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Prenatal Methylmercury Exposure in a South Carolina Coastal Cohort

Alexis Donohue
University of South Carolina

PRENATAL METHYLMERCURY EXPOSURE IN A SOUTH CAROLINA COASTAL COHORT

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Alexis Donohue

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Accepted by:

Sarah Rothenberg, Director of Thesis

Jim Burch, Reader

Sean Norman, Reader

Cheryl L. Addy, Vice Provost and Dean of the Graduate School

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ABSTRACT

***Objectives*:** Methylmercury is a neurotoxin that has the ability to cross the placenta and adversely affect the developing fetus. Our primary objective was to investigate whether prenatal methylmercury exposure varied longitudinally, and whether differences were associated with maternal characteristics.

***Methods*:** Pregnant mothers were recruited in Charleston, South Carolina (8-17 weeks gestation) (n=78). Blood samples were collected during both early (11.8 ± 1.7 weeks) and late (35.3 ± 2.0 weeks) gestation. Blood total mercury and methylmercury concentrations were analyzed. Upon enrollment in the study, mothers filled out a sociodemographic questionnaire that included questions regarding maternal age, race/ethnicity, and education level. Additionally, 47 mothers completed a semi-quantitative food frequency questionnaire of 161 items, which included five questions on seafood consumption.

***Results*:** The mean maternal blood total mercury concentration (n=156) was 0.88 ± 0.78 µg/L (range: 0.02-4.0 µg/L), and the mean maternal blood methylmercury concentration (n=156) was 0.54 ± 0.53 µg/L (range: 0.01-2.7 µg/L). Blood total mercury was positively correlated with blood methylmercury in early and late pregnancy (Spearman's rho=0.89-0.92, $p<0.01$, n=78). The average number of fish meals consumed was 0.84 ± 0.79 meals/wk (range: 0-3.5 meals/wk). In unadjusted bivariate analyses, blood total mercury and methylmercury were positively correlated with the number of fish meals consumed per week in both early and late pregnancy (Spearman's rho=0.41-0.63, $p<0.01$ for all, n=47), and blood total mercury and methylmercury were not significantly correlated with

other covariates, including race/ethnicity (Spearman's rho=-0.04, 0.19, p=0.11-0.98; Kruskal-Wallis, p=0.09-0.86, n=73-78). Using a paired t-test, blood methylmercury decreased from early to late pregnancy (paired t-test, p=0.04, n=78), while total mercury did not change (paired t-test, p=0.29, n=78). When normalized by hematocrit, the decrease in blood methylmercury was slightly attenuated (paired t-test, p=0.16, n=66). The reduction in blood methylmercury was not correlated with maternal characteristics, including race/ethnicity and education level (Spearman's rho=-0.13, 0.10, p=0.28-0.76; Kruskal-Wallis, p=0.33-0.96, n=73-78). In the mixed model for repeated measures, adjusted for hematocrit, race/ethnicity, and time (early or late pregnancy), the association between blood mercury and race/ethnicity was strengthened, where African Americans had higher blood total mercury and methylmercury than Caucasians and Hispanics (t-test, p=0.02-0.04).

***Conclusions*:** Blood methylmercury, but not total mercury, decreased significantly between early and late pregnancy in unadjusted models. This highlights the importance of mercury speciation, because often methylmercury is not measured. In adjusted models, African American mothers had significantly higher blood mercury compared to Caucasian and Hispanic mothers. Although nearly all values were low-level, there are still uncertainties regarding the health impacts due to prenatal methylmercury exposure.

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CHAPTER 1

INTRODUCTION

Mercury (Hg) is a toxic heavy metal and a global pollutant that poses a threat to human health (WHO, 1990). While there are both natural and anthropogenic sources of Hg, anthropogenic sources comprise most of the Hg in the environment. Combustion of fossil fuels, waste incineration, and mining and smelting of metals release elemental Hg into the atmosphere (USEPA, 1997). Once emitted into the atmosphere, elemental Hg is oxidized to Hg(II), which is water-soluble and is returned to the earth's surface in rainfall and via dry deposition (USEPA, 1997). Atmospheric Hg is deposited into oceans and freshwater systems, where anaerobic microbes in the sediment convert less toxic inorganic Hg to methylmercury (MeHg) (NRC, 2000). MeHg bioaccumulates in the aquatic food web, which results in high concentrations of MeHg in the tissues of large predatory fish (NRC, 2000).

MeHg is considered to be one of the most toxic Hg species, because it is readily absorbed in the body and has the ability to cross the placenta and the blood-brain barrier (Clarkson and Magos, 2006). MeHg targets the central nervous system and is capable of causing irreversible neurological damage (Clarkson and Magos, 2006). A developing fetus is most vulnerable to the adverse health outcomes caused by MeHg (Bakir et al., 1973; NRC, 2000; Clarkson and Magos, 2006). Chronic low-level exposure to MeHg during the prenatal period could negatively affect neurobehavioral function in early life

and has been known to be associated with deficits in visual-spatial abilities, fine-motor skills, attention, language, and verbal memory in children (NRC, 2000).

Consumption of fish and shellfish is considered the primary exposure pathway for MeHg (NRC, 2000). Consuming fish regularly throughout pregnancy is often recommended, because fish contain many nutrients that have been found to be beneficial to both maternal and fetal health (Oken et al., 2008, 2012; Strain et al., 2012). Fish is the main dietary source of omega-3 long-chain polyunsaturated fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid, which are not synthesized in the human body (Oken et al., 2012). Therefore, it is recommended that pregnant women consume seafood at least twice per week for adequate intake of these essential nutrients (Oken et al., 2012). The *Dietary Guidelines for Americans* provides recommendations regarding fish consumption during pregnancy (USDA, 2015). Types of fish that tend to have lower MeHg concentrations and higher levels of omega-3 long-chain polyunsaturated fatty acids, such as salmon, herring, and Pacific oysters are recommended for consumption during pregnancy (USDA, 2015). The guidelines from the Institute of Medicine (IOM) state that pregnant women may benefit from consuming two to three 3-ounce servings of low MeHg fish per week and can safely consume up to six ounces of white (albacore) tuna each week; however, pregnant women are advised against eating large carnivorous fish that tend to have high MeHg concentrations, such as swordfish, shark, king mackerel, and tilefish (IOM, 2006).

Blood is a biomarker that can be used to assess recent MeHg exposure (Kershaw et al., 1980). It has been reported that concentrations of MeHg in the blood tend to peak within 4-14 hours after consuming a fish meal (Kershaw et al., 1980). There are two

biological half-lives for MeHg in blood, which are 7.6 ± 0.8 hours and 52 ± 3.7 days (Kershaw et al., 1980). The concentration of MeHg in the blood is considered a good indicator of exposure dose and body burden of MeHg (Kershaw et al., 1980). It has also been shown that maternal blood is a useful biomarker for monitoring prenatal MeHg exposure during pregnancy, as it is predictive of MeHg levels in the fetal brain (Cernichiari et al., 1995).

Studies where blood MeHg concentrations were measured directly are lacking. Additionally, there are very few studies that have investigated longitudinal trends in blood Hg during pregnancy. The aim of this study is to investigate whether there are changes in maternal blood MeHg and THg concentrations from early pregnancy to late pregnancy and to determine whether covariates considered in this study, such as race/ethnicity, education level, maternal age, trimester one body-mass index (BMI), and serum vitamin D are associated with any possible changes in blood MeHg and THg during pregnancy.

CHAPTER 2

METHODS

2.1 Study Design

Study participants were recruited from a cohort of pregnant women enrolled in a vitamin D study at the Medical University of South Carolina (MUSC) in Charleston, SC. To be eligible for enrollment, the mother must have been within 18-45 years old and visited her obstetrician at MUSC with confirmation of a singleton pregnancy within 14 weeks of her last menstrual period. Exclusion criteria for this study included having one or more of the following pre-existing health conditions: uncontrolled thyroid disease, parathyroid conditions, sickle cell disease, sarcoidosis, Crohn's disease, and ulcerative colitis. Additionally, women taking medication including calcium channel blockers were not eligible for this study.

From October 27, 2014 to March 23, 2016, 137 pregnant women were recruited, including 78 women who gave blood twice and were the focus of the study. Women were between 8 and 17 weeks of gestation at enrollment. Because vitamin D production varies by race/ethnicity, approximately equal numbers of African American, Caucasian, and Hispanic women were recruited, and a stratified block randomization design was used. Upon enrollment in the study, women completed a sociodemographic questionnaire, which included questions regarding maternal age, race/ethnicity, parity, education level, smoking, and alcohol consumption. At the first study visit in early pregnancy (11.8 ± 1.7 weeks gestation), a blood sample was collected in a Vacutainer tube (Becton Dickinson,

K_2EDTA, Royal Blue, 368381). Height and weight of each mother were also recorded and used to calculate BMI. After the first visit, the women were randomized into one of two groups, where they either received a placebo or a vitamin D supplement. Women who were supplemented received 4400 IU/d of vitamin D, and those in the unsupplemented group received 400 IU/d of vitamin D (standard of care). A second blood sample was collected in late pregnancy (35.3 ± 2.0 weeks gestation). All blood was stored at -20ºC until analysis.

2.2 Food Frequency Questionnaire

Upon enrollment in this study, participants were asked to complete a semi-quantitative food frequency questionnaire (FFQ) of 161 items, which included five questions pertaining to seafood consumption. The FFQ asked mothers to report consumption frequency for the following categories of fish/shellfish: "oysters," "shellfish like shrimp, scallops, crabs," "tuna, tuna salad, tuna casserole," "fried fish or fish sandwich," and "other fish, not fried." While fish/shellfish consumption is the primary MeHg exposure pathway, dietary exposure to MeHg may also occur from rice ingestion, which is especially important in populations where rice is a staple food (Hong et al., 2016; Rothenberg et al., 2014). Therefore, frequency of rice consumption was also considered and was reported in the FFQ, which included one question regarding rice. For each type of food, there were nine possible responses for consumption frequency, which ranged from "never" to "every day." The responses were used to calculate daily consumption frequencies for seafood and rice as follows: 0=never, 0=a few times per year, 1/30.5=once per month, 2.5/30.5=two to three times per month, 1/7=once per week, 2/7=twice per week, 3.5/7=three to four times per week, 5.5/7=five to six times per week,

and 1=every day. Daily frequencies were multiplied by seven to calculate weekly consumption frequencies for each type of seafood.

