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A Longitudinal Analysis of Prenatal Exposure to Methylmercury and Fatty Acids in the Seychelles

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Abstract

Background—Maternal fish consumption during pregnancy exposes the fetus simultaneously to methyl mercury (MeHg) and long chain polyunsaturated fatty acids (LCPUFA). Data from the Seychelles Child Development Nutrition Study (SCDNS) showed a negative association of MeHg with child development when children were 30 months of age, only when controlling for LCPUFA. Concomitantly, n-3 LCPUFA were found to have a significant positive association only at 9 months. These findings suggest that the effects of MeHg and LCPUFA may vary with age over the first few years of life. We address this by including outcomes at two ages and adjusting for the child's age at testing.

Methods—A longitudinal analysis utilizing linear mixed models was performed to assess the associations of maternal hair total mercury (THg, a biomarker for MeHg) and maternal LCPUFA with children's Bayley Scales of Infant Development Psychomotor Developmental Index (BSID-II PDI) at 9 and 30 months of age, and to determine whether these associations change over time. Data from 228 children were included.

Results—Maternal hair MeHg had a negative effect on BSID PDI, while maternal n-3 LCPUFA had a positive effect. These effects did not change significantly from 9 to 30 months in this analysis.

Conclusions—The longitudinal analysis provides increased power for estimating the relationships of prenatal MeHg and LCPUFA exposures during child development. Significant associations of these exposures in opposite directions confirm the importance of LCPUFA in development and the need to adjust for maternal nutrition when studying prenatal MeHg exposure.

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Keywords

methylmercury; prenatal exposure; long chain polyunsaturated fatty acids; child development; fish consumption; maternal nutrition; Seychelles Child Development Study

1. Introduction

The Seychelles Child Development and Nutrition Study (SCDNS) has been testing the hypothesis that nutrients present in a maternal diet high in fish, such as maternal long chain polyunsaturated fatty acids (LCPUFA) exposure, may modify the toxicity of MeHg or may exert a beneficial influence on child development masking adverse effects of MeHg. To test this hypothesis, we enrolled a cohort of mother-infant pairs for a prospective study of prenatal exposure to both MeHg and nutrients including n-3 and n-6 LCPUFA. The cohort infants were assessed at 9 and 30 months of age and the data were analyzed separately at each age. In models that included MeHg, n-3 and n-6 LCPUFA, we found significant beneficial effects of n-3 LCPUFA at 9 months, but not at 30 months. We also found a significant adverse association with MeHg only at 30 months [5,9]. This paper reports a longitudinal model that allowed us to examine whether the association of MeHg and LCPUFA with neurodevelopment changed over time. In addition we examined interactions among MeHg, LCPUFA and time to test whether the effects of MeHg differed for subjects with varying LCPUFA status and whether the MeHg-LCPUFA interactions differed at the two time points.

2. Methods

2.1 Sample

We recruited a sample of 300 women who presented during the last four months of 2001 to one of nine antenatal clinics on Mahé, the main island of the Republic of Seychelles where more than ninety percent of the population resides. Attendance at antenatal clinics is typical in Seychelles where there are approximately 1,500 live births each year. To be included women had to be Seychellois, at least 16 years of age, and consent to participation. On average mothers in this cohort consumed fish at more than 9 meals per week. Shark and sea mammals are generally not part of the Seychellois diet. Details of the study setting and methods have been described previously [3,4,5,7,9].

There were 283 live births in the cohort representing about one-fifth of all births in Seychelles that year. Subsequently, two infants were excluded because they were born outside the Seychelles, four were excluded due to major congenital anomalies and one set of twins was excluded. Thirteen infants had birth weights <2,500 g. The smallest was 1870 g. There were no surviving LVBW infants. Similar to our previous analyses [5,9], the present analysis included only the 229 mother-children pairs with complete demographic, nutrition, and MeHg data, from which 1 mother-child pair was excluded due to a missing BSID PDI outcome at both ages. The study was reviewed and approved by the Seychelles Health Research Ethics Committee, and the Research Subjects Review Board of the University of Rochester.

2.2 Exposure measurement

Maternal hair samples covering as much of the gestational period as possible were obtained at delivery. Average maternal THg during pregnancy, the mean value from all available trimester segments, was used as a biomarker for prenatal MeHg exposure. These values

were not transformed. Further details of the laboratory methods used for THg estimation have been described previously [5].

