Published in final edited form as:
Clin Biochem. 2006 November ; 39(11): 1063–1070.

Maternal Erythrocyte Omega-3 and Omega-6 Fatty Acids, and Plasma Lipid Concentrations, are Associated with Habitual Dietary Fish Consumption in Early Pregnancy

Michelle A. Williams[1,2], **Ihunnaya O. Frederick**[1], **Chunfang Qiu**[1], **Lois J. Meryman**[1], **Irena B. King**[3], **Scott W. Walsh**[4], and **Tanya K. Sorensen**[1,5]

1 *Center for Perinatal Studies, Swedish Medical Center, Seattle, WA*

2 *Department of Epidemiology, University of Washington School of Public Health and Community Medicine, Seattle, WA*

3 *Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA*

4 *Department of Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA*

5 *Obstetrix Medical Group, Seattle, Washington, USA*

Abstract

Objectives—We investigated the relationship between selected maternal erythrocyte omega-3 and omega-6 polyunsaturated fatty acids (PUFA) and plasma lipids in early pregnancy and reported habitual fish consumption during the periconceptional period.

Design and Methods—This cohort study included 923 pregnant women who reported periconceptional dietary habits and provided a blood sample before 20 weeks gestation. PUFA was determined by gas chromatography and plasma lipids by standard enzymatic methods. Differences in erythrocyte PUFA and plasma lipid concentrations were estimated using linear regression.

Results—Mean erythrocyte eicosapentaeonic acid and other PUFA content (%/total) were positively associated with frequency of self-reported fish consumption. Arachidonic acid was inversely related with frequent fish consumption (p trend <0.001). Women who consumed fish > twice/week had lower plasma triglyceride (−11.5 mg/dl) and higher HDL-cholesterol (+2.8 mg/dl) concentrations than women consuming fish < once/week.

Conclusions—These results support findings of inverse relation between fish consumption and preeclampsia risk.

Keywords

fish; fatty acids; diet; lipids; and pregnancy

Introduction

Habitual consumption of fish, rich in marine omega-3 polyunsaturated fatty acids (PUFA), particularly eicosapentaeonic acid (EPA) and docosahexaenoic acid (DHA) fatty acids, is

Corresponding Author: Dr. Michelle A. Williams, Center for Perinatal Studies (Suite 4 North), wedish Medical Center, 747 Broadway, Seattle, WA 98122, Office Phone: (206) 386-3107; Facsimile (206) 386-3173., E-mail: Mwilliam@u.washington.edu

associated with reductions in preeclampsia risk in some (1,2), but not all populations (3). It is generally agreed that preeclampsia, a relatively common medical complication of pregnancy is characterized by diverse metabolic alterations that include hypertension, systemic chronic inflammation (4), oxidative stress or antioxidant deficiency (5–7), diffuse endothelial activation (8), and dyslipidemia, particularly hypertriglyceridemia and reductions in plasma high density lipoprotein-cholesterol (HDL-cholesterol) (9). Whether maternal fish intake or omega-3 fatty acid supplements influence preeclampsia risk through one or multiple pathophysiological pathways is not well known. At present, relatively few clinical metabolic studies of the physiological response to fish intake, or omega-3 fatty acid supplement use in pregnancy have been published. Available evidence, though sparse, suggests that the possible inverse relation between maternal fish consumption or omega-3 fatty acid supplementation use may, in part, be attributable to the hypotriglyceridemic effect of omega-3 PUFA (10–11). This thesis is supported by a much larger body of observational and intervention studies that consistently document inverse associations of plasma triglycerides with habitual fish consumption or omega-3 PUFA supplementation (12–17) in men and non-pregnant women. This same literature documents modest increase in plasma HDL-cholesterol associated with fish or fish oil intake.

We sought to update and extend the available literature by exploring the possible influence of maternal habitual fish consumption, during the preconception period (the three months prior and first three months of the index pregnancy), on early pregnancy plasma lipid and lipoprotein concentrations. We also explored relations of maternal erythrocyte phospholipids content for EPA and DHA, two metabolically important fatty acids that are primarily derived from fish consumption, with maternal self-reported habitual fish consumption and early pregnancy plasma lipids, respectively. We employed multivariable modeling techniques to adjust for possible confounders.

Materials and Methods

The Omega study

The Omega study is an on-going prospective cohort study of maternal dietary risk factors of preeclampsia and other complications of pregnancy (4,6). The study population is comprised of women attending prenatal care clinics affiliated with Swedish Medical Center and Tacoma General Hospital in Seattle and Tacoma, Washington, respectively. We began recruiting for the study in December of 1996. Women eligible for inclusion into the study were those who initiated prenatal care prior to 16 weeks gestation. Women were ineligible if they were younger than 18 years of age, did not speak and read English, did not plan to carry the pregnancy to term, did not plan to deliver at either of the two research hospitals, or were past 16 weeks gestation. We primarily approached and enrolled nulliparous women. The procedures used in this study were approved by the Institutional Review Boards of Swedish Medical Center and Tacoma General Hospital, respectively. All participants provided written informed consent.

