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TITLE Page

The effect of omega-3 supplementation on pregnancy outcomes by smoking status

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Condensation: Omega-3 supplementation may have a protective effect against spontaneous preterm delivery and low birth weight in pregnant smokers but not in nonsmokers.

Short title: Omega-3 supplementation and pregnancy outcomes in smokers and nonsmokers

ABSTRACT:

BACKGROUND: Smoking during pregnancy is associated with adverse maternal and neonatal outcomes such as preterm delivery, intrauterine growth restriction, stillbirth, and low birth weight. Since smoking causes oxidative stress, some have suggested using antioxidants to counteract the effects of oxidative stress. Smokers have lower serum levels of omega-3 fatty acids, an important antioxidant, and thus, investigating whether omega-3 supplementation in smokers reduces adverse maternal and neonatal outcomes represents an important area of research.

OBJECTIVE: To investigate whether the antioxidant effect of omega-3 fatty acid supplementation on the incidence of adverse pregnancy outcomes differs between smokers and nonsmokers.

STUDY DESIGN: Secondary analysis of a multicenter randomized controlled trial of omega-3 supplementation for preterm delivery prevention in women with a singleton pregnancy and a history of a prior singleton spontaneous preterm delivery. Subjects were randomized to begin omega-3 or placebo prior to 22 weeks which was continued until delivery. All women received 17 alpha-hydroxyprogesterone caproate intramuscularly weekly beginning between 16 to 20 weeks of gestation and continued until 36 weeks of gestation or delivery, whichever occurred first. The primary outcome was spontaneous preterm delivery. Secondary outcomes were indicated preterm delivery, any preterm delivery (spontaneous and indicated), pregnancy-associated hypertension (gestational hypertension and preeclampsia), a neonatal composite (retinopathy of prematurity, intraventricular hemorrhage grade III or IV, patent ductus

arteriosus, necrotizing enterocolitis, sepsis, respiratory morbidity, or perinatal death), low birth weight (<2500 grams), small for gestational age (less than the 10th percentile), and neonatal intensive care unit or intermediate nursery admission. The study population was stratified into smokers and nonsmokers, and the incidence of each outcome was compared by omega-3 supplementation versus placebo in each subgroup. Zelen tests were performed to test for homogeneity of effect in smokers and nonsmokers.

RESULTS: Of 851 subjects included in the analysis, 136 (16%) smoked. Baseline characteristics between omega-3 and placebo groups did not differ in smokers or nonsmokers. Omega-3 supplementation was associated with a lower risk of spontaneous preterm delivery in smokers (RR 0.56, 95% CI 0.36-0.87) but not in nonsmokers (RR 1.04, 95% CI 0.84-1.29); p-value for interaction = 0.013. Low birth weight was also less frequent in smokers receiving omega-3 supplementation (RR 0.57, 95% CI 0.36-0.90) compared to nonsmokers (RR 0.93, 95% CI 0.71-1.24); p-value for interaction = 0.047. The effect on other secondary outcomes did not differ significantly between smokers and nonsmokers.

CONCLUSION: Omega-3 supplementation in smokers may have a protective effect against recurrent spontaneous preterm delivery and low birth weight.

KEYWORDS: Preterm birth, preterm delivery, low birth weight, omega-3 supplementation, smoking, spontaneous preterm delivery, tobacco abuse

INTRODUCTION:

Smoking during pregnancy is associated with adverse maternal and neonatal outcomes. Simpson[1] reported in 1957 that women who smoked were at an increased risk of spontaneous preterm delivery. Since then, numerous studies have confirmed this relationship.[2-4] Further studies have led to the widely accepted principle that smoking during pregnancy causes an increased risk of spontaneous preterm labor, placental insufficiency, intrauterine growth restriction, stillbirth, low birth weight, and a higher rate of perinatal mortality.[2-7] Despite this, 8% of women in the United States continue to smoke during pregnancy.[8] Thus, significant efforts and resources have focused on strategies to mitigate adverse pregnancy outcomes in pregnant smokers. Studies of antioxidants to reduce preterm birth, regardless of smoking status, have yielded mixed results.[9-15] Some have suggested potential benefits for smokers, and hence, this represents an area requiring further investigation.