Maternal blood samples were collected at 28 weeks gestation and one day after delivery to measure levels of five major representatives of the n-3, and n-6 LCPUFA families: docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), alpha-linolenic acid (ALA), arachidonic acid (AA), and linoleic acid (LA). To avoid loss of data due to missing 8% of 28-week and 20% of delivery LCPUFA measurements, we imputed missing values as the predicted value conditional upon the value observed for that mother at the other observed time point. To estimate the level during the third trimester, the geometric mean of the values at the 28-week and delivery values was computed for each LCPUFA measured. For this analysis we used combined n-3 ($n-3 = \text{DHA} + \text{EPA} + \text{ALA}$) and n-6 ($n-6 = \text{AA} + \text{LA}$) as the measures of prenatal LCPUFA exposure, consistent with our previous cross-sectional analyses, where further details of the laboratory and statistical imputation methods are given [9].

2.3 Outcome measurements

For this longitudinal analysis we used the Bayley Scales of Infant Development Second Edition (BSID-II) Psychomotor Developmental Index (PDI) measured at 9 and 30 months [1]. 228 of the 229 children had a measurement of PDI, either at both ages ($n=222$) or at one age ($n=6$), and our longitudinal model is based on these 228 subjects. In our previous analyses, n-3 was found to have a significant beneficial relationship with PDI only at 9 months and MeHg was found to have a significant adverse relationship with PDI only at 30 months [9]. We did not find significant associations of the BSID-II Mental Developmental Index (MDI) with LCPUFA or maternal MeHg at either age, so MDI was not included in the present longitudinal analysis.

2.4 Statistical analysis

To examine the longitudinal relationship, we modeled the association of maternal hair MeHg, n-3 and n-6 LCPUFA with PDI over time using linear mixed models [10]. Each model included a random intercept term to account for the fact that an individual child may score consistently higher or consistently lower than the overall average. A binary variable for child's age was included to distinguish between 9 month and 30 month outcomes. All models included MeHg, n-3 and n-6 LCPUFA adjusted for the following covariates, chosen *a priori*, known to be associated with the developmental outcomes: socioeconomic status (SES) determined using the Hollingshead Four-Factor Socioeconomic Status modified for use in the Seychelles [4], home environment assessed using the Pediatric Review of Children's Environmental Support and Stimulation (PROCESS)[2], maternal intelligence measured using the Matrices subtest of the Kaufman Brief Intelligence Test (K-BIT)[6], maternal age (years), child's birth weight (g), child's sex, and an indicator of whether the child lived with both parents at 9-months of age. We did not adjust for child's age at testing since the BSID-II scores are already scaled for age. At the 9-month assessment, the mean (SD) age was 9.09 (0.51), with a range of 8-12 months. At the 30-month assessment, the mean (SD) age was 27.94 (1.22), with a range of 27 to 35 months.

The longitudinal analysis used PDI from the two ages (9 and 30 months) together in a linear mixed model. We used a backward elimination strategy for the interaction terms, starting with a model that included three-way interactions $\text{age} \times \text{MeHg} \times n-3$ and $\text{age} \times \text{MeHg} \times n-6$, two-way interactions of $\text{MeHg} \times n-3$, $\text{MeHg} \times n-6$, $\text{age} \times \text{MeHg}$, $\text{age} \times n-3$, $\text{age} \times n-6$, $\text{age} \times \text{sex}$, $\text{age} \times \text{birth-weight}$, $\text{age} \times \text{home-environment}$, and main effects of MeHg, n-3, n-6 and all covariates. For any models including interactions of MeHg with n-3 or with n-6 the LCPUFA variables were categorized into three groups defined by the sample tertiles to

avoid continuous-by-continuous interactions. If the final model did not include interactions of MeHg with n-3 or with n-6 the continuous LCPUFA variables were used.

The three-way interactions examined whether the MeHg x LCPUFA interactions changed as the child aged. The two-way interactions of age with MeHg, n-3, n-6, sex, birth weight and home environment were used to determine whether the association of these variables with PDI changed as the child aged. The two-way interactions of MeHg with n-3 and with n-6 examined whether the effect of prenatal MeHg exposure was different for children with different levels of prenatal LCPUFA exposure.

