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Original Contribution

Maternal Fish Intake during Pregnancy, Blood Mercury Levels, and Child Cognition at Age 3 Years in a US Cohort

Emily Oken[1], Jenny S. Radesky[1], Robert O. Wright[2], David C. Bellinger[3], Chitra J. Amarasiriwardena[4], Ken P. Kleinman[1], Howard Hu[5], and Matthew W. Gillman[1,6]

[1] Department of Ambulatory Care and Prevention, Harvard Medical School and Harvard Pilgrim Health Care, Boston, MA.
[2] Department of Medicine, Children's Hospital Boston and Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.
[3] Department of Neurology, Boston Children's Hospital and Harvard Medical School, Boston, MA.
[4] Department of Environmental Health, Harvard School of Public Health, Boston, MA.
[5] Department of Environmental Health, University of Michigan, Ann Arbor, MI.
[6] Department of Nutrition, Harvard School of Public Health, Boston, MA.

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The balance of contaminant risk and nutritional benefit from maternal prenatal fish consumption for child cognitive development is not known. Using data from a prospective cohort study of 341 mother-child pairs in Massachusetts enrolled in 1999–2002, the authors studied associations of maternal second-trimester fish intake and erythrocyte mercury levels with children's scores on the Peabody Picture Vocabulary Test (PPVT) and Wide Range Assessment of Visual Motor Abilities (WRAVMA) at age 3 years. Mean maternal total fish intake was 1.5 (standard deviation, 1.4) servings/week, and 40 (12%) mothers consumed >2 servings/week. Mean maternal mercury level was 3.8 (standard deviation, 3.8) ng/g. After adjustment using multivariable linear regression, higher fish intake was associated with better child cognitive test performance, and higher mercury levels with poorer test scores. Associations strengthened with inclusion of both fish and mercury: effect estimates for fish intake of >2 servings/week versus never were 2.2 (95% confidence interval (CI): −2.6, 7.0) for the PPVT and 6.4 (95% CI: 2.0, 10.8) for the WRAVMA; for mercury in the top decile, they were −4.5 (95% CI: −8.5, −0.4) for the PPVT and −4.6 (95% CI: −8.3, −0.9) for the WRAVMA. Fish consumption of ≤2 servings/week was not associated with a benefit. Dietary recommendations for pregnant women should incorporate the nutritional benefits as well as the risks of fish intake.

child development; fatty acids, omega-3; fishes; mercury; pregnancy

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PPVT, Peabody Picture Vocabulary Test; SD, standard deviation; WRAVMA, Wide Range Assessment of Visual Motor Abilities.

Fish and other seafood may contain beneficial nutrients such as n-3 fatty acids as well as harmful contaminants such as mercury. Methylmercury is a demonstrated neurotoxicant to which the fetal brain is particularly sensitive (1, 2). A well-designed cohort study in the Faroe Islands found that prenatal exposure to organic mercury from maternal fish and pilot whale consumption during pregnancy was associated with subtle neurodevelopmental deficits in children, such as poorer performance on some tests of language and intelligence (3, 4). In a cohort from the Seychelle Islands, however, investigators did not find evidence for a neurodevelopmental risk from prenatal methylmercury exposure resulting from

Correspondence to Dr. Emily Oken, Department of Ambulatory Care and Prevention, 133 Brookline Avenue, Boston, MA 02215 (e-mail: emily_oken@harvardpilgrim.org).

ocean fish consumption (5). Those studies did not examine the overall relation of fish intake with child cognitive development. In 2001, the US Environmental Protection Agency recommended that pregnant women avoid consuming fish types high in mercury and limit their total fish intake to no more than two 6-ounce servings per week (1 ounce = 28.3 g) (6). Following the advisory, some pregnant women decreased their fish intake (7).

Fish and other seafood are the primary dietary source for elongated polyunsaturated n-3 fatty acids including docosahexaenoic acid (DHA), an integral structural component of the brain. Prenatal or early postnatal DHA supplementation may improve child development (8–11). Because most pregnant women do not consume adequate dietary DHA, limiting fish consumption might further reduce access to this essential nutrient (12, 13).

The overall effect of fish consumption during pregnancy, incorporating the risks as well as the benefits, remains uncertain. A few recent studies, including a reanalysis of data from the Faroe Islands cohort, have found that, on balance, maternal fish intake is associated with improved child cognitive development (14–16). In the present study, we used prospectively collected information on maternal diet and mercury levels during pregnancy to examine the risks and benefits of maternal prenatal fish intake on child development at age 3 years.

MATERIALS AND METHODS

Population and study design

Subjects were participants in Project Viva, a prospective prebirth cohort study in Massachusetts (13, 14). Mothers provided informed consent at study recruitment (1999–2002), and the human subjects committee of Harvard Pilgrim Health Care approved all protocols. Of 2,128 women who delivered a live singleton infant, 1,579 were eligible for the age-3-year visit because they had completed a prenatal dietary questionnaire and had not disenrolled. We had information on maternal fish intake, stored maternal blood samples, and cognitive test results for children aged 3 years for 896 mother-child pairs. Available funding allowed measurement of erythrocyte mercury from 341 mothers. We selected women with an available maternal hair sample ($n = 98$) and who experienced preterm or small-for-gestational-age birth ($n = 45$) to ensure that the prevalence of these characteristics in the group of women studied in the present analysis closely mirrored the prevalence in the overall cohort. We also assayed a random sample of 198 of the remaining 753.