Analytical population

The analytical study population for this report is derived from participants who were enrolled in the Omega study between 1996 and 2000. During this period, 1,219 eligible women were approached and 1,000 (approximately 82%) agreed to participate. During this enrolment period, we primarily approached and enrolled nulliparous women. Multiparous women were approached and enrolled on a personnel available basis. Women found to have chronic hypertension (N=46), pre-gestational diabetes mellitus (N=5), and both conditions (N=1) were excluded. Also excluded were those women with missing dietary intake (i.e., fish consumption) information (N=25). A maximum sample of 923 women remained for analysis.

Interviews

Using structured questionnaires, interviewers collected information on maternal sociodemographic, behavioural, and medical characteristics. Covariate information included maternal age, height, pre-pregnancy weight, reproductive and medical histories, and medical histories of first-degree family members. We also collected information on maternal educational attainment, annual household income, occupation, prenatal vitamin supplementation use, as well as smoking and alcohol consumption before and during pregnancy. Maternal age at the time of interview was determined at the interview and was expressed in years. Parity was reported as the number of previous pregnancies lasting beyond 20 weeks gestation. All participants reported their maximum height and weight three months prior to the index pregnancy. Maternal race and educational attainment were based on self-reports made during the interview. Pre-pregnancy body mass index (BMI), used as a measure of overall maternal adiposity, was calculated as weight in kilograms divided by height in meters squared.

Dietary intake assessments

Detailed information about maternal habitual dietary intake during the periconceptional period and early pregnancy was ascertained at 12 weeks gestation, on average, using the self-administered, 121-item semi-quantitative FFQ used for the Women's Health Initiative Clinical Trial (18). Food composition values for vitamin C and other nutrients were obtained from the University of Minnesota Nutrition Coding Center nutrient database (Nutrition Coordinating Center, Minneapolis, MN) (19).

Blood sample collection, processing and storage

At or near the time of interview (13.2 weeks gestation, on average), a non-fasting blood sample was collected and protected from ultraviolet light. Blood was drawn into lavender-top vacutainer blood collection tubes containing K_3-EDTA (1 mg/ml). Tubes were centrifuged at 850g for 20 minutes at 4°C to separate plasma from erythrocytes; and erythrocytes were washed in a standard manner (20). Washed erythrocytes and plasma were divided into 0.5–1.0 ml aliquots that were stored frozen at −80°C until analysis.

Plasma lipid and lipoprotein determination

Maternal non-fasting blood samples, collected in 10 ml Vacutainer tubes at 13 weeks gestation, on average, were frozen at −80°C until analysis. Maternal plasma cholesterol and triglyceride concentrations were measured enzymatically on a Mira Plus Analyzer employing assays standardized by the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, GA (21). High density lipoprotein (HDL) was separated from low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol by a chemical precipitation technique using dextran sulfate of 50kDa. Analytical inter-assay coefficients of variation for cholesterol, triglyceride, and HDL-cholesterol were 1.5%, 2.5% and 3%, respectively. All assays were performed without knowledge of case-control status.

Erythrocyte omega-3 and omega-6 fatty acid determination

Erythrocyte phospholipids fatty acid content is considered a medium-term objective indicator of habitual dietary fatty acid intake (22). Because of the relatively high costs associated with erythrocyte phospholipids fatty acids analyses, we randomly selected approximately 60% of the study cohort and submitted their samples for assessments. Erythrocyte omega-3 and omega-6 fatty acids were determined using gas chromatography as previously described (2). Briefly, fatty acids were extracted from erythrocytes. Fatty acid methyl esters (FAME) were prepared by direct transesterification. Samples were then dissolved in hexane and individual fatty acids in erythrocytes were separated on a gas chromatograph (model 5890B, series II,

Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector (FID), automatic sampler (Hewlett-Packard 7673) and Chemstation software (Hewlett-Packard). FAMEs were separated on a 30m by 0.25mm ID wall-coated open-tubular fused silica column (DB23) with 0.25mm coating (J&W Scientific, Folsom, CA). The carrier gas, helium, was set at 60 pounds per square inch (PSI) at the tank; the make-up gas, nitrogen, was set at 60 PSI. At the detector end, hydrogen was set at 30 PSI and breathing air at 20 PSI. Column linear velocity was set at 33cm/sec (oven temperature of 200°C). The injector and detector port temperatures (T) were set at 250°C and 275°C, respectively. The initial oven temperature was set at 165°C with an initial hold of 10 minutes, and then the temperature was increased to 188°C at 10°/minute and held for 8 minutes. Quantitative precision and identification were evaluated using the model for mixtures of known FAME and an established specimen control pool. Erythrocyte fatty acids were expressed as the relative percent of total fatty acids in erythrocyte membranes (%/total). Inter-assay coefficients of variation were 2.8, 4.7, and 1.5, for eicosapentaeonic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA), respectively. All laboratory analyses were completed without knowledge of dietary fish consumption and other characteristics.

Statistical analyses

We examined frequency distributions of maternal sociodemographic, reproductive, and medical characteristics. We then examined the distributions of plasma triglyceride, HDL-C and total cholesterol and found them approximately normally distributed. We therefore compared differences in mean lipid concentrations across categorical levels of maternal characteristics (e.g., frequency of fish consumption or quartiles of erythrocyte fatty acids) using independent-sample t-tests, assuming unequal variances. Differences in mean lipid concentrations across measures of habitual fish consumption or erythrocyte fatty acid compositions (i.e., EPA, DHA, Σ omega-3 fatty acids, AA, Σ omega-6 fatty acids) were also examined.

