Maternal periconceptional seafood intake, pregnancy complications, and fetal growth

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Abstract

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Background: Seafood intake has well-known health benefits that include cardiometabolic benefits. However, associations of maternal seafood intake, particularly intake of different seafood subtypes, with course and outcomes of pregnancy have not been fully described. Findings have been inconsistent and the role of seafood-related healthy nutrients (e.g. n-3 long chain polyunsaturated fatty acids, eicosapentaenoic acid [EPA; 20:5n-3] and docosahexaenoic acid [DHA; 22:6n-3]) and adverse contaminants (e.g. mercury and cadmium, Cd) in these associations is largely unknown. Further, a significant gap remains in understanding of potential mechanisms underlying associations of maternal seafood intake with pregnancy complications and/or fetal growth.

Objectives: The specific aims of this dissertation project were to (1) investigate associations of maternal periconceptional seafood intake with pregnancy complications, (2) investigate associations of maternal periconceptional seafood intake with fetal growth, and (3) investigate associations of maternal Cd burden with sex-specific placental genome-wide DNA methylation.

Methods: Investigations to address specific aims #1 and #2 were conducted among pregnant women enrolled in the Omega study, a prospective cohort study of risk factors for pregnancy complications among Pacific Northwest residents (1996-2008). A food frequency questionnaire administered at 16 weeks gestation, on average, was used to assess intake of shell-, lean-, and fatty-fish, EPA and DHA.

Seafood intake was categorized into frequencies of: <0.5 ounces/month (oz/mo), 0.5 oz/mo-<1.5 oz/week (oz/wk), 1.5-3 oz/wk, and >3 oz/wk. Blood samples were analyzed for erythrocyte membrane fatty acids, including EPA and DHA, among a randomly selected subset (60%) of initial Omega study participants (enrolled from 1996-2000, N = 586). Using medical records, we ascertained diagnoses of preeclampsia (PE), pregnancy induced hypertension (PIH), gestational diabetes (GDM), and preterm birth (PTB) as well as fetal growth indices: birth weight (continuous, low birth weight [<2,500 g], or macrosomia [≥4,000 g]), birth length, ponderal index, and head circumference. We fit generalized linear models with a log link, Poisson family, and robust standard errors to estimate relative risks (RRs) and 95% confidence intervals (CIs) for pregnancy complications (PE, PIH, GDM, and PTB) and abnormal fetal growth indicators (low birth weight or macrosomia) across seafood intake categories. We used linear regression models to estimate mean differences and 95% CIs for continuous fetal growth indices across seafood intake categories. Investigations to address specific aim #3 were conducted among women who delivered at Swedish Medical Center and provided placental samples (N = 24). Placental Cd was quantified by inductively coupled plasma mass spectrometry. Placental genome-wide DNA methylation was profiled using the Infinium HumanMethylation 450 BeadChip. We used infant sex stratified ANOVA models to examine associations of Cd high/low status with methylation (at each CpG site or genomic region).

Results: Median (interquartile range) of shell-, lean-, and fatty-fish intake was 0.8 (0-2.8), 1.4 (0-2.9), and 1.5 (0.4-3.0) oz/wk, respectively. Lean fish intake of >3 oz/wk (versus <0.5 oz/mo) was associated with a higher risk of PTB (RR= 1.55, 95% CI: 1.04-2.30). In addition, lean fish intake of >3 oz/wk (versus <0.5 oz/mo) was associated with a 2.2-fold higher risk of low birth weight (RR= 2.23, 95% CI: 1.21-4.09). Shellfish intake of >3 oz/wk (versus <0.5 oz/mo) was associated with a higher mean ponderal index (0.64 kg/m^3 higher, 95% CI: 0.04-1.25 kg/m^3). There was no evidence for associations of shell-, lean-, or fatty-fish intake with other pregnancy complications or other fetal growth indices. Intake of total seafood (all subtypes combined), dietary EPA+DHA, or maternal erythrocyte EPA+DHA was not associated with pregnancy complications or fetal growth indices. Medians of placental Cd among female and male infants were 5 ng/g and 2 ng/g, respectively. Among female infants, high

placental Cd (≥5 ng/g) was associated with hypomethylation of three CpG sites (near *ARL9*, *SIAH3*, and *HS3ST4*) and one genomic region on chromosome 7 (included genes *CROT* and *TP53TG1*) (FDR adjusted p-value <0.10). Among male infants, high placental Cd (≥2 ng/g) was associated with differential methylation of three CpG sites, two (hypomethylated) near *MECOM* and one (hypermethylated) near *SALL1*, and two genomic regions (hypomethylated), one on chromosome 3 (included *MECOM* gene) and one on chromosome 8 (included *ARHGEF10* gene) (FDR adjusted p-value <0.10).

Conclusion: Our results suggest that associations of seafood intake with pregnancy complications, and fetal growth may vary by seafood subtype. Specifically, higher lean-fish intake was associated with higher risk of PTB and higher risk of low birth weight. Further, higher shellfish intake was associated with a higher mean ponderal index. Our pilot study provides suggestive evidence for sex-specific associations of placental Cd with differential placental DNA methylation. Replication efforts and mechanistic investigations are potential future areas of research. Such investigations can inform preventative activities to improve course and outcomes of pregnancy.

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Dedication

To my mother, for her sacrificial love and persevering faith

Introduction

Pregnancy complications and fetal growth are related to maternal and offspring long-term health, including cardiometabolic health[1-5]. Investigations of modifiable risk factors, such as dietary risk factors, that contribute to pregnancy complications and suboptimal fetal growth can help identify preventive/therapeutic targets to improve course and outcomes of pregnancy. Nutrients in seafood, such as n-3 long chain polyunsaturated fatty acids (LCPUFAs) eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), promote cardiometabolic and vascular health[6,7]. On the other hand, xenobiotics such as methylmercury (MeHg) and cadmium (Cd) can accumulate in some sources of seafood and may contribute to adverse cardiometabolic outcomes[8-11]. Available evidence supports the cardiometabolic health benefits of seafood intake, especially from fatty fish intake, among men and non-pregnant women[7]. However, associations of maternal seafood intake (including intake of different seafood subtypes) with pregnancy complications and fetal growth have not been fully described. Further, potential mechanisms and modifiers (including infant sex) of associations are under investigated.

Seafood, especially fatty or oily fish, is a major food source of EPA and DHA[7]. EPA and DHA intake may be related to lower risk of pregnancy complications and optimal fetal growth through several hypothesized mechanisms (see Figure 1.0). Consumption of marine n-3 LCPUFAs may lead to a more favorable eicosanoid profile that reduces vasoconstriction, platelet aggregation, cervical ripening, oxidative stress, and inflammation, while increasing uterine and vascular tone, and uteroplacental blood flow[6,12-18]. In addition EPA+DHA may decrease de novo lipogenesis (leading to decreases in plasma triglycerides[6,19]), improve insulin-glucose homeostasis[6,18], and reduce leptin synthesis[20]. Also, LCPUFAs form 10-15% per volume of the cell membrane lipid bilayer and are critical to normal cell membrane development and function[21]. Fetal n-3 LCPUFA status is heavily dependent upon maternal dietary n-3 LCPUFAs, which are actively transported across the placenta[21]. Dietary DHA requirements increase over the course of pregnancy to support fetal growth and development, particularly of the brain and retina[21,22]. On the other hand, heavy metals such as Cd that are present in seafood, especially shellfish[11], can lead to disruption of placental transfer of zinc[23-26], an important

micronutrient, endocrine dysfunction[27-31], increased oxidative stress[32,33], and vascular damage[33], potentially leading to harmful consequences for the pregnancy and the developing fetus.

The specific aims of this dissertation were to investigate (1) associations of maternal periconceptional seafood intake with pregnancy complications (preeclampsia [PE], pregnancy induced hypertension (PIH), gestational diabetes [GDM], or preterm birth [PTB]), (2) associations of maternal periconceptional seafood intake with fetal growth indices (birth weight [continuous, low birth weight or macrosomia], birth length, ponderal index, and head circumference), and (3) sex-specific associations of placental Cd with placental genome-wide DNA methylation. The study to address specific aims #1 and #2 was conducted among Omega study participants, a prospective cohort study of risk factors for pregnancy complications among residents of the Pacific Northwest (1996-2008). Participants were recruited from women attending prenatal care clinics affiliated with Swedish Medical Center and Tacoma General Hospital. The study to address specific aim #3 was conducted among participants of the Placental MicroArray Study that included women who delivered at Swedish Medical Center[34,35]. A complete description of the approaches used to address each of the study aims of this dissertation, study findings, and related discussion is provided in subsequent chapters: chapter 1 for investigations of associations of maternal periconceptional seafood with pregnancy complications, chapter 2 for investigations of associations of maternal periconceptional seafood intake with fetal growth indices, and chapter 3 for a cross-sectional pilot study of infant sex-specific associations of placental Cd with placental genome-wide DNA methylation. In the last section we provide a summary of the main findings from chapters 1-3, overall implications, and directions for future research.

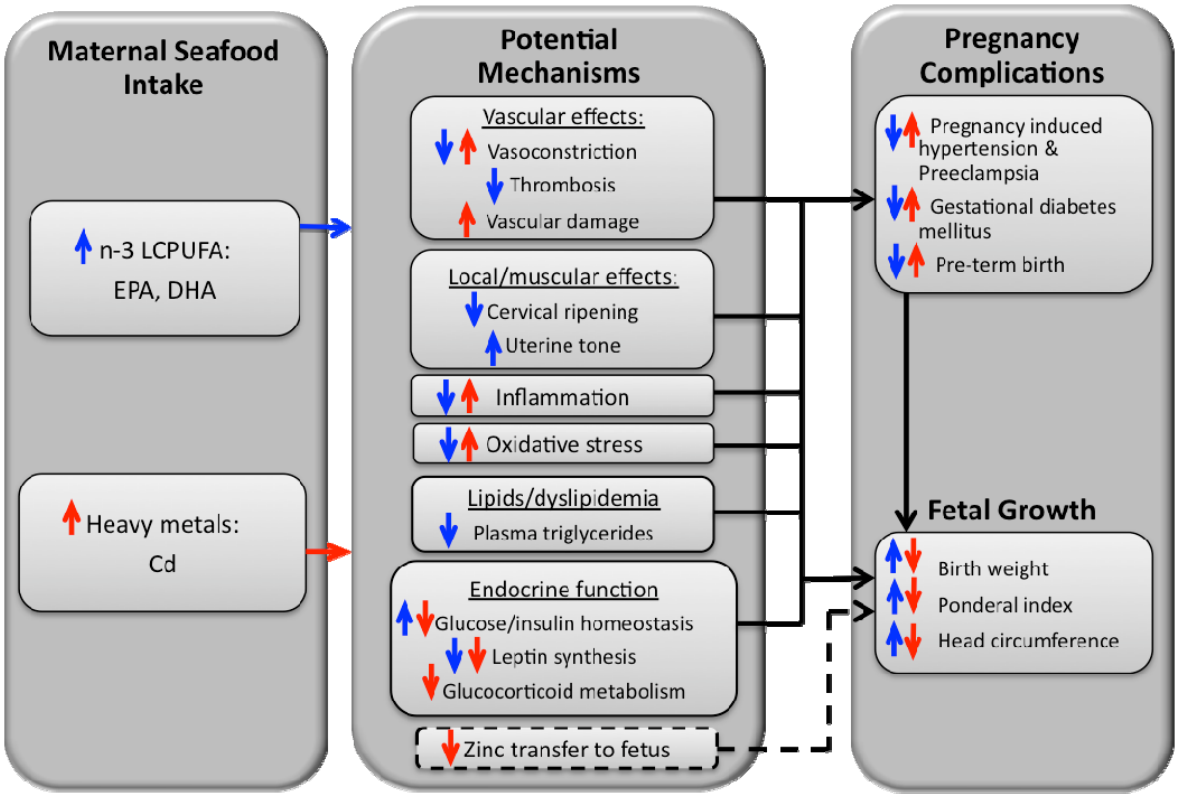


Figure 1.0 Conceptual Model: Maternal periconceptional seafood intake, pregnancy complications, and fetal growth

Chapter 1: Maternal periconceptional seafood intake and pregnancy complications

ABSTRACT

Background: While cardiometabolic benefits of seafood intake have been well-demonstrated, findings from previous studies of seafood intake and pregnancy complications have been inconsistent. Further, little is known whether associations differ by seafood subtype.

Methods: We investigated associations of maternal seafood intake with risk of pregnancy complications among participants (N =3,279) of the Omega study, a cohort study of risk factors for pregnancy complications among Pacific Northwest residents (1996-2008). A food frequency questionnaire was used to assess periconceptional intake of shell-, lean-, and fatty-fish, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Seafood intake was categorized into frequencies of: <0.5 ounces/month (oz/mo), 0.5 oz/mo-<1.5 oz/week (oz/wk), 1.5-3 oz/wk, and >3 oz/wk. We ascertained diagnoses of pregnancy induced hypertension (PIH), preeclampsia (PE), gestational diabetes (GDM), and preterm birth (PTB) using medical records. We also constructed a composite variable to indicate presence of any of these complications. We used generalized linear models with a log link, Poisson family, and robust standard errors, to estimate relative risks (RRs) and 95% confidence intervals (CIs) across seafood intake categories.

Results: Median (interquartile range) of shell-, lean-, and fatty-fish intake was 0.8 (0-2.8), 1.4 (0-2.9), and 1.5 (0.4-3.0) oz/wk, respectively. Women with intake of >3 oz/wk (versus <0.5 oz/mo) of lean fish had a 1.55-fold higher risk of PTB (RR= 1.55, 95% CI 1.04-2.30). RRs and 95% CIs for PTB corresponding to intake of 0.5 oz/mo-<1.5 oz/wk, 1.5-3.0 oz/wk, and >3 oz/wk of fatty fish versus intake of <0.5 oz/wk were 1.17 (0.83-1.66), 1.03 (0.72-1.48), and 0.74 (0.49-1.13), respectively (linear trend p-value = 0.1). Intake of fatty- and lean-fish was not associated with PE, PIH, GDM, or the composite of pregnancy complications. We did not observe associations across higher intakes of other seafood subtypes, total seafood, or EPA+DHA with pregnancy complications.

Conclusion: Our findings suggest that the risk of PTB may vary by subtype of seafood intake. Future replication studies and studies that consider potential mechanisms (including seafood preparation and nutrient/xenobiotic content) are warranted. If findings are replicated, they may have significance in the prevention of PTB, a common pregnancy complication.

BACKGROUND

Pregnancy complications, including preeclampsia (PE), pregnancy induced hypertension (PIH), gestational diabetes (GDM), and preterm birth (PTB), complicate 15-20% of pregnancies and are associated with maternal and infant cardiometabolic morbidity and mortality later in life[1-3,36-39]. These pregnancy complications co-occur and share many risk factors, including dietary and lifestyle risk factors[40-46]. While cardiometabolic benefits of seafood intake, especially from fatty or dark meat fish, have been demonstrated among men and non-pregnant women[6,7,47], results from investigations of seafood intake and course and outcomes of pregnancy have been inconsistent[48-63].

A number of randomized clinical trials and observational studies have investigated whether higher intakes of seafood or long chain n-3 polyunsaturated fatty acids (n-3 LCPUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from seafood are associated with lower risk of PE, PIH, GDM, and PTB[15,17,19,48-69]. In general, results from randomized clinical trials have suggested that higher intake of EPA and DHA (through supplementation or enriched foods) in mid- to late-pregnancy is associated with a modest reduction in risk of PTB, especially early PTB (<34 weeks of gestation)[64-67] but not PIH[15,17,19], PE[64,66,68], or GDM[68]. Few observational studies of seafood intake have supported inverse or U-shaped associations of seafood intake or maternal EPA and DHA status during pregnancy with pregnancy complications (PTB[53,54,57,58], PIH[59], PE[59,60,69] and GDM[61,62]), while other investigations did not support associations[48-52,63] (see Table 1.0).

Inconsistencies across findings of clinical trials and observational studies could be explained, at least partly, by the difference in the timing (early or late pregnancy) of exposure to EPA and DHA or differences in study population characteristics. Studies that focus on early pregnancy or the periconceptional period are particularly important since pathogenesis for these pregnancy complications often begin early in pregnancy[70-73]. The extent to which EPA and DHA in seafood accounts for potential associations of higher seafood intake with lower risk of pregnancy complications is not known. Investigations of cardiovascular disease in the general population provide evidence that the effects of seafood intake can be heterogeneous depending on several factors including the seafood

species and how the seafood is prepared[7,47,74]. However, little is known about the potential for varied effects of different types of seafood intake on pregnancy complications.

Our primary objective was to investigate the effect of seafood intake during the periconceptional period on the risk of pregnancy complications PE, PIH, GDM and PTB. We hypothesized that habitual higher intake of seafood is associated with lower risk of these pregnancy complications. We also hypothesized that the association between seafood intake and pregnancy complications varies by the type of seafood consumed.

METHODS

Overview and Study Setting

The Omega study is a prospective cohort study designed to examine dietary and metabolic risk factors associated with adverse pregnancy outcomes. Participants were recruited from women attending prenatal care clinics affiliated with Swedish Medical Center and Tacoma General Hospital in Seattle and Tacoma, Washington, respectively[19,75,76]. Eligible participants were pregnant women who initiated prenatal care and enrolled at or before 20 weeks gestation, were 18 years of age or older, were able to speak and read English, planned to carry the pregnancy to term and to deliver at either of the two hospitals. Participants were enrolled in this study from 1996-2008. The protocol for the study was approved by the Institutional Review Boards of Tacoma General Hospital and Swedish Medical Center. All study participants provided informed consent.

Study Population

During the study period 5,063 eligible women were approached and 4,000 (79%) women consented to participate and 3892 (97%) completed study follow-up. We excluded 118 multifetal pregnancies, 72 subjects with implausible total daily energy intake of <500 or >3500 kcal. After excluding these subjects, we additionally excluded 423 participants who were missing seafood intake information or had implausible seafood intake. A total of 3,279 participants remained for analyses.

Data Collection

At or near enrollment (16 weeks gestation on average), trained interviewers conducted in-person interviews (45-60 minutes in length) to collect data on socio-demographic characteristics, reproductive and medical histories, height and pre-pregnancy weight, recreational physical activity, alcohol and tobacco consumption, environmental tobacco smoke exposure, medication use, and family medical history. Participants completed a self-administered, validated, semi-quantitative food-frequency questionnaire (FFQ) to assess diet during the periconceptional period (the three months before and the first three months of index pregnancy)[19]. The FFQ had originally been developed for and used in the Women's Health Initiative Clinical Trial[77]. FFQs were analyzed using food composition values obtained from the University of Minnesota Nutrition Coding Center nutrient database[78]. Participants also completed a supplementary Seafood Intake Scale (SIS) FFQ[79]. The SIS FFQ included questions regarding usual intake frequency and serving size in the prior three-month period for 25 different types of finfish and 10 types of shellfish available in the Pacific Northwest (see Appendix Table A1.0). Participants also provided non-fasting peripheral blood samples at this initial visit. Blood samples were analyzed for erythrocyte membrane fatty acids, including EPA and DHA, among a randomly selected subset (60%) of initial participants (enrolled from 1996-2000 in the Omega study, N = 586)[19]. After delivery, trained personnel conducted maternal and infant medical records abstraction to ascertain course and outcomes of pregnancy.

Exposure Characterization

We calculated the amount of monthly seafood consumption for each seafood subtype using the SIS FFQ by multiplying the monthly frequency of intake by the serving size. The lowest amount of seafood that could be reported on the SIS FFQ was <0.5 ounces/month (oz/mo). Seafood was categorized into shell-, lean-, or fatty-fish groups as described in Appendix Table A1.0. Briefly, shellfish included crab, lobster, shrimp or prawns, clams, mussels, oysters, scallops, abalone, octopus, and squid. Lean fish included regular canned tuna, catfish, cod, flounder or sole, haddock, halibut, mahi mahi, snapper or rockfish, shark, imitation crab, imitation lobster, and fish sticks. Fatty fish included anchovies, herring (pickled or regular), kipper snacks, salmon (canned, fresh or smoked), sardines, albacore tuna, swordfish,

rainbow trout, smelt, and mackerel.

Using data from the US Department of Agriculture, we assigned average EPA and DHA values for each type of finfish and shellfish[80-83] and multiplied these values by the SIS FFQ reported amount and frequency for each type of seafood. We estimated overall intake of EPA+DHA by summing the values across all seafood subtypes (see Appendix Table A1.0). We defined quartiles of total EPA+DHA for individuals who consumed at least 0.5 oz/mo of seafood.

Outcome Definitions

We used published diagnostic criteria to define PIH and PE[73]. Briefly, PIH was defined as sustained blood pressure of ≥140/90 mmHg with readings measured ≥6 hours apart on or after 20 weeks gestation. PE was defined as sustained blood pressure of ≥140/90 mmHg with readings measured ≥6 hours apart on or after 20 weeks gestation with proteinuria based on urine protein concentrations of ≥30 mg/dL or 1+ reading on a urine dipstick from ≥2 urine specimens collected ≥4 hours apart. All women were screened for GDM according to the American Diabetes Association guidelines[84] between 24-28 weeks gestation using a 50 g glucose challenge test. Patients who failed the screening test (post-load glucose ≥140 mg/dL) were followed up within 1-2 weeks with a 100 g, 3-hour oral glucose tolerance test. Women were diagnosed with GDM if ≥2 of the following plasma glucose concentrations were abnormal: fasting ≥105 mg/dL; 1-h ≥190 mg/dL; 2-h ≥165 mg/dL; 3-h ≥145 mg/dL and they did not indicate a prior chronic diabetes diagnosis. PTB was defined as birth occurring before 37 completed weeks of gestation. Gestational age was estimated using the last menstrual period and ultrasound dates measured in early pregnancy, if available. Besides individual comparisons, we constructed a composite pregnancy complication variable indicating presence of any of these complications during pregnancy.

Statistical Analysis

We examined the frequency distributions of maternal characteristics across categories of seafood subtype intake and quartiles of EPA+DHA intake. For total seafood and seafood subtypes we chose categories of <0.5 oz/mo, 0.5 oz/mo-<1.5 oz/wk, 1.5-3 oz/wk, and >3 oz/wk, to allow for

approximately equal frequencies of subjects across seafood subtype categories above the reference (<0.5 oz/mo). According to the American Heart Association a typical single seafood serving is approximately 3 oz[85].

To estimate relative risks (RRs) and 95% confidence intervals (95% CIs) for each pregnancy complication and the composite pregnancy complication, associated with each category of seafood or quartile of EPA+DHA intake relative to the reference group, we fit generalized linear models with a log link, Poisson family (a "Log-Poisson" regression model), and robust standard errors. The Log-Poisson regression model with robust standard errors allows estimation of RRs for prospective studies with binary outcome data[86]. In these models, each non-reference category of seafood intake or EPA+DHA quartile was modeled as an indicator variable. Model fit was assessed by examination of regression residual diagnostics. Based on prior literature that suggests potential linear trends across higher seafood intake categories and EPA+DHA levels[50,87-89], we calculated Wald p-values for grouped linear terms of either seafood (or subtype) or EPA+DHA (i.e. β_1, see Example Equation A1.1 in Appendix Table A3.0b). All p-values were 2-sided and defined to be significant at $p<0.05$.

The following variables were identified *a priori* and considered as potential confounding variables and were included in all adjusted models including our primary adjusted model (Model 1.1). Non-Hispanic white race/ethnicity, high school or less education, unmarried marital status, nulliparity, habitual recreational physical activity during index pregnancy based on activity in the week prior to interview, alcohol intake, and cigarette smoking during index pregnancy were included as binary variables. We defined 4 categories of pre-pregnancy Body Mass Index (BMI), based on the National Institute of Health current definitions of underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2)[90], categories for normal weight and higher were included as indicator variables in all adjusted models. Maternal age (years), total energy consumed (kcal/day), and intake of red and processed meats (servings/day) were included in all adjusted models as continuous variables. Adjusted models for seafood subtypes as the exposure included non-referent indicator variables for intake of shell-, lean-, and fatty-fish simultaneously. For example, to observe

associations of shellfish with pregnancy complications, independent of fatty- or lean-fish, we included non-referent indicator variables for all three seafood subtypes (see Example Equation A1.2 in Appendix Table A3.0b). We additionally adjusted for enrollment year (Model 1.2) to examine whether our main findings were sensitive to potential confounding by the year the subject was enrolled. We defined 5 non-referent (1996-1997 was the referent) indicator variables for enrollment year: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007. Model 1.3 was used to examine whether associations of seafood/EPA+DHA intake and pregnancy complications were mediated by gestational weight gain (kg, modeled as a continuous variable). Model 1.4 was used to examine whether our results were materially altered by restrictions to nulliparous women, since they are at higher risk of having a pregnancy complicated by PE[73]. Finally, Model 1.5 was used to examine whether our results were altered upon restriction to women who did not indicate use of fish oil supplements. A detailed description of the variables included in each of the models and how they were parameterized is given in Appendix Table A3.0a. Analyses were carried out using Stata Software (version 10.1; StataCorp LP, College Station, TX).

RESULTS

Selected characteristics of women included in our analyses are described in Table 1.1a. The majority of our study participants were non-Hispanic white (88%), married (90%), nulliparous (61%), and had higher educational attainment (97% post-high school education). A minority of subjects smoked (6%) or consumed alcohol (26%) during the index pregnancy and very few indicated taking fish oil supplements (2%). Mean (standard deviation, SD) maternal age was 32.7 (4.4) years while mean (SD) of pre-pregnancy BMI was 23.5 (4.8) kg/m^2. Mean (SD) and median total seafood intake was 6.1 (5.7) and 4.8 oz/wk, respectively, and the interquartile range (IQR) was from 2.1 to 8.4 oz/wk. Most women (87%) consumed less than 12 oz/wk of seafood, consistent with current Environmental Protection Agency and the Food and Drug Administration recommendations[85]. There were 89 (3%) cases of PE, 375 (12%) cases of PIH, 160 (5%) cases of GDM and 259 (8%) cases of PTB. There were 800 (26%) subjects who had at least one pregnancy complication.

There were some differences in the subjects who were excluded due to missing seafood intake information or implausible seafood/energy intake (N = 495) versus those who were included in this study. Compared to included subjects, excluded subjects were less likely to be non-Hispanic white (73% versus 88%), nulliparous (39% versus 61%), married (59% versus 90%), and have higher mean (SD) pre-pregnancy BMI (25.1 (6.4) kg/m^2 versus 23.5 (4.8) kg/m^2) and occurrence of PTB (12% versus 8%).

Spearman rank correlations of maternal erythrocyte membrane EPA+DHA (expressed as %/total fatty acids) with weekly oz of seafood (all subtypes combined), shellfish, lean fish, and fatty fish were $r = 0.43$, $p<0.001$; $r = 0.34$, $p<0.001$; $r = 0.20$, $p<0.001$; $r = 0.46$, $p<0.001$, respectively. These are in agreement with another pregnancy study (N = 701) that compared erythrocyte EPA+DHA collected at 16-21 weeks gestation to frequency of total fish (lean and fatty fish) ($r = 0.22$, $p<0.01$)[57]. Among participants who had information on both erythrocyte EPA+DHA and dietary fat (N = 547), the correlation for total dietary fat (g) adjusted dietary EPA+DHA with erythrocyte membrane EPA+DHA (%/total fatty acids) was $r = 0.53$, $p<0.001$.

Tables 1.1b-e describe selected maternal characteristics across categories of seafood intake. Higher intake of seafood, regardless of type, was associated with non-white race (except for total seafood and fatty fish), post high school education (except for lean fish), and alcohol consumption (except for total seafood) during pregnancy. Women who either consumed more total seafood or fatty fish were more likely to be married and less likely to be overweight or obese pre-pregnancy. Women who consumed more total seafood gained less weight during pregnancy. Women who consumed more shellfish were less likely to be nulliparous. Women who enrolled in earlier study years consumed higher lean fish. Women who consumed higher fatty fish intake were less likely to smoke during pregnancy and were more likely to take fish oil supplements, engage in recreational physical activity during early pregnancy and had later gestational ages at delivery. Higher intake of total seafood or seafood subtypes corresponded to higher dietary EPA+DHA, total energy, fat, and red and processed meat intake, and erythrocyte EPA+DHA.

Tables 1.2a-e and Figures 1.1-1.3, display relative risks for PE, PIH, GDM, PTB and composite pregnancy complication associated with total seafood intake, EPA+DHA intake, and intake of seafood subtypes. We did not observe evidence for associations of higher total seafood (all subtypes combined), shellfish or marine derived EPA+DHA intake across non-referent categories or quartiles for any of the pregnancy complications, including the composite pregnancy complication, in our primary adjusted models (Tables 1.2a-c, Model 1.1). On the other hand, pregnant women who consumed >3 oz/wk of lean fish had a 1.55-fold higher risk of PTB compared with pregnant women who consumed <0.5 oz/mo of lean fish (RR = 1.55 95% CI: 1.04-2.30) (Table 1.2d, Model 1.1 and Figure 1.2). Relative risks (95% CI) for PTB corresponding to intake of 0.5 oz/mo-<1.5 oz/wk, 1.5-3.0 oz/wk, >3 oz/wk of fatty fish compared with intake of <0.5 oz/mo of fatty fish were 1.17 (0.83-1.66), 1.03 (0.72-1.48), and 0.74 (0.49-1.13), respectively (linear trend p-value = 0.1). However, among women who consumed at least 0.5 oz/mo fatty fish, higher intake of fatty fish was associated with lower risk of PTB (linear trend p-value = 0.02) (Table 1.2e, Model 1.1 and Figure 1.3). Lean and fatty fish were not associated with PE, PIH, or GDM in our adjusted models (Tables 1.2d and e). Also, lean and fatty fish intakes were not associated with the composite pregnancy complication outcome in our adjusted models (Tables 1.2d and e).

Except for the association of total seafood with PTB, our main findings were not materially altered by further adjustment for study year (Tables 1.2a-e, Model 1.2), gestational weight gain (Tables 1.3a-e, Model 1.3), restriction to nulliparous pregnancies (Tables 1.3a-e, Model 1.4), or exclusion of women who took omega 3 fish oil supplements (Tables 1.3a-e, Model 1.5). In sensitivity analyses, when we excluded women who indicated that they took omega 3 fish oil supplements, all categories of seafood intake above the reference category of <0.5 oz/mo were associated with higher risk of PTB (Table 1.3a, Model 1.5). RRs and (95% CIs) for total seafood intake of 0.5/mo-<1.5/wk, 1.5-3/wk, and >3/wk oz compared to intake of <0.5 oz/mo were 2.26 (1.20-4.26), 1.88 (1.02-3.45), and 1.76 (1.00-3.09) (linear trend across higher intake categories p-value = 0.37).

We explored model fit using Deviance Residuals and Cooks Distance and identified several potential outlying and influential points. Further examination of these observations did not suggest erroneous or

implausible characteristics for these subjects and our main findings were robust to the exclusion of these observations.

DISCUSSION

Our study suggests that associations of seafood intake with pregnancy complications may vary by seafood subtype. The evidence for this variation was strongest for PTB where we observed an association of higher lean-fish intake with higher risk of PTB and possible association of higher fatty fish intake with lower risk of PTB.

A previous study examined the potential for varied effects by different seafood subtypes and pregnancy complications[51]. The study by Guldner *et al.* investigated associations of maternal salt-water fish (other than shellfish) and shellfish intake with PTB among 2,398 pregnant women from Brittany, France. The investigators did not find strong evidence that either type of seafood was associated with PTB (the p-values for the linear trends were 0.30 for both types of seafood). However, the investigators did report evidence for an association of gestational length with seafood intake. Each additional monthly meal of fish (fatty or lean) was associated with a 0.02 wk increase in gestational length 95% CI (0.002-0.035). Guldner *et al.* did not further characterize fish intake into categories of lean or fatty fish intake. As a secondary analysis, we also examined associations of seafood subtypes and mean differences in gestational length (Table 1.3f). There was suggestive evidence, though not statistically significant, that higher intake of shell- and fatty-fish was associated with a longer gestational length. Compared to women who consumed <0.5 oz/mo of shellfish, intake of 1.5-3 oz/wk of shellfish was associated with a 0.16 wk longer mean gestational length 95% CI: -0.03, 0.35 wk. Also, compared to women with intake of <0.5 oz/mo of fatty fish compared to intake of >3 oz/wk of fatty fish was associated with a 0.16 wk longer mean gestational length 95% CI: -0.06, 0.38 wk.

Several studies among men and non-pregnant women provide evidence that the effects of dietary seafood can be heterogeneous[7,47,74]. Mozaffarian *et al.* reported that modest consumption of tuna or other broiled or baked fish, which tend to include fatty fish species (e.g. salmon or trout) that are

higher in EPA+DHA levels, was associated with a lower risk of ischemic heart disease (IHD) death[47]. Intake of fried fish/fish sandwich, which tend to include lean fish species (e.g. cod or halibut) and are lower in EPA+DHA levels, was associated with a higher risk of IHD death[47]. We did not have information on how the different seafood subtypes were prepared and were unable to further examine this potential mechanism. Xue, *et al*. reported higher maternal MeHg levels were associated with higher risk of very preterm birth (birth <35 weeks gestation)[91]. Lean fish included shark in our definitions, which is a predatory fish that the Environmental Protection Agency and the Food and Drug Administration advisory recommends pregnant women to avoid consuming due to higher levels of MeHg[85]. On the other hand, our definition of fatty fish included mackerel and swordfish, also predatory fish that the advisory recommends avoiding, and fatty fish intake was not associated with higher risk of PTB. We were unable to determine the level of MeHg exposure (or exposure to other contaminants e.g. PCBs) due to seafood intake. In sum, were unable to determine whether the association between higher intake of lean fish and higher risk of PTB in our study could be explained by how the fish was prepared, levels of MeHg or other contaminants, or whether the association could be attributed to residual confounding.

Previous observational studies of seafood intake with pregnancy complications have mainly focused on total seafood intake or dietary EPA and DHA. Studies have most consistently reported an inverse[53,54,58] or a U-shape[57] association between seafood intake or EPA and DHA in early or mid-pregnancy and PTB. Contrary to the literature, our study did not find an inverse or U-shape association between total seafood or EPA+DHA with PTB. In our sensitivity analyses we did observe an association of higher total seafood intake and higher risk of PTB among women who indicated that they did not consume fish oil supplements (Table 1.3a, Model 1.5). The association of higher total seafood and higher risk of PTB among fish oil non-consumers may have been driven by the association of higher lean fish intake and higher risk of PTB, which was evident among both fish oil non-consumers and consumers (Table 1.3d, Models 1.1 and 1.5). Given observed seafood subtype-specific differences in associations, assessment of total seafood intake with PTB risk may not be an ideal way to describe the associations.

Observational studies of maternal seafood intake or EPA+DHA status with risk of PE, PIH, and GDM have been less consistent; some studies have reported direct[92], inverse[60,61] or U-shape[59] associations and others have reported no associations[48,49,56]. Our study does not provide strong and/or consistent evidence for an association between total seafood intake, seafood subtypes, or EPA+DHA and risk of PE, PIH, or GDM.

Our findings should be interpreted in view of several potential limitations. First, we cannot rule out the potential for exposure measurement error. This may partly account for the differential findings from our study versus those from clinical trials. Calculated exposure to EPA+DHA from self-reported seafood intake is likely to be less precise than studies of EPA and/or DHA supplementation. Most clinical trials support an inverse association between n-3 LCPUFA, especially DHA, and PTB[64,66]. While our results suggest an association of higher fatty fish intake with lower risk of PTB, which have higher levels of EPA+DHA, we did not observe evidence for higher dietary EPA+DHA and lower PTB risk. Since seafood intake was evaluated prospectively, we suspect that the potential exposure measurement error for seafood intake and EPA+DHA to have been non-differential with respect to pregnancy complications and may have led to estimates that were attenuated toward the null. Second, our results should be interpreted with caution since we did not adjust for multiple comparisons. We evaluated associations between multiple exposures and outcomes**,** which will increase our type I error. Third, our overall study statistical power may be limited, particularly for the seafood subtypes and rare pregnancy complication analyses. For example, there was some evidence, though not statistically significant, of an association of higher fatty fish intake with lower risk of PE (Table 1.2e, Model 1.1). However, given the limited number of PE cases (N = 89) our power to detect a relative risk of 0.60 (α = 0.05, two sided) was approximately 40%. Fourth, although we modeled seafood intake categorically to allow for some flexibility to observe non-linear associations, we did not statistically examine threshold effects. Threshold effects have been observed for modest seafood intake (or up to approximately 250-500 mg/d EPA+DHA) and cardiovascular outcomes in men and non-pregnant women[7]. Our analyses findings, however do not suggest a threshold effect. Fifth, although our study examined the potential for varying effects of different types of seafood on pregnancy complications, we did not

consider the role of other factors, such as exposure to MeHg or how the seafood was prepared, in the relationships. Exposure to MeHg or other xenobiotics, often found in longer-living predatory fish species, have been suggested to potentially counteract the beneficial effects of n-3 LCPUFAs and be associated with greater risk of pregnancy complications[91]. In addition, some methods of seafood preparation, such as frying, can lead to oxidation and lower levels of n-3 LCPUFAs[74]. The association we observed between higher risk of PTB and higher intake of lean fish, which is typically prepared as fried fish, may result from this. Sixth, although we adjusted for multiple confounding variables, we cannot exclude the possibility of residual confounding due to unmeasured or imprecisely measured confounding factors. Seventh, there is a potential for selection bias since those who were missing seafood intake information, or who had indicated implausible seafood/energy intake and therefore excluded from our study, differed from included subjects on factors that were related both to the exposure (e.g. race, marital status, parity) and the outcome (e.g. PTB). Finally, our study included participants who were mostly non-Hispanic white, married, highly educated, with normal pre-pregnancy BMI, and who registered for prenatal care early in pregnancy and participated in regular annual medical examinations. Therefore, our results may not be generalizable to women of different race/ethnicity or socioeconomic status, or pre-pregnancy BMI.

Seafood intake, especially fatty fish intake, as a major dietary source of EPA+DHA, may have important implications on the course of pregnancy. It is plausible that EPA+DHA intake is related to lower risk of PTB through (1) alterations in eicosanoid profiles and (2) through hypotriglyceridemic effects. Diets rich in n-3 LCPUFAs favor production of prostaglandins, such as PGI_2 and PGI_3, which inhibit myometrial contractility and promote uterine and vascular tone[13,15]. Intake of n-3 LCPUFAs also tends to lower production of eicosanoids that increase platelet aggregation and vasoconstriction (e.g. TXB_2) as well as eicosanoids that stimulate cervical ripening and uterine contractions (e.g. $PGF_{2\alpha}$ and PGE_2)[6,13,16]. A previous investigation conducted among a subset of the subjects from the Omega cohort, reported results that support that higher seafood intake and maternal erythrocyte EPA+DHA are associated with lower plasma triglycerides[19] and elevated maternal plasma triglycerides have been associated with PTB[93]. Although lean fish are sources of n-3 LCPUFAs, since the levels of n-3 LCPUFAs tend to be lower

in these species, their beneficial effects may be offset by contaminants, such as MeHg, or by the method in which they are prepared. Often lean fish are consumed as fried fish whereas fatty fish tend to be prepared for consumption using other methods, such as baking, broiling, or poaching[74]. Besides, introducing less healthy fats such as trans-fats that may be associated with PTB, frying fish lowers n-3 LCPUFA levels. The lack of evidence for an association with dietary EPA+DHA may be explained, at least in part, by the heterogeneity of seafood sources contributing to dietary EPA+DHA. It may also be that there are some other constituents of fatty fish (e.g. Vitamin D, selenium) that lowers the risk of PTB[94]. Additional research is needed to confirm our findings of seafood subtype-specific differences in risk of PTB and to further elucidate potential biologic mechanisms.

Our study has several strengths. First, it is one of the few studies of maternal periconceptional seafood intake and EPA+DHA. Pathogenesis of the pregnancy complications we investigated often begins early in pregnancy. Our study offers insight to effects of seafood intake during this critical period of time. Our study included detailed information on seafood intake, much more detailed than traditional food frequency questionnaires, which we used to address a research gap on the potential varied effects of seafood subtypes. Access to blood samples in a subset permitted us to assess the correlation of EPA+DHA with our self-reports of seafood intake and EPA+DHA. Correlations were as high or higher than with those cited in previous studies[56]. Our cohort is well characterized and had high rates of participation and follow-up, which allowed us to control for many previously reported confounding variables.

