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ABSTRACT

FISH CONSUMPTION AND MATERNAL HAIR MERCURY LEVELS IN RELATION TO THE RISK OF PRETERM DELIVERY

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Preterm delivery (PD) has continued to be the leading cause of fetal mortality and morbidity despite extensive etiological studies and advancements in medical technologies. Recently, fish consumption has been hypothesized to be related to decreased risk of PD through the effect of omega-3 fatty acids (n-3 FA). However, other researchers have indicated that mercury, which comes mainly from fish consumption for many human populations has adverse effects on fetal development. This prospective cohort study was based on the Pregnancy Outcome & Community Health (POUCH) Study and assessed the association between maternal fish consumption during the first half of pregnancy and the risk of PD among 1326 pregnant women, and the association between fish consumption and hair mercury levels and the influence of hair mercury levels on the risk of PD among 1031 women who agreed to give hair samples. Results showed that the top 10^{th} percentile of hair mercury levels was significantly associated with increased risk of very PD (<35 weeks) and the 4^{th} quintile was significantly associated with increased risk of moderately PD (36-37 weeks). Total fish consumption, consumption of canned fish and sport caught fish were significantly associated with increased hair mercury levels. Other maternal characteristics including age, ethnicity, Medicaid status and community of enrollment were also associated with hair mercury levels after adjusting for fish consumption. Total fish consumption was not found to be associated with the risk of PD.

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LIST OF ABBREVIATIONS

WHO.....World Health Organization

PD..... Preterm Delivery

BW..... Birth Weight

LBW..... Low Birth Weight

IUGR..... Intrauterine Growth Retardation

PIH.....Pregnancy Induced Hypertension

HPA.....Hypothalamic-Pituitary-Adrenal

CRH.....Corticotropin-Releasing hormone

PPROM.....Preterm Premature Rupture of Membrane

MLCK.....Myosin Light Chain Kinase

IVF.....In-Vitro Fertilization

GIFT.....Gamete Intrafallopian Transfer

n-3 FA.....Omega-3 Fatty Acids

n-6 FA.....Omega-6 Fatty Acids

EPA.....Eicosapentaenoic

DHA.....Docosahexaenoic

PGE2.....Prostaglandin E2

PGF2a.....Prostaglandin F2a

PGI2.....Prostacyclin

PGI3.....Prostaciclina

AA.....Arachidonic Acid

AAS.....Atomic Absorption Spectrometry

CVAAS.....Cold Vapor Atomic Absorption Spectrometry

NAA.....Neuron Activation and Analysis

XRF.....X-Ray Fluorescence

GC.....Gas Chromatography

MSAFP.....Maternal Serum Alpha-Fetoprotein

POUCH.....Pregnancy Outcome and Community Health

LMP.....Last Menstrual Period

GLM.....General Linear Models

USA EPA.....United States Environmental Protection Agency

RfD.....Reference Dose

PPM.....Parts Per Million

DDT..... Dichloro Diphenyl Trichloroethane

PCB..... Polychlorinated Biphenyl

INTRODUCTION

The Problem of Preterm Delivery

Preterm Delivery (PD), defined by the World Health Organization (WHO) as birth before 37 completed weeks of gestation (1), remains the leading cause of perinatal morbidity and mortality in obstetric practice in developed countries. PD is commonly classified by clinical presentations as birth occurring after spontaneous preterm labor (in approximately 50% of cases), spontaneous rupture of the membrane (in approximately 30%), and delivery of a premature infant as indicated for the benefit of either the infant or the mother (in around 20%) (28). The prevalence of PD varies from 6% to 15% of all deliveries across populations with different geographic and demographic features (2-5). Three factors make PD a public health concern in the US: the substantial emotional and economic costs to families and communities produced by prematurely delivered infants, the higher rate of PD in low socio-economic status and ethnic minority patients, and the continually increasing incidence despite of extensive efforts to predict and prevent PD. Over the period from 1987 to 1998, the rate of PD increased from 10.2% to 11.6% of live births in the US (6). Factors that have been suggested to contribute to the rising rate include increased obstetric intervention (10), increased multiple birth driven by the use of assisted reproduction techniques (11-13), raised prevalence of substance misuse in urban areas (14), adverse socioeconomic factors (14-16) and changes in the better dating of gestation with the use of ultrasound (16). Over the twenty years between 1975 and 1995, PD increased by 3.6% among African-American women (from 15.5% to 16.0%), and by 22.3% among Whites (from 6.9% to 8.4%) (7). Though the racial gap is diminishing, it is more likely to be explained by increased rate among whites.

Around 75% of perinatal deaths happen in prematurely delivered infants and more than two thirds of these occur in infants delivered before 32 weeks' gestation (3, 8-9). The survival rate of preterm and Low Birth Weight (LBW) infants has been increased in recent years, especially for those delivered extremely early in gestation. However, because 80% of preterm infants are born at 32-36 weeks' gestation, though their mortality and morbidity are lower than extremely PD, the sheer number of this group makes the largest contribution to the total perinatal mortality following preterm birth (46). Moreover, the increased short-term morbidity and long-term physical and mental disability in survivors of premature infants have compromised the overall reduction in perinatal mortality. Hence, reducing the incidence of all PD through etiological research seems to be a more powerful way of improving perinatal health despite the advances we have made in neonatal medicine. Unfortunately, current approaches to predict and prevent PD are unsuccessful and it is mainly due to an inadequate understanding of the underlying pathogenesis. Four pathogenic processes have been supported by clinical and experimental evidences: activation of the maternal or fetal hypothalamic-pituitary-adrenal (HPA) axis, decidual-chorioamniotic or systemic inflammation, decidual hemorrhage, and pathologic distension of the uterus (17). A variety of potential predictors of PD have been researched in each of the pathogenic processes: maternal and fetal stress (18-19), corticotropin-releasing hormone (CRH) (20) and increased estrogen concentration (21) for the hypothesis of HPA activation; activation of the cytokine network leading to increased uterotonin expression (22) and increased placental and membrane apoptosis (23) for the inflammation hypothesis; thrombin and the genesis of Preterm Premature Rupture of Membrane (PPROM) for the hypothesis of decidual hemorrhage (24-25); and

expression of myometrial gap junctions (connexin 43) (26) and increased myosin light chain kinase (MLCK) activity (27) for the hypothesis of uterine distention. It is suggested that each of these four pathologic processes may have unique biochemical and biophysical pathway and distinct epidemiological profiles. However, they converge on a final common mechanism, which lead to cervical dilation, membrane rupture and uterine contraction.

Major risk factors for preterm birth include socioeconomic status, ethnicity, age, multiple pregnancy, past reproductive history, substance misuse and infection. Social disadvantage, usually evaluated by occupation, income and education level, is suggested to be associated with an increased risk of PD (14, 8, 29-30). Possible explanations are worse nutritional status, higher frequency of substance misuse and genital-tract infection, limited access to qualified antenatal care, physically demanding work, and more frequent cigarette smoking. Though part of the racial difference can be accounted for by socioeconomic status, other factors such as maternal body size, customs, behaviors, age distribution, exposure to racism and discrimination and some neighborhood level factors may contribute to the PD gap attributed to race. Results from a study indicate that the incidence of PD among whites is lowest in women aged 20-24 for the first birth, and 25-29 for subsequent births (7). While among African-American women, the lowest rates of PD for both first birth and subsequent births arise between 25 and 29 years of age (7). Multiple pregnancy accounts for 12%-27% of all preterm births (2, 31). Increased use of assisted conception technologies and increased intervention to deliver twins early in third trimester resulted in increased PD rate due to multiple pregnancy over the past 20 years (32-33). A previous history of preterm birth or delivery of a LBW infant is an important

risk factor for PD (34-35). Second (but not first) trimester pregnancy loss (36) and in-vitro fertilization (IVF) and gamete intrafallopian transfer (GIFT) (37-39) have also been linked with an increased risk of PD. Substance misuse, such as cocaine consumption during pregnancy has been linked to increased risk of PD. However, this association maybe confounded by lifestyle and other factors, e.g., high alcohol intake, cigarette smoking and congenital infections (14, 40-41). Systemic maternal infections may increase the risk of PD (42), and the association between spontaneous PD and genital-tract infection, particularly intrauterine infections has been extensively researched. However, the exact frequency of PD related to intrauterine infection, and the specific organisms involved, are poorly understood.

Current therapeutic interventions to prevent PD include tocolytics, glucocorticoids, antibodies, cervical cerclage and enhanced social support. To date, no method has been shown to be associated with lowering rates of PD (43). Four types of nutritional interventions have been widely studied: counseling, protein supplementation, caloric supplementation and vitamin and or mineral supplementation. No evidence has been provided that nutritional counseling changes diet habits of pregnant women, not to mention the pregnancy outcomes (44). The provision of protein supplementation was shown to be consistently associated with adverse outcomes (45). Also no compelling evidence has been found that caloric supplementation is related to a reduction in preterm births in developed countries (44). Studies on the supplementation of iron, zinc, folate and combined vitamin have produced conflicting results. Up to now, no effective intervention has been found in preventing PD.

Fish Consumption and Adverse Pregnancy Outcomes:

High consumption of fish and seafood, rich in long-chain omega-3 fatty acids (Ω-3 FA) eicosapentaenoic (EPA, 20:5 Ω-3) and docosahexaenoic (DHA, 22:6 Ω-3) has been found to explain the reduced rate of cardiovascular diseases in Greenland Inunits (47-48). This fact has inspired researchers to explore the effect of fish oil on other problems of human health. The risk of many adverse pregnancy outcomes, particularly PD, LBW, Intrauterine Growth Retardation (IUGR), preeclampsia and Pregnancy Induced Hypertension (PIH) have been assessed in relation to the consumption of fish and seafood or the intake of fish oil supplements.

There is some biological basis for the hypothesis that fish consumption may reduce the risk of PD because prostaglandins can play a role in the onset of labor in humans and other primates (49). Prostaglandin E2 (PGE2) and Prostaglandin F2a (PGF2a), derived from omega-6 fatty acids (Ω-6 FA), bring about contraction of myometrium, and cervical ripening. These prostaglandins can trigger the initiation of labor throughout pregnancy, and a small increase in these prostaglandins is detected in the amniotic fluid and plasma right before the onset of parturition (50). Prostacyclin (PGI2) is found to have the opposite effect, inhibition of myometrial contractility (51). Long chain Ω-3 FA can delay the initiation of parturition by down-regulating the formation of PGE2 and PGF2a, promoting the synthesis of PGI2 and PGI3 and thus leading to a more relaxed myometrium.

There is an extensive biologic basis for the fish oil to decrease the risk of preeclampsia, eclampsia and PIH. The exact cause of PIH is unclear, but a possible explanation is that the impaired maternal-placental circulation is aggravated by an interrupted balance in the production of vasoactive prostaglandin (thromboxane and PGI2)

during pregnancy (57). PGI2, a potent vasodilator and platelet aggregation inhibitor produced by endothelium, predominates over thromboxane A2, a potent vasoconstrictor and platelet aggregator produced by platelets in normal pregnancy. In PIH, the ratio of PGI2 to thromboxane A2 is reversed (58-59) and thus induces vasoconstriction of small arteries and the activation of platelets (60). EPA (Ω-3 FA) derived thromboxane A3 is physically less active than Arachidonic Acid (AA) (Ω-6 FA) derived thromboxane A2, while Prostaciclina (PGI3) is equipotent with its AA derived counterpart PGI2, suggesting that fish oil might be effective in preventing preeclampsia and PIH (61).

The long chain Ω-3 FA may increase birth weight (BW) either by prolonging pregnancy (54) or by enhancing fetal growth rate (55-56). Besides delaying initiation of spontaneous delivery resulting from altered balance between prostaglandin, Ω-3 FA lower thromboxane/prostacyclin ratio (55) and decrease blood viscosity (56) through the similar pathway to prevent preeclampsia, resulting in improved placental blood flow and increased fetal growth rate. The hypothesis that fish oil may reduce the risk of IUGR is also based on the mechanism for Ω-3 FA to increase fetal growth rate.

Most of epidemiological studies evaluating fish consumption or fish oil supplement intake in relation to the risk of pregnancy outcomes were performed in Northern European countries where the fish consumption levels are generally higher than the levels in the United States and these studies produced inconsistent results. Table 1 is a brief review of previous literature regarding the association between consumption of fish, seafood or Ω-3 FA supplements and the risk of adverse pregnancy outcomes, with an emphasis on PD. These studies were collected by searching PUBMED using keywords such as "fish" or "omega-3 fatty acids" together with "preterm delivery", "preterm birth",

"birth weight", "gestation" or "pregnancy", and all the studies evaluating either gestational age or BW as outcome variables were selected.

