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Bisgaard, Hans; Stokholm, Jakob; Chawes, Bo L.; Vissing, Nadja H.; Bjarnadóttir, Elin; Schoos, Ann-Marie M.; Wolsk, Helene M; Vinding, Rebecca K.; Thorsteinsdóttir, Sunna; Følsgaard, Nilofar V.

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ORIGINAL ARTICLE

Fish Oil–Derived Fatty Acids in Pregnancy and Wheeze and Asthma in Offspring

Hans Bisgaard, M.D., D.M.Sc., Jakob Stokholm, M.D., Ph.D.,
Bo L. Chawes, M.D., Ph.D., D.M.Sc., Nadja H. Vissing, M.D., Ph.D.,
Elin Bjarnadóttir, M.D., Ann-Marie M. Schoos, M.D., Ph.D.,
Helene M. Wolsk, M.D., Tine M. Pedersen, M.D., Rebecca K. Vinding, M.D.,
Sunna Thorsteinsdóttir, M.D., Nilofar V. Følsgaard, M.D., Ph.D.,
Nadia R. Fink, M.D., Jonathan Thorsen, M.D., Anders G. Pedersen, Ph.D.,
Johannes Waage, Ph.D., Morten A. Rasmussen, Ph.D., Ken D. Stark, Ph.D.,
Sjurdur F. Olsen, M.D., D.M.Sc., and Klaus Bønnelykke, M.D., Ph.D.

ABSTRACT

From COPSAC (Copenhagen Prospective Studies on Asthma in Childhood), Herlev and Gentofte Hospital, University of Copenhagen (H.B., J.S., B.L.C., N.H.V., E.B., A.-M.M.S., H.M.W., T.M.P., R.K.V., S.T., N.V.F., N.R.F., J.T., J.W., M.A.R., K.B.), and the Center for Fetal Programming, Statens Serum Institut (S.F.O.), Copenhagen, the Department of Pediatrics, Naestved Hospital, Naestved (J.S., E.B., T.M.P., R.K.V.), DTU Bioinformatics, Technical University of Denmark, Kongens Lyngby (A.G.P.), and Faculty of Science, Chemometrics and Analytical Technology, University of Copenhagen (M.A.R.) — all in Denmark; the Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada (K.D.S.); and the Department of Nutrition, Harvard School of Public Health, Boston (S.F.O.). Address reprint requests to Dr. Bisgaard at COPSAC, Herlev and Gentofte Hospital, University of Copenhagen, Ledreborg Alle 34, DK-2820 Gentofte, Copenhagen, Denmark, or at bisgaard@copsac.com.

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BACKGROUND

Reduced intake of n–3 long-chain polyunsaturated fatty acids (LCPUFAs) may be a contributing factor to the increasing prevalence of wheezing disorders. We assessed the effect of supplementation with n–3 LCPUFAs in pregnant women on the risk of persistent wheeze and asthma in their offspring.

METHODS

We randomly assigned 736 pregnant women at 24 weeks of gestation to receive 2.4 g of n–3 LCPUFA (fish oil) or placebo (olive oil) per day. Their children formed the Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$) cohort and were followed prospectively with extensive clinical phenotyping. Neither the investigators nor the participants were aware of group assignments during follow-up for the first 3 years of the children's lives, after which there was a 2-year follow-up period during which only the investigators were unaware of group assignments. The primary end point was persistent wheeze or asthma, and the secondary end points included lower respiratory tract infections, asthma exacerbations, eczema, and allergic sensitization.

RESULTS

A total of 695 children were included in the trial, and 95.5% completed the 3-year, double-blind follow-up period. The risk of persistent wheeze or asthma in the treatment group was 16.9%, versus 23.7% in the control group (hazard ratio, 0.69; 95% confidence interval [CI], 0.49 to 0.97; P=0.035), corresponding to a relative reduction of 30.7%. Prespecified subgroup analyses suggested that the effect was strongest in the children of women whose blood levels of eicosapentaenoic acid and docosahexaenoic acid were in the lowest third of the trial population at randomization: 17.5% versus 34.1% (hazard ratio, 0.46; 95% CI, 0.25 to 0.83; P=0.011). Analyses of secondary end points showed that supplementation with n–3 LCPUFA was associated with a reduced risk of infections of the lower respiratory tract (31.7% vs. 39.1%; hazard ratio, 0.75; 95% CI, 0.58 to 0.98; P=0.033), but there was no statistically significant association between supplementation and asthma exacerbations, eczema, or allergic sensitization.