Multivariable linear regression analyses with robust variances were performed to evaluate the association between various fish intake or erythrocyte fatty acid content (i.e., the dependent variables) and plasma lipid concentrations (i.e., the independent variables). To assess confounding, we entered covariates (i.e., additional independent variables) into a linear regression model sequentially. We then compared the unadjusted and adjusted regression coefficients for plasma lipid concentrations (23). Final models included covariates that altered unadjusted coefficients for plasma lipid concentrations by 10% or more, as well as covariates of *a priori* interest (i.e. maternal age and parity). The following covariates were considered as possible confounders: maternal age (<20, 20–34, 35–39, ≥40 years), race/ethnicity (White, African, Asian, and other), marital status (married vs. other), educational attainment (≤12 vs. >12 years), parity (nulliparous vs. multiparous), smoking during pregnancy (yes vs. no), gestational age (weeks) at blood collection (continuous), pre-pregnancy body mass index (BMI) and BMI at the time of blood collection (each BMI variable specified as follows; <20, 20 to <25, 25 to <30, ≥30 kg/m^2). All analyses were performed using Stata 7.0 statistical software (Stata, College Station, Texas, USA). All continuous variables are presented as mean ± SE. All reported probability values are two-tailed.

Results

Selected sociodemographic and lifestyle characteristics of the study cohort are summarized in Table 1 (far left column). The proportions of women who reported consuming fish less than once per week, once per week, twice per week, and more than twice per week were 20.2%, 38.0%, 23.1% and 18.7%, respectively. We examined maternal erythrocyte omega-3 and omega-6 fatty acid composition in relation to maternal fish consumption categories (Table 2). As the frequency of maternal self-reported fish consumption increased, mean erythrocyte

omega-3 fatty acid content increased (all trend p-values were <0.001). Strong linear increases in mean erythrocyte EPA and DHA were evident across for each successively higher category of the frequency of fish consumption. Erythrocyte EPA values were 55.9% higher among women who consumed fish more than twice per week as compared with those who consumed fish less than once per week. DHA values were 30.0% higher among women who consumed fish more than twice per week as compared with women who consumed fish less than once per week. A similar pattern was noted when the sum of long-chain omega-3 fatty acids (Σ LC omega-3 PUFA) were assessed. Statistically significant inverse associations were observed between the frequencies of maternal reported fish consumption and the sum of long chain omega-6 fatty acids (Σ LC omega-6 PUFA) (p for trend were all <0.001). Mean erythrocyte AA values were 4.7% lower among women who consumed fish more than twice per week, as compared with those who were infrequent fish consumers (i.e., those women who reported eating fish less than once per week). We evaluated the relationship between maternal reported fish consumption and plasma triglyceride, HDL-cholesterol and total cholesterol concentrations in early pregnancy. The results of these analyses are summarized in Table 3. Maternal plasma triglyceride and total cholesterol concentrations were highest among women who consumed fish less than once per week, as compared with more frequent fish eaters. Trends in mean plasma concentrations, however, did not reach statistical significance (p-values for trend were 0.11, and 0.60, respectively). Plasma HDL-cholesterol concentrations were statistically significantly and positively associated with increasing frequency of maternal fish consumption (p for trend=0.04). Women who reported consuming fish more than twice per week had mean HDL-cholesterol concentrations that were 3.9% higher than values observed among those who consumed fish less than once per week (69.7 versus 67.1 mg/dl, p=0.04).

After adjusting for confounders, we found that women who reported consuming fish more than twice per week tended to have lower plasma triglyceride (−8.1 mg/dl) and higher HDL-cholesterol (+2.8 mg/dl) concentrations than women consuming fish less than once per week, although only the trend in HDL-cholesterol reached statistical significance (p for trend=0.03). There was no clear evidence of a linear trend in plasma total cholesterol concentrations with increasing frequency of fish consumption in multivariate analyses (p for trend=0.86).

We next evaluated maternal plasma lipid and lipoprotein concentrations in relation to erythrocyte fatty acid composition in the sub-sample of 565 cohort members (Table 4) for whom we measured erythrocyte phospholipids fatty acids. We used linear regression procedures to adjust for potential confounding factors. Consistent with the findings for fish consumption, mean plasma triglyceride concentrations were inversely related with tertiles of EPA, and Σ LC omega-3 PUFA, respectively, although the trends were not statistically significant. Mean plasma HDL-cholesterol concentrations were increased with increasing EPA, DHA and Σ LC omega-3 PUFA, respectively. The association between plasma HDL-C and erythrocyte EPA, however did not reach statistical significance (p for trend=0.43). We noted that erythrocyte EPA content was inversely associated with plasma total cholesterol concentrations (β=−8.3 mg/dl), though the association did not reach statistical significance (p=0.35). However, mean plasma total cholesterol concentrations were statistically significantly associated with erythrocyte DHA (β=3.7 mg/dl, p=0.04).

