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MATERNAL METHYLMERCURY EXPOSURE THROUGH RICE INGESTION AND OFFSPRING NEURODEVELOPMENT: A PROSPECTIVE COHORT STUDY

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Abstract

Background—Dietary methylmercury intake can occur not only through fish ingestion but also through rice ingestion; however, rice does not contain the same beneficial micronutrients as fish.

Objectives—In rural China, where rice is a staple food, associations between prenatal methylmercury exposure (assessed using maternal hair mercury) and impacts on offspring neurodevelopment were investigated.

Methods—A total of 398 mothers were recruited at parturition at which time a sample of scalp hair was collected. Offspring (n=270, 68%) were assessed at 12 months using the Bayley Scales of Infant Development-II, yielding age-adjusted scores for the Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI).

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Results—Among 270 mothers, 85% ingested rice daily, 41% never or rarely ingested fish/shellfish and 11% ingested fish/shellfish at least twice/weekly. Maternal hair mercury averaged 0.41 µg/g (median: 0.39 µg/g, range: 0.079–1.7 µg/g). In unadjusted models, offspring neurodevelopment (both MDI and PDI) was inversely correlated with hair mercury. Associations were strengthened after adjustment for fish/shellfish ingestion, rice ingestion, total energy intake (kcal), and maternal/offspring characteristics for both the MDI [Beta: −4.9, 95% Confidence Interval (CI): −9.7, −0.12] and the PDI (Beta: −2.7, 95% CI: −8.3, 2.9), although confidence intervals remained wide for the latter.

Conclusions—For 12-month old offspring living in rural China, prenatal methylmercury exposure was associated with statistically significant decrements in offspring cognition, but not psychomotor development. Results expose potential new vulnerabilities for communities depending on rice as a staple food.

Keywords

mercury; prenatal; Bayley Scales; neurodevelopment

INTRODUCTION

Fish consumption is the primary exposure pathway for methylmercury (MeHg), a potent neurotoxin, which impairs fetal brain development [Clarkson and Magos 2006; National Research Council (NRC) 2000]. Dietary MeHg intake also occurs through rice ingestion (Rothenberg et al. 2013, 2014); however, less is known concerning the impacts of MeHg exposure from maternal rice ingestion on offspring neurodevelopment.

Fish tissue is a source for MeHg as well as micronutrients that benefit fetal brain development and retinal development, including long-chain polyunsaturated omega-3 (N-3) fatty acids, selenium (Se), choline, iodine and iron (Fe) (Choi et al. 2008; Davidson et al. 2008; Georgieff and Innis 2005; Oken et al. 2005, 2008; Strain et al. 2008). Although rice MeHg concentrations are lower compared to fish tissue (Rothenberg et al. 2014), rice consumption is substantial in regions of the world where rice is a staple food [Food and Agriculture Organization of the United Nations (FAO) 2015]. Furthermore, rice does not contain the same beneficial micronutrients as fish (Rothenberg et al. 2011a). This is partially due to the rice polishing process, which simultaneously removes micronutrients concentrated in the outer bran layer (Villareal et al. 1991). For example, rice-eating communities are often zinc (Zn)-deficient (Allen et al. 2006) because polished white rice contains lower Zn concentrations compared to unpolished brown rice (e.g., 66% less) (Villareal et al. 1991). Conversely, polishing does not reduce MeHg because MeHg accumulates in the rice endosperm and not the outer bran layer (Rothenberg et al. 2011b). Zn deficiency is associated with delays in attention and motor development (Allen et al. 2006) supporting the notion that MeHg exposure from rice may have a different confounding pattern than is characteristic of MeHg exposure from fish ingestion.

Half the world's population subsists on polished rice as a staple food, mostly in Asia (FAO 2015), where global Hg emissions are highest (Zhang et al. 2016), and where locally grown rice may therefore be susceptible to Hg contamination. Atmospheric Hg may be deposited to

flooded rice paddies, where anaerobes convert inorganic Hg to MeHg, which is efficiently accumulated in rice grain (Rothenberg et al. 2014).

In 2013, we initiated a prospective birth and child cohort study in rural China, where rice is a staple food, to investigate associations between maternal rice ingestion during pregnancy, biomarkers of MeHg exposure, and offspring neurodevelopment. In this study population, prenatal MeHg exposure during the third trimester (assessed using hair Hg and blood Hg) was similar to other cohorts of pregnant mothers, where fish consumption was the primary MeHg exposure pathway, although dietary MeHg intake was primarily through rice ingestion (average: 71%, median: 87%) and to a lesser extent fish/shellfish ingestion (average: 29%, median: 13%) (Hong et al. in press).

METHODS

Recruitment and Data Collection

Adult women were recruited at parturition at the Maternal and Child Health Hospital in Daxin County, Guangxi, China. Eligible mothers were in good general health, resided in Daxin County during the three previous months, and planned to remain for the next year. The recruitment goal was 400 to ensure sufficient number of children at the follow-up. Residency in Daxin County was important to ensure higher follow-up at 12 months, and consistency in environmental Hg exposures and food consumption patterns. Protocols were reviewed and approved by the Institutional Review Boards at the University of South Carolina (USA) and XinHua Hospital (China). Eligible mothers provided written informed consent prior to enrollment in the study.

After enrollment, ~50 strands of maternal hair (for Hg analyses) were collected from the occipital region and stored at room temperature in a plastic bag. A non-fasting blood sample was collected by venipuncture (6 mL total) into two vials, including one with lithium heparin anticoagulant [for Hg and lead (Pb) analyses], and a second vial for separation of serum by centrifugation [selenium (Se) and Zn analyses]. Whole blood and serum were stored frozen at the hospital at -26°C for up to 10 months, then transported to Shanghai where samples were archived at -80°C until analysis.

During their hospital stay, mothers completed a questionnaire eliciting information about demographics, maternal and paternal education and occupation, monthly household income, maternal pregnancy history (including smoke and alcohol exposure), family health history, and child's birth length and weight.