CONCLUSIONS

Supplementation with n–3 LCPUFA in the third trimester of pregnancy reduced the absolute risk of persistent wheeze or asthma and infections of the lower respiratory tract in offspring by approximately 7 percentage points, or one third. (Funded by the Lundbeck Foundation and others; ClinicalTrials.gov number, NCT00798226.)

THE INCIDENCE OF ASTHMA AND WHEEZ-ing disorders has more than doubled in westernized countries in recent decades.[1] These conditions often originate in early childhood[2] and currently affect one in five young children.[3] Concomitantly, the increased use of vegetable oils in cooking and of grain in the feeding of livestock has resulted in an increase in the intake of n–6 polyunsaturated fatty acids and a decrease in the intake of n–3 polyunsaturated fatty acids, especially the long-chain polyunsaturated fatty acids (LCPUFAs) — eicosapentaenoic acid (20:5n–3, EPA) and docosahexaenoic acid (22:6n–3, DHA) — found in cold-water fish.[4] Observational studies have suggested an association between a diet that is deficient in n–3 LCPUFA during pregnancy and an increased risk of asthma and wheezing disorders in offspring,[5,6] whereas randomized, controlled trials of n–3 LCPUFA supplementation in pregnant women have generally been underpowered and have produced ambiguous results.[7-10] Therefore, we conducted a double-blind, randomized, controlled trial of n–3 LCPUFA supplementation during the third trimester of pregnancy to assess the effect on the risk of persistent wheeze and asthma in offspring. The children were followed prospectively for the first 3 years of life with comprehensive clinical phenotyping,[11] during which time both the investigators and the participants were unaware of group assignments. For an additional 2 years of follow-up, only the investigators were unaware of group assignments.

METHODS

STUDY DESIGN

In this single-center, double-blind, placebo-controlled, parallel-group trial, pregnant women between 22 and 26 weeks of gestation were recruited into the Copenhagen Prospective Studies on Asthma in Childhood_{2010} (COPSAC_{2010}) pregnancy cohort.[11] Women taking more than 600 IU of vitamin D per day and women with any endocrine, heart, or kidney disorder were excluded. The trial was approved by the local ethics committee and the Danish Data Protection Agency. Both parents of each child provided oral and written informed consent before enrollment.

At week 24 of pregnancy, women were randomly assigned in a 1:1 ratio to receive 2.4 g per day of n–3 LCPUFA (55% EPA and 37% DHA) in triacylglycerol form (Incromega TG33/22, Croda

Health Care) or placebo (in the form of olive oil, containing 72% n–9 oleic acid and 12% n–6 linoleic acid [Pharma-Tech A/S]). (For complete information on the components of Incromega TG33/22, see Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. The trial protocol is also available at NEJM.org.) Both n–3 LCPUFA and placebo were administered in four identical 1-g capsules, with both investigators and participants unaware of group assignments. Neither the fish oil nor the placebo nor any medications, diagnostic equipment, or other materials used in the trial were donated. Similarly, no funding agency played any role in the design or conduct of the trial, the collection, management, or interpretation of the data, the preparation, review, or approval of the manuscript for publication, or the decision to submit the manuscript for publication. In addition, no pharmaceutical company and no company that produces n–3 LCPUFA was involved in the trial. The intervention was funded solely by COPSAC.