Omega-3 fatty acids are polyunsaturated fatty acids that have an important role in anti-oxidation. Omega-3 fatty acid supplementation has been reported to have cardioprotective effects, is safe, and has a narrow side effect panel with the most common being nausea (4% when the daily dose is < 2 grams).[16, 17] Smoking leads to peroxidation of omega-3 fatty acids and further oxidative stress,[18] and women who smoke have lower serum levels of omega-3 fatty acids compared to nonsmokers because smoking has an inhibitory effect on the metabolism, bioavailability, and absorption of omega-3 fatty acids.[9, 19, 20] Therefore, we sought to determine if omega-3 supplementation in smokers confers a protective effect against adverse pregnancy outcomes. We hypothesize omega-3 supplementation has a protective effect against adverse

pregnancy outcomes, including spontaneous preterm delivery, in smokers but not in nonsmokers.

MATERIALS AND METHODS:

We performed a secondary analysis of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units Network randomized controlled trial, "Omega-3 Fatty Acid Supplementation to Prevent Recurrent Preterm Birth."[14] The multicenter study was performed between January 2005 and October 2006 across 13 Maternal-Fetal Medicine Units Network sites. Patients between $16^{0/7}$ and $21^{6/7}$ weeks of gestation with a singleton gestation who had a prior history of at least one singleton spontaneous preterm delivery between $20^{0/7}$ to $36^{6/7}$ weeks of gestation were eligible for enrollment. Exclusion criteria included a major fetal anomaly, fish oil consumption (≥ 500 mg/day) in the month prior to enrollment, anticoagulation use, chronic hypertension, pregestational diabetes White's classification ≥ class D, substance abuse, epilepsy, uncontrolled thyroid disorder, clotting disorder, current or planned cerclage, or a medical indication for delivery to occur prior to 37 weeks of gestation. Gestational age was assigned by either last menstrual period or earliest ultrasound as described by Dombrowski et al.[21] All patients had an ultrasound prior to randomization to rule out a major fetal anomaly. Patients in both arms received weekly intramuscular 17 alpha-hydroxyprogesterone caproate for the prevention of recurrent spontaneous preterm delivery, and this was continued until 36 weeks of gestation or delivery, whichever occurred first.[22] Patients were randomized to either omega-3 supplementation or identical appearing placebo capsules. The omega-3 supplementation

consisted of 1200 mg eicosapentaenoic acid (EPA, 20:5n-3) and 800 mg docosahexaenoic acid (DHA, 22:6n-3) for a total of 2000 mg of omega-3 long-chain polyunsaturated fatty acids daily. Patients received study medication until $36^{6/7}$ weeks of gestation or delivery, whichever came earlier. Institutional review board approval was obtained at each clinical center and the data coordinating center. The current analysis is performed on the publicly released dataset of the trial under IRB-waiver by our institution (N160429005).

For this secondary analysis, patients who participated in the trial and had their smoking status recorded were included. Patients were divided into two subgroups, smokers and nonsmokers, and the effect of omega-3 supplementation versus placebo on the outcomes of interest was compared. Smoking status was classified as "any use" during pregnancy and was recorded at the conclusion of the pregnancy by patient interview and chart review. Exposure amount (e.g. number of cigarettes per day), duration of exposure, and whether the patient stopped smoking during pregnancy were not recorded in the original trial.

The main outcomes of interest were maternal and neonatal outcomes that may be influenced by smoking and omega-3 supplementation during pregnancy. Preterm delivery was defined as delivery occurring prior to $37^{0/7}$ weeks of gestation. The maternal outcomes included spontaneous preterm delivery (primary outcome), indicated preterm delivery, any preterm delivery (indicated and spontaneous), and pregnancy-associated hypertension (gestational hypertension or preeclampsia). Spontaneous preterm delivery was defined as patients who delivered spontaneously after entering preterm labor or prematurely rupturing membranes and those who underwent induction of labor between $34^{0/7}$ and $36^{6/7}$ for preterm premature

