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Prenatal exposure to methylmercury and LCPUFA in relation to birth weight

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Abstract

Background—Epidemiologic studies have been inconclusive regarding the impact of co-exposure to long chain polyunsaturated fatty acids (LCPUFA) and methyl mercury (MeHg) from fish consumption during pregnancy on measures of fetal development.

Objectives—We evaluated the association between birth weight and prenatal maternal LCPUFA status and MeHg exposure in the Republic of Seychelles.

Methods—We measured LCPUFA in maternal whole blood collected at 28 weeks of gestation and following delivery, and MeHg in maternal hair obtained at delivery. There were 230 births with complete data on birth weight and covariates. Multiple linear regression models controlled for infant sex, gestational age, maternal age, smoking during pregnancy, intrapartum weight gain, pre-pregnancy body mass index, maternal socioeconomic status, parity, gestational diabetes, and alcohol use during pregnancy.

Results—The average birth weight was 3,252 g (range 1654–4450) and the average gestational age was 39 weeks (range 34–41). Prenatal MeHg exposure and maternal LCPUFA status were not associated with birth weight. Infant sex and length of gestation were the only predictors, with male sex and increased gestational age consistently associated with greater birth weight.

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Conclusions—These findings do not support a relationship between prenatal exposure to LCPUFA and/or MeHg from fish consumption and birth weight.

Keywords

methylmercury compounds; fatty acids; unsaturated; birth weight; maternal exposure

Introduction

Many communities around the world depend on the nutritional properties of seafood, as it may be the only protein source that is reasonably available and affordable (1). Seafood contains nutrients such as long chain polyunsaturated fatty acids (LCPUFA) which are essential for maternal and fetal health, as they serve a crucial role in neurologic, visual, and immune function (2). However, there is concern that toxicants present in marine fish, such as methylmercury (MeHg) may affect fetal well-being and child development (3, 4).

Research on the potential effect of LCPUFA and MeHg has focused on neurobehavioral development. MeHg is especially toxic to the developing nervous system at high levels of exposure as demonstrated when children were born with severe neurological deficits in populations consuming seafood or seed grain heavily contaminated with MeHg. However, the extent to which or even if low-level exposure from fish consumption affects child development is uncertain. Numerous studies of neurobehavioral development have been conducted, but fetal growth has also received substantial attention. Assessing the relationship with birth weight is of particular interest as low birth weight has been associated with an increased risk of chronic illnesses later in life (5), including adult coronary artery disease (6), elevated adult body mass index (7), and all-cause mortality in adulthood (8). Biological mechanisms have been proposed to support beneficial influences of LCPUFA as well as adverse consequences of MeHg on birth weight (9). For example, n-3 LCPUFA may promote fetal growth by raising the ratio of active prostaglandins to thromboxanes which may result in increased blood supply to the fetus (9). On the other hand, MeHg may adversely affect fetal growth through the increased production of free radicals (9, 10). However, evidence from human observational and experimental studies has been inconclusive to date.

Several meta-analyses and systematic reviews of randomized controlled trials (RCT) of n-3 LCPUFA supplementation indicate the absence of an effect on birth weight in normal or high risk pregnancies although there may be a beneficial influence on gestational age (11–13). Observational studies relying on self-reported fish or seafood intake have been inconclusive, with some studies showing no association (14–16) and others that do support a beneficial relationship (9, 17) or find an adverse influence of increased intake on birth weight (18). Similarly, some observational studies using biomarkers of LCPUFA status report an increased birth weight in relation to higher n-3 LCPUFA intake (19–21), while others find an inverse relationship (22, 23) or no association (24–26). The literature on MeHg and birth weight is sparse and equivocal (9, 16, 17, 19, 27–31).

It is increasingly recognized that the net result of consuming seafood on maternal and child health is dependent upon the biological interplay between and among toxicants and nutrients (32) and the amount of seafood consumed. However, there is a dearth of literature describing the extent of the combined effect of concomitant exposures in human populations based on biological markers of exposure, information which would enhance the scientific basis for fish consumption advisories and provide insight into their most effective communication to consumers. Therefore, using the Seychelles Child Development Nutrition

Study (SCDNS) we evaluated whether prenatal exposure to methyl mercury and maternal LCPUFA status, alone and in combination, is associated with birth weight.