Mothers also filled out a modified semi-quantitative 102-item food frequency questionnaire (FFQ) (Cheng et al. 2009), reflecting food intake during the third trimester. Food categories included rice, seven commonly consumed varieties of fish and shellfish (freshwater fish, ocean fish, shrimp, eel, snails, crab, and other shellfish), and other foods (e.g., pork, eggs, tofu, fruits and vegetables). For each food item, the FFQ provided eight options, ranging from "never or rarely" to "2 times/day." Food frequencies were converted to servings per day as follows: 0=never or rarely, $1/30.5$ = monthly, $2.5/30.5$ =two to three times/month, $1/7$ =once per week, $2.5/7$ = two to three times/week, $5/7$ =four to six times/week, 1=once per day,

and 2.5—at least two times per day. Servings per day were summed for each food group (e.g., fish/shellfish, pork, tofu, fruits and vegetables). For rice, mothers indicated quantity per serving by selecting one of three bowls from a picture or actual bowls. The daily rice ingestion rate (grams per day) was calculated by multiplying servings per day $\times$ quantity per serving. To calculate fish/shellfish ingestion (grams per day), we assumed 170 g/serving for ocean fish and freshwater fish [170 g = 6 ounces, the recommended serving size from the U.S. Food and Drug Administration (USFDA) 2001], and 100 g/serving for other categories (Cheng et al. 2009). For the remaining food groups, portion sizes were assigned based on previous studies among Chinese pregnant mothers in rural western China (Cheng et al. 2009). Nutrient intakes, including energy and the proportion of calories from fat, carbohydrates and protein, were calculated from the Chinese Food Composition Tables, including 89 foods from 2009 tables (Yang 2009) and 13 foods from 2005 tables (Yang et al. 2005).

Offspring Assessment at 12 Months

At the 12-month visit, parents or caregivers provided information on breastfeeding duration and the child's general health. The primary outcome measure was children's cognitive and psychomotor abilities, assessed using the Bayley Scales of Infant Development (BSID)-II, which yields age-adjusted scores for the Mental Developmental Index (MDI) and the Psychomotor Developmental Index (PDI) (Bayley 1993). The BSID-II underwent extensive pre-testing, and a few items were slightly modified to make the BSID-II more culturally appropriate (e.g., image of a Chinese-style home rather than a western-style home), which was consistent with the approach used in other studies in developing countries where the BSID-II was implemented (e.g., India, Pakistan and Zambia, from Carlo et al. 2012). Only one evaluator administered the BSID-II, who spoke the local dialect and completed extensive training in BSID-II administration, and was unaware of the children's prenatal MeHg exposure level. Examiner reliability was assessed throughout the follow-up period by videotaping a subset of children ($n=8$, 3.4%). Both MDI and PDI sections were rescored by co-author FJB, a developmental psychologist; the scores were the same 95% of the time and differences in scoring were minor.

Lab Analyses

Hair total Hg (THg) concentrations corresponding to trimester 3 (proximal 3.4 cm for Asian women, Loussouarn et al. 2005) were analyzed directly without digestion by atomic absorption spectrometry (AAS) using a Lumex Model RA-915+/PYRO-915+ (St. Petersburg, Russia) using U.S. Environmental Protection Agency (USEPA) Method 7473 [USEPA 2007]. Prior to analysis exogenous Hg was removed by washing hair samples in 0.1% 2-mercaptoethanol, samples were triple-rinsed in double-distilled water (DDI-H₂O), and air-dried in a biosafety cabinet (Baker Company, Sanford, USA). Hair MeHg was extracted in 2 mL of 25% (w/v) sodium hydroxide:DDI-H₂O for 3 hours at 75°C, then diluted, and extracts were analyzed following U.S. EPA 1630 (USEPA 2001) using gas chromatography cold vapor atomic fluorescence spectrometry (Brooks Rand Model III, Seattle, WA, USA). Blood THg was analyzed as described above for hair THg using a DMA-80 (Milestone, Inc., Shelton, CT, USA), and following U.S. EPA 7473 (USEPA 2007). Blood Pb levels were analyzed directly without pretreatment using graphite furnace-

AAS (PinAAcle 900Z, PerkinElmer, Waltham, Mass., USA) (Cao et al. 2014). Serum Zn and Se concentrations were analyzed by inductively coupled plasma-mass spectrometry (Agilent 7500CE, USA) following U.S. EPA 3050B (USEPA 1996).

Quality assurance/quality control included analysis of standard reference materials, matrix spike recoveries, and analysis of replicates (Table A.1). Average percent recoveries from matrix spikes and standard reference materials recoveries ranged from 78% to 115%. Relative percent difference between sample replicates averaged 5.5% and 8.4% for hair THg and MeHg, respectively, and <20% for blood THg, blood Pb, serum Se, and serum Zn measures. The limits of detection were: hair THg (0.0095 µg/g), hair MeHg (0.0001 µg/g), blood THg (0.14 µg/L), blood Pb (0.1 µg/dL), serum Se (1.6 µg/L) and serum Zn (30 µg/L). All measurements exceeded the limits of detection.

N-3 fatty acids [docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and alpha-linolenic acid] and N-6 fatty acids (arachidonic acid and linoleic acid) were extracted following methods from Baylin et al. (2005) and assessed by gas-liquid chromatography (Agilent 6890N-5975B with flame ionization detector). Peak retention times were identified by injecting known standards of >99% purity.

Maternal hair THg and MeHg were analyzed at the University of South Carolina, USA, and blood and serum metals were analyzed at the State Key Lab for Children's Environmental Health in Shanghai, China, and serum fatty acids were analyzed at the State Key Laboratory of Nutrition and Metabolism in Shanghai, China.

Statistics

Univariate and bivariate statistics were used to assess the distribution of all variables. Unadjusted associations were determined using Spearman's or Pearson's correlation, one-way analysis of variance (ANOVA), Student's two-tailed t-test, Wilcoxon rank sum test, or the Kruskal-Wallis test, depending on the distribution and properties of the variables.

Multivariable linear regression was used to model the impacts of prenatal MeHg exposure on offspring cognition and psychomotor development at 12 months. Prenatal MeHg exposure was estimated using maternal hair THg, hair MeHg, and blood THg. Blood THg represents both MeHg and inorganic Hg exposure (Berglund et al. 2005), while hair THg represents MeHg exposure, as follows. MeHg is accumulated in the hair shaft during keratinization (Zareba et al. 2008). Some MeHg is demethylated to inorganic Hg after incorporation into the hair shaft; therefore hair inorganic Hg does not reflect inorganic Hg exposure (Berglund et al. 2005). Several researchers reported associations between hair THg and dietary MeHg intake through fish ingestion (NRC 2000), as well as rice ingestion (e.g., Rothenberg et al. 2013). Maternal hair THg is also correlated with fetal brain MeHg (Cernichiari et al. 1995). Few studies have included hair THg, hair MeHg, and blood THg concentrations; the biomarker most strongly correlated with outcome measures was retained in the final regression models.