To our knowledge this is the first investigation of the effects of maternal periconceptional consumption of shellfish, lean fish, and fatty fish separately and in combination on risk of pregnancy complications. Our study suggests that seafood intake can have varied effects on PTB depending on the type. In contrast to shellfish or lean fish, fatty fish, which tend to be higher in n-3 LCPUFAs, was associated with a lower risk of PTB. Future studies of maternal seafood consumption and pregnancy complications may consider more detailed characterizations of seafood intake, including how the seafood is prepared and levels of contaminants, such as MeHg, and nutrients, such as n-3 LCPUFAs.

Table 1.0 Selected previous investigations of maternal seafood intake or marine n-3 LCPUFA status and pregnancy complications from the literature

Author, Year	Location	Design	Exposures	Outcomes	N	Significant Associations [a]
Makrides, 2009[66]	Several	Review of 6 RCTs	Suppl. versus placebo	PE, PTB	PE: 1683 PTB: 1916	No statistically significant assoc.
Oken, 2007[49]	US	PC	FFQ: fish	PIH, PE	1718	No statistically significant assoc.
Olafsdottir, 2006[59]	Iceland	PC	FFQ: fish & suppl.	PIH/PE	488	PIH/PE: U-shape curve
Williams, 1995[60]	US	CS, CaCtrl	Maternal blood: EPA, DHA	PE	62	PE: inverse
Klebanoff, 2011[57]	US	RCT-ancillary	FFQ: fish Maternal blood: EPA & DHA	PTB	Fish: 852 EPA & DHA: 701	Fish, PTB: U-shape curve
Oken, 2004[50]	US	PC	FFQ: fish	PTB	2109	No statistically significant assoc.
Olsen, 2002[58]	Denmark	PC	FFQ: fish	PTB	8729	PTB: inverse
Bowers, 2012[48]	US	PC	FFQ: n-3 LCPUFA	GDM	13475	No statistically significant assoc.
Chen, 2010[61]	US	CaCtrl (nested in PC)	Average of 3 24-h recalls: EPA, DHA Maternal blood: EPA, DHA	GDM	227	FFQ, DHA, GDM: inverse Blood, EPA, GDM: inverse

Abbreviations: RCT, randomized controlled trial; PC, prospective cohort; CS, cross sectional; CaCtrl, case control; FFQ, food frequency questionnaire; Suppl., n-3 LCPUFA/fish oil supplement; PIH, pregnancy induced hypertension; PE, preeclampsia; PTB, preterm birth; GDM, gestational diabetes mellitus.
Footnotes: [a] Statistically significant associations (two sided p-value < 0.05).

Table 1.1a Selected characteristics of the analytic population versus excluded subjects due to missing SIS FFQ information or implausible seafood/energy intake

Maternal characteristics and pregnancy outcomes	Included (N = 3279)			Excluded, missing SIS FFQ or implausible seafood/energy intake (N = 495)		
	N		%	N		%
Non-Hispanic white race/ethnicity [a, d]	2870		87.8	348		72.7
High school or less [a, d]	111		3.4	34		6.9
Unmarried [a, d]	338		10.3	203		41.0
Nulliparous [a, d]	2012		61.4	195		39.4
Pre-pregnancy Body Mass Index (kg/m^2) [a, d]						
<18.5	138		4.2	24		4.8
18.5-<25	2337		71.3	284		57.4
25-<30	529		16.1	91		18.4
≥30.0	269		8.2	84		17.0
Smoked during pregnancy [a, d]	179		5.5	37		7.5
Any alcohol intake during pregnancy [d]	855		26.1	99		20.0
No recreational physical activity during early pregnancy [a, d]	591		18.0	92		18.6
Fish oil supplements	48		1.5	5		1.0
Family history of hypertension [a]	1613		49.2	248		50.1
Family history of diabetes [a, d]	449		13.7	105		21.2
Enrollment year [a, d]						
1996-1997	178		5.4	14		2.8
1998-1999	505		15.4	31		6.3
2000-2001	613		18.7	76		15.4
2002-2003	869		26.5	131		26.5
2004-2005	690		21.0	128		25.9
2006-2007	382		11.6	73		14.7
Preeclampsia [a]	89		2.7	18		3.6
Pregnancy induced hypertension [a, b]	375		11.4	53		10.7
Gestational diabetes [a, b]	160		4.9	27		5.5
Preterm birth [a, d]	259		7.9	59		11.9
	mean		SD	mean		SD
Maternal age (yrs) [a, d]	32.7		4.4	31.6		5.7
Pre-pregnancy Body Mass Index (kg/m^2) [a, d]	23.5		4.8	25.1		6.4
	median	interquartile range		median	interquartile range	
Gestational weight gain (kg) [a]	15.9	12.7	19.5	15.9	11.8	20.4
Gestational age at delivery (weeks) [a, d]	39	38	40	39	38	40
Weekly intake EPA+DHA (g) [a, d]	0.5	0.2	1.0	0.3	0.1	1.2
Erythrocyte EPA+DHA (%/total fatty acids) [c]	5.3	4.5	6.1	4.9	4.1	5.6
Daily intake						
Total energy (kcal) [a, d]	1641.7	1317.5	2058.0	1867.8	820.8	3915.8
Total fat (g) [a, d]	57.2	43.1	74.5	65.2	33.0	141.5
Red and processed meats (servings) [a, d]	0.6	0.3	0.9	0.6	0.2	1.5

[a] Maternal age, marital status, parity, and first degree family histories of hypertension and diabetes missing for 2 included and 6 excluded subjects. Maternal race missing for 10 included and 16 excluded subjects. Education status missing for 91 included and 136 excluded subjects. Pre-pregnancy Body Mass Index missing for 6 included and 12 excluded subjects. Smoking status missing for 100 included and 144 excluded subjects. Recreational physical activity during early pregnancy missing for 91 included and 140 excluded subjects. Enrollment year missing for 42 included and 42 excluded subjects. PE status missing for 64 included and 20 excluded subjects. GDM status missing for 37 included and 15 excluded subjects. PTB status missing for 103 included and 30 excluded subjects. Gestational weight gain missing for 54 included and 33 excluded subjects. Gestational age of delivery missing for 104 included and 30 excluded subjects. Intake of EPA+DHA missing for 423 excluded subjects. Daily energy, fat, and red and processed meat intake missing for 31 included and 378 excluded subjects

[b] PIH excludes women with history of chronic hypertension (143 among included and 29 among excluded subjects). GDM excludes women with history of pre-pregnancy diabetes (37 among included and 12 among excluded subjects).

[c] Erythrocyte EPA+DHA measured among 529 included and 40 excluded subjects.

[d] P-value <0.05 for Pearson's Chi-square test for comparisons across inclusion status for categorical or binary variables or one-way ANOVA test for differences in means across inclusion status for continuous variables.

Table 1.1b Selected characteristics of participants according to amount of **total seafood (all subtypes combined) intake**, Omega study

Maternal characteristics	Seafood intake (ounces)							
	<0.5/mo (N = 302)		0.5/mo-<1.5/wk (N = 320)		1.5-3/wk (N = 541)		>3/wk (N = 2116)	
	N	%	N	%	N	%	N	%
Non-Hispanic white race/ethnicity [a]	272	90.1	283	88.4	474	87.6	1841	87.0
High school or less [a, c]	21	7.0	19	5.9	25	4.6	46	2.2
Unmarried [c]	45	14.9	31	9.7	45	8.3	217	10.3
Nulliparous	195	64.6	179	55.9	317	58.6	1321	62.4
Pre-pregnancy Body Mass Index (kg/m^2) [a, c]								
<18.5	22	7.3	12	3.8	18	3.3	86	4.1
18.5-<25	199	65.9	219	68.4	399	73.8	1520	71.8
25-<30	55	18.2	56	17.5	76	14.0	342	16.2
≥30.0	26	8.6	32	10.0	48	8.9	163	7.7
Smoked during pregnancy [a]	24	7.9	17	5.3	28	5.2	110	5.2
Any alcohol intake during pregnancy	69	22.8	70	21.9	126	23.3	590	27.9
No recreational physical activity during early pregnancy [a]	59	19.5	65	20.3	108	20.0	359	17.0
Fish oil supplements	1	0.3	6	1.9	7	1.3	34	1.6
Family history of hypertension	140	46.4	163	50.9	265	49.0	1045	49.4
Family history of diabetes	44	14.6	39	12.2	65	12.0	301	14.2
Enrollment Year [a]								
1996-1997	17	5.6	13	4.1	25	4.6	123	5.8
1998-1999	49	16.2	48	15.0	65	12.0	343	16.2
2000-2001	49	16.2	65	20.3	102	18.9	397	18.8
2002-2003	73	24.2	79	24.7	150	27.7	567	26.8
2004-2005	67	22.2	70	21.9	131	24.2	422	19.9
2006-2007	41	13.6	36	11.2	63	11.6	242	11.4
	mean	SD	mean	SD	mean	SD	mean	SD
Maternal age (yrs) [c]	31.4	5.0	32.1	4.6	32.5	4.2	33.0	4.2
	med	(IQR)	med	(IQR)	med	(IQR)	med	(IQR)
Gestational weight gain (kg) [a, c]	16.8	(13.2-20.0)	15.9	(12.7-19.5)	15.9	(12.7-19.1)	15.9	(12.7-19.1)
Gestational age at delivery (weeks) [a]	39	(38-40)	39	(38-40)	39	(38-40)	39	(38-40)
Weekly intake EPA+DHA (g) [c]	0.0	(0.0-0.0)	0.1	(0.0-0.1)	0.3	(0.1-0.4)	0.9	(0.6-1.3)
Erythrocyte EPA+DHA (%/total fatty acids) [b, c]	4.0	(3.3-4.9)	4.6	(4.2-5.1)	4.8	(4.4-5.4)	5.5	(4.8-6.3)
Daily intake								
Total energy (kcal) [a, c]	1499.7	(1185.1-1959.7)	1471.3	(1131.3-1872.0)	1551.6	(1244.9-1911.2)	1720.3	(1388.1-2105.9)
Total fat (g) [a, c]	52.8	(39.4-68.6)	49.8	(36.2-66.0)	52.2	(39.1-69.3)	60.5	(45.7-77.7)
Red and processed meats (servings) [a, c]	0.4	(0.0-0.7)	0.5	(0.3-0.8)	0.5	(0.3-0.8)	0.6	(0.3-0.9)

[a] Missing maternal age, marital status, parity, family histories of hypertension and diabetes for 2 subjects. For each category: 1 (0.5 oz/mo-<1.5 oz/wk), 1 (1.5-3 oz/wk) were missing.
Missing race/ethnicity for 10 subjects. For each seafood category: 1 (<0.5 oz/mo), 4 (0.5 oz/mo-<1.5 oz/wk), 4 (1.5-3 oz/wk), 1 (>3 oz/wk) were missing.
Missing education information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 6 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (0.5 oz/mo-<1.5 oz/wk), 2 (1.5-3 oz/wk), 2 (>3 oz/wk) were missing.
Missing smoking information for 100 subjects. For each seafood category: 33 (<0.5 oz/mo), 16 (0.5 oz/mo-<1.5 oz/wk), 22 (1.5-3 oz/wk), 29 (>3 oz/wk) were missing.

Missing recreational physical activity information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing enrollment year for 42 subjects. For each seafood category: 19 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 8 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
Missing gestational weight gain for 54 subjects. For each seafood category: 21 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 12 (1.5-3 oz/wk), 12 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 104 subjects. For each seafood category: 44 (<0.5 oz/mo), 19 (0.5 oz/mo-<1.5 oz/wk), 24 (1.5-3 oz/wk), 17 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 31 subjects. For each seafood category: 13 (<0.5 oz/mo), 5 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
[b] Among 529 subjects with erythrocyte EPA+DHA (1996-2000). 176 (<0.5 oz/mo), 111 (0.5 oz/mo-<1.5 oz/wk), 130 (1.5-3 oz/wk), 112 (>3 oz/wk) were excluded.
[c] P-value <0.05 for Pearson's Chi-square test for comparisons across highest versus lowest seafood intake categories for categorical or binary variables or one-way ANOVA test for differences in means across highest versus lowest seafood intake categories for continuous variables.

Table 1.1c Selected characteristics of participants according to amount of **shellfish intake**, Omega study

Maternal characteristics	Shellfish intake (ounces)							
	<0.5/mo (N = 1099)		0.5/mo-<1.5/wk (N = 705)		1.5-3/wk (N = 772)		>3/wk (N = 703)	
	N	%	N	%	N	%	N	%
Non-Hispanic white race/ethnicity [a, c]	999	90.9	614	87.1	680	88.1	577	82.1
High school or less [a, c]	60	5.5	17	2.4	18	2.3	16	2.3
Unmarried	121	11.0	63	8.9	75	9.7	79	11.2
Nulliparous [c]	633	57.6	416	59.0	504	65.3	459	65.3
Pre-pregnancy Body Mass Index (kg/m^2) [a]								
<18.5	47	4.3	36	5.1	29	3.8	26	3.7
18.5-<25	764	69.5	527	74.8	556	72.0	490	69.7
25-<30	190	17.3	84	11.9	126	16.3	129	18.3
≥30.0	97	8.8	57	8.1	59	7.6	56	8.0
Smoked during pregnancy [a]	63	5.7	31	4.4	43	5.6	42	6.0
Any alcohol intake during pregnancy [c]	235	21.4	196	27.8	217	28.1	207	29.4
No recreational physical activity during early pregnancy [a]	215	19.6	134	19.0	129	16.7	113	16.1
Fish oil supplements	13	1.2	12	1.7	10	1.3	13	1.8
Family history of hypertension	522	47.5	349	49.5	396	51.3	346	49.2
Family history of diabetes	150	13.6	76	10.8	118	15.3	105	14.9
Enrollment Year [a]								
1996-1997	68	6.2	31	4.4	41	5.3	38	5.4
1998-1999	166	15.1	107	15.2	136	17.6	96	13.7
2000-2001	219	19.9	132	18.7	147	19.0	115	16.4
2002-2003	275	25.0	211	29.9	192	24.9	191	27.2
2004-2005	231	21.0	142	20.1	152	19.7	165	23.5
2006-2007	121	11.0	73	10.4	96	12.4	92	13.1
	mean	SD	mean	SD	mean	SD	mean	SD
Maternal age (yrs) [c]	32.3	4.6	32.7	4.2	32.8	4.2	33.1	4.3
	med	(IQR)	med	IQR	med	IQR	med	IQR
Gestational weight gain (kg) [a]	15.9	(12.7-19.5)	15.9	(12.7-19.1)	15.4	(12.7-19.1)	15.9	(12.7-19.5)
Gestational age at delivery (weeks) [a]	39	(38-40)	39	(38-40)	39	(38-40)	39	(38-40)
Weekly intake EPA+DHA (g) [c]	0.2	(0.0-0.5)	0.4	(0.2-0.8)	0.7	(0.4-1.1)	1.2	(0.8-1.8)
Erythrocyte EPA+DHA (%/total fatty acids) [b, c]	4.7	(4.2-5.5)	5.2	(4.4-5.9)	5.5	(4.9-6.3)	5.8	(5.0-6.5)
Daily intake								
Total energy (kcal) [a, c]	1599.3	(1265.3-2000.5)	1543.8	(1217.3-1943.1)	1660.8	(1359.4-2061.0)	1784.1	(1477.4-2197.8)
Total fat (g) [a, c]	54.5	(42.0-71.1)	54.0	(39.4-71.6)	59.8	(44.7-75.5)	63.7	(47.9-81.9)
Red and processed meats (servings) [a, c]	0.5	(0.2-0.8)	0.5	(0.3-0.8)	0.6	(0.3-0.9)	0.7	(0.4-1.0)

[a] Missing maternal age, marital status, parity, family histories of hypertension and diabetes for 2 subjects. For each category: 1 (0.5 oz/mo-<1.5 oz/wk), 1 (1.5-3 oz/wk) were missing.
Missing race/ethnicity for 10 subjects. For each seafood category: 1 (<0.5 oz/mo), 4 (0.5 oz/mo-<1.5 oz/wk), 4 (1.5-3 oz/wk), 1 (>3 oz/wk) were missing.
Missing education information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 6 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (0.5 oz/mo-<1.5 oz/wk), 2 (1.5-3 oz/wk), 2 (>3 oz/wk) were missing.
Missing smoking information for 100 subjects. For each seafood category: 33 (<0.5 oz/mo), 16 (0.5 oz/mo-<1.5 oz/wk), 22 (1.5-3 oz/wk), 29 (>3 oz/wk) were missing.

Missing recreational physical activity information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing enrollment year for 42 subjects. For each seafood category: 19 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 8 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
Missing gestational weight gain for 54 subjects. For each seafood category: 21 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 12 (1.5-3 oz/wk), 12 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 104 subjects. For each seafood category: 44 (<0.5 oz/mo), 19 (0.5 oz/mo-<1.5 oz/wk), 24 (1.5-3 oz/wk), 17 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 31 subjects. For each seafood category: 13 (<0.5 oz/mo), 5 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
[b] Among 529 subjects with erythrocyte EPA+DHA (1996-2000). 176 (<0.5 oz/mo), 111 (0.5 oz/mo-<1.5 oz/wk), 130 (1.5-3 oz/wk), 112 (>3 oz/wk) were excluded.
[c] P-value <0.05 for Pearson's Chi-square test for comparisons across highest versus lowest seafood intake categories for categorical or binary variables or one-way ANOVA test for differences in means across highest versus lowest seafood intake categories for continuous variables.

Table 1.1d Selected characteristics of participants according to amount of **lean fish intake**, Omega study

Maternal characteristics	Lean fish intake (ounces)							
	<0.5/mo (N = 835)		0.5/mo-<1.5/wk (N = 819)		1.5-3/wk (N = 857)		>3/wk (N = 768)	
	N	%	N	%	N	%	N	%
Non-Hispanic white race/ethnicity [a, c]	755	90.4	716	87.4	742	86.6	657	85.5
High school or less [a]	34	4.1	33	4.0	26	3.0	18	2.3
Unmarried	96	11.5	73	8.9	86	10.0	83	10.8
Nulliparous	529	63.4	458	55.9	544	63.5	481	62.6
Pre-pregnancy Body Mass Index (kg/m^2) [a]								
<18.5	48	5.7	32	3.9	31	3.6	27	3.5
18.5-<25	577	69.1	599	73.1	616	71.9	545	71.0
25-<30	142	17.0	130	15.9	142	16.6	115	15.0
≥30.0	68	8.1	57	7.0	67	7.8	77	10.0
Smoked during pregnancy [a]	57	6.8	38	4.6	48	5.6	36	4.7
Any alcohol intake during pregnancy [c]	192	23.0	207	25.3	234	27.3	222	28.9
No recreational physical activity during early pregnancy [a]	167	20.0	160	19.5	140	16.3	124	16.1
Fish oil supplements	9	1.1	12	1.5	15	1.8	12	1.6
Family history of hypertension	413	49.5	402	49.1	397	46.3	401	52.2
Family history of diabetes	105	12.6	99	12.1	134	15.6	111	14.5
Enrollment Year [a, c]								
1996-1997	41	4.9	34	4.2	45	5.3	58	7.6
1998-1999	123	14.7	106	12.9	150	17.5	126	16.4
2000-2001	138	16.5	152	18.6	163	19.0	160	20.8
2002-2003	222	26.6	235	28.7	220	25.7	192	25.0
2004-2005	186	22.3	186	22.7	176	20.5	142	18.5
2006-2007	113	13.5	91	11.1	96	11.2	82	10.7
	mean	SD	mean	SD	mean	SD	mean	SD
Maternal age (yrs)	32.4	4.6	32.6	4.2	32.9	4.3	32.8	4.3
	med	(IQR)	med	(IQR)	med	(IQR)	med	(IQR)
Gestational weight gain (kg) [a]	15.9	(12.7-19.5)	15.9	(12.7-19.1)	15.9	(12.7-18.6)	16.3	(12.7-19.5)
Gestational age at delivery (weeks) [a]	39	(38-40)	39	(38-40)	39	(38-40)	39	(38-40)
Weekly intake EPA+DHA (g) [c]	0.1	(0.0-0.5)	0.4	(0.2-0.8)	0.7	(0.4-1.1)	1.1	(0.7-1.6)
Erythrocyte EPA+DHA (%/total fatty acids) [b, c]	4.7	(4.0-5.8)	5.0	(4.5-5.9)	5.4	(4.7-6.1)	5.6	(4.8-6.4)
Daily intake								
Total energy (kcal) [a, c]	1567.0	(1261.8-1969.3)	1581.4	(1251.9-1989.3)	1623.7	(1290.3-2038.8)	1830.5	(1504.2-2198.3)
Total fat (g) [a, c]	53.7	(40.5-69.9)	55.2	(40.8-71.5)	56.5	(43.0-73.5)	65.0	(49.0-81.6)
Red and processed meats (servings) [a, c]	0.5	(0.2-0.8)	0.5	(0.3-0.9)	0.6	(0.3-0.9)	0.6	(0.4-1.0)

[a] Missing maternal age, marital status, parity, family histories of hypertension and diabetes for 2 subjects. For each category: 1 (0.5 oz/mo-<1.5 oz/wk), 1 (1.5-3 oz/wk) were missing.
Missing race/ethnicity for 10 subjects. For each seafood category: 1 (<0.5 oz/mo), 4 (0.5 oz/mo-<1.5 oz/wk), 4 (1.5-3 oz/wk), 1 (>3 oz/wk) were missing.
Missing education information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 6 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (0.5 oz/mo-<1.5 oz/wk), 2 (1.5-3 oz/wk), 2 (>3 oz/wk) were missing.
Missing smoking information for 100 subjects. For each seafood category: 33 (<0.5 oz/mo), 16 (0.5 oz/mo-<1.5 oz/wk), 22 (1.5-3 oz/wk), 29 (>3 oz/wk) were missing.

Missing recreational physical activity information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing enrollment year for 42 subjects. For each seafood category: 19 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 8 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
Missing gestational weight gain for 54 subjects. For each seafood category: 21 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 12 (1.5-3 oz/wk), 12 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 104 subjects. For each seafood category: 44 (<0.5 oz/mo), 19 (0.5 oz/mo-<1.5 oz/wk), 24 (1.5-3 oz/wk), 17 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 31 subjects. For each seafood category: 13 (<0.5 oz/mo), 5 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
[b] Among 529 subjects with erythrocyte EPA+DHA (1996-2000). 176 (<0.5 oz/mo), 111 (0.5 oz/mo-<1.5 oz/wk), 130 (1.5-3 oz/wk), 112 (>3 oz/wk) were excluded.
[c] P-value <0.05 for Pearson's Chi-square test for comparisons across highest versus lowest seafood intake categories for categorical or binary variables or one-way ANOVA test for differences in means across highest versus lowest seafood intake categories for continuous variables.

Table 1.1e Selected characteristics of participants according to amount of **fatty fish intake**, Omega study

Maternal characteristics	Fatty fish intake (ounces)							
	<0.5/mo (N = 775)		0.5/mo-<1.5/wk (N = 832)		1.5-3/wk (N = 889)		>3/wk (N = 783)	
	N	%	N	%	N	%	N	%
Non-Hispanic white race/ethnicity [a]	666	85.9	721	86.7	793	89.2	690	88.1
High school or less [a, c]	57	7.4	29	3.5	12	1.3	13	1.7
Unmarried [c]	114	14.7	76	9.1	61	6.9	87	11.1
Nulliparous	478	61.7	505	60.7	550	61.9	479	61.2
Pre-pregnancy Body Mass Index (kg/m^2) [a, c]								
<18.5	40	5.2	27	3.2	34	3.8	37	4.7
18.5-<25	507	65.4	615	73.9	663	74.6	552	70.5
25-<30	140	18.1	127	15.3	129	14.5	133	17.0
≥30.0	87	11.2	62	7.5	62	7.0	58	7.4
Smoked during pregnancy [a, c]	57	7.4	50	6.0	35	3.9	37	4.7
Any alcohol intake during pregnancy [c]	176	22.7	223	26.8	235	26.4	221	28.2
No recreational physical activity during early pregnancy [a, c]	148	19.1	167	20.1	165	18.6	111	14.2
Fish oil supplements [c]	5	0.6	13	1.6	16	1.8	14	1.8
Family history of hypertension	368	47.5	429	51.6	421	47.4	395	50.4
Family history of diabetes	104	13.4	125	15.0	104	11.7	116	14.8
Enrollment Year [a]								
1996-1997	46	5.9	39	4.7	46	5.2	47	6.0
1998-1999	119	15.4	134	16.1	132	14.8	120	15.3
2000-2001	144	18.6	155	18.6	165	18.6	149	19.0
2002-2003	198	25.5	196	23.6	249	28.0	226	28.9
2004-2005	163	21.0	186	22.4	181	20.4	160	20.4
2006-2007	91	11.7	107	12.9	108	12.1	76	9.7
	mean	SD	mean	SD	mean	SD	mean	SD
Maternal age (yrs) [c]	31.5	4.7	32.6	4.3	33.0	4.0	33.6	4.2
	med	(IQR)	med	(IQR)	med	(IQR)	med	(IQR)
Gestational weight gain (kg) [a]	15.9	(12.7-19.5)	15.9	(12.7-19.1)	15.9	(12.7-19.1)	15.9	(12.7-19.1)
Gestational age at delivery (weeks) [a, c]	39	(38-40)	39	(38-40)	39	(38-40)	39	(38-40)
Weekly intake EPA+DHA (g) [c]	0.0	(0.0-0.1)	0.3	(0.2-0.5)	0.8	(0.6-0.9)	1.4	(1.1-2.0)
Erythrocyte EPA+DHA (%/total fatty acids) [b, c]	4.5	(3.9-5.1)	5.2	(4.4-5.7)	5.6	(4.8-6.4)	5.8	(5.2-6.7)
Daily intake								
Total energy (kcal) [a, c]	1542.7	(1211.7-1959.3)	1556.7	(1230.1-1928.7)	1685.3	(1364.2-2085.6)	1796.9	(1458.9-2205.3)
Total fat (g) [a, c]	53.5	(40.5-69.5)	53.0	(40.0-70.4)	58.9	(45.0-75.2)	63.6	(48.1-84.9)
Red and processed meats (servings) [a, c]	0.5	(0.2-0.9)	0.5	(0.3-0.8)	0.6	(0.3-0.9)	0.6	(0.3-0.9)

[a] Missing maternal age, marital status, parity, family histories of hypertension and diabetes for 2 subjects. For each category: 1 (0.5 oz/mo-<1.5 oz/wk), 1 (1.5-3 oz/wk) were missing.
Missing race/ethnicity for 10 subjects. For each seafood category: 1 (<0.5 oz/mo), 4 (0.5 oz/mo-<1.5 oz/wk), 4 (1.5-3 oz/wk), 1 (>3 oz/wk) were missing.
Missing education information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 6 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (0.5 oz/mo-<1.5 oz/wk), 2 (1.5-3 oz/wk), 2 (>3 oz/wk) were missing.
Missing smoking information for 100 subjects. For each seafood category: 33 (<0.5 oz/mo), 16 (0.5 oz/mo-<1.5 oz/wk), 22 (1.5-3 oz/wk), 29 (>3 oz/wk) were missing.

Missing recreational physical activity information for 91 subjects. For each seafood category: 27 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-3 oz/wk), 28 (>3 oz/wk) were missing.
Missing enrollment year for 42 subjects. For each seafood category: 19 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 8 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
Missing gestational weight gain for 54 subjects. For each seafood category: 21 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 12 (1.5-3 oz/wk), 12 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 104 subjects. For each seafood category: 44 (<0.5 oz/mo), 19 (0.5 oz/mo-<1.5 oz/wk), 24 (1.5-3 oz/wk), 17 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 31 subjects. For each seafood category: 13 (<0.5 oz/mo), 5 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-3 oz/wk), 6 (>3 oz/wk) were missing.
[b] Among 529 subjects with erythrocyte EPA+DHA (1996-2000). 176 (<0.5 oz/mo), 111 (0.5 oz/mo-<1.5 oz/wk), 130 (1.5-3 oz/wk), 112 (>3 oz/wk) were excluded.
[c] P-value <0.05 for Pearson's Chi-square test for comparisons across highest versus lowest seafood intake categories for categorical or binary variables or one-way ANOVA test for differences in means across highest versus lowest seafood intake categories for continuous variables.

Table 1.2a Associations of **total seafood intake (all subtypes combined)** with risk of pregnancy complications

	Seafood intake (ounces)				
	<0.5/mo (N = 302)	0.5/mo-<1.5/wk (N = 320)	1.5-3/wk (N = 541)	>3/wk (N = 2116)	*P* value [d]
PE					
No. events (89/3215) [b]	7/292	15/306	16/531	51/2086	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	2.04 (0.85-4.94)	1.26 (0.52-3.02)	1.02 (0.47-2.23)	0.20
Model 1.1 [f]	1.0 (referent)	1.89 (0.76-4.73)	1.48 (0.59-3.67)	1.25 (0.55-2.84)	0.83
Model 1.2 [g]	1.0 (referent)	2.01 (0.80-5.05)	1.48 (0.60-3.62)	1.16 (0.50-2.65)	0.57
PIH					
No. events (375/3136) [c]	36/287	36/307	51/517	252/2025	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.93 (0.61-1.44)	0.79 (0.53-1.18)	0.99 (0.72-1.38)	0.70
Model 1.1 [f]	1.0 (referent)	1.12 (0.71-1.75)	0.91 (0.60-1.40)	1.13 (0.79-1.62)	0.44
Model 1.2 [g]	1.0 (referent)	1.12 (0.72-1.75)	0.92 (0.60-1.41)	1.10 (0.77-1.57)	0.61
GDM					
No. events (160/3205) [b, c]	14/293	14/303	28/534	104/2075	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.97 (0.47-1.99)	1.10 (0.59-2.05)	1.05 (0.61-1.81)	0.82
Model 1.1 [f]	1.0 (referent)	0.87 (0.42-1.80)	1.06 (0.57-1.97)	0.95 (0.55-1.65)	0.95
Model 1.2 [g]	1.0 (referent)	0.77 (0.36-1.67)	1.03 (0.55-1.94)	0.95 (0.55-1.65)	0.93
PTB					
No. events (259/3176) [b]	15/288	30/304	46/524	168/2060	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	**1.89 (1.04-3.45)**	1.69 (0.96-2.97)	1.57 (0.94-2.62)	0.42
Model 1.1 [f]	1.0 (referent)	**2.06 (1.11-3.83)**	1.73 (0.96-3.12)	1.64 (0.96-2.83)	0.41
Model 1.2 [g]	1.0 (referent)	**2.05 (1.10-3.79)**	1.72 (0.96-3.10)	1.60 (0.93-2.75)	0.47
Composite [e]					
No. events (800/3123) [b]	66/278	88/295	127/517	519/2033	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.26 (0.96-1.65)	1.03 (0.80-1.34)	1.08 (0.86-1.34)	0.87
Model 1.1 [f]	1.0 (referent)	**1.35 (1.02-1.79)**	1.13 (0.87-1.48)	1.17 (0.93-1.48)	0.64

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{seafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Composite is defined as one or more diagnosis of PE, PIH, GDM, or PTB.

[f] Model 1.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day)

[g] Model 1.2 adjusts for the same variables in Model 1.1 but additionally adjusts for study year (5 non-referent indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007)

Table 1.2b Associations of dietary **EPA+DHA quartile** from seafood with risk of pregnancy complications among subjects with at least 0.5 oz/mo of seafood intake

	Quartile 1 (N=744)	Quartile 2 (N=745)	Quartile 3 (N=744)	Quartile 4 (N=745)	*P* value [a]
Range EPA+DHA (g/month)	0.02-2.06	2.06-4.31	4.31-7.56	7.56-56.93	
Mean, SD EPA+DHA (g/month)	1.02, 0.57	3.12, 0.67	5.84, 0.92	12.64, 5.99	
PE					
No. events (82/2924) [b]	32/721	17/735	17/738	16/730	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	**0.52 (0.23-0.93)**	**0.52 (0.29-0.93)**	**0.49 (0.27-0.89)**	**0.02**
Model 1.1 [f]	1.0 (referent)	0.74 (0.41-1.36)	0.69 (0.37-1.29)	0.63 (0.33-1.21)	0.16
Model 1.2 [g]	1.0 (referent)	0.71 (0.39-1.28)	0.63 (0.33-1.19)	0.58 (0.31-1.12)	0.10
PIH					
No. events (339/2850) [c]	75/707	90/713	86/716	88/714	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.19 (0.89-1.59)	1.13 (0.85-1.52)	1.16 (0.87-1.55)	0.40
Model 1.1 [f]	1.0 (referent)	1.20 (0.90-1.62)	1.10 (0.81-1.48)	1.14 (0.84-1.54)	0.55
Model 1.2 [g]	1.0 (referent)	1.20 (0.90-1.61)	1.09 (0.81-1.47)	1.12 (0.83-1.51)	0.65
GDM					
No. events (146/2913) [b, c]	35/716	34/736	36/736	41/725	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.95 (0.60-1.50)	1.00 (0.64-1.58)	1.16 (0.75-1.79)	0.49
Model 1.1 [f]	1.0 (referent)	0.97 (0.60-1.55)	1.15 (0.72-1.85)	1.16 (0.73-1.83)	0.42
Model 1.2 [g]	1.0 (referent)	1.03 (0.63-1.67)	1.23 (0.75-2.01)	1.20 (0.74-1.95)	0.35
PTB					
No. events (244/2889) [b]	64/712	62/731	60/727	58/719	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.94 (0.68-1.32)	0.92 (0.66-1.29)	0.90 (0.64-1.26)	0.52
Model 1.1 [f]	1.0 (referent)	0.98 (0.69-1.37)	0.94 (0.66-1.33)	0.88 (0.60-1.27)	0.47
Model 1.2 [g]	1.0 (referent)	0.93 (0.65-1.31)	0.93 (0.66-1.32)	0.87 (0.60-1.27)	0.51
Composite [e]					
No. events (734/2846) [b]	189/695	183/722	178/722	184/707	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.93 (0.78-1.11)	0.91 (0.76-1.08)	0.96 (0.80-1.14)	0.58
Model 1.1 [f]	1.0 (referent)	0.99 (0.83-1.18)	0.96 (0.80-1.15)	0.98 (0.82-1.18)	0.76

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{seafoodGroup}+Confounding\ Variables,\ for\ Y = pregnancy\ complication$

[b] 54/2978 subjects missing PE status, 32/2978 subjects missing GDM status, 89/2978 subjects missing PTB status, 132/2978 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 128 subjects with chronic hypertension GDM outcome additionally excludes 33 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Composite is defined as one or more diagnosis of PE, PIH, GDM, or PTB.

[f] Model 1.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day)

[g] Model 1.2 adjusts for the same variables in Model 1.1 but additionally adjusts for study year (5 non-referent indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007)

Table 1.2c Associations of **shellfish intake** with risk of pregnancy complications

	Shellfish intake (ounces)				
	<0.5/mo (N = 1099)	0.5/mo-<1.5/wk (N = 705)	1.5-3/wk (N = 772)	>3/wk (N = 703)	*P* value [a]
PE					
No. events (89/3215) [b]	32/1071	26/691	14/762	17/691	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.26 (0.76-2.09)	0.61 (0.33-1.14)	0.82 (0.46-1.47)	0.20
Model 1.1 [f]	1.0 (referent)	1.37 (0.78-2.42)	0.78 (0.39-1.55)	0.97 (0.48-1.96)	0.65
Model 1.2 [g]	1.0 (referent)	1.54 (0.88-2.72)	0.82 (0.42-1.62)	1.17 (0.58-2.35)	1.00
PIH					
No. events (375/3136) [c]	128/1038	76/682	79/742	92/674	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.90 (0.69-1.18)	0.86 (0.66-1.12)	1.11 (0.86-1.42)	0.67
Model 1.1 [f]	1.0 (referent)	0.93 (0.70-1.24)	0.85 (0.64-1.13)	1.04 (0.78-1.40)	0.97
Model 1.2 [g]	1.0 (referent)	0.96 (0.72-1.27)	0.86 (0.65-1.14)	1.16 (0.87-1.55)	0.59
GDM					
No. events (160/3205) [b, c]	51/1068	29/690	36/760	44/687	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.88 (0.56-1.37)	0.99 (0.65-1.50)	1.34 (0.91-1.98)	0.18
Model 1.1 [f]	1.0 (referent)	0.97 (0.61-1.53)	1.06 (0.68-1.66)	1.17 (0.75-1.83)	0.47
Model 1.2 [g]	1.0 (referent)	0.90 (0.56-1.44)	1.04 (0.66-1.63)	1.11 (0.70-1.76)	0.60
PTB					
No. events (259/3176) [b]	81/1055	67/686	53/749	58/686	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.27 (0.93-1.73)	0.92 (0.66-1.29)	1.10 (0.80-1.52)	0.95
Model 1.1 [f]	1.0 (referent)	1.17 (0.84-1.62)	0.86 (0.60-1.23)	1.01 (0.69-1.47)	0.67
Model 1.2 [g]	1.0 (referent)	1.20 (0.86-1.67)	0.87 (0.61-1.26)	1.04 (0.71-1.53)	0.78
Composite [e]					
No. events (800/3123) [b]	265/1034	178/674	167/741	190/674	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.03 (0.88-1.21)	0.88 (0.74-1.04)	1.10 (0.94-1.29)	0.33
Model 1.1 [f]	1.0 (referent)	1.06 (0.89-1.26)	0.90 (0.75-1.07)	1.07 (0.89-1.29)	0.92

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{seafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Composite is defined as one or more diagnosis of PE, PIH, GDM, or PTB.

[f] Model 1.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day), and alternate seafood subtypes (indicator variables for non-reference categories).

[g] Model 1.2 adjusts for the same variables in Model 1.1 but additionally adjusts for study year (5 non-referent indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007)

Table 1.2d Associations of **lean fish intake** with risk of pregnancy complications

	Lean fish intake (ounces)				
	<0.5/mo (N= 835)	0.5/mo-<1.5/wk (N= 819)	1.5-3/wk (N= 857)	>3/wk (N= 768)	*P* value [a]
PE					
No. events (89/3215) [b]	22/811	24/804	16/842	27/758	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.10 (0.62-1.95)	0.70 (0.37-1.32)	1.31 (0.75-2.29)	0.63
Model 1.1 [f]	1.0 (referent)	1.23 (0.65-2.32)	0.94 (0.46-1.93)	1.67 (0.87-3.20)	0.23
Model 1.2 [g]	1.0 (referent)	1.17 (0.62-2.20)	0.80 (0.40-1.63)	1.27 (0.67-2.38)	0.70
PIH					
No. events (375/3136) [c]	96/800	82/790	109/825	88/721	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.86 (0.66-1.14)	1.10 (0.85-1.42)	1.02 (0.78-1.33)	0.50
Model 1.1 [f]	1.0 (referent)	0.92 (0.69-1.23)	1.09 (0.83-1.43)	0.97 (0.71-1.33)	0.83
Model 1.2 [g]	1.0 (referent)	0.91 (0.68-1.21)	1.03 (0.79-1.36)	0.89 (0.66-1.21)	0.70
GDM					
No. events (160/3205) [b, c]	42/813	33/799	43/843	42/750	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.80 (0.51-1.25)	0.99 (0.65-1.49)	1.08 (0.72-1.64)	0.54
Model 1.1 [f]	1.0 (referent)	0.72 (0.46-1.16)	0.83 (0.54-1.28)	0.88 (0.56-1.40)	0.74
Model 1.2 [g]	1.0 (referent)	0.76 (0.47-1.22)	0.86 (0.55-1.34)	0.94 (0.59-1.49)	0.92
PTB					
No. events (259/3176) [b]	53/805	68/790	70/836	68/745	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.31 (0.93-1.85)	1.27 (0.90-1.79)	1.39 (0.98-1.96)	0.09
Model 1.1 [f]	**1.0 (referent)**	**1.41 (0.97-2.05)**	**1.34 (0.92-1.95)**	**1.55 (1.04-2.30)**	**0.05**
Model 1.2 [g]	1.0 (referent)	1.37 (0.94-2.00)	1.32 (0.90-1.92)	**1.50 (1.01-2.24)**	0.07
Composite [e]					
No. events (800/3123) [b]	196/783	191/780	216/827	197/733	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.98 (0.82-1.16)	1.04 (0.88-1.23)	1.07 (0.91-1.27)	0.32
Model 1.1 [f]	1.0 (referent)	1.00 (0.84-1.20)	1.05 (0.88-1.25)	1.07 (0.88-1.30)	0.42

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{seafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Composite is defined as one or more diagnosis of PE, PIH, GDM, or PTB.