Methods

Study Population

Participants were recruited for an ongoing longitudinal study evaluating the association of high maternal fish intake during pregnancy with neonatal and developmental outcomes among mother-child pairs residing in the Republic of Seychelles (33, 34). The Seychelles is an archipelago of over 100 islands in the Indian Ocean about 1500 km off the coast of East Africa. In 2001, 300 pregnant women were recruited into the study during their first antenatal visit on the island of Mahé, where approximately 90% of the population of Seychelles resides. Of the women recruited with apparently healthy pregnancies at their initial visit, 24 were excluded prior to or at birth (4 were not pregnant, 14 had a miscarriage, termination of pregnancy or stillbirth, one withdrew, one delivered premature twins, two delivered abroad, one mother had preeclampsia and one baby had trisomy 21). Therefore, there were 276 subjects eligible for this specific study. The research protocols were reviewed and approved by the Institutional Review Boards of the University of Rochester and the Ministry of Health in Republic of Seychelles. The procedures followed were in accordance with the Helsinki Declaration, and all participants gave informed consent.

Anthropometric and Exposure Measures

Birth weight was measured in the hospital using routine clinical procedures and standardized scales, and was subsequently recorded on a neonatal form along with other information such as gestational age, birth length, head circumference, and Apgar score. Birth outcome data were available for 275 of the 276 subjects. Birth weight was only considered as a continuous variable because of the modest sample size and relatively low prevalence of low birth weight (<2500 grams; 6.1%).

As previously described, prenatal exposure to MeHg was estimated by measuring total maternal hair mercury during pregnancy in the sample that best recapitulated prenatal exposure using cold vapor atomic absorption spectroscopy with quality control procedures (35). Over 80% of Hg in hair is known to be organic (35). We have assumed a hair growth rate of 1.1 cm per month and a delay of 20 days between current blood concentrations and appearance of Hg in the first centimeter of scalp hair.

For the assessment of maternal LCPUFA status, non-fasting blood samples (30 ml) were taken from mothers at 28 weeks of gestation, and again one day after delivery. Samples were maintained at -80 degree Celsius until assayed using a modified method of Folch and colleagues (36). Absolute amounts of PUFA were determined using GC-MS as described previously (37). Maternal serum LCPUFA concentrations (in mg/ml), including both n-3 (docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), alpha-linolenic acid (ALA)) and n-6 (arachidonic acid (AA), linoleic acid (LA)) fatty acids, were determined using the geometric mean of the values measured at these two time points. As described elsewhere (33), missing LCPUFA values at each time point were imputed based on the relationship between each LCPUFA at the two time points in the observed data before calculation of geometric means.

Covariates

We controlled for the following covariates known to be associated with child development: child sex, gestational age (continuous; in weeks), maternal age (continuous; in years), alcohol and tobacco use during pregnancy (yes or no for each), intrapartum weight gain (in

kg), pre-pregnancy body mass index (in kg/m²), maternal socioeconomic status (continuous score), parity (continuous), and gestational diabetes (yes or no). Information on child sex, gestational age, intrapartum weight gain, pre-pregnancy body mass index, and gestational diabetes were obtained from hospital records, whereas data on tobacco and alcohol use, maternal socioeconomic status, and parity were obtained from a questionnaire. We operationalized maternal socioeconomic status through the use of the Hollingshead Social Status Index (38) which is a measure of social status based on educational attainment (seven codes; no education to postgraduate) and occupational prestige (nine codes; menial service to higher executive). Higher codes indicate higher educational attainment or higher occupational status. The Hollingshead Social Status Index was modified for the use of codes relevant to the Republic of Seychelles (39). We combined occupational and educational codes through a weighted formula into a continuous Hollingshead Index (39). Previous studies on birth weight, nutrients and toxicants in fish have controlled for similar covariates (9, 18, 22, 23, 26, 40). Given the comprehensive *a priori* selection of covariates, a total of 230 out of 275 mother-child pairs had complete covariate data and were available for the primary analysis. Data were primarily missing for one or more LCPUFA measures at both 28 weeks and delivery (n=8), maternal socioeconomic status (n=14), and parity (n=23) (Table 1).

