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Running title: Hg exposure from seafood and fetal growth

SUMMARY

Background: 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 mercury (Hg). **Objectives:** To determine associations between Hg contamination and both maternal seafood consumption and fetal growth in French pregnant women. **Design:** Pregnant women included in the "EDEN mother-child" cohort study answered food frequency questionnaires on their usual diet in the year before and during the last three 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. **Results:** 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 relation 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 (birthweight: +101g for a difference of 1SD in seafood consumption, $p=0.008$). **Conclusions:** 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.

Keywords: Mercury, seafood consumption, prenatal exposure**,** fetal growth.

INTRODUCTION

Human exposure to Methylmercury (MeHg) occurs mainly via consumption of fish[1-5]. As MeHg is transferred to the children through placenta, maternal exposition represents a risk for the offspring[6-7]. Adverse health effects following prenatal exposure to MeHg have been described from several prospective cohort studies conducted in fish-eating population: low mean birthweight in Greenland[8], adverse neuropsychological and behavioural effects in Faroe Islands [9-10], risk of preterm delivery in Michigan[11].

Nevertheless, in the Seychelles Child development Study[12-14], no adverse effects of MeHg exposure had 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 kind of fish consumed which resulting in differences in nutrients intake (n-3 Fatty Acids (FA))[12], selenium and other contaminants exposure (PCBs).

Fish is also known to have beneficial effects on fetal growth since it provides PUFA, especially n-3FA. In both epidemiologic[15-19] and intervention studies[20-21] mainly performed in women from Denmark and the Faroe Islands, intake of seafood or marine n-3FA by pregnant women was associated with an increased birthweight 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[22].

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

Objectives

To further explore the relationship between seafood consumption prior to pregnancy and fetal growth reported in the "EDEN mother-child" cohort[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 was modified after taking into account Hg and selenium exposure.

METHODS

Population and study design

Pregnant (n=2002) women were recruited in the University Hospitals of Nancy and Poitiers before 24 weeks of amenorrhea (WA). Standard ultrasound fetal measurements were recorded from routine examinations at 20-24 and 30-34 WA. Prepregnancy body mass index (pBMI) was computed as reported weight (kg) / measured height squared (m^2). According to references of the International Obesity Task Force, overweight was defined as a BMI $\leqq$25 kg/m^2 or more and obesity as a BMI $\leqq$ 30kg/m^2. Birthweight and length were extracted from the hospital record. Head circumference (in duplicate) and tricipital and subscapular skinfolds (in triplicate) were performed on the newborn after delivery (1.8 days (range 0-16)), and averaged. Standard ultrasound fetal measurements were recorded from routine examinations performed between 20-24 and 30-34 WG. Measurements included biparietal diameter, head and abdominal circumferences and femur length.

The study was approved by the Ethic 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 food frequency questionnaires: at inclusion, about diet in the year prior to pregnancy; after birth, about their diet in the last three 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, and (6) shellfish. We generated an average frequency of seafood servings per month for each woman, by weighing each frequency with the midpoint of the category (i.e. 2 for the category 1-3 servings/month). Information about the type of fish was asked only in women who were regular eaters (more than 1 time/month). Regular fish eaters consumed both fatty and lean fish and we were not able to contrast women according to this characteristic.

Determination of Hg exposure

Determination of heavy metal exposure was planned for the first 700 women included in the cohort for cost reason. 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 Perkin-Elmer AA600) for 691 mothers and only 87 newborns due to low hair mass. When hair mass was under 10mg for mothers and 7mg for newborns, measures were considered too inaccurate and were not taken into account. For samples of 82 women and 66 newborns with Hg levels too low to be detected, we arbitrarily attributed half of the limit level detectable with the hair mass. Hg concentration (expressed in micrograms per gram) was log transformed because of a skewed distribution.

Determination of Se concentration

Frozen samples at -80°C were thawed for Se measurements. Se concentrations in blood (expressed in micrograms per liter) were determined by fluorometric method which involves the reaction of 2,3-diaminonapthalene (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 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 impact of extreme values on the relationships; total hair-Hg level was studied in classes to separate the 15% lower levels (N1: Hg<0.23µg/g) and the 15% higher levels (N4: Hg≥0.82µg/g) and two middle categories (N2: [0.23-0.52[; N3: [0.52-0.82[µg/g). As BMI modified relationships between seafood intake and fetal growth (p for interaction=0.0001 for birthweight), we studied separately non-overweight and overweight women (BMI<25 vs. ≥25 kg/m²). Adjustments for selenium concentration or educational level were also made.

All analyses were performed with SAS version 9.1 (Cary, N.C., USA).

RESULTS

Subjects characteristics

Among the 691 first women included in the study, 26 were excluded because of a hair's samples <10 mg, 15 because seafood consumption was unknown and 5 because delay between birth and newborn anthropometric measures were greater than one week.

Mean pBMI 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 was no differences in gestational age (39.2 weeks both) or parity (54% multipare both) between included and excluded women, except that included women were slightly younger (28.7 vs. 29.2y) and more often smokers (31% vs. 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 times per month (SD=7.75) before pregnancy in both groups. No difference for newborns' measures was observed between the two groups (mean birthweight=3280g).

