

Clinical Communications

Fish oil supplementation during pregnancy is protective against asthma/wheeze in offspring

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Clinical Implications

- Early-life asthma represents a significant public health burden. Prenatal supplementation with fish oil—derived fatty acids (ie, $n - 3$ long chain polyunsaturated fatty acids) and vitamin D3 could modulate the risk of asthma/recurrent wheeze in offspring.

TO THE EDITOR:

The diagnosis of asthma in childhood has doubled in recent decades and is now estimated to affect as many as 1 in 5 young children across the United States and Europe.^{1,2} Attempts at early intervention and prevention, such as encouraging breastfeeding, administering probiotics, and reducing exposure to allergens, have not lowered the incidence.² Prenatal supplementation with vitamin D₃³ and fish oil—derived fatty acids (ie, $n - 3$ long chain polyunsaturated fatty acids [LCPUFAs])⁴ such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have recently been shown to reduce asthma/recurrent wheeze in children.⁵ An inverse association has also been reported between low maternal EPA, DHA, and total $n - 3$ fatty acid levels during pregnancy with increased risk of nonatopic persistent wheeze in their offspring by the age of 6 years.⁵ However, a complete understanding of how these nutritional interventions and their potential interactions may affect asthma development remains limited. The aims of this current study were 2-fold: (1) to assess the relationship between fish oil supplementation during pregnancy (derived using a food frequency questionnaire [FFQ]) with asthma/recurrent wheeze in offspring by age 3; and (2) to evaluate a possible interaction effect between prenatal fish oil supplementation and prenatal vitamin D₃ levels on the development of asthma/recurrent wheeze. We used children from the vitamin D Antenatal Asthma Reduction Trial (VDAART) that enrolled 881 pregnant women at 10 to 18 weeks of gestation, randomized them to 400 IU versus 4400 IU of vitamin D₃, then followed these women and their offspring for the development of asthma/recurrent wheeze by age 3, as described previously.^{3,6} Of these 881 pregnant women, a total of 804 (91.26%) mother-child pairs completed the FFQ at 10 to 18 weeks of gestation (first trimester); of these, 776 (88.08%) mother-child pairs also completed the FFQ at 32 to 38 weeks of gestation (third trimester).

Univariate and multivariable logistic regression models were used to evaluate the effect of fish oil on asthma/wheeze. Specifically, we used fish oil supplementation measures from the FFQ that were taken in the form of capsules (yes/no) during the first

and third trimester of pregnancy. This measure of maternal fish oil supplementation and an interaction term for fish oil supplementation and baseline 25-hydroxyvitamin D (25OHD) (deficient concentration <30 ng/mL vs sufficient 25OHD concentration ≥ 30 ng/mL)^{3,7} were used as predictors in a model to assess their association with the incidence of physician-diagnosed asthma/recurrent wheeze at age 3 years in the offspring, as defined in the primary VDAART paper³ and in this article's Online Repository at www.jaci-inpractice.org. Although we have previously shown that vitamin D supplementation in pregnancy reduced the risk of asthma and recurrent wheeze in 3-year-old children,⁶ we observed that the effect was dependent on the maternal level of 25OHD at entry into the trial.⁵ Thus, this analysis only used 25OHD levels at baseline as the index of exposure to prenatal vitamin D. Fish oil dosage was defined by the strength (in mg) of each capsule mother takes per week. We considered fish oil intake in both the first and the third trimester of pregnancy. Survival analysis using a Weibull regression model⁸ was also used to assess the fish oil and asthma/recurrent wheeze relationship in the first 3 years of life. Ultra-performance liquid chromatography—tandem mass spectroscopy-based metabolomic profiling conducted at the Broad Institute (Massachusetts Institute of Technology, Cambridge, Mass) was performed on plasma samples from a subset of women⁹ at the first trimester ($n = 107$, including 7 mothers [6.5%] who reported taking fish oil supplements) and the third trimester ($n = 103$, including 5 mothers [4.9%] who reported taking fish oil supplements). Two $n - 3$ LCPUFA metabolites, EPA and DHA, were evaluated using univariate regression models for an association with asthma/recurrent wheeze and with fish oil intake. Given the prior literature, we hypothesized that fish oil and vitamin D reduce the risk of asthma/wheeze in offspring and therefore used 1-sided P values and confidence intervals to test these associations. Analyses were conducted using statistical software R version 3.2.3 and the CRAN survival package.