Mean plasma triglyceride (β=−7.9 mg/dl, p<0.01), HDL-cholesterol (β=−1.6 mg/dl, p=0.03), and total cholesterol (β=−5.3 mg/dl, p<0.01) concentrations were statistically significantly and inversely associated with erythrocyte AA content. This general trend in plasma lipid and lipoprotein concentrations was similar when Σ LC omega-6 PUFAs was evaluated.

Discussion

In this cross-sectional study, we noted that EPA and DHA (two omega-3 fatty acids) content in maternal erythrocyte phospholipids were significantly and positively associated with maternal self-reported frequency of fish consumption. Conversely, AA (an omega-6 fatty acid) was strongly inversely related with frequent fish consumption. We observed statistically significant inverse associations between habitual fish consumption and plasma triglyceride concentrations. We found similar, though generally weaker, associations between plasma triglyceride in relation to omega-3 fatty acids measured in erythrocytes. Erythrocyte omega-6 fatty acids, particularly AA, were also inversely associated with plasma triglyceride concentrations in this population.

Several investigators have evaluated maternal self-reports of fish intake during pregnancy with objective measures of dietary intake including erythrocyte membrane or plasma phospholipids omega-3 fatty acid content (10,24–27). Overall, findings from these previous studies are in agreement with our findings. Parra et al, in their cross-sectional study of 146 pregnant women in Mexico City, reported that erythrocyte cell membrane phospholipids were positively related with dietary intake of omega-3 fatty acids (26). In their study of 162 Spanish women interviewed at term and asked to report total dietary intake for three typical days during pregnancy, Matorras et al, noted that omega-3 long chain fatty acid intake was statistically significantly correlated with plasma omega-3 levels (expressed as percentages r=0.22) in plasma phospholipids. Importantly, the authors did not observe statistical significant correlations between reported omega-3 fatty acid intake and erythrocyte phospholipids values (%/total membrane fatty acids) (24). They attributed the absence of an association to increased tissue apposition and mobilization, and placental transfer that is known to be considerable during the third trimester of pregnancy. Our findings, based on blood samples collected early in pregnancy, cannot be explained by these late pregnancy-related alterations in fatty acid metabolism.

On balance, results from our observational study and those of others (10,24–27), are consistent with intervention studies (28–30) that have documented increased omega-3 fatty acids in maternal plasma and/or erythrocyte after omega-3 fatty acid supplementation during pregnancy. We could find no published reports of maternal habitual fish consumption and plasma lipid and lipoprotein concentrations in early pregnancy. We were, however, able to find one published study that evaluated omega-3 fatty acid status and lipid profile in pregnant women (10). In their cross-sectional of Spanish women, the authors noted that omega-3 fatty acids in third-trimester plasma phospholipids were positively correlated with plasma HDL-C and inversely correlated with plasma triglyceride concentrations (10). Although we assessed maternal PUFA and lipids in early pregnancy, our findings concerning associations between maternal erythrocyte plasma phospholipids omega-3 fatty acids content and pregnancy lipid profile are consistent with those of Matorras et al (10). However, in contrast to our observations of associations between maternal dietary fish intake and plasma lipid concentrations, the authors found no association between maternal fish intake and maternal lipid concentrations among Spanish women. Differences in study populations, analytical approaches (the authors did not adjust for confounders), and the timing of fatty acid and plasma lipid assessments (early versus late pregnancy) may have contributed to the divergent findings. Of note, our findings are consistent with previously published observational studies that have evaluated the impact of habitual fish consumption on plasma lipid concentrations in men and non-pregnant women (12–17).

Our present study has several important strengths including our high participation rate and relatively large sample size. The high follow-up rate (>95%) minimized possible selection bias, and the large sample size allowed for dose-response analyses. Additionally, because specimens

were analyzed without knowledge of pregnancy outcome, the likelihood of systemic error in determining maternal plasma lipid concentrations and fatty acid content of erythrocyte phospholipids was reduced. However, several limitations merit discussion and consideration. First, the cross-sectional design of our study limited our ability to infer the temporal relationship between fish consumption and plasma lipid concentrations in early pregnancy. Longitudinal studies of pregnant women are needed to demonstrate more conclusively these potential causal relationships. Second, a single measurement of plasma lipids may be susceptible to short-term variations, and thus is not likely to provide a time-integrated measure of maternal status during early pregnancy. Longitudinal studies with serial measurements of maternal plasma lipid concentrations are needed. Third, measurement error from the use of self-reported fish consumption is likely to have occurred. However, this error is unlikely to have systematically biased our findings, because the reporting error is not associated with the laboratory determination of maternal plasma lipid concentrations. Misclassification of maternal fish intake (unrelated to our laboratory measures of maternal plasma lipid concentrations) would have served to underestimate the true association between the two covariates. Fourth, use of non-fasting lipids concentration may have influenced our results. Because subjects were recruited and enrolled while they attended obstetric clinics to receive standard prenatal care, and because prolonged fasting is contraindicated during pregnancy, we were restricted to measuring lipids in non-fasting blood samples. In anticipation of concerns related to measuring lipids and lipoproteins in non-fasting blood, we queried participants about elapsed time since eating at the time of blood draw. Pair-wise correlations between elapsed time since eating and each of the plasma lipids in this population were small, ranging from −0.01 to 0.04 ($0.29<p\text{-values}<0.75$). We also controlled for elapsed time since eating and blood draw in multivariable regression analyses. Furthermore, available data indicate high correlations between fasting and postprandial plasma lipids, ranging from 0.90 to 0.99 ($p<0.001$) (31); and suggest that non-fasting triglyceride concentrations may be useful in clinical practice (32;33).