We used a two-sided p-value ≤ 0.05 to determine significance of the interactions. This level was chosen a priori to be consistent with previous SCDNS analyses. If the three-way interactions were not significant the model was re-analyzed with only the two-way interactions and main effects. Then the significance of the two-way interactions was assessed and non-significant interactions were removed. Finally, a model including only the significant interactions and all the main effects was analyzed and is presented here.

3. Results

For the 229 mother-child pairs analyzed in this study, maternal hair MeHg ranged from 0.2 to 18.5 ppm (mean 5.7 ppm) and was similar (mean 5.2 ppm) for pairs excluded from the analysis due to missing covariates. Summary statistics of prenatal LCPUFA exposure, PDI, and covariates are shown in Table 1 for the 229 mother-child pairs included in the study as well for the subjects excluded due to missing covariates.

Only the age $\times$ sex interaction term was significant, and results from the model with this single interaction and all main effects are reported here. No other interaction term was close to significant ($p > 0.20$). In this model n-3 and n-6 were treated as continuous variables. PDI decreased between 9 to 30 months, on average 13 points for girls and 19 points for boys. The difference between girls' and boys' scores increased as the children aged ($p = 0.004$). Girls scored on average 3 points higher at 9-months and 8 points higher at 30-months. PDI increased with increasing birth weight ($p = 0.001$), on average 4 points for each additional kg. Effect estimates and 95% confidence limits for other covariates are shown in Table 2. Linear assumptions were checked and were appropriate for the continuous covariates. The model intraclass correlation indicated about 20 percent of the error variation was due to subject specific variation.

After adjusting for model covariates, significant associations were found such that larger maternal MeHg values are associated with lower PDI scores, while larger maternal n-3 values were associated with improved PDI scores. Maternal n-6 had a negative but non-significant association with PDI. The coefficient estimates and corresponding 95% confidence limits from the linear mixed model are compared with the estimates from the separate cross-sectional models from 9 and 30 months [9] also shown in Table 2. Using the data from both ages together in the longitudinal model effectively increased the sample size producing tighter confidence intervals for the effects compared with the cross-sectional models. In addition, the longitudinal model allows us to address the question of whether MeHg, LCPUFA, and/or covariate effects on the PDI change over time from 9 to 30 months. We found that only the effect of sex on the PDI was significantly different at these two ages. Maternal n-3 is composed mostly of DHA (mean DHA = 0.17, mean n-3 = 0.20, correlation = 0.99), and it is likely that the n-3 effect is driven by DHA. Conclusions from models which adjust for DHA in place of n-3 are the same (data not shown). In addition, the overall conclusions from the longitudinal model which does not adjust for birth weight are the same; specifically MeHg and n-3 coefficients remain significant predictors of the PDI.

4. Discussion

The present study showed a significant adverse association between maternal hair MeHg and BSID-II PDI, and a significant beneficial association between n-3 and PDI. Neither association was changed significantly as children aged. There was no significant difference in the effect of maternal hair MeHg exposure between children with different levels of prenatal LCPUFA exposure. Thus, it appears that at the exposure levels in the Seychelles population, the combined effect of LCPUFA and MeHg on children's psychomotor development does not change from 9 to 30 months of age, and that the association between MeHg and the PDI did not differ by levels of LCPUFA.

These findings confirm and extend the results from separate cross-sectional models at 9 and 30 months, as reported in [9]. Unlike separate cross-sectional models, these longitudinal analyses can assess whether prenatal MeHg and LCPUFA exposure effects or other covariate effects were significantly different at the two ages studied. We found that assuming common effects at the two time points was reasonable for all model covariates except for sex. The standard error was considerably smaller in the longitudinal model as compared to separate models, and both maternal MeHg and n-3 were significant predictors in the longitudinal model.

Our earlier reports [5,9] stressed that the small numbers of subjects were probably under powering each cross-sectional analysis. We speculated that similar effect sizes with a larger number of subjects might yield different results. Modeling the outcomes from the two ages simultaneously in a longitudinal model substantially increases the effective sample size, and in doing so increases the power to detect effects of prenatal MeHg or LCPUFA exposures. Although the improvement in confidence intervals turned out to be relatively small, the difference between the current report and our earlier reports is probably a reflection of this added power.

This study has some limitations. There may be other nutrients from fish or the overall diet, such as selenium, iodine, folate, vitamin B-12, or vitamin E that affect development which were not included in this analysis. These, so far unmeasured, covariates could also interact with the effects of prenatal MeHg and LCPUFA. In order to study the interactions of prenatal MeHg exposure with LCPUFA and other nutrients to which an infant is exposed *in utero*, as well as the effect of postnatal MeHg exposure and nutrition, a larger cohort is needed. The small sample size precluded studying any interactions between or among sex, MeHg and nutrients, which may be important.

