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Associations of prenatal maternal blood mercury concentrations with early and mid-childhood blood pressure: A prospective study

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ABSTRACT

Background: Childhood blood pressure (BP) is an important determinant of adult cardiovascular disease. Prenatal exposure to methylmercury through maternal fish consumption has been reported to increase the BP of children years later.

Methods: Mother–child pairs were enrolled from Project Viva, a prospective cohort study in Massachusetts. From second trimester maternal blood samples, we measured erythrocyte mercury concentration. Systolic BP in children, measured up to 5 times per visit in early and mid-childhood (median ages 3.2 and 7.7 years), was the primary outcome. We used mixed-effect regression models to account for variation in the number of BP measurements and to average effects over both time points.

Results: Among 1103 mother–child pairs, mean (SD) second trimester total erythrocyte mercury concentration was 4.0 (3.9) ng/g among mothers whose children were assessed in early childhood and 4.0 (4.0) ng/g for children assessed in mid-childhood. Mean (SD) offspring systolic BP was 92.1 (10.4) mm Hg in early childhood and 94.3 (8.4) mm Hg in mid-childhood. After adjusting for mother and infant characteristics, mean second trimester blood mercury concentration was not associated with child systolic BP (regression coefficient, 0.1 mm Hg; 95% CI, −1.3 to 1.5 for quartile 4 vs. quartile 1) at either time period. Further adjusting for second trimester maternal fish consumption, as well as docosahexaenoic acid and eicosapentaenoic acid consumption, did not substantially change the estimates.

Conclusions: The results of this study demonstrate an absence of association between childhood blood pressure and low-level mercury exposure typical of the general US population.

1. Introduction

Fish is the primary dietary source of omega-3 ($n-3$) polyunsaturated fatty acids, which may promote cardiovascular health by lowering resting heart rate and blood pressure (BP), improving endothelial function, increasing cardiac filling and myocardial efficiency, and decreasing vascular inflammation (Mozaffarian and Wu, 2011).

In fact, fish consumption, especially of species higher in omega-3 fatty acids, is associated with a markedly reduced risk of cardiovascular disease and sudden cardiac death (Chowdhury et al., 2012; Mozaffarian and Rimm, 2006; Mozaffarian and Wu, 2011). However, fish may also be contaminated with methylmercury, a toxic heavy metal that bioaccumates in the food chain and concentrates in larger, predatory fish. Prenatal exposure to methylmercury from seafood consumption may impair the cardiovascular health in children (Mone et al., 2004).

Mercury readily crosses the placenta and enters fetal circulation, where it has adverse neurocognitive effects (National Research Council, 2000). However, the tissue-specific effects of mercury on the fetal heart and vasculature are unknown (Castoldi et al., 2003; Clarkson, 2002). A longitudinal cohort study in the Faroe Islands reported that higher prenatal methylmercury exposure was associated with greater mean systolic and diastolic BP at age 7 years (higher by 14.6 mm Hg and 13.9 mm Hg, respectively, for 10 vs. 1 μg/L of cord blood mercury concentrations). In

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addition, mercury had a greater effect on children with low birth weights (Sørensen et al., 1999). A study in the Republic of Seychelles found that higher prenatal methylmercury exposure was associated with higher diastolic BP (0.36 mm Hg per 1 ppm increase in prenatal methylmercury exposure) but not systolic BP, and only among boys at age 15 years (Thurston et al., 2007). No effect was seen in girls or at age 12 (Thurston et al., 2007). Information on fish consumption was not available in these studies.

The biologic plausibility of an association between methylmercury exposure and BP is supported by the fact that mercury promotes oxidative stress, mitochondrial dysfunction, and lipid peroxidation (Salonen et al., 1995; Shenker et al., 1999; Yin et al., 2007). Mercury also decreases vascular endothelial repair, reduces the availability of nitric oxide, induces endothelial dysfunction, and promotes vascular smooth muscle proliferation, all of which may theoretically increase the risk of cardiovascular dysfunction (Aguado et al., 2013; Furieri et al., 2011; Lemos et al., 2012; Wiggers et al., 2008).

Thus, the balance between the potential harms for the cardiovascular system from methylmercury in fish and the potential benefit from nutrients is unclear. We sought to determine whether prenatal maternal blood concentrations of methylmercury among US women were associated with the offspring's BP in childhood years later.

2. Materials and methods

The Institutional Review Board of Harvard Pilgrim Health Care approved all study protocols, and all procedures were conducted in accordance with established ethical standards (Declaration of Helsinki, 2008). Mothers provided written informed consent at the time of recruitment and again for their children's participation at each visit after delivery, including early- and mid-childhood. Children provided verbal assent at the mid-childhood visit.

2.1. Participants

Participants were enrolled in Project Viva, a prospective pre-birth cohort study in Massachusetts. Between April 1999 and July 2002, we recruited pregnant women at their initial prenatal visit to Harvard Vanguard Medical Associates, a multispecialty group practice in eastern Massachusetts (Gillman et al., 2004). Recruitment and retention procedures for this longitudinal cohort have been described elsewhere (Oken et al., 2014). Women were eligible to enroll if they presented to their initial prenatal visit at <22 weeks of gestation, had a singleton pregnancy, did not plan to move away from the study area prior to delivery, and could complete study forms in English. To be included in this analysis, women had to have second trimester blood samples collected.

2.2. Data collection

2.2.1. Red blood cell mercury concentrations

At the second study visit, we collected maternal blood samples in Vacutainer tubes (Becton, Dickinson and Company) containing ethylenediaminetetraacetic acid. The tubes were centrifuged at 2000 rpm for 10 min at 4 °C to separate plasma from erythrocytes, which were then washed with chilled saline. Erythrocyte aliquots were stored at −70 °C until analysis.