Compared with the 1,238 mothers not included in this study, the 341 mothers included had similar fish intakes (1.5 vs. 1.7 servings/week) and were slightly older (32.6 vs. 31.9 years), were more likely to be White (82 vs. 65 percent), were better educated (41 vs. 30 percent with a graduate degree), were less likely to smoke (8 vs. 13 percent), and had higher Peabody Picture Vocabulary Test (PPVT) scores (108.8 vs. 104.6). For included children compared with those not included, gestational length (39.4 vs. 39.5 weeks) and fetal growth (0.24 vs. 0.18 (z value)) were similar, but breastfeeding duration was longer (7.0 vs. 6.0 months).

Exposures

Maternal fish intake. At the second-trimester study visit, participants completed a semiquantitative food frequency questionnaire, which we modified for pregnancy from a well-validated instrument used in several large cohort studies (17, 18). We previously calibrated this questionnaire against erythrocyte levels of elongated n-3 fatty acids (19). The questionnaire quantified average frequency of consumption of more than 140 foods and beverages during the previous 3 months, including four questions on intake of fish: canned tuna fish (3–4 ounces); shrimp, lobster, scallops, clams (1 serving); dark-meat fish (e.g., mackerel, salmon, sardines, bluefish, swordfish (3–5 ounces)); and other fish (e.g., cod, haddock, halibut (3–5 ounces)). Six frequency response options ranged from "never/less than 1 per month" to "1 or more servings per day." We combined responses to the four questions to estimate average total fish intake.

Red blood cell mercury. At the second-trimester visit, we obtained blood specimens in Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey) containing ethylenediaminetetraacetic acid. We centrifuged tubes at 2,000 rpm for 10 minutes at 4°C to separate plasma from erythrocytes, which we then washed with chilled saline. We stored erythrocyte aliquots at −70°C. Because of inhomogeneity of the erythrocyte sample, we diluted the thawed sample 1:1 by weight with deionized water, froze samples overnight, then centrifuged thawed samples at 30,000 rpm for 30 minutes at 20°C (L8-M Ultracentrifuge with Ti-70; Beckman Coulter, Inc., Fullerton, California) to remove cell membranes. We performed total mercury assays by using the Direct Mercury Analyzer 80 (Milestone Inc., Monroe, Connecticut). Results were reported as mercury content in the original red cell sample. The detection limit is 0.5 ng/ml of sample, and the percentage recovery for quality control standards is about 90–110 percent. Reproducibility for duplicate analysis was approximately 20 percent.

Fatty acids. We summed contributions to intake of the elongated marine fatty acids DHA and eicosapentaenoic acid (EPA) from all foods and supplements. We also estimated intake of DHA + EPA from fish alone. To obtain estimates of fatty acid content, we used the Harvard nutrient database, which is based on US Department of Agriculture publications as well as other published sources and personal communications, and it has been used in other studies of n-3 fatty acid intake (13, 20, 21).

We also analyzed the fatty acid content of packed maternal erythrocytes with gas-liquid chromatography following a single thaw, to determine levels of DHA and EPA. Methods have been described in detail elsewhere (22, 23). Coefficients of variation were 6.5 percent for DHA and 4 percent for EPA.

Cognitive outcomes in children aged 3 years

Trained research assistants administered two cognitive tests to enrolled children in either the child's home or a research office. The PPVT evaluates receptive vocabulary among children aged 2 years or older based on a nationally stratified reference sample (24). PPVT scores are strongly correlated ($r \geq 0.90$) with verbal and full-scale intelligence quotient on the Wechsler Intelligence Scale for Children-III (24). Mothers also completed the PPVT. The Wide Range Assessment of Visual Motor Abilities (WRAVMA) (25) evaluates three domains of visual motor development: visual-spatial (matching test), visual-motor (drawing test), and fine-motor skills (pegboard test), which are used to generate a total standard score. This test has norms for children aged 3 years or older, is moderately correlated with intelligence quotient ($r \sim 0.60$), and is sensitive to other neurotoxicants such as lead (25, 26). We calculated child age at testing in months and days.

Covariates

Using interviews and questionnaires, we collected self-reported maternal race/ethnicity, age, education, parity, smoking, alcohol consumption, marital status, household income, prepregnancy weight and height, and paternal education. We determined a Western diet score according to intake of red and processed meats, refined grains, desserts, and snack foods and a prudent diet score according to intake of fruits, vegetables, legumes, fish, and poultry (27). We obtained child sex, birth weight, and delivery date from the medical record. We determined birth-weight-for-gestational-age z value (''fetal growth'') based on US national reference data (28). We collected hair samples at delivery from 211 mothers (14), of whom 98 were included in the present analysis. On postpartum questionnaires, mothers reported duration of breastfeeding and the child's primary language. We obtained clinical screening lead levels for 139 children.

Statistical analysis

We studied bivariate associations of participant characteristics with maternal fish intake by using t tests and chi-square analysis. We examined associations among the primary exposures by using Spearman's correlation and simple linear regression.

We used multivariate linear regression to examine associations of participant characteristics and exposures of interest with child cognitive test scores. Because current guidelines (29) recommend that pregnant women consume up to two servings a week of fish and shellfish, we categorized fish intake as never, $\leq$2, and $>$2 servings per week. We categorized mercury at the top decile or below because our measure of total mercury in packed erythrocytes does not easily translate to categorization according to the benchmark dose for whole-blood mercury (2). We analyzed associations of fish intake and mercury levels individually with each outcome, and then we included both measures in prediction models.