rupture of membranes without contractions. Indicated preterm delivery included maternal and fetal indications for delivery. Examples of maternal indications included severe preeclampsia, placental abruption, placenta previa, and poorly controlled diabetes mellitus. Examples of fetal indications included abnormal antenatal testing, oligohydramnios, and intrauterine growth restriction. Pregnancy-associated hypertension was diagnosed in women who had two blood pressure measurements ≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic on two separate occasions at least four hours apart and were diagnosed with gestational hypertension or preeclampsia by the managing physician. Neonatal outcomes of interest included a neonatal composite, low birth weight (< 2500 grams), small for gestational age (less than the 10th percentile),[23] and neonatal intensive care unit or intermediate nursery admission. Only live-born infants were included in the analysis of neonatal outcomes with the exception of the composite outcome, which included perinatal death. The neonatal composite included retinopathy of prematurity, intraventricular hemorrhage grade III or IV, patent ductus arteriosus, necrotizing enterocolitis, culture proven sepsis, respiratory morbidity, and perinatal death. Respiratory morbidity included the diagnosis of respiratory distress syndrome, bronchopulmonary dysplasia, transient tachypnea of the newborn, or the requirement of surfactant administration at any point during the neonatal admission. The diagnostic requirements for each neonatal condition comprising the neonatal composite outcome were explained in the original study.[14]

Baseline characteristics between patients receiving omega-3 supplementation or placebo were compared in the smokers and nonsmokers groups using the Student t-test, Wilcoxon rank-sum test or *Chi-squared* test as appropriate. Univariate analyses were then used

to compare the outcomes of interest and exposure to omega-3 supplementation in the smokers and nonsmoker groups. Incidence and relative risks (RR) with 95% confidence intervals (CI) were then computed to determine the treatment effect of omega-3 supplementation in the smoker and nonsmoker groups. Zelen tests for homogeneity of odds ratios were used to compare the smoker and nonsmoker groups to determine if a treatment effect of omega-3 supplementation differed between smokers and nonsmokers. A p-value less than 0.05 was considered statistically significant.

RESULTS:

All 851 patients randomized in the original trial had their smoking status recorded and were included in the analysis; 136 (16.0%) were smokers. Of the women receiving omega-3 supplementation, 64 were smokers and 370 were nonsmokers. In the placebo group there were 72 smokers and 345 nonsmokers (Figure 1).

Baseline characteristics of patients assigned to omega-3 supplementation versus placebo were similar in smokers and nonsmokers (Table 1). Thus, multivariate analyses were not performed. Study medication and 17 alpha-hydroxyprogesterone caproate compliance rates were similar between women receiving omega-3 supplementation and placebo in the smoker and nonsmoker groups.

In smokers, omega-3 supplementation compared to placebo was associated with a 44% reduction in spontaneous preterm delivery (29.7% compared to 52.8%, RR 0.56, 95% CI 0.36-0.87); whereas, there was no difference in nonsmokers (33.5% compared to 32.2%, RR 1.04, 95% 0.84-1.29). The smoker and nonsmoker groups differed due to heterogeneity as identified

by the Zelen test (p=0.0.013). Thus, omega-3 supplementation in smokers had a protective effect against spontaneous preterm delivery but not in nonsmokers. Omega-3 supplementation did not have a differential treatment effect in smokers and nonsmokers for the other maternal outcomes (indicated preterm birth, any preterm delivery, and pregnancy-associated hypertension) (Table 2).

In regards to neonatal outcomes, smokers receiving omega-3 supplementation had a 43% reduction in delivering a low birth weight infant compared to placebo (28.3% compared to 50.0%, RR 0.57, 95% CI 0.36-0.90) whereas there was no difference in nonsmokers (21.0% compared to 22.5%, RR 0.93, 95% CI 0.71-1.24). The smoker and nonsmoker groups differed due to heterogeneity as identified by the Zelen test (p=0.047). The remaining neonatal outcomes did not statistically differ between the smoker and nonsmoker groups (Table 2).