Total mercury concentration was measured using the Direct Mercury Analyzer 80 (Milestone Inc.). Results were reported as mercury concentration in the original red cell sample. The detection limit was 0.5 ng/mL of sample. Blood samples from the interlaboratory study program from INSPQ/Laboratoire de Toxicologie, Quebec, were used as the quality control samples to monitor the accuracy and interday and intraday repeatability of the analysis. Concentrations of the quality control samples ranged from 3 ng/mL to 30.09 ng/mL. Percentage recoveries of these samples were between 87% and 104%. The interday repeatability ranged from 1.5% to 11.7%, and the intraday repeatability ranged from 0.2% to 11.4%. Percentage differences for duplicate analysis of quality control samples ranged from 0.04% to 12.4%.

2.2.2. Blood pressure in children at early and mid-childhood

At the early and mid-childhood study visits, trained research assistants measured each child's BP up to five times, at 1-minute intervals, using biannually calibrated Dinamap Pro 100 or Pro 200 (Critikon Inc.) automated BP monitors. The conditions of measurement were recorded, including the activity of the child (sleeping, quiet awake, active awake, or crying at the early childhood visit and quiet, still, talking, or moving at the mid-childhood visit); cuff size (child, small adult, adult, large adult); arm used for the measurement; and position (sitting, semi-reclining or standing).

2.2.3. Covariates

We studied covariates that were of *a priori* interest as independent predictors of child cardiovascular health. Using questionnaires and interviews, we collected information at study enrollment on maternal age, race/ethnicity, education, prenatal smoking and alcohol consumption, marital status, pre-pregnancy height and weight (from which we calculated body mass index [BMI]), and history of hypertension.

Maternal second trimester fish intake, measured on an ordinal scale of servings per week, was assessed with a food-frequency questionnaire (FFQ). The FFQ is modeled on one that has been extensively used in several other cohort studies and was previously validated for erythrocyte fatty acid content during pregnancy (Donahue et al., 2009; Fawzi et al., 2004; Rimm et al., 1992; Willett et al., 1985). The FFQ assessed average frequency of consumption of over 140 foods and beverages, as well as vitamin and supplement use, over the past 3 months. We multiplied a weighted value assigned to the frequency of consumption on the FFQ by the nutrient composition of each item to obtain specific nutrient intake. We derived nutrient estimates from the Harvard nutrient composition database (Hu et al., 2002; Iso et al., 2001). We used the nutrient residuals method to energy adjust the estimates of micronutrient intake (Willett, 1998).

Infant birth weight and date was obtained from the hospital clinical record, and gestational age was calculated using the last menstrual period. If the estimate of gestational age from the second trimester ultrasound differed by more than 10 days, we used the ultrasound measurement instead. Z scores for gestational age-adjusted birth weight (a measure of fetal growth) were calculated from US national natality data (Oken et al., 2003). The duration of breast-feeding was determined from questionnaires administered 6 and 12 months postpartum.

At the early and mid-childhood study visits, trained research assistants measured child weight (early childhood: Seca model 881, Seca Corp; mid-childhood: Tanita model TBF-300A, Tanita Corporation of America, Inc.) and height (Shorr stadiometer, Shorr Productions) using standard techniques. We calculated BMI and age- and sex-specific BMI z-scores from Centers for Disease Control and Prevention reference data (National Center for Health Statistics, 2000).

2.3. Statistical methods

We assessed bivariate associations of maternal and child characteristics with child systolic BP in early and mid-childhood using separate linear regression models with the outcome as the mean of the (up to) five BP measurements at each visit. The associations between predictors and covariates with child BP were similar at both outcome time points, and BP in early childhood was correlated with BP in mid-childhood ($r=0.28$). To improve power and prediction, we incorporated the two outcome time points in the same analysis using mixed-effect regression models, with an indicator for time as both a fixed- and random-effect covariate (Laird and Ware, 1982).

Each BP measurement was treated as a repeated measure. In all models, we adjusted for BP measurement conditions (child state and position, arm used, and measurement sequence number) to minimize measurement error, as well as for child exact age and sex. Systolic BP was the main outcome because it predicts later outcomes better than does diastolic BP and is measured more accurately with the Dinamap (Chobanian et al., 2003; Whincup et al., 1992). In all multivariate models, we examined second trimester mercury concentration in quartiles.

We created four multivariate models. Model 1 was adjusted for visit (early or mid-childhood), measurement conditions, and child age and sex. In Model 2, we also adjusted for potential confounders, including maternal age, race/ethnicity, education, marital status, pre-pregnancy BMI, smoking status, and second trimester BP, as well as child BMI z-score and fetal growth z-score. In model 3, we adjusted Model 2 for maternal second trimester fish intake, and in Model 4, for docosahexaenoic acid + eicosapentaenoic acid (DHA + EPA), as measured in the food frequency questionnaire intake.

Not all participants had complete data, although most were missing only one or two measures. We therefore used multiple imputation to generate several plausible values for each missing characteristic (Horton and Kleinman, 2007; Rubin, 1987). A "completed" data set comprised the observed data and one imputed value for each missing value. We replicated this analysis across completed data sets and then combined them in a structured manner that reflects the true amount of information in the observed data. This process recovers information in participants with missing data without presuming that the imputed values are known true values. We generated 50 complete data sets and combined multivariable modeling results (Proc MIANALYZE) in SAS version 9.3 (SAS Institute, Cary NC).

From these multiple imputation results, we report adjusted differences estimated from regression coefficients and 95% confidence intervals (CI). The data met the assumptions of all statistical tests. Alpha was set at 0.05, and all tests were two-tailed.