Prenatal mercury contamination: relationship with maternal seafood consumption during pregnancy and fetal growth in the ‘EDEN mother–child’ cohort

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Maternal seafood intake is of great health interest since it constitutes an important source of n-3 fatty acids, but provides also an important pathway for fetal exposure to Hg. The objective of the present study was to determine associations between Hg contamination and both maternal seafood consumption and fetal growth in French pregnant women. Pregnant women included in the ‘EDEN mother–child’ cohort study answered FFQ on their usual diet in the year before and during the last 3 months of pregnancy, from which frequencies of seafood intake were evaluated. Total hair-Hg level was determined for the first 691 included women. Associations between Hg level, seafood intake and several neonatal measurements were studied using linear regressions adjusted for confounding variables. The median Hg level for mothers was 0.52 μg/g. Maternal seafood intake was associated with Hg level (r=0.33; P=0.0001). There was no association between Hg level and fetal growth in the whole sample of women, except for an early negative relationship with biparietal diameter. A positive association was found between seafood intake and fetal growth in overweight women only which remained unchanged after adjustment for Hg level (birth weight: +101 g for a difference of 1 SD in seafood consumption; P=0.008). Although seafood intake was associated with Hg contamination in French pregnant women, the contamination level was low. There was no consistent association between Hg level and fetal growth. Taking into account Hg level did not modify associations between seafood intake and fetal growth.

Mercury: Seafood consumption: Prenatal exposure: Fetal growth

Human exposure to methylmercury occurs mainly via consumption of fish[1–5]. As methylmercury is transferred to the infant through the placenta, maternal exposure represents a risk for the offspring[6,7]. Adverse health effects following prenatal exposure to methylmercury have been described from several prospective cohort studies conducted in fish-eating populations: low mean birth weight in Greenland[8]; adverse neuropsychological and behavioural effects in Faroe Islands[9,10]; risk of pre-term delivery in Michigan[11].

Nevertheless, in the Seychelles Child Development Study[12–14], no adverse effects of methylmercury exposure have been found. The first hypothesis to explain these controversial results was the different level of contamination of the study populations; the second was a difference in the

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kind of fish consumed which resulted in differences in nutrient intake (n-3 fatty acids)\textsuperscript{12}, Se and other contaminant exposure (polychlorinated biphenyls; PCB).

Fish is also known to have beneficial effects on fetal growth since it provides PUFA, especially n-3 fatty acids. In both epidemiological\textsuperscript{15–19} and intervention studies\textsuperscript{20,21} mainly performed in women from Denmark and the Faroe Islands, intake of seafood or marine n-3 fatty acids by pregnant women was associated with an increased birth weight, explained by both a prolonged duration of pregnancy and increased fetal growth rate. In the ‘EDEN mother–child’ cohort, we have found a positive association between seafood consumption before pregnancy and fetal growth limited to overweight women\textsuperscript{22}.

The public is faced with seemingly conflicting reports on the risks and benefits of seafood intake, resulting in controversy and confusion over the place of fish consumption in a healthy diet. Only recently, a few studies in this field have focused on contaminant risks at the same time as nutrient benefits related to fish intake, although both risks affect the same outcomes and are derived from the same foods\textsuperscript{23–26}. Some recent studies hypothesised a confounding role of maternal nutrition in the assessment of Hg risk\textsuperscript{27,28}, suggesting opposite effects of maternal seafood intake and Hg exposure\textsuperscript{29,30}.

**Objectives**

To further explore the relationship between seafood consumption before pregnancy and fetal growth reported in the ‘EDEN mother–child’ cohort\textsuperscript{22,31}, the aim of the present analysis was, in the same French population, to study: (1) the association between seafood consumption and Hg contamination; (2) potential risks of Hg exposure on fetal growth; (3) whether relationships between seafood consumption and fetal growth were modified after taking into account Hg and Se exposure.