2.3 Blood Mercury Analysis

Blood samples were analyzed for THg following procedures from EPA Method 1631, using the cold digestion method (USEPA, 2001). Approximately 0.5 g of thawed blood was aliquoted into a 40 mL borosilicate vial with a Teflon-lined cap. Blood was digested in 4 mL of concentrated hydrochloric acid (HCl) (OmniTrace) and 1 mL of concentrated nitric acid (HNO_3) overnight at room temperature. Then 1% 0.2N bromine monochloride (BrCl) solution was added at least 12 hours prior to analysis to oxidize all Hg to Hg(II).

Just before analysis, sample was transferred to another 40 mL borosilicate vial. To neutralize the BrCl, 0.1 mL of 30% hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) was added, followed by 0.1 mL of tin (II) chloride ($SnCl_2$) to reduce Hg(II) to volatile Hg(0). The reagents in the vial were allowed to react for at least 20 minutes before they were analyzed. An autosampler was used to analyze THg concentrations (Merx-T, Brooks Rand Instruments, Seattle, WA). Samples were purged with N_2 gas directly into the vials, and the elemental Hg was collected on gold traps. Heating of the collection trap transferred the Hg to the analytical trap, which was then heated to desorb the Hg. THg concentrations were quantified using cold vapor atomic fluorescence spectrometry (CVAFS) (Model III Detector, Brooks Rand Instruments, Seattle, WA).

Blood MeHg was analyzed using ethylation-gas chromatography and CVAFS, following alkaline digestion-solvent extraction (Liang et al., 2000). Frozen blood samples were allowed to thaw at room temperature for about two to three hours and were shaken

to mix the water and red blood cells before aliquoting. Approximately 0.5 g of blood were dried in an oven overnight at 70°C. The dried blood was digested in 2 mL of 25% potassium hydroxide: methanol (w/v) in an oven for 3 hours at 75°C, and then 10 mL dichloromethane (CH_2Cl_2) and 2 mL of concentrated HCl were added to each sample. Samples were put on a shaker (IKA HS 501 Digital Shaker) at about 190 rpm for 30 minutes and were stored overnight to allow for complete phase separation. Approximately 8 mL of the CH_2Cl_2 layer were extracted and transferred to a sterile Falcon tube, and double-distilled (DDI)-H_2O (>18.0 MΩ cm^{-1}) was added to bring the total volume in the tube to 30 mL. Samples were put into a water bath (60-70°C) for 1.5 hours to evaporate the CH_2Cl_2 layer and back-extract the MeHg into DDI-H_2O.

The procedures for aqueous ethylation, purging, and MeHg quantification via gas chromatography and CVAFS followed US EPA Method 1630 (USEPA, 2001). Samples were added to borosilicate reaction bubblers, and the pH was adjusted to 4.9 by adding 0.3 mL of 2 M acetate buffer. Then 0.04 mL of 1% sodium tetraethyl borate ($NaBEt_4$) solution was added and allowed to react for 15 minutes to convert MeHg to volatile methylethyl mercury. The samples were then purged with N_2 gas for 15 minutes, and the ethylated Hg was absorbed onto Tenax traps. After purging, the methylethyl mercury was thermally desorbed from the Tenax traps, and MeHg concentrations were quantified via gas chromatography and CVAFS (Model III Detector, Brooks Rand Instruments, Seattle, WA).

2.4 Quality Assurance and Quality Control

Quality assurance and quality control parameters for THg and MeHg are presented in Table 2.1. The daily calibration curve had a minimum of five standard points

and an R-squared value of at least 0.99. Average percent recoveries for standard reference materials (NIST 955c, TORT-2, NIST 1568b, IAEA-086) (n=34) and matrix spikes (n=30) ranged from and 93-108% for THg. For MeHg, mean recoveries ranged from 81-118% for standard reference materials (NIST 955c, TORT-2, ERM CC580) (n=66) and matrix spikes (n=43). The average relative standard deviation (100 × standard deviation/mean) between replicates ranged from 3.3-12% for THg (n=33) and MeHg (n=50). The limit of detection for each method was calculated as 3 × standard deviation of the blanks and was 0.0001 µg/L and 0.001 µg/L for MeHg and THg, respectively (40 CFR 136, Appendix B). All blood MeHg and THg concentrations were above the limit of detection.

2.5 Covariates

Race/ethnicity, maternal age, education level, and parity were obtained from the sociodemographic questionnaire. Categories for education level included high school or less, college (some college and college graduate), and graduate school. Weight class groups, which were based on BMI, included underweight (<18.5 kg/m^2), normal weight (18.5-24.5 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2) (WHO, 2000). Additionally, categories for weight gain guidelines from the Institute of Medicine (IOM) were used and consisted of groups for below, within, and above recommended healthy weight gain during pregnancy. Because the women were participating in a vitamin D supplementation study, serum vitamin D concentrations were also included as a covariate. Information regarding antibiotic use and flu vaccination during pregnancy was collected as well. Use of antibiotics may change the composition of the gut microbiome (Dethlefsen et al., 2008), which could affect the metabolism of MeHg during pregnancy.

Antibiotic use was categorized into groups for number of times antibiotics were taken between early and late pregnancy, which included zero, one, and at least two times. Flu vaccines in multi-dose vials contain thimerosal, an ethylmercury-containing preservative (CDC, 2013), which has been shown to result in increased blood THg concentrations after vaccination (Barregard et al., 2011; Pichichero et al., 2008).

Hematocrit was also included as a covariate, because about 80% of MeHg in blood binds to red blood cells (Clarkson and Magos, 2006). It is known that plasma volume increases from early to late pregnancy, which results in a decrease in percentage of red blood cells in whole blood (Hytten, 1985). Therefore, change in hematocrit was taken into account and blood THg and MeHg concentrations were adjusted using the following equation previously used to adjust blood lead levels for hematocrit: (blood Hg/hematocrit) × 100 (Schell et al., 2000).

2.6 Statistical Analysis

Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). Bivariate associations of blood THg and MeHg concentrations (continuous) at early and late pregnancy with fish consumption frequency and maternal characteristics were analyzed. Blood inorganic Hg concentrations were also estimated (THg-MeHg). Spearman's and Pearson's correlation were used for continuous covariates. Untransformed variables were used to calculate Spearman's correlation, and a $\log_{10}$-tranformation was applied to positively-skewed variables for Pearson's correlation. Wilcoxon rank-sum test was used to test for differences between categorical variables with two groups, and one-way analysis of variance (ANOVA) and Kruskal-Wallis (for skewed variables) were used for three or more groups. For comparisons between two

categorical variables, Chi-square test and Fisher's exact test were used. Paired t-tests were used to determine whether there were differences in mean blood THg, MeHg, and estimated inorganic Hg between early and late pregnancy.

The SAS Proc Mixed procedure for repeated measures was used to model blood Hg by calculating maximum likelihood estimates for covariates as fixed effects using an autoregressive covariance matrix. Separate models were fit for blood MeHg and blood THg. To adjust for positive skew and normalize the residuals, blood MeHg and blood THg were both $\log_{10}$ transformed. The variable for "time" (early pregnancy, late pregnancy) was forced into the model to examine whether the time at which the blood sample was collected was predictive of blood Hg after controlling for other covariates. Hematocrit was also included in the model to adjust for hemodilution effects that occur as blood plasma increases from early to late pregnancy. Additional variables were assessed for inclusion in the model one at a time via forward selection, and type III sums of squares were used to evaluate the statistical significance of each effect included in the model. Bivariate analyses were assessed to determine whether there were any multicollinear covariates. In instances of multicollinearity, the variable that was most predictive of blood Hg remained in the model. Diagnostics for model fit included comparison of BIC values and examination of residual plots and Cook's distance. An alpha-level of 0.05 was used as a guide for statistical significance for all analyses.

Table 2.1 Quality assurance/quality control for analyses of blood total mercury (THg) and blood methylmercury (MeHg) concentrations, including percent recovery (%) and relative standard deviation (RSD) between replicates.

	Standard Reference Material							
Analyte	**NIST 955c % (n)**	**TORT-2 % (n)**	**NIST 1568b % (n)**	**IAEA-086 % (n)**	**ERM CC580 % (n)**	**Matrix Spike % (n)**	**Matrix Duplicate RSD % (n)**	**Analytical Duplicate RSD % (n)**
THg	107 (16)	N/A	93 (16)	108 (2)	N/A	96 (30)	3.3 (33)	6.1 (41)
MeHg	81 (26)	107 (38)	N/A	N/A	118 (2)	95 (43)	12 (50)	N/A

CHAPTER 3

RESULTS

3.1 Study Population Characteristics

Characteristics of the 78 women who provided paired blood samples are presented in Table 3.1. Sixty percent of mothers received an education above the high school level. Education level varied significantly by ethnicity (Fisher's exact test, $p<0.01$, n=78), where a higher percentage of Caucasian mothers (94%) were educated at the college or graduate school level compared to African American (50%) and Hispanic mothers (18%). A lower education level was also associated with a higher parity (Fisher's exact test, p=0.02, n=78). BMI averaged 29.3 ± 8.4 kg/m^2 (16.8-59.7 kg/m^2), and 63% of women were overweight or obese (BMI ≥ 25.0 kg/m^2), which is similar to trends observed in U.S. non-pregnant women from 2009-2010 (mean BMI: 28.7 kg/m^2; overweight or obese: 64.5%, n=3037) (Flegal et al., 2012). Only one mother (1.3%) was considered underweight (BMI<18.5 kg/m^2). BMI was significantly higher among African American mothers compared to Caucasian and Hispanic mothers (ANOVA pairwise, $p<0.01$ for both, n=78). Thirty-two mothers (41%) were within the IOM guidelines for healthy weight gain during pregnancy, while 22 (28%) were below and 23 (30%) exceeded recommended weight gain. Twenty-two mothers (28%) received a flu vaccine, and 27 mothers (35%) used antibiotics at least once between early and late pregnancy. Frequency of antibiotic use (0, 1, ≥2 times) during pregnancy varied by race/ethnicity, where Caucasian and Hispanic mothers used antibiotics less frequently than African

American mothers (Fisher's exact test, $p<0.01$, $n=78$). One mother (1.3%) reported drinking alcohol while pregnant, and two mothers (2.6%) reported smoking during pregnancy.

3.2 Fish/Shellfish Consumption

Food frequency questionnaires were available for 47 mothers (60%). A higher percentage of Caucasian mothers completed a FFQ compared to African American and Hispanic mothers (Chi-square, $p=0.03$, $n=78$), and mothers who were more educated (≥some college) were more likely to provide a FFQ than mothers who had an education level of high school or less (Chi-square, $p<0.01$, $n=78$) (Table 3.2). Additionally, a higher proportion of women who were within or above the IOM guidelines for healthy weight gain during pregnancy completed a FFQ compared to those who were below weight gain guidelines; although these differences were not significant (Chi-square, $p=0.07$, $n=77$). Other covariates did not differ between mothers who did and did not provide a FFQ (Chi-square, $p=0.16$-0.74; ANOVA, $p=0.17$-0.94, $n=73$-78).

