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Varying coefficient function models to explore interactions between maternal nutritional status and prenatal methylmercury toxicity in the Seychelles Child Development Nutrition Study ☆, ☆☆

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ABSTRACT

Maternal consumption of fish during the gestational period exposes the fetus to both nutrients, especially the long-chain polyunsaturated fatty acids (LCPUFAs), believed to be beneficial for fetal brain development, as well as to the neurotoxicant methylmercury (MeHg). We recently reported that nutrients present in fish may modify MeHg neurotoxicity. Understanding the apparent interaction of MeHg exposure and nutrients present in fish is complicated by the limitations of modeling methods. In this study we fit varying coefficient function models to data from the Seychelles Child Development Nutrition Study (SCDNS) cohort to assess the association of dietary nutrients and children's development. This cohort of mother–child pairs in the Republic of Seychelles had fish consumption averaging 9 meals per week. Maternal nutritional status was assessed for five different nutritional components known to be present in fish (n-3 LCPUFA, n-6 LCPUFA, iron status, iodine status, and choline) and associated with children's neurological development. We also included prenatal MeHg exposure (measured in maternal hair). We examined two child neurodevelopmental outcomes (Bayley Scales Infant Development-II (BSID-II) Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI)), each administered at 9 and at 30 months. The varying coefficient models allow the possible interactions between each nutritional component and MeHg to be modeled as a smoothly varying function of MeHg as an effect modifier. Iron, iodine, choline, and n-6 LCPUFA had little or no observable modulation at different MeHg exposures. In contrast the n-3 LCPUFA docosahexaenoic acid (DHA) had beneficial effects on the BSID-II PDI that were reduced or absent at higher MeHg exposures. This study presents a useful modeling method that can be brought to bear on questions involving interactions between covariates, and illustrates the continuing importance of viewing fish consumption during pregnancy as a case of multiple exposures to nutrients and to MeHg. The results encourage more emphasis on a holistic view of the risks and benefits of fish consumption as it relates to infant development.

1. Introduction

Fish consumption during pregnancy exposes the fetus to the neurotoxicant methylmercury (MeHg). All fish naturally take up MeHg and it is biomagnified up the aquatic food chain. Maternal dietary fish consumption also provides an important source of nutrients, most notably the long-chain polyunsaturated fatty acids (LCPUFA). These nutrients are important precursors to normal development of the central nervous system, and studies of cognitive and retinal development following maternal supplementation have shown benefits (Helland et al., 2003; Daniels et al., 2004; Dunstan et al., 2006; Judge et al., 2007). Recent reports from the

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Seychelles Child Development Nutrition Study (SCDNS) cohort suggested an adverse impact of MeHg exposure, accompanied by a simultaneous beneficial effect of n-3 LCPUFA on children's development (Davidson et al., 2008; Strain et al., 2008). It is unclear whether this result was evidence for independent influences of MeHg and n-3 LCPUFA or effect modification. Bellinger (2008) has distinguished between these two types of confounding effects, suggesting that effect modification can be tested through including statistical interaction terms in a regression model, whereas independent effects can be tested with main effect terms. The primary analyses reported by Davidson et al. (2008) and Strain et al. (2008) employed ordinary multivariate regression to examine four primary endpoints and found an adverse association between prenatal MeHg exposure and the BSID-II PDI at 30 months which was statistically significant only when the model contained the LCPUFA (Strain et al., 2008). However, the nutrition variables did not have a significant association with that outcome (Davidson et al., 2008). For one outcome (BSID-II MDI at 9 months), overall model significance was not achieved, but secondary analyses indicated a beneficial association with n-3 LCPUFA. Davidson et al. (2008) utilized ordinary linear model interactions between MeHg and the n-3 LCPUFA docosahexaenoic acid (DHA), and the n-6 LCPUFA arachidonic acid (AA). The study did not find significant interactions between MeHg and AA and DHA ($p=0.53$ and $p=0.30$ for a 2 df test for MeHg*DHA and MeHg*AA, respectively). Those models utilized indicator variables to distinguish between tertiles of the sample distribution of the LCPUFAs, and examination of the interactions is limited by this specific, discretized form. Interactions present in other forms might not be visible in this discretized setting.

Effect modification has been defined as variation in the magnitude of an association between an exposure and an outcome measure across strata of a separate, effect modifying factor (Bellinger, 2000). Modeling statistical interaction between exposures and other measurable characteristics by including interaction terms in the regression models is the standard means to identify effect modification. Simple regression interaction terms, however, can fail to capture the variation in association between exposure and outcome when the change in that association differs in a complicated, perhaps nonlinear, fashion across levels of the effect modifying factor. Within the framework of linear models, methods for characterizing not just the presence but also the form and features of covariate interactions have been lacking. To address this need, the semiparametric varying coefficient (VC) model (Hastie and Tibshirani, 1993) has been proposed as a means of capturing features of covariate-by-covariate interactions between continuously varying covariates. Such models are uniquely suited to examining covariate interactions as they model the regression coefficient representing the relationship between a covariate and an outcome variable as a nonparametric smooth function of a separate, effect modifying covariate. Conditional on a fixed value of an effect modifier, the model for an outcome is linear in the modified predictors. In this study, we utilize the varying coefficient model to explore how the relationships between maternal nutritional covariates and a series of neurodevelopment outcomes are potentially modulated at different maternal MeHg exposures. We applied the VC models to data from the SCDNS cohort using the two child neurodevelopment outcomes measured at both 9 and 30 months that showed an association in prior analyses. The VC models examined here provide a more complete portrait of the interactions under consideration, allowing continuous-by-continuous covariate interactions to be represented via smooth functions. These models provide an important extension of previous analyses by helping to define the possible role of covariate interactions in this cohort, and illustrate a valuable methodology for examining complex biological exposures that have a wide applicability to a variety of data settings in which continuous covariate interactions might play a role in influencing outcome.