Supplementation continued until 1 week after delivery, and both investigators and participants remained unaware of group assignments until the youngest child in the trial reached 3 years of age. The trial was continued for an additional 2 years, during which the investigators determined whether children with persistent wheeze had asthma. Follow-up is still ongoing, with the investigators only (not the participants) remaining unaware of group assignments. A subgroup of 623 women also participated in a trial with a nested, factorial design in which they took 2400 IU of vitamin D_{3} per day during the third trimester of pregnancy,[12] a subgroup of 51 women participated in a trial in which they were vaccinated against influenza A during pregnancy,[11,13] and a subgroup of 72 children with persistent wheeze participated in a trial in which they took azithromycin or placebo during asthmatic episodes.[14]

The participating women completed a validated 360-item food frequency questionnaire so that their dietary intake in the 4 weeks before randomization could be assessed.[15-17] Maternal whole-blood levels of EPA and DHA were assessed at the time of randomization and 1 week after birth.[18,19] Samples of breast milk were obtained 1 month after birth and were analyzed for EPA and DHA levels. Maternal variation in the genes encoding fatty acid desaturase (FADS) was tagged by genotyping the single-nucleotide polymor-

phism (SNP) rs1535 in mothers of European descent (with genotyping performed by LGC Group). Adherence to the intervention was assessed by comparing the number of capsules returned with the number expected.

END POINTS

Pediatricians collected information during clinical visits scheduled at 1 week after birth, at 1, 3, 6, 12, 18, 24, 30, and 36 months after birth, and yearly thereafter. Additional visits for acute care were arranged whenever a child had symptoms related to the lungs, an allergy, or the skin. Asthma, allergy, and eczema were diagnosed and treated by COPSAC pediatricians in accordance with predefined algorithms.[11]

Diary cards were completed daily by parents from the birth of their child for the purpose of monitoring troublesome lung-related symptoms, including cough, wheeze, and dyspnea that severely affected the well-being of the child,[11] skin-related symptoms, and symptoms related to infections of the lower respiratory tract. The diary cards were reviewed by COPSAC pediatricians, and the data were checked after being entered into the database.

Persistent wheeze or asthma (the primary end point) was diagnosed on the basis of a previously described quantitative diagnostic algorithm[20,21] that included diary recordings of five episodes of troublesome lung symptoms within the preceding 6 months, each lasting for at least 3 consecutive days; symptoms typical of asthma; the rescue use of inhaled beta$_2$-agonist; and response to a 3-month course of inhaled glucocorticoids followed by relapse after the end of treatment.[20] Remission was defined as a period of 12 months without relapse. The diagnosis was termed "persistent wheeze" until a child reached 3 years of age and was termed "asthma" thereafter.

Infection of the lower respiratory tract was defined as a diagnosis of pneumonia or bronchiolitis on the basis of symptoms and clinical presentation (i.e., without confirmation by means of pathogen identification or radiologic or laboratory findings).[22-24] Allergic sensitization was determined at 6 months and 18 months as a wheal larger than 2 mm in response to any skin-prick test (ALK-Abelló) or a specific IgE level of 0.35 kU per liter or higher against milk, egg, dog, or cat allergens (ImmunoCAP tests, Thermo Fisher Scientific).[11] Allergic rhinoconjunctivitis was diagnosed longitudinally by COPSAC pedia-

tricians on the basis of systematic interviews and was defined as allergic rhinitis, allergic conjunctivitis, or both. Lung function at 5 years of age was assessed by means of spirometry, specific airway resistance by means of whole-body plethysmography, and the lung-clearance index by means of multiple-breath washout.[11] A diagnosis of eczema was based on the criteria defined by Hanifin and Rajka.[25-27]

STATISTICAL ANALYSIS

Power calculations were based on the 695 newborns included in the trial. With an assumed hazard ratio of 0.5 for the treatment group and an expected frequency of persistent wheeze or asthma of 12% for the control group, the design had a power of 73% for the detection of a difference, with a significance level of 0.05 (two-sided test). The effect on the development of persistent wheeze or asthma and eczema was assessed by means of Kaplan–Meier curves and quantified by means of Cox proportional hazards regression (P values correspond to Wald tests). Post hoc analyses were performed with the use of Bayesian statistical modeling to evaluate the importance of covariates and to quantify the degree of certainty for estimates of hazard ratios and risks, and multiple imputation was used to avoid issues related to missing data in these specific analyses. Additional methodologic details are outlined in the Supplementary Appendix and in the COPSAC$_{2010}$ design paper.[11]

RESULTS

BASELINE CHARACTERISTICS

A total of 736 pregnant women were randomly assigned to receive the intervention or placebo between November 2008 and November 2010, and the final study included 695 children, including five pairs of twins (Fig. S1 in the Supplementary Appendix). The baseline characteristics of the pregnant women and their children showed that randomization was not biased; similarly, there were no significant differences in pregnancy end points, including the rate of preterm birth (P>0.1 for all comparisons) (Table S2 in the Supplementary Appendix). Information on maternal dietary intake before the intervention was available for 594 mothers (Table S3 in the Supplementary Appendix). The intake and blood levels of EPA and DHA were associated (r=0.32, P<0.001).