[f] Model 1.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day), and alternate seafood subtypes (indicator variables for non-reference categories).

[g] Model 1.2 adjusts for the same variables in Model 1.1 but additionally adjusts for study year (5 non-referent indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007)

Table 1.2e Associations of **fatty fish intake** with risk of pregnancy complications

	Fatty fish intake (ounces)				
	<0.5/mo (N= 775)	0.5/mo-<1.5/wk (N= 832)	1.5-3/wk (N= 889)	>3/wk (N= 783)	*P* value [a]
PE					
No. events (89/3215) [b]	30/753	24/812	18/883	17/767	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	0.74 (0.44-1.26)	**0.51 (0.29-0.91)**	0.56 (0.31-1.00)	0.02
Model 1.1 [f]	1.0 (referent)	0.87 (0.49-1.54)	0.60 (0.30-1.18)	0.59 (0.29-1.18)	0.09
Model 1.2 [g]	1.0 (referent)	0.86 (0.49-1.51)	0.63 (0.32-1.24)	0.56 (0.27-1.13)	0.08
PIH					
No. events (375/3136) [c]	84/729	96/804	101/857	94/746	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.04 (0.79-1.36)	1.02 (0.78-1.34)	1.09 (0.83-1.44)	0.57
Model 1.1 [f]	1.0 (referent)	1.15 (0.85-1.57)	1.11 (0.81-1.51)	1.15 (0.82-1.62)	0.51
Model 1.2 [g]	1.0 (referent)	1.18 (0.87-1.61)	1.11 (0.81-1.52)	1.10 (0.78-1.55)	0.74
GDM					
No. events (160/3205) [b, c]	37/749	44/814	38/880	41/762	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.09 (0.71-1.68)	0.87 (0.56-1.36)	1.09 (0.71-1.68)	0.98
Model 1.1 [f]	1.0 (referent)	1.26 (0.81-1.96)	1.00 (0.61-1.68)	1.14 (0.69-1.88)	0.88
Model 1.2 [g]	1.0 (referent)	1.32 (0.84-2.07)	1.02 (0.61-1.72)	1.20 (0.72-2.00)	0.79
PTB					
No. events (259/3176) [b]	59/747	75/806	72/866	53/757	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.18 (0.85-1.63)	1.05 (0.76-1.46)	0.89 (0.62-1.27)	0.39
Model 1.1 [f]	1.0 (referent)	1.17 (0.83-1.66)	1.03 (0.72-1.48)	0.74 (0.49-1.13)	0.10[h]
Model 1.2 [g]	1.0 (referent)	1.14 (0.80-1.63)	1.02 (0.71-1.46)	0.74 (0.48-1.12)	0.10
Composite [e]					
No. events (800/3123) [b]	189/724	218/795	207/861	186/743	
RR (95% CI) [d]					
Unadjusted	1.0 (referent)	1.05 (0.89-1.24)	0.92 (0.77-1.09)	0.96 (0.81-1.14)	0.39
Model 1.1 [f]	1.0 (referent)	1.14 (0.95-1.37)	1.00 (0.83-1.22)	0.97 (0.79-1.20)	0.45

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{SeafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Composite is defined as one or more diagnosis of PE, PIH, GDM, or PTB.

[f] Model 1.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day), and alternate seafood subtypes (indicator variables for non-reference categories).

[g] Model 1.2 adjusts for the same variables in Model 1.1 but additionally adjusts for study year (5 non-referent indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007)

[h] Wald p-value for a grouped linear term (excluding group < 0.5 oz/mo) was statistically significant for PTB, p =0.02 for Model 1.1

Table 1.3a Sensitivity analyses: associations of **total seafood intake (all subtypes combined)** with risk of pregnancy complications

	Seafood intake (ounces)				
	<0.5/mo (N = 302)	0.5/mo-<1.5/wk (N = 320)	1.5-3/wk (N = 541)	>3/wk (N = 2116)	*P* value [d]
PE					
No. events (89/3215) [b]	7/292	15/306	16/531	51/2086	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.89 (0.76-4.73)	1.48 (0.59-3.67)	1.25 (0.55-2.84)	0.83
Model 1.3 [f]	1.0 (referent)	2.04 (0.79-5.25)	1.53 (0.60-3.89)	1.35 (0.57-3.19)	0.97
Model 1.4 [g]	1.0 (referent)	2.57 (0.89-7.38)	1.38 (0.46-4.12)	1.19 (0.45-3.19)	0.47
Model 1.5 [h]	1.0 (referent)	1.77 (0.70-4.48)	1.38 (0.55-3.47)	1.26 (0.55-2.87)	0.96
PIH					
No. events (375/3136) [c]	36/287	36/307	51/517	252/2025	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.12 (0.71-1.75)	0.91 (0.60-1.40)	1.13 (0.79-1.62)	0.44
Model 1.3 [f]	1.0 (referent)	1.17 (0.75-1.83)	0.94 (0.61-1.43)	1.18 (0.82-1.69)	0.34
Model 1.4 [g]	1.0 (referent)	1.02 (0.62-1.70)	0.70 (0.43-1.15)	0.95 (0.64-1.42)	0.94
Model 1.5 [h]	1.0 (referent)	1.10 (0.70-1.73)	0.90 (0.59-1.38)	1.13 (0.80-1.61)	0.43
GDM					
No. events (160/3205) [b, c]	14/293	14/303	28/534	104/2075	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.87 (0.42-1.80)	1.06 (0.57-1.97)	0.95 (0.55-1.65)	0.95
Model 1.3 [f]	1.0 (referent)	0.84 (0.41-1.74)	1.02 (0.55-1.89)	0.90 (0.53-1.55)	0.79
Model 1.4 [g]	1.0 (referent)	0.71 (0.24-2.12)	1.15 (0.50-2.68)	1.12 (0.54-2.32)	0.51
Model 1.5 [h]	1.0 (referent)	0.95 (0.45-1.99)	1.15 (0.61-2.17)	1.03 (0.58-1.82)	0.91
PTB					
No. events (259/3176) [b]	15/288	30/304	46/524	168/2060	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	**2.06 (1.11-3.83)**	1.73 (0.96-3.12)	1.64 (0.96-2.83)	0.41
Model 1.3 [f]	1.0 (referent)	**1.99 (1.06-3.74)**	1.73 (0.95-3.18)	1.56 (0.89-2.74)	0.60
Model 1.4 [g]	1.0 (referent)	**1.77 (0.83-3.76)**	1.82 (0.92-3.63)	1.62 (0.86-3.06)	0.34
Model 1.5 [h]	1.0 (referent)	**2.26 (1.20-4.26)**	**1.88 (1.02-3.45)**	**1.76 (1.00-3.09)**	0.37

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{SeafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Model 1.1 (Primary Model) is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day)

[f] Model 1.3 adjusts for the same variables in Model 1.1 but additionally adjusts for gestational weight gain (kg, continuous)

[g] Model 1.4 adjusts for the same variables in Model 1.1 but is restricted to nulliparous women

[h] Model 1.5 adjusts for the same variables in Model 1.1 but excludes fish oil supplement users

Table 1.3b Sensitivity analyses: associations of **dietary EPA+DHA quartile** from seafood with risk of pregnancy complications for subjects with ≥ 0.5 oz/mo of seafood intake

	Quartile 1 (N=744)	Quartile 2 (N=745)	Quartile 3 (N=744)	Quartile 4 (N=745)	*P* value [a]
Range EPA+DHA (g/month)	0.02-2.06	2.06-4.31	4.31-7.56	7.56-56.93	
Mean, SD EPA+DHA (g/month)	1.02, 0.57	3.12, 0.67	5.84, 0.92	12.64, 5.99	
PE No. events (81/2924) [b]	32/721	17/735	17/738	16/730	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.74 (0.41-1.36)	0.69 (0.37-1.29)	0.63 (0.33-1.21)	0.16
Model 1.3 [f]	1.0 (referent)	0.78 (0.42-1.43)	0.69 (0.37-1.31)	0.66 (0.34-1.27)	0.19
Model 1.4 [g]	1.0 (referent)	0.72 (0.36-1.47)	0.73 (0.35-1.51)	0.55 (0.25-1.20)	0.16
Model 1.5 [h]	1.0 (referent)	0.73 (0.39-1.35)	0.72 (0.38-1.35)	0.67 (0.35-1.28)	0.23
PIH No. events (339/2850) [c]	75/707	90/713	86/716	88/714	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.20 (0.90-1.62)	1.10 (0.81-1.48)	1.14 (0.84-1.54)	0.55
Model 1.3 [f]	1.0 (referent)	1.22 (0.91-1.63)	1.09 (0.81-1.48)	1.16 (0.86-1.57)	0.50
Model 1.4 [g]	1.0 (referent)	1.17 (0.82-1.66)	1.15 (0.81-1.63)	1.16 (0.81-1.65)	0.47
Model 1.5 [h]	1.0 (referent)	1.24 (0.92-1.67)	1.11 (0.82-1.51)	1.15 (0.85-1.57)	0.53
GDM No. events (146/2913) [a, b]	35/716	34/736	36/736	41/725	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.97 (0.60-1.55)	1.15 (0.72-1.85)	1.16 (0.73-1.83)	0.42
Model 1.3 [f]	1.0 (referent)	0.94 (0.59-1.51)	1.13 (0.71-1.83)	1.14 (0.72-1.80)	0.45
Model 1.4 [g]	1.0 (referent)	1.19 (0.62-2.26)	1.42 (0.73-2.76)	1.27 (0.67-2.41)	0.40
Model 1.5 [h]	1.0 (referent)	0.96 (0.60-1.55)	1.15 (0.71-1.84)	1.16 (0.73-1.84)	0.42
PTB No. events (244/2889) [a, b]	64/712	62/731	60/727	58/719	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.98 (0.69-1.37)	0.94 (0.66-1.33)	0.88 (0.60-1.27)	0.47
Model 1.3 [f]	1.0 (referent)	0.94 (0.67-1.32)	0.91 (0.64-1.29)	0.87 (0.61-1.26)	0.47
Model 1.4 [g]	1.0 (referent)	0.84 (0.55-1.28)	0.90 (0.59-1.37)	0.82 (0.53-1.28)	0.47
Model 1.5 [h]	1.0 (referent)	0.97 (0.69-1.37)	0.91 (0.64-1.29)	0.88 (0.61-1.27)	0.44

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{SeafoodGroup}+Confounding\ Variables,\ for\ Y = pregnancy\ complication$

[b] 54/2978 subjects missing PE status, 32/2978 subjects missing GDM status, 89/2978 subjects missing PTB status, 132/2978 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 128 subjects with chronic hypertension GDM outcome additionally excludes 33 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Model 1.1 (Primary Model) is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day)

[f] Model 1.3 adjusts for the same variables in Model 1.1 but additionally adjusts for gestational weight gain (kg, continuous)

[g] Model 1.4 adjusts for the same variables in Model 1.1 but is restricted to nulliparous women

[h] Model 1.5 adjusts for the same variables in Model 1.1 but excludes fish oil supplement users

Table 1.3c Sensitivity analyses: associations of **shellfish intake** with risk of pregnancy complications

	Shellfish intake (ounces)				
	<0.5/mo (N = 1099)	0.5/mo-<1.5/wk (N = 705)	1.5-3/wk (N = 772)	>3/wk (N = 703)	*P* value [a]
PE					
No. events (89/3215) [b]	32/1071	26/691	14/762	17/691	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.37 (0.78-2.42)	0.78 (0.39-1.55)	0.97 (0.48-1.96)	0.65
Model 1.3 [f]	1.0 (referent)	1.42 (0.80-2.51)	0.80 (0.40-1.59)	1.00 (0.49-2.02)	0.71
Model 1.4 [g]	1.0 (referent)	1.08 (0.54-2.16)	0.80 (0.32-1.46)	0.80 (0.34-1.93)	0.42
Model 1.5 [h]	1.0 (referent)	1.25 (0.70-2.22)	0.77 (0.39-1.54)	0.95 (0.47-1.93)	0.65
PIH					
No. events (375/3136) [c]	128/1038	76/682	79/742	92/674	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.93 (0.70-1.24)	0.85 (0.64-1.13)	1.04 (0.78-1.40)	0.97
Model 1.3 [f]	1.0 (referent)	0.94 (0.71-1.25)	0.88 (0.67-1.18)	1.08 (0.81-1.44)	0.53
Model 1.4 [g]	1.0 (referent)	0.88 (0.63-1.23)	0.78 (0.56-1.10)	1.10 (0.79-1.55)	0.81
Model 1.5 [h]	1.0 (referent)	0.96 (0.72-1.27)	0.86 (0.64-1.15)	1.06 (0.79-1.43)	0.95
GDM					
No. events (160/3205) [b, c]	51/1068	29/690	36/760	44/687	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.97 (0.61-1.53)	1.06 (0.68-1.66)	1.17 (0.75-1.83)	0.47
Model 1.3 [f]	1.0 (referent)	0.93 (0.59-1.48)	1.05 (0.67-1.64)	1.17 (0.75-1.83)	0.47
Model 1.4 [g]	1.0 (referent)	1.14 (0.62-2.12)	0.99 (0.52-1.88)	1.26 (0.67-2.36)	0.58
Model 1.5 [h]	1.0 (referent)	0.97 (0.61-1.55)	1.06 (0.68-1.67)	1.17 (0.75-1.83)	0.48
PTB					
No. events (259/3176) [b]	81/1055	67/686	53/749	58/686	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.17 (0.84-1.62)	0.86 (0.60-1.23)	1.01 (0.69-1.47)	0.67
Model 1.3 [f]	1.0 (referent)	1.08 (0.77-1.51)	0.81 (0.57-1.17)	0.94 (0.64-1.38)	0.44
Model 1.4 [g]	1.0 (referent)	1.29 (0.86-1.95)	0.98 (0.63-1.53)	1.10 (0.68-1.78)	0.96
Model 1.5 [h]	1.0 (referent)	1.16 (0.83-1.62)	0.86 (0.60-1.23)	0.98 (0.67-1.44)	0.59

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{SeafoodGroup}+Confounding\ Variables,\ for\ Y = pregnancy\ complication$

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Model 1.2 (Primary Model) is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day), and alternate seafood subtypes (indicator variables for non-reference categories).

[f] Model 1.3 adjusts for the same variables in Model 1.1 but additionally adjusts for gestational weight gain (kg, continuous)

[g] Model 1.4 adjusts for the same variables in Model 1.1 but is restricted to nulliparous women

[h] Model 1.5 adjusts for the same variables in Model 1.1 but excludes fish oil supplement users

Table 1.3d Sensitivity analyses: associations of **lean fish intake** with risk of pregnancy complications

	Lean fish intake (ounces)				
	<0.5/mo (N= 835)	0.5/mo-<1.5/wk (N= 819)	1.5-3/wk (N= 857)	>3/wk (N= 768)	*P* value [a]
PE					
No. events (89/3215) [b]	22/811	24/804	16/842	27/758	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.23 (0.65-2.32)	0.94 (0.46-1.93)	1.67 (0.87-3.20)	0.23
Model 1.3 [f]	1.0 (referent)	1.26 (0.67-2.38)	0.98 (0.48-2.01)	1.72 (0.90-3.31)	0.20
Model 1.4 [g]	1.0 (referent)	1.95 (0.95-4.01)	1.06 (0.45-2.51)	1.76 (0.77-4.00)	0.46
Model 1.5 [h]	1.0 (referent)	1.32 (0.69-2.50)	0.94 (0.45-1.95)	1.77 (0.91-3.42)	0.20
PIH					
No. events (375/3136) [c]	96/800	82/790	109/825	88/721	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.92 (0.69-1.23)	1.09 (0.83-1.43)	0.97 (0.71-1.33)	0.83
Model 1.3 [f]	1.0 (referent)	0.93 (0.70-1.25)	1.12 (0.85-1.47)	0.97 (0.71-1.33)	0.81
Model 1.4 [g]	1.0 (referent)	0.78 (0.55-1.11)	1.04 (0.76-1.42)	0.97 (0.68-1.38)	0.72
Model 1.5 [h]	1.0 (referent)	0.94 (0.70-1.27)	1.12 (0.85-1.48)	1.02 (0.75-1.40)	0.61
GDM					
No. events (160/3205) [b, c]	42/813	33/799	43/843	42/750	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.72 (0.46-1.16)	0.83 (0.54-1.28)	0.88 (0.56-1.40)	0.74
Model 1.3 [f]	1.0 (referent)	0.72 (0.45-1.15)	0.82 (0.53-1.26)	0.86 (0.54-1.37)	0.67
Model 1.4 [g]	1.0 (referent)	0.76 (0.41-1.40)	0.81 (0.46-1.43)	0.75 (0.40-1.42)	0.45
Model 1.5 [h]	1.0 (referent)	0.74 (0.46-1.18)	0.84 (0.54-1.31)	0.90 (0.57-1.43)	0.81
PTB					
No. events (259/3176) [b]	53/805	68/790	70/836	68/745	
RR (95% CI) [d]					
Model 1.1 [e]	**1.0 (referent)**	**1.41 (0.97-2.05)**	**1.34 (0.92-1.95)**	**1.55 (1.04-2.30)**	**0.05**
Model 1.3 [f]	1.0 (referent)	1.36 (0.94-1.97)	1.27 (0.87-1.86)	1.46 (0.97-2.19)	0.12
Model 1.4 [g]	1.0 (referent)	1.36 (0.87-2.13)	1.21 (0.77-1.90)	1.39 (0.85-2.27)	0.29
Model 1.5 [h]	**1.0 (referent)**	**1.42 (0.98-2.08)**	**1.36 (0.93-1.99)**	**1.59 (1.06-2.37)**	**0.04**

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{SeafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Model 1.1 (Primary Model) is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day), and alternate seafood subtypes (indicator variables for non-reference categories).

[f] Model 1.3 adjusts for the same variables in Model 1.1 but additionally adjusts for gestational weight gain (kg, continuous)

[g] Model 1.4 adjusts for the same variables in Model 1.1 but is restricted to nulliparous women

[h] Model 1.5 adjusts for the same variables in Model 1.1 but excludes fish oil supplement users

Table 1.3e Sensitivity analyses: associations of **fatty fish intake** and risk of pregnancy complications

	Fatty fish intake (ounces)				
	<0.5/mo (N= 775)	0.5/mo-<1.5/wk (N= 832)	1.5-3/wk (N= 889)	>3/wk (N= 783)	*P* value [a]
PE					
No. events (89/3215) [b]	30/753	24/812	18/883	17/767	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	0.87 (0.49-1.54)	0.60 (0.30-1.18)	0.59 (0.29-1.18)	0.09
Model 1.3 [f]	1.0 (referent)	0.87 (0.50-1.53)	0.58 (0.29-1.14)	0.57 (0.28-1.16)	0.08
Model 1.4 [g]	1.0 (referent)	0.92 (0.46-1.82)	0.67 (0.30-1.50)	0.55 (0.23-1.30)	0.14
Model 1.5 [h]	1.0 (referent)	0.80 (0.45-1.43)	0.61 (0.31-1.20)	0.58 (0.29-1.18)	0.11
PIH					
No. events (375/3136) [c]	84/729	96/804	101/857	94/746	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.15 (0.85-1.57)	1.11 (0.81-1.51)	1.15 (0.82-1.62)	0.51
Model 1.3 [f]	1.0 (referent)	1.16 (0.85-1.58)	1.09 (0.80-1.48)	1.16 (0.82-1.63)	0.53
Model 1.4 [g]	1.0 (referent)	1.13 (0.80-1.61)	1.03 (0.72-1.46)	1.00 (0.67-1.47)	0.82
Model 1.5 [h]	1.0 (referent)	1.13 (0.83-1.54)	1.07 (0.79-1.47)	1.11 (0.79-1.56)	0.67
GDM					
No. events (160/3205) [b, c]	37/749	44/814	38/880	41/762	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.26 (0.81-1.96)	1.00 (0.61-1.68)	1.14 (0.69-1.88)	0.88
Model 1.3 [f]	1.0 (referent)	1.23 (0.79-1.90)	1.00 (0.60-1.67)	1.13 (0.69-1.85)	0.90
Model 1.4 [g]	1.0 (referent)	1.08 (0.58-2.04)	1.16 (0.58-2.34)	1.24 (0.62-2.51)	0.54
Model 1.5 [h]	1.0 (referent)	1.29 (0.83-2.01)	1.03 (0.61-1.73)	1.16 (0.70-1.92)	0.84
PTB					
No. events (259/3176) [b]	59/747	75/806	72/866	53/757	
RR (95% CI) [d]					
Model 1.1 [e]	1.0 (referent)	1.17 (0.83-1.66)	1.03 (0.72-1.48)	0.74 (0.49-1.13)	0.10
Model 1.3 [f]	1.0 (referent)	1.18 (0.82-1.70)	1.13 (0.78-1.63)	0.81 (0.53-1.22)	0.25
Model 1.4 [g]	1.0 (referent)	1.32 (0.86-2.02)	1.06 (0.68-1.66)	0.85 (0.51-1.42)	0.32
Model 1.5 [h]	1.0 (referent)	1.17 (0.82-1.67)	1.03 (0.72-1.49)	0.76 (0.50-1.15)	0.13

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0+beta1*X_{SeafoodGroup}+Confounding\ Variables$, *for Y = pregnancy complication*

[b] 64/3279 subjects missing PE status, 37/3279 subjects missing GDM status, 103/3279 subjects missing PTB status, 156/3279 missing composite pregnancy outcome status.

[c] PIH outcome additionally excludes 143 subjects with chronic hypertension GDM outcome additionally excludes 37 subjects with pre-pregnancy diabetes.

[d] Relative risks calculated using generalized linear model with a log link, Poisson family, and robust standard errors.

[e] Model 1.1 (Primary Model) is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), marital status, pre-pregnancy BMI (3 indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2) total energy (continuous, kcal/day), recreational physical activity (yes/no), smoking during pregnancy (yes/no), alcohol intake during pregnancy (yes/no) nulliparous (yes/no), intake of red and processed meats (continuous, servings/day), and alternate seafood subtypes (indicator variables for non-reference categories).

[f] Model 1.3 adjusts for the same variables in Model 1.1 but additionally adjusts for gestational weight gain (kg, continuous)

[g] Model 1.4 adjusts for the same variables in Model 1.1 but is restricted to nulliparous women

[h] Model 1.5 adjusts for the same variables in Model 1.1 but excludes fish oil supplement users

Table 1.3f Associations of total seafood and seafood subtype intake with **mean change in gestational week of delivery**

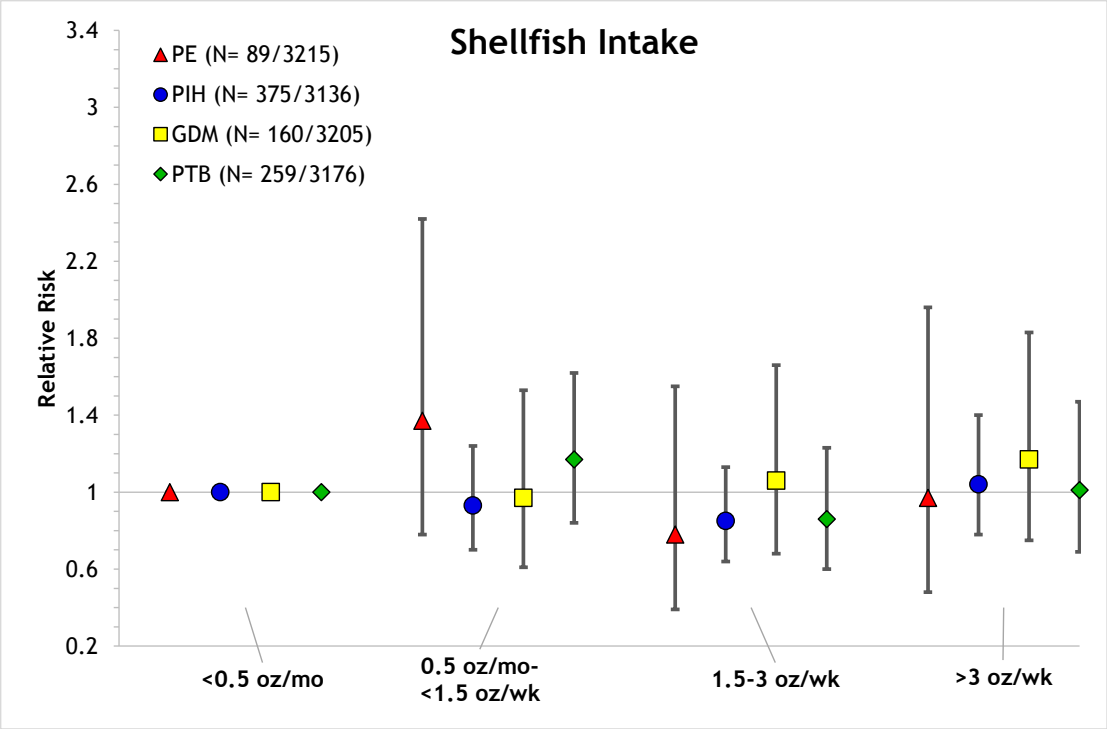
	Seafood intake (ounces)				
	<0.5/mo	0.5/mo-<1.5/wk	1.5-3/wk	>3/wk	*P* value [a]
Total seafood intake	288	304	524	2060	
Gestational wk (mean, std)	38.8, 2.0	38.8, 1.8	38.8, 1.8	38.9, 2.2	
Change (wk) (95% CI) [b]					
Unadjusted	(referent)	-0.03 (-0.36-0.30)	0.05 (-0.25-0.35)	0.14 (-0.11-0.39)	0.11
Model 1.1 [c]	(referent)	-0.04 (-0.35-0.26)	0.04 (-0.23-0.32)	0.09 (-0.15-0.33)	0.25
Shellfish intake	1055	686	749	686	
Gestational wk (mean, std)	38.8,1.9	38.8, 1.8	39.1, 2.5	38.9, 1.9	
Change (wk) (95% CI) [b]					
Unadjusted	(referent)	0.03 (-0.17-0.23)	**0.27 (0.07-0.45)**	0.09 (-0.11-0.29)	0.09
Model 1.1 [c]	(referent)	0.02 (-0.16-0.21)	0.16 (-0.03-0.35)	0.04 (-0.17-0.25)	0.40
Lean fish intake	805	790	836	745	
Gestational wk (mean, std)	38.9, 1.8	38.8, 2.0	38.9, 1.7	38.9, 2.7	
Change (wk) (95% CI) [b]					
Unadjusted	(referent)	-0.09 (-0.29-0.12)	0.05 (-0.15-0.24)	0.05 (-0.15-0.26)	0.37
Model 1.1 [c]	(referent)	-0.11 (-0.30-0.09)	-0.01 (-0.21-0.18)	-0.08 (-0.30-0.13)	0.67
Fatty fish intake	747	806	866	757	
Gestational wk (mean, std)	38.8, 1.9	38.9, 2.5	38.8, 1.9	39.0, 1.8	
Change (wk) (95% CI) [b]					
Unadjusted	(referent)	0.11 (-0.09-0.32)	0.06 (-0.15-0.26)	**0.21 (0.00-0.42)**	0.10
Model 1.1 [c]	(referent)	-0.03 (-0.23-0.17)	-0.03 (-0.23-0.17)	0.16 (-0.06-0.38)	0.17

[a] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory} + Confounding\ Variables$, for Y = gestational week

[b] Changes in mean gestational week were calculated using linear regression, with non-robust standard errors

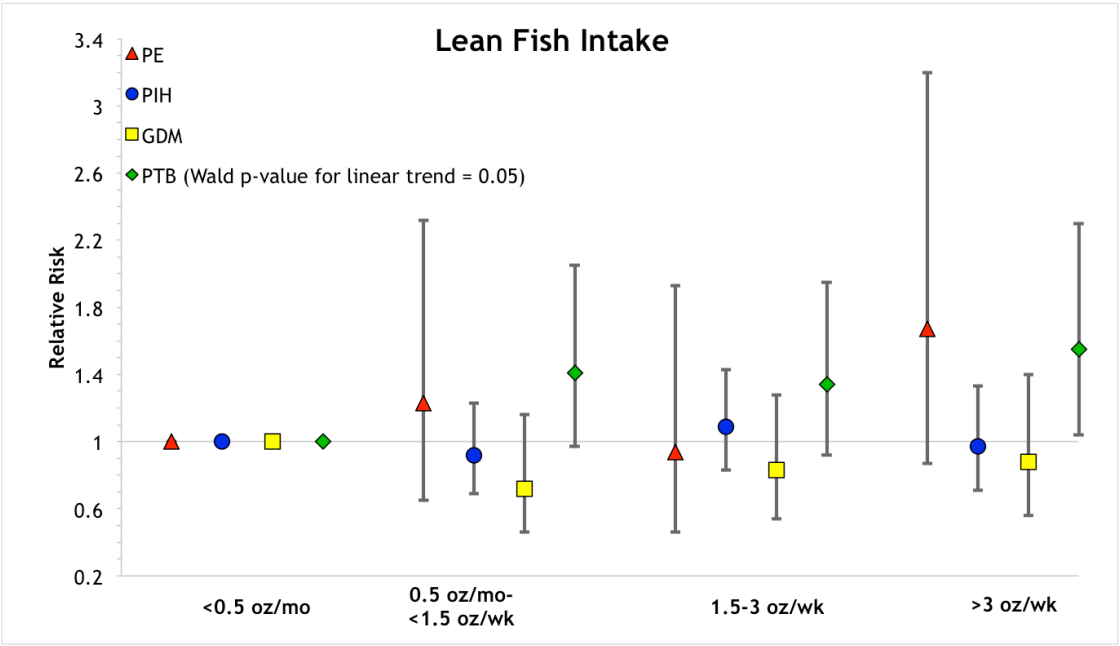
[c] Model 1.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex. For seafood subtypes (shell-, lean- and fatty-fish, alternate seafood subtypes are also included in Model 1.1 as indicator variables for non-reference categories.

Figure 1.1 Associations of **shellfish intake** with risk of pregnancy complications [a]



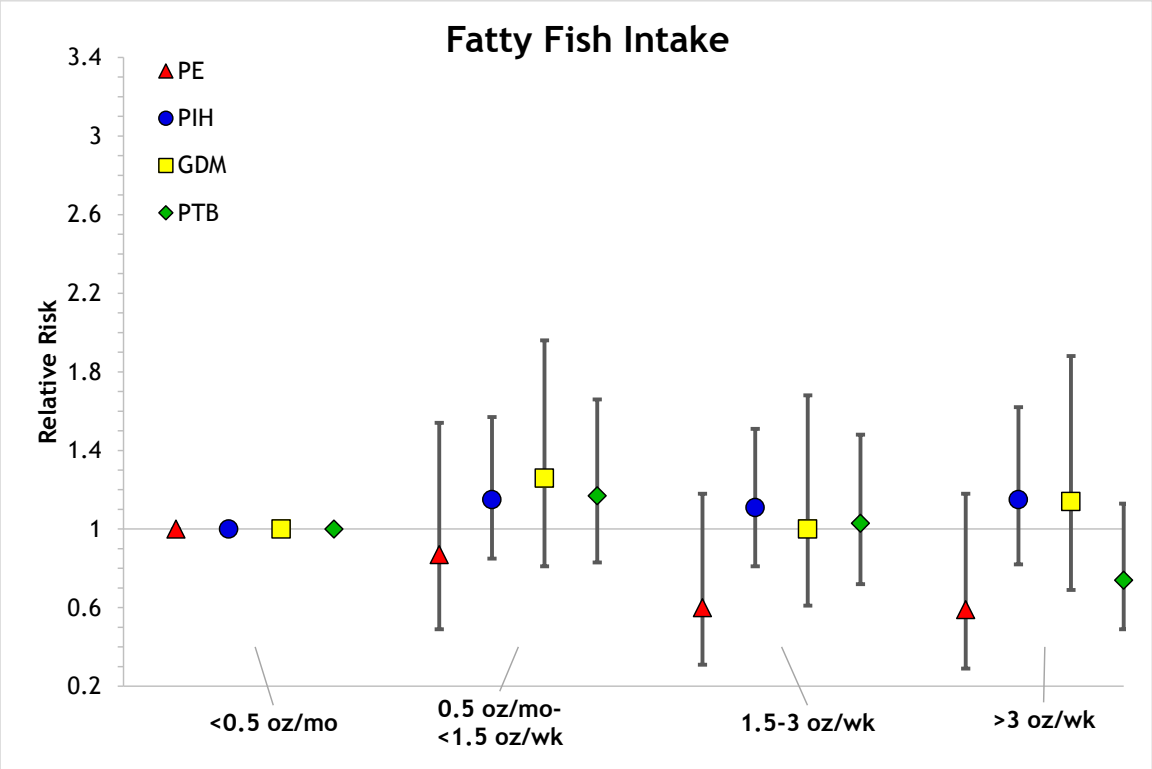
[a] Table 1.2c, Model 1.1

Figure 1.2 Associations of **lean fish intake** with risk of pregnancy complications [a]



[a] Table 1.2d, Model 1.1

Figure 1.3 Associations of **fatty fish intake** with risk of pregnancy complications [a]



[a] Table 1.2e, Model 1.1, Wald p-value for a grouped linear term among women who consumed at least 0.5 oz/mo was statistically significant for PTB, p-value =0.02

Chapter 2: Maternal periconceptional seafood intake and fetal growth

ABSTRACT

Background: Previous reports of associations of maternal seafood intake with fetal growth have been inconsistent and few investigations have been conducted among US populations. Further, little is known whether these associations differ across seafood subtypes, fetal growth indices, or infant sex.

Methods: We investigated associations of maternal periconceptional seafood intake with fetal growth among participants of the Omega study (N = 3,141), a prospective cohort study of risk factors for pregnancy complications and outcomes. Periconceptional shell-, lean-, and fatty-fish intakes, ascertained using a food frequency questionnaire, were categorized according to frequencies of: <0.5 ounces/month (oz/mo), 0.5 oz/mo-<1.5 oz/week (wk), 1.5-3 oz/wk, and >3 oz/wk. Information on fetal growth indices including birth weight (continuous, low birth weight [<2500 g], or macrosomia [≥4000 g]), birth length, ponderal index, and head circumference was abstracted from medical records. We fit generalized linear models with a log link, Poisson family, and robust standard errors to estimate relative risks (RR) and 95% confidence intervals (CIs) for low birth weight or macrosomia across seafood intake categories. We also used linear regression models, adjusted for confounders, to estimate mean differences and 95% CIs for continuous fetal growth indices across seafood intake categories.

Results: Medians (interquartile ranges) of shell-, lean-, and fatty-fish intake were 0.9 (0-2.8), 1.4 (0-2.9), and 1.5 (0.4-3.0) oz/wk, respectively. Lean fish intake of >3 oz/wk (versus <0.5 oz/mo) was associated with a 2.2-fold higher risk of low birth weight (95% CI: 1.21-4.09). Shellfish intake of >3 oz/wk (versus <0.5 oz/mo) was associated with higher mean ponderal index (0.64 kg/m^3 higher, 95% CI: 0.04-1.25 kg/m^3). There was no evidence for associations of shell-, lean-, or fatty-fish intake with other fetal growth indices. In secondary, infant sex-stratified analyses, higher lean fish intake (3 oz/wk versus <0.5 oz/mo) was associated with higher risk of low birth weight among male (RR= 3.26, 95% CI: 1.36-7.82) but not among female (RR= 1.77, 95% CI: 0.76-4.14) infants (interaction p-value = 0.43). Similarly, higher shellfish intake (>3 oz/wk versus <0.5 oz/mo) was associated with higher mean ponderal index among female (1.10 kg/m^3 higher 95% CI: 0.31 to 1.89 kg/m^3) but not among male (0.25 kg/m^3 95% CI: -0.67 to 1.17 kg/m^3) infants (interaction p-value = 0.17).

Conclusion: Higher intakes of lean- and shell-fish were associated with a higher risk of low birth weight and higher mean ponderal index, respectively. Findings also suggest that associations of seafood intake

with fetal growth indices may vary by infant sex, though we did not observe statistically significant interactions. Replication efforts, larger studies to assess interactions, and mechanistic investigations are possible areas of future research.

BACKGROUND

Early observations in the 1980s of higher birth weight and birth length infants among residents of a fish-eating community of the Faroe Islands, compared with residents of Denmark, led to the hypothesis that seafood intake during pregnancy may influence fetal growth[16,95,96]. Seafood is a primary dietary source of n-3 long chain polyunsaturated fatty acids (LCPUFA), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 20:6n-3) that influence the regulation of numerous biological pathways. Diets containing EPA and DHA can lead to alterations in eicosanoid hormone profiles, which are important for regulating inflammation, and maternal-fetal blood flow[12,13,97]. In addition, LCPUFAs form 10-15% per volume of the cell membrane lipid bilayer and are critical to normal cell membrane development and function[21]. It has been hypothesized that EPA and DHA may support offspring insulin-glucose homeostasis[6,18] through effects on cell membrane development *in utero*[21]. Therefore, seafood and/or EPA+DHA intake may have diverse beneficial effects on fetal growth. On the other hand, seafood intake is also associated with consumption of xenobiotics and contaminants (such as heavy metals) that may have adverse consequences on the intrauterine environment and the developing fetus.

Higher maternal seafood or EPA+DHA intake has been associated with modestly higher fetal growth indices including higher birth weight[88,89,98,99], birth length[99] and head circumference[88,89,99], and related lower risk of low birth weight or small for gestational age[51,55,88,100-102] status in some studies. However, other investigators have reported associations of higher intake of seafood or EPA+DHA with lower fetal growth indices (or small for gestational age status)[50,87,103]. Further, some investigators have reported no associations[104,105] of maternal seafood or EPA+DHA intake with fetal growth. Besides these inconsistences, findings also suggest possibilities of different relationships based on the type of seafood intake[51,87,88,104,106,107]. For example, investigators have reported that higher intake of fatty fish, or large oily fish[87,106], shellfish[51,104], or crustaceans[107] was associated with lower birth weight (or higher risk of low birth weight or small for gestational age status). Associations of higher intake of canned tuna have been inconsistent, some investigators have reported associations with higher birth weight[106], while others have reported associations with small for gestational age status[107]. The beneficial or adverse effects of seafood intake during pregnancy on fetal growth may depend on a number of factors that

include the timing of intake (early or late pregnancy), seafood species, and/or underlying population characteristics. These factors were heterogeneous across previous studies and most factors are highly dependent on geographic location. Few investigators have examined the associations of early pregnancy seafood and seafood subtype intake with fetal growth in the US[50,105], where intake is generally low to modest relative to intake among most of the previously studied populations. Most previous investigations have focused on birth weight and little is known about the associations of seafood and EPA+DHA intake with other indices of fetal growth, including ponderal index, a measure of asymmetric growth.

Despite the consistent observation that intrauterine growth and development of male and female fetuses[108-112] differ[108-112], as reflected by sex differences in birth weight, prior studies of maternal seafood intake and fetal growth have not examined potential differences in associations by infant sex. Accumulating evidence supports potential sex-specific differences in associations of other *in utero* exposures and fetal growth[113,114], including nutrients (i.e. DHA[115], Vitamin D[116]) and contaminants (i.e. cadmium[117], polychlorinated biphenyls [PCBs][118]) found in seafood. In addition, evidence from animal experimental studies suggests that perinatal n-6/n-3 PUFA ratio may have long-term effects on cardiometabolic parameters, primarily among male offspring[20], and bone parameters, primarily among female offspring[119] that persist into adulthood. To our knowledge, no prior study has investigated sex-specific differences in seafood intake and fetal growth associations.

Our primary objective was to investigate associations of maternal periconceptional seafood intake on fetal growth indices: birth weight, birth length, ponderal index, and head circumference. We hypothesized that associations between seafood intake and fetal growth indices vary by seafood subtype. In secondary analyses, we examined whether these associations differ by infant sex.