Of the 804 mothers with FFQ data from the first trimester, 50 mothers (6.2%) took fish oil supplements and 175 mothers (21.8%) had sufficient levels of 25OHD (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). The univariate regression models found that both fish oil supplementation (odds ratio [OR] = 0.41; 95% confidence interval [CI]: $-\infty$, 0.81; 1-sided $P = .016$) and sufficient 25OHD levels (OR = 0.61; 95% CI: $-\infty$, 0.87; 1-sided $P = .010$) in the first trimester were significantly associated with a reduced risk of asthma/recurrent wheeze among offspring (Table I). The Weibull analysis gave similar results: the risk of asthma/recurrent wheeze was significantly lower among children of mothers who took prenatal fish oil (14% vs 28%, $N = 804$, Figure 1, hazard ratio = 0.45; 95% CI: $-\infty$, 0.84; 1-sided $P = .018$). There was no statistically significant interaction of fish oil supplementation with 25OHD levels (OR = 0.94; 95% CI: 0.12, 5.25; $P = .946$) (Table I).

Analysis of fish oil into dosage categories suggested that the protective effect of fish oil was driven by the highest doses, with the greatest decrease in risk seen in those women who took more than 500 mg/week ($n = 28$) relative to those women who took none (OR = 0.19; 95% CI: $-\infty$, 0.66; 1-sided $P = .014$) (Table I). A test for trend also supported the fish oil intake

TABLE I. Association of fish oil and baseline 25OHD in mothers at first and third trimester with asthma/recurrent wheeze in children by age 3 years

Models for outcome asthma/wheeze	N	OR	One-sided (95% CI)	One-sided <i>P</i> value	Test for trend
<i>First trimester</i>	804				
Univariate models					
Fish oil	47	0.41	−∞, 0.81	.016	
Maternal 25OHD	163	0.61	−∞, 0.87	.010	
DHA	107	0.43	−∞, 0.96	.042	
EPA	107	0.80	−∞, 1.52	.288	
Univariate dose response					
Fish oil dose (test for trend)	747	0.64	−∞, 0.88		0.010
No fish oil	701	1.00			
Fish oil dose <250 mg	11	0.88	−∞, 2.69	.424	
Fish oil dose ≥250 and <500 mg	9	0.67	−∞, 2.52	.308	
Fish oil dose ≥500 mg	26	0.19	−∞, 0.66	.014	
Multivariate interaction model					
Fish oil	47	0.45	−∞, 1.03	.056	
Maternal 25OHD	163	0.64	−∞, 0.91	.020	
Fish oil × 25OHD*	19	0.94	0.12, 5.25	.946	
<i>Third trimester</i>	776				
Univariate models					
Duration of fish oil supplementation (test for trend)	722	0.65	−∞, 0.97		0.039
No fish oil intake	661	1.00			
Either time point	39	0.72	−∞, 1.36	.196	
Intake in both first and third trimester	22	0.38	−∞, 1.06	.060	
Maternal 25OHD	395	0.68	−∞, 0.89	.010	
DHA	103	0.49	−∞, 1.07	.067	
EPA	103	0.44	−∞, 0.92	.034	

25OHD, 25-Hydroxyvitamin D; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio.

N, subjects with available data for the predictor and the outcome asthma/recurrent wheeze in models.

Missing data: asthma/wheeze status was missing for 57 mothers in the first trimester and 54 mothers analyzed in the third trimester. Baseline 25OHD was missing for 4 mothers in the first trimester and 9 mothers in the third trimester.