Table 1. Effects of n–3 LCPUFA on the Primary End Point.*

End Point	Children with Available Data (no. with primary end point/total no.)	n–3 LCPUFA (N = 346)	Control (N = 349)	Odds Ratio	Hazard Ratio	95% CI	P Value
		percent					
Follow-up to 3–5 yr of age							
All	136/695	16.9	23.7		0.69	0.49–0.97	0.035
Stratified according to maternal pre-treatment blood levels of EPA + DHA as % of total fatty acids							
Lowest third (<4.3%)	49/203	17.5	34.1		0.46	0.25–0.83	0.011
Middle third (4.3–5.3%)	31/202	14.4	16.9		0.85	0.42–1.73	0.656
Highest third (>5.3%)	41/203	19.3	21.5		0.85	0.46–1.57	0.605
Follow-up from birth to age of 5 yr							
All	142/695	17.4	24.6		0.68	0.49–0.95	0.024
Stratified according to maternal pre-treatment blood levels of EPA + DHA as % of total fatty acids							
Lowest third (<4.3%)	51/203	17.6	34.3		0.47	0.26–0.84	0.011
Middle third (4.3–5.3%)	33/202	14.4	18.9		0.76	0.38–1.52	0.441
Highest third (>5.3%)	42/203	19.3	22.5		0.82	0.44–1.50	0.511
Follow-up from birth to date of submission (5–7 yr; mean age, 6.0 yr)							
All	152/695	19.0	29.2		0.66	0.47–0.91	0.011
Post hoc analyses (follow-up from birth to age of 5 yr)							
Stratified according to maternal pre-treatment intake of EPA + DHA							
Lowest third (<321 mg/day)	48/198	18.5	32.4		0.55	0.30–0.98	0.043
Middle third (321–584 mg/day)	28/198	13.8	14.6		0.95	0.45–2.00	0.894
Highest third (>584 mg/day	41/202	18.5	23.1		0.77	0.42–1.43	0.415
Stratified according to maternal *FADS* genotype							
GG genotype	21/85	15.2	37.8		0.37	0.15–0.91	0.031
AG genotype	55/291	15.4	23.5		0.63	0.37–1.09	0.099
AA genotype	58/284	20.0	21.8		0.91	0.54–1.52	0.712
Yearly prevalence at 1–5 yr of age	142/695			0.68		0.47–0.98	0.038

* Percentages represent Kaplan–Meier estimates. DHA denotes docosahexaenoic acid, EPA eicosapentaenoic acid, and LCPUFA long-chain polyunsaturated fatty acids.

FADS GENOTYPES

The maternal *FADS* genotype (rs1535) was identified in 660 mothers. Post hoc analyses showed that the minor allele (G) was associated with lower levels of EPA and DHA before the intervention and with higher levels of the upstream substrates of linoleic acid and alpha-linolenic acid before the intervention[28] (Fig. S2 in the Supplementary Appendix).

ADHERENCE

According to capsule count, 493 of 695 (70.9%) of the women had an intervention compliance of more than 80%, with no significant differences between the treatment and control groups. Supplementation resulted in increased blood levels of EPA and DHA (mean [±SD] relative percent of fatty acids measured in blood) from 4.9±1.3% to 6.1±2.0% as compared with a decrease from

4.9±1.2% to 3.7±1.1% in the control group (Fig. S3 in the Supplementary Appendix). The between-group difference in mean postintervention levels of EPA and DHA was 2.5 percentage points (95% CI, 2.2 to 2.7). Clinical follow-up for the initial 3-year double-blind period was completed for 664 children (95.5%), whereas clinical follow-up for the 5-year follow-up period was completed for 647 children (93.1%). The average age of the children for whom double-blind follow-up was completed was 4.0±0.6 years (Table 1, and Fig. S4 in the Supplementary Appendix), and the average age of the children at the time of statistical analysis in March 2016 was 6.0±0.6 years.