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Clinical Trials & Animal Experiments:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Olsen (2000) (101)	Hospital based, N=898.	Prophylactic trial: 2.7g fish oil/day Therapeutic trial: 6.1 g fish oil/day	1. Preterm delivery 2. Intrauterine growth retardation 3. Pregnancy induced hypertension	1. Gestational age at randomisation 2&3. Gestational age at delivery	Prophylactic trial: 1. OR=0.54, 95% CI 0.38, 0.98 2. OR=1.26, 95% CI 0.74, 2.12 3. OR=0.98, 95% CI 0.63, 1.53
Borod (1999) (102)	Hospital based, N=53.	Eggs from chicken fed with DHA-rich microalgae: 1 dozen/week.	1. DHA in maternal and cord blood 2. Low birthweight 3. Preterm delivery 4. Weight of placenta	NA	1. Significant positive association between DHA intake and DHA in plasma and RBC lipids 2. Intervention vs. 2 controls: 0% vs. 13 and 26% 3. Intervention vs. 2 controls: 6% vs. 25 and 26% 4. Intervention vs. 2 controls: 760g vs. 658 and 663g
Baguma-Nibasheka (1999) (53)	ewes, N=12	Infusion of fish oil concentration: 3ml/day.	1. Onset of labour 2. Tme of delivery 3. Maternal estradiol 4. Maternal&fetal PGE2 in plasma 5. Fetal body and organ weight.	NA	1. Interv. vs. cont.: 53±16 vs. 20±7 post-beta (significantly different) 2. Interv. vs. cont: 68±14 vs.30±11 post-beta (significantly different) 3&4. Rose in controls but not in intervention group 5. No difference

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Clinical Trials & Animal Experiments:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Onwude (1995) (103)	Women at risk of developing PIH or IUGR, N=223	MaxEpa (1.62 g EPA and 1.08g DHA): 2.7g/day	1. Proteinuric and non proteinuric pregnancy Induced Hypertension (PIH) 2. Birthweight <3rd centile 3. Duration of pregnancy	NA	No difference in all three outcome between intervention and control group.
Olsen (1992) (104)	Hospital based: N=533	Intervention: n-3 FA: 2.7g/day Control 1: olive oil Control 2: no supplement	1. Gestational age 2. Birthweight 3. Birth length	2&3: Sex, parity, duration of gestation	1. Mean value is highest in intervention group and lowest in control 1(P=0.0006), 4.0 days (95% CI 1.5, 6.4) longer in intervention than control 1. 2. Mean value is highest in intervention group and lowest in control 1(P=0.07), 107 g (95% CI 1, 214) heavier in intervention than control 1. 3. Mean value is highest in intervention group and lowest in control 1 (P=0.1)

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Clinical Trials & Animal Experiments:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Olsen (1990) (52)	Lewis rats, N=53	FO: 15% MaxEpa fish oil AO: 15% Arachis oil ST: Rat chow only	1. Gestation age 2. Birthweight 3. Maternal weight 4. Food consumption 5. Fatty acids composition of total lipids from kidney (FAC) 6. Fatty acids composition of total lipids from pup rat (FAP)	NA	1. AO<either FO or ST (P<0.01) 2. FO<AO (P<0.05), FO<ST (P<0.0005) AO<ST (P<0.05) 3. day 16: FO<ST (P<0.01) 4. FO<AO (P<0.01) 4. n-3/n-6: FO (1.15)>ST (0.24)> AO (0.17) (P=0.0001) 5. n-3/n-6: FO (3.30)>ST (1.03)> AO (0.67) (P=0.0001)
Olsen (1990) (105)	Hospital based, N=5150.	Vitamin, EPA and DHA (estimated 0.1g/day EPA and DHA)	1. Preeclampsia 2. Hypertension 3. Pregnancy duration<40wks 4. Birthweight 5. Fetal & infant mortality	NA	1. Treatment group reduced 31.1% OR(P=0.021) 2. Treatment group reduced 28.3% OR(P=0.026) 3. Treatment group reduced 20.4% OR(P=0.00083) 4. No difference 5. No difference

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Cohort Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Olsen (2002) (106)	Hospital based, N=8729	Levels of consumption of seafood in early pregnancy	1. Preterm delivery 2. Low birthweight 3. IUGR	Fetal sex, smoking, alcohol consumption, maernal age, parity, height, pre-pregnant weight, length of education, whether the mother had a cohabitant	Using the highest 5th quintile (qtl) of fish consumption as reference: 1. 1st qtl: OR=3.22, 95% CI 4.73, 6.00 2. 1st qtl: OR=2.69, 95% CI 1.49, 4.84 4. 1st qtl: OR=1.14, 95% CI 0.67, 1.98
Grandjean (2001) (107)	Hospital based, N=182.	1. Frequency of main meals with fish 2. Fatty acids (FA) in both maternal serum and fetal blood	1. Gestational age 2. Birthweight 3. placental weight	Maternal height, maternal weight, smoking during pregnancy, diabetes, parity, gestational length, sex of child	No association between fish consumption and outcomes. Cord blood EPA increased with maternal consumption of marine food (P=0.002). 1% increment of serum FA in relation to the change of outcomes: 1. DHA: increased by 1.5 days, 95% CI 0.7, 2.2 2. EPA: increased by 246 g, 95% CI 16, 476

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Cohort Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Bjerregaard (1996) (108)	Hospital based, N=1106.	1. Consumption of seal, whale or walrus 2. Total mercury level in maternal and infant blood 3. Smoking	Birthweight	Population group, inunit group, smoking.	1. Maternal blood mercury concentration were correlated with intake of local meat (R=0.15, P<0.0001). 2. Only in west Greenlanders (n=597), an increase in blood mercury of 10ug/l was accompanied by a decrease in birthweight of 56g (P=0.04)
Reddy (1994) (109)	Non-pregnant S. Asian vegetarians from 2 General Practices in North London, N=146	1. Being vegetarian.	1. FA levels in non-pregnant women's blood and cord blood 2. Early onset of labor and emergency Caesarean Section 3. Birthweight 4. Birth length 5. Head circumstance length.	Gestation age, maternal age, parity, and fetal gender	1. Greater Linoleic acids (n-6) (P<0.0001)and lower EPA and DHA (P<0.0001) in plasma among vegetarian women 2. 5.6 days shorter in vegetarian Asians than white omnivores (unadjusted p<0.0024) 3. 240 g lighter in Asian vegetarians (P<0.01) 4. 1.32 cm shorter in Asian vegetarians (P<0.05) 5. 0.87 cm shorter in Asian vegetarians (P<0.001)

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Cohort Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Olsen (1989) (110)	Hospital based, N=29	The ratio of n-3 FA to AA (n-6) (n-3/n-6 ratio) in phosphatidylcholine (PC), phosphatidylethanolamine (PE), and total lipids (TL) of red cells sampled during pregnancy.	Gestation age	NA	Gestational age was associated with the n-3/n-6 ratio quantified in PC (beta= 0.50, P=0.035) only.

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Case-Control Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Olsen (1993) (111)	Population based, N=1362	Consumption of seafood during pregnancy.	1. Birthweight 2. Birth length 3. Pregnancy duration 4. Placenta weight	Maternal height, weight, parity, age, marital status, and smoking	1. Significantly different across 7 consumption groups (P=0.02), tend to level off with further fish consumption, significant in the second degree polynomial model (P=0.005) 2. Significantly different across 7 consumption groups (P=0.002), tend to level off with further fish consumption, significant in the second degree polynomial model (P=0.001) 3. not significant 4. not significant
Kesmodel (1997) (112)	Population based, N=1002.	Consumption of marine diets during pregnancy	1. Preeclampsia 2. PIH 3. IUGR 4. Pretem delivery 5. Posterm delivery	Maternal smoking habits, maternal height, maternal pregnant weight, parity, maternal social status.	For all the five outcomes, none of the confounder-adjusted or comparing higher intake levels with the lowest level were significant.

Table 1. Literature review of studies on consumption of fish/fish oil supplements in relation to pregnancy outcomes including gestational age, birthweight, IUGR or PIH.

Case-Control Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Olsen (1991) (113)	Hospital based, N=99.	n-3/n-6 ratio in erythrocyte obtained within 2 days of delivery and levels of each FA	Gestational age	Maternal prepregnant weight, height, age, parity, marital status, smoking, and employment during pregnancy	Significant association with n-3/ n-6 ratio was found among Denmark women (P=0.02) but not among Faroes. The association with FAs was weaker but with the same trend.

Seven clinical trials, five cohort studies and three case-control studies have been included in Table 1. All of the 13 studies performed on humans were conducted in European countries with high consumption levels of fish or other seafood. Three associations were evaluated: consumption of fish, seafood or Ω-3 FA supplements in relation to the risk of PD or other pregnancy outcomes; consumption of fish, other seafood or Ω-3 FA supplements in relation to FA levels in blood; and FA levels in blood in relation to the risk of PD or other pregnancy outcomes. Thus, a hypothesis that fish consumption decreases the risk of PD or other adverse pregnancy outcomes by increasing blood FA levels could be tested by these studies.

Among the five clinical trials in humans (101-105), four (101-102,104-105) detected a significantly decreased risk of PD and/or increased gestational age in the intervention group given supplements containing fish oil or Ω-3 FA during pregnancy. Both of the animal experiments (52-53) found increased gestational age among animals treated with fish oil supplements, while only in one study (52) the increase was statistically significant, because a statistical test was not performed in the other one (53). In three out of the four cohort studies (106,109-110) evaluating gestational age or the risk of PD as the outcome variable, a significantly decreased risk of PD and elongated gestational age were related to higher levels of fish consumption or enhanced long chain Ω-3 FA levels in maternal/cord blood. Two of the three case-control studies (111-112) did not observe the association between gestational age and consumption of fish and seafood. Another case-control study (113) reported increased gestational age in relation to increased Ω-3/ Ω-6 ratio among Denmark but not Faroe Island women, who consumed higher levels of seafood. Both of the two studies evaluating fish consumption on maternal or cord blood

levels of FA (107, 109) were cohort studies, and they found either a significant increase in blood Ω-3 FA levels among women with higher levels of fish (107) or higher Ω-6 FA (109) among women with lower fish consumption levels.

The results from cohort studies and clinical trials were compatible with the hypothesis that intake of fish oil during pregnancy, either in the form of supplements or fish meals, elevates blood levels of Ω-3 FA and elongates the pregnancy duration and reduce the risk of PD. Two studies by Olsen et al (111, 113) observed a "ceiling effect" such that above a certain high fish consumption level, enhanced intake of fish or seafood would not result in a further increase in gestation age. This phenomenon may be explained by a limited capacity for human gastro-intestinal system to digest and absorb fish oil or a balance of PGE and PGI production, which is unchangeable by further Ω-3 FA intake after reaching a very high level. In addition, nutritional variable is likely to have misclassification error when used as a surrogate of Ω-3 FA intake, and this misclassification is likely to be greater at higher range of scales (e.g. Women are more likely to accurately remember whether they ate 1-2 fish meals than to remember whether they ate 10-15 fish meals during the first half of pregnancy) Thus, the dose-dependent trend would tend to flatten artificially at the highest range of fish consumption. Further studies are needed to measure and define this "threshold level" in order to guide pregnant women to eat fish in appropriate amount considering both the nutritional and potentially toxic contents in fish.

Major outcomes concerning fetal growth including IUGR, BW, birth length, placental weight and head circumference were assessed in 13 studies (101-109, 52-53, 111-112). Seven of the ten studies (102, 104, 52, 106, 107, 109, 111) evaluating BW observed significantly increased BW or reduced risk of associated with enhanced maternal/cord

blood Ω-3 FA levels or intake of fish, marine food or fish oil supplements. Three studies (101, 106, 112) assessed the effect of fish or fish oil supplement intake on the risk of IUGR and none of them reported any significant findings. Four studies (101, 103, 105, 112) researched the risk of PIH or preeclampsia and only one (105) found a weak protective effect from EPA and DHA intake. In summary, BW is the fetal growth-related factor most consistently related to intake of fish or fish oil supplement. Nonetheless, since BW increases almost linearly with gestational age during the later period of pregnancy (61), whether the fish consumption-BW association is confounded by gestational age needs further clarification, especially because only three (104, 107, 109) of current 13 studies controlled for gestational age in the analysis.

Fish Consumption, Mercury Exposure and Fetal Development

Mercury is a naturally existing element that is found in air, water and soil, and it is generally released to the natural environment in an inorganic form by both natural and anthropogenic sources (62-65). There is much concern about the potential for human intoxication because of exposure to mercury in foodstuffs particularly fish. The most serious toxicities to mercury arise from methylmercury residues in food (114). During methylation inorganic mercury is converted into methylmercury by microbial action, primarily in sediments of fresh and ocean water. Methylmercury readily enters the aquatic food chain and bioaccumulates in predatory fish such as swordfish, pike and ocean tuna (66). Larger and more long-lived fish tend to contain more methylmercury and the contamination can be significant, for example, the total mercury in the edible tissue of shark and swordfish can average as high as 1200 μg/kg (64). Consumption of

fish and marine food, though not the only source of methylmercury, is a very important exposure to methylmercury for humans, especially for those populations near seawater and rely on fish as main food source. Methylmercury is rapidly and extensively absorbed through the gastrointestinal tract, and it is distributed throughout the body and easily penetrates the blood-brain and placental barriers in humans and animals (66).

The critical target for methylmercury toxicity is the nervous system (66) and the developing fetus may be at particular risk from the exposure to methylmercury. Studies about epidemic poisonings in Japan (67) and Iraq (68) showed that maternal toxicity may or may not be present during pregnancy for those offspring exhibiting adverse effects. Offspring exposed to methylmercury in utero showed a variety of neurological abnormalities including delayed walking, delayed onset of talking, cerebral palsy, altered muscle tone and deep tendon reflexes, and reduced neurological test scores (67,68). The pathological mechanism for the methylmercury-induced developmental neurotoxicity is unknown, but several hypotheses were raised: changes in intracellular cytoskeletal structure (69-71), oxidative stress (72-74), alternations to membrane function and signal transduction (75), decreased protein production (76), and changes in neurotransmission (77).

Besides the adverse effect on fetal neurodevelopment, animal experiments also showed reduced neonatal weight gain among animals treated with high does of methylmercury (78-80). To explore a possible intrauterine growth inhibiting effect from mercury for human, Sikosky et al (81) studied the inorganic mercury levels in newborn hair samples and reported a statistically significant inverse correlation between mercury concentration in fetal hair and BW. However, no the analyses lacked other covariates and the result may be explained by

confounding. A study in Greenland reported high maternal and offspring methylmercury blood concentrations associated with low mean BW (82). Other studies detected no association between BW and methylmercury levels in hair or blood (83-85). Because BW and gestational age are correlated and most previous studies did not control for gestational age (81, 82, 84, 85), the detected BW-mercury association can at least partially be due to the confounding from gestational age.

The exact pathological mechanism linking gestation age with mercury intake is unknown, but if such relationship exists, the "threshold effect" detected in the fish consumption-gestational age association could be explained by the accumulated toxicity from mercury when the fish consumption level is very high. Studies investigating the influence of mercury level on gestational age are few and none of them observed significant results (82, 83, 87). The only study assessing the risk of PD (87) evaluated the difference in the incidence of PD between the group occupationally exposed to 0.06-0.1 mg/m3 of metallic mercury and a non-exposed group, and did not report a significant result.

Table 2 is a literature review of studies investigating BW and gestational age in relation to exposure to mercury. Except the study measuring metallic mercury exposure in relation to the risk of PD (87), all the other studies evaluated methylmercury in blood or hair samples. No convincing evidence was provided for gestational age or BW to be related to maternal or fetal mercury level in blood or hair. Noticeably, five of the six cohort studies have a small sample size (81-84, 86) and they may not have had enough power to detect subtle but important differences. In addition, various kinds of samples including maternal and cord blood, maternal hair and infant hair, were used to quantify mercury levels and this also may have contributed to the inconsistency of the results concerning levels of mercury exposure in association with

gestational age at delivery or BW. The measurement of mercury exposure will be discussed in more details in the next section. Two studies evaluated the association between intake of seafood and mercury levels (82, 85) and both found marine food consumption to be significantly related to elevated maternal and cord blood levels of methylmercury. All three studies assessing the correlation between maternal and fetal mercury levels (81, 82, 86) concluded that fetal mercury levels in blood or hair are significantly associated with maternal levels. This result supports the previous reports that methylmercury can easily cross placental barriers in humans and animals (66).