**Methods**

**Population and study design**

Pregnant women (n = 2002) were recruited in the University Hospitals of Nancy and Poitiers before 24 weeks of amenorrhoea. Standard ultrasound fetal measurements were recorded from routine examinations at 20–24 and 30–34 weeks of amenorrhoea. Pre-pregnancy BMI was calculated by dividing reported body mass (kg) by the square of measured height (m\textsuperscript{2}). According to references of the International Obesity Task Force, overweight was defined as BMI \( \geq 25 \) kg/m\textsuperscript{2} and obesity as BMI \( \geq 30 \) kg/m\textsuperscript{2}. Birth weight and length were extracted from the hospital records. Head circumference (in duplicate) and tricipital and subscapular skinfolds (in triplicate) were performed on the newborn after delivery (aged 1–8 (range 0–16) d), and averaged. Standard ultrasound fetal measurements were recorded from routine examinations performed at 20–24 and 30–34 weeks of gestation. Measurements included biparietal diameter, head and abdominal circumferences and femur length.

The study was approved by the Ethics Committee of the Bicêtre Hospital. Written consents were obtained from the mother for herself at inclusion and for her newborn child after delivery.

**Dietary assessment**

Mothers completed two FFQ: at inclusion, about diet in the year before pregnancy; after birth, about their diet in the last 3 months of pregnancy. We combined responses to the six questions that inquired about seafood consumption: ‘At which frequency did you eat’: (1) fresh or frozen fish (bought unprocessed); (2) oily fish; (3) smoked or salted fish; (4) breaded fish; (5) dishes containing fish; (6) shellfish.

We generated an average frequency of seafood servings per month for each woman, by weighting each frequency with the midpoints of the category (i.e. two for the category of 1–3 servings/month). Information about the type of fish was asked only in women who were regular eaters (more than once per month). Regular fish eaters consumed both fatty and lean fish and we were not able to contrast women according to this characteristic.

**Determination of mercury exposure**

Determination of heavy metal exposure was planned for the first 700 women included in the cohort for cost reasons. Hair samples were stored until analysis at room temperature. Chemical analyses were carried out at TOXILABO (Nantes, France), by cold-vapor atomic absorption spectrometry (Zeeman PerkinElmer AA600; PerkinElmer, Wellesley, MA, USA) for 691 mothers and only eighty-seven newborns due to low hair mass. When hair mass was under 10 mg for mothers and 7 mg for newborns, measures were considered too inaccurate and were not taken into account. For samples of eighty-two women and sixty-six newborns with Hg levels too low to be detected, we arbitrarily attributed half of the limit level detectable with the hair mass. Hg concentration (\( \mu g/g \)) was log transformed because of a skewed distribution.

**Determination of selenium concentration**

Frozen samples at −80°C were thawed for Se measurements. Se concentrations in blood (\( \mu g/l \)) were determined by a fluorimetric method which involves the reaction of 2,3-diaminonaphthalene (DAN) with Se (IV) to form a fluorescent Se–DAN piazselenol.

**Variable description and statistical analyses**

Comparisons between groups were studied by Student’s \( t \) test and correlations by Pearson’s and Spearman’s correlations. We studied relationships between seafood consumption before pregnancy, as well as maternal Hg level and fetal growth, using multiple linear regressions adjusted for different sets of confounding variables. Most of the included women were from Poitiers because this centre started recruitment earlier; therefore, comparisons were performed with adjustment for centre. Seafood consumption and Hg level were studied separately, then in the same model. We performed more analyses to evaluate the impact of extreme values on the relationships; total hair-Hg level was studied in classes to separate the 15% lower levels (N1, Hg \( \leq 0.23 \) \( \mu g/g \)) and the 15% higher levels (N4, Hg \( \geq 0.82 \mu g/g \)) and the two middle categories (N2, 0.23–0.52 \mu g/g; N3, 0.52–0.82 \mu g/g). As BMI modified relationships between seafood intake and fetal growth.
(P for interaction=0.0001 for birth weight), we studied separately non-overweight and overweight women (BMI < 25 v. ≥ 25 kg/m²). Adjustments for Se concentration or educational level were also made.

All analyses were performed with SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

Subject characteristics

Among the 691 first women included in the study, twenty-six were excluded because of a hair samples < 10 mg, fifteen because seafood consumption was unknown and five because the delay between birth and newborn anthropometric measures was greater than 1 week.