Other independent variables considered for inclusion in our models were chosen based on their importance in other studies concerning prenatal MeHg exposure and offspring

neurodevelopment (e.g., maternal age, maternal education, alcohol consumption, second-hand smoke exposure, pre-pregnancy body mass index, breast-feeding duration, infant sex, birth weight for gestational age, and child's age at cognitive testing, from Oken et al. 2005, 2008) (see Table 1). In addition to hair and blood Hg, other biomarkers collected from mothers at parturition included serum fatty acids (DHA, EPA, N-6/N-3 fatty acids), serum Zn, serum Se and blood Pb (Table 2). These measures were considered in our models because of their potential to confound associations. For example, Pb is neurotoxic [Centers for Disease Control and Prevention (CDC) 2014], and Se is neuroprotective but often deficient in rice-eating communities (Williams et al. 2005). Dietary measures (energy intake, and %calories from fat, protein, and/or carbohydrates) were considered for inclusion because of their associations with rice/fish ingestion (Table 3). Maternal Fe status was estimated by maternal self-reported diagnosis of anemia during pregnancy. Birth weight for gestational age z-scores were calculated using the Intergrowth-21 Newborn Birth standards, which were based on a reference population of 20,486 births from eight countries including 17% (n=3551 births) from China (Villar et al. 2014). In regression models, rice ingestion was dichotomized (<once/day or once/day) and fish ingestion was categorized (rarely or never, or <twice/week, or twice/week).

Inclusion/exclusion of covariates was determined: 1) by the relationship between each covariate and outcome measures, 2) by examining added variable plots for each covariate, 3) by investigating confounding between each covariate and effect estimates for MeHg, 4) by comparing the Akaike Information Criterion between models with/without covariates (Akaike 1974), and 5) by prior association with MeHg exposure and offspring neurodevelopment (e.g., maternal age). For multicollinear covariates, the most predictive variable remained in the regression model; i.e., higher partial r-squared.

To examine whether dietary intake of rice or fish/shellfish modified the associations between MeHg and outcome measures, we constructed additional models based on Tsaih et al. (2004), as follows. Specifically, to optimize power, we estimated the impact of MeHg exposure (using log₁₀ hair THg) by categories of rice intake (<once/day, once/day) within a single model. We used the same approach to assess differences in MeHg impacts by fish intake categories (rarely or never, <twice/week, or twice/week). We then assessed the significance of differences in MeHg associations by rice or fish intake by modeling the main effects of MeHg and diet measures and their interaction.

The log₁₀-transformation was applied to right-skewed variables to improve normality of residuals. Cook's distance was used to assess influential observations, and assumptions for residuals were checked (mean=0, constant variance). An alpha-level of 0.05 was chosen as guide for significance. Stata 9.2 (College Station, Texas) and the R-platform were used for all statistical analyses.

For continuous covariates, percent missingness among mothers (n=398) was 4.3% for all variables except household income (8.8%) and the rice ingestion rate (grams per day) (5.5%). Additionally, 6.3% of mothers had dietary energy intake >5000 kcal or <500 kcal, indicating self-reported food frequencies were likely biased high or low, respectively. Missing data, as well as outlier values for energy intake, and the proportional intake from

fat, protein and carbohydrates, were imputed using multiple imputation based on the multivariate normal distribution (Schafer 1997) conditional on maternal, paternal, and offspring characteristics (Table 1), and biomarker concentrations (THg, MeHg, other metals, and fatty acids) (Table 2). For fish/shellfish ingestion, missing values were assumed to be 0, which was appropriate for this inland rural area of China.

RESULTS

Participants

Between May 2013 and March 2014, 1261 women gave birth at the Maternal and Child Health Hospital in Daxin County, including 398 mothers who enrolled in the main cohort. A total of 318 (80%) participants/offspring returned for the 12-month assessment, however 48 (15%) children were 14 months and excluded, resulting in 270 mother/offspring pairs for this analysis (68% follow-up rate). Mother's age at the follow-up visit averaged 29 years (range: 18–45 years), offspring age averaged 12.5 months (range: 11.74–13.97 months), children were breastfed on average 8.5 months (range: 0–13.97 months), and additional characteristics are summarized in Table 1. For all biomarkers and most maternal, paternal and offspring characteristics, there were no differences between the main cohort (n=398) and those included in the 12-month follow-up (n=270) (2 tailed t-test, $p=0.25-0.99$). However in the main cohort five (1.3%) mothers smoked and there were no smokers included in the follow-up. In addition, the number of families with an older child in the same household was higher among participants in the follow-up (40%) compared to the main cohort (32%) (2-tailed t-test, $p=0.03$).

Rice and Fish Consumption

Mothers ingested on average 1.8 servings/day of rice (median: 2.5 servings/day, range: 0–2.5 servings/day). The rice ingestion rate averaged 226 g/day (median: 213 g/day, range: 0–650 g/day). Eighty-five percent of mothers ingested rice daily, while two mothers (<1%) never or rarely ingested rice (Table 4). Mothers consumed fish/shellfish on average 0.13 servings/day (median: 0.03 servings/day, range: 0–2.9 servings/day), including three mothers consuming 2 servings/day. Mothers ingested on average 19 g/day of fish/shellfish (median: 5.6 g/day, range: 0–470 g/day). Forty-one percent (n=111) of mothers never or rarely ate fish/shellfish, while 11% ingested fish/shellfish twice/weekly (n=29) (Table 4).

Fish/shellfish consumption (servings per day) was positively associated with mother's education (0.13 average servings/day for <high school education versus 0.14 average servings/day high school), and household income (0.11 average servings/day <2000 renminbi/month versus 0.18 average servings/day 2000 renminbi/month), while average fish/shellfish consumption was lower for farmers (0.12 average servings/day) compared to other occupations (0.18 average servings/day) (Kruskal-Wallis, $p<0.01$ for all). In contrast, rice ingestion (servings per day) was not strongly correlated with mother's education, household income, or mother's occupation (Kruskal-Wallis, $p=0.22-0.70$ for all).

Mothers ingesting more fish/shellfish also ingested more servings per day of pork, fruits, vegetables, eggs, and tofu (Spearman's $\rho=0.21-0.37$, $p<0.001$ for all) (Table 3). Likewise,

mothers consuming more rice also ingested more servings per day of pork, fruits, vegetables and eggs (Spearman's $\rho=0.16-0.35$, $p<0.01$ for all), and consumed slightly more tofu (Spearman's $\rho=0.11$, $p=0.08$).