Table 2. Literature review of previous studies on matern/fetal mrecury levels in relation to gestational age or birthweight.

Cohort Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Grandjean (2001) (83)	Hospital based, N=182	Cord serum concentration of seafood contaminants	1. Gestational age 2. Birthweight 3. Placental weight	Parity, maternal smoking, maternal height, fetal gender.	1-3. Serum mercury levels do not affect ay of the outcome parameters.
Bjerregaard (1996) (85)	Hospital based, N=1106	1. Mercury levels in cord/maternal blood. 2. consumption of marine mamels	Birthweight	Population group, inunit group, and smoking.	Maternal blood mercury concentration were correlated with intake of local meat (R=0.15, P<0.0001). Mercury levels in maternal blood and cord blood were closely correlated (R=0.93, P<0.0001). Only in west Greenlanders (n=597), an increase in blood mercury of 10ug/l was accompanied by decrease in birthweight of 56g (P=0.04)
Marsh (1995) (84)	Hospital based, N=131	Mother's mean hair mercury level: covers the 9 months of gestation.	1. Birthweight 2. Head circumference	Maternal age	1-2. In no instances is there a statistically significant relationship between maternal Methylmercury concentrations and a measure of infant development.

Table 2. Literature review of previous studies on matern/fetal mrecury levels in relation to gestational age or birthweight.

Cohort Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Fu (1993) (86)	Occupation based, N=704	Exposure to metallic mercury levels	1. Preterm delivery 2. Low birthweight	NA	1. Difference in incidence of preterm delivery between the group exposed to 0.06-0.1 mg/m3 of mercury and control group did not reach a significant level. 2. Incidence of low birthweight in offsprings ofthe exposed group was not significantly higher than in those of controls.
Foldspang (1990) (82)	Hospital based, N=376	Average weekly intake of marine food, and blood methymercury concentratios of mothers and offsprings	1. Gestational age 2. Birthweight	Mother's birth place. Town of residence, cigarette smoking, and sex of the offspring.	Intake of marine food is related to higher mercury levels in maternal&fetal blood (P=0.000). Maternal&fetal blood mercury levels were associated (Pearson's r=0.85, P=0.000) 1. Gestational age is not determined by either number of meals of local food or by the blood mercury concentration. 2. Low mean birthweight was associated with higher blood mercury of the offspring (P=0.01) and mother (P=0.01). The intake of marine food did not influence the mean birthweight.

Table 2. Literature review of previous studies on matern/fetal mrecury levels in relation to gestational age or birthweight.

Cohort Studies:					
Study (Author/Date)	Population/ Sample Size	Exposure/ Risk Factor	Outcome/ Disease	Confounders	Results
Sikorski (1986) (81)	Hospital based, N=141	Total mercury levels (TML) in maternal and neonatal hair.	Birthweight	NA	TML in mothers' hair and neonatal hair were statistically correlated. Significant inverse correlation between mercury concentration in the hair of a newborn and its birthweight: A 361g lower birthweight among 24 infants with lower concentrations.

Measurement of ExpFosure to Ω-3 FA and Mercury

Questionnaires and interviews concerning frequency of meals of fish or marine food during pregnancy are most often used to measure fish consumption. However, the estimate of Ω-3 FA intake by these methods is generally imprecise because of the portion size, distribution of fish species in meals, and food nutrient content are only approximations to the true value (106). Intake of supplements of fish oil or Ω-3 FA is sometimes taken into account as part of the sources of Ω-3 FA, but this part of Ω-3 FA intake is difficult to scale to levels of fish consumption in analysis. Classification according to types of fish and marine food is conducted in some but not all studies. Fish and sea mammals differ with respect to the chemical composition of their Ω-3 FA content (96) and considerable variation has been shown among fish living at different latitudes (97). These differences of Ω-3 FA in chemical composition may result in varied biological activities. Levels of fish or seafood consumption are usually categorized according to the range of the consumption level in each study population. Thus, cut points for the high or low levels of fish consumption in different studies vary and the results are difficult compare across populations. Furthermore, questionnaires and interviews concerning fish consumption may increase women's awareness of their intake of fish, and they alter their intake of fish in the duration of the pregnancy.

Direct measurement of biomarkers of Ω-3 FA in serum or erythrocytes is another approach to evaluating the exposure to Ω-3 FA. The most frequently used indicators include EPA, DHA and the ratio of Ω-3 FA to AA (Ω-6) (Ω-3/ Ω-6 ratio). Fatty acids concentrations in serum and erythrocytes are a result of dynamic interaction between absorption, degradation and for some of them, catabolism, and changes in transplacental passage (98-99). Levels of FA in cord serum seem to be stable during the last few weeks of normal gestation (99-100), though

physiological changes during pregnancy and the time of sampling and measurement should be taken into account in analysis.

Sources of mercury exposure can be categorized into environmental, industrial and agricultural (115). Mercury has a relatively high vapor pressure and the air over mercury and its ore deposits generally contains enhanced levels of mercury (115). The combustion of fossil fuels also releases traces of mercury into the atmosphere and these add to the much larger quantities present in the atmosphere than the natural vaporization processes from the earth's surface (116). Considerable evidence has shown that manmade pollution of rivers, lakes and estuaries has increased the mercury levels in fish, but such pollution has not contributed significantly to the mercury levels in ocean fish (115). Individual lakes or watercourses can also be contaminated by underlying mineral deposits containing mercury, which leach into the water under natural circumstances (117). The direct pollution of water by industrial sources is likely to affect fish more than other foods (115). The industrial sources that cause the transfer of mercury-containing wastes to water or mud in fishery areas constitute sources of most direct concern of mercury contamination. The principle industrial sources include the chlor-alkali industry using the mercury process, the pulp and paper industry and other industries using mercury (118). Other industrial sources such as mining, smelting and refining of ores are also significant but the effects are usually localized. The amount of mercury in the food supply from normal agricultural use has been usually small compared with that from other sources, but alkyl, alkoxyalkyl, aryl, and inorganic mercurial fungicides have been used for seed dressing, as turf fungicides and in orchards (115). Although in recent years, the use of alkyl and aryl mercurial fungicide has been restricted or forbidden in many countries, accidents have occurred from the misuse of seeds treated with alkyl mercury compounds

(115). Other sources of mercury include pharmaceuticals containing either inorganic or organic mercury compounds, and mercurials used as preservatives in cosmetics and toiletries (115).

The most common biological samples analyzed for mercury are blood, urine, and scalp hair. Blood, urine and exhaled air are common to assess occupational mercury exposure, which is primarily elemental mercury (87). Blood and scalp hair are the primary indicators used to assess methylmercury exposure (87). Blood is a good indicator medium for estimating methylmercury because methylmercury freely distributes throughout the body. However, because an individual's intake may vary, blood levels may not necessary reflect mercury intake over time (88-89). Methylmercury is incorporated into scalp hair at the hair follicle in proportion to its content in blood (90). Once incorporated into hair, the methylmercury is stable, and therefore, gives a longitudinal history of blood methylmercury levels (90). However, the analysis of hair mercury levels may be confounded by absorption of mercury vapor onto the hair strands (91). The hair-to-blood ratio in humans has been estimated as approximately 250:1 expressed as ug Hg/g hair to mg Hg/L blood, but some difficulties in measurements, interindividual variation in body burden and metabolism, differences in hair growth rates, and variations in fresh and saltwater fish intake have led to varying estimates (92-93).

The most common methods used to determine mercury levels in blood, urine and hair of humans and animals include atomic absorption spectrometry (AAS), neuron activation and analysis (NAA), X-ray fluorescence (XRF) and gas chromatography (GC). Among these methods, only GC is able to distinguish methylmercury from the total mercury content (94-95). The Magos and Clarkson method of AAS can estimate methylmercury by subtracting the

inorganic mercury content from the total mercury. However, hair contains primarily methylmercury from consumption of fish or other aquatic foods and thus the content of inorganic mercury is very low or absent, so the measurement of hair methylmercury levels should not be largely influenced if the method cannot distinguish methylmercury from inorganic mercury.

MATERNAL FISH CONSUMPTION IN RELATION TO PRETERM DELIVERY

Fei Xue, Claudia Holzman, Larry Fischer, Hossein Rahbar

Introduction:

PD, which occurs in 11% of U.S. birth, continues to be a leading cause of infant mortality and morbidity and excess healthcare costs. Lowering the rate of PD has been an elusive goal, in part because there are many clues regarding the underlying causes of PD, but few answers. Wide variations in PD rates across countries and between subgroups within the U.S. have prompted investigators to consider maternal diet as one potential factor affecting the risk of delivering prematurely. Though total caloric intake in developed countries (131), and diet supplementation (132) have not been associated with lower PD rates, it is possible that components of the diet, such as vitamins, minerals, or essential fatty acids may be protective for certain pathways leading to PD.

Researchers have hypothesized that intake of high levels of Ω-3 FA may be protective for preterm birth because Ω-3 FA inhibit the synthesis of Ω-6 eicosanoids from AA (112-113), a 20-carbon unsaturated fatty acid produced from membrane phospholipids. These Ω-6 ecosanoid derivatives are thought to play a role in the initiation and completion of term and preterm labor (114). Fish and marine food are the main source of Ω-3 FA in many human populations. The reduced rates of LBW in fish-eating Northern European populations, such as those in Faroe Islands, offer ecological support for the protective effects of Ω-3 FA on the risk of delivering LBW babies, many of whom are preterm (54, 124).

Studies of fish consumption during pregnancy and risk of PD are conducted mainly in regions near seawater with high levels of fish intake. These studies have produced mixed results, and do not provide insights into populations with lower levels of fish intake. In some regions, it may also be important to weigh the potentially harmful effects posed by fish contaminants (e.g. mercury) against the potential benefits of fish consumption as a source of Ω-3 FA.

In this prospective study from five Michigan communities, we evaluate fish intake in the first half of pregnancy and the risk of very preterm (<35 weeks) and moderately preterm (35-36 weeks) delivery. Our study offers an opportunity to assess the variability of fish intake in pregnancy in relation to maternal characteristics (e.g. age, ethnicity, education, Medicaid insurance status, and community of residence) and link these findings to the rates of PD. In this way we examine the impact of fish consumption on length of gestation and the potential for varying levels of fish consumption to contribute to ethnic and social class disparity in PD rate.

Methods:

Population:

The sample includes participants in the (Pregnancy Outcome and Community Health) POUCH Study who were enrolled from September 8, 1998 through July 31, 2001during the 17^{th} to 26^{th} weeks of pregnancy (130). Participants were recruited from 52 clinics in five Michigan communities 1, 2, 3, 4 and 5 (include five major cities and their surrounding areas). Eligibility criteria are maternal age greater than 14 years, participation in screening for maternal serum alpha-fetoprotein (MSAFP) levels between 15-20 weeks' gestation, proficiency in English and singleton pregnancy with no known

congenital or chromosomal abnormalities at the time of recruitment. Women with a history of diabetes prior to pregnancy were excluded. Approximately one third of all eligible women participated in the POUCH Study. Among the 1,336 women enrolled in this time period, ten were excluded (five lost to follow-up, five infants with congenital anomalies identified at birth), leaving a total of 1,326 women.

Fish consumption:

Participants provided dietary information about their fish consumption during the previous six months through an in-person interview at the time of enrollment. Since women were enrolled at 16-26 weeks' gestation, this period of recall approximately coincided with the first half of pregnancy. Women were asked about frequency of consumption of shellfish, canned fish, sport caught fish, purchased fish and other fish. Consumption of types of fish was reported as number of meals per day, week, month or six months and scaled to meals per six months.

Gestational Age at Delivery:

Gestational age at delivery was determined by the date of delivery and the gestational age estimated at the time of MSAFP screening. This estimate was based on the date of the first day of the last menstrual period (LMP), or on an early ultrasound (< 20 weeks) estimated gestational age, the latter given preference when the two estimates disagreed by more than 2 weeks. Very preterm was defined as delivery before 35 weeks' gestation and moderately preterm was delivery at 35-36 weeks' gestation.

Analytic Strategy:

Total fish consumption was calculated by summing up the frequency of consumption of all types of fish. The frequency of fish consumption was assessed both as continuous

variables and as quartiles. Descriptive statistics including mean, standard deviation, range and quartiles were used to describe the distribution of total fish consumption and consumption of subtypes of fish. The General Linear Models (GLM) and mixed procedure was used to assess associations between maternal characteristics and maternal fish consumption expressed on a continuous scale. Univariate and multivariate logistic regression models were used to test the association between maternal total fish consumption and the risk of all PD (<37 weeks), moderately PD (35-36 weeks) and very PD (<35 weeks). Maternal characteristic, which were found to be significantly associated with total fish consumption, were controlled for in the multivariate logistic regression model. To explore the influence of high levels of fish consumption on the risk of PD, cut points at the top 10^{th} and 5^{th} percentiles, and at thresholds where effects were noted in two previous studies (≥26 meals/6 months (106) and ≥104 meals/6 months (112)) were used. All analyses were conducted using the SAS software (133).

Results:

In this study sample, 44% of women were less than 25 years of age, 24% were African-American, 48% had education of less than or equal to 12 years, 46% were Medicaid insured, 28% were primiparous, 41% were from community four, 18% were from community five, 17% were from community two, 15% were from community one and 9% were from community three (Table 3).

Data concerning consumption of shellfish, canned fish, bought fish, sport caught fish or other fish were missing for some women respectively, therefore fewer women (N=1302) were included in calculation of frequency of total fish consumption. The mean total fish consumption was 19.7 meals/6 months. Three women reported unusually high

fish consumption, ranging from 364-548 fishmeals per six months. After removing these three outliers, the mean level of total fish consumption was 19.6 meals/6 months, or about three meals of fish per month. The mean levels of consumption of shell fish, canned fish, bought fish, sport caught fish and other fish were 3.7, 8.5, 6.3, 0.7 and 0.4 meals/6 months respectively, suggesting that canned fish and bought fish contributed most to total fish consumption (Table 4). During the first six months of pregnancy, 10.9% of women did not consume any fish and 50% ate at least 9.0 meals of fish. Only 9.2% reported consuming sport caught fish.