Mean pre-pregnancy BMI was 23 kg/m²; overweight women accounted for 26.7% of included women (n 645) and 28.1% of non-included women (n 1251). There were no differences in gestational age (39.2 weeks both) or parity (54% multiparous both) between included and excluded women, except that included women were slightly younger (28.7 vs. 29.2 years) and more often smokers (31 v. 27.6%) than the others. Sex ratio (boys:girls) was similar (1.1 and 1.2) in excluded and included women, respectively. Mean consumption of seafood was on average 8.4 (SD 7.75) times 1.2) in excluded and included women, respectively. Mean than the others. Sex ratio (boys:girls) was similar (1.1 and

Table 1. Fetal growth in relation to maternal total hair mercury level and average seafood intake before pregnancy in 159 overweight women

<table>
<thead>
<tr>
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<th>Model 1*</th>
<th>Model 2*</th>
<th>Model 3*</th>
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<td>Seafood</td>
<td>Mercury</td>
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<td>n</td>
<td>β†</td>
<td>P‡</td>
<td>β†</td>
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<tr>
<td>Ultrasound measures at 20–24 WG</td>
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<tr>
<td>Biparietal diameter (mm)</td>
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<td>Head circumference (mm)</td>
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<tr>
<td>Abdominal circumference (mm)</td>
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<td>0.16</td>
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<tr>
<td>Femoral length (mm)</td>
<td>154</td>
<td>0.18</td>
<td>0.32</td>
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<tr>
<td>Ultrasound measures at 30–34 WG</td>
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<tr>
<td>Biparietal diameter (mm)</td>
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<td>Femoral length (mm)</td>
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<td>Birth length (cm)</td>
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<td>Head circumference (cm)</td>
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<td>Gestational length (WA)</td>
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<td>Placental weight (g)</td>
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<td>0.75</td>
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</table>

WG, weeks of gestation; WA, weeks of amnorrhoea; MeHg, methylmercury.

* Adjusted for centre, maternal age and height, smoking during pregnancy, parity (yes/no), gestational length (at ultrasound measures or at delivery), delay between birth and anthropometric measures (except for ultrasound measures and gestational length) and newborn's sex. Model 1: association with MeHg level. Model 2: association with seafood intake. Model 3: association with MeHg level and seafood intake, mutually adjusted on each other.

†β corresponds to variation of the outcome variable for 1 sd of MeHg level (2.60) or seafood intake (7.75).

‡ Linear regression test.

Correlations between maternal total mercury level and maternal characteristics

Total hair-Hg levels were higher with age and university level, in Poitiers, and in non-smokers during pregnancy. BMI was not associated with Hg level. Spearman correlations between Hg and seafood consumption before pregnancy were 0.33 (P<0.0001) and 0.29 (P<0.0001) in the last 3 months of pregnancy. When the different items contributing to global seafood intake before pregnancy were considered separately, correlations with Hg contamination were stronger for 'fresh or frozen fish', 'smoked or salted fish', 'oily fish' and shellfish (r 0.39; 0.28; 0.20 and 0.17, respectively; P<0.0001).

Mercury exposure, seafood consumption and fetal growth

In the whole sample of women, there was no association between maternal level of total hair-Hg and ultrasound measures as well as newborn anthropometric measures (data not shown). Only a negative association was observed between total hair-Hg level and biparietal diameter measured at 20–24 weeks of gestation (decrease of 0.24 mm by 1 sd of hair-Hg; P=0.06). Seafood intake was not associated with fetal growth in all women. When both seafood consumption and Hg level were included in the model, the results did not change.

In overweight women, total hair-Hg level and seafood intake before pregnancy were both associated with higher...
newborn anthropometric measures in separate regression models. Seafood intake was also associated with increased placental weight and lower gestational length (Table 1). However, when adjusted on seafood intake, total hair-Hg level was no longer associated with newborn anthropometric measures, whereas the relationship with seafood intake remained the same. Excluding shellfish intake from the computation of seafood intake did not change the results. The association between seafood intake and lower gestational length was reinforced when adjusted for total hair-Hg level but it became non-significant after exclusion of the eight pre-term births.