Hair Hg and Bayley Scores

MeHg biomarker concentrations are summarized in Table 2. A total of nine mothers (3.3%) had hair THg levels that exceeded the concentration value ($>1.1 \mu\text{g/g}$), which corresponds to the U.S. EPA reference dose for MeHg intake of $0.1 \mu\text{g/kg}$ body weight/day (USEPA 1997).

The average ($\pm$ SD) age-adjusted scores for the MDI and the PDI were 99 ± 9.8 (range: 66–120) and 88 ± 11 (range: 60–121), respectively (Table 2). MDI scores were similar to the standardized mean (100 ± 15), while PDI scores averaged 12 points lower (Bayley 1993). Pearson's correlation between the MDI and PDI scores was 0.38, which was identical to the correlation coefficient reported for 12-month old offspring in test standardization data for U.S. children (Bayley 1993).

In unadjusted and adjusted models, biomarkers for prenatal MeHg exposure ($\log_{10}$ hair THg, $\log_{10}$ hair MeHg, and $\log_{10}$ blood THg) were inversely correlated with offspring neurodevelopment (Table 5). We focused on $\log_{10}$ hair THg in adjusted models because this biomarker was most strongly correlated with MDI and PDI scores. This is consistent with the premise that hair THg is a particularly robust biomarker of MeHg exposure (Berglund et al. 2005).

The inverse association between the MDI and $\log_{10}$ hair THg was strengthened after adjustment for fish/shellfish consumption and other covariates (Table 5). In the adjusted model, a doubling in hair THg corresponded to a 1.5-point decrease ($-1.5 = -4.9 \times \log_{10}2$) in the MDI score [95% Confidence Interval (CI): $-2.9, -0.03$] (Figure 1a). The inverse association of $\log_{10}$ hair THg with PDI was more modest compared to MDI, even after adjustment (Table 5, Figure 1b). A doubling in hair THg was associated with a 0.82-point decrease in the PDI and the confidence limits included the null (95% CI: $-2.5, 0.86$).

Higher fish/shellfish ingestion was positively correlated with outcome measures (both MDI and PDI); this trend was strongest for the MDI model. On average, the MDI and PDI scores were 4.1 points higher (95% CI: 0.04, 8.2) and 2.2 points higher (95% CI: $-2.6, 6.9$), respectively, for mothers ingesting fish/shellfish twice/weekly, compared to mothers who rarely or never ingested fish/shellfish. Conversely, daily rice ingestion was associated with lower MDI and PDI scores, compared to mothers ingesting rice less than once/day, although this trend was not statistically significant for either outcome measure (MDI Beta: -2.7 , 95% CI: $-6.7, 1.2$; PDI Beta: -0.23 , 95% CI: $-4.8, 4.3$).

Using stratified models, we investigated whether the associations of hair THg with offspring neurodevelopment were modified by the rice ingestion rate ($<\text{once/day}$ or once/day) or the fish/shellfish ingestion rate (0/week, $<\text{twice/week}$, or twice/week) (Figure 2, Table A.2). Associations between $\log_{10}$ hair THg and outcome measures (both MDI and PDI) were negative, regardless of the rice ingestion rate, with stronger adverse THg associations among mothers consuming less rice (MDI: $<\text{once/day}$ Beta: -9.2 , 95% CI: $-23, 4.5$; once/day

Beta: -4.3, 95% CI: -9.5, 0.88; PDI: <once/day Beta -6.3, 95% CI: -22, 9.5, once/day Beta -2.2, 95% CI: -8.2, 3.8). Similarly, adverse, albeit statistically non-significant, associations of $\log_{10}$ hair THg with outcomes were observed among women consuming 0 servings/week or <2 servings/week of fish/shellfish, whereas there was no evidence of an adverse association between $\log_{10}$ hair THg with outcomes among mothers ingesting fish/shellfish twice/week (MDI Beta: 2.1, 95% CI: -14, 18; PDI Beta: 2.1, 95% CI: -17, 21), although confidence intervals were wide. None of the interactions of $\log_{10}$ hair THg with diet were statistically significant ($p=0.51-0.70$) (Table A.2).

Relation of Other Covariates with Bayley Scores

Using WHO and FAO guidelines for non-pregnant adults, 97 mothers (36%) were Zn deficient (<700 $\mu\text{g/L}$), while just nine mothers (3.3%) were Se deficient (<87 $\mu\text{g/L}$) (Allen et al. 2006). Zn and Se associations were assessed using continuous measures and categorical variables (dichotomized at 700 $\mu\text{g/L}$ and 87 $\mu\text{g/L}$, respectively). Neither continuous nor categorical measures of Se intake contributed to MDI and PDI models, and therefore Se was excluded from further analyses. There were no differences in average MDI scores among infants of mothers who were Zn replete compared to those who were deficient (MDI = 99 for both). In our final multivariable analyses serum Zn (continuous) was predictive of MDI scores with an interquartile range increase in serum Zn (295 $\mu\text{g/L}$) corresponding to a 6.3-point increase in the MDI (95% CI: 1.1, 12).

Although most mothers (98%) had blood Pb below the U.S. reference value (<5 $\mu\text{g/dL}$) (CDC 2014), $\log_{10}$ blood Pb was associated with decrements in both the MDI and PDI. For PDI, associations were significant: for a doubling in blood Pb, the PDI decreased by 3.3 points (95% CI: -6.3, -0.36). When mothers with blood Pb $\geq 5 \mu\text{g/dL}$ were excluded ($n=6$), results did not differ (data not shown). Blood Pb was weakly inversely correlated with hair THg (Spearman's $\rho = -0.16$, $p<0.01$), but was not associated with dietary factors in Table 3, including ingestion of rice and fish/shellfish (Spearman's $\rho = |<0.01-0.10|$, $p=0.11-0.92$ for all).

Dietary measures included $\log_{10}$ energy intake and the proportion of calories from protein and carbohydrates (Table 2). All three variables were multicollinear (Table A.3); $\log_{10}$ energy intake was most strongly correlated with outcome measures and remained in the models (Table 5). $\log_{10}$ energy intake was more strongly correlated with PDI scores than MDI, although trends were positive for both. For a doubling in energy intake the PDI increased on average by 4.1 points (95% CI: 1.1, 7.0), while the MDI increased on average by 2.2 points (95% CI: -0.37, 4.7).