In univariate analyses women 25 years and older had a significantly higher mean total fish consumption compared to that of women less than 25 years of age (Difference=5.2 meals/6 months, 95% CI 1.5, 8.9). Using women enrolled from community one as reference, women from community five had a significantly higher mean total fish consumption (Difference=8.4 meals/6 months, 95% CI 0.5, 16.3). Mean levels of total fish consumption were also higher in African-Americans compared with whites and others (Difference=3.6 meals/6 months, 95% CI -0.7, 8.0) and the women with more than 12 years' education compared to women with 12 or few years (Difference=3.6 meals/6 months, 95% CI -0.1, 7.3), though the results were not statistically significant at P=0.05. After adjusting for all other maternal characteristics, the adjusted mean total fish consumption level continued to be significantly higher in women 25 years and older (Difference=5.9 meals/6 months, 95% CI 1.6, 10.2). In the adjusted model, mean levels of total fish consumption were suggested to be higher among African-American women (Difference=4.3, 95% CI -0.5, 9.2) and among women enrolled from community 5

(Difference=7.7, 95% CI -0.2, 15.6), though the result is no longer significant after controlling for other maternal characteristics (Table 5).

Overall, 10.6% of women delivered before 37 weeks' gestation and 4.1% delivered before 35 weeks' gestation. Modeling fish consumption thresholds at >2.0 (25^{th} percentile), >9.0 (50^{th} percentile), >25.0 (75^{th} percentile), ≥ 26.0 (Olsen study), ≥52 (top 10^{th} percentile), ≥76 (top 5^{th} percentile), and ≥104 (Kesmodel study) fishmeals per six months failed to reveal a statistically significant association between maternal fish consumption and PD (Table 6). In separate analyses assessing the association between PD and maternal consumption of bought fish, sport caught fish, canned fish, shellfish and other fish, high intake of any one subtype of fish did not appear to be significantly associated with the risk of PD.

Table 3. Maternal characteristics of study sample (N=1326)

Maternal Characteristics	% (N)
Age:	
<25	44 (580)
>=25	56 (746)
Ethnicity:	
white	68 (902)
Afr-American	24 (318)
Other	8 (106)
Education (yrs):**	
<=12	48 (631)
>12	52 (691)
Medicaid*:	
Yes	46 (613)
No	54 (712)
Community:	
1	15 (198)
2	17 (231)
3	9 (117)
4	41 (536)
5	18 (244)
Total Fish Consumption*:**	
(meals/6 months) <6	38 (495)
6-24	35 (452)
>=24	27 (355)
Primiparous*:	
Yes	28 (364)
No	72 (961)

* Data missing for 1 woman.
** Data missing for 4 women.
*** Data missing for 24 women.

Table 4. Number of fish meals* (total fish and subtypes) in the first half of pregnancy.

Consumption of Fish	Mean	Std Deviation	Range	25th percentile	50th percentile	75th percentile
Total Fish (everyone)	19.7	33.9	0-547.5	2.0	9.0	25.0
Total Fish (removed outliers)**	19.6	28.2	0-214.5	2.0	9.0	26.0
Shell Fish* **	3.7	10.1	0-182.5	0.0	1.0	3.0
Canned fish* **	8.5	16.5	0-182.5	0.0	2.0	12.0
Bought fish* **	6.3	18.5	0-365.0	0.0	1.0	6.0
Sport caught fish* **	0.7	4.9	0-90.0	0.0	0.0	0.0
Other fish * **	0.4	6.0	0-182.5	0.0	0.0	0.0

* Reported as meals per day, week, month or 6 months, and scaled to number of meals per 6 months.

** 3 women with total fish consumption more than 300 meals per 6 months.

*** Data missing for 5-8 women.

Table 5. Maternal Characteristics in relation to total fish consumption.

Maternal Characteristics		Frequency of Total Fish Consumption			
		Mean, 95% CI		Difference from the Referent, 95% CI	
		Crude	Adjusted***	Crude	Adjusted***
Age (years):	**<25**	16.7 (14.0, 19.5)	17.7 (14.4, 21.0)	--	--
	>=25	22.0 (19.5, 24.4)	23.6 (20.5, 26.8)	**5.2 (1.5, 8.9)***	**5.9 (1.6, 10.2)***
Ethnicity:	**Whites & other**	18.8 (16.7, 20.9)	18.5 (16.1, 20.9)	--	--
	African-American	22.5 (18.7, 26.2)	22.8 (18.6, 27.1)	**3.6 (-0.7, 8.0)****	**4.3 (-0.5, 9.2)****
Education:	**<=12 yrs**	17.8 (15.1, 20.4)	18.9 (15.7, 22.1)	--	--
	>12 yrs	21.3 (18.8, 23.9)	22.5 (19.2, 25.8)	**3.6 (-0.1, 7.3)****	3.6 (-0.8, 7.9)
Medicaid:	**Yes**	19.9 (17.2, 22.6)	22.0 (18.9, 25.1)	--	--
	No	19.5 (17.0, 22.0)	19.3 (15.8, 22.9)	-0.4 (-4.1, 3.3)	-2.7 (-7.2, 1.9)
Communities:	**1 (ref)**	15.9 (11.2, 20.6)	17.2 (12.3, 22.2)	--	--
	2	21.9 (17.5, 26.3)	22.0 (17.6, 26.5)	6.0 (-2.1, 14.0)	4.8 (-3.4, 13.0)
	3	19.1 (12.9, 25.2)	20.2 (13.7, 26.6)	3.1 (-6.5, 12.8)	2.9 (-6.8, 12.6)
	4	18.2 (15.3, 21.1)	19.0 (15.7, 22.3)	2.3 (-4.6, 9.2)	1.7 (-5.2, 8.7)
	5	24.3 (20.0, 28.6)	24.9 (20.5, 29.4)	**8.4 (0.5, 16.3)***	**7.7 (-0.2, 15.6)****

* P<0.05

** P<0.10

*** Adjusted for all the other variables in the table

Table 6. Percentage and Odds Ratio of term of preterm deliveries in fish consumption groups evaluated at multiple thresholds

		Term	All Preterm	Moderately Preterm	Very Preterm	<37 wks		35-36 wks		<35 wks	
Fish Consumption Groups		≥37 wks	<37 wks	35-37 wks	<35 wks	Crude	Adjusted*	Crude	Adjusted*	Crude	Adjusted*
Total Fish Consumption**		89.4 (1162)	10.6 (137)	6.5 (84)	4.1 (53)	P=0.48	P=0.50	P=0.53	P=0.54	P=0.71	P=0.71
Thresholds (meals/6months)		Column% (N)				OR (95% CI)					
25th	≤2.0	24.7 (287)	22.5 (31)	16.7 (14)	31.5 (17)	--	--	--	--	--	--
percentile	>2.0	75.3 (877)	77.5 (107)	83.3 (70)	68.5 (37)	1.1 (0.7, 1.7)	1.2 (0.8, 1.8)	1.6 (0.9, 2.9)	1.8 (1.0, 3.3)	0.7 (0.4, 1.3)	0.7 (0.4, 1.3)
50th	≤9.0	51.5 (600)	50.0 (69)	46.4 (39)	55.6 (30)	--	--	--	--	--	--
percentle	>9.0	48.5 (564)	50.0 (69)	53.6 (45)	44.4 (24)	1.1 (0.7, 1.5)	1.1 (0.8, 1.6)	1.2 (0.8, 1.9)	1.3 (0.8, 2.1)	0.9 (0.5, 1.5)	0.8 (0.5, 1.4)
75th	≤25.0	75.5 (879)	75.4 (104)	73.8 (62)	77.8 (42)	--	--	--	--	--	--
percentile	>25.0	24.5 (285)	24.6 (34)	26.2 (22)	22.2 (12)	1.0 (0.7, 1.5)	1.0 (0.7, 1.5)	1.1 (0.7, 1.8)	1.1 (0.7, 1.9)	0.9 (0.5, 1.7)	0.8 (0.4, 1.6)
Olsen's	<26	77.7 (905)	76.8 (106)	75.0 (63)	79.6 (43)	--	--	--	--	--	--
Study****	≥26	22.3 (259)	23.2 (32)	25.0 (21)	20.4 (11)	1.1 (0.7, 1.6)	1.1 (0.7, 1.6)	1.2 (0.7, 1.9)	1.2 (0.7, 2.0)	0.9 (0.5, 1.8)	0.9 (0.4, 1.7)
Top 10th	<52	91.0 (1059)	92.7 (128)	92.9 (78)	92.6 (50)	--	--	--	--	--	--
percentile	≥52	9.0 (105)	7.3 (10)	7.1 (6)	7.4 (4)	0.8 (0.4, 1.5)	0.8 (0.4, 1.5)	0.8 (0.3, 1.8)	0.8 (0.3, 1.8)	0.8 (0.3, 2.3)	0.8 (0.3, 2.2)

* Adjusted for maternal age, ethnicity and community.

** Excluding 3 outliers who consumed more than 300 fish meals per 6 months.

*** Cut point used in U. Kesmodel et al. (2002) (112).

**** Cut point used in S. F. Olsen et al (2002) (106).

Table 6. Percentage and Odds Ratio of term of preterm deliveries in fish consumption groups evaluated at multiple thresholds

		Term	All Preterm	Moderately Preterm	Very Preterm	<37 wks		35-36 wks		<35 wks	
Fish Consumption Groups		≥37 wks	<37 wks	35-37 wks	<35 wks	Crude	Adjusted*	Crude	Adjusted*	Crude	Adjusted*
Total Fish Consumption**		89.4 (1162)	10.6 (137)	6.5 (84)	4.1 (53)	P=0.48	P=0.50	P=0.53	P=0.54	P=0.71	P=0.71
Thresholds (meals/6months)		Column% (N)				OR (95% CI)					
Top 5th	<76	95.1 (1107)	94.9 (131)	95.2 (80)	94.4 (51)	--	--	--	--	--	--
						1.0	1.0	1.0	1.0	1.1	1.1
percentile	≥76	4.9 (57)	5.1 (7)	4.8 (4)	5.6 (3)	(0.5, 2.3)	(0.5, 2.3)	(0.3, 2.7)	(0.3, 2.8)	(0.3, 3.8)	(0.3, 3.6)
Kesmodel's	<104	97.7 (1137)	97.8 (135)	100.0 (84)	94.4 (51)	--	--	--	--	--	--
						0.9	0.9	NA	NA	2.5	2.3
Study***	≥104	2.3 (27)	2.2 (3)	0.0 (0)	5.6 (3)	(0.3, 3.1)	(0.3, 3.1)	NA	NA	(0.7, 8.4)	(0.7, 8.0)

* Adjusted for maternal age, ethnicity and community.

** Excluding 3 outliers who consumed more than 300 fish meals per 6 months.

*** Cut point used in U. Kesmodel et al. (2002) (112).

**** Cut point used in S. F. Olsen et al (2002) (106).

Discussion:

This study found that older women, African-American women and women enrolled from Community five had higher levels of total fish consumption in the first half of pregnancy. Evidences showed that maternal fish consumption was higher in women with more years of education in the unadjusted model but the result did not sustain after adjusting for other maternal characteristics. Maternal fish consumption did not differ between women with Medicaid insurance and those without. Levels of total fish consumption and consumption of types of fish were not associated with the risk of all PD, very preterm or moderately PD. High levels of total fish consumption, including the top 10^{th} percentile, the top 5^{th} percentile and the same cut points as the other two previous studies ($\geq$26 meals/6 months and $\geq$104 meals/6 months) was not associated with the risk of PD.

Researchers have hypothesized that intake of high levels of Ω-3 FA may be protective for preterm birth, possibly because Ω-3 FA inhibit the synthesis of eicosanoids from AA (112-113), whose derivatives play a role in the initiation and completion of term and preterm labor (114). Since fish and marine food are the main source of Ω-3 FA in many human populations, this study have considered frequency of fish consumption during the first 6 months of pregnancy as a potential predictor of PD. However, some other factors such as the portion size, fish preparation, fish types that were not assessed in this study and use of fish oil supplement could affect levels of n3-FA intake, so using fish consumption as a proxy for Ω-3 FA intake may have limitations. Direct measurement the blood level of Ω-3 FA in maternal or fetal blood would be preferred to test this hypothesis.

Generally low levels of fish consumption in this study population also reduced the power to detect an association between fish consumption and the risk of PD. Despite the small number of women who delivered before 35 weeks' gestation and consumed high levels of fish, the analysis showed hints of a protective effect for very PD from increased levels of total fish consumption from the 25% percentile to the top 10^{th} percentile (OR ranged from 0.7 to 0.9). At levels beyond the cut point of the study by Kesmodel et al ($\geq$104 meals/6 months), fish consumption seemed to be positively related to the risk of very PD (OR=2.3), however the sample size at high consumption levels was small and confidence interval was wide (0.7, 8.0). Data also showed consistently protective effects for PD using the cut point at the top 10^{th} percentile ($\geq$52 fish meals/6 months). However, the results were not statistically significant. This may be explained by the fact that only ten women with total fish consumption levels as high as over 52 fishmeals per six months delivered before 37 weeks of gestation. For example, with a current sample size of 1302 and using the prevalence of PD in this study, we have only a power of 41% to detect the difference in the risk of PD at the cut point of 52 fishmeals per 6 months at the significance of 0.05. To reach a power of 0.80, a sample size of 3287 would be required.

Several previous studies have been undertaken to explore whether maternal fish consumption, intake of Ω-3 FA supplement or levels of Ω-3 FA in blood during pregnancy could change the risk of premature labor. Most of them were conducted in European countries, such as Denmark, Finland, Faroe Islands and England where levels of fish consumption are generally high and these studies produced mixed results.

Studies Assessing Fish Consumption in Relation to Preterm Delivery:

In 1993, S. F. Olsen et al (111) conducted a case-control study and found that BW and birth length of the newborn increased with the frequency of seafood dinner meals during pregnancy, but such an effect tended to disappear with more than 3 meals/week. The same analyses for pregnancy duration were not significant though exhibited the same trend. In a more recent cohort study (106), Olsen et al estimated daily intakes of fish and Ω-3 FA from frequency of fish consumption and found that the decreasing occurrence of LBW, preterm birth and IUGR was associated with increasing fish consumption in early pregnancy. They also found that these associations were mainly apparent at the lower end of the exposure, particularly for mean duration of gestation. Both studies detected a less significant association at higher levels of fish consumption. It might be related to the "ceiling effect", which means after reaching a certain high level of exposure, incremental increases in exposure will no longer bring substantial changes to outcomes. The more recent cohort study (106) has a similar study deign as our study, however the population in Olsen's study has a higher general consumption of fishmeals (76.8% versus 60.7% women consume at least 1 fish meals/month). In addition, the fish types in Olsen's study were mainly seafood, which may contain higher levels of Ω-3 FA.