Data Analysis

Descriptive analyses of endpoints, primary predictors and covariates included the mean, standard deviation and median for continuous measures, and proportions for nominal variables. Measures of central tendency (mean, median) and variability (standard deviation) were reported for the whole study population.

We considered three primary models adjusted for the *a priori* selected covariates. Model 1 included the sum of DHA and EPA (as a measure of the n-3 LCPUFA found in fish), and AA. Model 2 evaluated total n-3 (the sum of DHA, EPA, and ALA) and n-6 (the sum of AA and LA) LCPUFA. We compared results with and without adjustment for MeHg. In Model 3, we evaluated the association of MeHg with birth weight without adjustment for LCPUFA. All models were repeated without adjustment for gestational age to examine previously reported findings that LCPUFA primarily affects birth weight by increasing gestational age, i.e., the association between LCPUFA and birth weight disappears after accounting for gestational age (23, 41). We repeated these models controlling for significant predictors only, thereby omitting covariates with potentially missing data and increasing the sample size to 267. This approach allowed us to examine how excluding participants with missing observations influenced the regression findings. In secondary analyses we examined models that included MeHg by EPA+DHA, MeHg by AA, MeHg by total n-3 and MeHg by total n-6 interactions while accounting for all other covariates. In these interaction models, LCPUFA variables were included as indicator variables based on tertiles of their sample distribution, following the same approach as our analyses for other outcomes (33). We also examined models that included MeHg by child sex and LCPUFA by child sex interactions. Within each model we used a two-tailed alpha- level of 0.05 to determine the significance of interactions and independent variable effects. We did not observe any statistically significant interactions; therefore, we present the estimates of parsimonious models without interaction terms. All models predicted birth weight adequately (model p value <0.05).

Regression assumptions were checked for each model, and the assumption of normally distributed errors with constant variance appeared reasonable for these models. We checked for collinearity among variables using variance inflation factors (VIFs) (42), and all VIFs were found to be well below two in models without interactions indicating that collinearity was not a concern. Additionally, each model was checked for statistical outliers (defined as

observations with standardized residuals greater than 3 in absolute value) and influential points (defined as observations with a Cook's distance larger than 0.50).

Results

Descriptive data of the study sample are presented in Table 1. Among the 230 subjects with complete covariate data, there were 113 males and 117 females. The average maternal hair MeHg level was 5.9 ppm. The mean age of mothers at birth was 28.3 years (range: 16–43). The average gestational age was 38.7 weeks (range: 34–41), with a mean birth weight of 3,252 grams (range: 1654–4450); 14 infants (6.1%) had a birth weight of less than 2,500 grams. Tobacco use during pregnancy was rare (3%), however, 46% of mothers reported using some alcohol during pregnancy. Participants excluded from primary analyses had a slightly lower birth weight and MeHg exposure, and mothers were slightly younger and had lower pre-pregnancy BMI. However, they were similar in terms of LCPUFA measures and other covariates.

The correlation between various LCPUFA measures and MeHg concentrations was weak to modest, with Spearman's correlation coefficients of 0.05 ($p=0.42$) for AA, 0.11 ($p=0.10$) for total n-6, and 0.28 ($p=0.00$) for EPA+DHA, and 0.28 ($p=0.00$) for total n-3. DHA was the main contributor to total n-3 concentrations, with EPA and ALA contributing only minimally.

Regression modeling results are summarized in Table 2. Maternal LCPUFA status was not associated with birth weight in either model 1 or model 2. The addition of MeHg as a covariate did not materially change the interpretation of the LCPUFA results, and MeHg was not independently predictive of birth weight in any of the models (Table 2). Sex and gestational age were significant predictors of birth weight in all models. Omitting gestational age from the models did not substantively affect LCPUFA and MeHg associations with birth weight, with the possible exception of a somewhat stronger adverse association with AA in MeHg-adjusted models ($\beta = -4195.6$, $p=0.05$; $\beta = -3731.6$, $p=0.08$ when one outlier was removed). Findings for LCPUFA and MeHg were not meaningfully different when models were repeated adjusting for sex and gestational age only in the larger dataset (data not shown).