Although children ≤ 14 months were excluded from this analysis, both MDI and PDI scores were positively correlated with child's age at the follow-up, which was consistent with residual age effects despite using age standardized measures. The MDI was higher for mothers with a university education compared to less education, and for increasing birth weight for gestational age. The MDI was higher for increasing BMI (but lower for obese mothers), and was lower for male compared to female offspring. Similar to the MDI model, PDI increased with higher birth weight for gestational age, and increased with maternal BMI (however the trend was increasing for all BMI categories). Unlike the MDI, PDI scores were

higher for male compared to female offspring, and were higher for mothers with less education compared to mothers with a university education.

Sensitivity Analyses

Regression models were run without imputed values (n=230 without imputed data), and the same trends were observed. Similarly our multivariable regression models were run using raw MDI and PDI scores (including adjustment for child's age at assessment), and the same direction of correlation was observed for all covariates (Table A.4).

DISCUSSION

In rural China, where 85% of mothers ingested rice daily and 41% of mothers rarely or never ingested fish, statistically significant inverse associations were observed for 12-month old offspring between MDI scores and $\log_{10}$ hair THg, after adjustment for fish/shellfish ingestion, rice ingestion, maternal energy intake, and maternal/offspring characteristics.

Most studies investigating prenatal MeHg exposure were conducted among fish-eaters because fish ingestion is considered the most important exposure pathway for MeHg among the general population (Clarkson and Magos 2006). However dietary MeHg intake through rice ingestion may be more important than fish ingestion in regions where rice is a staple food (Hong et al. in press; Rothenberg et al. 2014). One of our study's questions concerned whether the effects of MeHg on outcome measures depended on whether MeHg exposure was predominantly from rice versus fish. In our cohort, 41% of mothers did not ingest fish; however, all mothers ingesting fish also ingested rice (Table 4), and thus we were unable to completely isolate the impacts of fish MeHg from rice MeHg. We were able to use stratified analyses to assess the more general question of whether differences in diet (vis-a-vis fish versus rice intake) impacted MeHg-Bayley associations. Results from these analyses (Figure 2, Table A.2) suggested that adverse associations of $\log_{10}$ hair THg with Bayley measures were more adverse among mothers who ate less rice. As rice ingestion correlated with intake of other healthful foods (Table 3), this observation may reflect enhanced vulnerability related to poor quality diet. Conversely, no adverse associations of $\log_{10}$ hair THg with Bayley scores were observed among mothers consuming fish twice/weekly, when compared to mothers ingesting fish less often, although confidence intervals included the null (Table A.2). Persistence of adverse associations between $\log_{10}$ hair THg and Bayley scores among high rice but not high fish consumers were not likely due to mother's nutritional status because mothers ingesting more rice and more fish both had better diets (Table 3), suggesting other nutrients associated with fish ingestion (but not rice ingestion) potentially modified the impact of MeHg.

We compared our findings with other studies among fish-consumers, which used the BSID-II to evaluate offspring neurodevelopment. In most cases, biomarkers for prenatal MeHg exposure had higher concentrations compared with values reported in the present study. In the Seychelles, where maternal hair THg averaged 5.9 $\mu\text{g/g}$ (i.e., 14 times higher than our study) significant inverse associations were observed between prenatal MeHg exposure (maternal hair THg) and the PDI (but not the MDI) among 30-month old children after adjustment for DHA and other nutrients; however associations for both outcome measures

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were non-significant for 9-month old offspring (n=229) (Davidson et al. 2008; Strain et al. 2008). Similarly, for a New York City cohort recruited after the World Trade Center collapse (n=112–151), prenatal MeHg exposure (ln cord blood THg) was associated with significant decrements in the MDI and/or PDI at 24- and 36-months but not at 12-months of age, after adjustment for maternal fish consumption (Lederman et al. 2008). The authors also reported the geometric mean for maternal blood THg (1.6 µg/L, Lederman et al. 2008), which was 1.3 times higher compared to the present study (Table 2). In Spain (n=1683, Llop et al. 2012) and in the Arctic (n= 94, Boucher et al. 2014), where children were a similar age or younger compared to our cohort (Spain: average 14 months; Arctic: 6.5 months and 11 months), researchers reported a lack of correlation between MeHg exposure (ln cord blood THg for both) and the MDI and PDI scores, after adjustment for maternal fish intake (Boucher et al. 2014; Llop et al. 2012). In Spain the geometric mean for cord blood THg was 8.4 µg/L (Llop et al. 2012), and in the Arctic the median cord blood THg was 17 µg/L (Boucher et al. 2014). Assuming cord blood THg is on average 66% higher than maternal blood THg (Mahaffey et al. 2009), the estimated geometric mean for maternal blood THg in Spain was 5.06 µg/L (= 4.2 times higher than the present study), and the estimated median maternal blood THg in the Arctic was 10.2 µg/L (i.e., 8.5 times higher than the present study, where median blood THg = 1.2 µg/L = the geometric mean in Table 2). In contrast to these studies, among a Polish cohort of 374 children, cord blood THg (dichotomized at the median, 0.90 µg/L) was inversely correlated with both MDI and PDI scores at 12-months, although associations were not significant at 24- and 36-months after adjustment for confounders (but not fish consumption) (Jedrychowski et al. 2007). To the best of our knowledge, the latter was the only study with lower prenatal MeHg exposure compared to our cohort. In Poland, the estimated median maternal blood THg was 0.54 µg/L, which was 2.2 times lower compared to the median blood THg in our cohort (1.2 µg/L). A number of factors, for example, residual confounding, differential loss to follow-up, or use of a dichotomized exposure measure, may have contributed to potential enhanced Hg sensitivity among Polish infants compared to other populations. More generally, for a rural Chinese population, rice ingestion likely contributed substantially to prenatal MeHg exposure (Hong et al. in press). In this context, where confounding and potential effect modification by beneficial nutrients in fish are less prominent, adverse MeHg impacts on offspring cognition (assessed using the MDI) may be ascertainable at a younger age and at lower MeHg exposure levels than has been observed in most previous studies.