Another cohort study by Olsen et al investigated the influence of dietary nutrient density of marine Ω-3 FA at 30^{th} week of pregnancy and found no difference in quintiles of Ω-3 FA nutrient density and gestational age at delivery, BW and birth length (125). Other case-controls studies on the effect of consumption of fish and seafood on the risk of PD have likewise not detected a significant association with these birth outcomes. U. Kesmodel et al (112) detected no association between fish intake or intake of fish oil and the risk of PD or posterm delivery. Bjerregarrd et al found in Denmark that consumption

of marine mammals was not significantly associated with LBW (108). Philippe Grandjean et al reported that in Faroe Island, frequencies of main meals with fish, whale meat and whale blubber during pregnancy were not related to gestational age, BW or placental weight (107).

The studies assessing the dietary intake of fish in relation to gestational age at delivery produced mixed but largely negative results. Five possibilities might contribute to the explanation of the inconsistency of the results. First, other Ω-3 FA sources than fish may exist in some populations. Second, retrospective questionnaire is not an accurate method to measure the frequency of fish consumption. Third, the amount of fish intake during each fishmeal varies among individuals and areas. Fourth, fish type and fish preparation may influence the content of Ω-3 FA in each fishmeal. Fifth, other maternal characteristics which differ over different populations, such as ethnicity, socioeconomic status, physical activity and diet other than fish may influence the risk of PD.

Studies Assessing Blood Ω-3 FA Levels in Relation to Preterm Delivery:

Evidences have shown that fish and seafood are main n3-FA source for many human populations. S. Reddy et al proved that the proportion of EPA and DHA were lower in plasma phospholipids, plasma free FA and total plasma lipids of the vegetarians compared with those from omnivores (109). In the Olsen's study (113), the average ratio of Ω-3 FA and Ω-6 FA among Faroese women who consumed higher levels of marine fat was significantly greater than that of Danish women. Grandjean et al also observed that the maternal and cord serum concentration of EPA increased with maternal marine food intake, though the tendency was less clear for DHA (107).

In contrast to the largely negative results from studies measuring dietary fish intake, more studies correlating maternal or cord blood Ω-3 FA levels and PD detected significant associations, and most of them were cohort studies. Because of the contradictory biological effects of Ω-3 FA and Ω-6 FA on the initiation of spontaneous labor, the ratio of levels of Ω-3 FA to Ω-6 FA (the (3/6) ratio) is commonly used as a reliable single biochemical measure of Ω-3 FA level in blood. In an early cohort study by Olsen et al (110), results showed that gestational age was significantly related to the (3/6) ratio in phosphatidylethanolamine among 18 women with spontaneous onset of delivery after controlling for maternal age, weight, marital status or smoking. And in the study by Olsen et al in 1991 (113), the association between gestation age and the (3/6) ratio was significant in Danish women but not in Faroese women though marine food consumption was higher in Faroe Island. The result of this study reinforced the possibility of a "ceiling effect" in the fish consumption-PD association. A recent cohort study by Grandjean et al (107) reported that an increase in the relative concentration of DHA in cord serum phospholipids by 1% resulted in an increased duration of gestation by 1.5 days (95% CI 0.7-2.2), though BW adjusted for gestational age may decrease at high intake level. Another cohort studies by Olsen et al (125) assessed both dietary fish intake and Ω-3 FA relative to AA (FA-ratio) and indicated no difference in mean gestation length, BW or birth length across quintiles of the FA-ratio. Possible explanation is that in-person interview to collect dietary fish consumption data could enhance the awareness of the participants to increase their intake of marine Ω-3 FA. Also the "ceiling effect" cannot be excluded because the mean fish consumption in this population was as high as 2.5 meals/week.

Although studies on blood levels of Ω-3 FA produced more exciting findings, it is difficult to interpret the results from the public health perspective. Whether or not to encourage pregnant women to eat fish in order to decrease the risk of PD cannot be decided solely by these results. Lower levels of fish consumption could be accompanied by a higher consumption of vegetable oil, which contains high levels of Ω-6 FA, so biomarkers such as blood Ω-3 FA levels and 3/6 ratio may just reflect levels of vegetable oil intake rather than fish consumption levels. Intake of fish oil supplement and individual absorption and metabolism potential can influence the correlation between fish intake and blood levels of Ω-3 FA. Additionally, blood levels of FA might have daily fluctuations and do not accurately reflected tissue levels.

Experimental Studies and Clinical Trials:

Experimental studies were performed to test the effect of fatty acids in preventing premature birth and most showed consistently significant results. In the experiment by Olsen et al (52), 15% MaxEPA supplement (FO) and 15% arachis oil supplement (AO) were given to Lewis rats. Results showed significantly higher levels of Ω-3 FA and lower levels of Ω-6 FA, longer gestational age ($P<0.01$) and interestingly, lower BW ($P<0.05$) in FO-rats than AC-rats. The decreased BW in the FO-rats may be explained by the lower maternal weight gain during pregnancy and the lower food consumption observed in FO-rats ($P<0.01$). Baguma-Nibasheka et al found that infusion of fish oil concentrate emulsion 3ml/kg per day from 124 days of gestation in sheep delayed both the onset of labor and the time of delivery (53).

Randomized clinical trial is generally deemed as the "golden standard" for epidemiological studies because of its advantage of controlling for potential confounders

and biases in study design. Most clinical trials on fish oil supplements detected consistently protective effect for premature birth. The earliest published controlled trial of fish oil was performed during 1938-1939 in London (126-129) and reinterpreted in 1990 by Olsen et al (105). 2510 of the 5022 participants were given an estimate of 0.1 g/day EPA plus DHA supplement for an average length of 20 weeks. The odds of delivering before 40 weeks of gestation was decreased by 20.4% (P=0.00083) in the treatment group. In 1992, Olsen et al (104) found that the average gestational age, BW and birth length were consistently the greatest in the group treated with 2.7 g/d Ω-3 FA (P=0.0006, 0.07 and 0.1, respectively) and lowest in the group treated with olive oil though the risk of preterm and posterm delivery did not differ significantly among the control group and the two treatment groups. Borod et al found in a clinical trial that DHA in each lipid class was increased among women who consumed 1 dozen eggs/week, each containing approximately 135 mg DHA during 24-28 weeks' gestation and decreased in those consuming 1 dozen regular eggs/week fewer (102). The results also showed fewer newborns with LBW or preterm birth in intervention group though the result is not statistically significant (102). Olsen et al did not detect significant effects of Ω-3 FA supplement starting from 33 weeks of gestation on the risk of PD, but they did find significantly decreased recurrence risk of PD among women who were given the supplement during the second half of pregnancy (P<0.05), and significantly delayed spontaneous delivery (P=0.002) in two intervention groups (101). In a randomized double-blinded placebo controlled trial, Onwude et al (103) observed no difference in the occurrence of BW less than the 3rd centile and duration of pregnancy between intervention group and the control group. However, compared with other intervention

studies, the study by Onwude et al has a smaller sample size and might not be able to detect subtle but important differences.

Clinical trials are superior to cohort and case-control studies in controlling for confounders and testing casual relationship between exposure and outcome. However, clinical trials also have problems, such as the compliance of participants and the estimation of etiological relevant time window. Furthermore, most previous clinical trials used fish oil or Ω-3 FA supplement as the intervention assuming that Ω-3 FA would be the critical component in fish consumption that affects risk of PD. However fish oil pills may not be comparable to dietary fish intake in terms of type and amount of fatty acids and the combined effect of other nutrient contents in fish.

Conclusion:

The etiology and risk factors for PD has long been a field of active research. The present study investigated whether eating fish protects women from PD in Michigan area where general fish consumption is much lower than areas near seawater. We found no significant association between maternal fish consumption during the first half of pregnancy and the risk of PD. Most of previous studies were conducted in north European countries where general consumption of fish and seafood are higher, but few studies have been done in the USA. These studies provided evidence that fish consumption, intake of fish oil supplement and increased blood Ω-3 FA levels are associated with longer gestation duration, higher BW and decreased risk of PD, but the protective effect may not increase after reaching a certain high level of exposure. Future studies need to clarify etiological relevant time for fish consumption to influence

gestational age and take into concern effects of the fish type, fish preparation and intake of fish oil supplement.

MATERNAL MERCURY LEVELS IN HAIR IN RELATION TO FISH CONSUMPTION AND RISK OF PRETERM DELIVERY

Fei Xue, Claudia Holzman, Larry Fischer, Hossein Rhabar

Introduction:

PD has continued to be the leading cause of fetal mortality and morbidity in the US despite recent advancements in medical technology. Extensive epidemiological studies have been done to explore the etiological risk factors for PD, covering from infection, gene, stress, environmental exposure to diet and nutrition. However, effective prevention of PD is unfortunately still an unattainable objective. Data from epidemic poisonings in Japan (67) and Iraq (68) showed that infants with intrauterine exposure to methylmercury developed marked fetal developmental delays; especially neurodevelopmental disorders while their mothers experienced little or no overt signs of toxicity. Three forms of mercury including elemental mercury, inorganic mercury and methylmercury can present a human health hazard, but only methylmercury has been confirmed by the United States Environmental protection Agency (US EPA) as developmental toxicant in human. Clarkson et al indicated that the developing fetus might be 5 to 10 times more sensitive than the adults to the toxicity of mercury (135). Though previous researches have consistently suggested the influence of mercury on fetal neurodevelopment, epidemiological studies evaluating mercury levels in relation to BW and gestational age are scant and mixed results have been reached. Foldspang et al (82) reported that higher maternal and offspring methylmercury concentrations were associated with low mean BW. However, the same association was not detected in other studies (83-86, 136). Small

sample size, selection of various biological samples and methods of mercury measurement can contribute to the inconsistency of these research results.

Fish and seafood, especially the species high in the food chain, are an important source of methylmercury exposure. Previous studies have shown that approximately 95% of the methylmercury in fish ingested by volunteers is absorbed from the gastrointestinal tract (137-138). In recognition of the hazard of methylmercury exposure, the US EPA has established a reference dose (RfD) for methylmercury at 0.3 μg/kg/day, which is equivalent to consumption of 19 μg per day of Methylmercury for a 62 kg woman (139). Most previous studies on this topic were conducted in populations either exposed to catastrophic pollution or with a diet composed mainly of fish or seafood and thus the results can not be generalized to populations with moderate levels of fish consumption if a threshold effect is taken into account. To better understand the effect of fish consumption and mercury on pregnancy outcomes, we conducted a prospective cohort study among 1024 pregnant women from five Michigan communities to investigate fish consumption during the first half of pregnancy in relation to mercury levels in hair and the effect of mercury levels on the risk of PD.

Methods:

Population:

The POUCH Study, a prospective study enrolls pregnant women at 16 to 26 weeks' gestation from 52 participating clinics in five Michigan communities, including the major city and surrounding areas. Eligibility criteria for the POUCH Study include maternal age greater than 14 years, screening for MSAFP levels between 15-20 weeks' gestation, proficiency in English and singleton pregnancy with no known congenital or

chromosomal abnormalities at time of recruitment, and no history of diabetes mellitus prior to pregnancy. This study included POUCH participants who were enrolled from September 8, 1998 through July 31, 2001. Of the 1336 women enrolled in this study (approximately one third of all eligible women), five women were lost to follow-up and five infants had congenital anomalies identified and thus were excluded. At the time of enrollment women meet with a study nurse who conducts interviews and collects biologic samples including scalp hair. Women had to have loose hair (e.g. no woven hair) longer than 3 inches in order to have their hair sampled. Hair samples were not collected in 197 POUCH participants because they declined (N=69, 35%) or had hair that was too short or not loose (N=128, 65%). Resources for measuring hair mercury levels were available for the first 1,024 hair samples. The remaining 105 hair samples have not been assessed to date.

Fish Consumption:

Dietary information about fish consumption during the first half of pregnancy was collected from all 1336 participating women through in-person interview during the mid-pregnancy visit at 16-26 weeks' gestation. Women were asked about frequency of consumption of shellfish, canned fish, sport caught fish, bought fish and other fish. Consumption of types of fish was reported as number of meals per day, week, month or six months and scaled to meals per six months. Total fish consumption was calculated by summing up consumption of all types of fish.

Mercury Levels in Hair:

Approximately 100 strands or more of hair were sampled from each woman during the mid-pregnancy interview. The hair samples were cut close to the scalp from the posterior

vertex region and the length of hair that was analyzed was measured and represented the period of time that the subject was pregnant prior to sampling (calculated using an 1cm/month of hair growth). Cold Vapor Atomic Absorption Spectrometry (CVAAS) was used to quantify total mercury levels in hair.

Gestational Age at Delivery:

Gestational age at delivery was determined by the date of delivery and the gestational age estimated at the time of MSAFP screening. This estimate was based on the date of the first day of the LMP, or on an early ultrasound (< 20 weeks) estimated gestational age, the latter given preference when the two estimates disagreed by more than 2 weeks. Very preterm was defined as delivery before 35 weeks' gestation and moderately preterm was delivery at 35-36 weeks' gestation.

Analytical Strategy:

Chi-square test was used to assess the differences in maternal characteristics in women with hair sampled and those without. The distribution of total fish consumption and consumption of types of fish were described using descriptive statistics including mean, standard deviation, range and quartiles. Levels of total fish consumption in relation to maternal characteristics were measured using GLM. Univariate and multivariate logistic regression models were applied to assess the association between maternal characteristics and mercury levels in hair. Mercury levels were categorized into quintiles and the first quintile was used as reference in the analysis. GLM was used to evaluate fish consumption in relation to mercury levels in hair. In the analysis, mercury levels were transformed to natural log (log ppm) to adjust for the right skewness, and the results were transformed back to mercury levels parts per million (ppm) for easier interpretation. Total

fish consumption was analyzed in relation to mercury levels after controlling for maternal characteristics, and consumption of each type of fish was analyzed after controlling for maternal characteristics and consumption of other types of fish. Total fish consumption was included in a logistic regression model when assessing the association between maternal characteristics and quintiles of mercury levels to determine if mercury originated from sources other than fish consumption. The risk of PD, moderately PD and very PD in relation to quintiles and the top 10^{th} percentile of mercury levels in hair was evaluated using logistic regression model. Total fish consumption and maternal characteristics, which were found to be significantly related to mercury levels in hair, were controlled for as potential confounders in the adjusted logistic regression model. All analyses were conducted using the SAS software (133).