COMMENT:

In our cohort of women at high risk of a recurrent spontaneous preterm delivery, omega-3 supplementation was associated with a reduction in recurrent spontaneous preterm delivery in smokers but not in nonsmokers. Similarly, omega-3 supplementation reduced the risk of low birth weight in smokers but not in nonsmokers, which was most likely related to the reduced risk of spontaneous preterm delivery. Smokers and nonsmokers taking omega-3 supplementation were at similar risks of experiencing an indicated preterm delivery, any preterm delivery, pregnancy-associated hypertension, small for gestational age, neonatal intensive care unit admission or intermediate care nursery, and the composite adverse neonatal outcome.

A proposed mechanism of action of omega-3 fatty acids reducing the risk of spontaneous preterm birth and therefore low birth weight is by altering the balance of prostaglandins PGE_2 and $PGF_{2\alpha}$ and prostacyclin (PGI_2).[11, 12, 24] Omega-3 fatty acids decrease the production of prostaglandins PGE_2 and $PGF_{2\alpha}$, which are important for initiating labor, while they increase the production of prostacyclin (PGI_2), which is important for uterine quiescence.[25] Since smokers have lower serum levels of omega-3 fatty acids, [19] the physiology of supplementation leading to uterine quiescence makes biological plausibility.

Our study is a secondary analysis of the largest randomized controlled trial performed in the United States investigating the effects of omega-3 supplementation on the prevention of recurrent preterm delivery. The authors of the original trial concluded omega-3 supplementation did not reduce the risk of recurrent preterm delivery (RR 0.91, 95% CI 0.77-1.07).[14] To the contrary, a similar study performed in Denmark found omega-3 supplementation reduced the risk of recurrent preterm delivery by 46% (RR 0.54, 95% CI 0.30-0.98), [10] which was similar to our findings in smokers (RR 0.56, 95% CI 0.36-0.87). Interestingly, the smoking rates between the United States and Denmark studies were vastly different. The smoking rate in the United States study was nearly 1/3 of that in the Denmark study (16 versus 43%). The Denmark study was published three years before the Meis trial[22] established 17 alpha-hydroxyprogesterone caproate as the standard of care for the prevention of recurrent preterm delivery. Thus, it would have been unethical to withhold 17 alpha-hydroxyprogesterone caproate from participants in the United States study, and therefore, all patients received weekly 17 alpha-hydroxyprogesterone caproate. The authors of the United States study suggested 17 alpha-hydroxyprogesterone caproate may have blunted the effect of omega-3

supplementation on recurrent preterm delivery. Our findings, instead, suggest omega-3 supplementation has different effects in smokers and nonsmokers.

Seven meta-analyses have reported conflicting results on the effects of omega-3 supplementation on pregnancy outcomes; [13, 24, 26-30] however, none of the included randomized-controlled studies or the meta-analyses evaluated the differences in outcomes between smokers and nonsmokers receiving omega-3 supplementation. We propose the differences are explained by the antioxidant effects of omega-3 supplementation combating the oxidative stress caused by cigarette toxins. This is further supported by the similar findings of Abramovici et al.[31] who showed supplementation with vitamins C and E, which are antioxidants, reduced the risk of preterm birth in smokers but not nonsmokers. Since smokers have lower serum levels of omega-3 fatty acids and vitamins C and E, [19, 32] this may explain why supplementation has a preferential effect in smokers compared to nonsmokers.

Our study is not without limitations. First, due to the way data were collected on smoking status, we were unable to account for whether patients quit smoking during pregnancy or the amount of exposure (e.g. number of cigarettes per day). We would expect if a larger proportion of women quit during pregnancy, our results would have been biased towards the null; however, it is possible the dosage and duration of exposure may influence our results in ways that we are unable to define given the limitations. Further, the number of smokers in each group was overall too small to delineate the subgroups for some of the outcomes. Secondly, most interactions are likely due to chance, and we performed multiple comparisons which increase the likelihood of chance findings. For example, while not statistically significant,

the relative risk of having pregnancy-associated hypertension and an indicated preterm delivery appears more than doubled in smokers receiving omega-3 supplementation compared to those receiving placebo. These findings are likely due to chance and not attributable to omega-3 supplementation. Also, our results may not be generalizable to the wider population since our study population was a high risk group of women who had previously experienced a spontaneous preterm delivery.