METHODS

Overview and Study Setting

The Omega study is a prospective cohort study designed to examine dietary and metabolic risk factors associated with pregnancy complications and adverse pregnancy outcomes. Participants were recruited among women attending prenatal care clinics affiliated with Swedish Medical Center and Tacoma General Hospital in Seattle and Tacoma, Washington, respectively[19,75,76]. Eligible participants, pregnant women who initiated prenatal care at or before 20 weeks gestation, were 18 years of age or older, were able to speak and read English, planned to carry the pregnancy to term, and to deliver at either of the two hospitals. Participants were enrolled in this study from 1996-2008. The protocol for the study was approved by the Institutional Review Boards of Tacoma General Hospital and Swedish Medical Center. All study participants provided informed consent.

Study Population

During the study period 5,063 eligible women were approached and 4,000 (79%) women, who consented to participate, were enrolled in the study. Of these, 3,892 (97%) women completed study follow-up. Among participants who completed the study,118 had multifetal pregnancies, 72 had implausible total daily energy intake of <500 or >3500 kcal, 34 had pregnancies that were complicated by fetal demise (after 20 weeks of gestation), 124 were missing labor and delivery information, and 14 were missing information on all fetal growth indices. These were excluded from the current study. In addition, women with missing seafood intake information (N = 389) were excluded. A total of 3,141 participants remained for analyses.

Data Collection

At or near enrollment (16 weeks gestation on average), trained interviewers conducted in-person interviews (45-60 minutes in length) to collect data on socio-demographic characteristics, reproductive and medical histories, height and pre-pregnancy weight, recreational physical activity, alcohol and tobacco intake, environmental tobacco smoke exposure, medication use, and family medical history. Participants completed a self-administered, validated, semi-quantitative food-frequency questionnaire (FFQ) to assess diet during the periconceptional period (the three months before and the first three months of index pregnancy)[19]. The FFQ had originally been developed for and used in the Women's

Health Initiative Clinical Trial[77]. FFQs were analyzed using food composition values obtained from the University of Minnesota Nutrition Coding Center nutrient database[78]. Participants also completed a supplementary Seafood Intake Scale (SIS) FFQ[79]. The SIS FFQ included questions regarding usual frequency and serving size in the prior three-month period for 25 different types of finfish and 10 types of shellfish available in the Pacific Northwest (see Appendix Table A1.0). Participants also provided non-fasting peripheral blood samples at this initial visit. Blood samples were analyzed for erythrocyte membrane fatty acids, including EPA and DHA, among a randomly selected subset (60%) of initial participants (enrolled from 1996-2000 in the Omega study, N = 586)[19]. After delivery, trained personnel conducted maternal and infant medical records abstraction to ascertain course and outcomes of pregnancy.

Exposure Characterization

Seafood subtypes were categorized into fatty-, lean-, or shell-fish groups as described in Appendix, Table A1.0. Briefly, shellfish included crab, lobster, shrimp or prawns, clams, mussels, oysters, scallops, abalone, octopus, and squid. Lean fish included regular canned tuna, catfish, cod, flounder or sole, haddock, halibut, mahi mahi, snapper or rockfish, shark, imitation crab, imitation lobster, and fish sticks. Fatty fish included anchovies, herring (pickled or regular), kipper snacks, salmon (canned, fresh or smoked), sardines, albacore tuna, swordfish, rainbow trout, smelt, and mackerel. Using information from the SIS FFQ, we calculated the amount of monthly intake of each seafood subtype by multiplying the monthly frequency of intake by the serving size. The lowest amount of seafood that could be reported on the SIS FFQ was <0.5 ounces/month (oz/mo).

Outcome Definitions

Information on infant birth weight, birth length, and head circumference was abstracted from medical records. Categories of low birth weight (<2500 g), normal birth weight (2500-3999 g), and macrosomia (≥4000 g) were defined according to the World Health Organization recommendation[120]. Infant ponderal index was calculated as birth weight (kg) divided by the birth length cubed (m^3)[121].

Statistical Analysis

We examined frequency distributions of maternal characteristics across categories of seafood subtype intake and across quartiles of maternal erythrocyte membrane EPA+DHA. For total seafood, and seafood subtypes, we chose categories of <0.5 oz/mo, 0.5 oz/mo-<1.5 oz/wk, 1.5-3 oz/wk, and >3 oz/wk, to allow for approximately equal frequencies of subjects across seafood subtype categories above the reference (<0.5 oz/mo). A typical single serving of seafood is about 3 oz according to the American Heart Association[85].

To estimate mean differences and 95% confidence intervals (95% CIs) for continuous indices of fetal growth across each category of seafood or EPA+DHA quartile relative to the reference group, we fit linear regression models. To estimate relative risks (RRs) and 95% CIs for low birth or macrosomia relative to normal birth weight infants associated with each category of seafood intake (or EPA+DHA quartile), we fit generalized linear models with a log link, Poisson family (a "Log-Poisson" regression model), and robust standard errors. In the low birth weight analyses, macrosomic infants were excluded while in the macrosomia analyses, low birth weight infants were excluded. The Log-Poisson regression model with robust standard errors allows estimation of RRs for prospective studies with binary outcome data[86]. In these models, each non-reference category of seafood intake or EPA+DHA quartile was modeled as an indicator variable. Model fit was assessed by examination of regression residual diagnostics. Based on prior literature that suggests potential linear trends across higher seafood intake categories and EPA+DHA levels[50,87-89], we calculated Wald p-values for grouped linear terms of either seafood subtype or EPA+DHA (i.e. β_1, see Example Equation A2.1 in Appendix Table A3.0b). All p-values were 2-sided and defined to be significant at $p<0.05$.

The following variables were identified *a priori* and considered as potential confounding variables and were included in all adjusted models including our primary adjusted model (Model 2.1). Non-Hispanic white race/ethnicity, high school or less education, unmarried marital status, nulliparity, habitual recreational physical activity during index pregnancy based on activity in the week prior to interview, alcohol intake, and cigarette smoking during index pregnancy were included as binary variables. Three

non-referent indicator variables for pre-pregnancy body mass index (BMI), based on the National Institute of Health current definitions of underweight (referent, <18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2)[90] status, were included in all adjusted models. Maternal age (years), total energy consumed (kcal/day), and intakes of red and processed meats (servings/day) were included in all adjusted models as continuous variables. Adjusted models for seafood subtypes as the exposure included non-referent indicator variables for intake of shell-, lean-, and fatty-fish simultaneously. For example, to observe associations of shellfish with fetal growth indices, independent of fatty- or lean-fish, we included non-referent indicator variables for all three seafood subtypes (see Example Equation A2.2 in Appendix Table A3.0b). We additionally adjusted for enrollment year (Model 2.2) to examine whether our main findings were sensitive to potential confounding by the year the subject was enrolled. We defined five non-referent (1996-1997 was the referent) indicator variables for enrollment year: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007. Model 2.3 was used to examine whether associations of seafood intake/EPA+DHA status with fetal growth indices were mediated by gestational age at delivery (weeks, modeled continuously)[16,58,96]. Model 2.4, also a sensitivity analysis, was used to examine whether our results were materially altered by exclusion of subjects who experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth (birth <37 weeks gestation). A detailed description of the variables included in each of the models and how they were parameterized is given in Appendix Table A3.0a.

To examine whether associations of seafood intake with fetal growth indices varied across infant sex, in secondary analyses, we repeated our analyses described above among strata defined by infant sex. The Wald test was used to calculate p-values for the seafood (or seafood subtype)-infant sex interaction terms. Analyses were carried out using Stata Software (version 10.1; StataCorp LP, College Station, TX).

RESULTS

Selected study population characteristics are described in Table 2.1a. Participants in our study tended to be non-Hispanic white (88%), married (90%), nulliparous (62%), and have higher educational attainment (97% post-high school education). A minority of subjects smoked cigarettes (5%) or consumed alcohol (26%) during the index pregnancy and few indicated intake of fish oil supplements (1%). Mean (standard deviation, SD) maternal age was 32.7 (4.3) years. Median (interquartile range, IQR) total seafood intake was 4.7 (2.1-8.4) oz/wk. Medians (IQRs) of shell-, lean-, and fatty-fish intake were 0.9 (0-2.8), 1.4 (0-2.9), and 1.5 (0.4-3.0) oz/wk, respectively. Means (SDs) infant birth weight for all, female, and male infants were 3460.4 (534.6) g, 3391.2 (515.2) g, and 3528.4 (544.4) g, respectively. Among all infants, 123 (4%) and 449 (14%) were low birth weight and macrosomic, respectively. Among female infants (N = 1552), 63 (4%) and 164 (11%) were low birth weight and macrosomic, respectively. Among male infants (N = 1588), 60 (4%) and 285 (18%) were low birth weight and macrosomic, respectively. Means (SD) for birth length, infant head circumference, and ponderal index were 50.9 (3.1) cm, 34.7 (2.2) cm and 26.5 (5.3) kg/m^3, respectively.

There were some differences among subjects who were excluded due to missing seafood intake information or implausible seafood/energy intake (N = 461) and those who were included in this study (Table 2.1a). Compared to included subjects, excluded subjects were less likely to be non-Hispanic white (71% versus 88%), nulliparous (40% versus 62%), or married (59% versus 90%). Excluded subjects were more likely to be obese (17% versus 8%). Subjects were similar across indices of fetal growth.

Selected baseline maternal and infant characteristics across intake categories of total seafood and seafood subtypes, and maternal erythrocyte EPA+DHA quartiles (for the subset of participants who had these measurements, N = 534) are described in Tables 2.1b-f. Higher intake of all seafood subtypes or maternal EPA+DHA was associated with post high school education and some alcohol intake during pregnancy. Higher intake of lean fish was associated with non-white race, recreational physical activity during early pregnancy, and enrollment in earlier study years. Women with higher intake of shellfish or who were in the higher EPA+DHA quartiles were more likely to be nulliparous compared women with lower intake of shellfish or who were in the lower quartiles for erythrocyte EPA+DHA,

respectively. Women with higher fatty fish intake or EPA+DHA were less likely to have smoked during the index pregnancy. Women with seafood intake (across all subtypes) of >3 oz/wk compared to <0.5 oz/mo reported higher daily total energy, fat, red, and processed meat intake.

Tables 2.2a-b and Figures 2.1-2.2 display RRs and associated 95% CIs for low birth weight and macrosomia corresponding to categories of seafood subtype or total seafood (all subtypes combined) intake. We found evidence for an association of higher lean fish intake with higher risk of low birth weight (linear trend p-value = 0.02) (Table 2.2a, Figure 2.1). Lean fish intake of >3 oz/wk compared to the reference category (<0.5 oz/mo) was associated with a 2.2-fold higher risk of low birth weight (95% CI: 1.21-4.09). Intakes of shellfish, fatty fish, and total seafood were not associated with risk of low birth weight (Table 2.2a); and, intakes of seafood subtypes and total seafood were not associated with risk of macrosomia (Table 2.2b).

Maternal periconceptional intake of seafood (all subtypes combined) and maternal erythrocyte EPA+DHA were not associated with continuous fetal growth indices (Tables 2.2c-d). Tables 2.2e-g display mean differences and Figures 2.3a-l display box and whisker plots for continuous fetal growth indices across intake categories of shell-, lean-, and fatty-fish. Except for associations of shellfish intake with ponderal index, we found limited evidence for associations of seafood subtypes with continuous fetal growth indices. Intake of >3 oz/wk of shellfish compared to intake of <0.5 oz/mo (reference) was associated with a mean ponderal index that was 0.64 kg/m^3 (95% CI: 0.04 to 1.25 kg/m^3, linear trend p-value = 0.07) (Model 2.1, Table 2.2e).

Additional adjustment for study year (Tables 2.2a-g, Model 2.2) and exclusion of infants (N = 785) born to mothers who experienced a pregnancy complication during the index pregnancy (Tables 2.2a-g, Model 2.4) did not materially alter the results. Except for the association of total seafood and risk of low birth weight, our main findings were robust to further adjustment for gestational week of delivery (Tables 2.2a-g, Model 2.3). In sensitivity analyses, when we further adjusted for gestational week of delivery, all categories of seafood intake above the reference category of <0.5 oz/mo were associated

with a higher risk of low birth weight (Table 2.2a, Model 2.3). RRs and (95% CIs) for total seafood intake of 0.5/mo-<1.5/wk, 1.5-3/wk, and >3/wk oz compared to intake of <0.5 oz/mo were 5.40 (1.37-21.21), 5.14 (1.39-19.05), and 3.64 (1.05-12.68) (linear trend across higher intake categories p-value = 0.30).

Tables 2.3a-b display our secondary analyses of infant-sex stratified associations of seafood subtype intake and risk of low birth weight or mean change in ponderal index. Adjusted RRs (and 95% CIs) for associations of lean fish intake of >3 oz/wk compared to the reference with low birth weight, were 3.26 (1.36-7.82) and 1.77 (0.76-4.14) for male and female infants, respectively (Table 2.3a, Model 2.1). However, the Wald p-value for lean fish (grouped linear variable)-infant sex interaction was 0.43. Associations of shell- and fatty-fish intake with risk of low birth weight did not appear to vary across infant sex (Table 2.3a). Among female infants, compared to the reference category (intake of <0.5 oz/mo), intake of >3 oz/wk of shellfish was associated with a higher mean ponderal index (1.10 kg/m^3 higher 95% CI: 0.31 to 1.89 kg/m^3) (linear trend p-value = 0.02) (Table 2.3b Model 2.1). The corresponding mean change in ponderal index (and 95% CI) among male infants was 0.25 kg/m^3 higher (95% CI: -0.67 to 1.17 kg/m^3). The Wald p-value for the shellfish (grouped linear variable)-infant sex interaction term was 0.17 (Model 2.1). Associations of lean- and fatty-fish intake with mean ponderal index did not vary across infant sex.

We explored model fit using residual diagnostic procedures and identified several potential outlying and influential points. Except shellfish intake and ponderal index, further examination of these observations did not suggest erroneous or implausible characteristics for these subjects and our main study findings were robust to the exclusion of these observations. Of note, we found that the 23 subjects with ponderal indices of 40 kg/m^3 or higher were influencing the association of higher shellfish intake and higher mean ponderal index. After we excluded these subjects (N = 23) and compared to intake of <0.5 oz/mo of shellfish, mean differences in ponderal index (95% CIs) corresponding to intake of 0.5 oz/mo-<1.5 oz/wk, 1.5 – 3.0 oz/wk, >3 oz/wk were 0.28 higher (-0.06 to 0.62), 0.22 higher (-0.12 to 0.57), and 0.24 higher (-0.14 to 0.62) kg/m^3, linear trend p-value = 0.25 (Model 2.1). Exclusion

of subjects with ponderal indices of at least 80 kg/m^3 (N = 7) led to mean differences in ponderal index (95% CIs) corresponding to intake of 0.5 oz/mo-<1.5 oz/wk, 1.5 – 3.0 oz/wk, >3 oz/wk compared to <0.5 oz/mo of shellfish intake were 0.36 higher (-0.08 to 0.79), 0.26 higher (-0.18 to 0.70), and 0.32 higher (-0.16 to 0.81) kg/m^3, linear trend p-value = 0.21 (Model 2.1).

DISCUSSION

In the current study, we found associations of higher maternal periconceptional lean fish intake with higher risk of low birth weight and associations of higher shellfish intake with higher mean ponderal index. Women with lean fish intake of >3 oz/wk, compared with those with intake of <0.5 oz/mo, had a 2.2-fold higher risk of low birth weight (95% CI: 1.21-4.09) (linear trend p-value= 0.02). Women with shellfish intake of >3 oz/wk, compared with women with intake of <0.5 oz/mo, had infants with higher mean ponderal index (0.64 kg/m^3 higher 95% CI: 0.04 to 1.25 kg/m^3) (linear trend p-value = 0.07). We also found suggestive evidence that associations of maternal periconceptional seafood intake and fetal growth may vary by infant sex, although our tests for interaction were not statistically significant.

To our knowledge, only two previous US cohort studies of maternal fish/seafood intake and fetal growth. In Project Viva (N = 2,109), Oken *et al*. examined associations of total seafood and EPA+DHA intake during the 1st, 2nd and 3rd trimesters of pregnancy with fetal growth indices: birth weight, birth-weight-for-gestational-age (fetal growth) z value, low birth weight, and small for gestational age[50]. They reported a 70 g (95% CI: -18 to 158 g, p-value for trend = 0.05) higher birth weight comparing the lowest category of first-trimester seafood intake (<1 serving/mo) to the highest category of intake (2> servings/wk) and a 94 g (95% CI: 23 to 166 g, p-value for trend = 0.01) higher birth weight comparing the lowest (mean = 0.02 g/day) to the highest (mean = 0.36 g/day) quartiles of EPA+DHA intake. Risk of low birth weight and small for gestational age were not associated with total seafood or EPA+DHA intake in Project Viva. Unlike the findings in the Project Viva study, we did not observe associations of early pregnancy total seafood intake or EPA+DHA with mean birth weight. In our sensitivity analyses we did observe an association of higher total seafood intake and higher risk of low birth weight among women when we additionally adjusted for gestational age at delivery (Table 2.2a, Model 2.3). The

association of higher total seafood and higher risk of low birth weight may have been driven by the association of higher lean fish intake and higher risk of low birth weight, which was evident when we did and did not adjust for gestational age at delivery (Table 2.2a, Models 2.1 and 2.3). The Omega and Project Viva studies were similar in the total amount of seafood and EPA+DHA intake. Mean seafood intakes were about 5-6 oz/wk, assuming 1 serving = 3 oz in both studies[122]. Intake of EPA+DHA ranged from 0-2.5 g/day in Project Viva[50] and from 0-1.2 g/day in the Omega cohort. In addition, few women, 1% or less, in both cohorts reported intake of fish oil supplements. Despite these similarities, the contribution of various seafood subtypes to overall intake differed and may account for some of the differences in findings across the two studies. For example, intake of canned tuna was higher among Project Viva versus Omega participants, 36% versus 19% of total seafood, respectively[122]. Also, shellfish and fatty fish intake was lower among Project Viva participants, compared with Omega study participants, 25% and 17% versus 31% and 25%, respectively.

Results from the New York State Angler Cohort Study (N = 2,716) suggested that the number of years prior to the index pregnancy of maternal intake of sport caught fish was not associated with birth weight, birth length, ponderal index, or head circumference[105]. While the assessment period for this study of maternal life-time fish intake differs from ours (periconceptional period), it may be a better approximation for the accumulated effect of adverse fish-related exposure such as heavy metals or PCBs.

We found associations of higher lean fish intake with higher risk of low birth weight. Unlike our findings, previous investigations of maternal lean fish intake with fetal growth found associations of higher intake with higher birth weight[88] or no associations[87,106,107]. In studies of men and non-pregnant women, adverse cardiovascular outcomes have been associated with fried fish[47,74]. Generally fried fish tends to include lean fish species rather than fatty fish or shellfish and there is evidence that trans-fats often used in frying are associated with low birth weight[123]. Also, frying can lead to oxidation and lower levels EPA and DHA[74]. We did not have information on how the different seafood subtypes were prepared and were unable to further examine this potential mechanism. Higher maternal

methylmercury (MeHg) exposure has been associated with lower mean birth weight in previous studies[124,125]. Lean fish did include shark in our definitions, which is a predatory fish that the Environmental Protection Agency and the Food and Drug Administration advisory recommends pregnant women to avoid consuming due to higher levels of MeHg[85]. On the other hand, our definition of fatty fish included mackerel and swordfish, also predatory fish that the advisory recommends avoiding, and fatty fish was not associated with higher risk of low birth weight. We were unable to determine the level of MeHg exposure (or exposure to other contaminants e.g. PCBs) due to seafood intake. In sum, were unable to determine whether the association between higher intake of lean fish and higher risk of low birth weight in our study could be explained by how the fish was prepared, levels of MeHg or other contaminants, or whether the association could be attributed to residual confounding.

We found suggestive evidence of higher intake of shellfish with higher ponderal index. No prior study has investigated associations of seafood subtypes with ponderal index. Ponderal index is commonly used to capture infant body composition or proportionality and is similar to BMI[126]. Ponderal index at birth has been positively associated with BMI at adolescence[127] and early adulthood[128], and C-reactive protein in adult males (16% higher CRP, 95% CI: 8-23% per 1 kg/m^3 lower ponderal index)[129]. High ponderal index has been used to identify disproportionately large infants, those with excessive fetal fat accumulation versus those, which are genetically large[130]. The difference in mean ponderal index observed in our study was modest 0.64 kg/m^3 higher comparing shellfish intakes of >3 oz/wk to <0.5 oz/mo, and it is unclear whether these results have clinical significance.

Importantly, it should be noted that in our examination of model fit for the association of higher shellfish intake and higher mean ponderal index using regression residual diagnostics, we found that the 23 subjects with ponderal indices of 40 kg/m^3 or higher were influencing the association. Compared to shellfish intake of <0.5 oz/mo, mean differences in ponderal index (95% CI) corresponding to intake of >3 oz/wk were 0.24 kg/m^3 higher (-0.14 to 0.62) kg/m^3 when the 23 subjects were excluded compared to 0.64 kg/m^3 higher (0.04 to 1.25 kg/m^3) when the 23 subjects were included. Although the ponderal indices for these 23 subjects were high, ranging from 40.1 to 100.1 kg/m^3, the

birth weights and birth lengths were within plausible ranges (see Figure 2.4). Since we could not be certain whether these values of ponderal index were erroneous, we retained them in the analyses.

While available evidence strongly supports different intrauterine growth patterns, trajectories, and birth size differences between male and female infants[108-112], prior studies of early pregnancy seafood intake and fetal growth have adjusted for infant sex without examining the potential for differences or effect modification by infant sex. Recent evidence supports the possibility for fetal sex specific differences in environmental (including the intrauterine environment) risk factors, such as nutrient and xenobiotic exposure, on fetal growth and adulthood disease[20,113,114,119]. One study by Kilari *et al*. examined infant-sex stratified associations of maternal erythrocyte DHA at delivery with low birth weight status[115]. Kilari *et al*. reported that DHA levels were significantly lower among low birth weight infants versus normal birth weight infants only among female infants (p-value <0.05), although the p-value for infant sex interaction was not reported. Associations of higher lean fish intake with low birth weight were statistically significant only among male infants. We also observed statistically significant associations for higher intake of shellfish and higher mean ponderal index only among female infants. However our sex-specific findings should be interpreted with caution since our tests for interaction were not statistically significant and our current study may be underpowered to formally assess statistical interaction.

Our findings should be interpreted in view of several potential limitations. First, we cannot rule out the possibility of exposure measurement error. Since seafood intake was evaluated prospectively, we suspect that the potential exposure measurement error for seafood intake to be non-differential with respect to fetal growth indices and may have led to estimates that were attenuated toward the null. Second, we evaluated associations between multiple exposures and outcomes, which leads to increased type I error. Therefore, our results should be interpreted with caution since we have not adjusted for multiple comparisons. Third, our overall study statistical power may be limited, particularly for the maternal erythrocyte EPA+DHA or infant sex-interactions analyses. Fourth, although we modeled seafood intake categorically to allow for some flexibility to observe non-linear associations, we did not

statistically examine threshold effects. Threshold effects have been observed for modest intake of seafood (or up to approximately 250-500 mg/day EPA+DHA) and cardiovascular outcomes in the general population[7]. Our analyses findings, however, do not suggest a threshold effect. Fifth, we did not consider the role of other factors, such as xenobiotic exposure or how the seafood was prepared, when we examined the relationships of seafood intake with fetal growth indices. Sixth, although we adjusted for multiple confounding variables, we cannot exclude the possibility of residual confounding due to unmeasured or imprecisely measured confounding factors. Finally, our study participants were mostly non-Hispanic white, married, highly educated, with normal pre-pregnancy BMI, and who registered for prenatal care early in pregnancy and participated in regular annual medical examinations. Therefore, our results may not be generalizable to women of different race/ethnicity or socioeconomic status.

Our study has several strengths. First, to our knowledge, this is the first US cohort study to comprehensively examine associations of periconceptional intake of seafood subtypes with multiple fetal growth indices. Our study included detailed information on seafood intake, much more detailed than traditional food frequency questionnaires, which we used to address a research gap on the potential varied effects of seafood subtypes. Access to blood samples in a subset of participants permitted us to assess associations of maternal erythrocyte EPA+DHA status with fetal growth. These biomarker data also allowed us to evaluate the correlation of EPA+DHA with self-reports of intakes of seafood and EPA+DHA, covering the periconceptional period (EPA+DHA $r = 0.53$, p-value <0.001). Correlations were as high or higher than previous reports[57]. Our cohort is well characterized with high participation and follow-up rates, which allowed us to control for many previously reported confounding variables and to determine that our results were robust in several sensitivity analyses. Our results were not materially altered by further adjustments for study year or gestational week of delivery, or when complicated pregnancies were excluded.

In sum, our study adds to the growing body of research investigating the potential heterogeneous effects of maternal seafood intake on pregnancy outcomes, and is the first to examine seafood

subtypes in a US cohort in relation to fetal growth. Our findings suggest that higher maternal periconceptional lean fish intake is associated with higher risk of low birth weight, and higher maternal periconceptional shellfish intake is associated with higher mean ponderal index. Findings also suggest that associations of seafood intake with fetal growth indicators may vary by infant sex, though our sex-specific associations should be interpreted with caution since we did not find statistically significant interactions. Future studies in different study populations as well as studies that examine the role of seafood preparation, contaminants, infant sex, and potential mechanisms for observed associations are warranted.

Table 2.0 Selected previous investigations of maternal seafood intake or marine n-3 LCPUFA status and fetal growth from the literature

Author, Year	Location	Design	Exposures	Outcomes	N	Significant Associations [a]
Heppe, 2011[104]	Netherlands	PC	FFQ: fish	BW, HC, BL	3380	No statistically significant assoc.
Courville, 2011[131]	US	RCT	DHA-FF versus placebo	PI	47	Inverse
Makrides, 2009[66]	Several	Review of 6 RCTs	Suppl. versus placebo	BW, LBW, BL	BW: 2440, LBW: 2302, BL: 824	BL, BW: positive
Drouillet, 2009[100, b]	France	PC	FFQ: fish	BWa, HC, BL	1805, pBMI ≥25: 464	BWa, HC, BL: positive (among pBMI ≥25 only)
Ramon, 2009[106]	Spain	PC	FFQ: total fish, canned tuna, lean, or large oily fish [c]	BWa, BL, SGA	554	Canned tuna, BWa: positive Large oily fish, SGA risk: positive
Halldorsson, 2007[87]	Denmark	PC	FFQ: total, lean, or fatty fish [d]	BWa, HC, BL	44824	Total fish, HC, BL: inverse Fatty fish, BWa, HC, BL: inverse
Guldner, 2007[51]	France	PC	FFQ: fish, shellfish	BWa, LBW, SGA	2278	Shellfish, SGA risk: positive
Olafsdottir, 2005[132]	Iceland	PC	FFQ: fish, suppl.	BWa	435	Suppl., BWa: positive
Oken, 2004[50]	US	PC	FFQ: fish, EPA+DHA	BWa, LBW	2109	Fish, BWa: inverse EPA+DHA, BWa: inverse

Abbreviations: *RCT, randomized controlled trial; PC, prospective cohort; Suppl., n-3 LCPUFA/fish oil supplement; FFQ, food frequency questionnaire; DHA-FF, DHA functional food; BW, birth weight; BWa, birth weight adjusted for gestational age; LBW, birth weight <2500g; SGA, small for gestational age = BW < 10th percentile of BW distribution for a given gestational age and sex in the given population; HC, head circumference; BL, birth length; pBMI, prepregnancy body mass index (kg/m^2).*

Footnotes: *[a] Statistically significant associations (two sided p-value < 0.05). [b] Interaction p-value for overweight (pBMI ≥ 25) < 0.05 [c] Large oily fish included swordfish, bonito, and fresh tuna [d] Fatty fish included salmon, herring, mackerel, trout, and Greenland Halibut.*

Table 2.1a Selected characteristics of the analytic population versus excluded subjects due to missing SIS FFQ information or implausible seafood/energy intake

Maternal characteristics, pregnancy and fetal growth outcomes	Included (N = 3141)			Excluded, missing SIS FFQ or implausible seafood/energy intake (N = 461)		
	N	%		N	%	
Non-Hispanic white race/ethnicity [a, e]	2746	87.6		326	70.7	
High school or less [a, e]	99	3.2		30	6.5	
Unmarried [e]	317	10.1		188	40.8	
Nulliparous [e]	1941	61.8		184	39.9	
Pre-pregnancy Body Mass Index (kg/m^2) [a, e]						
<18.5	130	4.1		23	5.0	
18.5-<25	2240	71.3		264	57.3	
25-<30	512	16.3		88	19.1	
≥30.0	257	8.2		80	17.4	
Smoked during pregnancy [a, e]	171	5.4		31	6.7	
Any alcohol intake during pregnancy [e]	813	25.9		96	20.8	
No recreational physical activity during early pregnancy [a, e]	566	18.0		88	19.1	
Fish oil supplements	44	1.4		5	1.1	
Family history of hypertension	1552	49.4		235	51.0	
Family history of diabetes [e]	434	13.8		98	21.3	
Pregnancy complication [a, d, e]	785	25.0		134	29.1	
Enrollment Year [a, e]						
1996-1997	168	5.3		14	3.0	
1998-1999	485	15.4		29	6.3	
2000-2001	571	18.2		67	14.5	
2002-2003	836	26.6		125	27.1	
2004-2005	680	21.6		126	27.3	
2006-2007	364	11.6		70	15.2	
Male infant sex [a]	1588	50.6		247	55.6	
Infant birth weight [a, e]						
Low birth weight (<2500 g)	123	3.9		31	6.8	
Normal birth weight (2500-3999 g)	2569	81.8		362	78.9	
Macrosomia (≥4000 g)	449	14.3		66	14.4	
	mean	SD		mean	SD	
Maternal age (yrs) [e]	32.7	4.3		31.6	5.7	
Birth weight (g) [a]	3460.4	534.6		3415.4	588.0	
Birth length (cm) [a, e]	50.9	3.1		50.4	3.2	
Head circumference (cm) [a]	34.7	2.2		34.6	2.3	
Ponderal index (kg/m^3) [a]	26.5	5.3		26.9	5.6	
	med	IQR		med	IQR	
Gestational age at delivery (weeks) [a, e]	39	38	40	39	38	40
Weekly intake EPA+DHA (g) [c]	0.5	0.2	1.0	0.4	0.1	1.2
Erythrocyte EPA+DHA (%/total fatty acids) [b, e]	5.3	4.5	6.1	4.9	4.1	5.6
Total energy intake (kcal/day) [a, e]	1641.7	1318.0	2058.0	1895.3	939.6	3936.5
Total fat intake (g/day) [a, e]	57.2	43.3	74.5	66.2	34.0	144.5
Red and processed meats intake (servings/day) [a, e]	0.6	0.3	0.9	0.7	0.2	1.5
Birth weight (g) [a]	3470.0	3154.0	3796.0	3455.0	3102.0	3740.0
Birth length (cm) [a, e]	51.0	49.0	53.0	51.0	49.0	52.0
Head circumference (cm) [a]	35.0	33.0	36.0	35.0	33.0	36.0
Ponderal index (kg/m^3) [a]	26.0	23.9	28.3	26.2	24.1	28.7

[a] Maternal race missing for 6 included and 9 excluded subjects. Education status missing for 83 included and 124 excluded subjects. Pre-pregnancy BMI missing for 2 included and 6 excluded subjects. Smoking status missing for 92 included and 130 excluded subjects. Recreational physical activity during early pregnancy missing for 83 included and 126 excluded subjects. Composite pregnancy complication missing for 55 included and 14 excluded subjects. Enrollment year missing for 37 included and 30 excluded subjects. Infant sex ambiguous/missing for 1 included and 2 excluded subjects. Gestational age of delivery missing for 2 included subjects. Weekly intake of EPA+DHA missing for 391 excluded subjects. Daily energy, fat, and red and processed meat intake missing for 29 included and 347 excluded subjects. Infant birth weight missing for 2 excluded subjects. Infant head circumference missing for 78 included and 7 excluded subjects. Infant birth length and ponderal index missing for 40 included and 5 excluded subjects.

[b] Erythrocyte EPA+DHA measured among 509 included and 40 excluded subjects.

[c] Missing SIS FFQ information precluded the calculation of dietary EPA+DHA.

[d] Pregnancy complication defined as any of the following: PE, PIH, GDM, or PTB.

[e] P-value <0.05 for Pearson's Chi-square test for comparisons across inclusion status for categorical or binary variables or one-way ANOVA test for differences in means across inclusion status for continuous variables.

Table 2.1b Selected characteristics of participants according to amount of **total seafood intake (all subtypes combined),** Omega study

Characteristic	Total seafood intake (ounces)											
	<0.5/mo (N = 286)			0.5/mo-<1.5/wk (N =300)			1.5-<3/wk (N = 521)			>3/wk (N = 2034)		
	N		%	N		%	N		%	N		%
Maternal race/ethnicity [a]												
Non-Hispanic White	259		90.6	266		88.7	456		87.5	1765		86.8
African American	2		0.7	4		1.3	6		1.2	28		1.4
Asian	15		5.2	15		5.0	34		6.5	151		7.4
Other	10		3.5	15		5.0	22		4.2	87		4.3
High school or less [a]	17		5.9	17		5.7	23		4.4	42		2.1
Unmarried	37		12.9	29		9.7	44		8.4	207		10.2
Nulliparous	186		65.0	174		58.0	308		59.1	1273		62.6
Pre-pregnancy Body Mass Index (kg/m^2) [a]												
<18.5	21		7.3	11		3.7	17		3.3	81		4.0
18.5-<25	187		65.4	210		70.0	386		74.1	1457		71.6
25-<30	53		18.5	51		17.0	73		14.0	335		16.5
≥30.0	25		8.7	28		9.3	45		8.6	159		7.8
Smoked during pregnancy [a]	23		8.0	14		4.7	28		5.4	106		5.2
Alcohol intake during pregnancy	64		22.4	66		22.0	122		23.4	561		27.6
No recreational physical activity during pregnancy [a]	58		20.3	59		19.7	103		19.8	346		17.0
Fish oil supplements	1		0.3	5		1.7	7		1.3	31		1.5
Family history of hypertension	136		47.6	152		50.7	258		49.5	1006		49.5
Family history of diabetes	41		14.3	37		12.3	65		12.5	291		14.3
Pregnancy complication [b]	64		22.4	86		28.7	125		24.0	510		25.1
Enrollment year [a]												
1996-1997	15		5.2	12		4.0	25		4.8	116		5.7
1998-1999	45		15.7	45		15.0	64		12.3	331		16.3
2000-2001	46		16.1	58		19.3	94		18.0	373		18.3
2002-2003	71		24.8	76		25.3	144		27.6	545		26.8
2004-2005	66		23.1	67		22.3	129		24.8	418		20.6
2006-2007	37		12.9	34		11.3	61		11.7	232		11.4
Male infant sex [a]	153		53.5	150		50.0	258		49.5	1027		50.5
	mean		SD	mean		SD	mean		SD	mean		SD
Maternal age (yrs)	31.6		4.8	32.0		4.6	32.5		4.2	33.0		4.2
	med	IQR		med	IQR		med	IQR		med	IQR	
Maternal age (yrs)	32	29	35	32	29	35	32	30	35	33	30	36
Pre-pregnancy Body Mass Index (kg/m^2) [a]	22.1	20.5	25.2	22.4	20.5	25.1	22.3	20.6	24.7	22.3	20.6	25.0
Gestational age at delivery (weeks) [a]	39	38	40	39	38	40	39	38	40	39	38	40
Weekly intake EPA+DHA (g)	0.0	0.0	0.0	0.1	0.0	0.1	0.3	0.1	0.4	0.9	0.6	1.3
Erythrocyte EPA+DHA (%/total fatty acids) [c]	4.0	3.3	4.9	4.6	4.3	5.1	4.8	4.4	5.4	5.5	4.8	6.3
Daily intake												

Total energy (kcal) [a]	1514.6	1192.6	1963.6	1471.3	1124.8	1870.9	1545.0	1256.4	1909.9	1720.0	1386.6	2106.0
Total fat (g) [a]	52.8	39.7	68.8	49.8	36.0	66.5	52.4	39.4	69.3	60.3	45.9	77.7
Red and processed meats (servings) [a]	0.4	0.1	0.7	0.5	0.3	0.8	0.5	0.3	0.8	0.6	0.3	0.9

[a] Missing race/ethnicity for 6 subjects. For each seafood category: 3 (1.5-<3 oz/wk), 3 (>3 oz/wk) were missing.
Missing education information for 83 subjects. For each seafood category: 6 (<0.5 oz/mo), 7 (0.5 oz/mo-<1.5 oz/wk), 13 (1.5-<3 oz/wk), 57 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 2 subjects. For each seafood category: 2 (>3 oz/wk) were missing.
Missing smoking information for 92 subjects. For each seafood category: 8 (<0.5 oz/mo), 7 (0.5 oz/mo-<1.5 oz/wk), 15 (1.5-<3 oz/wk), 62 (>3 oz/wk) were missing.
Missing recreational physical activity information for 83 subjects. For each seafood category: 6 (<0.5 oz/mo), 7 (0.5 oz/mo-<1.5 oz/wk), 13 (1.5-<3 oz/wk), 57 (>3 oz/wk) were missing.
Missing composite pregnancy complication for 55 subjects. For each seafood category: 11 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-<3 oz/wk), 28 (>3 oz/wk) were missing.
Missing enrollment study year information for 37 subjects. For each seafood category: 6 (<0.5 oz/mo), 8 (0.5 oz/mo-<1.5 oz/wk), 4 (1.5-<3 oz/wk), 19 (>3 oz/wk) were missing.
Missing infant sex for 1 subject. For each seafood category: 1 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 2 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 29 subjects. For each seafood category: 3 (<0.5 oz/mo), 14 (0.5 oz/mo-<1.5 oz/wk), 5 (1.5-<3 oz/wk), 7 (>3 oz/wk) were missing.
[b] Pregnancy complication defined as occurrence of any of the following during the index pregnancy: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.
[c] Among 509 subjects with erythrocyte EPA+DHA (1996-2000). 42 (<0.5 oz/mo), 44 (0.5 oz/mo-<1.5 oz/wk), 67 (1.5-<3 oz/wk), 356 (>3 oz/wk) were included.