Results:

Maternal Characteristics in Relation to Sampling:

The distribution of maternal characteristics including maternal age, ethnicity, education, Medicaid status, smoking during pregnancy and community were found to be significantly different between the group of women with hair sampled (N=1024) and those without (N=197). Compared with the women who did not give hair samples, women with samples were more likely to be older than 25 years of age (59% vs. 45%), whites (73% vs. 33%) and other ethnicity (non-African-American) (9% vs. 6%), have more than 12 years education (58% vs. 35%), not smoke during pregnancy (84% vs. 75%), and enrolled from community 2 (18% vs. 8%), 3 (10% vs. 9%) and 5 (19% vs. 15%) (Table 7).

Fish Consumption:

The mean level of total fish consumption in this study population was 20.4 meals/6 months. After removing two outliers whose total fish consumption levels were as high as 364 and 547.5 meals/6 months, the mean level of total fish consumption was reduced to 19.6 meals/6 months. The mean consumptions of types of fish from the highest to the lowest were 9.1 meals/6months for canned fish, 6.3 meals/6 months for bought fish, 3.8 meals/6 months for shellfish, 0.8 meals/6 months for sport caught fish and 0.3 meals/6 months for other fish. The distribution of total fish consumption and the consumption of types of fish were slightly right skewed and mean levels were all higher than median levels. Half of the women consumed at least 3 meals/6 months of canned fish, at least 1 meal/6 months of shellfish and bought fish. 10.5% of the women did not consume any fish and more than 75% of them did not consume any sport caught fish or other fish during the first 6 months of pregnancy (Table 8).

Total Fish Consumption in Relation to Maternal Characteristics:

Compared with women less than 25 years of age, mean levels of total fish consumption were 5.6 meals/6 months (95% CI 1.3, 9.9) higher among women $\geq$ 25 years of age in the unadjusted model and 5.8 meals/6 months (95% CI 0.8, 10.7) higher after adjusting for all the other maternal characteristics. Mean total fish consumption level was 5.7 meals/6 months (95% CI 0.1, 11.2) higher among African-American women and 7.5 meals/6 months (95% CI 1.3, 13.6) higher after adjusting for all the other maternal characteristics. Using gestational age of less than 20 weeks at enrollment as reference, women who were enrolled at 20-24 weeks' and $\geq$24 weeks' gestation had lower levels of total fish consumption both in the unadjusted model (Mean Difference=-11.0 meals/6 months, 95% CI -18.1, -4.0; and Mean Difference=-14.9, 95% CI-22.6, -7.1,

respectively) and after controlling for all the other maternal characteristics (Adjusted Mean Difference=-9.8 meals/6 months, 95% CI -16.9, -2.7; and Adjusted Mean Difference=-13.7, 95% CI -21.5, -5.8, respectively). Mean total fish consumption among women enrolled from community 5 was 10.3 meals/6 months (95% CI 1.1, 19.4) higher than that of women from community 1 in the unadjusted model and 9.2 meals/6 months (95% CI 0.1, 18.4) higher after adjusting for all the other maternal characteristics (Table 9).

Mercury Levels in Hair in Relation to Maternal Characteristics:

The range of mercury levels in hair was from 0.013 ppm to 2.50 ppm. After adjusting for all the other maternal characteristics and using the first quintile of mercury level as reference, women 25 years and older were more likely to be in the fourth quintile (Adjusted OR=1.96, 95% CI 1.21, 3.17) and the fifth quintile (Adjusted OR=2.13, 95% CI 1.22, 3.71) as compared to women under 25 years of age. Compared with African-American women, whites and women of other ethnicities were more likely to be in the third quintile (Adjusted OR=1.89, 95% CI 1.10, 3.26), fourth quintile (Adjusted OR=1.93, 95% CI 1.08, 3.44) and fifth quintile (Adjusted OR=1.94, 95% CI 1.00, 3.73). Women not insured by Medicaid were at significantly increased risk of being in the second quintile (Adjusted OR=1.93, 95% CI 1.15, 3.25), third quintile (Adjusted OR=1.82, 95% CI 1.09, 3.05), fourth quintile (Adjusted OR=1.90, 95% CI 1.09, 3.32) and fifth quintile (Adjusted OR=3.05, 95% CI 1.69, 5.49). The distributions of hair mercury levels and fish consumption levels in women from community one and three were very similar to each other. They are also geographically close, so community one and three were combined into one group in the analysis. Using community five, the community with the lowest hair

mercury levels as reference, and after adjusting for all the other maternal characteristics, women enrolled from community two were consistently more likely to be in the second quintile (Adjusted OR=3.93, 95% CI 1.76, 8.77), third quintile (Adjusted OR=2.38, 95% CI 1.05, 5.36), fourth quintile (Adjusted OR=5.73, 95% CI 2.39, 13.72) and fifth quintile (Adjusted OR=7.34, 95% CI 2.52, 21.37) of hair mercury level. In the adjusted model, women enrolled from community one and three were at increased risk of being in the second quintile (Adjusted OR=2.21, 95% CI 1.10, 4.42), the fourth quintile (Adjusted OR=3.50, 95% CI 1.64, 7.44) and the fifth quintile (Adjusted OR=6.00, 95% CI 2.33, 15.45) of hair mercury levels than community five. Interestingly, after adjusting for all the other maternal characteristics, women from community four were at significantly reduced risk of being in the third quintile of hair mercury levels (Adjusted OR=0.47, 95% CI 0.27, 0.82) than community 5 though the mean mercury level in community 5 was lower. Mercury levels in hair were associated with education, smoking before and during pregnancy and gestation age at enrollment in the univariate analysis, but the statistical significance of the association disappeared after controlling for other maternal characteristics (Table 10).

Mercury Levels in Relation to Fish Consumption:

Using total fish consumption of less than 6 meals/6 months as reference, mean mercury levels were significantly increased among women with 6-24 meals/6 months ($P<0.0001$) or $\geq$ 24 meals/6 months ($P<0.0001$) of total fish consumption after controlling for all maternal characteristics. Mean mercury levels were higher among women who consumed 6-24 meals/6 months ($P<0.0001$) or $\geq$ 24 meals/6 months ($P<0.0001$) of canned fish, women who consumed 6-24 meals/6 months of bought fish

(P=0.02) and women who consumed ≥24 meals/6 months of sport caught fish (P=0.007) after adjusting for all maternal characteristics and the consumption of other types of fish. Increase mercury levels in hair were found to be significantly related to consumption of 6-24 meals/6 months of shellfish (P<0.0001), ≥24 meals/6 months of bought fish (P=0.03) and 6-24 meals/6 months of sport caught fish (P=0.02), but the relationships disappeared after controlling for all the other maternal characteristics and consumption of other fish types (Table 11).

Mercury Levels in Hair in Relation to Maternal Characteristics after Controlling for Total Fish Consumption:

After adjusting for other maternal characteristics and levels of total fish consumption, the association between mercury levels in hair and maternal age, ethnicity, Medicaid status and community remained to be significant. Women ≥ 25 years old were at elevated risk of being in the fourth quintile (OR=1.83, 95% CI 1.12, 3.02) and fifth quintile (OR=2.05, 95% CI 1.15, 3.66) of hair mercury levels compared with African-American. Whites and women of other ethnicity were at higher risk of being in the third quintile (OR=2.14, 95% CI 1.22, 3.74), the fourth quintile (OR=2.58, 95% 1.38, 4.85) and fifth quintile (OR=2.72, 95% CI 1.33, 5.54) of hair mercury levels. Women not insured with Medicaid were found to be consistently at increased risk of being in the second to the fifth quintile of mercury levels in hair (OR=1.96, 95% CI 1.16, 3.30; OR=1.87, 95% CI 1.11, 3.14; OR=1.90, 95% CI 1.08, 3.37; OR=3.21, 95% CI 1.73, 5.98, respectively for the second to the fifth quintile). The risk of being in the second to the fifth quintile of mercury levels in hair was significantly higher among women enrolled from community two than community five (OR=3.97, 95% CI 1.78, 8.86; OR=2.53, 95% CI 1.10, 5.79;

OR=6.56, 95% CI 2.65, 16.27; OR=8.69, 95% CI 2.83, 26.67, respectively for the second to the fifth quintile). Women enrolled from community one and three continued to be at increased risk of being in the second quintile (OR=2.36, 95% CI 1.17, 4.79), fourth quintile (OR=4.59, 95% CI 2.05, 10.29) and the fifth quintile (OR=10.65, 95% CI 3.58, 31.70) compared with women enrolled from community five. Women enrolled from community four were at significantly lower risk of being in the third quintile of mercury level (OR=0.51, 95% CI 0.29, 0.91) than women from community five (table 12).

Mercury Levels in Hair in Relation to the Risk of Preterm Delivery:

The association between mercury levels in hair and the risk of PD was assessed both in univariate and multicovariate models that adjusted for total fish consumption and maternal characteristics that were found to be significantly associated with hair mercury levels (i.e. maternal age, ethnicity, Medicaid status and community). Compared with women in the first quintile of hair mercury level, women in the fourth quintile of mercury level were at significantly greater risk of delivering moderately preterm in the adjusted model (Adjusted OR=2.80, 95% CI 1.03, 7.59). Women in the top 10^{th} percentile of hair mercury levels were at significantly higher risk of delivering very preterm when compared to women in the lowest 90% in both the univariate model (OR=2.35, 95% CI 1.10, 5.05) and the adjusted model (Adjusted OR=2.95, 95% CI 1.30, 6.69). Interestingly, the third quintile was associated with significantly decreased risk of very PD in both univariate model (OR=0.20, 95% CI 0.06, 0.73) and adjusted model (OR=0.22, 95% CI 0.06, 0.81), however, the confidence intervals were wide (table 13).

Table 7. Some demographic characteristics in the 1024 POUCH participants with mercury assessments in sampled hair and in 197 participants with no hair sampled.

Maternal Characteristics		Hair Sampled % (N) (N=1024)	No Hair Sampled % (N) (N=197)
Maternal Age+:	**<25 yrs**	41 (422)	55 (109)
	>=25 yrs	59 (602)	45 (88)
Maternal Ethnicity+:	**White, Non-Latino**	73 (752)	33 (66)
	African-American	18 (183)	61 (120)
	Others	9 (89)	6 (11)
Education+:	**<=12 yrs**	42 (430)	65 (129)
	>12 yrs	58 (590)	35 (68)
Medicaid* +:	**Yes**	43 (436)	65 (129)
	No	57 (587)	35 (72)
Smoking before Pregnancy*:	**Yes**	26 (268)	33 (64)
	No	74 (751)	67 (133)
Smoking during Pregnancy* +:	**Yes**	16 (161)	25 (48)
	No	84 (859)	75 (149)
Preterm Delivery:	**<35 wks**	4 (44)	5 (9)
	35-36 wks	6 (57)	9 (18)
	>=37 wks (Ref)	90 (923)	86 (170)
Gestation Age at Enrollment:	**<20 wks**	14 (141)	11 (21)
	20-24 wks	59 (606)	61 (121)
	>=24 ks	27 (277)	28 (55)
Total Fish Consumption*:	**<6 meals/6 months**	37 (376)	38 (74)
	6-24 meals/6 months	34 (347)	34 (67)
	>=24 meals/6 months	29 (288)	28 (55)
Community+:	**1**	15 (148)	23 (46)
	2	18 (185)	8 (16)
	3	10 (100)	9 (17)
	4	38 (393)	45 (88)
	5	19 (198)	15 (30)

+ The difference is statistically significant between women with hair sampled and those without at $P<0.05$.

* Data missing for 1-13 women.

Table 8. Number of fish meals*** (total fish and subtypes) in the first half of pregnancy.

Consumption of Fish (N)	Mean	Std Deviation	Range	25th percentile	50th percentile	75th percentile
Total Fish (including outliers)* (1011)	20.4	34.4	0-547.5	3.0	9.0	26.0
Total Fish (outliers removed) (1009)**	19.6	28.2	0-214.5	3.0	9.0	26.0
Shell Fish* (1018)	3.8	10.6	0-182.5	0.0	1.0	4.0
Canned Fish* (1017)	9.1	17.6	0-182.5	0.0	3.0	12.0
Bought Fish* (1020)	6.3	16.7	0-182.5	0.0	1.0	6.0
Sport Caught Fish* (1022)	0.8	5.2	0-90.0	0.0	0.0	0.0
Other Fish * (1023)	0.3	3.7	0-78.0	0.0	0.0	0.0

* Data missing for 13 women for total fish consumption, and for 1-7 women for each fish subtype.

** 2 women with total fish consumption more than 300 meals per 6 months were removed.

*** Reported as per day, week, month or 6 months and scaled to 6 months.

Table 9. Total fish consumption (meals/6 months) in relation to maternal characteristics.

Maternal Characteristics		Total Fish Consumption			
		Mean, 95% CI		Difference From the Referent	
		Crude	Adjusted*	Crude	Adjusted***
Age (years):	**<25**	17.1 (13.8, 20.4)	22.4 (17.6, 27.1)	--	--
	>=25	22.7 (20.0, 25.5)	28.2 (23.7, 32.7)	**5.6 (1.3, 9.9)***	**5.8 (0.8, 10.7)***
Ethnicity:	**Whites & others**	19.4 (17.1, 21.7)	21.6 (18.1, 25.1)	--	--
	African-American	25.0 (20.0, 30.1)	29.0 (22.9, 35.1)	**5.7 (0.1, 11.2)***	**7.5 (1.3, 13.6)***
Education:	**<=12 yrs**	19.2 (16.0, 22.5)	24.7 (20.1, 29.4)	--	--
	>12 yrs	21.2 (18.4, 24.0)	25.9 (21.1, 30.6)	1.9 (-2.4, 6.3)	1.1 (-4.0, 6.3)
Medicaid:	**Yes**	20.2 (17.0, 23.5)	25.5 (21.3, 29.7)	--	--
	No	20.5 (17.7, 23.3)	25.1 (19.8, 30.4)	0.3 (-4.0, 4.6)	-0.4 (-6.0, 5.1)
Smoking before Pregnancy:	**Yes**	20.6 (18.1, 23.0)	26.1 (20.8, 31.4)	--	--
	No	20.2 (16.0, 24.4)	24.5 (19.4, 29.6)	-0.4 (-5.2, 4.5)	-1.7 (-8.5, 5.2)
Smoking during Pregnancy:	**Yes**	20.0 (17.7, 22.3)	22.4 (18.1, 26.7)	--	--
	No	22.8 (17.5, 28.2)	28.2 (21.4, 34.9)	2.8 (-3.0, 8.7)	5.7 (-2.5, 13.9)
Gestation Age at Enrollment:	**<20 wks(Ref)**	31.0 (25.3, 36.7)	33.1 (26.6, 39.6)	--	--
	20-24 wks	19.9 (17.2, 22.7)	23.4 (19.2, 27.5)	**-11.0 (-18.1, -4.0)****	**-9.8 (-16.9, -2.7)****
	>=24 wks	16.1 (12.1, 20.2)	19.4 (14.5, 24.4)	**-14.9 (-22.6, -7.1)****	**-13.7 (-21.5, -5.8)****
Communities:	**1 (Ref)**	15.8(10.2, 21.3)	21.8 (15.3, 28.2)	--	--
	2	21.3 (16.3, 26.3)	24.4 (18.8, 30.0)	5.5 (-3.7, 14.8)	2.7 (-6.8, 12.1)
	3	18.0 (11.2, 24.8)	24.3 (16.6, 32.0)	2.2 (-8.6, 13.1)	2.6 (-8.3, 13.4)
	4	19.6 (16.1, 23.0)	25.0 (20.2, 29.9)	3.8 (-4.3, 11.9)	3.3 (-4.8, 11.4)
	5	26.0 (21.2, 30.9)	31.0 (25.2, 36.7)	**10.3 (1.1, 19.4)***	**9.2 (0.1, 18.4)***

* P<0.05.