PRIMARY END POINT

During the prespecified, double-blind follow-up period, which covered children from birth to between 3 and 5 years of age, 136 of 695 children (19.6%) received a diagnosis of persistent wheeze or asthma, and this condition was associated with reduced lung function by 5 years of age, with parental asthma, and with a genetic risk of asthma (Table S4 in the Supplementary Appendix). The risk of persistent wheeze or asthma was 16.9% in the treatment group and 23.7% in the control group (hazard ratio, 0.69; 95% confidence interval [CI], 0.49 to 0.97; P=0.035), with

a relative reduction in risk of 30.7% (Table 1). The effect estimate and significance level remained largely unchanged in adjusted analyses (i.e., with adjustment for twin status, sex, supplementation with a high dose of vitamin D_3, and EPA and DHA level before randomization) (Table S5 in the Supplementary Appendix).
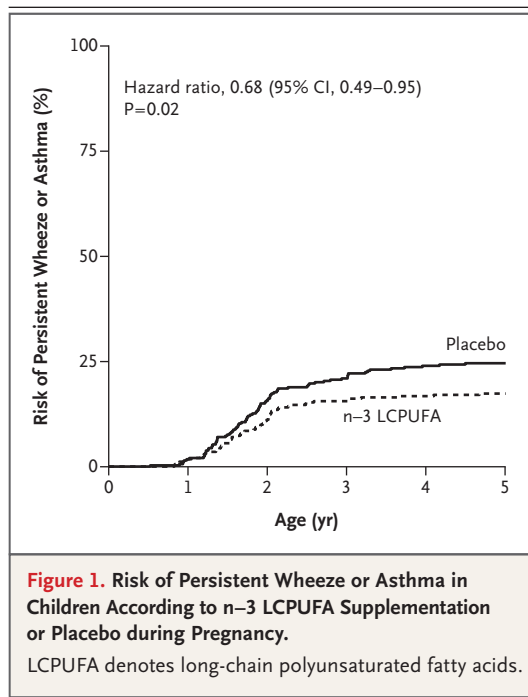
The preventive effect of supplementation appeared to be driven primarily by children of mothers who had low blood levels of EPA and DHA at randomization (the lowest third of the trial population), for whom the risk of persistent wheeze or asthma was 17.5% in the treatment group as compared with 34.1% in the control group (hazard ratio, 0.46; 95% CI, 0.25 to 0.83; P=0.011), corresponding to a relative reduction of 54.1% (Table 1).

There was no statistically significant interaction between supplementation with n−3 LCPUFA and high-dose vitamin D_3 with regard to persistent wheeze (P=0.065). However, exploratory analyses stratified according to randomization to receive high-dose vitamin D_3 suggested that the strongest effect of n−3 LCPUFA supplementation occurred in children of mothers who did not receive high-dose vitamin D_3 supplementation (Table S6 in the Supplementary Appendix).

The number needed to treat to prevent a case of persistent wheeze or asthma was 14.6 among the entire cohort and 5.6 among women in the lowest third with regard to EPA and DHA levels before the intervention. The safety profiles for n−3 LCPUFA supplementation and olive oil appeared to be similar (Table S7 in the Supplementary Appendix).

During the continued follow-up period for children from birth to the age of 5 years, there was a reduced risk of persistent wheeze or asthma among the children in the treatment group as compared with the control group (Table 1 and Fig. 1 and 2). A similar difference in risk between groups was also found when statistical analyses were conducted in March 2016 that covered the period from birth to the ages of 5 to 7 years (mean age, 6.0 years) (Table 1).

In a post hoc analysis, the effect of supplementation seemed strongest in the children of mothers whose dietary intake of EPA and DHA was in the lowest third of that in the trial population (<321 mg per day) before the intervention and in the children of mothers who carried the

Hazard ratio, 0.68 (95% CI, 0.49–0.95)
P=0.02

Placebo

n−3 LCPUFA

Figure 1. Risk of Persistent Wheeze or Asthma in Children According to n−3 LCPUFA Supplementation or Placebo during Pregnancy.

LCPUFA denotes long-chain polyunsaturated fatty acids.