In regression models, frequent fish consumption (twice/weekly) was associated with higher offspring developmental scores, which was consistent with other studies (Budtz-Jørgensen et al. 2007; Choi et al. 2008; Daniels et al. 2004; Davidson et al. 2008; Lederman et al. 2008; Oken et al. 2005, 2008; Strain et al. 2008). However, serum fatty acids (DHA, EPA, and N-6/N-3 fatty acids) were not correlated with the MDI or the PDI (data not shown), and were thus excluded from our models. Similarly, Oken et al. (2008) reported DHA + EPA intake from dietary sources and maternal erythrocyte DHA + EPA concentrations were not associated with cognition among 3-year old children. In the present study, maternal biomarkers were collected at a single time point (parturition), which possibly resulted in less precise measurements of fatty acids due to depletion of maternal stores at parturition (Georgieff and Innis 2005). Other nutrients related to fish ingestion may be more important

than N-3 fatty acids to children's cognition (e.g., choline) (Choi et al., 2008). Alternatively, the BSID-II may not target areas of the brain sensitive to the effects of N-3 fatty acids (Colombo et al. 2013).

There are several limitations of this study. First, there may be measurement error in the outcome (MDI or PDI) because the Bayley Scales were standardized among a U.S. population (Bayley 1993), which would lead to imprecision in our effect estimates. This was likely particularly true for the PDI model because the average PDI score was 12 points below the standardized mean, suggesting that our population differed from the standardization sample. That said, other researchers have reported lower average BSID-II MDI and/or PDI scores among non-U.S. cohorts (Davidson et al. 2008, Hamadani et al. 2002). The likely confounding structure in these data is complex because women who eat more fish are also better educated and have higher incomes. Thus mothers with potentially higher MeHg exposures (see Table 3) also have developmental advantages; we adjusted for these factors in our models but potential residual negative confounding by unmeasured aspects of developmental advantage may lead to underestimation of MeHg associations. Lastly, the population's characteristics, i.e., poor, rural, largely farmers, is a challenge as there may be a number of potential confounding factors that diet correlates with that are not known or measured in the study.

CONCLUSIONS

In communities depending on rice as a staple food, women of childbearing age are often deficient in essential micronutrients (e.g., Zn), which contribute to decrements in offspring growth and development (Allen et al. 2006). After accounting for measures of maternal nutritional status in our rural Chinese cohort, a biomarker of MeHg exposure (i.e., log₁₀ hair THg) (the majority of which was attributable to rice ingestion) was associated with decrements in offspring cognition, despite low hair THg levels. These results expose additional potential vulnerabilities among rice-eaters.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

The appendix includes quality assurance/quality control parameters (Table A.1), regression coefficients for stratum-specific estimates and p-values for interaction terms (Table A.2), Spearman's correlation matrix for dietary variables (Table A.3), and multivariable regression results using the raw Bayley scores (Table A.4).

Figure 1.

Partial regression plots from the fully adjusted regression models relating outcome measures versus $\log_{10}$ maternal hair total mercury (THg), including a) Bayley Scales of Infant Developmental (BSID)-II Mental Developmental Index and b) BSID-II Psychomotor Developmental Index ($n=270$ mother/offspring pairs). Models were also adjusted for maternal characteristics (pre-pregnancy BMI, education, age at parturition, fish ingestion (3 categories), rice ingestion (2 categories), total energy intake [kcal], and peripartum serum Zn and blood Pb levels) and child characteristics (sex, birth weight for gestational age z-score, and age at Bayley testing).

Figure 2. The modifying effect of rice consumption (<daily and daily) and fish/shellfish consumption (0/week, 0/week < ingestion < twice/week, and twice/week) on the Bayley Scales of Infant Development-II with a doubling of hair mercury concentrations for the a) Mental Developmental Index (MDI) and b) Psychomotor Developmental Index (PDI). Error bars indicate 95% confidence intervals for the Beta coefficients (see Table A.2). In addition to hair mercury, models were adjusted for maternal characteristics (pre-pregnancy BMI, education, age at parturition, total energy intake, and peripartum serum Zn and blood Pb levels) and child characteristics (sex, birth weight for gestational age z-score, and age at Bayley testing).

Table 1

Maternal, paternal and offspring characteristics (N, %), and associations with outcome measures for each category (n=270 mother/offspring pairs).

	N (%)	MDI Mean $\pm$ SD	PDI Mean $\pm$ SD
Mother's Age (years)			
Age <20	23 (8.5)	100 $\pm$ 8.6	90 $\pm$ 8.0
20 Age <30	156 (58)	99 $\pm$ 10	87 $\pm$ 11
30 Age <45	91 (34)	100 $\pm$ 8.9	89 $\pm$ 11
	<i>p-value</i>	<i>0.58</i>	<i>0.30</i>
Mother's Ethnicity			
Zhuang	235 (87)	99 $\pm$ 10	88 $\pm$ 11
Han	29 (11)	99 $\pm$ 8.0	89 $\pm$ 9.4
Other	6 (2.2)	105 $\pm$ 8.1	96 $\pm$ 11
	<i>p-value</i>	<i>0.28</i>	<i>0.19</i>
Mother's Education Completed			
< High School	215 (80)	99 $\pm$ 9.3	89 $\pm$ 11
High School	40 (15)	96 $\pm$ 11	86 $\pm$ 11
University	15 (5.6)	102 $\pm$ 11	85 $\pm$ 11
	<i>p-value</i>	<i>0.09</i>	<i>0.31</i>
Father's Education Completed			
< High School	209 (77)	99 $\pm$ 9.4	88 $\pm$ 10
High School	45 (17)	101 $\pm$ 10	89 $\pm$ 14
University	16 (5.9)	98 $\pm$ 12	83 $\pm$ 9.8
	<i>p-value</i>	<i>0.45</i>	<i>0.19</i>
Mother's occupation			
Farmer	196 (73)	99 $\pm$ 9.5	88 $\pm$ 11
Worker ^a	27 (10)	101 $\pm$ 13	89 $\pm$ 12
Unemployed	35 (13)	98 $\pm$ 9.2	88 $\pm$ 13
Other	12 (4.4)	96 $\pm$ 9.4	86 $\pm$ 8.5
	<i>p-value</i>	<i>0.53</i>	<i>0.82</i>
Father's occupation			
Farmer	193 (71)	99 $\pm$ 9.5	88 $\pm$ 10
Worker ^a	42 (16)	100 $\pm$ 11	88 $\pm$ 12
Unemployed	20 (7.4)	98 $\pm$ 9.6	89 $\pm$ 13
Other	15 (5.6)	96 $\pm$ 11	86 $\pm$ 13
	<i>p-value</i>	<i>0.52</i>	<i>0.84</i>
Household Income (RMB)^b			
Income <2000	180 (67)	99 $\pm$ 9.7	87 $\pm$ 11