Physiological mechanisms by which omega-3 fatty acids may lower plasma triglyceride have been suggested. Fatty acids from fish oils might exert their effects on triglyceride via their capacity to reduce hepatic triglyceride synthesis and by decreasing the release of triglyceride-rich lipoproteins into peripheral circulation (34,35). Notably, EPA and DHA may exerts their hypotriglyceridemic effects via interactions with peroxisome proliferators activated receptor (PPAR) and sterol regulating element binding protein (SREBP) (36,37), two transcription factors known to be involved in fatty acid oxidation and inhibition of triglyceride synthesis.

In conclusion, results from our study suggest that dietary fish consumption (assessed either using a semi-quantitative FFQ or using erythrocyte measures of omega-3 PUFAs) is inversely related with plasma triglyceride and positively related to HDL-cholesterol (though to a lesser degree) in early pregnancy. Taken together with previously published literature, these results may help to explain, in part, findings of an inverse relation between fish consumption and preeclampsia risk in some populations. If confirmed by other studies, our finding supports efforts aimed at exploring additional dietary approaches to lower hypertriglyceridemia in early pregnancy. Interpretation of these and other data concerning maternal fish consumption during pregnancy must be balanced with concerns regarding possible adverse health consequences of consuming large amounts of fish and other seafood possibly contaminated with mercury (38), pesticides and herbicides (39).

Acknowledgements

The authors are indebted to the participants of the Omega Study for their cooperation. They are also grateful for the technical expertise contributed by the staff of the Center for Perinatal Studies, Swedish Medical Center.

Grant Support: This research was supported in part by an award from the National Institutes of Health (HD 32562).

References

1. Olsen SF, Secher NJ. A possible preventive effect of low-dose fish oil on early delivery and pre-eclampsia: indications from a 50-year-old controlled trial. Br J Nutr 1990;64:599–609. [PubMed: 2265175]
2. Williams MA, Zingheim RW, King IB, Zebelman AM. Marine polyunsaturated fatty acids in maternal erythrocytes and risk of preeclampsia. Epidemiology 1995;6:232–237. [PubMed: 7619928]
3. Kesmodel U, Olsen SF, Salvig JD. Marine n-3 fatty acid and calcium intake in relation to pregnancy induced hypertension, intrauterine growth retardation, and preterm delivery. A case-control study. Acta Obstet Gynecol Scand 1997;76:38–44. [PubMed: 9033242]
4. Qiu C, Luthy DA, Zhang C, Walsh SW, Leisenring WM, Williams MA. A prospective study of maternal serum C-reactive protein concentrations and risk of preeclampsia. Am J Hypertens 2004;17:154–160. [PubMed: 14751658]
5. Walsh SW. Eicosanoids in preeclampsia. Prostaglandins Leukot Essent Fatty Acids 2004;70:223–232. [PubMed: 14683695]
6. Zhang C, Williams MA, King IB, et al. Vitamin C and the risk of preeclampsia--results from dietary questionnaire and plasma assay. Epidemiology 2002;13:409–16. [PubMed: 12094095]
7. Zhang C, Williams MA, Sanchez SE, et al. Plasma concentrations of carotenoids, retinol, and tocopherols in preeclamptic and normotensive pregnant women. Am J Epidemiol 2001;153:572–580. [PubMed: 11257065]
8. Vadachkoria S, Sanchez SE, Qiu C, Muy-Rivera M, Malinow MR, Williams MA. Hyperhomocyst(e) inemia and elevated soluble vascular cell adhesion molecule-1 concentrations are associated with an increased risk of preeclampsia. Gynecol Obstet Invest 2004;58:133–139. [PubMed: 15205565]
9. Enquobahrie DA, Williams MA, Butler C, Frederick IO, Miller RS, Luthy DA. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. Am J Hypertens 2004;17:574–581. [PubMed: 15233976]
10. Matorras R, Perteagudo L, Sanjurjo P, Sasieta M, Ruiz JI. Long chain W3 polyunsaturated fatty acids and lipid patterns in the mother and the newborn infant. J Perinat Med 1998;26:313–319. [PubMed: 9846307]
11. Glueck CJ, Streicher P, Wang P, Sprecher D, Falko JM. Treatment of severe familial hypertriglyceridemia during pregnancy with very-low-fat diet and n-3 fatty acids. Nutrition 1996;12:202–205. [PubMed: 8798226]
12. Mori TA, Beilin LJ. Long-chain omega -3 fatty acids, blood lipids and cardiovascular risk reduction. Curr Opin Lipidol 2001;12:1211–1217.
13. Angerer P, von Schacky C. N-3 polyunsaturated fatty acids and the cardiovascular system. Curr Opin Lipidol 2000;11:57–63. [PubMed: 10750695]
14. Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71:228S–231S. [PubMed: 10617976]
15. Djousse L, Hunt SC, Arnett DK, Province MA, Eckfeldt JH, Ellison RC. Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study. Am J Clin Nutr 2003;78:1098–1102. [PubMed: 14668270]
16. Harris WS. N-2 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65:1645S–1654S. [PubMed: 9129504]
17. Harris WS. Extending cardiovascular benefits of omega-3 fatty acids. Curr Atheroscler Rep 2005;7:375–380. [PubMed: 16105481]
18. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Ann Epidemiol 1999;9:178–187. [PubMed: 10192650]
19. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc 1988;88:1268–1271. [PubMed: 3171020]
20. Rose HG, Oklander M. Improved procedure for the extraction of lipids from human erythrocytes. J Lipid Res 1965;6:428–431. [PubMed: 14336214]