FADS gene variant associated with low levels of EPA and DHA (the G allele at rs1535) (Table 1, and Fig. S5 in the Supplementary Appendix). The interaction between the intake of EPA and DHA and the *FADS* genotype on the effect of treatment is illustrated in Fig. S6 in the Supplementary Appendix.

The yearly prevalence of persistent wheeze or asthma was lower among the children of mothers who received supplementation, and this effect did not appear to change between the ages of 2 and 5 years (Table 1 and Fig. 3). A threshold analysis suggested that supplementation was effective up to a preintervention blood level of EPA and DHA of approximately 5.0 to 5.5% of total blood fatty acids (Fig. S7 in the Supplementary Appendix). In the children of mothers in the control group, there was a significant association between low maternal blood levels of EPA and DHA and the risk of persistent wheeze or asthma in their children (Table S8 in the Supplementary Appendix). There was no association between levels of EPA and DHA in breast milk collected when the child was 1 month old and later development of persistent wheeze or asthma (Table S9 in the Supplementary Appendix). Bayesian analyses confirmed the effect of the intervention with similar estimates for hazard ratios and for absolute and relative risks (see Supplementary Methods and Results and Tables S10 through 13 in the Supplementary Appendix).

SECONDARY AND POST HOC END POINTS

Supplementation was associated with a reduced risk of lower respiratory tract infections in the first 3 years of life and up to 5 years of follow-up (Table 2, and Fig. S8 in the Supplementary Appendix) but not with other secondary end points, including the risk of asthma exacerbations, ecze-

Figure 2. Effect of n-3 LCPUFA Supplementation during Pregnancy on Persistent Wheeze or Asthma in Children According to Mother's EPA and DHA Levels before Intervention.

The effect of n–3 LCPUFA supplementation in pregnancy is shown in children born to women who were in the lowest third of the study population regarding blood levels of EPA and DHA (Panel A, <4.30%), the middle third (Panel B, 4.30 to 5.33%), and the highest third (Panel C, >5.33%). Blood levels of EPA and DHA were measured in women on the day of randomization, at week 24 of pregnancy.

Figure 3. Prevalence of Persistent Wheeze or Asthma in Children Whose Mothers Received n–3 LCPUFA Supplementation or Placebo during Pregnancy.
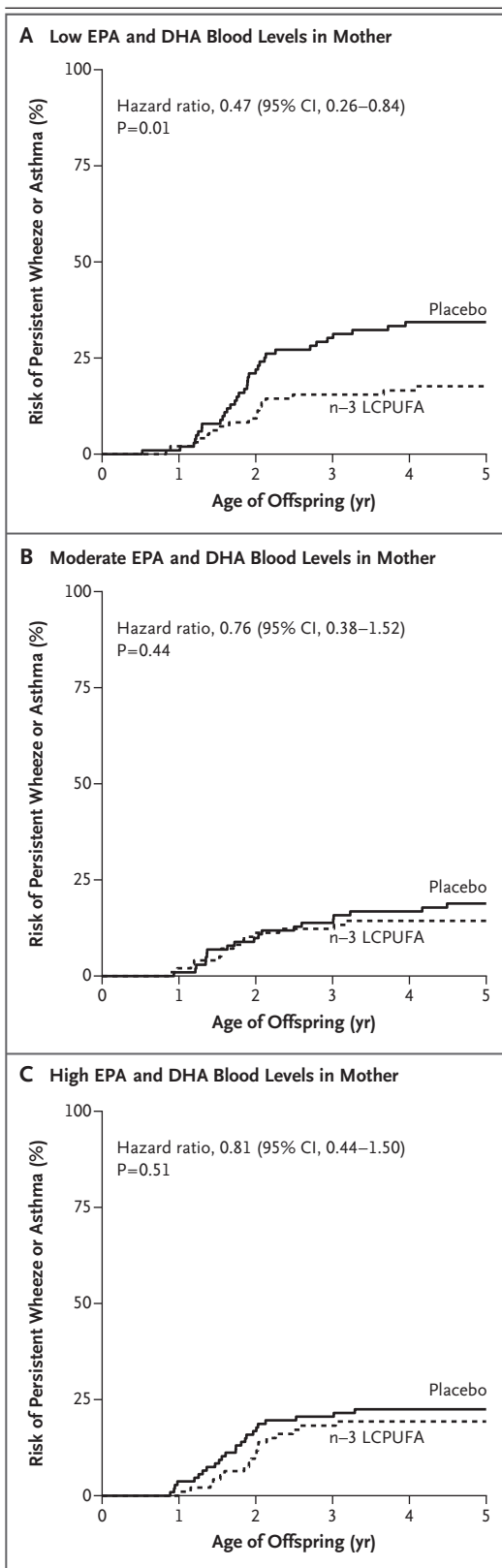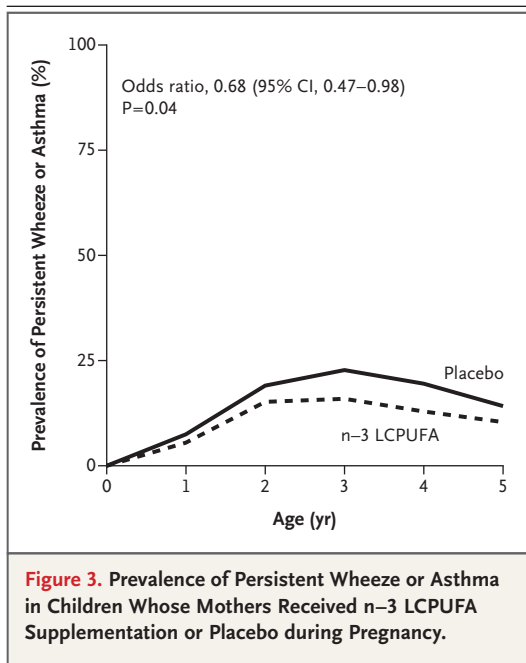
ma, and allergic sensitization at 6 months and 18 months. There was also no statistically significant association between supplementation and the post hoc end points of allergic rhinoconjunctivitis and lung function at the age of 5 years.