We began collecting maternal hair samples in February 2002 and continued through the end of the study. During this period, 409 participants delivered, of whom 302 were approached for collection of a hair sample and 34 were ineligible (hair too short or in braids); 211 of the remaining 270 (78 percent) consented to provide a hair sample. In the subset of mothers whose hair was collected at delivery, 10 percent had hair mercury levels of $>$1.2 ppm (14). Thus, we anticipate that erythrocyte mercury in the top decile of our population likely corresponds to a hair mercury level of $>$1.2 ppm, the level used to determine the US Environmental Protection Agency reference dose (2). Ninety-eight women with hair mercury results also had blood available for mercury assay.

In secondary analyses, we studied fish intake and mercury levels simultaneously, using the following five categories: low mercury/fish intake $>$2 weekly servings; low mercury/fish intake $\leq$2 weekly servings; mercury top decile/fish intake $>$2 weekly servings; mercury top decile/fish intake $\leq$2 weekly servings; and never fish intake. Exclusion of the one woman who reported never consuming fish but was in the top decile of mercury levels did not alter results. We also studied intake of canned tuna fish, fish types excluding canned tuna, and fish types excluding shellfish. We also modeled dietary and erythrocyte levels of DHA + EPA and maternal blood mercury as continuous predictors.

We included covariates that were of a priori interest as independent predictors of child cognition, namely, maternal age, prepregnancy body mass index, prenatal smoking and alcohol consumption, race/ethnicity, marital status, and education; paternal education; and child sex, fetal growth, gestation length, duration of breastfeeding, primary language, and age at testing. We modeled all covariates as presented in tables 1 and 2; we also included missing categories for maternal education (2 percent), smoking (1 percent), and paternal education (5 percent). Additional adjustment for household income, maternal Western or prudent dietary pattern, depression at 6 months postpartum, child body mass index, or test administrator did not substantially change estimates for fish or mercury, so we did not include these factors in our final models. We performed all analyses by using SAS version 9.1 software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Mean maternal fish intake was 1.5 (standard deviation (SD), 1.4; range, 0–7.5) weekly servings, and 40 (12 percent) mothers consumed more than two weekly fish servings, whereas 47 (14 percent) never consumed fish (table 1). Mean erythrocyte total mercury was 3.8 (SD, 3.8; range, 0.03–21.9) ng/g, with 35 mothers above the 90th percentile of 9.1 ng/g. Mean child age at testing was 38.4 (SD, 2.2) months, and child test scores were 105.7 (SD, 13.8) for the PPVT, 99.9 (SD, 10.3) for drawing, 99.8 (SD, 10.3) for pegboard, 107.8 (SD, 14.1) for matching, and 103.2 (SD, 10.5) for the WRAVMA total score.

Maternal fish intake was directly correlated with erythrocyte total mercury (Spearman's $r = 0.42$, $p < 0.0001$), with an unadjusted increase of 0.94 (95 percent confidence interval (CI): 0.66, 1.21) ng/g of mercury for each weekly fish serving. The likelihood of being in the top decile of erythrocyte mercury was 2 percent for those who never consumed

TABLE 1. Parental and child characteristics* according to maternal second-trimester fish intake among 341 mother-child pairs in Project Viva, Massachusetts, 1999–2002

	Overall (n = 341)	Maternal second-trimester fish intake		
		Never (n = 47; 14%)	≤2 servings/week (n = 254; 74%)	>2 servings/week (n = 40; 12%)
Maternal characteristics				
Age (years)	32.6 (4.7)	31.7 (4.8)	32.8 (4.6)	32.3 (4.7)
Race/ethnicity				
Black	6	2	7	8
Hispanic	2	0	3	3
Other	9	13	9	5
White	82	85	82	85
Education				
High school	6	6	5	8
Some college	14	13	15	10
College graduate	40	49	37	43
Graduate degree	41	32	43	40
Partner status				
Married/cohabiting	96	96	96	93
Single	4	4	4	8
PPVT† score	108.8 (14.3)	109.0 (16.4)	109.5 (13.9)	103.6 (14.2)
Smoking in pregnancy				
Former smoker	22	24	21	23
During pregnancy	8	17	6	10
Never	70	59	73	67
Alcohol consumption				
Any during pregnancy	76	81	76	70
None	24	19	24	30
Prepregnancy body mass index (kg/m^2)				
<25	70	77	70	65
25–<30	20	17	19	30
≥30	10	6	11	5

Table continues

fish but 23 percent for those who consumed fish more than twice weekly (table 1). Otherwise, maternal or child characteristics did not differ significantly according to maternal fish intake ($p > 0.05$ for all characteristics in table 1). Among 98 mothers for whom data were available, mean hair mercury level was 0.53 (SD, 0.47; range, 0–2.3) mcg/g. Hair mercury was correlated with erythrocyte total mercury (Spearman's $r = 0.46$, $p < 0.0001$) and with fish intake (Spearman's $r = 0.49$, $p < 0.0001$). An increase of 1 ppm of hair mercury was associated with an increase of 4.5 (95 percent CI: 3.3, 5.8) ng/g in erythrocyte mercury.

Participant characteristics were generally associated with child test scores in the anticipated directions (table 2). For example, scores were higher among children who were born with higher fetal growth (birth weight adjusted for gestational age), were girls, were firstborn, and were older at testing (table 2).