21. Caudill SP, Cooper GR, Smith SJ, Myers GL. Assessment of current National Cholesterol Education Program guidelines for total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol measurements. Clin Chem 1998;44:1650–1658. [PubMed: 9702951]
22. Willett, W. Nutritional Epidemiology. 2. New York: Oxford University Press; 1998.
23. Rothman, KJ.; Greenland, S. Modern Epidemiology. 2. Philadelphia, Pennsylvania: Lippincott-Raven Publishers; 1998. p. 311-316.
24. Matorras R, Perteagudo L, Sanjurjo P, Ruiz JI. Intake of long chain w3 polyunsaturated fatty acids during pregnancy and the influence of levels in the mother on newborn levels. Eur J Obstet Gynecol Reprod Biol 1999;83:179–184. [PubMed: 10391529]
25. De Vriese SR, Matthys C, De Henauw S, De Backer G, Dhont M, Christophe AB. Maternal and umbilical fatty acid status in relation to maternal diet. Prostaglandins Leukot Essent Fatty Acids 2002;67:389–396. [PubMed: 12468259]
26. Parra MS, Schnaas L, Meydani M, Perroni E, Martinez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. Public Health Nutr 2002;5:931–937. [PubMed: 12633518]
27. Olsen SF, Hansen HS, Sandstrom B, Jensen B. Erythrocyte levels compared with reported dietary intake of marine n-3 fatty acids in pregnant women. Br J Nutr 1995;73:387–395. [PubMed: 7766562]
28. Van Houwelingen AC, Sorensen JD, Hornstra G, et al. Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy. Br J Nutr 1995;74:723–731. [PubMed: 8541278]
29. Conner WE, Lowensohn R, Hatcher L. Increased docosahexaenoic acids levels in human newborn infants by administration of sardines and fish oil during pregnancy. Lipids 1996;31:S183–187. [PubMed: 8729116]
30. Sanjurjo P, Ruiz-Sanz JI, Jimeno P, et al. Supplementation with docosahexaenoic acid in the last trimester of pregnancy: maternal-fetal biochemical findings. J Perinat Med 2004;32:132–136. [PubMed: 15085888]
31. Schaefer EJ, Audelin MC, McNamara JR, Shah PK, Tayler T, Daly JA. Comparison of fasting and postprandial plasma lipoproteins in subjects with and without coronary heart disease. Am J Cardiol 2001;88:1129–1133. [PubMed: 11703957]
32. Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979;60:473–485. [PubMed: 222498]
33. Castro Cabezas M, Erkelens DW. Triglycerides and atherosclerosis: to feast or fast. Neth J Med 2000;56:110–118. [PubMed: 10759022]
34. Roche HM, Gibney MJ. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am J Clin Nutr 2000;71:232S–237S. [PubMed: 10617977]
35. Zampelas A, Murphy M, Morgan LM, Williams CM. Postprandial lipoprotein lipase, insulin and gastric inhibitory polypeptide responses to test meals of different fatty acid composition: comparison of saturated, n-6 and n-3 polyunsaturated fatty acids. Eur J Clin Nutr 1994;48:849–858. [PubMed: 7889893]
36. Clarke SD, Thuillier P, Baillie RA, Sha X. Peroxisome proliferator-activated receptors: a family of lipid-activated transcription factors. Am J Clin Nutr 1999;70:566–571. [PubMed: 10500027]
37. Clarke SD, Jump DB. Polyunsaturated fatty acid regulation of hepatic gene transcription. J Nutr 1996;126:1105S–1109S. [PubMed: 8642441]
38. Virtanen JK, Voutilainen S, Rissanen TH, et al. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. Arterioscler Thromb Vasc Biol 2005;25:228–233. [PubMed: 15539625]
39. Hites RA, Foran JA, Carpenter DO, Hamilton MC, Knuth BA, Schwager SJ. Global assessment of organic contaminants in farmed salmon. Science 2004;303:226–229. [PubMed: 14716013]

Table 1

Selected Socio-demographic and Lifestyle Characteristics of the Entire Cohort and According to Participants' Self-report of the Frequency of Fish Consumption During Early Pregnancy, Seattle and Tacoma, Washington, 1996–2000.