Discussion

Our current analysis using biological markers of maternal nutritional status and prenatal toxicant exposure did not find either LCPUFA or MeHg to be predictive of birth weight, and there was no evidence of an LCPUFA-MeHg interaction. Adjusting for gestational age did not substantially alter our results. In a previous cohort of Seychellois mother-child pairs, we found a positive association between prenatal MeHg exposure and birth weight, but only in boys (43). One can speculate that the earlier finding may have reflected a beneficial influence of nutrients found in fish rather than an effect attributable to MeHg.

Fish is an important source of protein and contains a variety of nutrients, in particular n-3 (primarily DHA) and n-6 (primarily AA) LCPUFA which are important for normal fetal development (44). The human fetus is strongly dependent on placental transfer of both the n-6 LCPUFA, AA, and the n-3 LCPUFA, DHA (45, 46). The exact mechanisms by which LCPUFA might influence fetal growth have not been established. One proposed mechanism suggests that n-3 LCPUFA provided by fish might improve fetal growth due to their effect on endothelium (20, 44). That is, n-3 LCPUFA may promote fetal growth by raising the ratio of active prostaglandins to thromboxanes which inhibits platelet aggregation and reduces blood viscosity, thereby enhancing blood supply to the fetus (9). The n-6 LCPUFA

AA is the precursor of prostaglandins PGE₂ and PGF_{2α}, which are responsible for placental blood flow and play an important role in the physiology of cervical ripening and initiation of labor. A high intake of n-3 LCPUFA may decrease the concentrations of AA, and an imbalance between n-3 and n-6 fatty acids may influence the production of prostaglandins thereby possibly affecting fetal development and labor initiation (9, 11). However, while marine-derived n-3 LCPUFA and also n-6 LCPUFA (which are mainly derived from non-marine sources) are believed to beneficially influence fetal growth, MeHg is believed to counteract LCPUFA effects on platelet aggregation and prostaglandin production. In addition, MeHg may accelerate the production of free radicals and impede the action of glutathione (GSH), which can adversely affect fetal growth (9, 10). Because these are complex interrelated biological processes, human studies of the effects of fish consumption during pregnancy on fetal development should evaluate the integrated effect of both toxicants and nutrients found in fish.

Human experimental and observational studies have not provided consistent evidence for an association between LCPUFA intake during pregnancy and birth weight. Randomized controlled trials of LCPUFA supplementation have generally not found an effect on birth weight in normal or high risk pregnancies (11–13). However, one recent RCT in Mexico found a possible association of DHA on birth weight in a subgroup of first-time mothers, and the authors suggested that the effect may only be apparent in populations with very low background DHA intake (47). Observational studies relying on self-reported fish or seafood intake have been inconclusive. Some have reported beneficial influences on birth weight (9, 17), while others reported no relationship (14, 15) or an adverse association (18). Observational studies using biomarkers of LCPUFA status have also shown conflicting results, with some demonstrating beneficial effects (19–21), which has been suggested to be particularly relevant to low fish-eating populations (20), and others reporting no effect (24–26) or adverse associations (22, 23). The assessment of LCPUFA status in the different studies was variable, however, with sampling of maternal or cord serum and reporting of LCPUFA as absolute or relative quantities.

In observational studies that measured fish-related LCPUFA as well as MeHg, adjusting for MeHg did not meaningfully change the results (15, 17, 19, 23). A few studies reported an association (9, 30), but the preponderance of evidence thus far suggests that MeHg exposure is not an important predictor of birth weight (9, 17, 19, 27–29).