For all fish/shellfish categories combined, the average number of fish meals consumed was 0.84 ± 79 meals/wk (median: 0.57 meals/wk, range: 0-3.5 meals/wk) (Table 3.3). Nine mothers (19%) never or rarely ate fish/shellfish, and four mothers (8.5%) reported consuming fish/shellfish at least twice per week (Table 3.4). Of the five categories of fish/shellfish, the average weekly consumption of "shellfish like shrimp, scallops, crabs," was greatest, followed by "tuna, tuna salad, tuna casserole," "other fish, not fried," "fried fish or fish sandwich," and "oysters." BMI was higher, although not significantly, for women who consumed more fish/shellfish meals per week (Spearman's rho=0.21, $p=0.15$). Hispanic mothers did not consume any tuna or oysters, and a higher

percentage did not eat any seafood compared to other races/ethnicities (Figure 3.1). On average, African American mothers consumed fish/shellfish most frequently (mean: 0.96 meals/wk, median: 0.84 meals/wk, n=16), followed by Caucasian mothers (mean: 0.89 meals/wk, median: 0.57 meals/wk, n=24), and Hispanic mothers (mean: 0.36 meals/wk, median: 0.22 meals/week, n=7); however, differences were not significant (Kruskal-Wallis, p=0.11, n=47).

3.3 Blood Hg and Maternal Characteristics

Descriptive statistics for maternal blood THg, MeHg, and %MeHg (of THg) are summarized in Table 3.5. Blood THg concentrations in this study (mean: 0.88 ± 0.78 µg/L, median: 0.62 µg/L) were 1.4-1.6 times lower compared to adult women who were NHANES study participants (2009-2010) (mean: 1.4 µg/L, median THg: 0.84 µg/L, n=1786) (USEPA, 2013). The reference dose for blood Hg is based on cord blood (5.8 ug/L) (NRC, 2000); however, blood Hg is more concentrated in cord blood compared to maternal blood (WHO, 1990). The estimated corresponding dose for maternal blood is 3.5 ug/L (Mahaffey et al., 2009). Only 2.3% of adult women in NHANES (2009-2010) had blood THg concentrations exceeding 5.8 µg/L (USEPA, 2013). In the present study, two blood samples (1.3%) had THg levels above 3.5 µg/L, and no blood THg concentrations exceeded 5.8 µg/L. The %MeHg (of THg) in blood averaged 61.5 ± 23.0% (median: 59.0%, range: 12.0-116.7%), which is slightly lower than the 80% estimated by the NRC (NRC, 2000). Concentrations of blood THg and blood MeHg were significantly correlated in both early and late pregnancy (Spearman's rho=0.89-0.92, $p<0.01$, n=78) (Figure 3.2).

Descriptive characteristics for blood Hg by categories of covariates are presented in Tables 3.6-3.9. Number of weekly fish meals was significantly positively correlated with blood THg and blood MeHg concentrations in both early and late gestation (Spearman's rho=0.41-0.63, $p<0.01$ for all, n=47) (Figure 3.3). Similar to fish consumption trends, blood THg and MeHg concentrations were higher, although not significantly, for African American mothers compared to other Caucasian and Hispanic mothers (Kruskal-Wallis, p=0.09-0.13, n=78). For rice, mothers reported consuming an average of 1.2 ± 1.4 cups/wk (median: 0.57 cups/wk, range: 0-7.0 cups/wk); however, cups of rice consumed per week were not associated with blood THg (Spearman's rho=-0.01, 0.09, p=0.53-0.94, n=47) and MeHg (Spearman's rho=0.02-0.19, p=0.20-0.91, n=47) in early and late pregnancy.

Associations between blood Hg and sociodemographic factors, such as age, education level, and parity have been found in previous studies (Basu et al., 2014; Golding et al., 2016; Miranda et al., 2011; Morrissette et al., 2004; Razzaghi et al., 2014; Vahter et al., 2000). Positive associations have been found between blood Hg and maternal age and education level in the U.S. (Miranda et al., 2011; Razzaghi et al., 2014), Canada (Morrissette et al., 2004), Mexico (Basu et al., 2014), and Europe (Golding et al., 2016; Vahter et al., 2000). An inverse relationship between maternal blood Hg and parity has also been found (Golding et al., 2016; Vahter et al., 1999), although some studies did not find an association (Basu et al., 2014; Hsu et al., 2007). We did not find any significant relationships between blood THg or MeHg and maternal age, education level, or parity (Spearmans's rho=-0.04, 0.03, p=0.74-0.85; Kruskal-Wallis, p=0.12-0.76, n=78). It has previously been reported that women who were older and more educated

consumed fish more frequently (Miranda et al., 2011). In our current study, weekly fish consumption frequency was not associated with age or education level (Spearman's rho=0.07, p=0.64; Kruskal-Wallis, p=0.22, n=47).

Previously, an inverse relationship was found between blood Hg and BMI after controlling for dietary MeHg intake through fish consumption in an NHANES (2007-2010) study of children and non-pregnant adults (Rothenberg et al., 2015). In the current study, there were weak positive trends, although not significant, between BMI and blood THg and MeHg during pregnancy (Spearman's rho=0.10-0.18, p=0.11-0.36, n=78); however, this analysis did not include adjustment for dietary MeHg intake. Research regarding whether there are associations between Hg and vitamin D is limited. A study investigating interactions between MeHg and the vitamin D pathway in dolphin cells found that MeHg exposure was associated with alterations of the vitamin D pathway, resulting in downregulation of vitamin D target genes (Ellis et al., 2010). Whether similar interactions occur between Hg and vitamin D in humans is uncertain. No association was found between maternal serum vitamin D and cord blood THg in a Spanish cohort (Llop et al., 2012). In this study, blood THg and MeHg were not related to serum vitamin D levels (Spearman's rho=0.004-0.03, p=0.79-0.98, n=73-76). Maternal blood THg and MeHg levels in late pregnancy also did not differ by frequency of antibiotic use between early and late pregnancy (Kruskal-Wallis, p=0.69-0.86, n=78). Studies of mice have shown that the demethylation of MeHg and elimination of inorganic Hg decreased in antibiotic-treated mice compared to controls (Rowland et al., 1984; Seko et al., 1981). For these studies, mice were dosed with MeHg-chloride either two days before the start of antibiotics (Seko et al., 1981) or seven days after antibiotic treatment started (Rowland

et al., 1984). On average, mothers in the current study ended antibiotic treatment 66.9 ± 49.3 days (range: 1-173 days) before the second blood sample was collected, and only eight mothers took antibiotics less than 30 days before blood sample collection. Additionally, it is uncertain whether fish consumption coincided with antibiotic treatment.

Blood THg and MeHg concentrations in late pregnancy were similar between mothers who received a flu vaccine (n=22) and mothers who were not vaccinated (n=54) (Wilcoxon rank-sum test, p=0.20-0.35, n=76). Multi-dose vaccines, including flu vaccines, contain thimerosal, which is an ethylmercury-based preservative (CDC, 2013). Blood THg levels have been known to peak 12-24 hours after receiving a vaccination that contains thimerosal (Pichichero et al., 2008). The half-life for ethylmercury has been estimated as 3.7 days (95% CI: 2.9, 4.5 days) in newborns and infants and 5.6 days (95% CI: 4.8, 6.3 days) in adults, and blood THg concentrations returned to pre-vaccination levels within 30 days after vaccination (Barregard et al., 2011; Pichichero et al., 2008). On average, mothers in this study received a flu vaccine 90.1 ± 56.7 days (range: 7-205 days) before their second blood sample was collected, and only one mother was vaccinated less than 30 days before blood sample collection. Two women received a flu vaccine on the same day their second blood sample was collected and were excluded from flu vaccine analyses, because it is uncertain whether vaccination occurred before or after blood sample collection. However, results were not significantly different when these women were included and classified as either receiving the flu vaccine (Wilcoxon rank-sum test, p=0.18-0.39, n=78) or not being vaccinated (Wilcoxon rank-sum test, p=0.20-0.33, n=78).

3.4 Blood Hg between Early and Late Gestation

There was a statistically significant decrease in blood MeHg from early to late pregnancy (paired t-test, p=0.04, n=78) (Figure 3.4), but there was not a significant change in blood THg (paired t-test, p=0.29, n=78) (Figure 3.5) or estimated blood inorganic Hg (THg-MeHg) (paired t-test p=0.69, n=78) (Figure 3.6). The mean change in blood THg and blood MeHg during pregnancy were -0.09 ± 0.78 µg/L (range: -2.6, 3.0 µg/L) and -0.12 ± 0.50 µg/L (range: -1.9, 1.1 µg/L), respectively. There were five women (6.4%) who were an exception to the mean and had a large increase in blood MeHg during pregnancy, which averaged 0.98 ± 0.11 µg/L (range: 0.80-1.1 µg/L). These women were African American (n=2) and Caucasian (n=3) and were educated at the college (n=3) and graduate school (n=2) level. The fish/shellfish consumption frequencies for these mothers were 0, 0.46, 1.4, 1.7, and 1.8 meals/wk. However, these frequencies reflect fish consumption reported in early pregnancy when the FFQ was completed, and it is possible fish consumption changed as pregnancy progressed.

Women who reported consuming more fish meals per week tended to have a larger decrease (more negative change) in blood MeHg; however, this trend was not significant (Spearman's rho=-0.25, p=0.10, n=47). The difference in blood MeHg from early to late pregnancy was not associated with maternal sociodemographic factors, such as race/ethnicity (Kruskal-Wallis, p=0.96, n=78), education level (Kruskal-Wallis, p=0.48, n=78), maternal age (Spearman's rho=-0.03, p=0.76), or parity (Kruskal-Wallis, p=0.79, n=78). The change in blood MeHg was also not related to frequency of antibiotic use between early and late pregnancy (Kruskal Wallis, p=0.33, n=78), trimester one BMI (Spearman's rho=0.10, p=0.40), IOM weight gain categories (Kruskal-Wallis, p=0.18,

n=77), or serum vitamin D in early or late pregnancy (Spearman's rho=-0.13,-0.02, p=0.28-0.88, n=73-76).