Table 2.1c Selected characteristics of participants according to amount of **shellfish intake**, Omega study

Characteristic	Shellfish intake (ounces)											
	<0.5/mo (N = 1038)			0.5/mo-<1.5/wk (N =685)			1.5-<3/wk (N = 739)			>3/wk (N = 679)		
	N		%	N		%	N		%	N		%
Maternal race/ethnicity [a]												
Non-Hispanic White	945		91.0	597		87.2	649		87.8	555		81.7
African American	9		0.9	11		1.6	5		0.7	15		2.2
Asian	43		4.1	40		5.8	55		7.4	77		11.3
Other	41		3.9	34		5.0	28		3.8	31		4.6
High school or less [a]	52		5.0	16		2.3	18		2.4	13		1.9
Unmarried	107		10.3	62		9.1	72		9.7	76		11.2
Nulliparous	604		58.2	408		59.6	484		65.5	445		65.5
Pre-pregnancy Body Mass Index (kg/m^2) [a]												
<18.5	44		4.2	34		5.0	28		3.8	24		3.5
18.5-<25	725		69.8	513		74.9	531		71.9	471		69.4
25-<30	181		17.4	81		11.8	123		16.6	127		18.7
≥30.0	88		8.5	57		8.3	57		7.7	55		8.1
Smoked during pregnancy [a]	60		5.8	30		4.4	42		5.7	39		5.7
Alcohol intake during pregnancy	221		21.3	189		27.6	205		27.7	198		29.2
No recreational physical activity during pregnancy [a]	200		19.3	133		19.4	125		16.9	108		15.9
Fish oil supplements	12		1.2	12		1.8	10		1.4	10		1.5
Family history of hypertension	499		48.1	342		49.9	375		50.7	336		49.5
Family history of diabetes	146		14.1	73		10.7	112		15.2	103		15.2
Pregnancy complication [b]	257		24.8	178		26.0	164		22.2	186		27.4
Enrollment year [a]												
1996-1997	64		6.2	29		4.2	38		5.1	37		5.4
1998-1999	159		15.3	105		15.3	128		17.3	93		13.7
2000-2001	199		19.2	125		18.2	139		18.8	108		15.9
2002-2003	262		25.2	203		29.6	186		25.2	185		27.2
2004-2005	226		21.8	141		20.6	150		20.3	163		24.0
2006-2007	112		10.8	73		10.7	92		12.4	87		12.8
Male infant sex [a]	523		50.4	337		49.2	390		52.8	338		49.8
	mean		SD	mean		SD	mean		SD	mean		SD
Maternal age (yrs)	32.3		4.5	32.7		4.2	32.8		4.2	33.1		4.2
	med	IQR		med	IQR		med	IQR		med	IQR	
Maternal age (yrs)	32	29	35	32	30	35	33	30	35	33	30	36
Pre-pregnancy Body Mass Index (kg/m^2) [a]	22.5	20.7	25.1	22.1	20.4	24.2	22.3	20.5	25.0	22.5	20.6	25.2
Gestational age at delivery (weeks) [a]	39	38	40	39	38	40	39	38	40	39	38	40
Weekly intake EPA+DHA (g)	0.2	0.0	0.5	0.4	0.2	0.8	0.7	0.4	1.1	1.2	0.8	1.8
Erythrocyte EPA+DHA (%/total fatty acids) [c]	4.8	4.2	5.6	5.2	4.4	5.9	5.5	4.9	6.2	5.8	5.1	6.5
Daily intake												

Total energy (kcal) [a]	1599.5	1273.4	1997.1	1543.2	1211.3	1944.6	1671.0	1360.1	2061.3	1779.4	1469.6	2196.2
Total fat (g) [a]	54.8	42.1	71.3	53.8	39.2	71.3	59.7	44.9	75.5	62.9	47.5	81.9
Red and processed meats (servings) [a]	0.5	0.2	0.8	0.5	0.3	0.8	0.6	0.3	0.9	0.7	0.4	1.0

[a] Missing race/ethnicity for 6 subjects. For each seafood category: 3 (0.5 oz/mo-<1.5 oz/wk), 2 (1.5-<3 oz/wk), 1 (>3 oz/wk) were missing.
Missing education information for 86 subjects. For each seafood category: 25 (<0.5 oz/mo), 14 (0.5 oz/mo-<1.5 oz/wk), 19 (1.5-<3 oz/wk), 28 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 4 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (1.5-<3 oz/wk), 2 (>3 oz/wk) were missing.
Missing smoking information for 95 subjects. For each seafood category: 31 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 20 (1.5-<3 oz/wk), 29 (>3 oz/wk) were missing.
Missing recreational physical activity information for 86 subjects. For each seafood category: 25 (<0.5 oz/mo), 14 (0.5 oz/mo-<1.5 oz/wk), 19 (1.5-<3 oz/wk), 28 (>3 oz/wk) were missing.
Missing enrollment study year information for 39 subjects. For each seafood category: 17 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-<3 oz/wk), 6 (>3 oz/wk) were missing.
Missing infant sex for 13 subjects. For each seafood category: 5 (<0.5 oz/mo), 5 (1.5-<3 oz/wk), 3 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 12 subjects. For each seafood category: 6 (<0.5 oz/mo), 5 (1.5-<3 oz/wk), 1 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 29 subjects. For each seafood category: 11 (<0.5 oz/mo), 5 (0.5 oz/mo-<1.5 oz/wk), 7 (1.5-<3 oz/wk), 6 (>3 oz/wk) were missing.
[b] Pregnancy complication defined as occurrence of any of the following during the index pregnancy: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.
[c] Among 511 subjects with erythrocyte EPA+DHA (1996-2000). 169 (<0.5 oz/mo), 108 (0.5 oz/mo-<1.5 oz/wk), 125 (1.5-<3 oz/wk), 109 (>3 oz/wk) were included.

Table 2.1d Selected characteristics of participants according to amount of **lean fish intake,** Omega study

Characteristic	Lean fish intake (ounces)											
	<0.5/mo (N = 800)			0.5/mo-<1.5/wk (N =781)			1.5-<3/wk (N = 825)			>3/wk (N = 735)		
	N		%	N		%	N		%	N		%
Maternal race/ethnicity [a]												
Non-Hispanic White	724		90.5	681		87.2	712		86.3	629		85.6
African American	6		0.8	9		1.2	11		1.3	14		1.9
Asian	45		5.6	57		7.3	57		6.9	56		7.6
Other	24		3.0	31		4.0	43		5.2	36		4.9
High school or less [a]	29		3.6	31		4.0	22		2.7	17		2.3
Unmarried	86		10.8	69		8.8	83		10.1	79		10.7
Nulliparous	512		64.0	441		56.5	526		63.8	462		62.9
Pre-pregnancy Body Mass Index (kg/m^2) [a]												
<18.5	47		5.9	29		3.7	29		3.5	25		3.4
18.5-<25	552		69.0	573		73.4	594		72.0	521		70.9
25-<30	136		17.0	127		16.3	137		16.6	112		15.2
≥30.0	65		8.1	52		6.7	65		7.9	75		10.2
Smoked during pregnancy [a]	55		6.9	36		4.6	46		5.6	34		4.6
Alcohol intake during pregnancy	184		23.0	197		25.2	222		26.9	210		28.6
No recreational physical activity during pregnancy [a]	165		20.6	150		19.2	135		16.4	116		15.8
Fish oil supplements	8		1.0	12		1.5	15		1.8	9		1.2
Family history of hypertension	394		49.2	387		49.6	386		46.8	385		52.4
Family history of diabetes	100		12.5	97		12.4	132		16.0	105		14.3
Pregnancy complication [b]	193		24.1	186		23.8	212		25.7	194		26.4
Enrollment year [a]												
1996-1997	38		4.8	33		4.2	43		5.2	54		7.3
1998-1999	115		14.4	100		12.8	147		17.8	123		16.7
2000-2001	130		16.2	139		17.8	150		18.2	152		20.7
2002-2003	217		27.1	224		28.7	213		25.8	182		24.8
2004-2005	183		22.9	184		23.6	174		21.1	139		18.9
2006-2007	107		13.4	86		11.0	93		11.3	78		10.6
Male infant sex [a]	405		50.6	395		50.6	429		52.0	359		48.8
	mean		SD	mean		SD	mean		SD	mean		SD
Maternal age (yrs)	32.4		4.5	32.6		4.3	32.9		4.3	32.8		4.2
	med	IQR		med	IQR		med	IQR		med	IQR	
Maternal age (yrs)	32	30	35	33	30	35	33	30	36	33	30	35
Pre-pregnancy Body Mass Index (kg/m^2) [a]	22.1	20.4	25.0	22.1	20.5	24.8	22.6	20.7	25.0	22.5	20.7	25.1
Gestational age at delivery (weeks) [a]	39	38	40	39	38	40	39	38	40	39	38	40
Weekly intake EPA+DHA (g)	0.1	0.0	0.5	0.4	0.2	0.8	0.7	0.4	1.1	1.1	0.7	1.6
Erythrocyte EPA+DHA (%/total fatty acids) [c]	4.7	4.0	5.8	5.0	4.6	5.9	5.4	4.7	6.1	5.6	4.8	6.4
Daily intake												

Total energy (kcal) [a]	1567.0	1266.2	1970.2	1584.5	1251.9	1976.7	1620.9	1292.1	2034.5	1837.3	1501.5	2201.1
Total fat (g) [a]	54.3	40.5	70.1	55.2	40.8	71.4	56.4	43.2	73.2	64.5	48.6	81.6
Red and processed meats (servings) [a]	0.5	0.2	0.8	0.5	0.3	0.9	0.6	0.3	0.9	0.6	0.4	1.0

[a] Missing race/ethnicity for 6 subjects. For each seafood category: 1 (<0.5 oz/mo), 3 (0.5 oz/mo-<1.5 oz/wk), 2 (1.5-<3 oz/wk) were missing.
Missing education information for 83 subjects. For each seafood category: 18 (<0.5 oz/mo), 25 (0.5 oz/mo-<1.5 oz/wk), 19 (1.5-<3 oz/wk), 21 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 2 subjects. For each seafood category: 2 (>3 oz/wk) were missing.
Missing smoking information for 92 subjects. For each seafood category: 20 (<0.5 oz/mo), 28 (0.5 oz/mo-<1.5 oz/wk), 21 (1.5-<3 oz/wk), 23 (>3 oz/wk) were missing.
Missing recreational physical activity information for 83 subjects. For each seafood category: 18 (<0.5 oz/mo), 25 (0.5 oz/mo-<1.5 oz/wk), 19 (1.5-<3 oz/wk), 21 (>3 oz/wk) were missing.
Missing composite pregnancy complication for 55 subjects. For each seafood category: 23 (<0.5 oz/mo), 10 (0.5 oz/mo-<1.5 oz/wk), 9 (1.5-<3 oz/wk), 13 (>3 oz/wk) were missing.
Missing enrollment study year information for 37 subjects. For each seafood category: 10 (<0.5 oz/mo), 15 (0.5 oz/mo-<1.5 oz/wk), 5 (1.5-<3 oz/wk), 7 (>3 oz/wk) were missing.
Missing infant sex for 1 subject. For each seafood category: 1 (1.5-<3 oz/wk) were missing.
Missing gestational age at delivery information for 2 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 29 subjects. For each seafood category: 3 (<0.5 oz/mo), 14 (0.5 oz/mo-<1.5 oz/wk), 5 (1.5-<3 oz/wk), 7 (>3 oz/wk) were missing.
[b] Pregnancy complication defined as occurrence of any of the following during the index pregnancy: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.
[c] Among 590 subjects with erythrocyte EPA+DHA (1996-2000). 108 (<0.5 oz/mo), 108 (0.5 oz/mo-<1.5 oz/wk), 148 (1.5-<3 oz/wk), 145 (>3 oz/wk) were included.

Table 2.1e Selected characteristics of participants according to amount of **fatty fish intake,** Omega study

Characteristic	Fatty fish intake (ounces)							
	<0.5/mo (N = 742)		0.5/mo-<1.5/wk (N = 797)		1.5-<3/wk (N = 855)		>3/wk (N = 747)	
	N	%	N	%	N	%	N	%
Maternal race/ethnicity [a]								
Non-Hispanic White	639	86.1	692	86.8	759	88.8	656	87.8
African American	13	1.8	8	1.0	8	0.9	11	1.5
Asian	50	6.7	62	7.8	52	6.1	51	6.8
Other	39	5.3	32	4.0	35	4.1	28	3.7
High school or less [a]	53	7.1	24	3.0	10	1.2	12	1.6
Unmarried	104	14.0	71	8.9	59	6.9	83	11.1
Nulliparous	464	62.5	493	61.9	526	61.5	458	61.3
Pre-pregnancy Body Mass Index (kg/m^2) [a]								
<18.5	38	5.1	26	3.3	33	3.9	33	4.4
18.5-<25	486	65.5	594	74.5	635	74.3	525	70.3
25-<30	135	18.2	121	15.2	127	14.9	129	17.3
≥30.0	83	11.2	56	7.0	60	7.0	58	7.8
Smoked during pregnancy [a]	55	7.4	46	5.8	34	4.0	36	4.8
Alcohol intake during pregnancy	166	22.4	215	27.0	223	26.1	209	28.0
No recreational physical activity during pregnancy [a]	140	18.9	160	20.1	158	18.5	108	14.5
Fish oil supplements	5	0.7	12	1.5	14	1.6	13	1.7
Family history of hypertension	359	48.4	412	51.7	405	47.4	376	50.3
Family history of diabetes	100	13.5	119	14.9	102	11.9	113	15.1
Pregnancy complication [b]	186	25.1	214	26.9	202	23.6	183	24.5
Enrollment year [a]								
1996-1997	43	5.8	37	4.6	44	5.1	44	5.9
1998-1999	114	15.4	128	16.1	124	14.5	119	15.9
2000-2001	133	17.9	143	17.9	158	18.5	137	18.3
2002-2003	192	25.9	189	23.7	240	28.1	215	28.8
2004-2005	160	21.6	184	23.1	180	21.1	156	20.9
2006-2007	86	11.6	104	13.0	101	11.8	73	9.8
Male infant sex [a]	387	52.2	416	52.2	419	49.0	366	49.0
	mean	SD	mean	SD	mean	SD	mean	SD
Maternal age (yrs)	31.6	4.8	32.0	4.6	32.5	4.2	33.0	4.2
	med	IQR	med	IQR	med	IQR	med	IQR
Maternal age (yrs)	32	29 35	32	30 35	33	30 36	34	31 36
Pre-pregnancy Body Mass Index (kg/m^2) [a]	22.5	20.6 25.7	22.3	20.5 24.6	22.3	20.6 24.7	22.3	20.5 25.0
Gestational age at delivery (weeks) [a]	39	38 40	39	38 40	39	38 40	39	38 40
Weekly intake EPA+DHA (g)	0.0	0.0 0.1	0.3	0.2 0.5	0.8	0.6 0.9	1.4	1.1 2.0
Erythrocyte EPA+DHA (%/total fatty	4.5	3.9 5.1	5.1	4.4 5.7	5.6	4.8 6.4	5.8	5.2 6.7

acids) [c]												
Daily intake												
Total energy (kcal) [a]	1542.7	1211.7	1955.7	1557.7	1237.1	1931.2	1685.5	1363.9	2085.6	1796.9	1455.6	2205.3
Total fat (g) [a]	53.7	40.5	69.6	53.2	40.0	70.4	58.9	45.1	75.1	63.5	48.2	85.0
Red and processed meats (servings) [a]	0.5	0.2	0.9	0.5	0.3	0.8	0.6	0.3	0.9	0.6	0.3	1.0

[a] Missing race/ethnicity for 6 subjects. For each seafood category: 1 (<0.5 oz/mo), 3 (0.5 oz/mo-<1.5 oz/wk), 1 (1.5-<3 oz/wk), 1 (>3 oz/wk) were missing.
Missing education information for 83 subjects. For each seafood category: 24 (<0.5 oz/mo), 20 (0.5 oz/mo-<1.5 oz/wk), 16 (1.5-<3 oz/wk), 23 (>3 oz/wk) were missing.
Missing pre-pregnancy BMI for 2 subjects. For each seafood category: 2 (>3 oz/wk) were missing.
Missing smoking information for 92 subjects. For each seafood category: 26 (<0.5 oz/mo), 22 (0.5 oz/mo-<1.5 oz/wk), 19 (1.5-<3 oz/wk), 25 (>3 oz/wk) were missing.
Missing recreational physical activity information for 83 subjects. For each seafood category: 24 (<0.5 oz/mo), 20 (0.5 oz/mo-<1.5 oz/wk), 16 (1.5-<3 oz/wk), 23 (>3 oz/wk) were missing.
Missing composite pregnancy complication for 55 subjects. For each seafood category: 24 (<0.5 oz/mo), 11 (0.5 oz/mo-<1.5 oz/wk), 6 (1.5-<3 oz/wk), 14 (>3 oz/wk) were missing.
Missing enrollment study year information for 37 subjects. For each seafood category: 14 (<0.5 oz/mo), 12 (0.5 oz/mo-<1.5 oz/wk), 8 (1.5-<3 oz/wk), 3 (>3 oz/wk) were missing.
Missing infant sex for 1 subject. For each seafood category: 1 (>3 oz/wk) were missing.
Missing gestational age at delivery information for 2 subjects. For each seafood category: 1 (<0.5 oz/mo), 1 (>3 oz/wk) were missing.
Missing total energy, fat, red and processed meat information for 29 subjects. For each seafood category: 6 (<0.5 oz/mo), 9 (0.5 oz/mo-<1.5 oz/wk), 9 (1.5-<3 oz/wk), 5 (>3 oz/wk) were missing.
[b] Pregnancy complication defined as occurrence of any of the following during the index pregnancy: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.
[c] Among 509 subjects with erythrocyte EPA+DHA (1996-2000). 123 (<0.5 oz/mo), 129 (0.5 oz/mo-<1.5 oz/wk), 128 (1.5-<3 oz/wk), 129 (>3 oz/wk) were included.

Table 2.1f Selected characteristics of participants according to **quartile of maternal erythrocyte membrane EPA+DHA (%/total fatty acids)**, Omega study

Characteristics	Total (N = 534)		Quartile 1 (N =133)		Quartile 2 (N =134)		Quartile 3 (N =133)		Quartile 4 (N =134)	
	N	%	N	%	N	%	N	%	N	%
Non-Hispanic White race/ethnicity [a]	467	87.5	122	91.7	117	87.3	119	89.5	109	81.3
High school or less [a]	24	4.5	12	9.0	6	4.5	2	1.5	4	3.0
Unmarried	78	14.6	31	23.3	19	14.2	9	6.8	19	14.2
Nulliparous	467	87.5	108	81.2	115	85.8	120	90.2	124	92.5
Pre-pregnancy Body Mass Index (kg/m^2) [a]										
<18.5	22	4.1	6	4.5	6	4.5	5	3.8	5	3.7
18.5-<25	375	70.2	84	63.2	93	69.4	91	68.4	107	79.9
25-<30	87	16.3	22	16.5	22	16.4	26	19.5	17	12.7
≥30.0	49	9.2	21	15.8	13	9.7	11	8.3	4	3.0
Smoked during pregnancy [a]	36	6.7	17	12.8	9	6.7	5	3.8	5	3.7
Alcohol intake during pregnancy	177	33.1	29	21.8	50	37.3	50	37.6	48	35.8
No recreational physical activity during pregnancy [a]	67	12.5	22	16.5	16	11.9	18	13.5	11	8.2
Fish oil supplements	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Family history of hypertension	241	45.1	64	48.1	67	50.0	58	43.6	52	38.8
Family history of diabetes	70	13.1	16	12.0	18	13.4	19	14.3	17	12.7
Pregnancy complication [b]	176	33.0	55	41.4	41	30.6	43	32.3	37	27.6
Enrollment Year										
1996-1997	174	32.6	47	35.3	40	29.9	40	30.1	47	35.1
1998-1999	302	56.6	76	57.1	80	59.7	75	56.4	71	53.0
2000-2001	58	10.9	10	7.5	14	10.4	18	13.5	16	11.9
Male infant sex [a]	250	46.8	63	47.4	65	48.5	63	47.4	59	44.0
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Maternal age (yrs)	32.6	4.5	29.8	4.7	31.8	4.2	32.6	4.0	33.3	4.4
	median, IQR		median, IQR		median, IQR		median, IQR		median, IQR	
Maternal age (yrs)	33, 30-36		30, 27-33		32, 29-35		32, 30-35		33, 30-36	
Pre-pregnancy Body Mass Index (kg/m^2) [a]	22.4, 20.6-25.1		22.8, 20.4-25.8		22.7, 20.8-25.8		22.2, 20.2-25.6		21.8, 20.3-23.7	
Gestational age at delivery (wks) [a]	39.0, 38.0-40.0		39.0, 38.0-40.0		39.0, 38.0-40.0		39.0, 38.0-40.0		39.5, 38.0-40.0	
Weekly intake EPA+DHA (g)	0.5, 0.2-1.0		0.2, 0.0-0.5		0.4, 0.1-0.8		0.7, 0.4-1.1		1.0, 0.6-1.4	
Erythrocyte EPA+DHA (%/total fatty acids)	5.3, 4.5-6.1		4.0, 3.6-4.3		4.8, 4.7-5.0		5.6, 5.4-5.8		6.7, 6.3-7.2	
Daily intake										
Total energy (kcal) [a]	1640.9, 1317.8-2057.2		1562.3, 1284.4-1979.4		1591.9, 1240.0-1948.0		1600.4, 1205.6-2019.4		1514.8, 1233.6-2068.4	
Total fat (g) [a]	57.2, 43.3-74.4		55.8, 41.7-74.5		54.4, 38.7-70.8		51.7, 35.5-68.4		49.3, 36.2-68.2	
Red and processed meats (servings) [a]	0.6, 0.3-0.9		0.6, 0.3-1.0		0.4, 0.3-0.7		0.5, 0.3-0.8		0.4, 0.2-0.7	

[a] Missing race/ethnicity for 1 subject from Quartile (Q) 3. Missing education and recreational physical activity information for 8 subjects. For each Quartile: 2 (Q1), 2 (Q2), 1 (Q3), 3 (Q4) were missing. Missing pre-pregnancy BMI for 1 subject from Q4. Missing smoking information for 9 subjects. For each Quartile: 2 (Q1), 2 (Q2), 2 (Q3), 3 (Q4) were missing. Missing composite pregnancy complication for 13 subjects. For each Quartile: 4 (Q1), 3 (Q2), 2 (Q3), 4 (Q4). Missing infant sex information for 1 subjects from Q3. Missing dietary EPA+DHA for 25 subjects. For each Quartile: Quartile: 11 (Q1), 6 (Q2), 5 (Q3), 3 (Q4) were missing. Missing total energy, fat, red and processed meat information for 26 subjects. For each Quartile: 10 (Q1), 7 (Q2), 5 (Q3), 4 (Q4) were missing.
[b] Pregnancy complication defined as occurrence of any of the following during the index pregnancy: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.2a Associations of seafood intake with **risk of low birth weight**

	Seafood intake (ounces)								
	<0.5/mo	0.5/mo-<1.5/wk		1.5-3/wk		>3/wk		*P* value [a]
Shellfish									
LBW/Normal Weight (N=123/2569)	44/843	31/565		19/617		29/544			
Unadjusted, RR (95% CI) [b]	(referent)	1.05	(0.67-1.64)	0.60	(0.36-1.02)	1.02	(0.65-1.61)	0.54
Model 2.1 [c]	(referent)	0.97	(0.59-1.59)	**0.54**	**(0.31-0.93)**	0.83	(0.48-1.44)	0.20
Model 2.2 [d]	(referent)	0.98	(0.60-1.62)	**0.52**	**(0.29-0.91)**	0.84	(0.48-1.46)	0.20
Model 2.3[e]	(referent)	0.68	(0.40-1.16)	**0.51**	**(0.29-0.89)**	0.77	(0.48-1.23)	0.17
Model 2.4 [f]	(referent)	0.51	(0.16-1.61)	0.32	(0.09-1.07)	0.37	(0.10-1.39)	0.07
Lean Fish									
LBW/Normal Weight (N=123/2569)	25/671	31/628		31/678		36/592			
Unadjusted, RR (95% CI) [b]	(referent)	1.31	(0.78-2.19)	1.22	(0.73-2.04)	1.60	(0.97-2.63)	0.10
Model 2.1 [c]	(referent)	1.58	(0.87-2.84)	1.51	(0.83-2.74)	**2.23**	**(1.21-4.09)**	**0.02**
Model 2.2 [d]	(referent)	1.56	(0.86-2.83)	1.51	(0.83-2.76)	**2.21**	**(1.20-4.07)**	**0.02**
Model 2.3[e]	(referent)	1.68	(0.86-3.30)	**2.24**	**(1.22-4.14)**	**1.94**	**(1.02-3.68)**	**0.02**
Model 2.4 [f]	(referent)	4.97	(0.92-27.03)	5.27	(0.99-28.07)	2.78	(0.47-16.57)	0.14
Fatty Fish									
LBW/Normal Weight (N=123/2569)	32/605	30/654		36/701		25/609			
Unadjusted, RR (95% CI) [b]	(referent)	0.87	(0.54-1.42)	0.97	(0.61-1.55)	0.78	(0.47-1.31)	0.47
Model 2.1 [c]	(referent)	0.89	(0.51-1.55)	1.01	(0.59-1.73)	0.65	(0.34-1.21)	0.26
Model 2.2 [d]	(referent)	0.84	(0.49-1.46)	0.97	(0.57-1.66)	0.62	(0.33-1.16)	0.22
Model 2.3[e]	(referent)	1.32	(0.71-2.47)	1.33	(0.69-2.55)	1.03	(0.54-1.95)	0.97
Model 2.4 [f]	(referent)	0.78	(0.15-4.03)	1.62	(0.46-5.73)	0.39	(0.07-2.21)	0.57
Total Seafood									
LBW/Normal Weight (N=123/2569)	7/245	19/234		21/431		76/1659			
Unadjusted, RR (95% CI) [b]	(referent)	**2.70**	**(1.16-6.32)**	1.67	(0.72-3.88)	1.58	(0.74-3.38)	0.87
Model 2.1 [c]	(referent)	**3.52**	**(1.29-9.57)**	2.19	(0.81-5.91)	2.02	(0.80-5.05)	0.92
Model 2.2 [d]	(referent)	**3.65**	**(1.35-9.83)**	2.19	(0.82-5.86)	1.95	(0.79-4.84)	0.93
Model 2.3[e]	(referent)	**5.40**	**(1.37-21.21)**	**5.14**	**(1.39-19.05)**	**3.64**	**(1.05-12.68)**	0.30
Model 2.4 [f]	(referent)	2.85	(0.29-27.87)	1.93	(0.20-19.02)	1.31	(0.15-11.18)	0.59

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0 + beta1*X_{SeafoodCategory\ (includes\ reference)} + Confounding\ Variables$, for Y = fetal growth outcome

[b] Relative risks calculated using generalized linear model with a log link from the Poisson family, with robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, years), white Non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, $\geq$30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 780/3141 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.2b Associations of seafood intake with **risk of macrosomia**

	Seafood intake (ounces)								
	<0.5/mo	0.5/mo-<1.5/wk		1.5-3/wk		>3/wk		*P* value [a]
Shellfish									
Macrosomia/Normal Weight (N=449/2569)	151/843	89/565		103/617		106/544			
Unadjusted, RR (95% CI) [b]	(referent)	0.90	(0.70-1.14)	0.94	(0.75-1.19)	1.07	(0.85-1.35)	0.62
Model 2.1 [c]	(referent)	0.86	(0.67-1.11)	0.89	(0.69-1.14)	1.04	(0.80-1.34)	0.91
Model 2.2 [d]	(referent)	0.87	(0.67-1.12)	0.90	(0.70-1.15)	1.06	(0.82-1.38)	0.77
Model 2.3[e]	(referent)	0.86	(0.67-1.09)	0.87	(0.68-1.11)	1.03	(0.80-1.33)	0.99
Model 2.4 [f]	(referent)	0.81	(0.61-1.08)	0.94	(0.71-1.23)	1.03	(0.76-1.38)	0.83
Lean Fish									
Macrosomia/Normal Weight (N=449/2569)	104/671	122/628		116/678		107/592			
Unadjusted, RR (95% CI) [b]	(referent)	1.21	(0.95-1.54)	1.09	(0.85-1.39)	1.14	(0.89-1.46)	0.48
Model 2.1 [c]	(referent)	1.22	(0.94-1.58)	1.05	(0.80-1.38)	1.10	(0.82-1.47)	0.83
Model 2.2 [d]	(referent)	1.19	(0.91-1.55)	1.04	(0.79-1.37)	1.09	(0.81-1.46)	0.84
Model 2.3[e]	(referent)	1.20	(0.94-1.55)	1.03	(0.79-1.34)	1.08	(0.81-1.45)	0.92
Model 2.4 [f]	(referent)	1.28	(0.95-1.71)	1.03	(0.76-1.40)	1.04	(0.74-1.45)	0.76
Fatty Fish									
Macrosomia/Normal Weight (N=449/2569)	105/605	113/654		118/701		113/609			
Unadjusted, RR (95% CI) [b]	(referent)	0.87	(0.54-1.42)	0.97	(0.61-1.55)	0.78	(0.47-1.31)	0.47
Model 2.1 [c]	(referent)	0.89	(0.51-1.55)	1.01	(0.59-1.73)	0.65	(0.34-1.21)	0.26
Model 2.2 [d]	(referent)	0.84	(0.49-1.46)	0.97	(0.57-1.66)	0.62	(0.33-1.16)	0.22
Model 2.3[e]	(referent)	1.32	(0.71-2.47)	1.33	(0.69-2.55)	1.03	(0.54-1.95)	0.97
Model 2.4 [f]	(referent)	0.78	(0.15-4.03)	1.62	(0.46-5.73)	0.39	(0.07-2.21)	0.57
Total Seafood									
Macrosomia/Normal Weight (N=449/2569)	34/245	47/234		69/431		299/1659			
Unadjusted, RR (95% CI) [b]	(referent)	1.37	(0.91-2.07)	1.13	(0.77-1.66)	1.25	(0.90-1.75)	0.36
Model 2.1 [c]	(referent)	1.40	(0.93-2.13)	1.18	(0.80-1.74)	1.26	(0.90-1.78)	0.42
Model 2.2 [d]	(referent)	1.39	(0.92-2.12)	1.15	(0.77-1.70)	1.24	(0.88-1.75)	0.46
Model 2.3[e]	(referent)	1.34	(0.90-2.02)	1.08	(0.74-1.59)	1.16	(0.83-1.62)	0.76
Model 2.4 [f]	(referent)	1.39	(0.87-2.21)	1.20	(0.78-1.85)	1.25	(0.85-1.82)	0.50

[a] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0 + beta1*X_{SeafoodCategory} + Confounding\ Variables$, for Y = fetal growth outcome

[b] Relative risks calculated using generalized linear model with a log link from the Poisson family, with robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, years), white Non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, $\geq$30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 780/3141 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.2c Associations of **total seafood intake** with mean change in fetal growth indices

	Seafood intake (ounces)								
	<0.5/mo (N=286)	0.5/mo-<1.5/wk (N=300)		1.5-3/wk (N=521)		>3/wk (N=2034)		*P* value [a]
Birth Weight (N=3141)	286	300		521		2034		
Change (g) (95% CI) [b]								
Unadjusted	(referent)	-4.80	(-91.46-81.85)	-20.56	(-97.73-56.60)	-8.68	(-74.89-57.54)	0.86
Model 2.1 [c]	(referent)	-12.33	(-99.26-74.60)	-19.87	(-97.69-57.96)	-14.95	(-82.35-52.46)	0.73
Model 2.2 [d]	(referent)	-14.62	(-102.41-73.16)	-21.47	(-99.77-56.83)	-16.82	(-84.75-51.11)	0.71
Model 2.3 [e]	(referent)	5.38	(-63.93-74.69)	-28.83	(-90.89-33.22)	-30.63	(-84.41-23.14)	0.14
Model 2.4 [f]	(referent)	44.26	(-39.95-128.48)	0.81	(-73.29-74.90)	-0.32	(-64.43-63.80)	0.55
Birth Length (N=3101)	283	295		515		2008		
Change (cm) (95% CI) [b]								
Unadjusted	(referent)	0.20	(-0.31-0.70)	0.25	(-0.20-0.70)	0.17	(-0.22-0.56)	0.62
Model 2.1 [c]	(referent)	0.17	(-0.34-0.69)	0.23	(-0.22-0.69)	0.11	(-0.29-0.51)	0.88
Model 2.2 [d]	(referent)	0.16	(-0.36-0.67)	0.23	(-0.23-0.69)	0.06	(-0.34-0.45)	0.85
Model 2.3 [e]	(referent)	0.26	(-0.18-0.71)	0.21	(-0.19-0.60)	0.03	(-0.31-0.38)	0.51
Model 2.4 [f]	(referent)	0.31	(-0.23-0.85)	0.42	(-0.06-0.89)	0.25	(-0.17-0.66)	0.53
Head Circumference (N=3063)	278	292		512		1981		
Change (cm) (95% CI) [b]								
Unadjusted	(referent)	-0.16	(-0.52-0.20)	-0.25	(-0.57-0.06)	-0.15	(-0.42-0.12)	0.53
Model 2.1 [c]	(referent)	-0.20	(-0.56-0.17)	-0.26	(-0.59-0.06)	-0.15	(-0.43-0.13)	0.64
Model 2.2 [d]	(referent)	-0.21	(-0.57-0.16)	-0.26	(-0.58-0.07)	-0.15	(-0.43-0.14)	0.67
Model 2.3 [e]	(referent)	-0.15	(-0.49-0.18)	-0.28	(-0.58-0.02)	-0.19	(-0.45-0.07)	0.25
Model 2.4 [f]	(referent)	0.07	(-0.33-0.47)	-0.12	(-0.47-0.23)	-0.02	(-0.32-0.28)	0.84
Ponderal Index (N=3101)	283	295		515		2008		
Change (kg/m^3) (95% CI) [b]								
Unadjusted	(referent)	-0.61	(-1.47-0.25)	-0.84	(-1.61--0.08)	-0.51	(-1.17-0.15)	0.50
Model 2.1 [c]	(referent)	-0.68	(-1.56-0.21)	-0.84	(-1.63--0.05)	-0.46	(-1.15-0.23)	0.61
Model 2.2 [d]	(referent)	-0.65	(-1.53-0.24)	-0.83	(-1.62--0.04)	-0.36	(-1.05-0.33)	0.89
Model 2.3 [e]	(referent)	-0.66	(-1.55-0.22)	-0.85	(-1.64--0.06)	-0.48	(-1.67-0.20)	0.54
Model 2.4 [f]	(referent)	-0.50	(-1.59-0.58)	-1.13	(-2.08--0.17)	-0.62	(-1.45-0.20)	0.30

[a] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory\ or\ EPA+DHA\ Quartile} + Confounding\ Variables$, for Y = fetal growth

[b] Change in mean fetal growth measures were calculated using linear regression, with non-robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, years), white Non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, $\geq$30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 780/3141 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.2d Associations of **maternal quartile of erythrocyte EPA+DHA** with mean change in fetal growth indices

	Quartile 1 (N=133)	Quartile 2 (N=134)	Quartile 3 (N=133)	Quartile 4 (N=134)	*P* value [a]
Median, Range %/Total Fatty Acids	4.01, 2.28-4.49	4.84, 4.49-5.25	5.63, 5.26-6.07	6.66, 6.07-9.55	
Birth Weight (N=534)	133	134	133	133	
Change (g) (95% CI) [b]					
Unadjusted	(referent)	-43.84 (-183.65-95.98)	59.11 (-80.97-199.19)	-48.23 (-188.05-91.58)	0.85
Model 2.1 [c]	(referent)	-58.97 (-207.52-89.58)	49.62 (-100.89-200.13)	-39.37 (-194.46-115.73)	0.99
Model 2.2 [d]	(referent)	-60.67 (-209.44-88.10)	43.27 (-107.80-194.34)	-45.00 (-200.54-110.53)	0.94
Model 2.3 [e]	(referent)	-44.65 (-158.06-68.77)	-18.45 (-133.57-96.68)	-66.51 (-184.95-51.92)	0.38
Model 2.4 [f]	(referent)	-4.34 (-179.18-170.50)	59.67 (-120.0-239.34)	-31.72 (-214.75-151.31)	0.91
Birth Length (N=526)	131	133	131	131	
Change (cm) (95% CI) [b]					
Unadjusted	(referent)	0.14 (-0.59-0.88)	0.11 (-0.62-0.85)	0.24 (-0.49-0.98)	0.55
Model 2.1 [c]	(referent)	-0.14 (-0.91-0.62)	-0.15 (-0.92-0.63)	-0.05 (-0.85-0.75)	0.92
Model 2.2 [d]	(referent)	-0.15 (-0.91-0.62)	-0.20 (-0.97-0.58)	-0.10 (-0.90-0.71)	0.81
Model 2.3 [e]	(referent)	-0.04 (-0.68-0.61)	-0.39 (-1.04-0.27)	-0.23 (-0.90-0.45)	0.34
Model 2.4 [f]	(referent)	0.24 (-0.66-1.13)	-0.06 (-0.98-0.86)	0.02 (-0.93-0.96)	0.83
Head Circumference (N=511)	129	133	126	123	
Change (cm) (95% CI) [b]					
Unadjusted	(referent)	0.17 (-0.37-0.71)	0.45 (-0.09-1.00)	0.05 (-0.50-0.60)	0.61
Model 2.1 [c]	(referent)	0.28 (-0.31-0.86)	0.49 (-0.10-1.09)	0.23 (-0.39-0.85)	0.38
Model 2.2 [d]	(referent)	0.26 (-0.32-0.85)	0.45 (-0.15-1.05)	0.19 (-0.44-0.81)	0.47
Model 2.3 [e]	(referent)	0.31 (-0.20-0.83)	0.32 (-0.21-0.85)	0.16 (-0.39-0.71)	0.63
Model 2.4 [f]	(referent)	0.28 (-0.40-0.95)	0.41 (-0.28-1.10)	0.17 (-0.53-0.87)	0.63
Ponderal Index (N=526)	131	133	131	131	
Change (kg/m^3) (95% CI) [b]					
Unadjusted	(referent)	-0.70 (-1.80-0.39)	0.59 (-0.51-1.68)	-0.71 (-1.81-0.39)	0.64
Model 2.1 [c]	(referent)	-0.31 (-1.50-0.88)	1.06 (-0.15-2.27)	0.03 (-1.22-1.28)	0.43
Model 2.2 [d]	(referent)	-0.30 (-1.50-0.89)	1.09 (-0.12-2.31)	0.06 (-1.19-1.32)	0.40
Model 2.3 [e]	(referent)	-0.27 (-1.46-0.91)	0.98 (-0.22-2.18)	-0.03 (-1.27-1.22)	0.53
Model 2.4 [f]	(referent)	-0.55 (-2.18-1.07)	1.45 (-0.18-3.09)	0.06 (-1.60-1.72)	0.38

[a] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory\ or\ EPA+DHA\ Quartile} + Confounding\ Variables$, for Y = fetal growth

[b] Change in mean fetal growth measures were calculated using linear regression, with non-robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, yrs), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (ind. variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-ref. categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 174/534 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth (N = 78, 94, 90, and 98 subjects included in quartiles 1-4, respectively).