** P<0.01.

*** Adjusted for all the other variables in the table.

Table 10. Mercury level (ppm range) in hair sample in relation to maternal characteristics after adjusting for all the other maternal characteristics.

Maternal Characteristics		Mean Mercury Level and 95% CI** (PPM)	Odds Ratio and 95% CI			
			2nd Quintile (0.12-0.19)	3rd Quintile (0.20-0.26)	4th Quintile (0.27-0.38)	5th Quintile (0.39-2.50)
			Adjusted	Adjusted	Adjusted	Adjusted
Age (years):	**<25 (Ref)**	0.18 (0.17, 0.19)	--	--	--	--
	>=25	0.26 (0.25, 0.28)	0.99 (0.62, 1.60)	1.48 (0.91, 2.38)	**1.96* (1.21, 3.17)**	**2.13* (1.22, 3.71)**
Ethnicity:	**Afri-American (Ref)**	0.17 (0.15, 0.18)	--	--	--	--
	White & Other	0.24 (0.23, 0.25)	1.25 (0.76, 2.06)	**1.89* (1.10, 3.26)**	**1.93* (1.08, 3.44)**	**1.94* (1.00, 3.73)**
Education:	**<=12 yrs(Ref)**	0.19 (0.18, 0.20)	--	--	--	--
	>12 yrs	0.25 (0.24, 0.27)	0.99 (0.61, 1.61)	1.00 (0.61, 1.65)	1.15 (0.69, 1.91)	1.52 (0.88, 2.63)
Medicaid**:**	**Yes (Ref)**	0.18 (0.17, 0.20)	--	--	--	--
	No	0.26 (0.25, 0.28)	**1.93* (1.15, 3.25)**	**1.82* (1.09, 3.05)**	**1.90* (1.09, 3.32)**	**3.05* (1.69, 5.49)**
Smoking before pregnancy*****	**No (Ref)**	0.23 (0.22, 0.24)	--	--	--	--
	Yes	0.21 (0.19, 0.23)	0.64 (0.35, 1.20)	0.83 (0.45, 1.54)	0.86 (0.45, 1.66)	0.63 (0.30, 1.31)

* Statistically significant at alpha=0.05.

** Mercury levels were transformed to natural log in the analysis to adjust for right skewness, and the results were transformed back to mercury levels as exp[mean of ln(mercury)].

*** Data missing for 4 women.

**** Data missing for 1 woman.

***** Data missing for 5 women.

****** Mean mercury levels in hair and total fish consumption levels among women from Grand Rapids and Kalamazoo were close and they are close geographically.

Table 10. Mercury level (ppm range) in hair sample in relation to maternal characteristics after adjusting for all the other maternal characteristics.

Maternal Characteristics		Mean Mercury Level and 95% CI** (PPM)	Odds Ratio and 95% CI			
			2nd Quintile (0.12-0.19)	3rd Quintile (0.20-0.26)	4th Quintile (0.27-0.38)	5th Quintile (0.39-2.50)
			Adjusted	Adjusted	Adjusted	Adjusted
Smoking during	**No (Ref)**	0.23 (0.22, 0.24)	--	--	--	--
pregnancy* **	**Yes**	0.21 (0.19, 0.23)	1.45 (0.69, 3.05)	1.89 (0.91, 3.91)	1.89 (0.88, 4.05)	1.20 (0.47, 3.03)
Gestation Age	**<20Wks (Ref)**	0.25 (0.23, 0.29)	--		--	--
At Enrollment	**20-24 Wks**	0.23 (0.21, 0.24)	0.82 (0.43, 1.56)	1.52 (0.74, 3.11)	0.96 (0.49, 1.90)	0.64 (0.33, 1.25)
	>=24 Wks	0.21 (0.19, 0.23)	0.90 (0.45, 1.82)	1.29 (0.56, 2.94)	0.88 (0.42, 1.84)	0.62 (0.29, 1.34)
Community:	**5**	0.20 (0.18, 0.22)	--	--	--	--
	4	0.21 (0.19, 0.22)	0.81 (0.46, 1.44)	**0.47*** **(0.27, 0.82)**	0.86 (0.46, 1.59)	1.03 (0.51, 2.09)
	2	0.25 (0.22, 0.28)	**3.93*** **(1.76, 8.77)**	**2.38*** **(1.05, 5.36)**	**5.73*** **(2.39, 13.72)**	**7.34*** **(2.52, 21.37)**
	1&3	0.26 (0.23, 0.28)	**2.21*** **(1.10, 4.42)**	1.56 (0.77, 3.15)	**3.50*** **(1.64, 7.44)**	**6.00*** **(2.33, 15.45)**

* Statistically significant at alpha=0.05.

** Mercury levels were transformed to natural log in the analysis to adjust for right skewness, and the results were transformed back to mercury levels as exp[mean of ln(mercury)].

*** Data missing for 4 women.

**** Data missing for 1 woman.

***** Data missing for 5 women.

****** Mean mercury levels in hair and total fish consumption levels among women from Grand Rapids and Kalamazoo were close and they are close geographicall

Table 11. Mercury levels in hair in relation to fish consumption.

Maternal Characteristics		Odds Ratio amd 95%CI, using the first quintile (0.013-0.13) as referent			
		2nd Quintile (0.12-0.19)	3rd Quintile (0.20-0.26)	4th Quintile (0.27-0.38)	5th Quintile (0.39-2.50)
Age (years):	**<25 (Ref)**	--	--	--	--
	>=25	0.97 (0.60, 1.56)	1.43 (0.88, 2.32)	**1.83*** **(1.12, 3.02)**	**2.05*** **(1.15, 3.66)**
Ethnicity:	**Afri-American (Ref)**	--	--	--	--
	White & Other	1.32 (0.79, 2.21)	**2.14*** **(1.22, 3.74)**	**2.58*** **(1.38, 4.85)**	**2.72*** **(1.33, 5.54)**
Education:**	**<=12 yrs (Ref)**	--	--	--	--
	>12 yrs	1.00 (0.62, 1.62)	0.99 (0.60, 1.64)	1.21 (0.71, 2.05)	1.44 (0.81, 2.54)
Medicaid:**	**Yes (Ref)**	--	--	--	--
	No	**1.96*** **(1.16, 3.30)**	**1.87*** **(1.11, 3.14)**	**1.90*** **(1.08, 3.37)**	**3.21*** **(1.73, 5.98)**
Smoking before pregnancy**	**No (Ref)**	--	--	--	--
	Yes	0.65 (0.35, 1.21)	0.81 (0.44, 1.51)	0.83 (0.42, 1.65)	0.63 (0.29, 1.35)

* Statistically significant at alpha=0.05.

** Data missing for 1-7 women.

Table 11. Mercury levels in hair in relation to fish consumption.

Maternal Characteristics		Odds Ratio amd 95%CI, using the first quintile (0.013-0.13) as referent			
		2nd Quintile (0.12-0.19)	**3rd Quintile (0.20-0.26)**	**4th Quintile (0.27-0.38)**	**5th Quintile (0.39-2.50)**
Smoking during pregnancy**	**No (Ref)**	--	--	--	--
	Yes	1.44	1.78	1.72	1.07
		(0.69, 3.03)	(0.85, 3.70)	(0.78, 3.75)	(0.41, 2.80)
Gestation Age At Enrollment**	**<20Wks (Ref)**	--	--	--	--
	20-24 Wks	0.81	1.56	1.02	0.68
		(0.42, 1.59)	(0.74, 3.27)	(0.50, 2.10)	(0.33, 1.38)
	>=24 Wks	0.90	1.20	0.89	0.64
		(0.44, 1.84)	(0.52, 2.77)	(0.41, 1.91)	(0.29, 1.42)
Community:	**5**	--	--	--	--
	4	0.82	**0.51***	1.05	1.32
		(0.46, 1.46)	**(0.29, 0.91)**	(0.55, 2.00)	(0.62, 2.82)
	2	**3.97***	**2.53***	**6.56***	**8.69***
		(1.78, 8.86)	**(1.10, 5.79)**	**(2.65, 16.27)**	**(2.83, 26.67)**
	1&3	**2.36***	1.95	**4.59***	**10.65***
		(1.17, 4.79)	(0.92, 4.12)	**(2.05, 10.29)**	**(3.58, 31.70)**

* Statistically significant at alpha=0.05.

** Data missing for 1-7

Table 12. Mercury level (ppm range) in hair sample in relation to maternal characteristics after adjusting for total fish consumption and other maternal characteristics.

Maternal Characteristics		Odds Ratio amd 95%CI, using the first quintile (0.013-0.13) as reference			
		2nd Quintile (0.12-0.19)	3rd Quintile (0.20-0.26)	4th Quintile (0.27-0.38)	5th Quintile (0.39-2.50)
Age (years):	**<25 (Ref)**	--	--	--	--
	>=25	0.97	1.43	**1.83***	**2.05***
		(0.60, 1.56)	(0.88, 2.32)	**(1.12, 3.02)**	**(1.15, 3.66)**
Ethnicity: Afri-American (Ref)		--	--	--	--
White & Other		1.32	**2.14***	**2.58***	**2.72***
		(0.79, 2.21)	**(1.22, 3.74)**	**(1.38, 4.85)**	**(1.33, 5.54)**
Education:**	**<=12 yrs (Ref)**	--	--	--	--
	>12 yrs	1.00	0.99	1.21	1.44
		(0.62, 1.62)	(0.60, 1.64)	(0.71, 2.05)	(0.81, 2.54)
Medicaid:**	**Yes (Ref)**	--	--	--	--
	No	**1.96***	**1.87***	**1.90***	**3.21***
		(1.16, 3.30)	**(1.11, 3.14)**	**(1.08, 3.37)**	**(1.73, 5.98)**
Smoking before	**No (Ref)**	--	--	--	--
pregnancy**	**Yes**	0.65	0.81	0.83	0.63
		(0.35, 1.21)	(0.44, 1.51)	(0.42, 1.65)	(0.29, 1.35)
Smoking during	**No (Ref)**	--	--	--	--
pregnancy**	**Yes**	1.44	1.78	1.72	1.07
		(0.69, 3.03)	(0.85, 3.70)	(0.78, 3.75)	(0.41, 2.80)
Gestation Age	**<20Wks (Ref)**	--	--	--	--
At Enrollment**	**20-24 Wks**	0.81	1.56	1.02	0.68
		(0.42, 1.59)	(0.74, 3.27)	(0.50, 2.10)	(0.33, 1.38)
	>=24 Wks	0.90	1.20	0.89	0.64
		(0.44, 1.84)	(0.52, 2.77)	(0.41, 1.91)	(0.29, 1.42)
Community:	**5**	--	--	--	--
	4	0.82	**0.51***	1.05	1.32
		(0.46, 1.46)	**(0.29, 0.91)**	(0.55, 2.00)	(0.62, 2.82)
	2	**3.97***	**2.53***	**6.56***	**8.69***
		(1.78, 8.86)	**(1.10, 5.79)**	**(2.65, 16.27)**	**(2.83, 26.67)**
	1&3	**2.36***	1.95	**4.59***	**10.65***
		(1.17, 4.79)	(0.92, 4.12)	**(2.05, 10.29)**	**(3.58, 31.70)**

* Statistically significant at alpha=0.05.

** Data missing for 1-7 women.

Table 13. The association between preterm delivery and mercury levels (ppm range) in hair.

Preterm Delivery wks (N)	Odds Ratio and 95% CI									
	2nd Quintile** (0.12-0.19)		3rd Quintile** (0.20-0.26)		4th Quintile** (0.27-0.38)		5th Quintile** (0.39-2.50)		Top 10th Percentile*** (0.55-2.50)	
	Crude	Adjusted*	Crude	Adjusted*	Crude	Adjusted*	Crude	Adjusted*	Crude	Adjusted*
Term >=37 (923)	--	--	--	--	--	--	--	--	--	--
All <37 (101) Preterm	0.77 (0.41, 1.44)	0.94 (0.49, 1.83)	0.54 (0.27, 1.08)	0.62 (0.29, 1.31)	0.98 (0.54, 1.79)	1.39 (0.68, 2.84)	0.64 (0.33, 1.24)	1.32 (0.56, 3.11)	1.12 (0.58, 2.17)	1.45 (0.72, 2.91)
Term >=37 (923) (923)	--	--	--	--	--	--	--	--	--	--
Mod. 35-36 (57) Preterm	1.09 (0.47, 2.56)	1.48 (0.60, 3.69)	0.97 (0.40, 2.34)	1.21 (0.46, 3.16)	1.39 (0.61, 3.14)	**2.80 (1.03, 7.59)**	0.61 (0.23, 1.63)	1.67 (0.44, 6.29)	0.33 (0.08, 1.39)	0.44 (0.10, 1.89)
Term >=37 (923) (923)	--	--	--	--	--	--	--	--	--	--
Very <35 (44) Preterm	0.52 (0.21, 1.28)	0.60 (0.23, 1.52)	**0.20 (0.06, 0.73)**	**0.22 (0.06, 0.81)**	0.67 (0.29, 1.56)	0.68 (0.25, 1.85)	0.67 (0.29, 1.56)	1.09 (0.37, 3.20)	**2.35 (1.10, 5.05)**	**2.95 (1.30, 6.69)**

* Adjusted for maternal charcateristics including matenral age, ethnicity, Medicaid status, community and total fish consumption.
** Compared to lowest quintile.
*** Test of threshold top10% compared to lowest 90%.