3.5 Hematocrit Adjustment

The distribution of Hg between red blood cells and plasma differs by Hg type. The red blood cell to plasma ratio for MeHg in blood is estimated to be 20 (Kershaw et al., 1980), whereas that for inorganic Hg is approximately one (Lundgren et al., 1967). Because blood plasma volume tends to increase during pregnancy (Hytten, 1985), it is possible that change in hematocrit contributed to the decrease in blood MeHg between early to late pregnancy. Blood THg and MeHg concentrations were adjusted for hematocrit using an equation previously used for hematocrit-adjustment of lead, most of which (~90%) also binds to red blood cells (blood Hg/hematocrit × 100) (Schell et al., 2000). After blood MeHg concentrations were normalized by hematocrit, a decrease in blood MeHg during pregnancy was still observed; however, this decrease was no longer statistically significant (paired t-test, p=0.16, n=66). When unadjusted blood MeHg concentrations were considered only for the 66 women who had hematocrit data in both early and late pregnancy, the decrease in blood MeHg was slightly attenuated and was near the significance level (paired t-test, p=0.07, n=66). Hematocrit-adjusted blood THg did not change from early to late pregnancy (paired t-test, p=0.97, n=66). Results were similar for unadjusted blood THg including only mothers with hematocrit data (paired t-test, p=0.59, n=66).

3.6 Mixed Model Analysis

A mixed model analysis for repeated measures was completed using the SAS Proc Mixed procedure, which accounted for within-subject covariability when modeling blood

THg and MeHg concentrations (both log_{10}-transformed) during pregnancy (Table 3.10). After adjusting for hematocrit and race/ethnicity, log_{10} blood THg and MeHg did not change significantly over time. However, after controlling for time and hematocrit, relationships between blood Hg and race/ethnicity were strengthened, and mean log_{10} blood THg and log_{10} blood MeHg concentrations were significantly higher among African American women compared to Caucasian and Hispanic women (t-test, p=0.02-0.04) (Table 3.11).

Table 3.1 Demographic data for 78 participants.

	Mean ± SD or n (%) Median (range)
Age (yr)	30.1 ± 5.3
	30.1 (18.3-42.3)
Race/ethnicity	
African American	30 (38.5)
Caucasian	31 (39.7)
Hispanic	17 (21.8)
Education	
≤ High school	31 (39.7)
College	30 (38.5)
Graduate school	17 (21.8)
Parity	
0	27 (34.6)
1	23 (29.5)
≥2	28 (35.9)
BMI (kg/m²)- early pregnancy	29.3 ± 8.4
	27.2 (16.8-59.7)
Weight class	
Normal[a]	29 (37.2)
Overweight	22 (28.2)
Obese	27 (34.6)
IOM weight gain guidelines	
Below	22 (28.2)
Within	32 (41.0)
Above	23 (29.5)
Missing	1 (1.3)
Hematocrit (%)- early pregnancy	36.7 ± 3.0
	36.5 (29.8-44.1)
Missing	9 (11.5)
Hematocrit (%)- late pregnancy	35.0 ± 3.3
	35.2 (27.1-41.4)
Missing	8 (10.3)
Vitamin D (ng/mL)- early pregnancy	28.8 ± 10.7
	27.5 (8.2-63.0)
Missing	5 (6.4)
Vitamin D (ng/mL)- late pregnancy	45.9 ± 23.1
	42.7 (9.3-131.2)
Missing	2 (2.6)

Table 3.1 continued.

	Mean ± SD or Median (range)	n (%)
Antibiotic use between early and late pregnancy		
0 times		51 (65.4)
1 time		17 (21.8)
≥2 times		10 (12.8)
Flu vaccine between early and late pregnancy[b]		
yes		22 (28.2)
no		54 (69.2)
Alcohol consumption during pregnancy		
yes		1 (1.3)
no		77 (98.7)
Smoking during pregnancy		
yes		2 (2.6)
no		76 (97.4)

[a]Normal weight class category includes one underweight mother.
[b]Two mothers were excluded from flu vaccine analyses due to uncertainties in whether vaccination occurred before or after their second blood samples were collected.

Table 3.2 Characteristics of women who completed a food frequency questionnaire (FFQ) (n=47) and women who did not complete a FFQ (n=31), including p-values for comparisons between the two groups.

	Mean ± 1 SD or n (%) Median (range)		
	Completed FFQ n=47	**No FFQ n=31**	**p-value**
Age (yr)	30.8 ± 5.0 31.3 (19.4-42.3)	29.1 ± 5.6 28.7 (18.3-40.7)	0.17
Race/ethnicity			0.03
African American	16 (34)	14 (45)	
Caucasian	24 (51)	7 (23)	
Hispanic	7 (15)	10 (32)	
Education			<0.01
≤ High school	11 (23)	20 (65)	
College	25 (53)	5 (16)	
Graduate school	11 (23)	6 (19)	
Parity			0.39
0	19 (40)	8 (26)	
1	12 (26)	11 (35)	
≥2	16 (34)	12 (39)	
Weight class			0.74
Normal[a]	19 (40)	10 (32)	
Overweight	13 (28)	9 (29)	
Obese	15 (32)	12 (39)	
IOM weight gain			0.07
Below	9 (19)	13 (43)	
Within	22 (47)	10 (33)	
Above	16 (34)	7 (23)	
Missing			
Hematocrit (%)- early pregnancy	37.0 ± 3.0 36.9 (30.4-44.1)	36.1 ± 3.0 35.9 (29.8-41.9)	0.17
Hematocrit (%)- late pregnancy	35.6 ± 2.8 35.4 (29.5-41.4)	34.0 ± 4.0 34.5 (27.1-40.6)	0.22
Vitamin D (ng/mL)- early pregnancy	29.7 ± 10.6 27.9 (13.0-63.0)	27.2 ± 11.0 26.3 (8.2-48.1)	0.52
Vitamin D (ng/mL)- late pregnancy	45.3 ± 22.1 43.1 (9.3-100.3)	46.9 ± 25.1 40.9 (14.7-131.2)	0.92

Table 3.2 continued

	Mean ± 1 SD or n (%) Median (range)		
	Completed FFQ n=47	**No FFQ n=31**	**p-value**
Antibiotic use between early and late pregnancy			0.28
0 times	29 (62)	22 (71)	
1 time	13 (28)	4 (13)	
≥2 times	5 (11)	5 (16)	
Flu vaccine between early and late pregnancy[b]			0.16
Yes	16 (35)	6 (20)	
No	30 (65)	24 (80)	

[a]Normal weight class category includes one underweight mother.
[b]Two mothers were excluded from flu vaccine analyses due to uncertainties in whether vaccination occurred before or after their second blood samples were collected.

Table 3.3 Average weekly fish/shellfish consumption by type for participants who completed a food frequency questionnaire (n=47).

Number of meals per week by fish type Mean ± 1 SD Median (range)					
Oysters	**Other shellfish**	**Tuna**	**Fried fish**	**Other fish, not fried**	**All seafood total**
0.02 ± 0.09 0 (0-0.57)	0.30 ± 0.33 0.23 (0-1.0)	0.22 ± 0.55 0 (0-3.5)	0.14 ± 0.21 0 (0-0.57)	0.17 ± 0.25 0 (0-1.0)	0.84 ± 0.79 0.57 (0-3.5)

Table 3.4 Frequencies of weekly fish/shellfish consumption among participants who completed a food frequency questionnaire (n=47).

	Frequency of fish consumption per week n (%)			
	0 times	**<1 time**	**≥1-<2 times**	**≥2 times**
Oysters	45 (95.7)	2 (4.3)	0 (0)	0 (0)
Other shellfish	22 (46.8)	21 (44.7)	4 (8.5)	0 (0)
Tuna	31 (66.0)	14 (29.8)	1 (2.1)	1 (2.1)
Fried fish	29 (61.7)	18 (38.3)	0 (0)	0 (0)
Other fish, not fried	28 (59.6)	18 (38.3)	1 (2.1)	0 (0)
All seafood total	9 (19.1)	19 (40.4)	15 (31.9)	4 (8.5)

Table 3.5 Descriptive statistics for blood total mercury (THg) and methylmercury (MeHg) concentrations and percent methylmercury (%MeHg) of THg at early and late pregnancy.

	Mean ± 1 SD Median (range)		
	Early pregnancy (n=78)	**Late pregnancy (n=78)**	**Total (n=156)**
THg (µg/L)	0.92 ± 0.81 0.65 (0.02-3.9)	0.83 ± 0.76 0.61 (0.06-4.0)	0.88 ± 0.78 0.62 (0.02-4.0)
MeHg (µg/L)	0.60 ± 0.62 0.40 (0.01-2.7)	0.48 ± 0.42 0.36 (0.01-2.1)	0.54 ± 0.53 0.38 (0.01-2.7)
%MeHg (%)	62.3 ± 24.0 60.4 (15.5-114.2)	60.8 ± 22.1 57.4 (12.0-116.7)	61.5 ± 23.0 59.0 (12.0 -116.7)

Table 3.6 Blood methylmercury (μg/L) in early pregnancy by categories of covariates.

	n (%)	Mean	Range	50th	75th	95th
All	78	0.60	0.01-2.7	0.40	0.78	2.3
Age[a] (yr)						
<30.1	39 (50.0)	0.59	0.01-2.7	0.39	0.71	2.5
≥30.1	39 (50.0)	0.61	0.03-2.4	0.42	0.82	2.3
Race/ethnicity						
African American	30 (38.5)	0.69	0.10-2.7	0.49	0.78	2.5
Caucasian	31 (39.7)	0.55	0.01-2.4	0.30	0.79	1.9
Hispanic	17 (21.8)	0.54	0.03-2.3	0.28	0.68	2.3
Education						
≤ High school	31 (39.7)	0.54	0.03-2.5	0.40	0.68	1.8
College	30 (38.5)	0.59	0.04-2.7	0.37	0.67	2.3
Graduate school	17 (21.8)	0.73	0.01-2.4	0.50	1.1	2.4
Parity						
0	27 (34.6)	0.62	0.01-2.7	0.30	0.82	2.4
1	23 (29.5)	0.77	0.04-2.5	0.61	1.1	2.3
≥2	28 (35.9)	0.45	0.03-1.8	0.34	0.51	1.6
Weight class						
Normal[b]	29 (37.2)	0.61	0.01-2.5	0.29	0.80	2.4
Overweight	22 (28.2)	0.53	0.03-1.9	0.37	0.63	1.8
Obese	27 (34.6)	0.65	0.04-2.7	0.47	0.82	1.9
IOM weight gain guidelines						
Below	22 (28.2)	0.86	0.13-2.5	0.59	1.1	2.3
Within	32 (41.0)	0.54	0.01-2.4	0.35	0.70	1.9
Above	23 (29.5)	0.44	0.04-2.7	0.34	0.45	0.82
Missing	1 (1.3)					
Fish consumption (meals/week)						
0	9 (11.5)	0.15	0.01-0.34	0.13	0.24	0.34
<1	19 (24.4)	0.30	0.03-1.0	0.28	0.34	1.0
≥1-<2	15 (19.2)	0.68	0.04-1.8	0.54	1.1	1.8
≥2	4 (5.1)	0.80	0.63-1.1	0.73	0.95	1.1
Missing	31 (39.7)	0.85	0.06-2.7	0.56	1.3	2.5
Hematocrit[a] (%)						
<36.5	33 (42.3)	0.63	0.01-2.7	0.42	0.71	2.5
≥36.5	36 (46.2)	0.58	0.03-2.3	0.31	0.90	1.9
Missing	9 (11.5)	0.60	0.08-1.6	0.63	0.68	1.6
Vitamin D[a] (ng/mL)						
<27.5	36 (46.2)	0.54	0.03-2.7	0.31	0.66	2.5
≥27.5	37 (47.4)	0.64	0.01-2.4	0.42	0.80	2.3
Missing	5 (6.4)	0.75	0.40-1.6	0.65	0.68	1.6