Table 2.2e Associations of **shellfish intake** with mean change in fetal growth indices

	Shellfish intake (ounces)									
	<0.5/mo (N=1038)	0.5/mo-<1.5/wk (N=685)		1.5-3/wk (N=739)		>3/wk (N=679)		*P* value [a]
Birth Weight (N=3141)	1038	685		739		679		
Change (g) (95% CI) [b]								
Unadjusted	(referent)	-32.82	(-84.42-18.79)	-7.89	(-58.34-42.56)	5.85	(-45.89-57.59)	0.76
Model 2.1 [c]	(referent)	-20.38	(-73.99-33.23)	-4.56	(-58.65-49.53)	23.68	(-35.76-83.11)	0.46
Model 2.2 [d]	(referent)	-22.95	(-77.02-31.12)	-2.07	(-56.47-52.33)	27.50	(-32.50-87.50)	0.38
Model 2.3[e]	(referent)	-15.02	(-57.73-27.70)	-32.36	(-75.49-10.77)	14.76	(-32.60-62.11)	0.91
Model 2.4 [f]	(referent)	-17.35	(-69.34-34.64)	-32.80	(-85.93-20.33)	11.74	(-45.60-69.08)	0.99
Birth Length (N=3101)	1025	674		732		670		
Change (cm) (95% CI) [b]								
Unadjusted	(referent)	-0.27	(-0.57-0.04)	-0.05	(-0.34-0.25)	-0.11	(-0.41-0.19)	0.68
Model 2.1 [c]	(referent)	-0.26	(-0.58-0.05)	-0.14	(-0.46-0.18)	-0.19	(-0.55-0.16)	0.34
Model 2.2 [d]	(referent)	-0.25	(-0.57-0.07)	-0.09	(-0.41-0.23)	-0.10	(-0.46-0.25)	0.67
Model 2.3[e]	(referent)	-0.25	(-0.53-0.02)	-0.26	(-0.54-0.01)	-0.23	(-0.54-0.07)	0.10
Model 2.4 [f]	(referent)	-0.17	(-0.50-0.17)	-0.09	(-0.43-0.24)	-0.11	(-0.51-0.27)	0.59
Head Circumference (N=3063)	1012	669		722		660		
Change (cm) (95% CI) [b]								
Unadjusted	(referent)	-0.09	(-0.30-0.12)	0.05	(-0.16-0.26)	-0.03	(-0.24-0.18)	0.94
Model 2.1 [c]	(referent)	-0.06	(-0.28-0.17)	0.07	(-0.15-0.30)	0.06	(-0.19-0.31)	0.48
Model 2.2 [d]	(referent)	-0.06	(-0.29-0.16)	0.07	(-0.15-0.30)	0.04	(-0.21-0.29)	0.53
Model 2.3[e]	(referent)	-0.05	(-0.26-0.16)	0.00	(-0.21-0.21)	0.04	(-0.19-0.27)	0.72
Model 2.4 [f]	(referent)	0.00	(-0.25-0.24)	0.02	(-0.22-0.27)	0.09	(-0.18-0.36)	0.53
Ponderal Index (N=3101)	1025	674		732		670		
Change (kg/m^3) (95% CI) [b]								
Unadjusted	(referent)	0.21	(-0.30-0.73)	-0.03	(-0.53-0.47)	0.23	(-0.28-0.75)	0.55
Model 2.1 [c]	(referent)	0.32	(-0.23-0.87)	0.17	(-0.38-0.72)	**0.64**	**(0.04-1.25)**	0.07
Model 2.2 [d]	(referent)	0.28	(-0.27-0.83)	0.09	(-0.46-0.64)	0.50	(-0.11-1.10)	0.19
Model 2.3[e]	(referent)	0.32	(-0.22-0.87)	0.14	(-0.41-0.69)	**0.64**	**(0.03-1.24)**	0.08
Model 2.4 [f]	(referent)	0.12	(-0.56-0.79)	-0.14	(-0.81-0.53)	0.58	(-0.16-1.33)	0.28

[a] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory\ or\ EPA+DHA\ Quartile} + Confounding\ Variables$, for Y = fetal growth

[b] Change in mean fetal growth measures were calculated using linear regression, with non-robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, years), white Non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, $\geq$30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 780/3141 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.2f Associations of **lean fish intake** with mean change in fetal growth indices

	Lean fish intake (ounces)									
	<0.5/mo (N=800)		0.5/mo-<1.5/wk (N=781)		1.5-3/wk (N=825)		>3/wk (N=735)		*P* value [a]
Birth Weight (N=3141)	800		781		825		735		
Change (g) (95% CI) [b]									
Unadjusted	(referent)	-18.95	(-71.69-33.79)	-6.05	(-58.07-45.97)	-25.00	(-78.57-28.57)	0.48	
Model 2.1 [c]	(referent)	-27.08	(-82.67-28.52)	-15.80	(-71.38-39.80)	-47.41	(-107.81-13.00)	0.19	
Model 2.2 [d]	(referent)	-27.62	(-83.62-28.38)	-13.94	(-69.86-41.98)	-49.08	(-110.05-11.89)	0.19	
Model 2.3[e]	(referent)	-16.36	(-60.66-27.93)	-14.39	(-58.68-29.90)	-27.00	(-75.16-21.15)	0.32	
Model 2.4 [f]	(referent)	3.65	(-51.28-58.59)	-11.77	(-65.22-41.67)	1.33	(-57.33-60.00)	0.88	
Birth Length (N=3101)	789		774		812		726		
Change (cm) (95% CI) [b]									
Unadjusted	(referent)	-0.21	(-0.52-0.09)	-0.11	(-0.41-0.19)	-0.09	(-0.40-0.23)	0.74	
Model 2.1 [c]	(referent)	-0.24	(-0.56-0.09)	-0.19	(-0.52-0.14)	-0.16	(-0.51-0.20)	0.47	
Model 2.2 [d]	(referent)	-0.26	(-0.59-0.07)	-0.24	(-0.57-0.09)	-0.24	(-0.60-0.12)	0.23	
Model 2.3[e]	(referent)	-0.20	(-0.49-0.08)	-0.18	(-0.47-0.10)	-0.08	(-0.39-0.23)	0.66	
Model 2.4 [f]	(referent)	-0.18	(-0.53-0.16)	-0.22	(-0.56-0.13)	-0.03	(-0.41-0.35)	0.81	
Head Circumference (N=3063)	783		764		801		715		
Change (cm) (95% CI) [b]									
Unadjusted	(referent)	0.01	(-0.20-0.23)	-0.01	(-0.22-0.20)	0.01	(-0.21-0.23)	0.97	
Model 2.1 [c]	(referent)	0.03	(-0.20-0.26)	-0.01	(-0.24-0.22)	0.05	(-0.20-0.30)	0.80	
Model 2.2 [d]	(referent)	0.05	(-0.19-0.28)	0.00	(-0.23-0.23)	0.05	(-0.20-0.31)	0.80	
Model 2.3[e]	(referent)	0.06	(-0.16-0.27)	-0.01	(-0.22-0.20)	0.09	(-0.14-0.32)	0.62	
Model 2.4 [f]	(referent)	0.21	(-0.05-0.46)	0.05	(-0.20-0.30)	0.22	(-0.05-0.50)	0.28	
Ponderal Index (N=3101)	789		774		812		726		
Change (kg/m^3) (95% CI) [b]									
Unadjusted	(referent)	0.36	(-0.16-0.88)	0.18	(-0.34-0.70)	-0.13	(-0.66-0.40)	0.53	
Model 2.1 [c]	(referent)	0.36	(-0.21-0.92)	0.26	(-0.31-0.83)	-0.14	(-0.75-0.48)	0.62	
Model 2.2 [d]	(referent)	0.42	(-0.15-0.98)	0.38	(-0.18-0.95)	0.01	(-0.60-0.63)	0.97	
Model 2.3[e]	(referent)	0.36	(-0.20-0.93)	0.26	(-0.31-0.83)	-0.13	(-0.74-0.49)	0.64	
Model 2.4 [f]	(referent)	0.49	(-0.20-1.19)	0.33	(-0.36-1.02)	0.02	(-0.73-0.78)	0.96	

[a] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory\ or\ EPA+DHA\ Quartile\ (includes\ reference)} + Confounding\ Variables$, for Y = fetal growth

[b] Change in mean fetal growth measures were calculated using linear regression, with non-robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, years), white Non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, $\geq$30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 780/3141 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.2g Associations of **fatty fish intake** with mean change in fetal growth indices

	Fatty Fish Intake (ounces)									
	<0.5/mo (N=742)		0.5/mo-<1.5/wk (N=797)		1.5-3/wk (N=855)		>3/wk (N=747)		*P* value [a]
Birth Weight (N=3141)	742		797		855		747		
Change (g) (95% CI) [b]									
Unadjusted	(referent)	-8.77	(-62.26-44.72)	-14.13	(-66.74-38.47)	5.34	(-49.01-59.68)	0.91	
Model 2.1 [c]	(referent)	4.96	(-51.55-61.47)	1.26	(-56.16-58.67)	21.29	(-41.14-83.71)	0.56	
Model 2.2 [d]	(referent)	7.62	(-49.39-64.62)	0.37	(-57.39-58.13)	16.47	(-46.36-79.30)	0.69	
Model 2.3[e]	(referent)	2.82	(-42.21-47.85)	-2.64	(-48.40-43.11)	-17.83	(-67.60-31.95)	0.46	
Model 2.4 [f]	(referent)	-22.86	(-79.85-34.13)	-3.62	(-60.07-52.83)	-14.17	(-75.41-47.07)	0.85	
Birth Length (N=3101)	736		785		843		737		
Change (cm) (95% CI) [b]									
Unadjusted	(referent)	0.15	(-0.16-0.46)	0.16	(-0.15-0.46)	0.17	(-0.14-0.50)	0.30	
Model 2.1 [c]	(referent)	0.23	(-0.10-0.57)	0.27	(-0.07-0.61)	0.31	(-0.06-0.68)	0.11	
Model 2.2 [d]	(referent)	0.15	(-0.21-0.50)	0.26	(-0.10-0.61)	0.22	(-0.16-0.61)	0.22	
Model 2.3[e]	(referent)	0.28	(-0.05-0.61)	0.27	(-0.07-0.61)	0.25	(-0.12-0.62)	0.24	
Model 2.4 [f]	(referent)	0.23	(-0.06-0.52)	0.24	(-0.06-0.53)	0.14	(-0.18-0.46)	0.44	
Head Circumference (N=3063)	721		781		830		731		
Change (cm) (95% CI) [b]									
Unadjusted	(referent)	-0.12	(-0.34-0.10)	-0.03	(-0.24-0.19)	-0.08	(-0.31-0.14)	0.67	
Model 2.1 [c]	(referent)	-0.15	(-0.39-0.08)	-0.05	(-0.29-0.19)	-0.10	(-0.36-0.16)	0.70	
Model 2.2 [d]	(referent)	-0.14	(-0.38-0.10)	-0.05	(-0.29-0.19)	-0.09	(-0.35-0.18)	0.74	
Model 2.3[e]	(referent)	-0.16	(-0.38-0.06)	-0.06	(-0.29-0.16)	-0.20	(-0.44-0.05)	0.23	
Model 2.4 [f]	(referent)	**-0.26**	**(-0.52-0.00)**	-0.13	(-0.39-0.13)	-0.28	(-0.57-0.00)	0.14	
Ponderal Index (N=3101)	736		785		843		737		
Change (kg/m^3) (95% CI) [b]									
Unadjusted	(referent)	-0.40	(-0.93-0.13)	-0.29	(-0.81-0.23)	-0.46	(-1.00-0.07)	0.14	
Model 2.1 [c]	(referent)	-0.49	(-1.07-0.08)	-0.40	(-0.98-0.18)	-0.59	(-1.22-0.05)	0.12	
Model 2.2 [d]	(referent)	-0.55	(-1.13-0.03)	-0.39	(-0.97-0.19)	-0.50	(-1.13-0.14)	0.22	
Model 2.3[e]	(referent)	-0.49	(-1.07-0.08)	-0.41	(-0.99-0.17)	-0.62	(-1.26-0.01)	0.09	
Model 2.4 [f]	(referent)	-0.65	(-1.36-0.06)	-0.55	(-1.27-0.16)	**-0.82**	**(-1.60--0.05)**	0.07	

[a] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory\ or\ EPA+DHA\ Quartile\ (includes\ reference)} + Confounding\ Variables$, for Y = fetal growth

[b] Change in mean fetal growth measures were calculated using linear regression, with non-robust standard errors.

[c] Model 2.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[d] Model 2.2 adjusts for the same variables in Model 2.1 but additionally adjusts for study year (5 indicator variables for the following years: 1998-1999, 2000-2001, 2002-2003, 2004-2005, 2006-2007).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] Model 2.4 adjusts for the same variables in Model 2.1 but excludes 780/3141 infants whose mothers experienced any of the following pregnancy complications: preeclampsia, pregnancy induced hypertension, gestational diabetes, or preterm birth.

Table 2.3a Associations of **seafood subtype intake** with risk of low birth weight, **stratified by infant sex** [a]

	Seafood intake (ounces)									
	<0.5/mo	0.5/mo-<1.5/wk		1.5-3/wk		>3/wk		*P* value [b]		
Shellfish										
Female: LBW/Normal Weight (N=63/1325)	23/431	19/300		6/308		15/286				
Unadjusted, RR (95% CI) [c]	(referent)	1.18	(0.65-2.12)	**0.38**	**(0.16-0.92)**	0.98	(0.52-1.85)	0.39		
Model 2.1 [d]	(referent)	1.00	(0.52-1.90)	**0.31**	**(0.12-0.78)**	0.74	(0.34-1.61)	0.12		
Model 2.3[e]	(referent)	0.71	(0.35-1.44)	**0.31**	**(0.11-0.87)**	0.80	(0.42-1.51)	0.17		
Male: LBW/Normal Weight (N=60/1243)	21/412	12/265		13/308		14/257				
Unadjusted, RR (95% CI) [c]	(referent)	0.89	(0.45-1.78)	0.83	(0.42-1.64)	1.07	(0.55-2.06)	0.99		
Model 2.1 [d]	(referent)	0.86	(0.39-1.92)	0.79	(0.39-1.60)	0.96	(0.44-2.10)	0.85		
Model 2.3[e]	(referent)	0.78	(0.28-2.16)	1.02	(0.43-2.43)	0.97	(0.40-2.33)	0.86		
Lean Fish [f]										
Female: LBW/Normal Weight (N=63/1325)	14/339	13/325		18/340		18/321				
Unadjusted, RR (95% CI) [c]	(referent)	0.97	(0.46-2.03)	1.27	(0.64-2.51)	1.34	(0.68-2.65)	0.30		
Model 2.1 [d]	(referent)	1.23	(0.54-2.78)	1.44	(0.66-3.17)	1.77	(0.76-4.14)	0.17		
Model 2.3[e]	(referent)	0.98	(0.41-2.36)	1.75	(0.89-3.41)	1.31	(0.61-2.81)	0.23		
Male: LBW/Normal Weight (N=60/1243)	11/332	18/302		13/338		18/271				
Unadjusted, RR (95% CI) [c]	(referent)	1.75	(0.84-3.66)	1.15	(0.52-2.54)	1.94	(0.93-4.05)	0.19		
Model 2.1 [d]	(referent)	2.19	(0.93-5.16)	1.63	(0.67-3.99)	**3.26**	**(1.36-7.82)**	**0.02**		
Model 2.3[e]	(referent)	2.59	(0.91-7.33)	**3.17**	**(1.04-9.63)**	**3.53**	**(1.09-11.48)**	**0.02**		
Fatty Fish										
Female: LBW/Normal Weight (N=63/1325)	14/296	12/332		23/370		14/327				
Unadjusted, RR (95% CI) [c]	(referent)	0.77	(0.36-1.64)	1.30	(0.68-2.48)	0.91	(0.44-1.88)	0.78		
Model 2.1 [d]	(referent)	0.80	(0.36-1.79)	1.30	(0.64-2.66)	0.71	(0.30-1.68)	0.83		
Model 2.3[e]	(referent)	1.08	(0.47-2.46)	1.43	(0.67-3.05)	0.85	(0.40-1.79)	0.99		
Male: LBW/Normal Weight (N=60/1243)	18/309	18/322		13/331		11/281				
Unadjusted, RR (95% CI) [c]	(referent)	0.96	(0.51-1.82)	0.69	(0.34-1.38)	0.68	(0.33-1.43)	0.20		
Model 2.1 [d]	(referent)	0.98	(0.45-2.12)	0.71	(0.31-1.64)	0.59	(0.23-1.46)	0.18		
Model 2.3[e]	(referent)	1.14	(0.43-3.08)	0.76	(0.27-2.14)	0.66	(0.21-2.12)	0.27		

[a] 1 infant (normal birth weight) was missing infant sex.

[b] Wald p-value for grouped linear term beta1 from the model: $log(P[Y=1|X]) = beta0 + beta1*X_{SeafoodCategory\ (includes\ reference)} + Confounding\ Variables$, for Y = fetal growth outcome

[c] Relative risks calculated using generalized linear model with a log link from the Poisson family, with robust standard errors.

[d] Model 2.1 is adjusted for maternal age (continuous, years), white non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, ≥30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] The Wald p-value for the infant_sexXweekly_lean_fish interaction term was 0.43 and 0.20 for Models 2.1 and 2.2, respectively

Table 2.3b Associations of **seafood subtype intake** with mean change in ponderal index, **stratified by infant sex** [a]

Seafood intake (ounces)	<0.5/mo	0.5/mo-<1.5/wk		1.5-3/wk		>3/wk		*P* value [b]
Shellfish [f]								
Female: No. Infants N=1528	507	341		344		336		
Change (kg/m^3) (95% CI) [c]								
Unadjusted	(referent)	**0.63**	**(-0.04-1.30)**	0.26	(-0.41-0.93)	0.58	(-0.09-1.25)	0.15
Model 2.1 [d]	(referent)	**0.73**	**(0.02-1.45)**	0.47	(-0.26-1.19)	**1.10**	**(0.31-1.89)**	**0.02**
Model 2.3[e]	(referent)	**0.76**	**(0.05-1.47)**	0.42	(-0.31-1.14)	**1.07**	**(0.28-1.86)**	**0.02**
Male: No. Infants N=1572	518	333		388		333		
Change (kg/m^3) (95% CI) [c]								
Unadjusted	(referent)	-0.20	(-0.98-0.57)	-0.29	(-1.04-0.45)	-0.10	(-0.88-0.68)	0.68
Model 2.1 [d]	(referent)	-0.09	(-0.92-0.75)	-0.09	(-0.92-0.74)	0.25	(-0.67-1.17)	0.68
Model 2.3[e]	(referent)	-0.10	(-0.93-0.74)	-0.10	(-0.93-0.73)	0.25	(-0.67-1.17)	0.68
Lean Fish								
Female: No. Infants N=1528	387	380		388		373		
Change (kg/m^3) (95% CI) [c]								
Unadjusted	(referent)	0.61	(-0.08-1.29)	0.32	(-0.36-1.01)	-0.11	(-0.80-0.58)	0.60
Model 2.1 [d]	(referent)	0.43	(-0.31-1.18)	0.33	(-0.42-1.08)	-0.34	(-1.15-0.46)	0.40
Model 2.3[e]	(referent)	0.43	(-0.31-1.17)	0.36	(-0.39-1.10)	-0.31	(-1.11-0.49)	0.46
Male: No. Infants N=1572	402	393		424		353		
Change (kg/m^3) (95% CI) [c]								
Unadjusted	(referent)	0.14	(-0.65-0.92)	0.05	(-0.72-0.82)	-0.16	(-0.96-0.65)	0.69
Model 2.1 [d]	(referent)	0.26	(-0.59-1.11)	0.13	(-0.72-0.98)	-0.05	(-0.99-0.89)	0.86
Model 2.3[e]	(referent)	0.26	(-0.59-1.12)	0.13	(-0.72-0.98)	-0.05	(-0.98-0.89)	0.86
Fatty Fish								
Female: No. Infants N=1528	353	373		429		373		
Change (kg/m^3) (95% CI) [c]								
Unadjusted	(referent)	-0.18	(-0.89-0.53)	-0.37	(-1.06-0.32)	-0.30	(-1.01-0.41)	0.34
Model 2.1 [d]	(referent)	-0.33	(-1.10-0.43)	-0.56	(-1.32-0.19)	-0.46	(-1.29-0.36)	0.25
Model 2.3[e]	(referent)	-0.32	(-1.08-0.44)	-0.57	(-1.32-0.19)	-0.48	(-1.31-0.34)	0.20
Male: No. Infants N=1572	383	412		414		363		
Change (kg/m^3) (95% CI) [c]								
Unadjusted	(referent)	-0.60	(-1.39-0.19)	-0.21	(-1.00-0.57)	-0.63	(-1.44-0.18)	0.23
Model 2.1 [d]	(referent)	-0.65	(-1.52-0.21)	-0.21	(-1.11-0.69)	-0.72	(-1.68-0.25)	0.31
Model 2.3[e]	(referent)	-0.66	(-1.52-0.21)	-0.21	(-1.11-0.69)	-0.73	(-1.70-0.23)	0.30

[a] 1 infant (normal birth weight) was missing infant sex.

[b] Wald p-value for grouped linear term beta1 from the model: $E(Y|X) = beta0 + beta1*X_{SeafoodCategory\ or\ EPA+DHA\ Quartile\ (includes\ reference)} + Confounding\ Variables$, for Y = fetal growth

[c] Change in mean fetal growth measures were calculated using linear regression, with non-robust standard errors.

[d] Model 2.1 is adjusted for maternal age (continuous, years), white Non-Hispanic race/ethnicity (yes/no), post high-school education (yes/no), married marital status (yes/no), pre-pregnancy BMI (indicator variables: 18.5-24.9, 25-29.9, $\geq$30 kg/m^2), recreational physical activity during pregnancy (yes/no), smoking during pregnancy (yes/no), any alcohol intake during pregnancy (yes/no), nulliparous (yes/no), total energy (continuous, kcal/day), red and processed meat intake (continuous, servings/day), male infant sex, and alternate seafood subtypes (indicator variables for non-reference categories).

[e] Model 2.3 adjusts for the same variables in Model 2.1 but additionally adjusts for gestational week of delivery (continuous).

[f] The Wald p-value for the weekly_shellfish-infant sex interaction term for Model 2.1 was 0.17.

Figure 2.1 Associations of seafood subtype intake and **risk of low birth weight**[a]

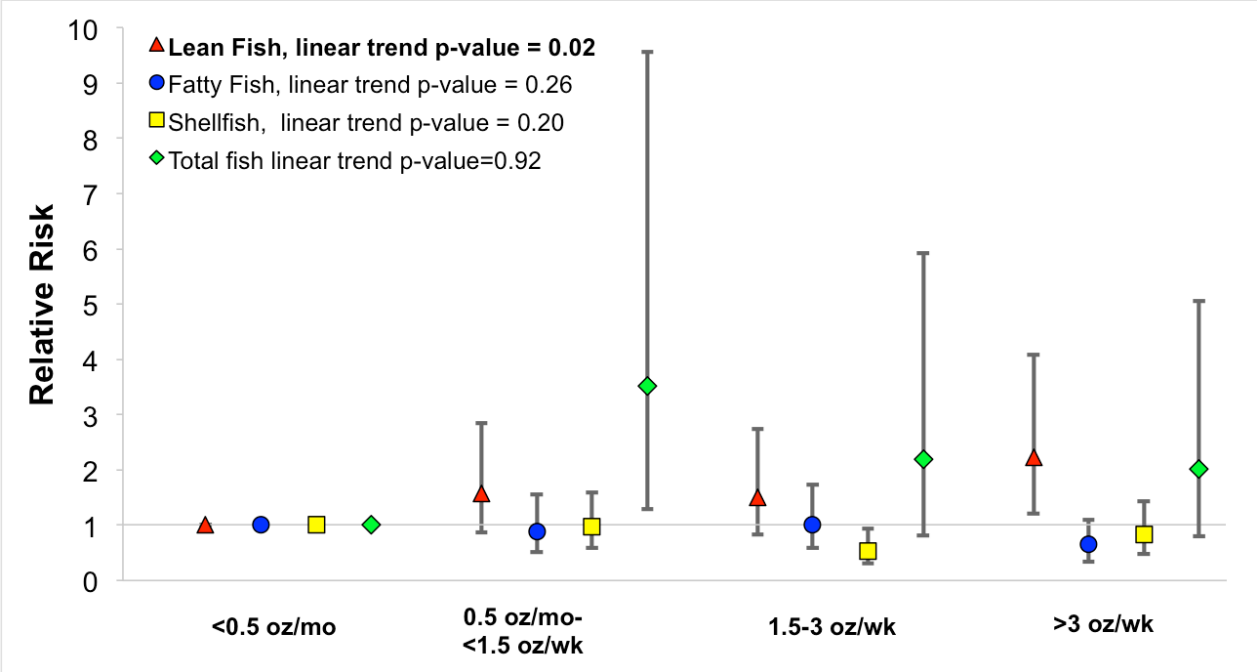
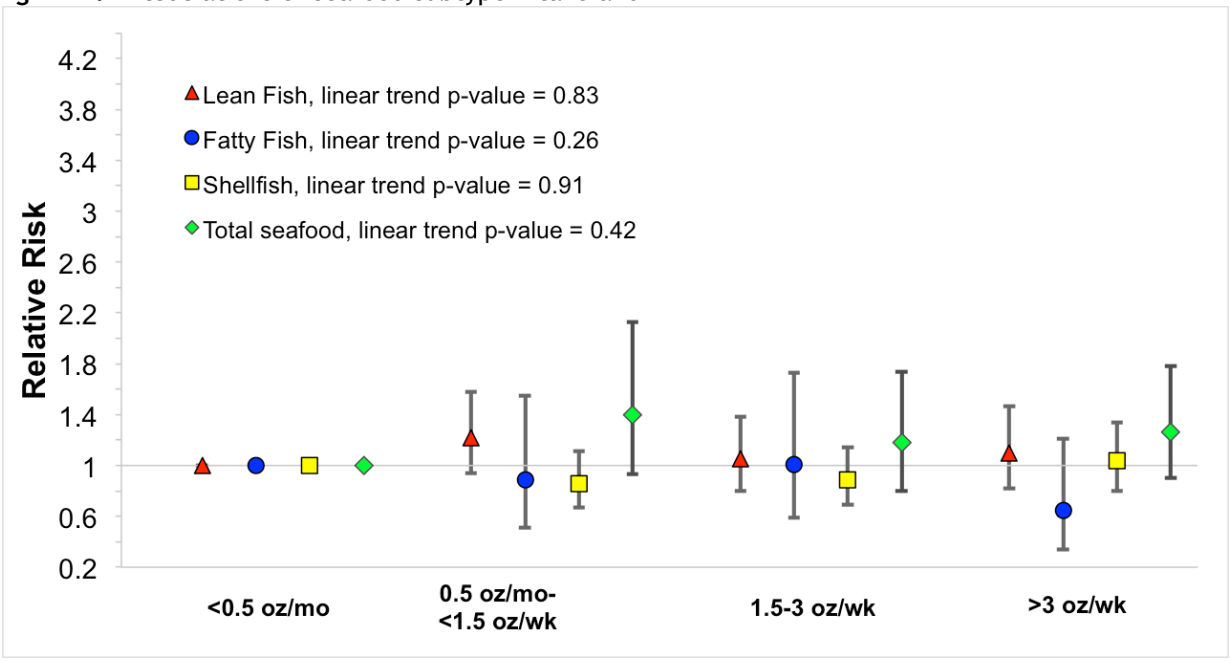


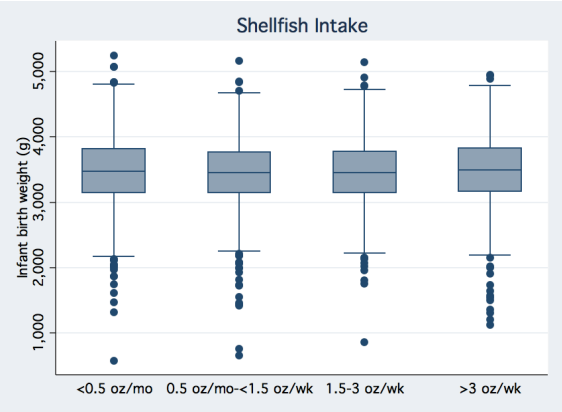
Figure 2.2 Associations of seafood subtype intake and **risk of macrosomia**[b]



[a]Primary adjusted Model (2.1) from Table 2.2a
Primary adjusted Model (2.1) from Table 2.2b

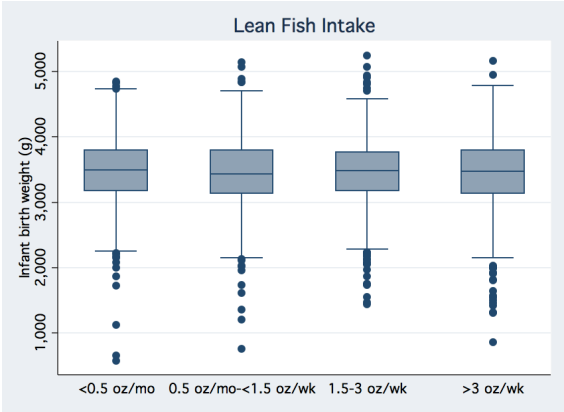
Figures 2.3a-c Box plots of **birth weight** in relation to seafood subtype intake

Figure 2.3a



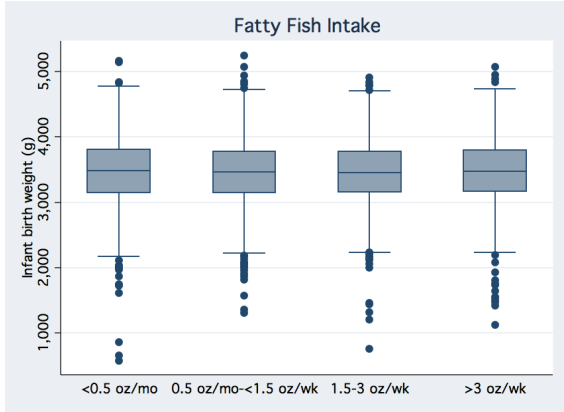
Median and range (g) for each level of shellfish intake, from lowest to highest were: 3475.5 (578.0-5250.0), 3459.0 (652.0-5160.0), 3450.0 (859.0-5140.0), 3490.0 (1118.0-4946.0).

Figure 2.3b



Median and range (g) for each level of lean fish intake, from lowest to highest were: 3496.5 (578.0-4845.0), 3437.0 (757.0-5140.0), 3489.0 (1440.0-5250.0), 3470.0 (859.0-5160.0).

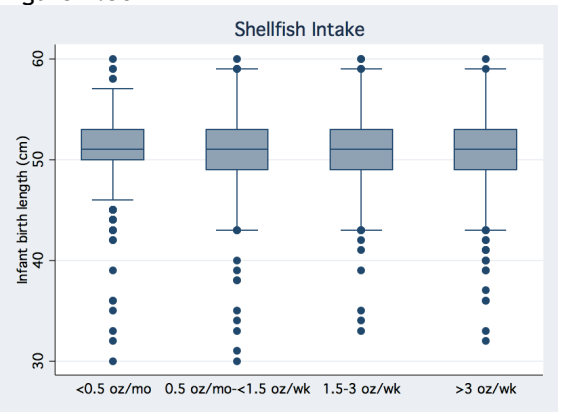
Figure 2.3c



Median and range (g) for each level of fatty fish intake, from lowest to highest were: 3482.5 (578.0-5160.0), 3461.0 (1310.0-5250.0), 3459.0 (757.0-4915.0), 3478.0 (1118.0-5070.0).

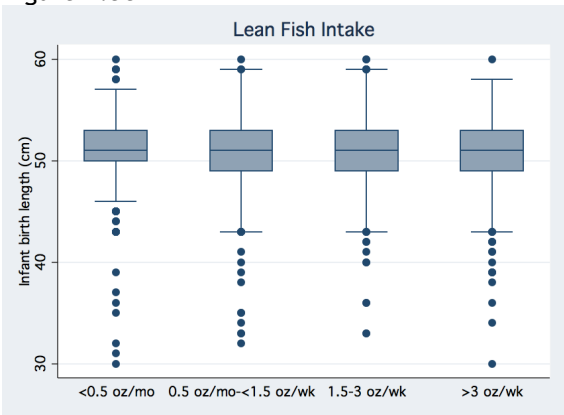
Figures 2.3d-f Box plots of **birth length** in relation to seafood subtype intake

Figure 2.3d



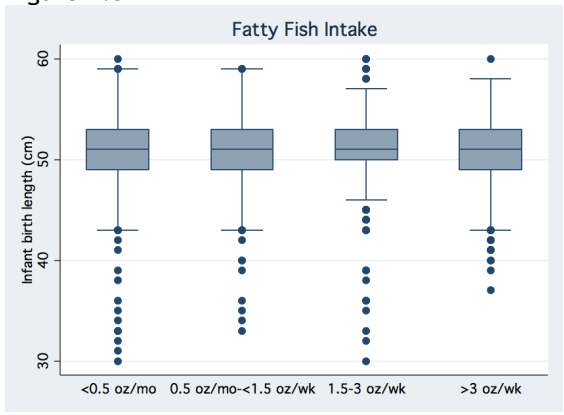
Median and range (cm) for each level of shellfish intake, from lowest to highest were: 51.0 (30.0-60.0), 51.0 (30.0-60.0), 51.0 (33.0-60.0), 51.0 (32.0-60.0).

Figure 2.3e



Median and range (cm) for each level of lean fish intake, from lowest to highest were: 51.0 (30.0-60.0), 51.0 (32.0-60.0), 51.0 (33.0-60.0), 51.0 (30.0-60.0).

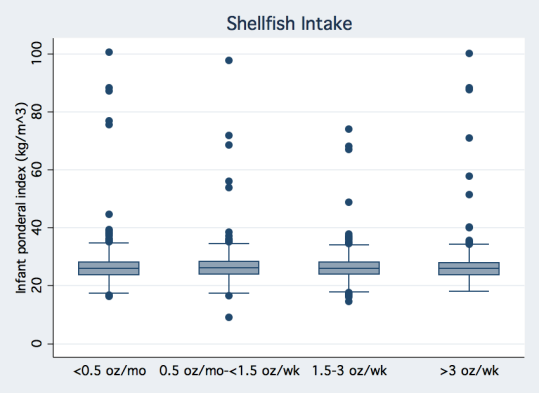
Figure 2.3f



Median and range (cm) for each level of fatty fish intake, from lowest to highest were: 51.0 (30.0-60.0), 51.0 (33.0-59.0), 51.0 (30.0-60.0), 51.0 (37.0-60.0).

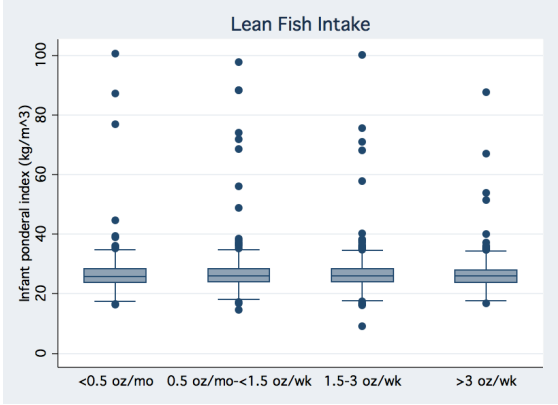
Figures 2.3g-i Box plots of **ponderal index** in relation to seafood subtype intake

Figure 2.3g



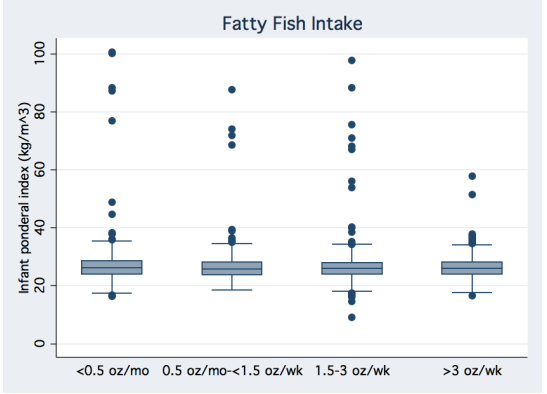
Median and range (kg/m^3) for each level of shellfish intake, from lowest to highest were: 25.9 (16.3-100.6), 26.2 (9.1-97.6), 25.9 (14.7-74.1), 25.9 (18.1-100.1).

Figure 2.3h



Median and range (kg/m^3) for each level of lean fish intake, from lowest to highest were: 25.9 (16.3-100.6), 26.0 (14.7-97.6), 26.1 (9.1-100.1), 25.9 (16.8-87.7).

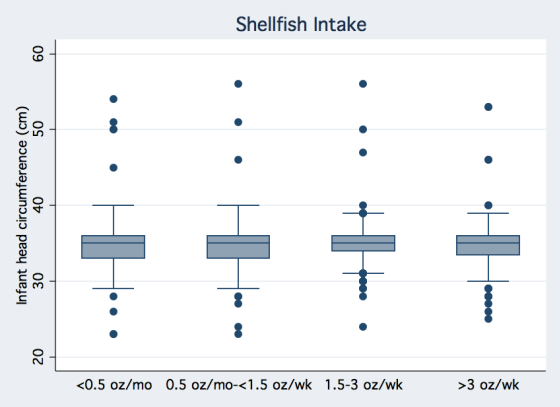
Figure 2.3i



Median and range (kg/m^3) for each level of fatty fish intake, from lowest to highest were: 26.2 (16.3-100.6), 25.8 (18.6-87.7), 25.9 (9.1-97.6), 25.9 (16.6-57.9).

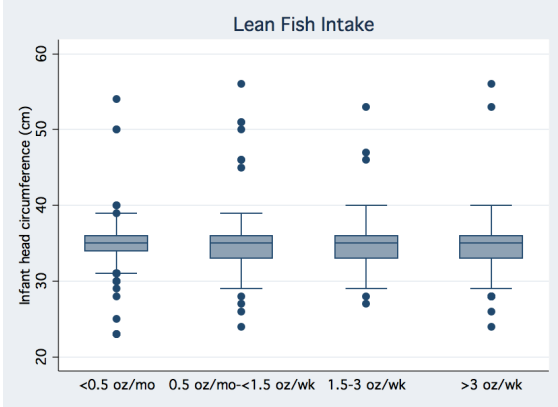
Figures 2.3j-l Box plots of **head circumference** in relation to seafood subtype intake

Figure 2.3j



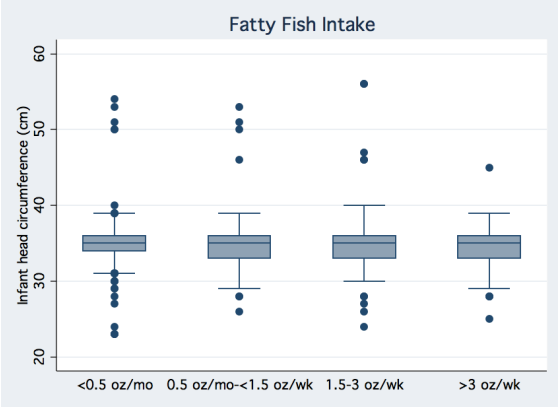
Median and range (cm) for each level of shellfish intake, from lowest to highest were: 35.0 (23.0-54.0), 35.0 (23.0-56.0), 35.0 (24.0-56.0), 35.0 (25.0-53.0).

Figure 2.3k



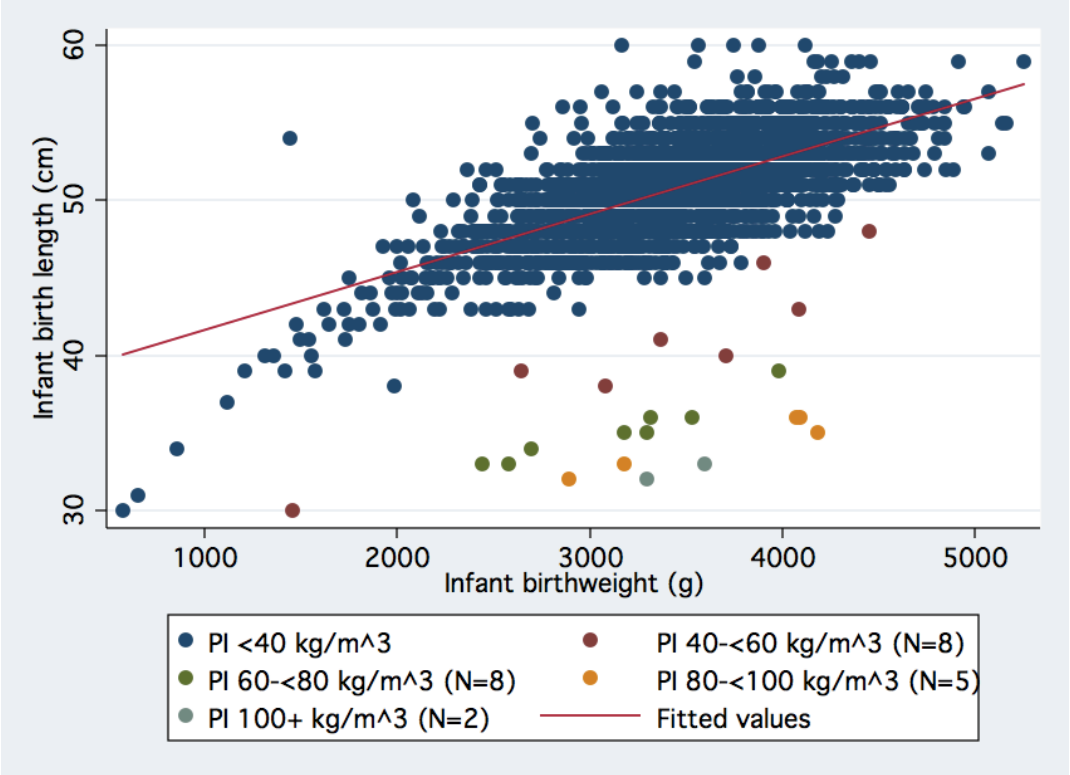
Median and range (cm) for each level of lean fish intake, from lowest to highest were: 35.0 (23.0-54.0), 35.0 (24.0-56.0), 35.0 (27.0-53.0), 35.0 (24.0-56.0).

Figure 2.3l



Median and range (cm) for each level of fatty fish intake, from lowest to highest were: 35.0 (23.0-54.0), 35.0 (26.0-53.0), 35.0 (24.0-56.0), 35.0 (25.0-45.0).