DISCUSSION

The risk of persistent wheeze or asthma was reduced by approximately 7 percentage points, or one third, in the first 5 years of life among children of women who received daily supplementation with n–3 LCPUFA during the third trimester of pregnancy. This effect was most prominent among children of women with low preintervention EPA and DHA blood levels. Supplementation was also associated with a reduced risk of infections of the lower respiratory tract but not with a reduced risk of eczema, allergic sensitization, or other secondary end points.

The trial results are strengthened by the use of centralized, longitudinal clinical follow-up, the daily recording of symptoms in a diary, and frequent visits to our clinical research unit for regular and acute care. COPSAC served as the de facto primary health care center for the birth cohort, thereby ensuring the use of a standardized approach to diagnosis and treatment. This approach greatly improves the reliability of the diagnoses.

The additional Bayesian analyses confirmed the results of the predefined analyses, thereby supporting the robustness of the results.

We used a high dose of n–3 LCPUFA — corresponding to an increase estimated to be 10 times the normal daily intake in Denmark[29] and 20 times that in most other countries, including Canada and the United States.[30,31] It is possible that a lower dose would have sufficed.

The FADS region harbors several genetic variants. The SNP rs1535 was chosen because this SNP and its proxies in close linkage disequilibrium have been associated with n–3 LCPUFA levels in a genomewide association study[32] and with blood levels of EPA and DHA during pregnancy.[28]

Several secondary and subgroup analyses were performed. These analyses should be considered as exploratory; the results need to be confirmed in subsequent studies. Furthermore, it is a limitation of our study that allergic rhinoconjunctivitis was not confirmed in tests of allergic sensitization by the age of 5 years.

We observed that supplementation had a greater effect on the children of mothers with low blood levels and low intake of EPA and DHA and on the children of mothers with a *FADS* genotype associated with low EPA and DHA blood levels. This pattern is biologically plausible and supports the supposition that the intervention had a causal effect. Furthermore, this pattern indicates that there is the potential for so-called "precision prevention" by targeting pregnant women with the *FADS* genotype associated with low levels of EPA and DHA and those with a low dietary intake of these fatty acids (Fig. S6 in the Supplementary Appendix).