Table 3.6 continued.

	n (%)	Mean	Range	50th	75th	95th
Antibiotic use between early and late pregnancy						
0 times	51 (65.4)	0.60	0.01-2.3	0.42	0.80	1.9
1 time	17 (21.8)	0.48	0.06-2.4	0.30	0.54	2.4
≥2 times	10 (12.8)	0.83	0.04-2.7	0.54	0.82	2.7
Flu vaccine between early and late pregnancy[c]						
Yes	22 (28.2)	0.65	0.06-2.4	0.35	0.83	1.9
No	54 (69.2)	0.59	0.01-2.7	0.41	0.68	2.3

[a]Continuous variables were dichotomized at the median.
[b]Normal weight class category includes one underweight mother.
[c]Two mothers were excluded from flu vaccine analyses due to uncertainties in whether vaccination occurred before or after their second blood samples were collected.

Table 3.7 Blood methylmercury (μg/L) in late pregnancy by categories of covariates.

	n (%)	Mean	Range	50th	75th	95th
All	78	0.48	0.01-2.1	0.36	0.60	1.4
Age[a] (yr)						
<30.1	39 (50.0)	0.49	0.01-2.1	0.34	0.59	1.4
≥30.1	39 (50.0)	0.48	0.01-1.6	0.39	0.60	1.4
Race/ethnicity						
African American	30 (38.5)	0.59	0.05-2.1	0.48	0.64	1.6
Caucasian	31 (39.7)	0.45	0.01-1.4	0.28	0.60	1.4
Hispanic	17 (21.8)	0.36	0.01-0.90	0.34	0.42	0.90
Education						
≤ High school	31 (39.7)	0.40	0.01-0.90	0.36	0.58	0.82
College	30 (38.5)	0.52	0.05-2.1	0.34	0.57	1.6
Graduate school	17 (21.8)	0.58	0.01-1.4	0.53	0.88	1.4
Parity						
0	27 (34.6)	0.51	0.01-2.1	0.35	0.60	1.4
1	23 (29.5)	0.61	0.05-1.6	0.44	0.82	1.4
≥2	28 (35.9)	0.35	0.05-0.90	0.32	0.54	0.69
Weight class						
Normal[b]	29 (37.2)	0.39	0.01-1.4	0.32	0.56	1.2
Overweight	22 (28.2)	0.52	0.05-1.4	0.47	0.62	1.4
Obese	27 (34.6)	0.55	0.05-2.1	0.38	0.64	1.6
IOM weight gain guidelines						
Below	22 (28.2)	0.57	0.11-1.4	0.54	0.75	1.36
Within	32 (41.0)	0.49	0.01-1.6	0.36	0.61	1.35
Above	23 (29.5)	0.39	0.01-2.1	0.32	0.43	1.4
Missing	1 (1.3)					
Fish consumption (meals/week)						
0	9 (11.5)	0.30	0.01-1.2	0.14	0.32	1.2
<1	19 (24.4)	0.32	0.08-1.2	0.25	0.38	1.2
≥1-<2	15 (19.2)	0.66	0.14-1.6	0.59	0.88	1.6
≥2	4 (5.1)	0.43	0.26-0.57	0.44	0.54	0.57
Missing	31 (39.7)	0.56	0.01-2.1	0.43	0.75	1.4
Hematocrit[a] (%)						
<35.2	35 (44.9)	0.45	0.01-1.6	0.36	0.60	1.4
≥35.2	35 (44.9)	0.54	0.05-2.1	0.36	0.69	1.4
Missing	8 (10.3)	0.39	0.08-0.90	0.37	0.41	0.90
Vitamin D[a] (ng/mL)						
<42.7	38 (48.7)	0.46	0.01-1.4	0.39	0.59	1.4
≥42.7	38 (48.7)	0.51	0.05-2.1	0.36	0.62	1.6
Missing	2 (2.6)	0.38	0.34-0.42	0.38	0.42	0.42

Table 3.7 continued.

	n (%)	Mean	Range	50th	75th	95th
Antibiotic use between early and late pregnancy						
0 times	51 (65.4)	0.49	0.01-1.4	0.38	0.66	1.4
1 time	17 (21.8)	0.46	0.05-1.6	0.35	0.54	1.6
≥2 times	10 (12.8)	0.49	0.05-2.1	0.33	0.51	2.1
Flu vaccine between early and late pregnancy[c]						
Yes	22 (28.2)	0.47	0.05-1.4	0.33	0.69	1.4
No	54 (69.2)	0.49	0.01-2.1	0.40	0.59	1.4

[a]Continuous variables were dichotomized at the median.

[b]Normal weight class category includes one underweight mother.

[c]Two mothers were excluded from flu vaccine analyses due to uncertainties in whether vaccination occurred before or after their second blood samples were collected.

Table 3.8 Blood total mercury (μg/L) in early pregnancy by categories of covariates.

	n (%)	Mean	Range	50th	75th	95th
All	78	0.92	0.02-3.9	0.65	1.2	2.6
Age[a] (yr)						
<30.1	39 (50.0)	0.89	0.02-3.9	0.62	0.97	2.6
≥30.1	39 (50.0)	0.96	0.05-3.0	0.70	1.3	2.6
Race/ethnicity						
African American	30 (38.5)	1.1	0.33-3.9	0.75	1.3	2.6
Caucasian	31 (39.7)	0.76	0.02-3.0	0.49	1.0	2.6
Hispanic	17 (21.8)	0.91	0.17-2.6	0.66	1.2	2.6
Education						
≤ High school	31 (39.7)	0.86	0.17-3.9	0.65	1.1	2.5
College	30 (38.5)	0.86	0.05-2.6	0.59	1.3	2.6
Graduate school	17 (21.8)	1.1	0.02-3.0	0.85	1.5	3.0
Parity						
0	27 (34.6)	0.89	0.02-3.0	0.65	1.0	2.6
1	23 (29.5)	1.1	0.05-3.9	0.81	1.5	2.6
≥2	28 (35.9)	0.74	0.09-2.5	0.49	0.97	2.4
Weight class						
Normal[b]	29 (37.2)	0.90	0.02-3.9	0.56	1.1	3.0
Overweight	22 (28.2)	0.75	0.09-2.6	0.60	0.97	1.8
Obese	27 (34.6)	1.0	0.05-2.6	0.80	1.3	2.6
IOM weight gain guidelines						
Below	22 (28.2)	1.3	0.17-3.9	0.81	2.1	2.6
Within	32 (41.0)	0.78	0.02-3.0	0.58	1.2	2.6
Above	23 (29.5)	0.74	0.05-2.6	0.63	0.85	2.4
Missing	1 (1.3)					
Fish consumption (meals/week)						
0	9 (11.5)	0.34	0.02-0.73	0.29	0.50	0.73
<1	19 (24.4)	0.50	0.10-1.3	0.38	0.66	1.3
≥1-<2	15 (19.2)	1.0	0.08-2.2	0.68	1.6	2.2
≥2	4 (5.1)	1.2	0.59-2.2	0.98	1.6	2.2
Missing	31 (39.7)	1.3	0.09-3.9	1.0	2.4	3.0
Hematocrit[a] (%)						
<36.5	33 (42.3)	0.97	0.02-3.9	0.62	1.3	3.0
≥36.5	36 (46.2)	0.87	0.08-2.6	0.59	1.1	2.6
Missing	9 (11.5)	0.98	0.10-2.5	0.98	1.1	2.5
Vitamin D[a] (ng/mL)						
<27.5	36 (46.2)	0.84	0.17-3.9	0.59	0.96	2.6
≥27.5	37 (47.4)	0.97	0.02-3.0	0.66	1.3	2.6
Missing	5 (6.4)	1.2	0.54-2.5	1.1	1.2	2.5

Table 3.8 continued.

	n (%)	Mean	Range	50th	75th	95th
Antibiotic use between early and late pregnancy						
0 times	51 (65.4)	0.90	0.02-2.6	0.59	1.3	2.6
1 time	17 (21.8)	0.83	0.29-3.0	0.66	0.93	3.0
≥2 times	10 (12.8)	1.0	0.05-3.9	0.71	0.85	3.9
Flu vaccine between early and late pregnancy[c]						
Yes	22 (28.2)	0.96	0.20-3.0	0.65	1.3	2.6
No	54 (69.2)	0.90	0.02-3.9	0.65	1.1	2.6

[a]Continuous variables were dichotomized at the median.

[b]Normal weight class category includes one underweight mother.

[c]Two mothers were excluded from flu vaccine analyses due to uncertainties in whether vaccination occurred before or after their second blood samples were collected.