After adjustment for parent and child characteristics, maternal fish intake of >2 weekly servings, compared with never, was directly associated with higher child WRAVMA drawing and total scores (table 3). Associations strengthened with adjustment for mercury levels, with the largest effects seen for the WRAVMA drawing (6.4, 95 percent CI: 2.1, 10.7) and total (6.4, 95 percent CI: 2.0, 10.8) scores, and generally positive associations were also found on the other tests (table 3 and figure 1). We saw no evidence for an advantage of fish consumption at or below two weekly servings compared with never (table 3). Exclusion of the four participants who reported taking prenatal fish oil supplements did not materially change results. The interaction between fish intake and breastfeeding duration was not significant ($p = 0.08$ for the PPVT model and $p = 0.38$ for the WRAVMA model).

The 28 mothers (8 percent) who reported eating canned tuna at least twice weekly had children with higher scores on the PPVT (3.7, 95 percent CI: −0.9, 8.3) and WRAVMA total (5.6, 95 percent CI: 1.4, 9.8) compared with the 130

TABLE 1. Continued

	Overall (*n* = 341)	Maternal second-trimester fish intake		
		Never (*n* = 47; 14%)	≤2 servings/week (*n* = 254; 74%)	>2 servings/week (*n* = 40; 12%)
Mercury level (ng/g)	3.8 (3.8)	1.9 (2.3)	3.9 (3.8)	5.6 (4.5)
Mercury top decile	10	2	10	23
DHA† + EPA† from fish (mg/day)	128 (128)	0 (0)	122 (97)	318 (160)
DHA + EPA total from diet (mg/day)	149 (154)	22 (77)	148 (142)	301 (159)
Paternal and child characteristics				
Paternal education				
High school	23	27	21	27
College graduate	41	33	42	41
Graduate degree	36	40	36	32
Birth order				
Firstborn	54	66	54	43
Not firstborn	46	34	46	58
Gestation length (weeks)	39.4 (1.8)	39.2 (2.5)	39.5 (1.7)	39.4 (1.4)
Fetal growth (*z* value)	0.24 (1.0)	0.29 (0.9)	0.23 (1.0)	0.25 (1.0)
Sex				
Boy	49	45	52	38
Girl	51	55	48	63
Breastfeeding (months)	7.0 (4.5)	7.2 (4.4)	7.0 (4.5)	6.8 (4.7)
Age at outcome (months)	38.4 (2.2)	38.9 (3.2)	38.4 (2.0)	38.5 (1.9)
Primary language				
English	95	98	94	93
Other	5	2	6	8

* Expressed as mean (standard deviation) or percentage. Some percentages do not total 100 because of rounding.

† PPVT, Peabody Picture Vocabulary Test; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

mothers (38 percent) who reported never eating tuna fish. The 11 mothers (3 percent) who reported consuming more than two weekly servings of fish other than canned tuna had children with higher scores on the WRAVMA total (6.1, 95 percent CI: −0.7, 12.8) but not on the PPVT (−1.4, 95 percent CI: −8.9, 6.1) compared with the 97 (28 percent) who reported eating no fish excluding tuna. Effect estimates for intake of more than 2 weekly servings of the three fish types excluding shellfish were 4.3 (95 percent CI: −0.5, 9.0) for the PPVT and 5.9 (95 percent CI: 1.6, 10.3) for the WRAVMA total score.

Among children for whom we obtained clinical lead results, lead levels were not correlated with either maternal fish consumption (Spearman's $r = -0.01$, $p = 0.88$) or mercury levels (Spearman's $r = 0.03$, $p = 0.76$). Additional adjustment for lead level in multivariable models did not alter effect estimates for fish or mercury on child cognitive test scores (results not shown).

Mean intake of DHA + EPA from fish was 128 (SD, 128; range, 0–843) mg/day, and mean intake from all sources was 149 (SD, 154; range, 0–1,605) mg/day. For each 100 mg of maternal daily DHA + EPA intake from fish, children had PPVT scores that were 0.5 (95 percent CI: −0.5, 1.5) points higher and WRAVMA total scores that were 1.1 (95 percent CI: 0.1, 2.0) points higher. Neither intake of DHA + EPA from all dietary sources nor the DHA + EPA content of maternal erythrocytes was associated with child cognition (data not shown).

Higher maternal erythrocyte mercury levels were associated with worse child test performance, with stronger associations after adjustment for fish intake (table 4). We observed the strongest adverse associations of mercury levels with the PPVT (−4.5, 95 percent CI: −8.5, −0.4), WRAVMA matching (−6.0, 95 percent CI: −10.9, −1.1), and WRAVMA total (−4.6, 95 percent CI: −8.3, −0.9) tests, with associations that were somewhat less strong but still suggestive of an inverse relation for the WRAVMA drawing and pegboard tests (table 4 and figure 1).