Our study has a number of strengths. First, it was a multicenter trial that included a racially diverse group of women from throughout the United States. Secondly, study medication compliance rate in the original study was high and did not differ between the treatment and placebo groups (omega-3: 85.1% compared to placebo: 84.8%, p=0.33).[14] Finally, the data was collected by trained research staff, and thus, we are confident in the accuracy of the collected data.

In sum, our findings suggest omega-3 supplementation may be protective against recurrent spontaneous preterm delivery and low birth weight in smokers but not in nonsmokers. Further studies should replicate and validate our findings. If these studies confirm our findings, we suggest evaluating the relationship between cotinine (an objective measure of quantity of smoking exposure) and serum omega-3 fatty acid concentrations in women who smoke to better explain the mechanisms of the protective effects of omega-3 supplementation.

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369 Table 1: Baseline characteristics of smokers and nonsmokers who received omega-3

370 supplementation or placebo

Characteristics	Smokers (n = 136)			Nonsmokers (n=715)		
	Omega-3 (n = 64)	Placebo (n = 72)	P value	Omega 3 (n = 370)	Placebo (n = 345)	P value
Maternal age (years)*	26.0 ± 5.3	25.4 ± 3.9	0.48	28.1 ± 5.4	28.0 ± 5.3	0.88
Gestational age at randomization (weeks)	19.9 (18-21)	19.3 (18.4-20.8)	0.39	19.4 (17.9-20.9)	19.6 (18-21)	0.48
Race			0.76			0.63
Black	36 (56.3)	34 (47.2)		108 (29.2)	109 (31.6)	
White	21 (32.8)	30 (41.7)		190 (51.4)	178 (51.6)	
Hispanic	6 (9.4)	7 (9.7)		58 (15.7)	50 (14.5)	
Other	1 (1.6)	1 (1.4)		14 (3.8)	8 (2.3)	
Marital status			0.50			0.22
Married	32 (50.0)	29 (40.3)		277 (74.9)	248 (71.9)	
Divorced, separated, or widowed	6 (9.4)	7 (9.7)		10 (2.7)	18 (5.2)	
Never Married	26 (40.6)	36 (50.0)		83 (22.4)	79 (22.9)	
Education level (years)	12 (11-13)	12 (11-12)	0.61	13 (12-16)	13 (12-16)	0.64
17 alpha-OHP compliance	93 (85-100)	100 (88-100)	0.23	100 (93-100)	100 (93-100)	0.90
Study drug compliance	89.5 (79-98.5)	86 (78-94.5)	0.11	93 (80-99)	93 (81-99)	0.89
Consumption of fish at baseline (servings/week)	0.5 (0-1.5)	0.5 (0-1.5)	0.93	1 (0-1.5)	0.5 (0-1.5)	0.17
Consumed at least one serving of fish/week during pregnancy	41 (64.1)	47 (65.3)	0.88	269 (72.7)	241 (69.9)	0.40
Number of previous PTDs	1 (1-2)	1 (1-2)	0.72	1 (1-2)	1 (1-2)	0.30
History of more than one PTD	22 (34.4%)	25 (34.7%)	0.97	109 (29.5%)	91 (26.1%)	0.31
History of more than one full-term births	17 (26.6%)	14 (19.4%)	0.32	41 (11.1%)	35 (10.1%)	0.68

OHP, hydroxyprogesterone; PTD, preterm delivery
Data are mean ± standard deviation, median (interquartile range), and n(%)
*Eighteen maternal ages removed due to exact ages not known.