Table 3.9 Blood total mercury (μg/L) in late pregnancy by categories of covariates.

	n (%)	Mean	Range	50th	75th	95th
All	78	0.83	0.06-4.0	0.61	1.1	2.6
Age[a] (yr)						
<30.1	39 (50.0)	0.78	0.09-4.0	0.58	1.1	2.5
≥30.1	39 (50.0)	0.87	0.06-3.4	0.61	1.2	3.0
Race/ethnicity						
African American	30 (38.5)	0.95	0.18-4.0	0.70	1.2	2.2
Caucasian	31 (39.7)	0.86	0.06-3.4	0.51	1.2	3.0
Hispanic	17 (21.8)	0.57	0.10-1.3	0.50	0.76	1.3
Education						
≤ High school	31 (39.7)	0.60	0.09-1.4	0.57	0.81	1.3
College	30 (38.5)	0.90	0.06-4.0	0.60	1.2	2.5
Graduate school	17 (21.8)	1.1	0.09-3.4	0.99	1.3	3.4
Parity						
0	27 (34.6)	1.0	0.09-4.0	0.61	1.2	3.4
1	23 (29.5)	0.90	0.06-2.5	0.68	1.2	2.2
≥2	28 (35.9)	0.59	0.09-1.4	0.49	0.85	1.3
Weight class						
Normal[b]	29 (37.2)	0.70	0.09-3.0	0.58	0.76	2.6
Overweight	22 (28.2)	0.91	0.14-3.4	0.75	1.1	2.5
Obese	27 (34.6)	0.90	0.06-4.0	0.61	1.2	2.2
IOM weight gain guidelines						
Below	22 (28.2)	0.86	0.15-2.0	0.81	1.2	1.5
Within	32 (41.0)	0.85	0.09-3.4	0.58	1.1	3.0
Above	23 (29.5)	0.78	0.06-4.0	0.41	1.0	2.5
Missing	1 (1.3)					
Fish consumption (meals/week)						
0	9 (11.5)	0.71	0.06-3.4	0.42	0.76	3.4
<1	19 (24.4)	6.0	0.14-3.0	0.37	0.61	3.0
≥1-<2	15 (19.2)	1.0	0.26-2.5	0.89	1.3	2.5
≥2	4 (5.1)	0.83	0.39-1.2	0.85	1.2	1.2
Missing	31 (39.7)	0.90	0.09-4.0	0.68	1.2	2.6
Hematocrit[a] (%)						
<35.2	35 (44.9)	0.79	0.06-3.4	0.61	1.2	2.2
≥35.2	35 (44.9)	0.91	0.09-4.0	0.64	0.99	3.0
Missing	8 (10.3)	0.65	0.23-1.4	0.48	0.95	1.4
Vitamin D[a] (ng/mL)						
<42.7	38 (48.7)	0.73	0.06-2.0	0.63	1.2	1.5
≥42.7	38 (48.7)	0.94	0.09-4.0	0.59	0.99	3.4
Missing	2 (2.6)	0.45	0.35-0.55	0.45	0.55	0.55

Table 3.9 continued.

	n (%)	Mean	Range	50th	75th	95th
Antibiotic use between early and late pregnancy						
0 times	51 (65.4)	0.83	0.09-3.4	0.61	1.1	2.5
1 time	17 (21.8)	0.77	0.09-2.6	0.61	0.99	2.6
≥2 times	10 (12.8)	0.95	0.06-4.0	0.60	1.2	4.0
Flu vaccine between early and late pregnancy[c]						
Yes	22 (28.2)	0.70	0.09-2.6	0.41	0.85	2.0
No	54 (69.2)	0.89	0.06-4.0	0.63	1.2	3.0

[a]Continuous variables were dichotomized at the median.

[b]Normal weight class category includes one underweight mother.

[c]Two mothers were excluded from flu vaccine analyses due to uncertainties in whether vaccination occurred before or after their second blood samples were collected.

Table 3.10 Summary of mixed model analysis for repeated measures, relating log_{10} blood total mercury (THg) and log_{10} blood methylmercury (MeHg) to time at which the blood sample was collected during pregnancy.

		β (95% CI)	p-value for β
Log_{10} blood THg			
	Time		
	Early pregnancy	Referent	0.89
	Late pregnancy	-0.006 (-0.09, 0.08)	
	Hematocrit	0.01 (-0.006, 0.03)	0.18
	Race/ethnicity		
	African American	0.27 (0.02, 0.53)	0.04
	Caucasian	0.04 (-0.21, 0.29)	0.74
	Hispanic	Referent	
Log_{10} blood MeHg			
	Time		
	Early pregnancy	Referent	0.45
	Late pregnancy	−0.033 (-0.12, 0.054)	
	Hematocrit	0.0052 (-0.016, 0.026)	0.62
	Race/ethnicity		
	African American	0.34 (0.048, 0.64)	0.02
	Caucasian	0.09 (-0.20, 0.38)	0.54
	Hispanic	Referent	

Table 3.11 Least squares means presented as geometric means of blood total mercury (THg) and blood methylmercury (MeHg) by race/ethnicity, after adjusting for hematocrit and time at which the blood sample was collected during pregnancy.

		Geometric mean ± 1 SD
Blood THg		
	African American	0.83 ± 1.2[a]
	Caucasian	0.49 ± 1.2
	Hispanic	0.44 ± 1.3
Blood MeHg		
	African American	0.48 ± 1.2[b]
	Caucasian	0.27 ± 1.2
	Hispanic	0.22 ± 1.3

[a]Mean $\log_{10}$ blood THg significantly higher for African Americans compared to Caucasians (p=0.02) and Hispanics (p=0.04), after controlling for hematocrit and time

[b]Mean $\log_{10}$ blood MeHg significantly higher for African Americans compared to Caucasians (p=0.03) and Hispanics (p=0.02), after controlling for hematocrit and time

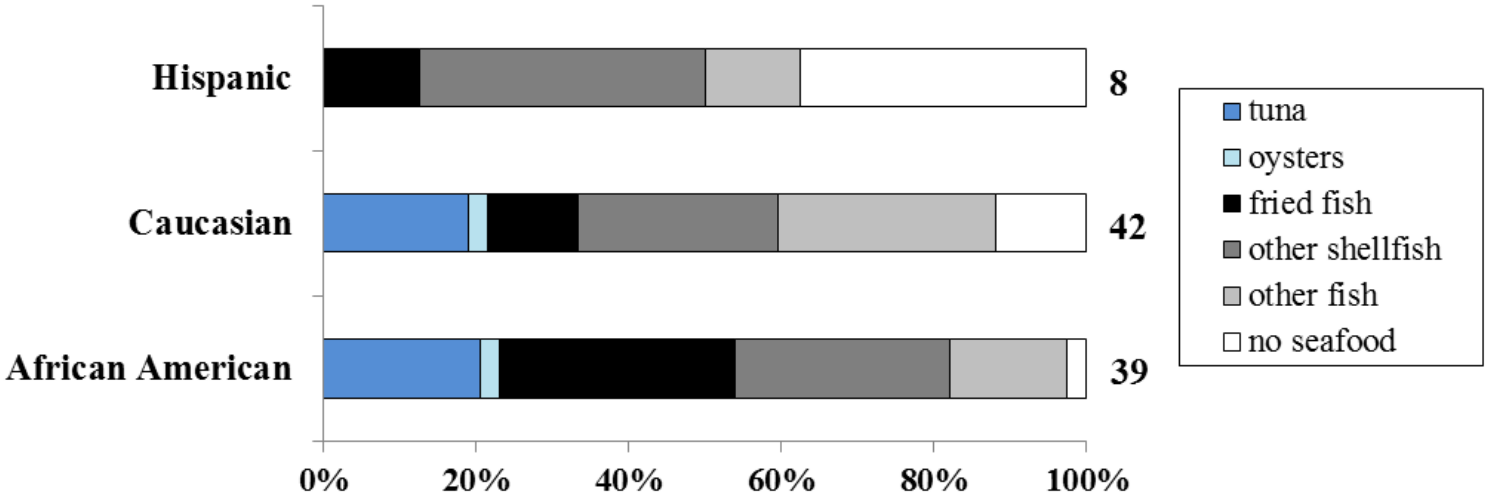

Figure 3.1 Fish/shellfish types consumed by race/ethnicity (n=47 mothers). Note that some mothers indicated eating more than one kind of fish/shellfish.

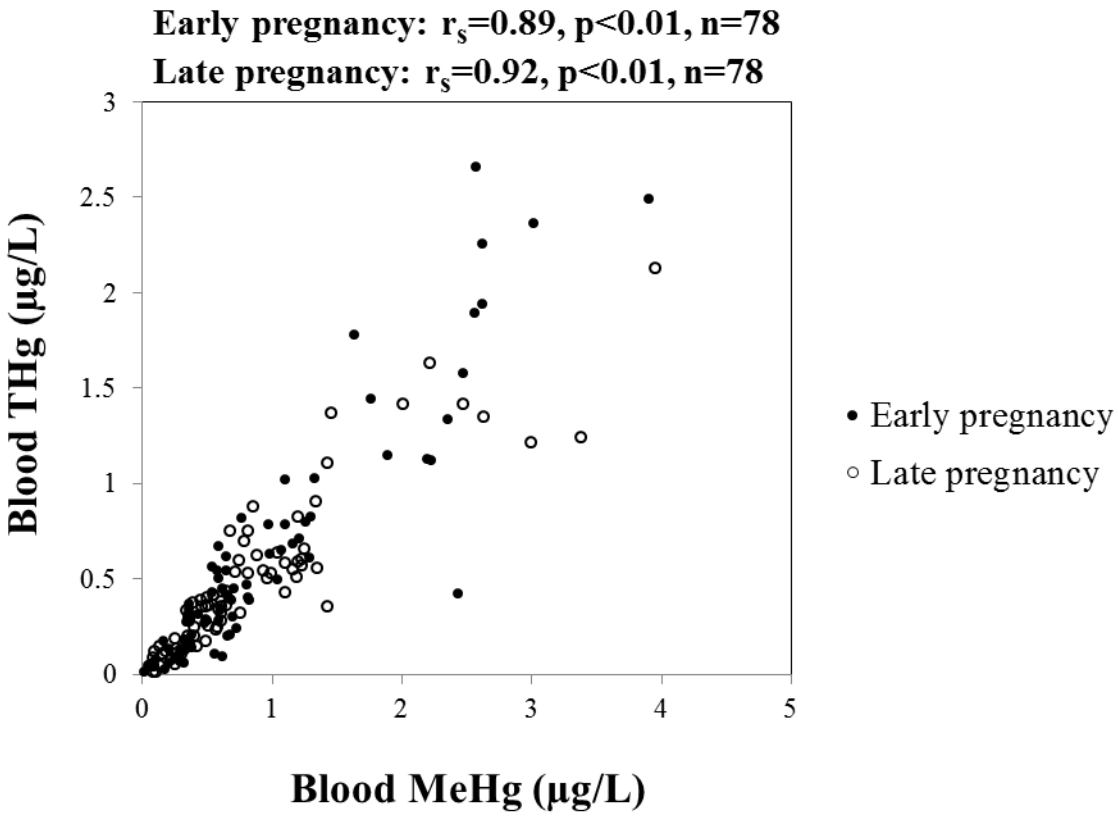

Figure 3.2 Bivariate scatterplot of blood total mercury (THg) and methylmercury (MeHg) for 78 women in both early and late pregnancy, which includes Spearman's correlation (r_s).