In non-overweight women (data not shown), total hair-Hg level tended to be negatively associated with biparietal and head circumferences at 20–24 weeks of gestation (decrease of 0·29 and −1·15 mm by 1 s.d. of hair-Hg, respectively; $P<0·06$) but not statistically significant for measures at 30–34 weeks of gestation. Adjustment for seafood intake did not change these results. Seafood intake was associated with a lower birth length and head circumference, with an average decrease of 0·19 and 0·17 cm, respectively, for an increase of 1 s.d. of seafood intake ($P<0·02$), even when adjusted for total hair-Hg level.

A similar trend was observed for associations with seafood intake in the last 3 months of pregnancy, but associations were weaker.

We performed further analyses to evaluate the impact of high maternal values of total hair-Hg on the relationships. The relationships reported above with total hair-Hg were consistent with linear relationships, with no threshold effects for extreme values (data not shown).

In the present study, correlations between Se and seafood consumption were 0·10 ($P=0·03$) and 0·14 ($P=0·001$) before and in the last 3 months of pregnancy. The correlation between maternal hair-Hg after pregnancy and blood Se during pregnancy at 24–28 weeks of amenorrhoea was 0·10 ($P=0·03$). Adjustment for Se level did not change the relationship observed between total hair-Hg, seafood and fetal growth in the whole sample of women as well as in non-overweight and overweight women (data not shown).

Discussion

In the present study, total hair-Hg level was associated with seafood intake, but was not associated with fetal growth.

The lack of association between maternal total hair-Hg level and birth weight could be explained by low mean hair-Hg level in our population (0·52 μg/g) compared with other studies: 12·7 μg/g in French Guiana(32), 12·8 μg/g in Amazonia(33) Studies where prenatal Hg exposure was associated with fetal growth were cases of massive intoxication. The negative relationship with biparietal diameter early in pregnancy may be a chance finding because it was not confirmed with measures at 30–34 weeks of amenorrhoea and at birth. Alternatively, it may disclose an early alteration in the neurological developmental process. Oken et al.(29) found a negative effect on infant cognition when mean hair-Hg level was close to ours (0·55 μg/g). As Lucas et al.(26), we did not find associations between Hg exposure and length of gestation or risk of pre-term delivery whereas Xue et al.(11) and Ramirez et al.(34) found a decrease of length of gestation and risk of pre-term delivery with increasing Hg contamination. However, low exposure induces low power and unstable results.

Hg was measured in hair; however, measured in blood, Hg could be a better estimation of fetal exposure, as hypothesised by Grandjean & Budtz-Jorgensen(35). Problems with undetectable values could explain the differences with other studies; however, similar results were found in the present study when we took into account only detectable measures.

Health effects of Hg may partly be due to selenoprotein activation, which may be moderated by an adequate intake of Se(36,37). A possible protective role of Se has been suggested in some studies to explain lack of negative effects of Hg(38). In the present study, relationships between total hair-Hg and fetal growth remained unchanged after adjustment for Se level.

In conclusion, our data do not support a detrimental effect of low maternal Hg contamination on birth weight or other newborn anthropometric measurements. Taking into account maternal Hg contamination and blood Se level did not change the observed increase in birth weight associated with seafood consumption in overweight women(22). The negative association between seafood intake and gestational length may be a chance finding, as in most studies, opposite or no results were reported(17–19), and was not found in our previous analysis, which was not restricted to women with Hg measurements(22).

Most of the adverse effects of Hg exposure are negative effects on brain development(33) and neuropsychological effects(32). Follow-up of the children included in the present study on visual and neurodevelopmental outcomes will allow us to evaluate the consequences for the child of maternal Hg exposure at levels currently found in France.

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P. D.-P. performed the study analysis and wrote the paper. G. H., R. S. and M. K. participated in the study design and analysis. G. H. supervised and J. S. performed some of the heavy metal measurements. A. F. was in charge of the coordination of the data file and analysis. B. F., G. M., V. G. and O. T. coordinated the EDEN study in Poitiers and Nancy. M.-A. C. participated in the design, coordinates the EDEN study and supervised the analysis with S. C. providing help. All co-authors reviewed the paper.

There are no conflicts of interest.
References


