2.0.

Five months

The primary analytical model of the VEXP failed the F-test in its entirety. It was significant how many saccades were anticipatory overall. Prenatal MeHg and the quantity of anticipatory saccades had no statistically significant relationship. There was a strong link between motherly TSH and the number of anticipatory saccades (p = 0.04).The number of anticipatory saccades decreased as the

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mother's TSH level increased, indicating that performance improved. A decrease of 0.22 points in the 5-month Percent Anticipatory Saccades was correlated with an increase of 1 mlU/l in the mother's TSH. After MeHg was taken into consideration in the secondary analysis, the relationship between TSH and Anticipatory Saccades was not shown to be significant. All other endpoints, both secondary and primary analyses showed that MeHg and any measures of motherly food intake and nutritional status either were not significant as well as did not have any real effects. At 9 months, neither the first nor the second models of a BSID-II MDI have been statistically significant. All of the BSID-II PDI's main as well as auxiliary models were statically important at p=0.01. The mother's diet, nutritional state, or the amount of mercury in her blood prior to giving birth were not statistically associated with the PDI. The first model showed that the PDI went up with low birthweight (p = 0.0008) but was higher throughout females than in males (p = 0.02). The secondary models showed that there was no statistically significant link between MeHg, food intake, or any other measure of nutritional status and the outcomes.The PDI analysis contained two outliers, but removing them had no impact on the outcomes. 25 months are equal to 3.1.3. No maternal nutrient, food consumption, or indicator of nutritional status was shown to have any meaningful influence on any endpoint at 25 months in either a primary or secondary model.

Thirty months

MeHg and the maternal food intake and nutritional status variables were not significant at 30 months of age in any of the total models for the primary or secondary analyses of the BSID-II MDI. The MDI at 30 months increased with both increased home stimulation (p = 0.0001) & increased birth wt. (p = 0.04). Table 3 summarises the significant outcomes from the primary & secondary analyses of the BSID-II PDI over 30 months.Prenatal MeHg exposure increased by 1 ppm when the PDI decreased by 0.55 points (p = 0.035, a negative effect). As stated previously, the association among MeHg & the PDI at 30 months was marginally significant (p =.07) when diet and nutritional status of the mother are considered. According to models fitted to the SCDS Main cohort, a 1-ppm rise in MeHg was associated with a 0.24-point reduction in PDI.According to Table 4, this was the only age-related outcome for which MeHg was significantly negative. In the first experiment, no connection could be seen between DHA (p = 0.34) & AA (p = 0.49) levels.On the other hand, these connections followed the expected pattern. The 30-month PDI increased 2.5 points when DHA levels increased by 0.1 mg/ml (p = 0.34), but dropped 0.6 points when AA levels increased by 0.1 mg/ml (p = 0.49). At 9 months, girls did better than boys on the PDI (p = 0.0001) the 30-month PDI went up as the age of the mother went up (p = 0.04)

Table 3: Regression coefficients (p values) * for the 30-month BSID PDI main analyses

Model component	**Primary analysis model**		**Secondary analysis**	
	All data, N = 226	**Excluding outlier, N = 225**	**MeHg Model (all data, N = 226)**	**Nutrition Model (all data , N = 226)**
Prenatal MeHg (ppm in maternal hair)	**-0.54 (0.03)**	**-0.60 (0.01)**	-0.45 (0.08)	-
DHA (mg/ml)	25.00 (0.35)	29.80 (0.25)	-	5.44 (0.83)
TSH (mlU/l)	-1.40 (0.36)	- 1.35 (0.35)	-	-1.25 (0.40)
AA (mg/ml)	-6.34 (0.50)	- 9.75 (0.30)	-	-2.95 (0.80)
Choline (mg/day)	0.01 (0.75)	0.01 (0.65)	-	0.004 (0.85)
Fish Intake (g/day)	0.02 (0.31)	0.03 (0.22)	-	0.02 (0.34)

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Iron (∑ body stores in mg/kg body weight)	0.07 (0.75)	0.15 (0.65)	-	0.07 (0.85)
Sex (girls)	**7.78 (<0.0001)**	**7.90 (<0.0001)**	**8.15 (<0.0001)**	**7.67 (<0.0001)**
Family status (<2 parents)	1.55 (0.38)	1.98 (0.25)	1.32 (0.42)	1.74 (0.31)
Maternal age (years)	**0.34 (0.03)**	**0.30 (0.05)**	**0.35 (0.02)**	**0.32 (0.05)**
Birth weight (g)	0.005 (0.07)	0.003 (0.10)	0.004 (0.07)	0.004 (0.08)
Maternal intelligence (K-BIT)	0.04 (0.60)	0.06 (0.38)	0.03 (0.68)	0.03 (0.70)

*Bold values for significant coefficients.

[a]In secondary analysis models, there were no outliers.

Table 4: Impacts of Methylmercury on endpoints of the Primary Model

Outcome	**Direction of improved score**	**N**	**Correlation with MeHg**	**MeHg coefficient in primary model**	**p***
5 months					
Mean Fixation Duration[a]	-	216	-0.001	0.01	0.41
Novelty preference	+	215	0.12	0.02	0.92
Mean Reaction Time[a]	-	183	- 0.01	- 0.005	0.19
Percent Anticipatory Saccades[a]	+	180	0.01	0.02	0.20
9 months					
MDI[b]	+	225	- 0.04	- 0.17	0.35
PDI[b]	+	226	0.09	- 0.18	0.40
Mean Fixation Duration[a]	-	221	- 0.14	- 0.005	0.44
Novelty preference	+	221	0.11	0.04	0.84
Mean Reaction Time[a]	-	198	- 0.06	- 0.0004	0.93
Percent Anticipatory Saccades[a]	+	198	0.06	0.003	0.92
25 months					
A not B percentage correct	+	217	- 0.02	- 0.04	0.79

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reaches					
A not B percentage lose stay errors	-	217	0.01	- 0.06	0.50
DSA percentage correct reaches	+	186	- 0.06	0.08	0.70
DSA percentage lose stay errors	-	186	0.05	- 0.20	0.37
30 months					
MDI[b]	+	228	0.03	- 0.16	0.34
PDI[b]	+	225	0.03	**- 0.55**	**0.04**

* Significant coefficients are bolded.

[a]Outcome variable transformed to log scale.

[b] Primary endpoints.

CONCLUSION

This study concludes that maternal diet and fish-specific nutrients may impede efforts to establish a link among prenatal MeHg exposure from fish consumption & later child outcomes. The functions played by MeHg & diet during childhood are not mutually exclusive, according to these findings. Consumption of fish containing MeHg may negate or reduce the protective effects of nutrients on the developing brain.These contradictory interpretations of our results merit additional research and, if validated, might have substantial implications for public health. The study included many assessments at multiple endpoints and several different outcomes. Only one statistical correlation between MeHg and any of the four major outcomes was discovered. Different findings could be found in later follow-up investigations of our sample. Some of these concerns may require a larger confirmatory study.

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