We next examined maternal fish intake and mercury levels simultaneously. Compared with children whose mothers reported never consuming fish, children of women with mercury levels below the top decile but fish intake above two weekly servings had higher WRAVMA total scores (5.9 points, 95 percent CI: 1.0, 10.9). Children whose mothers

TABLE 2. Associations of parental and child characteristics with child test scores at age 3 years, Project Viva, Massachusetts, 1999–2002

	PPVT*		WRAVMA*: total score	
	Beta coefficient†	95% CI*	Beta coefficient†	95% CI
Maternal characteristics				
Age (years)	0.4	0.1, 0.7	0.6	0.1, 1.1
Race/ethnicity				
Black	−11.2	−16.8, −5.6	−2.3	−7.9, 3.3
Hispanic	−15.4	−24.0, −6.8	−7.7	−16.2, 0.7
Other	−1.6	−6.3, 3.1	1.5	−2.7, 5.8
White	Referent			
Education				
High school	−7.4	−14.9, 0.2	−5.5	−12.4, 1.3
Some college	−3.0	−7.4, 1.4	−1.5	−5.6, 2.5
College graduate	−3.3	−6.1, −0.5	−1.9	−4.5, 0.7
Graduate degree	Referent		Referent	
Partner status				
Married/cohabiting	Referent		Referent	
Single	6.4	−10.3, 23.2	−4.8	−20.0, 10.5
PPVT score	0.12	0.02, 0.23	0.0	0.0, 0.1
Smoking in pregnancy				
Former smoker	−3.2	−6.2, −0.3	1.2	−1.5, 3.9
During pregnancy	−0.6	−5.5, 4.2	2.9	−1.7, 7.4
Never	Referent		Referent	
Alcohol consumption				
Any during pregnancy	2.8	−0.2, 5.8	0.8	−2.0, 3.6
None	Referent		Referent	
Prepregnancy body mass index (kg/m^2)				
<25	Referent		Referent	
25–<30	−1.9	−4.9, 1.2	−1.2	−4.0, 1.6
≥30	5.9	1.7, 10.0	0.1	−3.7, 3.9
Paternal and child characteristics				
Paternal education				
High school	−3.4	−7.2, 0.4	−3.5	−7.0, 0.1
College graduate	−1.2	−4.1, 1.7	1.5	−1.2, 4.1
Graduate degree	Referent		Referent	
Birth order				
Firstborn	4.8	2.1, 7.4	0.9	−1.5, 3.3
Not firstborn	Referent		Referent	
Gestation length (weeks)	0.05	−0.6, 0.7	−0.1	−0.7, 0.6
Fetal growth (*z* value)	2.4	1.1, 3.7	0.8	−0.4, 2.0
Sex				
Boy	Referent		Referent	
Girl	1.5	−0.9, 4.0	4.5	2.3, 6.8
Breastfeeding (months)	0.1	−0.2, 0.4	−0.1	−0.4, 0.2
Age at outcome (months)	0.7	0.1, 1.3	0.6	0.1, 1.1
Primary language				
English	Referent		Referent	
Other	−11.6	−18.0, −5.3	−2.2	−8.1, 3.6

* PPVT, Peabody Picture Vocabulary Test; WRAVMA, Wide Range Assessment of Visual Motor Abilities; CI, confidence interval.

† Estimates were obtained from multivariable linear regression models, adjusted for all characteristics in the table and also for maternal fish intake and mercury levels.

TABLE 3. Associations of maternal fish intake during pregnancy* with child cognitive test results at age 3 years among 341 mother-child pairs in Project Viva, Massachusetts, 1999–2002

Cognitive test and maternal fish intake	Unadjusted mean score	Adjusted for child age and sex		Multivariable†		Additionally adjusted for erythrocyte mercury	
		Beta	95% CI‡	Beta	95% CI	Beta	95% CI
PPVT							
>2 servings/week	106.3	−1.5	−7.3, 4.4	1.2	−3.5, 6.0	2.2	−2.6, 7.0
≤2 servings/week > never	105.2	−2.2	−6.5, 2.2	−2.1	−5.7, 1.4	−1.8	−5.4, 1.8
Never	107.5	0	Referent	0	Referent	0	Referent
WRAVMA‡ drawing							
>2 servings/week	104.0	5.1	0.9, 9.4	6.0	1.8, 10.2	6.4	2.1, 10.7
≤2 servings/week > never	99.5	1.3	−1.8, 4.5	1.2	−2.0, 4.4	1.3	−1.8, 4.5
Never	98.5	0	Referent	0	Referent	0	Referent
WRAVMA pegboard							
>2 servings/week	103.2	3.2	−1.2, 7.5	2.9	−1.4, 7.1	3.5	−0.8, 7.8
≤2 servings/week > never	99.2	−0.5	−3.7, 2.6	−0.7	−3.9, 2.4	−0.5	−3.7, 2.7
Never	100.1	0	Referent	0	Referent	0	Referent
WRAVMA matching							
>2 servings/week	107.9	0.6	−5.4, 6.5	2.8	−3.1, 8.6	4.1	−1.8, 10.0
≤2 servings/week > never	108.0	1.4	−3.1, 5.8	1.8	−2.6, 6.3	2.3	−2.1, 6.7
Never	107.0	0	Referent	0	Referent	0	Referent
WRAVMA total score							
>2 servings/week	106.4	3.7	−0.7, 8.1	5.3	0.9, 9.6	6.4	2.0, 10.8
≤2 servings/week > never	102.8	0.7	−2.5, 4.0	1.1	−2.2, 4.4	1.5	−1.8, 4.7
Never	100.1	0	Referent	0	Referent	0	Referent

* Of 341 participants, 47 (14%) reported never consuming fish, 254 (74%) reported ≤2 weekly servings, and 40 (12%) reported >2 weekly servings.

† Adjusted for child sex, age at testing, fetal growth, gestation length, breastfeeding duration, birth order, and primary language; maternal Peabody Picture Vocabulary Test (PPVT) score, age, prepregnancy body mass index, race/ethnicity, education, marital status, and alcohol consumption and smoking during pregnancy; and paternal education.