371 Table 2: Treatment effects of omega-3 supplementation and placebo in smokers and nonsmokers on pregnancy and neonatal

372 outcomes

Outcome	Smoker Omega-3 (n=64)	Smoker Placebo (n=72)	Nonsmoker Omega-3 (n=370)	Nonsmoker Placebo (n=345)	Smoker Treatment Effect RR(95% CI)	Nonsmoker Treatment Effect RR(95% CI)	P-Value for Interaction
Spontaneous PTD	19(29.7)	38(52.8)	124(33.5)	111(32.2)	**0.56(0.36-0.87)**	1.04(0.84-1.29)	**0.013**
Indicated PTD	6(9.4)	3(4.2)	15(4.1)	22(6.4)	2.25(0.59-8.63)	0.64(0.34-1.21)	0.125
PTD (indicated and spontaneous)	25(39.1)	41(56.9)	139(37.6)	133(38.6)	**0.69(0.48-0.99)**	0.98(0.81-1.18)	0.091
Pregnancy-associated HTN	5(7.8)	2(2.8)	15(4.1)	18(5.2)	2.81(0.57-14.0)	0.78(0.40-1.52)	0.204
Neonatal composite	14(21.9)	17(23.6)	78(21.1)	56(16.2)	0.93(0.50-1.73)	1.30(0.95-1.77	0.375
LBW (< 2500 grams) †	17(28.3)	36(50.0)	77(21.0)	76(22.5)	**0.57(0.36-0.90)**	0.93(0.71-1.24)	**0.047**
SGA†	8(13.3)	13(18.1)	27(7.4)	28(8.3)	0.74(0.33-1.66)	0.89(0.53-1.48)	0.783
NICU or intermediate nursery admission‡	13(22)	22(32.4)	97(26.5)	77(23)	0.68(0.38-1.23)	1.15(0.89-1.50)	0.130

PTD, preterm delivery; LBW, low birth weight; SGA, small for gestational age; NICU, neonatal intensive care unit
Neonatal composite: retinopathy of prematurity, grade III or IV intraventricular hemorrhage, patent ductus arteriosus, necrotizing enterocolitis, culture proven sepsis, respiratory morbidity, and perinatal death
Data are n(%); bold signifies statistical significance
* Zelen test for homogeneity of odds ratios comparing smokers to nonsmokers
†Only live born neonates included, ‡Only live born neonates included but excludes 9 infants that died in the delivery room

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Figure 1 legend page:

Title of figure 1: Flow diagram of included subjects

Description of figure 1: Flow diagram of smokers and nonsmokers who received omega-3 supplementation or placebo beginning between 16 to 22 weeks of gestation and continuing until 36 weeks of gestation.

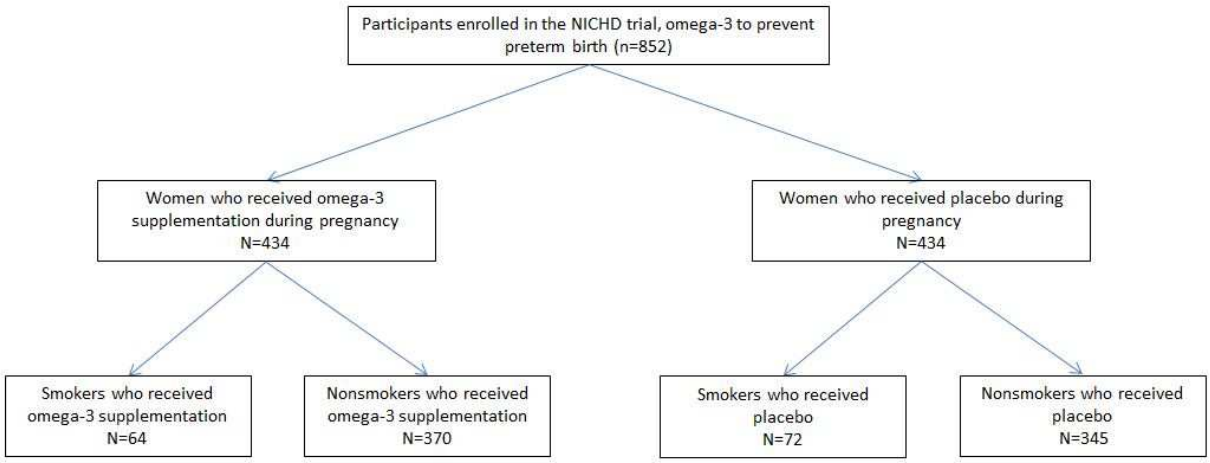

Figure 1: Flow diagram of included subjects