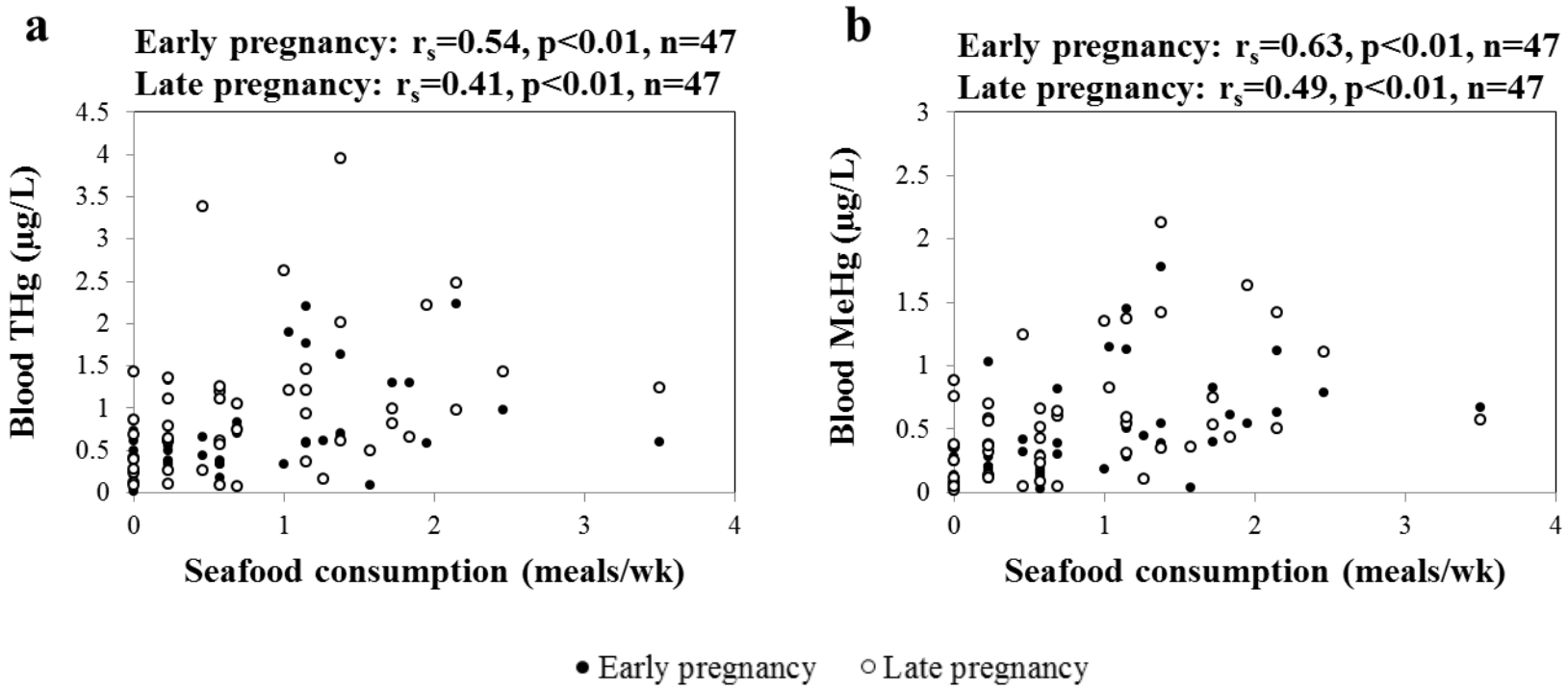

Figure 3.3 Bivariate scatterplots of the number of fish meals consumed per week and (a) blood total mercury (THg) and (b) blood methylmercury (MeHg) concentrations for 47 participants who completed a food frequency questionnaire. Includes Spearman's correlation (r_s).

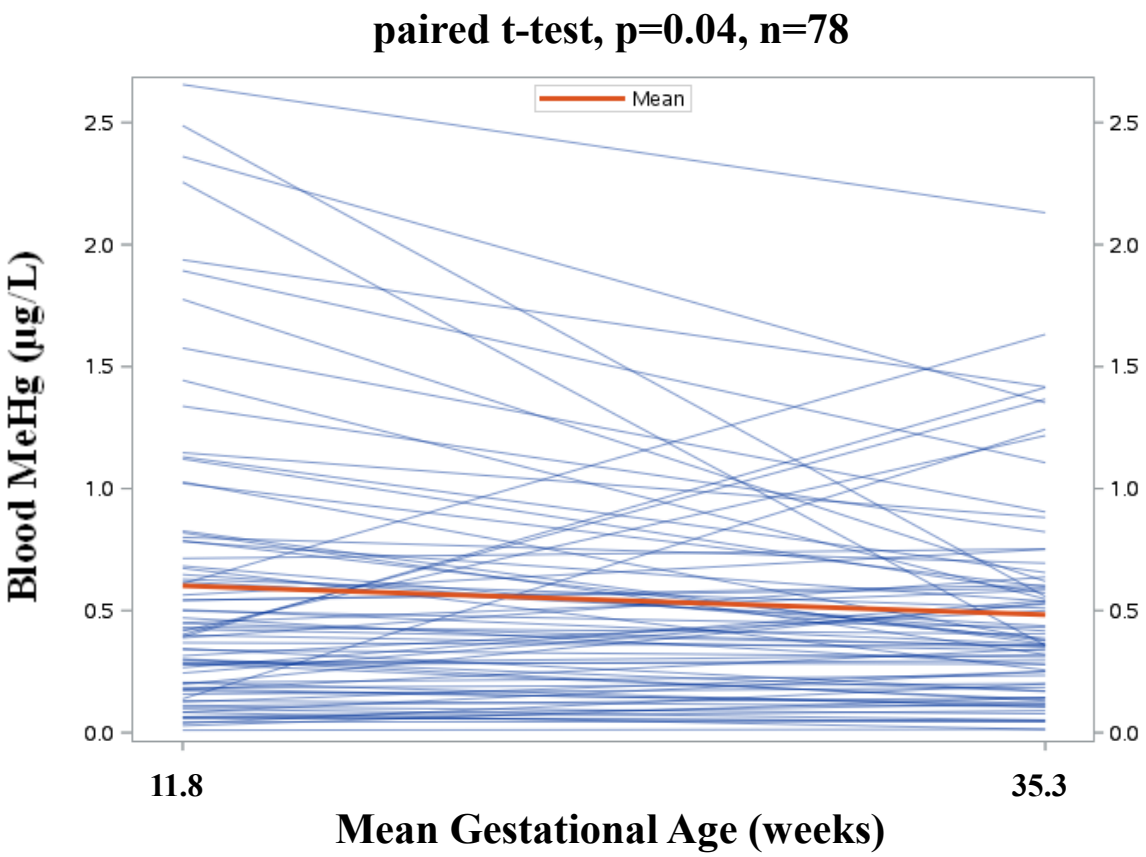

Figure 3.4 Change in mean blood methylmercury (MeHg) concentration from early to late pregnancy (n=78).

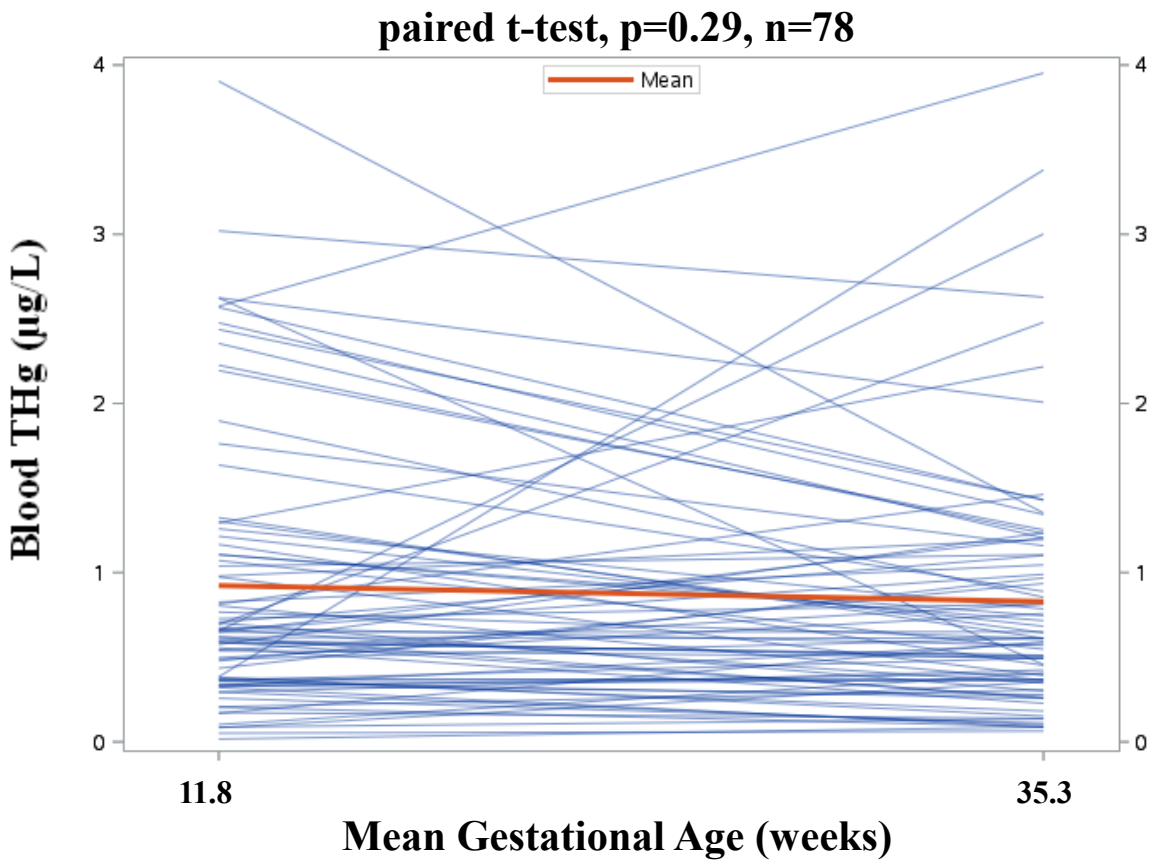

Figure 3.5 Change in mean blood total mercury (THg) concentration from early to late pregnancy (n=78).