*Two-sided *P* values and CIs for the interaction term. Significant and suggestive *P* values are highlighted in bold.

asthma/recurrent wheeze relationship, with an increased protective effect seen with increased dosage of fish oil (1-sided *P* = .010). Fish oil supplementation remained protective against asthma/wheeze after adjusting for potential confounders (maternal education and race) (Table E2, available in this article's Online Repository at www.jaci-inpractice.org; OR = 0.49; 95% CI: −∞, 0.99; 1-sided *P* = .047); however, because of low numbers in certain strata when accounting for these variables, these analyses were unstable (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Ninety-two percent of the 50 women who took fish oil supplements were college graduates compared with 60% of the nonusers, and 70% were white compared with 39% respectively (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Replication in a larger population is necessary to fully assess the effect of these variables on the outcome.

Of the 50 mothers, 24 continued to take fish oil supplements in the third trimester. We observed the greatest reduction in risk of asthma/recurrent wheeze among the offspring of these 24 mothers who took fish oil during both the first and third trimesters compared with none at all (OR = 0.38; 95% CI: −∞, 1.06; 1-sided *P* = .06, Table I). The increased reduction in risk with duration of supplementation was supported by a test for trend (1-sided *P* = .039).

In addition, although we were underpowered to investigate PUFAs in the mothers, higher levels of DHA in the mothers

during their first trimester were significantly associated with a reduced risk of asthma/recurrent wheeze among their offspring (OR = 0.43; 95% CI: −∞, 0.96; 1-sided *P* = .042, Table I). Similarly, higher levels of EPA also demonstrated a decreased risk, although this association did not meet statistical significance (OR = 0.80; 95% CI: −∞, 1.52; 1-sided *P* = .288, Table I). In the third trimester, there was evidence of a decreased risk of asthma/wheeze in offspring with increasing maternal PUFA levels: EPA (OR = 0.43; 95% CI: −∞, 0.92; 1-sided *P* = .034, Table I) and DHA (OR = 0.49; 95% CI: −∞, 1.07; 1-sided *P* = .067, Table I). Blood metabolite levels for both EPA (estimate: 0.91; 95% CI: −∞, 1.30; 1-sided *P* = 9.9×10^{-5}) and DHA (estimate: 0.68; 95% CI: −∞, 1.03; 1-sided *P* = 9.7×10^{-4}) reached statistical significance in the third trimester (Table E3, available in this article's Online Repository at www.jaci-inpractice.org); EPA's effect was also significant in the first trimester (estimate: 0.55; 95% CI: −∞, 0.91; 1-sided *P* = 5.7×10^{-3} , Table E3, available in this article's Online Repository at www.jaci-inpractice.org). Therefore, these findings are consistent with the FFQ analyses, one of the most frequently employed methods for nutritional epidemiological investigations.¹⁰ The main strength of this study is the assessment of the relationship between PUFA intake and asthma/wheeze using the FFQ. Our results suggest that there is a protective effect of maternal fish oil intake on the development of asthma/recurrent wheeze in offspring during the first 3 years of their life,