The median hair-Hg level for mothers and newborns were 0.52 (Interquartile Range (IR):0.30-0.82) (SD=2.6) and 0.38 (IR:0.30-0.43) (SD=0.32) µg/g respectively. As correlation was strong between levels in mothers and their offspring (r=0.43 p<0.0001 (n=87)), and fewer measures were available for newborns, analyses were made with maternal Hg level only. The median blood Se level for mothers was 97.4 µg/l (IR:81.4-114.4) (SD=26.2).

Correlations between maternal total-Hg 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 correlation between Hg and seafood consumption before pregnancy was 0.33 (p<0.0001) and 0.29 (p<0.0001) in the last three 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).

Hg 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 WG (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, 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 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 8 preterm 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 WG (decrease of 0.29 and -1.15 mm by 1SD of hair-Hg respectively, $p<0.06$) but not statistically significant for measures at 30-34 WG. 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 1SD 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 three months of pregnancy but associations were weaker.

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

In our study, correlation between selenium and seafood consumption were 0.10 (p=0.03) and 0.14 (p=0.001) before and in the last three months of pregnancy. Correlation between maternal hair-Hg after pregnancy and blood Se during pregnancy at 24-28WA was 0.10 (p=0.03). Adjustment for selenium level did not change the relation 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 our 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 birthweight could be explained by low mean hair-Hg level in our population (0.52µg/g) compared to other studies: 12.7µg/g in French Guiana[32], 12.8µg/g in Amazonia[33]. Studies where prenatal Hg exposition was associated with risk for fetal growth were cases of massive intoxication. The negative relationship with biparietal diameter early in pregnancy may be a chance finding because was not confirmed with measures at 30-34WA 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 (0.55µg/g). . As Lucas *et al.*[26], we did not find association between Hg exposition and length of gestation or risk of preterm delivery whereas Xue *et al.*[11] or Ramirez *et al.*[34] found a decrease of length of gestation and risk of preterm delivery with increasing Hg contamination. However, low exposition induces low power and unstable results.

Hg was measured in hair, however, measured in blood, Hg could be a better estimation of fetal exposition as hypothesized by Grandjean & Budtz-Jorgensen[35]. Problem with undetectable values could explain the differences with other studies; but similar results were found in our 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 adequate intake of selenium[36-37]. Possible protective role of Se has been suggested in some studies to explain lack of negative effects of Hg[38]. In our study, relationships between total hair-Hg and fetal growth remained unchanged after adjustment for selenium level.

In conclusion, our data do not support a detrimental effect of low maternal Hg contamination on birthweight or other newborn anthropometric measurements. Taking into account maternal Hg contamination and blood selenium impregnation did not change the observed increase in birthweight 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 were negative effects on brain development(33) and neuropsychological effects(32). Follow-up of the children included in our study on visual and neurodevelopmental outcomes will allow evaluating the consequences for the child of maternal Hg exposure at levels currently found in France.

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Contributors: PD performed the study analysis and wrote the paper. GH, RS and MK participated in the study design and analysis. GH supervised and JS performed some of the heavy metals measurements. AF was in charge of the coordination of the data file and analysis. BF, GM, VG and OT coordinated the EDEN study in Poitiers and Nancy. MAC participated in the design, coordinates the EDEN study and supervised the analysis with the help of SC. All co-authors reviewed the paper.

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

n=159	**n**	**Model 1*** **Mercury** β^{Δ}	$p^{\dagger}$	**Model 2*** **Seafood** β^{Δ}	$p^{\dagger}$	**Model 3*** **Mercury** β^{Δ}	$p^{\dagger}$	**Model 3*** **Seafood** β^{Δ}	$p^{\dagger}$
ULTRASOUND MEASURES at 20-24 WG									
Biparietal diameter (mm)	156	-0.08	0.75	-0.07	0.75	-0.03	0.92	-0.06	0.79
Head circumference (mm)	149	0.94	0.18	0.50	0.44	1.04	0.17	0.14	0.84
Abdominal circumference (mm)	153	1.26	0.16	1.35	0.10	0.93	0.34	1.04	0.24
Femoral length (mm)	154	0.18	0.32	0.24	0.16	0.12	0.53	0.20	0.28
ULTRASOUND MEASURES at 30-34 WG									
Biparietal diameter (mm)	149	0.17	0.57	0.22	0.42	0.15	0.64	0.17	0.56
Head circumference (mm)	145	0.64	0.71	-1.63	0.31	1.74	0.36	-2.19	0.20
Abdominal circumference (mm)	147	0.54	0.68	1.51	0.21	0.34	0.81	1.40	0.28
Femoral length (mm)	149	0.30	0.17	0.39	0.06	0.27	0.27	0.30	0.16
ANTHROPOMETRIC MEASURES									
Birthweight (g)	151	79.80	0.04	115.09	0.001	41.91	0.30	100.52	0.008
Birth length (cm)	147	0.34	0.05	0.39	0.01	0.23	0.21	0.32	0.07
Head circumference (cm)	150	0.17	0.09	0.22	0.02	0.09	0.41	0.19	0.06
Sum of skinfolds (mm)	150	0.24	0.15	0.39	0.01	0.10	0.57	0.35	0.04
OTHER MEASURES									
Gestational length (WA)	156	0.13	0.41	-0.30	0.03	0.30	0.07	-0.40	0.008
Placental weight (g)	109/123	4.05	0.75	31.58	0.005	0.43	0.97	31.48	0.007

$^{\Delta}$ *β corresponds to variation of the outcome variable for 1 SD of MeHg level (2.60) or seafood intake (7.75)*

† *Linear regression test*

* *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*