Discussion:

The mean level of total fish consumption was 19.6 meals/6 months after removing two outliers. 11% of women did not eat any fish and 90% of them did not eat any sport caught fish. Older women, African-American women, women enrolled earlier in gestation and women enrolled from community five were found to eat more fish during the first half of pregnancy after controlling for other maternal characteristics. Increased hair mercury levels were found to be significantly associated with older maternal age, ethnicities other than African-American, not being insured by Medicaid and enrollment from community 1-3 after adjusting for maternal characteristics. Mercury levels in hair were significantly related to higher total fish consumption and consumption of canned fish and sport caught fish. After adjusting for total fish consumption and other maternal characteristics, the association between hair mercury levels and maternal characteristics including maternal age, ethnicity, Medicaid status, and community persisted. The top 10^{th} percentile of hair mercury level was found to be significantly associated with increased risk of very PD. The fourth quintile was found to be significantly associated with enhanced risk of moderately PD.

Later gestational age at enrollment was observed to be significantly associated with lower total fish consumption levels, most likely due to maternal characteristics of women who delay prenatal screening or would be difficult to enroll into the study in a timely manner. Women enrolled from community five were found to have the highest levels of fish consumption. Below is a map regarding the location of the five communities in this study (Figure 1). Community five is the closest to a Great Lake among the five communities and perhaps this influences availabilities and preferences for fish. Culture

and food preference may account for the higher fish consumption levels among older women and African-American women.

Because tuna comprises a large part of canned fish, the detected association between the consumption of canned fish and increased hair mercury levels is compatible with previous reports that methylmercury readily enters the aquatic food chain and bioaccumulates in predatory fish such as swordfish, pike and ocean tuna (66). Though the study population generally ate very low levels of sport caught fish, consumption of sport caught fish continued to be significantly related to increased hair mercury levels after adjusting for consumption of other types of fish. The result may be explained by local pollution in nearby waters, but confounding from other factors such as life style and outdoor activities cannot be excluded.

Figure 1. Location of the five communities in this study

Similar to the association between maternal age and fish consumption, older women were also found to have higher hair mercury levels, and the association can not be completely explained by elevated fish consumption. The higher hair mercury levels in older women can not be explained by bioaccumulation as well because the half-life of

methylmercury is approximately 70-80 days in the human body (137) and so hair mercury level can not reflect methylmercury exposure over years. However, aging may have influence on the absorption, metabolism and excretion of mercury, though no previous reports on these mechanisms have been found. The association between Medicaid insurance and lower mercury levels in hair cannot be explained by ethnicity, age and fish consumption. Some factors related to higher socioeconomic status might contribute to an elevated exposure to mercury.

Higher fish consumption was found among African-American women, however non-African-American women had significantly higher hair mercury levels after controlling for all the other maternal characteristics. Similarly, women enrolled from community 5 were found to consume the highest levels of fish, but their hair mercury levels were the lowest among the 5 communities. In addition, all the relationships between maternal characteristics and hair mercury levels remained significant after controlling for total fish consumption levels. It is generally accepted that fish is the major source of methylmercury for human. These results suggest that there may be other sources of methylmercury in addition to fish, or more likely the measurement of fish intake or subtypes of fish by maternal diet recall for the past 6 months leads to some misclassification of fish consumption levels. The personal interview used in our study did not take into concern portion size, cooking methods, other fish types and the intake of fish supplements, thus the analyses may not be able to completely control for fish consumption. Besides this, other methylmercury sources may influence the mercury exposure in this study sample. Localized industrial, especially those chlor-alkali industry using the mercury process, the pulp and paper industry and other industries using

mercury (118) and agricultural pollution including alkyl, alkoxyalkyl, aryl, and inorganic mercurial fungicides have been used for seed dressing, as turf fungicides and in orchards (115) could contribute to mercury exposure, but how the exposure to the pollution varied with age, ethnicity and Medicaid status is unknown. The exposure to other sources of mercury including pharmaceuticals and preservatives in cosmetics and toiletries (115) are more likely to differ with maternal characteristics and further studies need to clarify these factors in relation to personal characteristics and their effect on human health.

One previous review of human data on the developmental effects of methylmercury exposure concluded that maternal hair levels of 10 to 20 ppm are potentially harmful to fetal development (66). The range of hair mercury levels among this study population were 0.013 ppm to 2.5 ppm, therefore were considerably lower. However, the top 10^{th} percentile of mercury levels in hair (0.55-2.50 ppm) was found to be significantly associated with increased risk of very PD after controlling for potential confounders. The biological mechanism for higher mercury exposure to be related to early delivery is unknown. Few studies have assessed mercury levels in relation to risk of PD, and all of them produced negative results (82, 83, 86). Foldspang et al (82) evaluated average weekly intake of marine food, and blood methylmercury concentrations of mothers and offsprings immediately after delivery, and concluded that gestational age at delivery was not related to either number of meals of local food or to the blood mercury concentration. Fu et al (86) measured exposure to metallic mercury levels in an occupation based study and reported that the difference in incidence of PD between the group exposed to 0.06-0.1 mg/m3 of mercury in air and control group did not reach a significant level.

Grandjean et al (83) found that cord blood total mercury levels did not vary by gestational age at delivery.

Three underlying factors may explain the inconsistency of results between our study and previous studies: First, most of previous studies were based on small sample size (range 182-704) (81-84, 86) and they might not have had enough power to detect subtle but important differences. Second, none of these studies measured hair mercury levels. Metallic mercury level in air is not equivalent to methylmercury exposure. Blood levels may not be a good measurement of mercury intake over time (88-89). In our study, we measured mercury levels in the length of hair that reflects the exposure during the first half of pregnancy. Once incorporated into hair follicles, the methylmercury is chemically stable, and therefore, gives a longitudinal history of blood methylmercury levels (90). Previous studies did not control for fish consumption. Fish intake is associated with mercury levels in hair, and previous studies have reported that Ω-3 FA in fish can delay the initiation of parturition by down-regulating the formation of PGE2 and PGF2a, promoting the synthesis of PGI2 and PGI3 and thus leading to a more relaxed myometrium (49-51). Thus, in previous studies fish consumption may have had a protective effect for PD and thereby the mercury-gestational age association toward null.

The study suggested a "U" shaped curve in the association between mercury levels in hair and the risk of PD. The possible explanation is that at lower levels of fish consumption, the effect of nutrients in fish, such as Ω-3 FA predominates, but when certain higher levels of fish consumption are attained the effect of methylmercury can overcome the effect of Ω-3 FA. Although current study adjusted for total fish

consumption in the analysis, the effect of fish consumption may not have been entirely controlled for because of the crude measurement of levels of fish consumption.

There were some maternal characteristics that differed between all POUCH enrollees and these with hair sampled. Women with hair sampled were older women, less likely to smoke during pregnancy, had higher education, were not insured with Medicaid, and more likely to be whites and other ethnicities than African-American. Among the 197 women without hair sampled, only 35% declined and the other 65% were due to either short hair or elaborated hairstyles such as woven hair, which were more prevalent among African-American women. African-American women in POUCH tended to be younger, lower educate, and insured by Medicaid. So this might explain some of the differences in characteristics noted between those with and without hair samples. However, the disproportional distribution of these maternal characteristics should not significantly bias the study results because of the sheer larger number of women with hair sampled compared to those without (1024 vs. 197). Furthermore, levels of total fish consumption, an important factor suggested to be associated with both mercury levels in hair and the risk of PD, did not differ significantly between women with hair sampled and those without.

We used CVAAS to measure mercury levels in hair, however this measurement cannot distinguish methylmercury from the total mercury content. Though hair is a good indicator medium for methylmercury exposure (90), analysis of hair mercury levels may be confounded by absorption of mercury vapor onto the hair strands (91). Nonetheless, this situation mostly occurs if women had occupational exposure as in a dentist' office or a factory containing metallic mercury. Since this is a community-based study sample of

women, the contamination of vapor mercury in hair is probably minimized compared with that found in occupational studies. In addition, all hair samples were washed before analyses and thus chances to be contaminated by mercury vapor were minimized. Also in our analysis, fish consumption, an important source of methylmercury exposure for humans, was found to be significantly associated with hair mercury levels, suggesting that the measurement of hair methylmercury levels in our study was not significantly confounded by the contamination of vapor mercury.

Conclusion:

In brief, this study showed that total fish consumption, and consumption of canned fish and sport caught fish were significantly associated with increased hair mercury levels. Maternal characteristics including age, ethnicity, Medicaid status and community of enrollment were significantly associated with mercury levels in hair after taking account of fish consumption. Women in the top 10^{th} percentile of methylmercury levels in hair (0.55-2.50 ppm) were at to increased risk of Delivery very preterm. The consumption of fish and other seafood may provide with beneficial nutrients such as Ω-3 FA and Selenium, and adverse substances such as methylmerucury. Studies need to consider effects from both exposures when interpreting evidence regarding the role of fish consumption during pregnancy. Though fish consumption is an important source of methylmercury for human, results of this study indicate other potential methylmercury sources, which distribute differentially with certain maternal characteristics, especially those related to socioeconomic status and place of residence. Further studies need to clarify these sources and their importance in increasing methylmercury in the human body and influencing human health.

SUMMARY

In order to evaluate the influence of fish consumption on the risk of PD, the two papers included in this thesis separately investigated two elements, which have been suggested by previous studies to have opposite effects on gestational age at delivery. The first paper measured frequency of fish consumption during the first half of pregnancy to provide indirect evidence that high levels of Ω-3 FA, which comes mainly from fish and seafood, may prevent PD. Our study did not show the risk of PD, moderately PD or very PD to be significantly associated with levels of fish consumption at. However, there were hints that the risk of very PD may be reduced with increased total fish consumption, and the risk of PD, moderately PD and very PD seemed to be lower among women in the top 10^{th} percentile of total fish consumption, but the results were not statistically significant.

The second study examined mercury levels in hair reflecting the growth during the first half of pregnancy, and the risk of PD, moderately PD and very PD. Results showed that women in the top 10^{th} percentile of hair mercury levels were significantly more likely to delivery very preterm.

Adjusted analyses were performed in both studies. Higher fish consumption levels were reported in older women, African-American women, women enrolled from community 5 and women enrolled after 20 weeks' gestation. Total fish consumption and consumption of canned fish and sport caught fish were found to be significantly associated with increased mercury levels in hair. Higher mercury levels in hair were found in older women, non-African-American women, women not insured with Medicaid and women enrolled from community 1-3 even after adjusting for levels of fish consumption. Adjustment for total fish consumption and other maternal characteristics

did not influence the significance of the detected association between hair mercury levels and the risk of PD.

The range of hair mercury levels in our study was much lower than the range of 10 to 20 ppm that is considered potentially harmful to fetal development (66). However, our study detected a significantly increased risk of very PD in women in the top 10^{th} percentile of hair mercury levels. No previous study has reported significant association between mercury exposure and gestational age at delivery. Our study may have been well-suited detect this association because it had a larger sample size than other studies and controlled for of fish consumption to test the persistence of the effect and attempt to control for the potential protective effects of fatty acids from fish.

The studies confirmed fish consumption, especially marine predators such as tuna as one of important sources of methylmercury intake for human being. The significance of sport caught fish can be accounted for by local mercury pollution released into nearby waters and accumulated in fish. However, some other maternal characteristics including maternal age, ethnicity, Medicaid status and community of enrollment were also found to be associated with mercury levels even after controlling for fish consumption. Some of the remained effect of maternal characteristics can be explained by inaccuracy of the measurement of fish consumption (106), others can be other resources of mercury exposure such as local pollution, pharmaceuticals and preservatives in cosmetics and toiletries, and occupational exposure. Based on these results, reducing fish consumption during gestation alone may not be enough for pregnant women to decrease methylmercury exposure. Further efforts need to clarify other methylmercury sources and establish effective methods to manage these sources.

Though there were hints of reduced risk of PD among women with elevated total fish consumption levels, fish consumption was not shown to be significantly associated with the risk of PD. This result is incompatible with the reported protective effect of Ω-3 FA in fish for PD (112-114) and findings from previous epidemiological studies (54, 124). Toxic effects of other contents in fish, inaccuracy in measurement of fish consumption, and generally low fish consumption levels in this study population may explain such inconsistency. Although fish is the main dietary source of Ω-3 FA, it also contains hazardous elements such as methylmercury, PCB and DDT, the toxic effects of which can compromise the benefits from Ω-3 FA. The contents of these toxics in fish tissues and the association between fish consumption and the risk of PD can vary according to geographic location and extent of local pollution of waters and fish tissues. Compared with previous studies conducted in European countries that found association between maternal fish intake during pregnancy and gestational age, fish consumption levels in this study population are considerably lower. Thus the number of women delivered prematurely and ate high levels of fish during pregnancy is small and enough power may not have been reached. Measurement of dietary fish intake has always been a challenge for researchers because most of these measurements rely on the recall of participants and inaccuracy in the recall is unavoidable, especially when the consumption levels are high or a long time has elapsed between fish intake and the interview. Although our personal interview inquired the consumption of types of fish, the processing and cooking methods, which can also influence the nutrient content, were too complex to be standardized and documented. Furthermore, our study only assessed the fish consumption levels of the first half of pregnancy, however, this period of pregnancy may not be the important etiologic

time window for fish consumption to influence the gestation age at delivery. Fish consumption levels before or after this period of time may also be important in deciding the risk of preterm delivery.

Overall, the two studies in this thesis provided evidences about moderately high levels of fish consumption and methylmercury intake on the risk of PD. Through the investigations, we hope to demonstrate the relative benefits and hazards of fish consumption during the first half of gestation on one of the most important pregnancy outcomes, PD. The results are not comparable to studies on populations taking seafood as main food resource or data from epidemic methylmercury poisonings. Our studies also showed that selected maternal characteristics are not only related to fish consumption levels, but also associated with methylmercury levels even after controlling for fish consumption. These findings are important because effective interventions cannot be taken until these potential methylmercury resources are better identified. In order to provide further insight into the role of Ω-3 FA and methylmercury in fish in on gestation age, future studies should include information on Ω-3 FA levels in maternal and fetal blood, intake of fish oil supplement, occupational mercury exposure and local pollution.

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