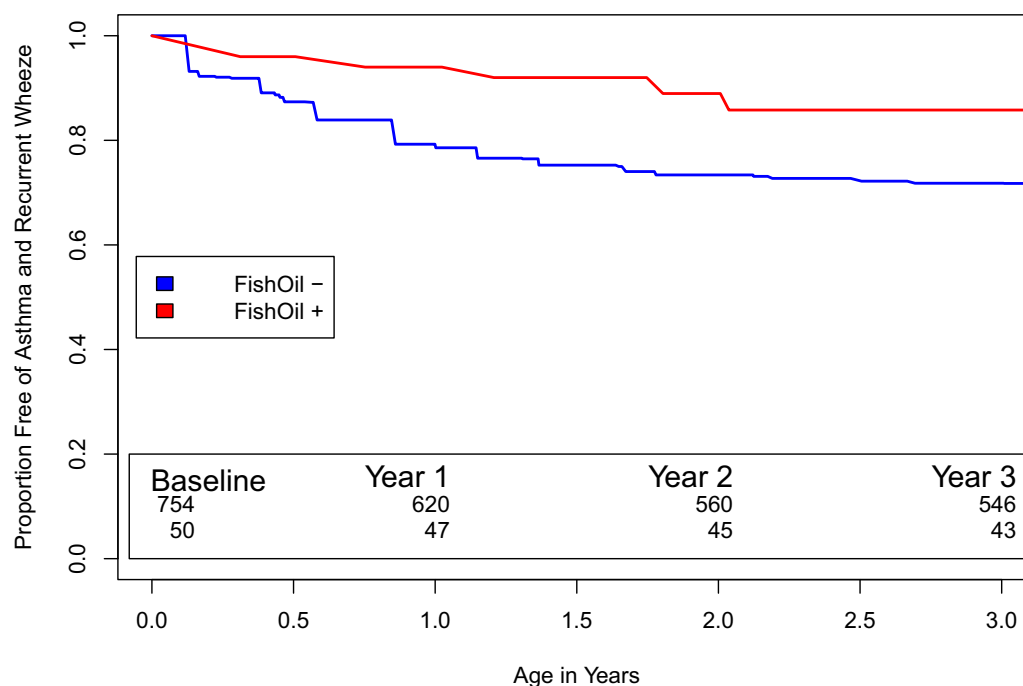


FIGURE 1. Kaplan-Meier survival estimates demonstrating offspring's asthma or recurrent wheeze free proportion by fish oil intake status. The red curve corresponds to offspring from mothers who took fish oil (fish oil positive) and the blue curve to those from mothers who did not take fish oil (fish oil negative). In this time to event analysis, the event corresponds to either the occurrence of asthma or recurrent wheeze. The hazard ratio for time to first event of either asthma or recurrent wheeze by age 3 years obtained using the Weibull regression model was 0.45 (1-sided 95% confidence interval, $-\infty$, 0.84; 1-sided $P = .018$).

which is in agreement with the study by Bisgaard et al.⁴ The results based on maternal PUFA levels also support this conclusion. Moreover, there is evidence of a dose-response protective effect against asthma/recurrent wheeze. We did not identify a significant interaction between fish oil supplementation and baseline 25OHD, which is again consistent with Bisgaard et al's findings.⁴ In conclusion, both fish oil and vitamin D₃ are recommended during pregnancy; however, a more comprehensive assessment of vitamin D₃ and fish oil supplementation during pregnancy may provide further insight into the biological mechanisms at play.

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ONLINE REPOSITORY

OUTCOME ASCERTAINMENT

The primary outcome was ascertained from offspring questionnaires administered every 3 months during the 3-year follow-up of the children. Doctor's diagnosis of asthma was ascertained from the parental report by a positive response to the question: "Since [birth/the last time we talked], was there a physician diagnosed asthma in child [CHILD]?"

In addition to doctor's diagnosis of asthma, symptoms or medications used in the past year were also reported.^{E1} Similarly, recurrent wheeze was mainly ascertained from parental reports of wheezing in either of the first 2 years of life and wheezing in the third year.^{E2} More precisely, wheeze was defined by the occurrence of at least one of the following 5 conditions:^{E3} (1) parental report of wheeze after child's second birthday preceded by at least 1 report of wheeze before second birthday; (2) report of child's use of asthma controller medication (defined below) after the second birthday, preceded by a report of wheeze before the second birthday; (3) 2 or more distinct parental reports of wheeze after the second birthday; (4) at least 1 parental report of wheeze and use of asthma controller medications at distinct visits, both subsequent to the second birthday; or (5) 2

distinct reports of the use of asthma controller medications after the second birthday.