	N (%)	MDI Mean $\pm$ SD	PDI Mean $\pm$ SD
2000 Income <5000	77 (29)	99 $\pm$ 9.9	89 $\pm$ 12
Income 5000	13 (4.8)	101 $\pm$ 10	89 $\pm$ 14
	<i>p-value</i>	<i>0.68</i>	<i>0.55</i>
Maternal Pre-Pregnancy BMI^c			
Underweight	72 (27)	97 $\pm$ 10	86 $\pm$ 10
Normal Weight	151 (56)	100 $\pm$ 9.1	89 $\pm$ 11
Overweight	38 (14)	99 $\pm$ 11	89 $\pm$ 12
Obese	9 (3.3)	97 $\pm$ 12	90 $\pm$ 12
	<i>p-value</i>	<i>0.11</i>	<i>0.32</i>
Smoking During Pregnancy			
Yes	0 (0)	NA	NA
No	270 (100)	99 $\pm$ 9.8	88 $\pm$ 11
	<i>p-value</i>	<i>NA</i>	<i>NA</i>
2nd-Hand Smoke During Pregnancy^d			
Yes	149 (55)	99 $\pm$ 10	88 $\pm$ 11
No	120 (45)	99 $\pm$ 9.0	88 $\pm$ 11
	<i>p-value</i>	<i>0.79</i>	<i>0.90</i>
Alcohol During Pregnancy^d			
Yes	3 (1.1)	109 $\pm$ 5.9	76 $\pm$ 13
No	264 (99)	99 $\pm$ 9.8	88 $\pm$ 11
	<i>p-value</i>	<i>0.07</i>	<i>0.06</i>
Anemia			
Yes	9 (3.3)	98 $\pm$ 12	87 $\pm$ 9.2
No	261 (97)	99 $\pm$ 9.7	88 $\pm$ 11
	<i>p-value</i>	<i>0.79</i>	<i>0.85</i>
Primipara^d			
Yes	137 (53)	98 $\pm$ 10	88 $\pm$ 10
No	122 (47)	100 $\pm$ 9.5	88 $\pm$ 12
	<i>p-value</i>	<i>0.09</i>	<i>0.98</i>
Cesarean Birth			
Yes	72 (27)	99 $\pm$ 10	87 $\pm$ 10
No	198 (73)	99 $\pm$ 9.7	88 $\pm$ 11
	<i>p-value</i>	<i>0.86</i>	<i>0.39</i>
Offspring Gender			
Male	127 (47)	98 $\pm$ 10	89 $\pm$ 11
Female	143 (53)	100 $\pm$ 9.2	87 $\pm$ 11
	<i>p-value</i>	<i>0.21</i>	<i>0.41</i>

	N (%)	MDI Mean $\pm$ SD	PDI Mean $\pm$ SD
Gestational Age (weeks)			
Gestational Age <37	6 (2.2)	95 $\pm$ 10	89 $\pm$ 14
37 Gestational Age <39	97 (36)	99 $\pm$ 9.6	86 $\pm$ 10
39 Gestational Age <41	148 (55)	100 $\pm$ 9.7	89 $\pm$ 11
Gestational Age 41	19 (7.0)	95 $\pm$ 11	88 $\pm$ 12
	<i>p-value</i>	<i>0.16</i>	<i>0.09</i>
Birth Weight for Gestational Age (centile)^e			
Centile <10th	33 (12)	99 $\pm$ 9.9	86 $\pm$ 9.8
10th Centile <90th	223 (83)	99 $\pm$ 9.8	88 $\pm$ 11
Centile 90th	14 (5.2)	97 $\pm$ 10	88 $\pm$ 8.6
	<i>p-value</i>	<i>0.68</i>	<i>0.43</i>
Breastfeeding Duration (months)			
Duration <3	10 (3.7)	96 $\pm$ 9.0	89 $\pm$ 9.9
3 Duration <6	22 (8.2)	97 $\pm$ 10	87 $\pm$ 8.0
6 Duration <9	101 (37)	99 $\pm$ 9.3	89 $\pm$ 9.9
Duration 9	137 (51)	100 $\pm$ 10	87 $\pm$ 12
	<i>p-value</i>	<i>0.47</i>	<i>0.42</i>
Offspring Living With Both Parents Since Birth			
Yes	259 (96)	99 $\pm$ 9.8	88 $\pm$ 11
No	11 (4.1)	97 $\pm$ 84	84 $\pm$ 9.5
	<i>p-value</i>	<i>0.45</i>	<i>0.22</i>
Older Child^f in the Same Household			
Yes	109 (40)	100 $\pm$ 9.4	89 $\pm$ 12
No	161 (60)	98 $\pm$ 10	87 $\pm$ 10
	<i>p-value</i>	<i>0.10</i>	<i>0.25</i>

p-value for ANOVA or two-tailed t-test.

BMI (body mass index), MDI (Mental Developmental Index), PDI (Psychomotor Developmental Index), RMB (renminbi)

^aFor mothers, workers (n=27) include: civil servant (n=2), white-collar worker (n=3), skilled worker (n=9), unskilled worker (n=1), and shopkeeper (n=12). For fathers, workers (n=42) include: civil servant (n=3), white-collar worker (n=3), skilled worker (n=17), unskilled worker (n=3), and shopkeeper (n=16).

^b2000 RMB = US\$327; 5000 RMB = US\$818

^cBMI for Asian populations: underweight (BMI < 18.5 kg/m²), normal weight (18.5 kg/m² BMI < 23 kg/m²), overweight (23 kg/m² BMI < 27.5 kg/m²), and obese (BMI $\geq$ 27.5 kg/m²) (World Health Organization (WHO) Expert Consultation 2004)

^dAlcohol, missing = 3, smoking, missing = 1, primipara, missing = 11

^eCentiles were calculated from an international reference population, including China (Villar et al. 2014)

^fOlder child includes sibling, cousin, etc.

Table 2

Geometric mean (GM) and range for maternal biomarkers, dietary factors, and outcome measures (n=270 mother/offspring pairs).