Figure 2.4 Infant birth weight and birth length plot with ponderal indices of 40 kg/m^3 or higher



Chapter 3: Placental cadmium and DNA methylation

ABSTRACT

Maternal cadmium (Cd) burden has been associated with fetal growth indicators and recent evidence suggests differences in infant sex-specific associations. However, mechanisms accounting for observed associations are largely unknown. Epigenetic mechanisms involving the placenta, a target organ of Cd accumulation and toxicity and a key component of the intrauterine environment, can potentially mediate the effect of maternal Cd burden on fetal growth. Among 24 maternal-infant pairs, we investigated sex-specific associations of placental Cd with placental genome-wide DNA methylation. Placental Cd was measured by inductively coupled mass spectrometry. Placental genome-wide methylation was profiled using the Infinium HumanMethylation 450 BeadChip. We used ANOVA models to examine associations of high/low placental Cd status with DNA methylation (at each CpG site or regions of CpG sites), stratified by infant sex. Statistical significance of associations was determined using false discovery rates (FDR) (cutoff FDR adjusted p-value <0.1). Medians of placental Cd levels among female and male infants were 5 ug/g and 2 ug/g, respectively. Among female infants, three individual CpG sites (near *ARL9*, *SIAH3*, and *HS3ST4*) and one genomic region on chromosome 7 (which included genes *CROT* and *TP53TG1*) were hypomethylated in placentas with high Cd levels, compared to placentas with low Cd levels. Among male infants, high placental Cd levels were associated with differential methylation of three individual CpG sites, two (hypomethylated) near *MECOM* and one (hypermethylated) near *SALL1*, and two genomic regions, both hypomethylated (one on chromosome 3 and one on chromosome 8, which included the *MECOM* and *ARHGEF10* genes, respectively). Our preliminary study provides suggestive evidence for infant sex-specific associations of placental Cd with placental DNA methylation of several genes, largely consistent with prior studies in the literature of sex-specific associations of Cd. Cd-related differentially methylated genes included those involved in cell damage (*SIAH3, HS3ST4, TP53TG1*) in female infants, and cell differentiation, angiogenesis and organ development (*MECOM, SALL1*) in male infants. Future larger studies have the potential to further our understanding of epigenetic mechanisms that potentially mediate associations of maternal Cd burden with suboptimal fetal growth.

BACKGROUND

Cadmium (Cd) is a heavy metal that accumulates naturally in the environment and as a pollutant due to its wide use in industrial and agricultural settings. Low-level environmental exposure to Cd, from cigarette smoke or diet (consumption of seafood and plants grown in Cd-contaminated soil)[8,133], is ubiquitous and has been associated with adverse health outcomes in adults including cardiovascular and kidney diseases, osteoporosis, and cancer[9,10,134-136]. A growing number of studies suggest that maternal Cd is associated with reduced fetal growth including lower birth weight[117,137-142], shorter birth length[141,143,144], and smaller head circumference[117,141,145,146]. Further, sex-specific differences in associations of maternal Cd burden with fetal growth indices have also been described[117,146]. Investigators recently reported associations of higher maternal Cd with lower birth weight, smaller chest and head circumference in female infants only[117]. These results parallel studies in adult populations that have described a higher body burden of Cd in females[147] and a higher risk of Cd-related bone fracture[148] and end-stage kidney disease[136] among females. Other investigators have reported Cd-related higher risk of cardiovascular diseases, cancer, and all-cause mortality among males[135].

Mechanisms underlying observed associations of maternal Cd burden with fetal growth indicators are largely unknown. During pregnancy, the placenta acts as an efficient, but partial, barrier to fetal Cd exposure, transferring as low as 10% of Cd[23,149-151] from the mother to the fetus. Increasingly, researchers have suggested that the main mechanism by which maternal Cd burden influences fetal growth may be through its impact on the intrauterine environment[24,27,28,138]. Placental function disturbances subsequent to Cd exposure may present a less than optimal intrauterine environment, influencing fetal programming, growth, and development. These disturbances include disruption of zinc transfer to the fetus[23-26], production of reactive oxidative species (ROS)[32], disruption to endocrine function including reduced placental glucocorticoid metabolism[29], leptin synthesis[27], and placental progesterone synthesis[28], changes to levels of insulin-like growth factor (IGF)[30,31], and vascular damage[33]. In addition, accumulating evidence supports that disruption of endocrine-related mechanisms may impact fetal growth in a sex-dependent manner[152-156]. DNA methylation, an

epigenetic mechanism, may mediate the adverse consequences of maternal Cd body burden on fetal growth[157-160]. Investigators have identified associations of Cd levels and global hypomethylation in peripheral blood[161] and cord blood[162], in non-pregnant women and in newborns, respectively. To date, only one study, by Kippler *et al.*, examined genome-wide associations of maternal Cd burden, DNA methylation, and infant birth weight[163]. Kippler *et al.* investigated infant-sex specific associations of maternal Cd burden (characterized using measurements in urine collected at 8 weeks gestation and blood collected at 16 weeks gestation)[11] with DNA methylation in mononuclear cells in cord blood among 127 maternal-infant pairs in Bangladesh. In this study, maternal blood Cd was associated with sex-specific DNA methylation in cord blood. In female infants, methylation of the top CpG sites including sites near genes, *GPR123, TTC40,* and *SLC45A4,* and other genes related to organ development, morphology and bone mineralization were inversely correlated with maternal Cd levels. In male infants, methylation of most of the top CpG sites including sites near genes *TBCD, STK10,* and *HRASLS2,* and other genes related to cell death were positively correlated with maternal Cd levels. In addition, investigators reported sex-specific associations of Cd-related differential methylation with birth weight. For instance, a CpG site near *TSH7DA* was associated with lower birth weight in female infants, but not in males.

To our knowledge, no previous study investigated Cd-related DNA methylation in the placenta, a key component of the intrauterine environment. Therefore, we investigated sex-specific associations of placental Cd with genome-wide DNA methylation in the placenta. In a secondary analysis, we examined whether identified placental DNA methylation changes were associated with birth weight.

METHODS

Study Setting and Study Population

The current study was conducted among participants of the Omega study and Placental MicroArray study[34,35]. Briefly, the Omega study (1996-2008) is a prospective cohort study investigating risk factors of pregnancy complications among residents of the Pacific Northwest. Participants were recruited from women attending prenatal care clinics affiliated with Swedish Medical Center and Tacoma

General Hospital. Women who initiated prenatal care before 20 weeks of gestation were eligible to participate. The Placental MicroArray study is a case-control study designed to examine differential placental gene expression that is related to pregnancy complications and included women who delivered at Swedish Medical Center.

Eligibility criteria for selection of participants (maternal-infant pairs) for the current study included the following: term delivery at Swedish Medical Center with available archived placental tissue, no history of chronic hypertension, or pre-pregnancy diabetes, singleton deliveries, and non-missing data for infant birth weight. For this pilot, due to the availability of resources and in an attempt to leverage previously generated gene-expression data, a total of 24 participants were included, 8 participants randomly selected from the Omega study and 16 (all) controls from a previous preeclampsia gene expression study among Placental MicroArray study participants[34]. The Swedish Medical Center Institutional Review Board approved study protocols and all participants provided written informed consent.

Data and Sample Collection

Trained personnel conducted medical records abstraction to obtain information on maternal socio-demographic and medical characteristics, and course and outcomes of pregnancy, including information on birth characteristics of the offspring. In addition, at-delivery, placenta samples were collected. Briefly, placenta specimens were double bagged, placed into coolers, and transported to a dedicated placenta-processing laboratory[34]. After removing the chorionic plate and overlying membranes, biopsy samples (approximately 0.5 cm^3 each) were collected from 16 sites (8 maternal and 8 fetal) adhering to a systematic technique to achieve uniformity and adequate sampling. Biopsy samples taken from the maternal side, that consist of villous tissue, uteroplacental ateries, and some decidua basalis, were evaluated for the current study. Abnormal morphology of these tissues has been associated with low birth weight and intrauterine growth restriction[164,165]. Biopsy samples were placed into cryotubes that contained RNAlater (Qiagen Inc, Valencia, CA)[166], at 10 µL/1 mg of tissue and stored at -80°C until processing.

Placental Cd Measurement

Briefly, 100 mg samples of placental tissue were thawed, partitioned, weighed and placed into 10 ml Teflon digestion vessels using acid-washed trace-metal free instruments and vessels. Tissues were further prepared for Cd measurement by microwave-assisted closed vessel digestion with nitric acid[167]. Cd was quantified on an Agilent 7500 CE ICP-MS in helium mode using internal standard calibration. Terbium (Tb, 20 ng) was used as a recovery standard for digestion. Spike recovery was performed for 4 additional placental samples for quality control. The reporting limit of Cd for this procedure was 2 ng/g of placental tissue. Placental Cd measurements were conducted at the Environmental Health Laboratory and Trace Organics Analysis Center at the University of Washington.

DNA Isolation and Genome-wide DNA Methylation Profiling Experiment

DNA was isolated using the QIAamp Tissue Kit (QIAGEN Inc. Chatsworth, CA). DNA purity was assessed by measuring OD260/280 and OD260/230 ratios. Samples with OD260/280 ratios >1.8 and OD260/230 ratios ≥2.0 were deemed of good quality DNA. DNA samples were archived at -80°C until further processing. DNA extraction procedures were conducted at the Center of Ecogenetics and Environmental Health (CEEH) Laboratories of the University of Washington. Isolated placental DNA samples were bisulphite-treated using EZ DNA Methylation Kit™, according to manufacturer's protocol (Zymo, D5001). Bisulphite-treated DNA was diluted with H_2O to 4 ul and hybridized to the Infinium HumanMethylation 450 BeadChip (Illumina, San Diego, CA)[163]. Briefly, each array measures methylation in approximately 450K CpGs per sample and is comprehensive in genome-wide coverage, including 99% of RefSeq genes, and 96% of CpG islands with additional coverage in island shores and regions flanking them. Each probe is designed to interrogate individual CpG sites within a given DNA sample. Genome-wide DNA methylation profiling experiments were conducted at the University of Texas Southwest.

Data Pre-processing

Data quality was checked using GenomeStudio™ Methylation Module software (2010.3). CpG sites (probes) with detection p-values > 0.01 in one or more samples were excluded. The raw data were

imported using the Bioconductor *minfi* package[168]. Raw intensity data were stratified by (type I or II), region (CpG Island, Shelf, Shore, or Other), and status (methylated or unmethylated) and sex, and then normalized using quantile normalization (QN) (Figure 3.1a). After normalization, the intensity values were converted to β-values, which estimate the proportion of DNA that is methylated at each CpG site. Density bean plots[169] of methylation β-values were examined for data anomalies and abnormal skews (Figure 3.1b). As a quality check, normalized data were visualized using a multi-dimensional scaling (MDS) plot after excluding allosomes (Figure 3.1c). β-values were converted to M-values ($\log_2(\beta/(1-\beta))$), which can be interpreted as the log ratio between methylated and unmethylated probe intensities[170].

Statistical Analysis

Frequency distributions of maternal and infant characteristics for all subjects and stratified by infant sex were examined. We defined an infant sex-specific placental Cd high/low cutoff value as greater than or equal to the median value specific to females or males. Two approaches were used to examine infant-sex stratified associations of placental Cd levels with placental genome-wide DNA methylation. The first approach used methylation of individual probes or CpG sites as the outcome while the second approach, a bump hunting method[171], used methylation over regions or clusters of probes as the outcome.

In the first approach, 482,422 separate ANOVA models were fit for each CpG site (stratified by infant sex) where methylation (M-value) was the dependent variable, infant-sex-specific Cd high/low status was the independent variable, and an indicator variable was included in the model for source population as an adjustment variable. False discovery rate adjusted p-values ("q" values), to account for multiple testing, were obtained using the Benjamini-Hochberg procedure[172]. We used p-value <0.1 as a cutoff to determine statistical significance.

The second or region analysis approach used the same infant-sex-stratified models as in the first set of analyses, with the M-values as the dependent variable (averaged over probe clusters/regions with a maximum gap of 300 bases between contiguous probes within a region) and sex-specific Cd high/low

status as the independent variable, and an indicator variable to adjust for source population. The Bioconductor bumphunter package was then used to identify candidate peaks in the data that were consistent with differential methylation of a region. These candidate regions were then restricted to the 99th percentile of all such regions, and statistical significance was determined for each of the remaining candidate regions by permutation (1000 permutations). This second smoothed analysis approach utilizes the high correlation of methylation status for probes within the same region to minimize selection of a region based on a single probe.

In the secondary analyses, CpG sites or regions associated with Cd, with (FDR adjusted p-value) <0.1, were evaluated for their associations with infant birth weight. We first examined Spearman's rank order correlation between methylation at each of these sites with birth weight, stratified by infant sex. Then we fit sex-stratified linear regression models examining associations of methylation at each site (or region) with birth weight. In these models, birth weight was the dependent variable, individual CpG site methylation or average methylation of all probes in the region (depending on the approach) was the independent variable, and models were adjusted for the source population (either the Omega study or the Placental MicroArray Study).

Analyses were conducted using Bioconductor and R packages (see Appendix A4.0).

RESULTS

The majority of participants were non-Hispanic white (71%), married (83%), and nulliparous (63%) (Table 3.0). Median maternal age of participants was 31.5 years. Median gestational age at delivery was 39 weeks. Median birth weight was 3516 g for females and 3341 g for males. Median placental Cd was 5 ng/g (wet weight) for females and 2 ng/g for males (Table 3.0). Placental Cd levels were <2 ng/g (the lower limit of detection) for 1 female infant and 6 male infants. There was negligible overlap in the distribution of Cd levels above the limit of detection in male and female infants (Figure 3.0). In subsequent analyses medians (≥5 ng/g for females and ≥2 ng/g for males) were used as cutoffs to determine high/low placental Cd groups.

Among female infants, in the individual probe analyses, we identified three differentially methylated CpG sites, all hypomethylated, in relation of high placental Cd levels. These sites were closest to *ARL9* (M-value: -3.52, FDR adjusted p-value = 0.01), *SIAH3* (M-value: -1.49, FDR adjusted p-value = 0.08) and *HS3ST4* (M-value: -1.28, FDR adjusted p-value = 0.08) genes (Table 3.1a, Figures 3.2a-c). In the genomic region analyses, among female infants, high placental Cd was associated with hypomethylation of one genomic region (region 86974674 to 86975244) on chromosome 7 which included genes *CROT* and *TP53TG1* (FDR adjusted p-value <0.1) (Table 3.1b, Figure 3.3a).

Among male infants, in the individual probe analyses, three CpG sites were differentially methylated in relation to Cd high/low status. The two genes closest to the three CpG sites associated with high Cd in males were *MECOM* (two hypomethylated sites, M-values: -3.39 and -3.26, FDR adjusted p-values <0.01) and *SALL1* (one hypermethylated site, M-value: 2.34, FDR adjusted p-value =0.08) (Table 3.1a, Figures 3.2d-f). In the genomic region analyses, in male infants, high placental Cd was associated with hypomethylation of two genomic regions, one on chromosome 3 (region 169379554 to 169380078) and one on chromosome 8 (region 1792758 to 1792758), which included the *MECOM* and *ARHGEF10* genes, respectively (FDR adjusted p-value <0.1) (Table 3.1b, Figures 3.3b-c).

We observed stronger correlations between methylation of CpG sites, significantly associated with Cd, and birth weight, among male infants (range -0.60 to 0.54) compared with respective correlations among female infants (range -0.33 to 0.22). Spearman rank correlations (unadjusted) were marginally significant for the CpG site and region analyses related to *MECOM* (CpG site correlation -0.60, p-value =0.04, region correlation -0.64, p-value =0.03) and *ARL9* (CpG site correlation 0.54, p-value =0.07) genes, among males (Tables 3.2a-b, Figures 3.4a-f). However, methylation at individual sites or genomic regions, identified in the Cd-methylation analyses, was not associated with infant birth weight.

DISCUSSION

Our study provides suggestive evidence for associations of placental Cd levels with placental DNA methylation among female and male infants. In female infants, high placental Cd (≥5 ng/g) was associated with hypomethylation of CpG sites near *ARL9*, *SIAH3*, and *HS3ST4, CROT and TP53TG1*. In male infants, high placental Cd (≥2 ng/g) was associated with hypomethylation of two CpG sites near *MECOM* and *ARHGEF10*, and hypermethylation of a CpG site near *SALL1*. Although we observed moderate to strong correlations (rho range = -0.60 to 0.54) between methylation at some of these identified target sites (e.g. *MECOM* and *ARL9*) and birth weight, particularly among male infants, associations were not statistically significant in this preliminary pilot study. Since the cutoffs for placental Cd were sex-specific and there was negligible overlap in the distributions of placental Cd in female and male infants, we were unable to contrast associations across infant sex.

To our knowledge, this is the first study to investigate associations of Cd and DNA methylation in placental tissue, a target organ of Cd accumulation and toxicity and a major component of the intrauterine environment. Our findings of infant-sex specific differences in Cd-methylation associations are consistent with those by Kippler *et al.*, who reported infant-sex specific associations of maternal peripheral blood Cd and cord blood DNA methylation of several genes[163]. In the study by Kippler *et al.*, the top 6 genes with Cd related-differential DNA methylation were *GPR123/TTC40, SLC45A4* and *GTF2A1,* all hypomethylated, in female infants and *TBCD, STK10*, and *HRASLS2,* all hypermethylated, in male infants. In that study, pathway analyses suggested strongest associations of Cd with differential methylation for genes related to bone morphology and mineralization, and organ development in female infants and genes related to cell death among male infants. In the current study, identified genes among female infants included those that have been associated with cancer (*HS3ST4*[173], *TP53TG1*[174], *SIAH3*[175]) while identified genes among male infants included those that have been associated with osteoporotic fracture *(MECOM)*[176], and kidney development *(SALL1)*[177] , outcomes or organs that have been related to Cd toxicity. However, genes identified in our study have not been previously linked to Cd or specific effects of Cd, warranting future replication and/or mechanistic studies.

Although our current study and the study by Kippler *et al.*, share similar methods, including use of the Infinium HumanMethylation450K BeadChip, there were several differences that deserve mention. Unlike our study, which used maternal placental tissue (median 39 weeks gestation, range 37-41 weeks) for all investigations, Kippler *et al.* examined Cd concentrations in maternal blood from early pregnancy (14 weeks gestation) and DNA methylation of mononuclear cells from cord blood (39 weeks gestation). Cd levels in maternal blood reflect more recent Cd exposure compared with placental tissue Cd, that bioaccumulates[11]. Although Cd levels in the two tissues have been positively correlated[144,178-181], Cd concentration in placental tissue tends to be much higher, as high as 10 times its concentration in maternal blood[178,181]. Second, DNA methylation can vary by developmental stage and by tissue[160]. Therefore, our assessment of placental Cd at-delivery may differ from the previous study's assessment of peripheral blood tissue in early pregnancy. Third, maternal Cd exposure may differ between our study populations. In the study by Kippler *et al.*, mean maternal urine concentration at 8 weeks gestation was 0.77 ug/L (5-95th percentiles: 0.25-2.40 ug/L) while among a subset of our study population, mean maternal urine concentration at 16 weeks gestation was 0.34 ug/L (5-95th percentiles: 0.13-0.63 ug/L). A study conducted among a similar population to the study population of Kippler *et al.*, in Matlab, Bangladesh, reported median placental Cd of 20 ng/g wet weight, also 4-fold higher than our median placental concentration of Cd, 5 ng/g wet weight[24]. These differences may contribute to differences in findings between the two studies.

Increasingly, studies have examined sex-specific associations of Cd and fetal growth. Romano *et al.*, investigated these associations among 472 randomly selected maternal-infant pairs from the Omega study, one of the population sources for subjects in the current study (unpublished). In their study, higher maternal urine Cd collected at 16 weeks gestation was associated with lower birth length among female infants and higher birth length among male infants (p-value for interaction = 0.03). Higher maternal Cd was also associated with higher ponderal index in females and lower ponderal index in males (p-value for interaction <0.01). The sex-specific associations of Cd and fetal growth indices in the Omega study were generally consistent with associations reported in the study conducted in Matlab, Bangladesh[117]. Kipper *et al.* reported higher urinary Cd collected at 8 weeks gestation to be associated

with lower birth weight, head and chest circumference that were statistically significant (p-value <0.05) in female infants only, although interaction p-values were not statistically significant. As in the Omega study, an association of higher Cd with lower and higher birth length was observed for female and male infants, respectively (interaction p-value = 0.11). In sum, these findings highlight the potential importance of evaluating sex-specific associations (and related mechanisms) of maternal Cd with fetal growth indices. While we only evaluated birth weight in the current study, future investigations on other indices of fetal growth, such as birth length, ponderal index and head circumference are warranted.

While Cd-related DNA methylation changes in several tissues (liver, prostate, and blood) have been well described[182-185], whether sex-specific associations of placental Cd and fetal growth can be explained by epigenetic mechanisms is unknown. However, accumulating research supports the thesis that the placenta (a target organ of Cd accumulation and toxicity) mediates fetal programming and growth responses to environmental stressors in a sex dependent manner[113,186-188]. Several studies suggest that placental responses to Cd that are associated with fetal growth, including placental 11ß-hydroxysteroid dehydrogenase type 2 *(11ß-HSD2)* gene activity[155,189,190], progesterone synthesis[28,191,192], and secretion of insulin like growth factors (IGF)[30,152,153], may be sex dependent. Further, investigators have reported infant sex-specific differential methylation in cord blood that is associated with other environmental toxins including cigarette smoke[193] and arsenic[194] in humans. Bisphenol A, another environmental exposure, has also been associated with offspring sex-specific gene methylation in brain tissue of mice[195]. However, sex-specific placental DNA methylation in relation to environmental stressors has yet to be investigated.

In the current study, we identified differential methylation of several target sites related to novel genes whose functions play critical roles in cellular metabolism, growth, and development. MDS1 and EVI1 (ecotropic virus integration site 1 protein homolog) complex locus (*MECOM*) is an oncogene that encodes a zinc finger transcription factor that may be involved in hematopoiesis, apoptosis, development, vascularization, angiogenesis, cell cycle regulation, differentiation, and proliferation[196-

[200]. *MECOM* is important for normal embryogenesis including development of kidney, heart, lung, and neural tissues[197-199]. Overexpression of *MECOM* can lead to the onset of leukemia and may be a predisposing factor of osteoporotic fracture[176,198]. Similar to *MECOM*, the protein encoded by sal-like 1 (*SALL1*), is a zinc finger transcription factor that may regulate angiogenesis, vascularization, embryonic stem cell differentiation, and organogenesis, especially kidney development[177,201-203]. Mutations or deletions of *SALL1* are associated with Townes-Brocks syndrome and branchio-oto-renal syndrome, developmental disorders with widespread adverse effects on anal, renal, heart, nervous system, limb, and ear development[204]. ADP-ribosylation factor-like 9 (*ARL9*) encodes a GTP binding member of the small GTPase protein family with a high degree of similarity to ARF proteins of the RAS superfamily[196]. SIAH E3 ubiquitin protein ligase member 3 (*SIAH3*) encodes a protein in the seven in absentia (Sina) family that regulates cellular responses to hypoxia, induction of apoptosis, and cell differentiation[196,205]. The heparan sulfate (glucosamine) 3-O-sulfotransferase 4 (*HS3ST4*) gene encodes an enzyme that generates 3-O-sulfated glucosaminyl residues in heparan sulfate[196]. Heparan sulfate interacts with a wide range of proteins influencing development, angiogenesis, blood coagulation, and tumorigenesis[173,206]. TP53 target gene 1 (non-protein coding) (*TP53TG1*) participates in the response to cellular damage and mutations in TP53 genes have been related to tumor development and progression in human cancers[174]. Genes *SIAH3, HS3ST4,* and *TP53TG1* all regulate responses to cell damage, which may have relevance to Cd-induced cell damage in placental tissue, although these genes have not been previously linked to Cd.

As a follow-up, we performed analyses to evaluate associations of differentially methylated sites, identified in our study, with genome-wide gene expression, using data from control participants (N=16) of a previously reported preeclampsia gene expression study[34]. These 16 participants were included in the current study. Our analyses showed that methylation of the six CpG sites associated with placental Cd in female infants were associated with down-regulation of two genes: *GPR160 (*related to CpG-site-methylation near *MECOM), HOPX (*related to CpG-site-methylation near *ARL9)*, and up-regulation of two genes: *SEC62* (related to CpG-site-methylation near *MECOM*) and *CPB2* (related to CpG-site-methylation near *SIAH3*) in placental tissue, p-value < 0.05 (Table 3.3, Figures 3.5a-e). Of note Cd-

related hypermethylation of a CpG site close to another member of the GPR gene family, *GPR123*, was the top hit among female infants in the study by Kippler *et al.*[163]. Related proteins are important for transmission of signals across cell membranes and promote the exchange of GDP for GTP on the alpha subunit of heterotrimeric G-proteins[207]. In males, expression of one gene, *KIAA1211* (down-regulated, p-value <0.05) was associated with CpG site methylation near *ARL9*.

Our results should be interpreted in view of several study limitations. These include limited statistical power to detect sex-specific associations of Cd with genome-wide DNA methylation and fetal growth, due to sample size constraints. Since the cutoffs for placental Cd were sex-specific and there was negligible overlap in the distributions of placental Cd in female and male infants, we were unable to examine sex interactions. We did not examine or control for potential confounding from placental exposure to other xenobiotics. Reduced fetal growth has been associated with prenatal exposure to other heavy metals, such as arsenic[138], mercury[124,125], and lead[208]. Exposure to these metals, including Cd, can also be correlated[179]. We measured mercury in placental tissue and found that most subjects (n = 19/24) had levels below the limit of detection (<2 ng/g placenta tissue) and placental mercury was not included in subsequent analyses. Therefore, we cannot completely rule out confounding by placental exposure to other xenobiotics or other potential confounding factors. We did not examine associations of genome-wide methylation with other indices of fetal growth, such as birth length or head circumference, indices that have been related to maternal Cd burden in prior studies[117,141,143-146] (Romano *et al.*). Birth weight was highly correlated with birth length in female (Spearman rho 0.75, p-value = 0.01) and male (Spearman rho 0.65, p-value = 0.02) infants in our study. Future investigations of associations of Cd related methylation with other fetal growth indices are warranted. Our study of mostly non-Hispanic white, married participants who initiated prenatal care early in pregnancy and participated in regular annual medical examinations may not be generalizable to women of other race/ethnicity or socioeconomic status.

Our study has several strengths. The placenta, a target organ of Cd accumulation and toxicity, plays a central role in the association between maternal Cd burden and fetal growth. Therefore,

measurements of placental Cd was of particular relevance. Second, DNA methylation is tissue specific[160] and use of placental tissue for genome-wide DNA methylation is pertinent for exploring mechanisms related to alterations to the intrauterine environment. We used a systematic sampling technique to collect placenta tissue from the maternal side to determine maternal Cd exposure since studies suggest that Cd may not be uniformly distributed across the placenta[209]. Fourth, we used two approaches to examine associations of placental Cd and genome-wide methylation (i.e. individual probes and genomic regions). Finally, we had an opportunity to examine associations between Cd-related DNA methylation and gene expression in a subset of the study population, allowing us to assess downstream effects of DNA methylation.

In sum, in the current study, among females, high Cd was associated with hypomethylation of top CpG sites near genes *SIAH3, HS3ST4,* and *TP53G1*, genes that have been linked to proteins that respond to cell damage[173-175,196,206]. Among male infants, high Cd was associated with hypomethylation of *MECOM*, and hypermethylation of *SALL1,* genes which have been linked to cell differentiation[176], angiogenesis, and organ development[177,196,201]. Our study provides suggestive evidence for sex-specific associations of placental Cd with placental DNA methylation of several genes. Similar larger studies can enhance our understanding of epigenetic mechanisms that may be linked to sex-specific associations, and investigations of potential sex-interactions, of maternal Cd burden with fetal growth.

Table 3.0 Characteristics for maternal-infant pairs included in the genome-wide DNA methylation experiment

Characteristic	All (N = 24)			Females (N = 12)			Males (N = 12)		
	N		%	N		%	N		%
Non-Hispanic white race/ethnicity [a]	17		70.8	10		83.3	7		58.3
Maternal unmarried status	4		16.7	0		0.0	4		33.3
Nulliparous	15		62.5	8		66.7	7		58.3
Sex-specific placenta high-Cd cutoff (≥2 ng/g for males and for ≥5 ng/g females	13		54.2	7		58.3	6		50.0
Placental MicroArray Study Subject	16		66.7	8		66.7	8		66.7
	median	min	max	median	min	max	median	min	max
Maternal age (yrs)	31.5	19.0	40.0	34.0	24.0	40.0	31.0	19.0	36.0
Maternal Pre-pregnancy BMI (kg/m^2)	22.6	18.0	39.5	22.9	20.2	39.5	22.6	18.0	38.7
Gestational age at delivery (weeks)	39	37	41	39	38	41	39	37	41
Maternal Placental Cd (ng/g) [b]	3.0	<2.0	7.0	5.0	<2.0	7.0	2.0	<2.0	5.0
Maternal Urinary Cd (ug/g creatinine) [a]	0.5	0.2	1.0	0.5	0.2	1.0	0.6	0.2	0.8
Maternal Creatinine (g) [a]	72.0	44.0	115.0	82.0	44.0	89.0	57.0	47.0	115.0
Infant birth weight (g)	3403.0	2610.0	4807.0	3516.0	2610.0	4320.0	3341.0	2696.0	4807.0

[a] Maternal race missing for 1 male infant. Maternal urinary Cd and creatinine missing for 7 female and 7 male infants.
[b] Placental Cd includes 1/12 females and 6/12 males with levels below the lower limit of detection (<2 ng/g).

Table 3.1a Cd high/low status and differential DNA methylation at **individual CpG sites**, stratified by infant sex[1]

Sex	Probe Order	CpG Site	logFC[2]	P-value	FDR adjusted p-value	Distance to TSS	CHR	Closet Gene Symbol/Name	Gene Ontology (Molecular Function)[2]
Females	**1**	**cg04528060**	**-3.52**	**<0.01**	**0.01**	**0**	**4**	***ARL9*/ ADP-ribosylation factor-like 9**	**GTP binding**
	4	cg07846311	-2.47	<0.01	0.29				
	5	cg15846316	-2.11	<0.01	0.29				
	2	**cg00613224**	**-1.49**	**<0.01**	**0.08**	**0**	**13**	***SIAH3*/siah E3 ubiquitin protein ligase family member 3**	**Metal ion binding**
	3	**cg03884018**	**-1.28**	**<0.01**	**0.08**	**0**	**16**	***HS3ST4*/ heparan sulfate (glucosamine) 3-O-sulfotransferase 4**	**[Heparan sulfate]-glucosamine 3-sulfotransferase 1 activity**
	6	cg11528069	-0.66	<0.01	0.29	16928	12	*uc009ztl.1*	
	7	cg10372829	0.90	<0.01	0.29	10346	13	*GJB2*/gap junction protein, beta 2, 26kDa	Gap junction channel activity
	8	cg26181490	-1.71	<0.01	0.35	0	5	*uc021ykd.1*	
	9	cg15543769	0.76	<0.01	0.35	92	1	*ZNF593*/zinc finger protein 593	DNA & metal ion binding, transcription corepressor activity
	10	cg10389032	-0.62	<0.01	0.38	0	2	*MGAT5*/mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetly-glucosaminyltransferase	alpha-1,6-mannosylglycoprotein 6-beta-N-acetlyglucosaminyltransferase activity
Males	**1**	**cg15958576**	**-3.39**	**<0.01**	**<0.01**	**0**	**3**	***MECOM*\MDS1 & EVI1 complex locus**	**DNA-, metal ion-, & protein- binding; protein homodimerization, & NOT protein homodimerization activity; sequence-specific DNA binding transcription factor activity**
	2	**cg10903116**	**-3.26**	**<0.01**	**<0.01**				
	4	cg24458896	-2.79	<0.01	0.12				
	3	**cg09728607**	**2.34**	**<0.01**	**0.08**	**4881**	**16**	***SALL1*/sal-like 1**	**DNA-, beta-catenin-, chromatin-, metal ion- & protein-binding; contributes to histone deacetylase activity; sequence-specific DNA binding transcription factor activity**
	5	cg01895398	-1.18	<0.01	0.60	24777	21	*PCBP3*/poly(rC) binding protein 3	DNA & RNA binding
	6	cg24686734	-1.55	<0.01	0.93	326382	13	*TEX29*/testis expressed 29	Integral to membrane
	7	cg25449412	-1.66	<0.01	0.93	0	8	*Uc003wpn.1*	
	8	cg16748433	-4.64	<0.01	1.00	0	8	*ARHGEF10*/Rho guanine nucleotide exchange factor 10	Rho guanyl-nucleotide exchange factor activity, kinase binding
	9	cg11917968	-0.92	<0.01	1.00	161066	6	*SMOC2*/SPARC related molecular calcium	Calcium and heparin binding; stimulates endothelial cell proliferation and migration,

								binding 2	angiogenic activity
10	cg03237316	0.64	<0.01		1.00	7848	4	*Uc003gdg.1*/	

[1] High Cd (≥5 ng/g females, ≥2 ng/g males)
[2] Difference in M-value (methylation) comparing high to low placental Cd.
[3] Gene ontology obtained from http://www.ncbi.nlm.nih.gov/gene

Table 3.1b Cd high/low status[1] and differential DNA methylation at **genomic regions**, stratified by infant sex

Infant Sex	Region	M-value[2] (mean)	FDR adjusted p-value	CHR	Gene(s) within Peak Symbol/Name	Gene Ontology (Molecular Function)[3]
Female	86974674 to 86975244	-1.82	0.06	7	*CROT*/carnitine O-octanoyltransferase *TP53TG1*/TP53 target 1 (non-protein coding)	Carnitine O-octanoyltransferase activity, receptor binding Molecular function
Male	169379554 to 169380078	-3.04	0.03	3	*MECOM*\MDS1 & EVI1 complex locus	DNA-, metal ion-, & protein- binding; protein homodimerization, & NOT protein homodimerization activity; sequence-specific DNA binding transcription factor activity
	1792758 to 1792758	-4.57	0.07	8	*ARHGEF10*/Rho guanine nucleotide exchange factor 10	Rho guanyl-nucleotide exchange factor activity, kinase binding

[1] High Cd (≥5 ng/g females, ≥2 ng/g males)
[2] Difference in M-value (methylation) comparing high to low placental Cd.
[3] Gene ontology obtained from http://www.ncbi.nlm.nih.gov/gene

Table 3.2a Correlations of Cd-related differential DNA methylation at **individual CpG sites** and infant birth weight, stratified by infant sex

Infant Sex	CpG site	CHR	Closest Gene	Distance to TSS	Methylation Birth Weight Association		Methylation Birth Weight Correlation	
					ß[1]	p-value	Rho	p-value
Female	cg04528060	4	*ARL9*	0	-78.1	0.33	-0.06	0.86
	cg00613224	13	*SIAH3*	0	-185.0	0.32	-0.13	0.70
	cg03884018	16	*HS3ST4*	0	-291.0	0.17	-0.12	0.72
	cg15958576	3	*MECOM*	0	-8.45	0.92	-0.33	0.30
	cg10903116	3	*MECOM*	0	-4.48	0.95	-0.12	0.72
	cg09728607	16	*SALL1*	4881	-36.74	0.74	0.22	0.49
Male	cg15958576	3	*MECOM*	0	-64.8	0.36	-0.48	0.12
	cg10903116	**3**	***MECOM***	**0**	**-66.4**	**0.37**	**-0.60**	**0.04**
	cg09728607	16	*SALL1*	4881	70.2	0.48	0.27	0.39
	cg04528060	4	***ARL9***	**0**	**2.5**	**0.98**	**0.54**	**0.07**
	cg00613224	13	*SIAH3*	0	326.0	0.20	-0.11	0.73
	cg03884018	16	*HS3ST4*	0	151.0	0.36	0.49	0.11

[1] ß for CpG site is equal to the mean change in birth weight (g) per unit increase in M-value for DNA methylation, adjusted for population source

Table 3.2b Correlations of Cd-related differential DNA methylation at **genomic regions** and infant birth weight, stratified by infant sex

Infant Sex	Region	CHR	Genes near region peak	Methylation Birth Weight Association		Methylation Birth Weight Correlation	
				ß[1]	p-value	Rho	p-value
Female	86974674 to 86975244	7	*CROT/TP53TG1*	-0.05	1.00	-0.15	0.65
Male	**169379554 to 169380078**	**3**	***MECOM***	**-43.3**	0.38	**-0.64**	**0.03**
	1792758 to 1792758	8	*ARHGEF10*	-40.6	0.27	-0.30	0.34

[1] ß for CpG site is equal to the mean change in birth weight (g) per unit increase in average regional M-value for DNA methylation, adjusted for population source

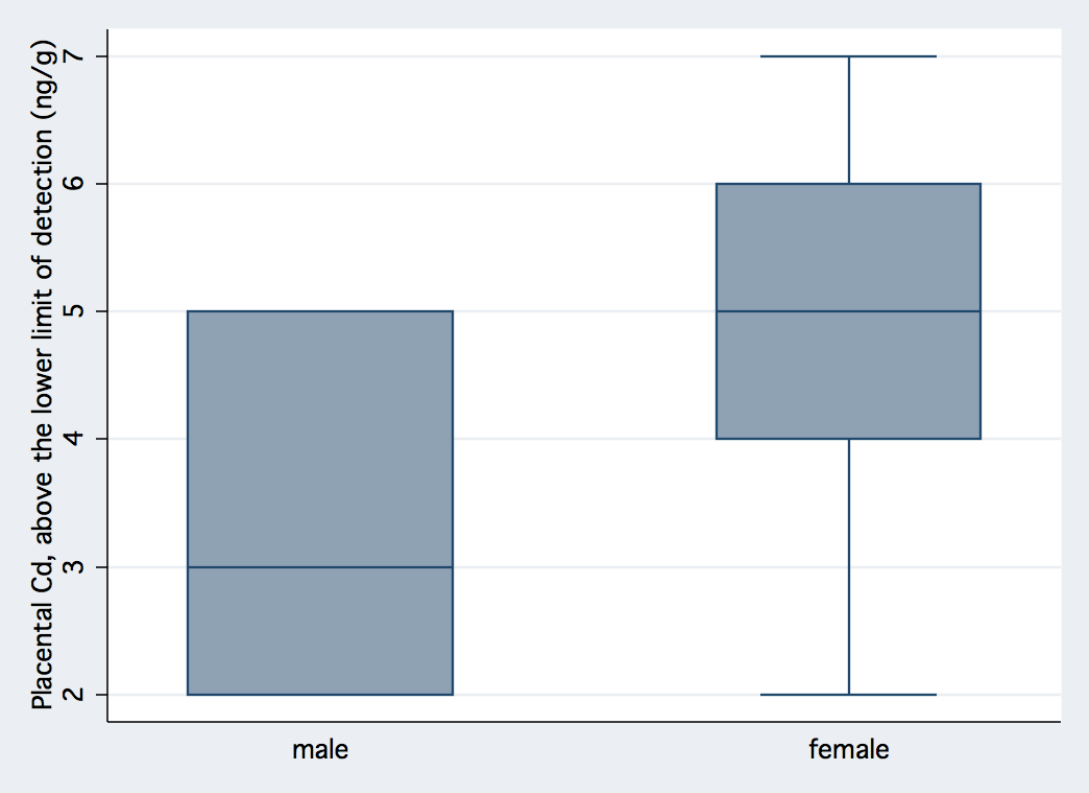
Table 3.3 Cd-related differential DNA methylation of individual CpG sites and **placental tissue gene expression**, stratified by infant sex

Gene symbol/Name	Gene ontology (molecular function)	CpG-site	Females		Males	
			ß[1]	p-value	ß[1]	p-value
HOPX/HOP homeobox	Sequence-specific DNA binding, sequence-specific DNA binding transcription factor activity	cg04528060	-1.84	0.04	0.10	0.95
KIAA1211/KIAA1211		cg04528060	-5.86	0.28	-9.65	0.04
CPB2/Carboxypeptidase B2 (plasma)	Metallocarboxypeptidase activity, zinc ion binding	cg00613224	0.58	0.05	-1.28	0.09
GPR160/G protein-coupled receptor 160	G-protein coupled receptor activity	cg15958576	-4.88	0.02	-0.86	0.74
		cg10903116	-5.38	0.03	-0.10	0.97
SEC62/SEC62 (S. cerevisiae)	Protein transporter activity, receptor activity	cg10903116	3.52	0.05	5.16	0.07

[1] B = fold change in gene expression

[2] Gene ontology obtained from http://www.ncbi.nlm.nih.gov/gene

Figure 3.0 Box plots of placental cadmium by infant sex [a]



[a] Above the lower limit of detection (2 ng/g), 6/12 male and 1/12 female infants had levels below 2 ng/g.