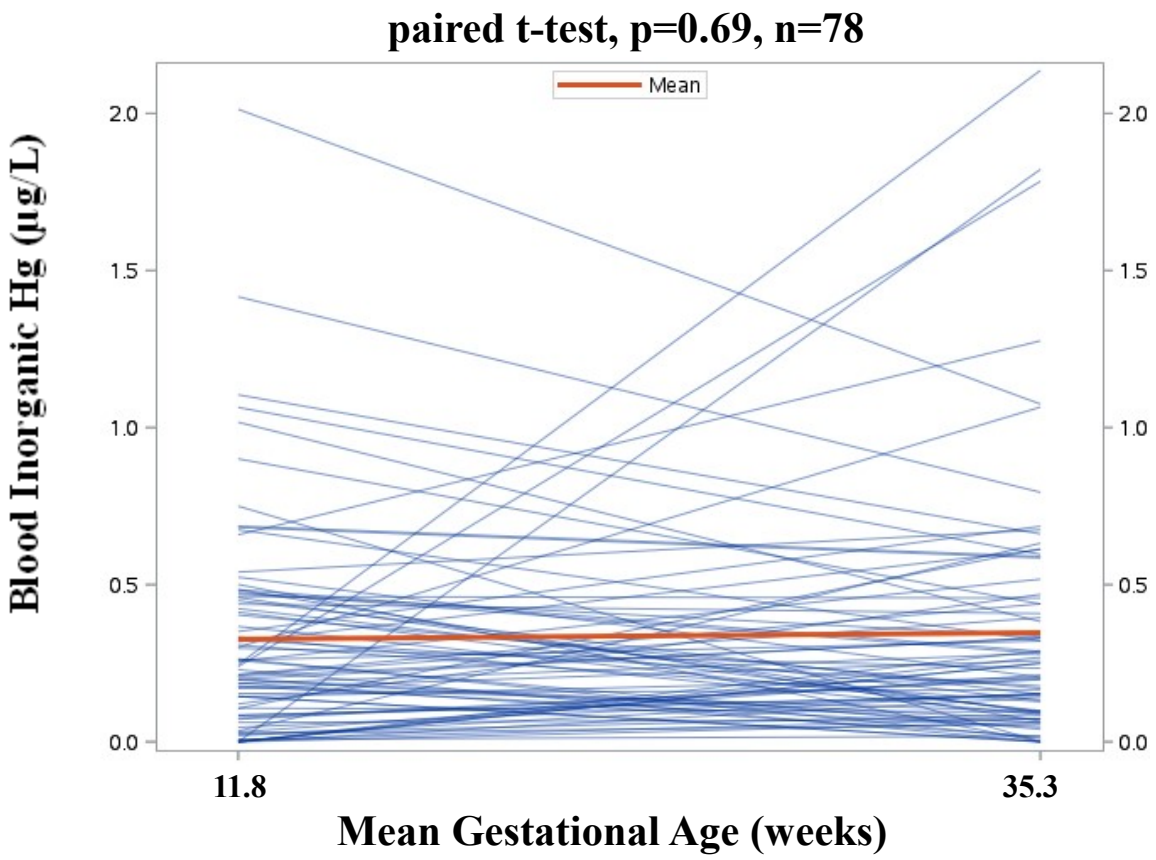

Figure 3.6 Change in estimated mean blood inorganic mercury (Hg) concentration from early to late pregnancy (n=78).

CHAPTER 4

DISCUSSION/CONCLUSION

Whole blood samples were collected from pregnant mothers during early (11.8 ± 1.7 weeks gestation) and late (35.3 ± 2.0 weeks gestation) gestation. In unadjusted bivariate analyses, blood THg and MeHg in early and late pregnancy were higher for African Americans compared to Caucasians and Hispanics; however, these trends were not significant (Kruskal-Wallis, p=0.09-0.13, n=78). Using a paired t-test, maternal blood MeHg was lower during late gestation compared to early gestation (paired t-test, p=0.04, n=78), while blood THg did not differ between early and late pregnancy (paired t-test, p=0.38, n=78). The decrease in blood MeHg was slightly attenuated when normalized by hematocrit (paired t-test, p=0.16, n=66). There were not any associations between the decrease in blood MeHg during pregnancy and maternal characteristics, including race/ethnicity, education level, trimester one BMI, weight gain during pregnancy, serum vitamin D, and antibiotic use (Spearman's rho=-0.03, 0.10, p=0.28-0.88; Kruskal-Wallis, p=0.18-0.96, n=73-78). In the adjusted models for repeated measures (adjusted for hematocrit, race/ethnicity, and time of blood sample collection), the association between blood Hg and race/ethnicity was strengthened, where African Americans had higher $\log_{10}$ blood THg and MeHg compared to Caucasians and Hispanics (t-test, p=0.02-0.04).

Few studies have investigated longitudinal trends in maternal blood Hg during pregnancy, which also measured or estimated blood MeHg. In a Swedish pregnant cohort, maternal blood MeHg and inorganic Hg levels decreased between trimesters one

(n=148) and three (n=112) (paired t-test, $p<0.001$) (Vahter et al., 2000). Decreases in maternal blood Hg were also found in a pregnant cohort along the St. Lawrence River in Canada, where there was a reduction in maternal blood THg, inorganic Hg, and estimated organic Hg (THg-inorganic Hg) between trimester two and delivery (Wilcoxon signed rank, $p<0.01$-0.01, n=159) (Morrissette et al., 2004). Women in both the Swedish and Canadian pregnant cohorts reported reductions in fish consumption frequency from before pregnancy to during pregnancy, which may have contributed to decreases in blood Hg (Vahter et al., 2000; Morrissette et al., 2004). In our study, it is uncertain whether mothers reduced fish/shellfish consumption during pregnancy, because information on food frequencies was collected just one time.

One difference between our study and the Canadian and Swedish studies is that we only observed a decrease in blood MeHg, and not blood inorganic Hg (or blood THg).This is important, because most studies, including NHANES, rely on THg. One reason we did not observe a decrease in inorganic Hg may be that mothers in this study reported consuming shellfish, such as shrimp, scallops, and crabs, most frequently, which tend to be low in MeHg and contain a higher proportion of inorganic Hg compared to other freshwater and ocean fish (FDA, 2017). A second reason may be due to ingestion of foods and drinks sweetened with high fructose corn syrup, which may contain inorganic Hg (Dufault et al., 2009). In the FFQ, there were a total 5 questions concerning how often mothers drank beverages potentially containing high fructose corn syrup, such as juices, iced teas, sodas, and other soft drinks. NHANES data (1999-2004) have shown that fructose-sweetened food and drinks are prominent in the U.S. diet (Marriott et al., 2009), and mothers in our South Carolina cohort reported drinking an average of 7.2 ± 9.6

servings/wk (range: 0-41 servings/wk) of beverages possibly sweetened with high fructose corn syrup. However, estimated blood inorganic Hg concentrations in early and late pregnancy were not correlated with consumption frequency of these drinks (Spearman's rho=-0.22, 0.08, p=0.15-0.60). In our study, intake of high fructose corn syrup could not be quantified from the FFQ alone, and it is not known how many foods/drinks included in the FFQ were actually made with high fructose corn syrup. Although it is uncertain whether the drinks we investigated contained inorganic Hg, differences in diet, including ingestion of fructose-sweetened food and drinks, may help explain why inorganic Hg did not decrease in our current study, as was observed in the Swedish and Canadian pregnant cohorts. Therefore, further research is needed to investigate whether fructose-sweetened food and drinks may be a dietary source of inorganic Hg.

In the mixed models adjusted for hematocrit, race/ethnicity, and time, African American mothers had higher $\log_{10}$ blood THg and MeHg concentrations compared to Caucasian and Hispanic mothers, which reflects fish consumption trends observed in our current study. In NHANES studies of adult women, the highest fish consumption frequency was reported among "other races" (Asian, Pacific Islander, American Indian, Alaska Native, multi-racial), followed by non-Hispanic black, non-Hispanic white, and Hispanic women (USEPA, 2013). Due to the current study design, few, if any, Asian mothers were recruited for this study. However, the fish consumption trends observed in our South Carolina cohort were consistent with national fish consumption trends among African American, Caucasian, and Hispanic women, including NHANES (1999-2008) (USEPA, 2013). In a study of adult women along the northeast Florida coast (n=703),

African American women consumed more fish meals per month and had higher hair THg levels than Caucasian women (Traynor et al., 2013). A similar trend was observed in a South Carolina study of 258 fishermen along the Savannah River, where African Americans reported consuming more fish meals per month and larger portion sizes relative to Caucasians (Burger et al., 1999). It has also been reported that African Americans in South Carolina, particularly the Gullah/Geechee communities along the coast, continue to depend on subsistence fishing, which is an important part of the heritage and culture of the Gullah/Geechee people (n=136) (Ellis et al., 2014).

In our current study, the decline in blood MeHg was not attributed to any maternal characteristics, including race/ethnicity, education level, and age, which suggested that fish consumption guidelines provided to pregnant women at the first prenatal visit were potentially successful in encouraging safer fish consumption habits among women of all sociodemographic levels. Future research should consider whether serum fatty acids also changed during pregnancy, because fish consumption guidelines (including those administered at MUSC) are designed to both minimize MeHg and maximize the benefits of fish consumption, i.e., co-consumption of omega-3 fatty acids.

There were some limitations of this study, which are worth noting. First, information concerning fish/shellfish consumption was limited. Only 47 mothers (60%) completed the FFQ, which included a greater proportion of women with a higher education level (≥some college) compared to those who did not complete the FFQ. However, frequency of fish/shellfish consumption did not differ by education level in our cohort. Whether mothers completed the FFQ also differed by race/ethnicity, where 41% of Hispanics, 53% of African Americans, and 77% of Caucasians provided a FFQ. On

average, African American mothers ate fish/shellfish most frequently, while Hispanic mothers consumed fish/shellfish less frequently than African American and Caucasian mothers. Results for fish/shellfish consumption may have been biased due to the relatively low response rates among African American and Hispanic mothers. However, it is uncertain whether potential bias would result in either an underestimation or overestimation of overall fish/shellfish consumption frequency, because African Americans were the most frequent fish/shellfish consumers, and Hispanics ate the least fish/shellfish. Additionally, mothers were only asked about fish/shellfish consumption one time during the study, and it is unknown whether mothers changed fish/shellfish consumption frequency and/or chose healthier seafood options during pregnancy.

Despite the limitations, a notable strength of this study is that both THg and MeHg were analyzed in maternal blood. Often Hg is not speciated when assessing prenatal MeHg exposure (e.g., Basu et al., 2014). Had we not measured MeHg, we would have concluded no change in blood Hg, and hence no change in MeHg exposure to the developing fetus. Many studies, including NHANES, rely on blood THg as the main biomarker; however our results underscore the need for measurement of blood MeHg in addition to THg.

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