‡ CI, confidence interval; WRAVMA, Wide Range Assessment of Visual Motor Abilities.

consumed more than two weekly fish servings and whose mercury levels were in the top decile also had somewhat higher WRAVMA scores, whereas children of mothers who consumed up to two weekly servings of fish and had mercury levels in the top decile had somewhat lower WRAVMA scores (table 5).

DISCUSSION

In this US cohort with moderate levels of fish consumption, pregnant women who ate more fish had higher erythrocyte mercury levels. Among their children, higher prenatal mercury exposure was associated with lower developmental test scores at age 3 years. Nevertheless, we observed no overall adverse effect upon child development with higher maternal fish intake. Rather, maternal fish intake more than twice a week was associated with improved performance on tests of language and visual motor skills.

These results support our earlier findings among a smaller subset of our study population that we assessed at age 6 months with a single outcome (14). The present results derive from a larger sample of children whom we assessed at age 3 years with well-validated developmental tests that are correlated with intelligence quotient. Our findings show broadly consistent results for tests of language as well as visual-spatial, visual-motor, and fine-motor skills, suggesting that the benefit of fish consumption spans these developmental domains.

These results also extend findings from other populations that have suggested benefits from maternal prenatal n-3 fatty acid or fish intake. Children of women randomized to receive prenatal fish oil supplementation had higher mental processing scores and better eye-hand coordination (8, 9). In a recent publication of data from the United Kingdom, Hibbeln et al. (15) found that children of mothers who ate three or more fish servings per week were less likely to have suboptimal scores on tests of intelligence quotient and behavior at 7–8 years of age, but the authors did not observe any benefit of seafood intake below compared with above three servings per week. In that study, there was no measure of mercury, although, in an earlier publication from a subset

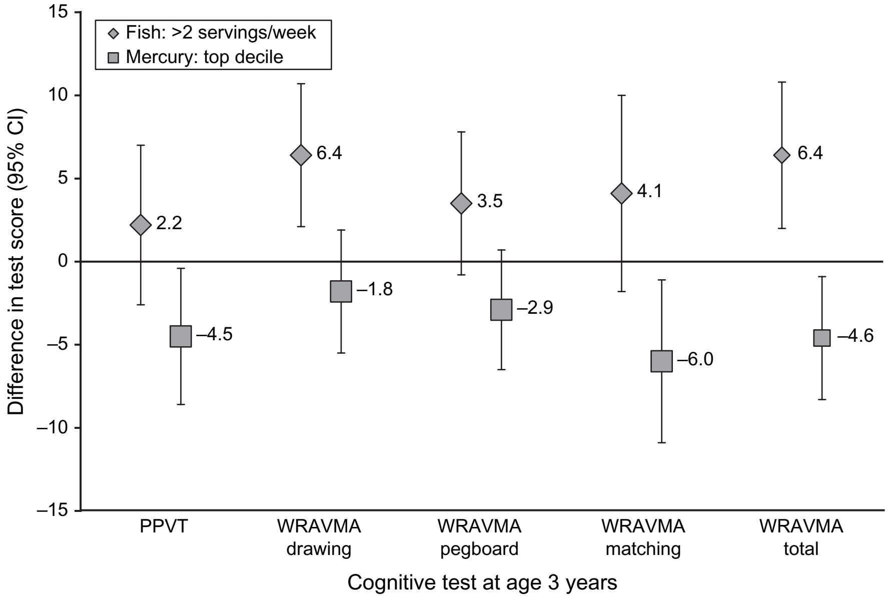

FIGURE 1. Associations of maternal second-trimester fish intake (>2 weekly servings vs. never) and erythrocyte mercury levels (top decile vs. below) with child cognitive test results at age 3 years, Project Viva, Massachusetts, 1999–2002. Effect estimates were adjusted for each other as well as for parent and child characteristics. CI, confidence interval; PPVT, Peabody Picture Vocabulary Test; WRAVMA, Wide Range Assessment of Visual Motor Abilities.

of the same population, levels of mercury in umbilical cord tissue were not associated with development in infancy (30). Additionally, in a reanalysis of data from the Faroe Islands cohort, whose seafood intake and mercury exposure are much higher, Budtz-Jorgensen et al. (16) reported that maternal fish intake during pregnancy was associated with somewhat higher performance on all seven outcomes studied at ages 7 and 14 years. Similar to our findings, in that study the effects of fish and mercury were each strengthened with mutual adjustment.

We observed associations of mercury levels with child cognition at exposure levels substantially lower than in populations previously studied. Our findings suggest that no lower threshold exists for the adverse effects of prenatal mercury exposure.

In previous cohort studies, biomarkers of mercury exposure included mercury in maternal hair collected at or after delivery (4, 5, 14, 31) and in umbilical cord blood (4). In Project Viva, we did not retain cord blood erythrocytes, and we collected hair from a small number of mothers. We measured total mercury in maternal erythrocytes based on evidence that more than 90 percent of the total blood mercury resides in the erythrocytes (2) and that about 95 percent of the mercury in red blood cells is methylmercury, with some of the inorganic mercury derived from demethylated methylmercury (32). Maternal first- or second-trimester blood mercury is highly correlated with blood mercury collected at delivery and with cord blood levels (33–37). Thus, good evidence exists that the sample we used is a suitable proxy for fetal methylmercury exposure.