	Entire Cohort (N=923)		Fish Consumption							
			Less Than Once per Week (N=186)		Once per Week (N=351)		Twice per Week (N=213)		More Than Twice per Week (N=173)	
	n	%	n	%	n	%	n	%	n	%
Maternal Age (years)										
<35	670	72.6	150	80.7	252	71.8	156	73.2	112	64.7
≥35	243	26.3	35	18.8	95	27.1	54	25.4	59	34.1
Missing	10	1.1	1	0.5	4	1.1	3	1.4	2	1.2
Pre-Pregnancy BMI kg/m^2)										
<20.0	179	19.4	36	19.4	74	21.1	36	16.9	33	19.1
20.0–24.9	507	54.9	93	50.0	194	55.3	125	58.7	95	54.9
25.0–29.9	145	15.7	32	17.2	49	14.0	34	16.0	30	17.3
≥30.0	78	8.5	21	11.3	30	8.6	14	6.6	13	7.5
Missing	14	1.5	4	2.2	4	1.1	4	1.9	2	1.2
<12 Years Education	38	4.1	11	5.9	16	4.6	7	3.3	4	2.3
Single Marital Status	97	10.5	22	11.9	34	9.7	29	13.6	12	6.9
Maternal Race/Ethnicity										
Non-Hispanic White	781	84.6	151	81.2	301	85.8	183	85.9	146	84.4
African-American	21	2.3	6	3.2	7	2.0	6	2.8	2	1.2
Other	109	11.8	26	14.0	40	11.4	21	9.9	22	12.7
Missing	12	1.3	3	1.6	3	0.8	3	1.4	3	1.7
Nulliparous	777	84.2	153	82.3	300	85.5	179	84.0	145	83.8
Smoked During Pregnancy	53	5.7	15	8.1	23	6.6	10	4.7	5	2.9
Family History of Hypertension	442	47.9	92	49.5	169	48.2	94	44.1	87	50.3
BMI @ Blood Collection (kg/m^2)										
<20.0	71	7.7	19	10.2	24	6.8	14	6.6	14	8.1
20.0–24.9	495	53.6	88	47.3	201	57.3	110	51.6	96	55.5
25.0–29.9	219	23.7	40	21.5	77	21.9	60	28.2	42	24.3
≥30.0	108	11.7	28	15.1	40	16.4	22	10.3	18	10.4
Missing	30	3.3	11	5.9	9	2.6	7	3.3	3	1.7
Gestational Age @ Blood Collection (weeks)	13.1±0.1		13.6±0.5		13.2±0.2		13.2±0.2		2.6±0.2	
Inactive During Pregnancy	142	15.4	37	20.0	59	16.8	24	11.3	22	12.7
Total Calories (kcal/day)	1638.3±19.8		1442.4 ±44.5		1514.4 ±28.2		1679.7 ±34.2		2048.8 ±48.8	
Percent Daily Calories From Fat (%)	32.2±0.2		32.0±0.6		31.9±0.3		32.6±0.5		32.7±0.5	

Note: Test for homogeneity (Chi-square test) among the four fish consumption categories were not statistically significant across all categorical characteristics; independent Student *t* test was employed to test the difference of mean between two groups using less than once per week as the reference groups, only total calories intake shows statistical significant difference.

Table 2

Maternal Erythrocyte Omega-3 and Omega-6 Fatty Acids According to Reported Frequency of Fish Consumption During Early Pregnancy, Seattle and Tacoma, Washington, 1996–2000.

	Fish Consumption				
	Less Than Once per Week (N=113)	Once per Week (N=208)	Twice per Week (N=132)	More Than Twice per Week (N=112)	
Fatty Acids (%/total)	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**P for Trend**
Eicosapentaenoic Acid (20:5n3, EPA)	0.34 ± 0.01	0.41 ± 0.01	0.44 ± 0.014	0.5344 ± 0.024	<0.001
Docosahexaenoic Acid (22:6n3, DHA)	4.27 ± 0.11	4.75 ± 0.06	5.21 ± 0.08	5.55 ± 0.09	<0.001
Σ Long-Chain Omega-3 Fatty Acids	6.82 ± 0.12	7.39 ± 0.07	7.89 ± 0.10	8.37 ± 0.12	<0.001
Eicosatetraenoic Acid (Arachidonic, 20:4n6, AA)	13.95 ± 0.09	13.75 ± 0.06	13.51 ± 0.08	13.32 ± 0.10	<0.001
Σ Long-Chain Omega-6 Fatty Acids	19.69 ± 0.12	19.31 ± 0.08	18.92 ± 0.11	18.38 ± 0.13	<0.001
Ratio of Σ Long-Chain Omega-6: Σ Long-Chain Omega-3 Fatty Acids	3.00 ± 0.06	2.68 ± 0.03	2.46 ± 0.04	2.26 ± 0.04	<0.001

Analysis restricted to 565 individuals that had available erythrocyte fatty acids values and dietary intake information.

Table 3

Distributions of Maternal Plasma Triglyceride, High Density Lipoprotein (HDL)-Cholesterol, and Total Cholesterol Concentrations According to Maternal Fish Consumption During early Pregnancy, Seattle and Tacoma, Washington, 1996–2000.