	GM (Range)
Hair THg (µg/g)	0.47 (0.078, 1.7)
Hair MeHg (µg/g)	0.26 (0.048, 1.4)
Hair %MeHg (of THg) (unitless)	65 (30, 108)
Blood THg (µg/L)	1.2 (0.32, 8.6)
Serum Zn (µg/L)	720 (340, 900)
Serum Se (µg/L)	150 (52, 540)
Blood Pb (µg/dL)	2.6 (0.96, 7.8)
Serum DHA (mg/mL)	0.089 (0.039, 0.33)
Serum EPA (mg/mL)	0.0074 (0.001, 0.092)
Serum N-6/N-3 (unitless)	12 (3.5, 25)
%Calories from Fat	34 (6.9, 81)
%Calories from Protein	11 (5.1, 25)
%Calories from Carbohydrates	52 (12, 82)
Energy Intake (kcal)	1940 (549, 4480)
BSID-II Mental Developmental Index	99 (66, 120)
BSID-II Psychomotor Developmental Index	88 (60, 121)

BSID-II (Bayley Scales of Infant Development-II), DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), MeHg (methylmercury), N-6 fatty acids (linoleic acid and arachidonic acid), N-3 fatty acids (DHA, EPA and alpha-linolenic acid), Pb (lead), Se (selenium), THg (total mercury), Zn (zinc)

Table 3

Spearman's correlation between rice and fish/shellfish ingestion, mercury exposure, and other dietary factors (n=270 mother/offspring pairs).

	Rice ingestion (servings per day)	Fish/shellfish ingestion (servings per day)
Hair THg (µg/g)	0.10	0.08
Hair MeHg (µg/g)	0.04	0.07
Blood THg (µg/L)	0.05	0.09
Serum Zn (µg/L)	−0.02	−0.10
Serum Se (µg/L)	<0.01	−0.02
Blood Pb (µg/dL)	<0.01	−0.07
Serum DHA (mg/mL)	0.05	0.13 *
Serum EPA (mg/mL)	−0.03	0.19 **
Serum N-6/N-3 (unitless)	−0.04	−0.16 **
Pork (servings per day)	0.33 ***	0.21 ***
Eggs (servings per day)	0.16 **	0.37 **
Tofu (servings per day)	0.11	0.24 ***
Fruit (servings per day)	0.27 ***	0.31 ***
Vegetables (servings per day)	0.35 ***	0.23 ***
%Calories from fat	−0.62 ***	−0.01
%Calories from protein	0.17 **	0.49 ***
%Calories from carbohydrates	0.53 ***	−0.14 *
Energy Intake (kcal)	0.65 ***	0.33 ***

p-value for Spearman's correlation:

* p<0.05,

** p<0.01,

*** p<0.001

DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), MeHg (methylmercury), N-6 fatty acids (linoleic acid and arachidonic acid), N-3 fatty acids (DHA, EPA and alpha-linolenic acid), Pb (lead), Se (selenium), THg (total mercury), Zn (zinc)

Table 4

Ingestion rates for rice and fish/shellfish (n=270 mother/offspring pairs).

		Fish/Shellfish Ingestion (n)			Total
		0/Weekly	<Twice/Weekly	Twice/Weekly	
	0/Day	2	0	0	2 (<1%)
Rice Ingestion (n)	<Once/Day	19	15	4	38 (14%)
	Once/Day	90	115	25	230 (85%)
	Total	111 (41%)	130 (48%)	29 (11%)	270

Associations between mercury and maternal/offspring characteristics and child's neurodevelopment (dependent variable) assessed at 12-months (n=270 mother/offspring pairs).

Table 5

	MDI Beta	(95% CI)	r ²	PDI Beta	(95% CI)	r ²
Unadjusted Models						
Log ₁₀ Hair THg (µg/g)	-4.4	(-9.4, 0.6)	0.01	-2.9	(-8.5, 2.7)	0.01
Log ₁₀ Hair MeHg (µg/g)	-2.4	(-6.7, 1.8)	0.01	-1.3	(-6.1, 3.6)	0.01
Log ₁₀ Blood THg (µg/L)	-2.5	(-7.7, 2.6)	0.01	-3.6	(-9.4, 2.1)	0.01
Fully Adjusted Model						
Log ₁₀ Hair THg (µg/g)	-4.9 *	(-9.7, -0.1)	0.20	-2.7	(-8.3, 2.9)	0.14
Maternal Pre-Pregnancy BMI (kg/m ²) ^a						
Underweight		Referent			Referent	
Normal Weight	3.6 **	(0.87, 6.2)		2.7	(-0.41, 5.8)	
Overweight	2.4	(-1.3, 6.1)		3.4	(-0.89, 7.7)	
Obese	0.57	(-5.9, 7.1)		3.9	(-3.6, 11)	
Mother's Education Completed						
<High School	-2.3	(-7.3, 2.6)		4.4	(-1.3, 10)	
High School	-4.1	(-9.6, 1.4)		2.3	(-4.0, 8.7)	
University		Referent			Referent	
Mother's Age at Parturition (yr)	0.015	(-0.18, 0.21)		-0.029	(-0.26, 0.20)	
Fish/Shellfish Ingestion						
Ingestion=0/Week		Referent			Referent	
0<Ingestion<Twice/Week	1.7	(-0.86, 4.2)		0.38	(-2.5, 3.3)	
Ingestion Twice/Week	4.1 *	(0.041, 8.2)		2.2	(-2.6, 6.9)	
Daily Rice Ingestion (Yes)	-2.7	(-6.7, 1.2)		-0.23	(-4.8, 4.3)	
Serum Zn (µg/L)	0.021 *	(0.0038, 0.039)		0.0041	(-0.016, 0.025)	
Log ₁₀ Blood Pb (µg/dL)	-3.7	(-12, 4.8)		-11 *	(-21, -1.2)	
Log ₁₀ Energy Intake (kcal)	7.1	(-1.3, 15)		14 **	(3.9, 23)	

	MDI Beta	(95% CI)	r ²	PDI Beta	(95% CI)	r ²
Offspring Sex (Males)	-1.8	(-4.0, 0.42)		0.59	(-2.0, 3.2)	
Birth Weight for Gestational Age (z-score) ^b	0.070	(-1.2, 1.4)		1.3	(-0.25, 2.8)	
Offspring Age at Follow-Up (months)	5.1 ***	(3.2, 7.0)		3.1 **	(0.91, 5.3)	

p-value for Beta coefficient:

* p<0.05,
** p<0.01,
*** p<0.001

BMI (body mass index), MeHg (methylmercury), CI (confidence interval), MDI (Mental Developmental Index), PDI (Psychomotor Developmental Index), Pb (lead), THg (total mercury), Zn (zinc)
^aBMI for Asian populations: underweight (BMI< 18.5 kg/m²), normal weight (18.5 kg/m² BMI < 23 kg/m² BMI < 27.5 kg/m² BMI < 27.5 kg/m² (World Health Organization Expert Consultation 2004)

^bZ-scores were calculated from an international reference population, including China (Villar et al. 2014)