The variability in LCPUFA findings across studies is more difficult to interpret. It could be due to a combination of differences between studies such as genetic heritage, measures of LCPUFA intake (i.e., variability in biomarker assessment, or reliance on self-reported intake), underlying health and nutritional status of pregnant mothers, or the presence of other concomitant exposures and nutrients potentially affecting fetal growth. Recently, Lee and colleagues found that a glutathione S-transferase (GST) polymorphism may modify the association between prenatal MeHg exposure and birth weight (40). Our results support those of previous studies reporting the absence of an association between birth weight and prenatal exposure to LCPUFA and MeHg.

Our study has a number of strengths. The subjects were enrolled early in pregnancy and LCPUFA were measured during pregnancy and at delivery, allowing for a prospective assessment. Maternal hair MeHg levels were about 12 times higher than in US women, which increased our ability to detect an association with MeHg, if one truly exists. We were able to account for many covariates which should make our findings less susceptible to the possibility of residual confounding. Finally, our study is one of the few to consider confounding and interaction effects between toxicants and nutrients found in fish. Only two

previous studies have relied on biological measures of both maternal LCPUFA status and prenatal MeHg exposure (19, 23).

However, our findings should be interpreted in light of several limitations. First, our sample size may have been too small to detect interactions between toxicants and nutrients, although our cohort was similar in size to one previous study assessing biological LCPUFA and MeHg measures (23). Second, there were a relatively small number of infants of low birth weight and the range in gestational age was quite narrow, precluding our ability to evaluate more clinically relevant endpoints using a binary approach. Third, our findings may have been affected by non-differential measurement error of LCPUFA. This could have resulted from selective oxidation of the more susceptible LCPUFA among a random subset of serum samples. If present, this measurement error would have resulted in bias towards the null, limiting our ability to detect associations with LCPUFA. Finally, mothers in Seychelles consumed large quantities of fish (nine fish meals per week). This may have resulted in higher LCPUFA concentrations which may have limited us from identifying LCPUFA associations. Previous literature suggests that the beneficial influences are limited to pregnant mothers with low LCPUFA consumption (20, 47). The potential lack of variability in exposure and outcome measures possibly hampered our ability to find associations between LCPUFA concentrations and birth weight in our study population.

In conclusion, we found no evidence for an association between birth weight and LCPUFA or MeHg exposure during pregnancy. Future studies should continue to include biomarkers of exposure and explore the extent to which maternal health and nutritional status, other nutrients found in fish, and genetic variability may influence the relationship between fish consumption during pregnancy and birth weight.

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Table 1

Descriptive statistics for exposures, outcome, and covariates: Seychelles Child Development Nutrition Study

	Total (n=230)	Excluded (n=46) ^a	
	Mean (SD); Median	N missing	Mean (SD); Median ^b
Primary Exposures			
EPA+DHA (mg/ml)	0.03 (0.01); 0.03	7	0.03 (0.01); 0.03
AA (mg/ml)	0.10 (0.02); 0.10	7	0.10 (0.02); 0.09
n-3 (mg/ml)	0.03 (0.01); 0.03	8	0.03 (0.01); 0.03
n-6 (mg/ml)	1.22 (0.21); 1.22	7	1.19 (0.22); 1.17
MeHg (ppm)	5.9 (3.9); 5.1	0	4.4 (3.1); 3.7
Primary Outcome			
Birth weight (g)	3252 (495); 3268	1	3158 (456); 3220
Covariates			
Male sex	49%	0	50%
Alcohol use during pregnancy (% , n)	46% (n=106)	0	48% (n=22)
Gestational diabetes (% , n)	3% (n=8)	0	0% (n=0)
Tobacco use during pregnancy (% , n)	3% (n=7)	1	7% (n=3)
Gestational age (weeks)	38.7 (1.4); 39.0	1	38.5 (1.2); 38.0
Maternal age (years)	28.3 (6.1); 28.0	0	24.2 (5.8); 23.0
Intrapartum weight gain (kg)	10.2 (5.1); 10.1	1	10.8 (4.6); 11.0
Pre-pregnancy body mass index	26.2 (6.5); 24.8	1	24.6 (5.3); 23.5
Hollingshead score	33.5 (11.0); 32.0	14	34.3 (11.6); 32.5
Parity	1.2 (1.2); 1.0	23	1.0 (1.2); 1.0

^aExcluded from primary analyses^bAmong those for whom data on respective variables were available