The differences in the geographical and temporal incidences of asthma and wheezing indicate that lifestyle plays a major role, and our trial suggests that diets low in n–3 LCPUFA could contribute to these differences. The mechanisms may involve an imbalance between n–6 and n–3 lipid mediators, which may promote a proinflammatory state.[33,34]

There was no statistically significant interaction related to concurrent supplementation with vitamin D_3 and n–3 LCPUFA, but there was a trend suggesting that n–3 LCPUFA supplementation was less effective in children receiving high-dose vitamin D_3. Such an interaction would indicate that these supplements target the same

Table 2. Effects of n−3 LCPUFA on Secondary and Post Hoc End Points.*

End point	No. of Children with Available Data	n−3 LCPUFA (N=346)	Control (N=349)	Odds Ratio	Hazard Ratio	Estimate of Between-Group Differences	95% CI	P Value
Secondary — no./total no. (%)								
Risk of lower respiratory infections†								
Follow-up to 3–5 yr of age	230/690	31.7	39.1		0.75		0.58 to 0.98	0.033
Follow-up to 5 yr of age	257/690	38.8	45.5		0.77		0.61 to 0.99	0.041
Risk of asthma exacerbations (%)†								
Follow-up to 3–5 yr of age	57/695	8.5	8.5		1.00		0.60 to 1.67	0.995
Follow-up to 5 yr of age	61/695	8.5	9.4		0.90		0.55 to 1.49	0.695
Eczema (%)†								
Follow-up to 3–5 yr of age	193/695	33.0	28.3		1.19		0.89 to 1.57	0.238
Follow-up to 5 yr of age	203/695	30.8	28.9		1.10		0.83 to 1.44	0.516
Risk of sensitization at 6 or 18 mo. of age — no./total no. (%)								
Skin-prick test	53/691	30/345 (8.7)	23/346 (6.6)	1.34			0.76 to 2.37	0.313
Specific IgE	50/678	31/337 (9.2)	19/341 (5.6)	1.72			0.96 to 3.15	0.074
Post hoc								
Risk of allergic rhinoconjunctivitis by 5 yr of age — no./total no. (%)	78/645	33/324 (10.2)	45/321 (14.0)	0.70			0.43 to 1.12	0.137
Lung function by 5 yr of age — no.								
FEV$_1$ (liters)	536	1.19	1.18			0	−0.02 to 0.03	0.699
MMEF (liters/sec)	536	1.51	1.51			−0.01	−0.07 to 0.06	0.864
Specific airway resistance (kPa×sec)	598	1.18	1.15			0.03	−0.02 to 0.07	0.207
Multiple-breath washout, lung-clearance index‡	549	6.80	6.77			0.03	−0.11 to 0.16	0.676

* FEV$_1$ denotes forced expiratory volume in 1 second, LCPUFA long-chain polyunsaturated fatty acids, and MMEF maximum midexpiratory flow.
† Percentages represent Kaplan–Meier estimates.
‡ The lung-clearance index is the total expired volume during the washout of an inert gas divided by the functional residual capacity and reflects the inhomogeneity of the ventilation distribution.

disease pathway, and this possibility should be addressed in future studies.

Analysis of the prevalence of current persistent wheeze or symptoms of asthma showed that the effect of the intervention was largely unchanged between the ages of 2 years and 5 years (Fig. 3). This finding suggests that the effect is not restricted to a "transient wheezing" phenotype.

The maternal baseline intake of n−3 LCPUFA was high in this Danish population relative to that of the global population (Table S14 in the Supplementary Appendix). It has been estimated that 80% of the global population consumes less than 250 mg of EPA and DHA per day,[35] — a level that is well below 321 mg per day, the level below which we observed the highest treatment effect in our trial. Our data therefore suggest that a sizeable effect may be expected from supplementation in other populations worldwide; however, this suggestion is speculative, since other factors may be at play in such populations.

The implications of our findings on health and disease may be wide. The number needed to treat to prevent one case of persistent wheeze

or asthma was 14.6 in the entire cohort and 5.6 among women with the lowest blood levels of EPA and DHA before intervention.

In conclusion, these findings show that n–3 LCPUFA supplementation during pregnancy was associated with a significantly diminished burden of wheezing and asthma in children in this Danish birth cohort. To address the question of whether similar effects could be seen in other populations, further studies are required.

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