Symptoms or medication use was ascertained from the following questions:

1. Since [birth/the last time we talked], has [CHILD] been given any medication for wheezing, or asthmatic bronchitis, or asthma?
 - a. No 0
 - b. Yes 1
 - c. Don't know 2
2. Since [birth/the last time we talked], which of the following types of medication has [CHILD] been given? (Refer to primary VDAART paper^{E2,E3} for more details or common examples of each medication category or other past conditions taken into consideration):

Yes No

- | | | |
|---|---|---|
| a. Bronchodilator inhalers/nebulizers, pills, or syrups | 1 | 0 |
| b. Steroid inhalers/nebulizers | 1 | 0 |
| c. Leukotriene modifiers | 1 | 0 |
| d. Steroid pills or liquids | 1 | 0 |

This composite definition of asthma/recurrent wheeze has been shown to be a reliable marker of asthma/recurrent wheeze in prior peer-reviewed published studies.^{E2,E3}

TABLE E1. Clinical characteristics of mothers who did (n = 50) vs those who did not take fish oil supplements in the first trimester (n = 754)

	No fish oil (n = 754)	Fish oil (n = 50)	<i>P</i> value*
Asthma/wheeze diagnosis in children (%)			.030
No	490 (70)	40 (85.1)	
Yes	210 (30)	7 (14.9)	
Maternal education (%)			1.5×10^{-6}
High school	298 (39.5)	4 (8)	
College graduate	456 (60.5)	46 (92)	
Maternal race, n (%)			1.9×10^{-5}
White	291 (38.6)	35 (70)	
Others	463 (61.4)	15 (30)	
Baseline 25OHD, mean (SD)	22.6 (10.3)	28.30 (7.8)	7.4×10^{-3}
Sufficient >30 ng/mL, n (%)	156 (20.8)	19 (38)	
Deficient <30 ng/mL, n (%)	593 (79.2)	31 (62)	

25OHD, 25-Hydroxyvitamin D; SD, standard deviation.

Children of those mothers with and without asthma/recurrent wheeze diagnosis as outcome have also been reported.

Missing data: asthma/wheeze status was missing for 57 subjects. Maternal 25OHD was missing for 5 subjects.

*Significance of difference was evaluated using Fisher's exact test for categorical variables and the 2-sample *t* test for continuous variables. Significant and suggestive *P* values are highlighted in bold.

TABLE E2. Adjusted analyses of effects of fish oil, 25OHD, and interaction term fish oil $\times$ 25OHD in mothers from first trimester on the development of recurrent wheeze at age 3 years

Models for outcome asthma/wheeze	OR	One-sided (95% CI)	One-sided <i>P</i> value
Univariate models			
Fish oil	0.49	$-\infty, 0.99$	.047
Maternal 25OHD	0.69	$-\infty, 0.99$	.047
Multivariate interaction model			
Fish oil	0.53	$-\infty, 1.23$	.108
Maternal 25OHD	0.71	$-\infty, 1.03$	.063
Fish oil $\times$ 25OHD*	0.87	0.11, 4.90	.884

25OHD, 25-Hydroxyvitamin D; CI, confidence interval; OR, odds ratio.

Missing data: asthma/wheeze status was missing for 57 subjects. Maternal 25OHD was missing for 5 subjects. Adjusted for maternal race (white or others [black or African American, Asian, Other]) and education.

*Two-sided *P* values and CIs for the interaction term. Significant and suggestive *P* values are highlighted in bold.

TABLE E3. Analyses of fish oil intake association with its metabolites EPA and DHA in first (N = 107 mothers, 7 with fish oil intake) and third trimester (N = 103, 5 with fish oil intake)

Models for fish oil intake predicting metabolite levels	Estimate	One-sided (95% CI)	One-sided <i>P</i> value
Univariate models in first trimester			
EPA	0.55	$-\infty, 0.91$	5.7×10^{-3}
DHA	0.11	$-\infty, 0.42$	.279
Univariate models in third trimester			
EPA	0.91	$-\infty, 1.30$	9.9×10^{-5}
DHA	0.68	$-\infty, 1.03$	9.7×10^{-4}

CI, Confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. Significant and suggestive *P* values are highlighted in bold.

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