Some aspects of the PDI score exhibited in this cohort need further study. The drop in PDI scores between 9 and 30 months is not expected for infants and toddlers with normal development at least in the US. A similar drop in scores was seen in an earlier Seychellois cohort [3] suggesting that either test performance characteristics or differences in experientially determined skills may have affected cohort scores. In addition, girls scored higher than boys on the PDI, and the difference in scores increased as the children got older. There was an expectation that the opposite result should have occurred, based upon our earlier report of precocity in motor development among Seychellois boys [3]. We lacked power to explore this sex effect, and believe it needs further study.

The results of this study support the importance of studying the simultaneous and confounding exposures that occur when fish is consumed. Determining the safety of fish containing MeHg is more a matter of risk-benefit assessment than a study of exposure to a single neurotoxicant. In the case of fish consumption, the neurotoxicant and the confounded nutrients may have simultaneous opposite influences on children's development. Further research in this area is needed to assist public health authorities in developing advice on fish

consumption during pregnancy that protects children from possible harmful effects of MeHg while not depriving them of beneficial nutrients important for brain growth.

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Table 1

Summary Statistics and comparison of subjects included and excluded from analysis

	A: subjects included in analysis			B: subjects excluded due to missing covariates			A vs B P (t-test)
	N	Mean	SD	N	Mean	SD	
Prenatal MeHg exposure							
Maternal THg (ppm in hair)	229	5.7	3.7	34	5.2	4.4	0.47
Maternal LCPUFA levels							
n-3 = DHA + EPA + ALA (mg/mL)	229	0.2	0.06	31	0.2	0.06	0.66
n-6 = AA + LA (mg/mL)	229	7.6	1.3	32	7.5	1.4	0.53
Developmental Outcome: BSID-II PDI							
9 months	225	105.7	16.4	33	102.5	13.7	0.11
30 months	225	89.8	13.7	31	90.1	14.1	0.91
Covariates - continuous							
Maternal age (years)	229	27.7	5.9	38	27.2	7.0	0.60
Maternal intelligence (K-BIT)	229	86.2	14.2	29	84.0	11.8	0.42
SES (modified Hollingshead)	229	33.9	11.0	33	31.5	11.0	0.23
Child's birth weight (g)	229	3238.9	471.0	37	3221.4	605.8	0.84
Home environment (PROCESS)	229	152.1	14.6	23	149.0	14.3	0.32
Covariates - binary	N	%		N	%		P (chi-square test)
Girls	229	49.3		38	50.0		0.94
Child living with both parents	229	51.1		33	48.0		0.78

Table 2

Adjusted Regression Coefficients and 95% Confidence Limits from Linear Mixed Effect Model 3 for PDI Compared with Cross-sectional Analyses. Significant ($p < 0.05$) coefficients are bolded.

Effect	Longitudinal Model		Cross Sectional Models ^a			
	Beta	95% CL	9 months		30 Months	
Age, 30 months ^b	-18.85	-21.58	-16.13	.	.	.
Maternal THg (ppm)	-0.37	-0.71	-0.04	-0.24	-0.62	0.14
n-3 (mg/ml)	25.5	1.5	49.5	33.3	6.0	60.5
n-6 (mg/ml)	-0.33	-1.34	0.69	-0.13	-1.29	1.02
girl, 30 months	8.48	5.39	11.57	.	.	8.17
girl, 9 months	2.81	-0.28	5.90	3.15	0.40	5.90
living with 2 parents	0.43	-1.97	2.82	-0.51	-3.21	2.20
Maternal Age (years)	0.20	-0.01	0.40	0.06	-0.18	0.29
Maternal Intelligence (K-BIT)	0.002	-0.09	0.09	-0.03	-0.13	0.07
SES (Mod. Hollingshead)	0.01	-0.11	0.12	-0.04	-0.17	0.10
Child's birth weight (kg)	4.32	1.74	6.90	5.06	2.14	7.98
Home environment (PROCESS)	0.07	-0.02	0.15	0.09	-0.01	0.19
				0.04	-0.09	0.17

^a Results from Strain, et al., 2008 [9]

^b baseline is 9 month