Plasma Lipids	Fish Consumption				
	Less Than Once per Week (N=183)	Once per Week (N=348)	Twice per Week (N=210)	More Than Twice per Week (N=171)	
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**P for Trend**
Triglyceride (mg/dl)	132.9 ± 4.8	**122.3 ± 3.1**[a]	**120.3 ± 3.6**[b]	123.0 ± 4.0	0.11
HDL-Cholesterol (mg/dl)	67.1 ± 1.0	68.0 ± 0.8	**69.9 ± 1.0**[b]	69.7 ± 1.2	0.04
Total Cholesterol (mg/dl)	194.8 ± 2.7	191.1 ± 2.0	191.7 ± 2.5	192.2 ± 2.9	0.60
	*Adjusted β ± SE	*Adjusted β ± SE	*Adjusted β ± SE	*Adjusted β ± SE	
Triglyceride (mg/dl)	Referent	−10.8 ± 5.7	−13.2 ± 6.0 (p=0.03)	−11.5 ± 6.3	0.06
HDL-Cholesterol (mg/dl)	Referent	1.0 ± 1.3	3.0 ± 1.4 (p=0.03)	3.0 ± 1.7	0.03
Total Cholesterol (mg/dl)	Referent	−3.9 ± 3.4	−3.5 ± 3.8	−3.7 ± 4.2	0.44
	**Adjusted β ± SE	**Adjusted β ± SE	**Adjusted β ± SE	**Adjusted β ± SE	P for Trend
Triglyceride (mg/dl)	Referent	−7.9 ± 5.8	**−12.2 ± 6.1 (p=0.05)**	−8.1 ± 6.5	0.14
HDL-Cholesterol (mg/dl)	Referent	0.7 ± 1.3	**3.1 ± 1.4 (p=0.03)**	2.8 ± 1.7	0.03
Total Cholesterol (mg/dl)	Referent	−2.2 ± 3.4	−2.5 ± 3.9	−0.7 ± 4.2	0.86

Analysis restricted to 912 individuals that had available plasma lipid profile and dietary intake information.

a, b Pairwise test: less than once per week as the referent group; a, b indicated that the t test between two groups were statistically significant.

* Model adjusted for total calories intake, p indicate if the coefficient is statistically significant using robust variance.

** Model adjusted for total calories intake, maternal age, gestational age at blood collection and body mass index at blood collection, p indicate if the coefficient is statistically significant using robust variance.

Table 4

Distributions of Maternal Plasma Triglyceride, High Density Lipoprotein (HDL)-Cholesterol, and Total Cholesterol Concentrations According to Maternal Erythrocyte Omega-3 and Omega-6 Fatty Acid Values in Early Pregnancy, Seattle and Tacoma, Washington, 1996–2000.

Maternal Erythrocyte Fatty Acids (%/Total)	Maternal Plasma Lipid Concentrations (mg/dl)		
	Triglyceride	**HDL-Cholesterol**	**Total Cholesterol**
Unadjusted	**β ± SE**	**β ± SE**	**β ± SE**
Eicosapentaenoic Acid (20:5n3, EPA)	−16.7 ± 10.6	2.1 ± 2.3	−11.8 ± 7.9
Docosahexaenoic Acid (22:6n3, DHA)	3.0 ± 2.2	2.6 ± 0.5 (p<0.001)	4.2 ± 1.4 (0.003)
Σ Long-Chain Omega-3 Fatty Acid	0.02 ± 1.8	1.8 ± 0.5 (p<0.001)	2.0 ± 1.2
ArachidonicAcid (20:4n6, AA)	−8.0 ± 2.6 (p=0.002)	−2.3 ± 0.6 (p<0.001)	−5.8 ± 1.4 (<0.001)
Σ of Long-Chain Omega-6 Fatty Acid	−3.0 ± 1.7	−1.5 ± 0.5 (p=0.002)	−2.5 ± 1.2 (p=0.03)
Ratio of Σ Long-Chain Omega-6: Σ Long-Chain Omega-3 Fatty Acids	−2.7 ± 4.4	−4.5 ± 1.0 (p<0.001)	−6.9 ± 2.6 (p=0.009)
Adjusted*	**Adj β ± SE**	**Adj β ± SE**	**Adj β ± SE**
Eicosapentaenoic Acid (20:5n3, EPA)	−11.1 ± 11.8	2.7 ± 3.4	−8.3 ± 8.9
Docosahexaenoic Acid (22:6n3, DHA)	2.7 ± 2.5	2.2 ± 0.6 (p<0.001)	3.7 ± 1.8 (0.04)
Σ Long-Chain Omega-3 Fatty Acid	−0.2 ± 2.1	1.5 ± 0.5 (p=0.002)	1.7 ± 1.4
ArachidonicAcid (20:4n6, AA)	−7.9 ± 2.9 (p=0.006)	−1.6 ± 0.7 (p=0.03)	−5.3 ± 1.9 (p=0.005)
Σ of Long-Chain Omega-6 Fatty Acid	−4.2 ± 1.9 (p=0.03)	−1.0 ± 0.5 (p=0.04)	−2.9 ± 1.3 (p=0.03)
Ratio of Σ Long-Chain Omega-6: Σ Long-Chain Omega-3 Fatty Acids	−3.8 ± 5.2	−4.1 ± 1.2 (p<0.001)	−7.7 ± 3.3 (p=0.02)

Analysis restricted to 565 individuals that had available erythrocyte fatty acids values and plasma lipid profile.

* Model adjusted for maternal age, gestational age at blood collection and body mass index at blood collection using robust variance.

P-values are reported if the estimated coefficient was statistically significant using robust variance.