Figure 3.1a Quantile normalization

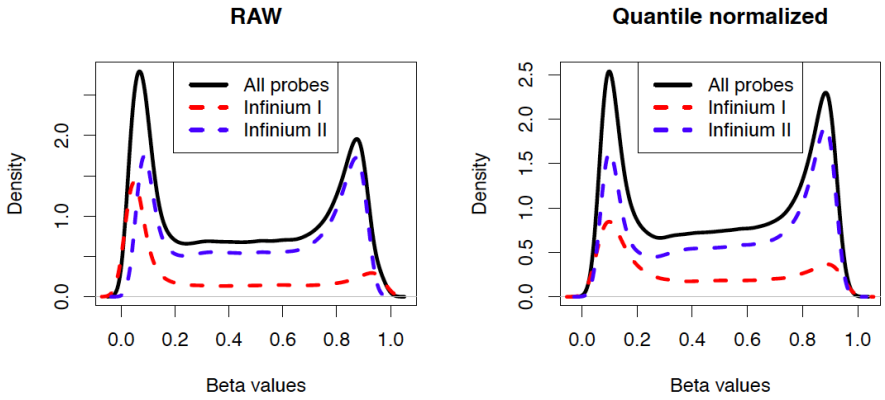


Figure 3.1b Bean plot

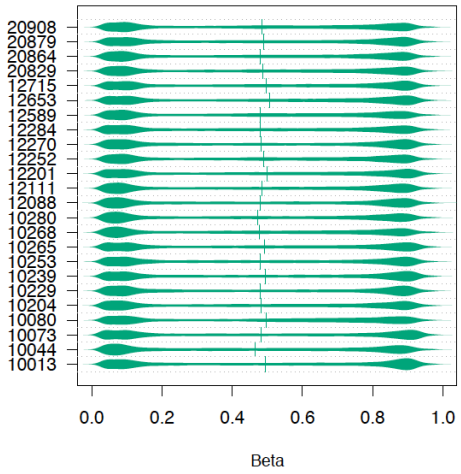
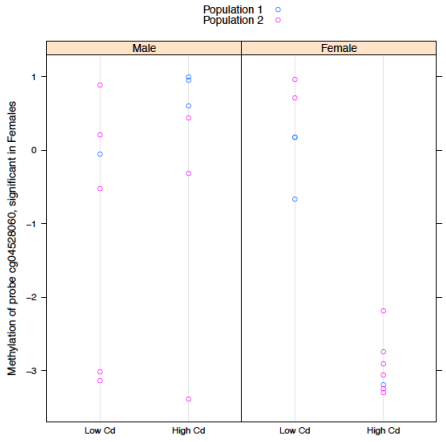


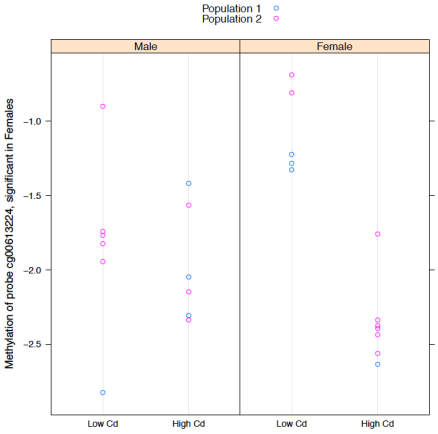
Figure 3.1c Multi-dimensional scaling (MSD) plot for normalized data, allosomes only

Figure 3.2a-f High/low Cd status and methylation at **individual CpG sites** stratified by infant sex: (a) cg04528060, (b) cg00613224, (c) cg03884018, (d) cg15958576, (e) cg10903116, and (f) cg09728607[a]

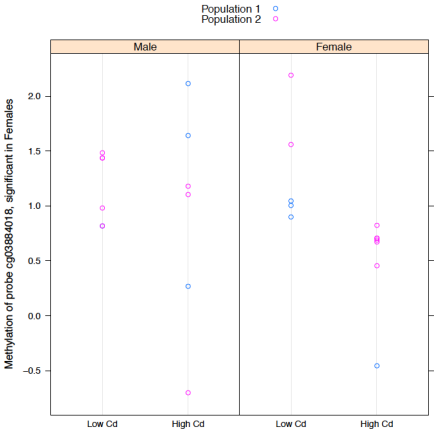
a.



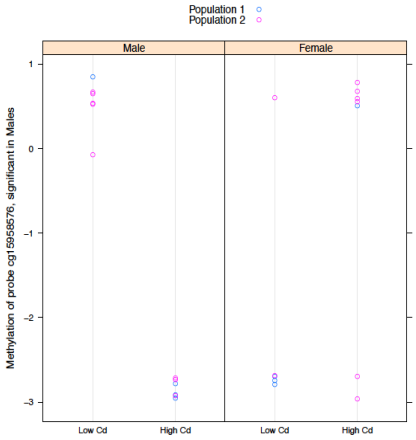
b.



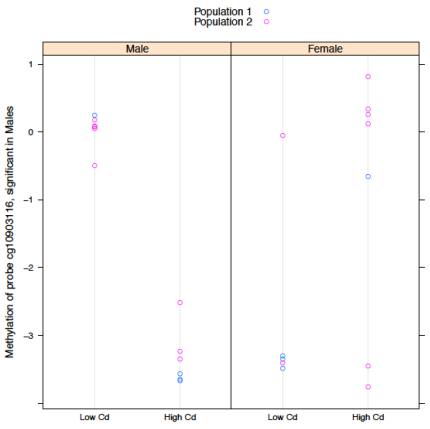
c.



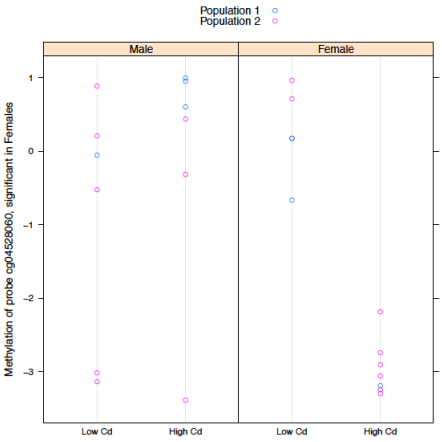
d.



e.



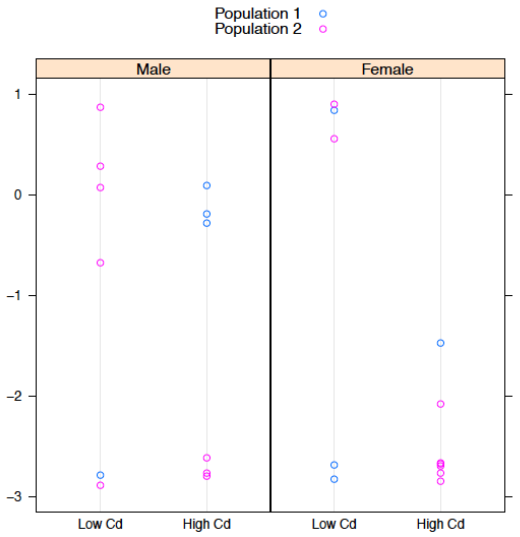
f.



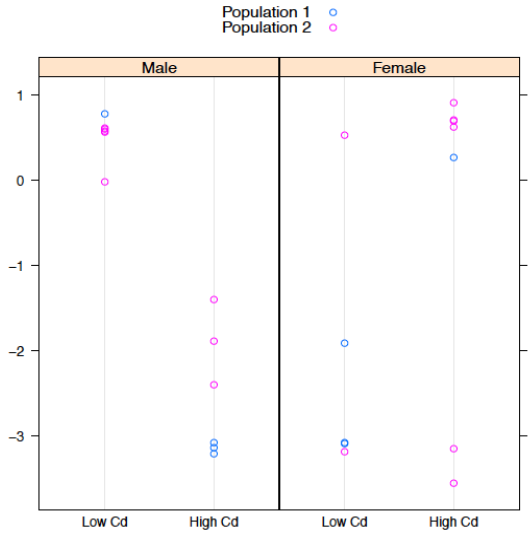
[a] High (≥5 ng/g females, ≥2 ng/g males)/low Cd status

Figure 3.3a-c High/low Cd status and methylation at **genomic regions** stratified by infant sex: (a) 86974674 to 86975244 of chromosome 7, (b) 169379554 to 169380078 of chromosome 3, and (c) 1792758 to 1792758 of chromosome 8[a]

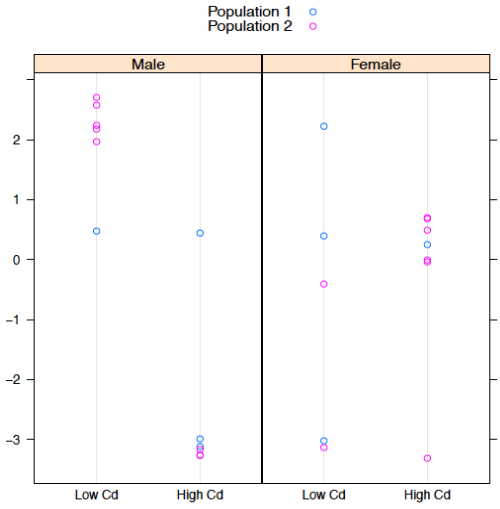
a.



b.



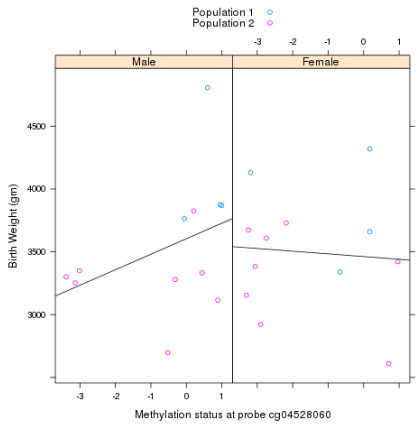
c.



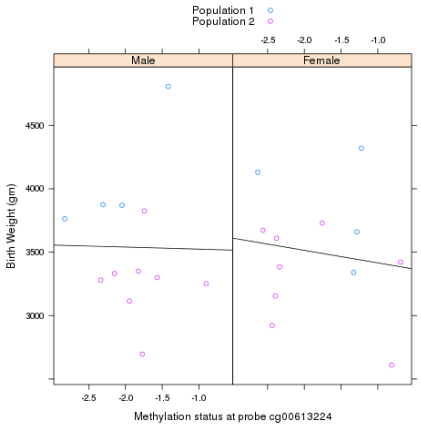
[a] High (≥5 ng/g females, ≥2 ng/g males)/low Cd status

Figures 3.4a-f Methylation of individual CpG sites (a) cg04528060, (b) cg00613224, (c) cg03884018, (d) cg15958576, (e) cg10903116, and (f) cg09728607 **and birth weight** stratified by infant sex[a]

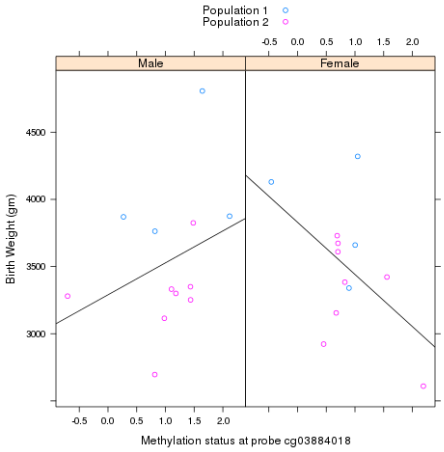
a.



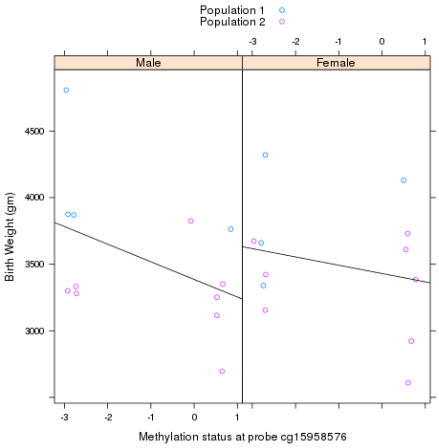
b.



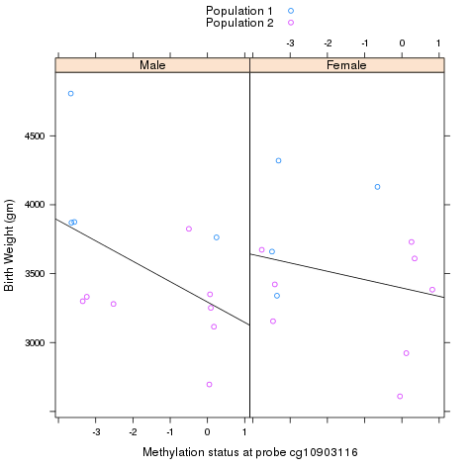
c.



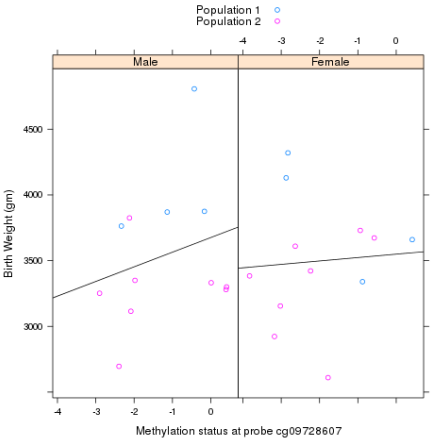
d.



e.



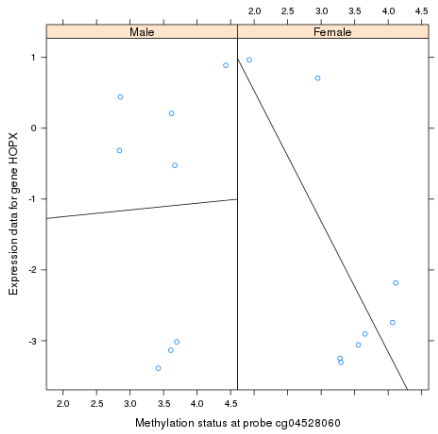
f.



[a] High (≥5 ng/g females, ≥2 ng/g males)/low Cd status

Figures 3.5a-e Methylation at (a) CpG site cg04528060 and HOPX gene expression, (b) CpG site cg04528060 and KIAA1211 gene expression, (c) CpG site cg00613224 versus CPB2 gene expression, (d) CpG site cg15958576 and GPR160 gene expression, and (e) at CpG site cg10903116 and SEC62 gene expression, stratified by infant sex

a.



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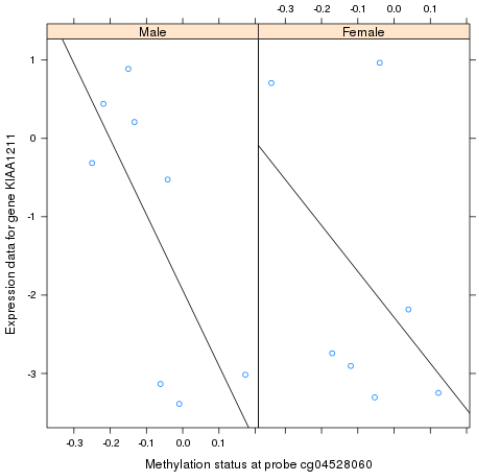
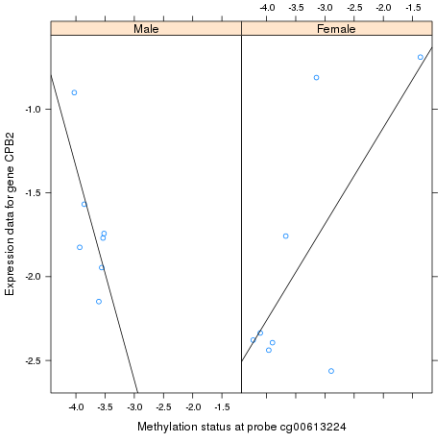
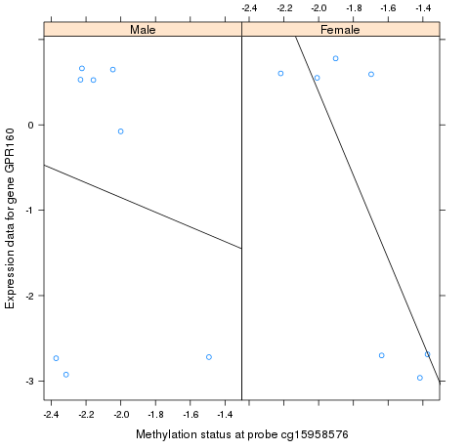


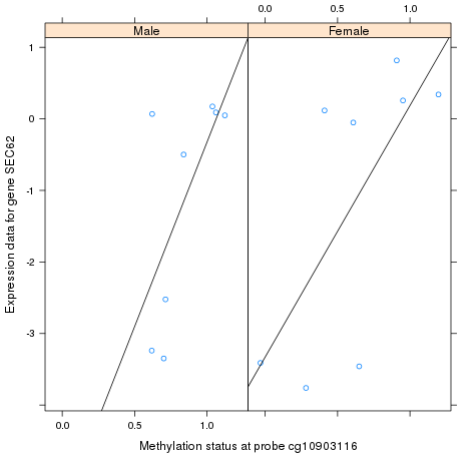
c.



d.



e.



Summary

We investigated associations of periconceptional seafood intake with pregnancy complications (preeclampsia (PE), pregnancy induced hypertension (PIH), gestational diabetes (GDM), and preterm birth (PTB)) and fetal growth. We also investigated sex-specific associations of placental cadmium (Cd) and placental genome-wide DNA methylation in a preliminary pilot study. Maternal periconceptional seafood intake was characterized as total seafood intake, intake of seafood subtypes (shell-, lean-, and fatty-fish), and intake of dietary EPA+DHA. Fetal growth was characterized using fetal growth indices: birthweight (including low birth weight and macrosomia), birth length, head circumference, and ponderal index. We addressed our study aims using data and biological samples collected from participants of the Omega study, a prospective cohort study of risk factors for pregnancy complications among residents of the Pacific Northwest (1996-2008) and participants of the Placental MicroArray Study, a case-control study of placental gene expression and pregnancy complications among women who delivered at Swedish Medical Center.

We observed associations of higher lean fish intake with higher risk of PTB. We also observed associations of higher lean fish intake with higher risk of low birth weight and associations of higher shellfish intake with higher mean ponderal index. Other seafood subtypes or total seafood (all subtypes combined) were not associated with these pregnancy outcomes or fetal growth indicators. We also observed evidence from our secondary analyses that suggests possible differences in associations of seafood and fetal growth across infant sex. In the placental Cd-DNA methylation study, we found suggestive evidence for sex-specific associations of placental Cd with placental DNA methylation of several sites related to *MECOM* and *SALL1* genes among males and *SIAH3* and *HS3ST4* among females.

Some strengths of the project deserve mention. The wealth of available information from a large prospective pregnancy cohort including information on socio-demographic characteristics, information on a wide range of dietary seafood intake, maternal erythrocyte EPA+DHA, course, and outcomes of pregnancy was a particular strength of the project. Our study addressed questions (e.g. seafood

subtype specific associations, different fetal growth indices, and sex-specific relationships) mostly overlooked by previous studies. Finally, integration of a mechanistic study, though at the preliminary pilot study level, is an additional strength of the project.

While we were able to conduct a detailed investigation of periconceptional seafood intake, pregnancy complications, and fetal growth, as well as potential epigenetic mechanisms related to placental Cd burden, a contaminant that can be found in seafood, these investigations do have several limitations. Given the number of tests that we conducted and absence of multiple testing correction, caution is warranted in interpreting our results. On the other hand, we may be underpowered to examine some of the associations (e.g. rare pregnancy complications or outcomes) and seafood- or sex-stratified relationships. Finally, we lacked information on other xenobiotic exposures (e.g. methylmercury (MeHg), polychlorinated byphenols (PCBs)) closely related to seafood intake.

Several questions are still outstanding as possible future areas of research. Whether differences in associations across seafood subtypes could be explained by differences in how the seafood is prepared or content of nutrients or contaminants, is unknown. For instance, future studies may consider a more detailed examination of whether the seafood is baked or fried, content of nutrients (EPA, DHA, vitamin D, selenium) and xenobiotics (Cd, MeHg, or PCBs). Future, larger studies of seafood intake and fetal growth are needed to thoroughly examine infant-sex interactions. Replication efforts and mechanistic investigations, particularly involving the placenta, are additional possible areas of future research.

To conclude, findings from our project indicate that associations of maternal periconceptional seafood intake with pregnancy complications and fetal growth may vary across intake of seafood subtypes. Future studies in this research area have the potential to inform preventative activities that may improve the course and outcomes of pregnancy.

APPENDIX

Table A1.0 Seafood Intake Scale FFQ, type of seafood, and assigned EPA and DHA values

	Seafood subtype	EPA (mg/100 g)	DHA (mg/100 g)
Anchovies	fatty fish	763	1292
Herring, Pickled or Regular	fatty fish	1242	883
Kipper Snacks	fatty fish	970	1179
Salmon, Canned	fatty fish	492	664
Sardines	fatty fish	611	993
Albacore Tuna, Canned	fatty fish	233	629
Swordfish	fatty fish	138	681
Rainbow Trout	fatty fish	334	820
Smelt	fatty fish	353	536
Mackerel	fatty fish	653	1195
Fresh Salmon	fatty fish	1010	727
Smoked Salmon	fatty fish	1010	727
Regular Tuna, Canned	lean fish	47	223
Catfish	lean fish	49	128
Cod	lean fish	103	173
Flounder or Sole	lean fish	119	136
Haddock	lean fish	76	162
Halibut	lean fish	91	374
Mahi Mahi	lean fish	26	113
Snapper or Rockfish	lean fish	48	273
Shark	lean fish	258	431
Unknown Lean Fish	lean fish	103	173
Imitation Crab	lean fish	185	283
Imitation Lobster	lean fish	185	283
Fish Sticks	lean fish	103	173
Crab	shellfish	281	113
Lobster	shellfish	53	31
Shrimp or Prawns	shellfish	171	144
Clams	shellfish	138	146
Mussels	shellfish	276	506
Oysters	shellfish	876	500
Scallops	shellfish	86	103
Abalone	shellfish	54	0
Octopus	shellfish	152	162
Squid	shellfish	162	380

Summary of Cross Tabulations of Seafood Subtypes

Tables A2.0a-d display cross tabulations of lean fish versus shellfish intake for each intake category of fatty fish. There is evidence that seafood intake subtypes are correlated. For example, the highest proportion of subjects for each fatty fish intake category is the cross tabulation where there is perfect agreement for intake of all subtypes: 285/745 for <0.5 oz/mo, 95/799 for 0.5 oz/mo-<1.5 oz/wk, 85/856 for 1.5-3.0 oz/wk, and 174/750 for >3 oz/wk of fatty fish intake. Nevertheless, there appears to be sufficient discordance across the cross tabulations to allow simultaneous adjustment for intake of seafood subtypes to evaluate associations of one subtype independent of the others.

Table A2.0a Cross Tabulation of Shellfish Versus Lean Fish Intake For Fatty Fish Intake <0.5 oz/mo (N = 745)

	Lean Fish Intake (ounces)								
Shellfish Intake	<0.5/mo		0.5/mo-<1.5/wk		1.5-3/wk		>3/wk		Total
(ounces)	**n**	col %	**n**	col %	**n**	col %	**n**	col %	
<0.5 oz/mo	**285**	74.6	**76**	46.9	**59**	45.7	**28**	38.9	**448**
row %	63.6		17.0		13.2		6.3		100.0
0.5 oz/mo-<1.5 oz/wk	**56**	14.7	**46**	28.4	**23**	17.8	**17**	23.6	**142**
row %	39.4		32.4		16.2		12.0		100.0
1.5-3 oz/wk	**31**	8.1	**29**	17.9	**27**	20.9	**15**	20.8	**102**
row %	30.4		28.4		26.5		14.7		100.0
>3 oz/wk	**10**	2.6	**11**	6.8	**20**	15.5	**12**	16.7	**53**
row %	18.9		20.8		37.7		22.6		100.0
Total	**382**	100.0	**162**	100.0	**129**	100.0	**72**	100.0	**745**

Chi2(9) = 91.31, p-value <0.001

Table A2.0b Cross Tabulation of Shellfish Versus Lean Fish Intake For Fatty Fish Intake 0.5 oz/mo-<1.5 oz/wk (N = 799)

	Lean Fish Intake (ounces)								
Shellfish Intake	<0.5/mo		0.5/mo-<1.5/wk		1.5-3/wk		>3/wk		Total
(ounces)	**n**	col %	**n**	col %	**n**	col %	**n**	col %	
<0.5 oz/mo	**87**	49.4	**91**	33.2	**61**	29.0	**26**	18.7	**265**
row %	32.8		34.3		23.0		9.8		100.0
0.5 oz/mo-<1.5 oz/wk	**49**	27.8	**95**	34.7	**67**	31.9	**31**	22.3	**242**
row %	20.2		39.3		27.7		12.8		100.0
1.5-3 oz/wk	**31**	17.6	**67**	24.5	**48**	22.9	**43**	30.9	**189**
row %	16.4		35.4		25.4		22.8		100.0
>3 oz/wk	**9**	5.1	**21**	7.7	**34**	16.2	**39**	28.1	**103**
row %	8.7		20.4		33.0		37.9		100.0
Total	**176**	100.0	**274**	100.0	**210**	100.0	**139**	100.0	**799**

Chi2(9) = 75.67, p-value <0.001

Table A2.0d Cross Tabulation of Shellfish Versus Lean Fish Intake For Fatty Fish Intake 1.5-3 oz/wk (N = 856)

Shellfish Intake (ounces)	Lean Fish Intake (ounces) <0.5/mo n	col %	0.5/mo-<1.5/wk n	col %	1.5-3/wk n	col %	>3/wk n	col %	Total
<0.5 oz/mo	**70**	44.3	**52**	24.8	**58**	22.1	**35**	15.6	**215**
row %	32.6		24.2		27.0		16.3		100.0
0.5 oz/mo-<1.5 oz/wk	**31**	19.6	**51**	24.3	**53**	20.2	**39**	17.3	**174**
row %	17.8		29.3		30.5		22.4		100.0
1.5-3 oz/wk	**34**	21.5	**69**	32.9	**85**	32.3	**70**	31.1	**258**
row %	13.2		26.7		32.9		27.1		100.0
>3 oz/wk	**23**	14.6	**38**	18.1	**67**	25.5	**81**	36.0	**209**
row %	11.0		18.2		32.1		38.8		100.0
Total	**158**	100.0	**210**	100.0	**263**	100.0	**225**	100.0	**856**

Chi2(9) = 62.10, p-value <0.001

Table A2.0e Cross Tabulation of Shellfish Versus Lean Fish Intake For Fatty Fish Consumption >3 oz/wk (N = 750)

Shellfish Intake (ounces)	Lean Fish Intake (ounces) <0.5/mo n	col %	0.5/mo-<1.5/wk n	col %	1.5-3/wk n	col %	>3/wk n	col %	Total
<0.5 oz/mo	**30**	35.3	**24**	17.4	**36**	16.1	**24**	3.2	**114**
row %	26.3		21.1		31.6		21.1		100.0
0.5 oz/mo-<1.5 oz/wk	**13**	15.3	**38**	27.5	**40**	17.9	**36**	4.8	**127**
row %	10.2		29.9		31.5		28.3		100.0
1.5-3 oz/wk	**21**	24.7	**38**	27.5	**66**	29.5	**69**	9.2	**194**
row %	10.8		19.6		34.0		35.6		100.0
>3 oz/wk	**21**	24.7	**38**	27.5	**82**	36.6	**174**	23.2	**315**
row %	6.7		12.1		26.0		55.2		100.0
Total	**85**	100.0	**138**	100.0	**224**	26.2	**303**	40.4	**750**

Chi2(9) =81.75, p-value <0.001

Table A3.0a Exposure and potential confounding, mediating, or precision variables included in models

Chapter	Included in All Models Unless Noted Below	Characteristic	Variable(s)	Parameterization
1, 2	All Models[1]	Shellfish intake Lean fish intake Fatty fish intake Total seafood intake	$X_{ShellGroup}$ $X_{LeanGroup}$ $X_{FattyGroup}$ $X_{SeafoodGroup}$	group linear: 0 = <0.5 oz/mo 1 = 0.5 oz/mo-<1.5 oz/wk 2 = 1.5-3 oz/wk 3 = >3 oz/wk
1, 2	All Models	Shellfish, lean fish, fatty fish, or total seafood intake of 0.5 oz/mo-<1.5 oz/wk	$X_{Shell0.5-<1.5}$ $X_{Lean0.5-<1.5}$ $X_{Fatty0.5-<1.5}$ $X_{Seaf0.5-<1.5}$	no, yes
1, 2	All Models	Shellfish, lean fish, fatty fish, or total seafood intake of 1.5-3 oz/wk	$X_{Shell1.5-3}$ $X_{Lean1.5-3}$ $X_{Fatty1.5-3}$ $X_{Seaf1.5-3}$	no, yes
1, 2	All Models	Shellfish, lean fish, fatty fish, or total seafood intake of >3 oz/wk	$X_{Shell>3}$ $X_{Lean>3}$ $X_{Fatty>3}$ $X_{Seaf>3}$	no, yes
1, 2	All Models[1]	Maternal EPA+DHA status	$X_{EPA+DHAQuart}$	group linear: 0 = Quartile 1 1 = Quartile 2 2 = Quartile 3 3 = Quartile 4
1, 2	All Models	Maternal EPA+DHA quartile 2	$X_{EPA+DHAQ2}$	no, yes
1, 2	All Models	Maternal EPA+DHA quartile 3	$X_{EPA+DHAQ3}$	no, yes
1, 2	All Models	Maternal EPA+DHA quartile 4	$X_{EPA+DHAQ4}$	no, yes
1, 2	All Models	Non-Hispanic White Race	$X_{WhiteRace}$	no, yes
1, 2	All Models	High school or less education	$X_{\leq HighSchool}$	no, yes
1, 2	All Models	Unmarried marital status	$X_{Unmarried}$	no, yes
1, 2	1.1, 1.2, 1.3, 2.1, 2.2, 2.3, 2.4	Nulliparous	X_{Nullip}	no, yes
1, 2	All Models	Any recreational physical activity during index pregnancy	$X_{PhysAct}$	no, yes
1, 2	All Models	Any alcohol intake during index pregnancy	X_{Alc}	no, yes
1, 2	All Models	Any cigarette smoking during index pregnancy	X_{Smoke}	no, yes
1, 2	All Models	Pre-pregnancy BMI 18.5-24.9 kg/m^2	$X_{BMINormal}$	no, yes
1, 2	All Models	Pre-pregnancy BMI 25-29.9 kg/m^2	$X_{BMIOver}$	no, yes
1, 2	All Models	Pre-pregnancy BMI ≥30.0 kg/m^2	$X_{BMIObese}$	no, yes
2	2.1, 2.2, 2.3, 2.4	Female infant sex	$X_{FemaleInfant}$	no, yes

1, 2	All Models	Mother's age (years)	X_{MatAge}	continuous
1, 2	All Models	Daily energy consumed (kcal/day)	$X_{TotEnergy}$	continuous
1, 2	All Models	Daily red & processed meat intake (servings/day)	$X_{Red\&ProcMeat}$	continuous
1, 2	1.2, 2.2	Enrolled into study from 1998-1999	$X_{1998-1999}$	no, yes
1, 2	1.2, 2.2	Enrolled into study from 2000-2001	$X_{2000-2001}$	no, yes
1, 2	1.2, 2.2	Enrolled into study from 2002-2003	$X_{2002-2003}$	no, yes
1, 2	1.2, 2.2	Enrolled into study from 2004-2005	$X_{2004-2005}$	no, yes
1, 2	1.2, 2.2	Enrolled into study from 2006-2007	$X_{2006-2007}$	no, yes
1	1.3	Gestational weight gain (kg)	$X_{GestWtGain}$	continuous
2	2.3	Gestational age at delivery (weeks)	$X_{GestAge}$	continuous

[1]Group linear exposure variables were only included in models used to obtain Wald p-values for a linear trend.

Table A3.0b Example primary models

Example Equation A1.1: Association of Shellfish (Grouped Linear) and Risk of Pregnancy Complication *(for Y = pregnancy complication)*

$$\log(P[Y=1|X]) = \beta_0 + \beta_1 X_{ShellGroup} + \beta_2 X_{Lean0.5-<1.5} + \beta_3 X_{Lean1.5-3} + \beta_4 X_{Lean>3} + \beta_5 X_{Fatty0.5-<1.5} + \beta_6 X_{Fatty1.5-3} + \beta_7 X_{Fatty>3} + \beta_8 X_{WhiteRace} + \beta_9 X_{\leq HighSchool} + \beta_{10} X_{Unmarried} + \beta_{11} X_{Nullip} + \beta_{12} X_{PhysAct} + \beta_{13} X_{Alc} + \beta_{14} X_{Smoke} + \beta_{15} X_{BMINormal} + \beta_{16} X_{BMIOver} + \beta_{17} X_{BMIObese} + \beta_{18} X_{MatAge} + \beta_{19} X_{TotEnergy} + \beta_{20} X_{Red\&ProcMeat}$$

Example Equation A1.2: Association of Shell-, Lean-, Fatty-fish and Risk of Pregnancy Complication *(for Y = pregnancy complication)*

$$\log(P[Y=1|X]) = \beta_0 + \beta_1 X_{Shell0.5-<1.5} + \beta_2 X_{Shell1.5-3} + \beta_3 X_{Shell>3} + \beta_4 X_{Lean0.5-<1.5} + \beta_5 X_{Lean1.5-3} + \beta_6 X_{Lean>3} + \beta_7 X_{Fatty0.5-<1.5} + \beta_8 X_{Fatty1.5-3} + \beta_9 X_{Fatty>3} + \beta_{10} X_{WhiteRace} + \beta_{11} X_{\leq HighSchool} + \beta_{12} X_{Unmarried} + \beta_{13} X_{Nullip} + \beta_{14} X_{PhysAct} + \beta_{15} X_{Alc} + \beta_{16} X_{Smoke} + \beta_{17} X_{BMINormal} + \beta_{18} X_{BMIOver} + \beta_{19} X_{BMIObese} + \beta_{20} X_{MatAge} + \beta_{21} X_{TotEnergy} + \beta_{22} X_{Red\&ProcMeat}$$

Example Equation A2.1: Association of Shellfish (Grouped Linear) and Fetal Growth *(for Y = fetal growth)*

$$E(Y|X) = \beta_0 + \beta_1 X_{ShellGroup} + \beta_2 X_{Lean0.5-<1.5} + \beta_3 X_{Lean1.5-3} + \beta_4 X_{Lean>3} + \beta_5 X_{Fatty0.5-<1.5} + \beta_6 X_{Fatty1.5-3} + \beta_7 X_{Fatty>3} + \beta_8 X_{WhiteRace} + \beta_9 X_{\leq HighSchool} + \beta_{10} X_{Unmarried} + \beta_{11} X_{Nullip} + \beta_{12} X_{PhysAct} + \beta_{13} X_{Alc} + \beta_{14} X_{Smoke} + \beta_{15} X_{BMINormal} + \beta_{16} X_{BMIOver} + \beta_{17} X_{BMIObese} + \beta_{18} X_{MatAge} + \beta_{19} X_{TotEnergy} + \beta_{20} X_{Red\&ProcMeat} + \beta_{21} X_{FemaleInfant}$$

Example Equation A2.2: Association of Shell-, Lean-, Fatty-fish and Risk of Low Birth Weight or Macrosomia *(for Y = low birth weight or macrosomia)*

$$\log(P[Y=1|X]) = \beta_0 + \beta_1 X_{Shell0.5-<1.5} + \beta_2 X_{Shell1.5-3} + \beta_3 X_{Shell>3} + \beta_4 X_{Lean0.5-<1.5} + \beta_5 X_{Lean1.5-3} + \beta_6 X_{Lean>3} + \beta_7 X_{Fatty0.5-<1.5} + \beta_8 X_{Fatty1.5-3} + \beta_9 X_{Fatty>3} + \beta_{10} X_{WhiteRace} + \beta_{11} X_{\leq HighSchool} + \beta_{12} X_{Unmarried} + \beta_{13} X_{Nullip} + \beta_{14} X_{PhysAct} + \beta_{15} X_{Alc} + \beta_{16} X_{Smoke} + \beta_{17} X_{BMINormal} + \beta_{18} X_{BMIOver} + \beta_{19} X_{BMIObese} + \beta_{20} X_{MatAge} + \beta_{21} X_{TotEnergy} + \beta_{22} X_{Red\&ProcMeat} + \beta_{23} X_{FemaleInfant}$$

Example Equation A2.3: Association of EPA+DHA and Risk of Low Birth Weight or Macrosomia *(for Y = low birth weight or macrosomia)*

$$\log(P[Y=1|X]) = \beta_0 + \beta_1 X_{EPA+DHAQ2} + \beta_2 X_{EPA+DHAQ3} + \beta_3 X_{EPA+DHAQ4} + \beta_4 X_{WhiteRace} + \beta_5 X_{\leq HighSchool} + \beta_6 X_{Unmarried} + \beta_7 X_{Nullip} + \beta_8 X_{PhysAct} + \beta_9 X_{Alc} + \beta_{10} X_{Smoke} + \beta_{11} X_{BMINormal} + \beta_{12} X_{BMIOver} + \beta_{13} X_{BMIObese} + \beta_{14} X_{MatAge} + \beta_{15} X_{TotEnergy} + \beta_{16} X_{Red\&ProcMeat} + \beta_{17} X_{FemaleInfant}$$

Table A4.0 R and Bioconductor Packages used for genome-wide DNA methylation analyses: individual probe analysis approach

```
R Under development (unstable) (2013-06-18 r62971)
Platform: x86_64-unknown-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=C                 LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] parallel  stats     graphics  grDevices utils     datasets  metho
[8] base

other attached packages:
 [1] xtable_1.7-1
 [2] ReportingTools_2.1.11
 [3] knitr_1.2
 [4] Homo.sapiens_1.1.1
 [5] TxDb.Hsapiens.UCSC.hg19.knownGene_2.9.2
 [6] org.Hs.eg.db_2.9.0
 [7] GO.db_2.9.0
 [8] RSQLite_0.11.4
 [9] DBI_0.2-7
[10] OrganismDbi_1.3.11
[11] GenomicFeatures_1.13.26
[12] AnnotationDbi_1.23.18
[13] IlluminaHumanMethylation450kannotation.ilmn.v1.2_0.1.3
[14] IlluminaHumanMethylation450kmanifest_0.4.0
[15] limma_3.17.21
[16] minfi_1.7.11
[17] bumphunter_1.1.11
[18] locfit_1.5-9.1
[19] iterators_1.0.6
[20] foreach_1.4.1
[21] Biostrings_2.29.14
[22] GenomicRanges_1.13.35
[23] XVector_0.1.0
[24] IRanges_1.19.21
[25] reshape_0.8.4
[26] plyr_1.8
[27] lattice_0.20-15
[28] Biobase_2.21.6
```

```
[29] BiocGenerics_0.7.3

loaded via a namespace (and not attached):
 [1] annotate_1.39.0          AnnotationForge_1.3.10   base64_1.1
 [4] beanplot_1.1             biomaRt_2.17.2           biovizBase_1.9.2
 [7] bitops_1.0-5             BSgenome_1.29.1          Category_2.27.2
[10] cluster_1.14.4           codetools_0.2-8          colorspace_1.2-2
[13] compiler_3.1.0           DESeq2_1.1.26            dichromat_2.0-0
[16] digest_0.6.3             doRNG_1.5.3              edgeR_3.3.6
[19] evaluate_0.4.4           formatR_0.9              genefilter_1.43.0
[22] ggbio_1.9.7              ggplot2_0.9.3.1          GOstats_2.27.1
[25] graph_1.39.3             grid_3.1.0               gridExtra_0.9.1
[28] GSEABase_1.23.0          gtable_0.1.2             Hmisc_3.12-2
[31] hwriter_1.3              illuminaio_0.3.5         itertools_0.1-1
[34] labeling_0.2             MASS_7.3-28              Matrix_1.0-12
[37] matrixStats_0.8.5        mclust_4.2               multtest_2.17.0
[40] munsell_0.4.2            nor1mix_1.1-4            PFAM.db_2.9.0
[43] preprocessCore_1.23.0    proto_0.3-10             RBGL_1.37.2
[46] RColorBrewer_1.0-5       RCurl_1.95-4.1           reshape2_1.2.2
[49] R.methodsS3_1.4.4        R.oo_1.13.9              rpart_4.1-1
[52] Rsamtools_1.13.28        rtracklayer_1.21.9       R.utils_1.26.2
[55] scales_0.2.3             siggenes_1.35.0          splines_3.1.0
[58] stats4_3.1.0             stringr_0.6.2            survival_2.37-4
[61] tools_3.1.0              VariantAnnotation_1.7.38 XML_3.98-1.1
[64] zlibbioc_1.7.0
```

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