Advantages of the present study include assessment of maternal diet during pregnancy with a validated dietary questionnaire, well-established outcome measures, and measurement of a number of parent and child characteristics, including maternal PPVT scores. Although women in our population were generally well educated and were employed, their fish and dietary DHA intake was similar to that reported in other general populations in North America (12, 38, 39). Nevertheless, this study has several limitations. It is possible that unmeasured confounding may account for at least part of the observed findings. In particular, we did not assess home environment. We did not measure other contaminants that may be found in fish, such as polychlorinated biphenyls. Accounting for the harms of polychlorinated biphenyls would be expected to suggest an even stronger benefit of the nutrients in fish but might weaken estimates for mercury. It is possible that women in other populations who consume fish types with different amounts of nutrients and contaminants may experience less of a benefit or even perhaps overall harm from higher prenatal fish intake. In addition, we did not measure other neurodevelopmental domains, such as overall intelligence, that might be differently associated with the nutrients or toxicants in fish.

We previously reported that mothers in Project Viva consumed less fish after dissemination of a federal mercury advisory in January 2001 and reduced their intake of even those fish types not included in the advisory (7). Subsequent advisories and expert reviews have emphasized the nutritional benefits of fish (29, 40), but they have continued to recommend that pregnant women consume no more than

TABLE 4. Associations of maternal second-trimester erythrocyte mercury levels with child cognitive test results at age 3 years among 341 mother-child pairs in Project Viva, Massachusetts, 1999–2002

	Unadjusted mean score	Adjusted for child age and sex		Multivariable*		Additionally adjusted for fish intake	
		Beta	95% CI†	Beta	95% CI	Beta	95% CI
PPVT							
Mercury top decile	100.9	−5.3	−10.1, −0.5	−4.0	−8.0, 0.05	−4.5	−8.5, −0.4
Mercury <90th percentile	106.2	0	Referent	0	Referent	0	Referent
Per ng/g of mercury		−0.5	−0.9, −0.2	−0.4	−0.7, −0.05	−0.4	−0.8, −0.1
WRAVMA† drawing							
Mercury top decile	99.7	−0.2	−3.8, 3.4	−0.7	−4.4, 3.0	−1.8	−5.5, 1.9
Mercury <90th percentile	99.9	0	Referent	0	Referent	0	Referent
Per ng/g of mercury		0.2	−0.04, 0.5	0.2	−0.1, 0.5	0.1	−0.2, 0.4
WRAVMA pegboard							
Mercury top decile	99.3	−0.5	−4.1, 3.0	−2.2	−5.8, 1.4	−2.9	−6.5, 0.7
Mercury <90th percentile	99.8	0	Referent	0	Referent	0	Referent
Per ng/g of mercury		0.2	−0.04, 0.5	0.1	−0.2, 0.4	0.03	−0.3, 0.3
WRAVMA matching							
Mercury top decile	102.1	−6.3	−11.1, −1.5	−5.4	−10.2, −0.5	−6.0	−10.9, −1.1
Mercury <90th percentile	108.5	0	Referent	0	Referent	0	Referent
Per ng/g of mercury		−0.2	−0.6, 0.1	−0.1	−0.5, 0.3	−0.2	−0.6, 0.2
WRAVMA total							
Mercury top decile	100.1	−3.4	−7.0, 0.2	−3.5	−7.2, 0.2	−4.6	−8.3, −0.9
Mercury <90th percentile	103.5	0	Referent	0	Referent	0	Referent
Per ng/g of mercury		0.05	−0.2, 0.3	0.03	−0.3, 0.3	−0.06	−0.4, 0.2

* Adjusted for child sex, age at testing, fetal growth, gestation length, breastfeeding duration, birth order, and primary language; maternal Peabody Picture Vocabulary Test (PPVT) score, age, prepregnancy body mass index, race/ethnicity, education, marital status, and alcohol consumption and smoking during pregnancy; and paternal education.

† CI, confidence interval; WRAVMA, Wide Range Assessment of Visual Motor Abilities.

two weekly fish servings (29). Our finding that the benefit of fish intake is strengthened with adjustment for mercury levels suggests that if mercury contamination were not present, the cognitive benefits of fish intake would be greater. Maternal consumption of fish lower in mercury and reduced environmental mercury contamination would allow for stronger benefits of fish intake. Recommendations for fish consumption during pregnancy should take into account the nutritional benefits of fish as well as the potential harms from mercury exposure.

TABLE 5. WRAVMA* total score for children aged 3 years according to maternal prenatal fish intake and mercury levels, Project Viva, Massachusetts, 1999–2002

Fish intake	Mercury ≤90th percentile			Mercury top decile		
	No.	Estimate†	95% CI*	No.	Estimate†	95% CI
>2 weekly servings	31	5.9	1.0, 10.9	9	4.1	−3.4, 11.7
≤2 weekly servings	229	1.8	−1.8, 5.3	25	−4.2	−9.6, 1.2
Never	47	0	Referent			

* WRAVMA, Wide Range Assessment of Visual Motor Abilities; CI, confidence interval.

† Adjusted for child sex, age at testing, fetal growth, gestation length, breastfeeding duration, birth order, and primary language; maternal Peabody Picture Vocabulary Test score, age, prepregnancy body mass index, race/ethnicity, education, marital status, and alcohol consumption and smoking during pregnancy; and paternal education.