SD = Standard deviation; DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; AA = Arachidonic Acid; MeHg = Methyl Mercury

Table 2

Regression coefficients (95% confidence interval) from multiple linear regression models evaluating the association between maternal LCPUFA status, prenatal MeHg exposure, and covariates in relation to birth weight from 230 subjects with complete covariate data: Seychelles Child Development Nutrition Study

Covariates	Model 1: EPA+DHA, AA		Model 2: n-3, n-6		Model 3: MeHg	
	Without MeHg adjustment	With MeHg adjustment	Without MeHg adjustment	With MeHg adjustment	Without LCPUFA adjustment	With LCPUFA adjustment
Primary Exposures						
EPA+DHA	5715.1 (-3059.2;14489.4)	4505.0 (-4609.7;13619.6)	-	-	-	-
AA	-2897.6 (-6675.6;880.4)	-2660.1 (-6469.5;1149.4)	-	-	-	-
n-3	-	-	407.5 (-6675.9;7490.9)	-504.5 (-7767.5;6758.5)	-	-
n-6	-	-	170.4 (-124.2;465.1)	165.4 (-129.2;460.1)	-	-
MeHg	-	7.7 (-7.9;23.3)	-	8.7 (-6.8;24.2)	9.3 (-5.7;24.3)	-
Covariates						
Child sex	176.4 (62.4;290.3)	178.1 (64.1;292.2)	166.8 (52.3;281.2)	169.4 (54.9;283.9)	175.1 (61.3;288.9)	175.1 (61.3;288.9)
Alcohol use during pregnancy	40.0 (-72.6;152.6)	46.4 (-67.0;159.8)	33.0 (-79.6;145.5)	41.0 (-72.4;154.4)	41.8 (-71.4;154.9)	41.8 (-71.4;154.9)
Gestational diabetes	268.1 (-55.2;591.4)	265.6 (-57.7;589.0)	214.5 (-106.3;535.4)	216.0 (-104.7;536.7)	226.1 (-89.9;542.1)	226.1 (-89.9;542.1)
Tobacco use during pregnancy	12.1 (-328.6;352.7)	10.6 (-330.1;351.3)	-51.5 (-392.0;289.0)	-48.8 (-389.1;291.6)	-24.5 (-361.4;312.5)	-24.5 (-361.4;312.5)
Gestational age	159.3 (116.6;202.0)	161.4 (118.5;204.4)	162.9 (120.3;205.5)	165.0 (122.3;207.7)	164.6 (121.9;207.2)	164.6 (121.9;207.2)
Maternal age	-2.3 (-14.2;9.7)	-2.3 (-14.3;9.6)	-1.9 (-13.9;10.1)	-2.0 (-14.0;10.0)	-2.4 (-14.3;9.6)	-2.4 (-14.3;9.6)
Intrapartum weight gain	8.4 (-3.5;20.3)	8.8 (-3.1;20.8)	8.7 (-3.3;20.6)	9.1 (-2.8;21.1)	8.7 (-3.1;20.6)	8.7 (-3.1;20.6)
Pre-pregnancy body mass index	4.8 (-5.1;14.8)	4.9 (-5.0;14.9)	4.3 (-5.6;14.3)	4.6 (-5.4;14.5)	3.5 (-6.1;13.1)	3.5 (-6.1;13.1)
Hollingshead score	4.4 (-1.1;9.9)	4.2 (-1.3;9.6)	3.1 (-2.6;8.7)	2.8 (-2.8;8.5)	3.6 (-1.8;9.0)	3.6 (-1.8;9.0)
Parity	50.1 (-9.4;109.6)	47.4 (-12.3;107.2)	48.1 (-11.7;107.9)	45.1 (-14.9;105.1)	47.8 (-11.0;106.7)	47.8 (-11.0;106.7)
Model fit statistics						
Adjusted R-square (p-value)	0.26 (0.00)	0.26 (0.00)	0.25 (0.00)	0.26 (0.00)	0.26 (0.00)	0.26 (0.00)

DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; AA = Arachidonic Acid; MeHg = Methyl Mercury