2. Methods

2.1. Data setting and developmental outcomes modeled

The Seychelles Child Development Study (SCDS) is an ongoing longitudinal study of neurodevelopmental effects arising from maternal MeHg exposure from a diet high in fish, conducted in the Republic of Seychelles (Myers et al., 2000). A cohort of 300 mother–infant pairs was recruited in 2001 to investigate the role that both MeHg exposure and maternal nutritional status have on developmental outcomes in the offspring. Mothers in this cohort were enrolled in the first trimester and completed a four day diet diary and a food use questionnaire at 28 weeks gestation. The primary developmental endpoints modeled were the Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) of the Bayley Scales of Infant Development-II (BSID-II), measured at 9 and 30 months. A total of $n=229$ children completed developmental testing at both time points and had complete covariate information.

2.1.1. Nutrition covariates

In the VC models for this cohort, we examined five primary nutrition indicators. These include two LCPUFA, (DHA, an n-3 fatty acid and AA, an n-6 fatty acid), maternal iodine status indicated by free thyroxine (T4), maternal iron status (Fe), and dietary choline (determined from the diet diary). These nutritional indicators were selected as they can be associated with fish consumption and child development (Strain et al., 2004). LCPUFA values were computed as geometric means of lipid values from nonfasting blood samples taken at 28 weeks gestation and 1 day post delivery. Data for some of the LCPUFA measurements missing at single time points were imputed prior to determination of the geometric means according to methods described in Davidson et al. (2008). Maternal iodine status T4 was determined at 28 weeks gestation using competitive immunoassay. Iron status was determined as the total body iron store derived from the soluble transferrin receptor and ferritin measured at enrollment (prior to Fe supplementation). Dietary choline was estimated from the food use questionnaires (FUQs) and a 4-day diet diary as described in Davidson et al. (2008).

2.1.2. Exposure assessment and model covariates

The biomarker for prenatal MeHg exposure was the average maternal hair total Hg (THg) in ppm measured in hair growing during pregnancy. The Hg was measured using atomic absorption spectroscopy as described earlier (Cernichiari et al., 1995; Davidson et al., 2008). All models containing these exposure and nutritional metrics additionally include maternal- and child-related covariates chosen for their potential association with child development as described by Davidson et al. (2008). These include the continuous covariates socioeconomic status (SES) measured by the Hollingshead Four-Factor method modified for use in the Seychelles (Davidson et al., 1998), the *Pediatric Review of Children's Environmental Support and Stimulation* (PROCESS), a measure of stimulation in the home environment, maternal intelligence measured using the Matrices subtest of the Kaufman Brief Intelligence Test (K-BIT) (Kaufman and Kaufman, 1990), maternal age, birth weight and weekly fish intake measured by the FUQ, as well as the categorical variables gender and family status (an indicator variable for presence of both parents in the home at age 9 months). Full details on the protocols for nutritional assessment and MeHg dosimetry are described in Davidson et al. (2008).

2.2. Models

We are using varying coefficient models to characterize the interaction of mercury and nutritional covariates (Hastie and Tibshirani, 1993). The varying coefficient model is a regression model which is additive in the regressors, but the relationship between each regressor and the outcome is allowed to vary as a smooth function of additional, effect modifying regressors in the model. By allowing the regression coefficients to vary nonparametrically as functions of an effect modifying covariate, the relationship between a regressor and the outcome can change at different levels of the effect modifier. The VC model in which only one covariate operates as the effect modifier has the general form

$$Y=\beta_0(R)+\beta_1(R)X_1+\beta_2(R)X_2+\ldots+\beta_p(R)X_p+\varepsilon$$

where X_1, X_2, …, X_p are explanatory covariates, R the single effect modifying explanatory covariate, $\beta_i(R)$ the smoothly varying coefficient function of R, for the $i=1, 2, \ldots, p$ other explanatory variables, and ε an error term. In this work, we are using MeHg as the effect modifier R, and the five nutritional status measurements as the modified explanatory covariates labeled as X_i in the above equation. Observe that if $\beta_i(R)=\beta_i$ (a nonzero constant) for each i, the model reduces to an ordinary linear regression model. The conditional relationship between the explanatory variables X_i and R thus characterizes a type of interaction between those two covariates. Note that the intercept term $\beta_0(R)$ represents a main effect of covariate R, and is also modeled as a smoothly varying function of R. The coefficient functions $\beta_i(R)$ thus characterize the manner in which the relationship between covariate X_i and outcome changes as levels of the effect modifier change.