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REFERENCES

1. Amin-Zaki L, Elhassani S, Majeed MA, et al. Intra-uterine methylmercury poisoning in Iraq. Pediatrics 1974;54:587–95.
2. Goyer R, Aposhian V, Arab L, et al. Toxicological effects of methylmercury. Washington, DC: National Academy Press, 2000.
3. Grandjean P, Weihe P, Burse VW, et al. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol Teratol 2001;23:305–17.
4. Debes F, Budtz-Jorgensen E, Weihe P, et al. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. Neurotoxicol Teratol 2006;28:536–47.
5. Myers GJ, Davidson PW, Cox C, et al. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 2003;361:1686–92.
6. Center for Food Safety and Applied Nutrition, US Food and Drug Administration. Consumer advisory: an important message for pregnant women and women of childbearing age who may become pregnant about the risks of mercury in fish. March 2001. (http://vm.cfsan.fda.gov/~dms/admehg.html). (Accessed July 17, 2007).
7. Oken E, Kleinman KP, Berland WE, et al. Decline in fish consumption among pregnant women after a national mercury advisory. Obstet Gynecol 2003;102:346–51.
8. Helland IB, Smith L, Saarem K, et al. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:e39–44.
9. Dunstan JA, Simmer K, Dixon G, et al. Cognitive assessment of children at age 2 1/2 years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 2008;93:F45–50.
10. Werkman SH, Carlson SE. A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until nine months. Lipids 1996;31:91–7.
11. Willatts P, Forsyth JS, DiModugno MK, et al. Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. Lancet 1998;352:688–91.
12. Denomme J, Stark KD, Holub BJ. Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations. J Nutr 2005;135:206–11.
13. Oken E, Kleinman KP, Olsen SF, et al. Associations of seafood and elongated n-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. Am J Epidemiol 2004;160:774–83.
14. Oken E, Wright RO, Kleinman KP, et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. Environ Health Perspect 2005;113:1376–80.
15. Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578–85.
16. Budtz-Jorgensen E, Grandjean P, Weihe P. Separation of risks and benefits of seafood intake. Environ Health Perspect 2007;115:323–7.
17. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of an expanded self-administered semi-quantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
18. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
19. Fawzi WW, Rifas-Shiman SL, Rich-Edwards JW, et al. Calibration of a semi-quantitative food frequency questionnaire in early pregnancy. Ann Epidemiol 2004;14:754–62.
20. US Department of Agriculture, Agricultural Research Service. USDA nutrient database for standard reference, release 13. Nutrient Data Laboratory Home Page. (http://www.ars.usda.gov/main/site_main.htm?modecode=12354500).
21. Hu FB, Bronner L, Willett WC, et al. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 2002;287:1815–21.
22. Sun Q, Ma J, Campos H, et al. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr 2007;86:74–81.
23. Baylin A, Kim MK, Donovan-Palmer A, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. Am J Epidemiol 2005;162:373–81.
24. Dunn LM, Dunn LM. Examiner's manual for the Peabody Picture Vocabulary Test. 3rd ed. Circle Pines, MN: American Guidance Service, 1997.
25. Adams W, Sheslow D. Wide range assessment of visual motor abilities. Wilmington, DE: Wide Range, Inc, 1995.
26. Bellinger DC, Hu H, Kalaniti K, et al. A pilot study of blood lead levels and neurobehavioral function in children living in Chennai, India. Int J Occup Environ Health 2005;11:138–43.
27. Hu FB, Rimm E, Smith-Warner SA, et al. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. Am J Clin Nutr 1999;69:243–9.
28. Oken E, Kleinman KP, Rich-Edwards J, et al. A nearly continuous measure of birth weight for gestational age using a United States national reference. BMC Pediatr 2003;3:6.
29. US Department of Health and Human Services. What you need to know about mercury in fish and shellfish. March 2004. (http://www.cfsan.fda.gov/~dms/admehg3.html). (Accessed July 20, 2004).
30. Daniels JL, Longnecker MP, Rowland AS, et al. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology 2004;15:394–402.
31. Crump KS, Kjellstrom T, Shipp AM, et al. Influence of prenatal mercury exposure upon scholastic and psychological test performance: benchmark analysis of a New Zealand cohort. Risk Anal 1998;18:701–13.
32. Berglund M, Lind B, Bjornberg KA, et al. Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ Health 2005;4:20.

33. Morrissette J, Takser L, St-Amour G, et al. Temporal variation of blood and hair mercury levels in pregnancy in relation to fish consumption history in a population living along the St. Lawrence River. Environ Res 2004;95: 363–74.
34. Vahter M, Akesson A, Lind B, et al. Longitudinal study of methylmercury and inorganic mercury in blood and urine of pregnant and lactating women, as well as in umbilical cord blood. Environ Res 2000;84:186–94.
35. Ask K, Akesson A, Berglund M, et al. Inorganic mercury and methylmercury in placentas of Swedish women. Environ Health Perspect 2002;110:523–6.
36. Sakamoto M, Kubota M, Liu XJ, et al. Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ Sci Technol 2004;38:3860–3.
37. Ramirez GB, Cruz MC, Pagulayan O, et al. The Tagum study I: analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium, and infants' hair. Pediatrics 2000;106:774–81.
38. Kris-Etherton PM, Taylor DS, Yu-Poth S, et al. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 2000;71(suppl):179S–88S.
39. Innis SM, Elias SL. Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women. Am J Clin Nutr 2003;77:473–8.
40. Nesheim M, Yaktine A. Seafood choices: balancing benefits and risks. Washington, DC: The National Academies Press, 2007.