Fish oil supplementation during pregnancy and allergic respiratory disease in the adult offspring

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Background: Maternal supplementation with long-chain n-3 polyunsaturated fatty acids can have immunologic effects on the developing fetus through several anti-inflammatory pathways. However, there is limited knowledge of the long-term programming effects.

Objective: In a randomized controlled trial from 1990 with 24 years of follow-up, our aim was to determine whether supplementation with 2.7 g of long-chain n-3 polyunsaturated fatty acids in pregnancy can reduce the risk of asthma in offspring and allergic respiratory disease.

Methods: The randomized controlled trial included 533 women who were randomly assigned to receive fish oil during the third trimester of pregnancy, olive oil, or no oil in the ratio 2:1:1. The offspring were followed in a mandatory national prescription register, with complete follow-up for prescriptions related to the treatment of asthma and allergic rhinitis as primary outcomes. Furthermore, the offspring were invited to complete a questionnaire (74% participated) and attend a clinical examination (47% participated) at age 18 to 19 years.

Results: In intention-to-treat analyses the probability of having had asthma medication prescribed was significantly reduced in the fish oil group compared with the olive oil group (hazard ratio, 0.54, 95% CI, 0.32-0.90; $P = .02$). The probability of having had allergic rhinitis medication prescribed was also reduced in the fish oil group compared with the olive oil group (hazard ratio, 0.70, 95% CI, 0.47-1.05; $P = .09$), but the difference was not statistically significant. Self-reported information collected at age 18 to 19 years supported these findings. No associations were detected with respect to lung function outcomes or allergic sensitization at 18 to 19 years of age.

Conclusion: Maternal supplementation with fish oil might have prophylactic potential for long-term prevention of asthma in offspring. (J Allergy Clin Immunol 2017;139:104-11.)

Key words: *Randomized controlled trial, fetal programming, asthma, allergies, long-chain n-3 polyunsaturated fatty acids*

There is increasing evidence that environmental factors in fetal life can affect a person's susceptibility to asthma and allergic diseases.[1] Particular focus has been on maternal intake of the long-chain n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which are abundant in seafood and fish oil. EPA and DHA can affect function of the fetal immune system through several anti-inflammatory mechanisms. Fatty acids cross the placenta,[2] and EPA and DHA might directly affect the development of the fetal immune system by influencing cell signaling and gene expression, competing with arachidonic acid for cyclooxygenase and lipoxygenase enzymes to produce less potent eicosanoids, and through production of anti-inflammatory resolvins.[3-5] This in turn might result in a reduced T_H2 allergic immune response,[6] which has been substantiated in 2 previous randomized controlled trials (RCTs) with maternal fish oil supplementation, leading to reduced formation of cytokines produced by T_H2 cells at birth.[7-9] Furthermore, maternal fish oil supplementation had a beneficial effect on skin sensitization and IgE-related allergic diseases in infants up to 12 months.[10]

In 2008, Olsen et al[11] published results from the first randomized placebo-controlled trial that investigated the long-term effect of fish oil supplementation in late pregnancy, and they reported a beneficial effect on offspring asthma discharge diagnoses from a mandatory national patient register. However, because only patients seen in hospitals are reported to the patient register,[12] asthma discharge diagnoses from this register are known to underestimate the true occurrence of asthma in the general population.[13]

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Abbreviations used
DHA: Docosahexaenoic acid
EPA: Eicosapentaenoic acid
HR: Hazard ratio
OR: Odds ratio
PUFA: Polyunsaturated fatty acid
RCT: Randomized controlled trial

Therefore in the same RCT we decided to extend the analyses with methods of asthma and allergic respiratory disease ascertainment that are more relevant for the general population and with follow-up up until 24 years of age. Our aim was to determine whether our previously reported reduced occurrence of asthma discharge diagnoses in offspring whose mothers had taken fish oil in pregnancy could be extended to a more varied phenotype that also included mild and moderate manifestations of allergic respiratory disease and might be more important for disease prevention on a population level.

METHODS

Study cohort

The Aarhus Trial recruited 533 Danish pregnant women (61% of those eligible) with singleton pregnancies through antenatal care clinics in 1990. The women were block randomized in the ratio 2:1:1 stratified by parity to 3 groups who received a daily supplementation of either fish oil, olive oil, or no oil from gestational week 30 until delivery. The study design and original aim have been described in detail elsewhere.[14] Briefly, 266 women were randomized to the intervention group and received four 1-g gelatin capsules with fish oil (32% EPA, 23% DHA, and 2 mg of tocopherol/mL; Pikasol, Lube Ltd, Hadsund, Denmark) daily, corresponding to 2.7 g/d long-chain n-3 PUFAs. An additional 136 women were randomized to the placebo group and given 4 similar-looking 1-g capsules with olive oil (72% oleic acid [18:1n-9] and 12% linoleic acid [18:2n-6]) per day. Women allocated to the 2 oil capsule groups and the study coordinators were blinded to treatment allocation. A third group of 131 women were randomized to receive no oil capsules but were informed about the purpose of the trial and the potential beneficial effects of supplementation with long-chain n-3 PUFAs, thus acting as a passive-intervention positive control group. Active fish oil supplementation was associated with longer gestation[14] when compared with placebo, and it increased maternal EPA-derived thromboxane and prostacyclin production[15] and increased the concentrations of long-chain n-3 PUFAs in umbilical blood and tissues[16] and in early breast milk[17] when compared against a combination of both control groups.

In 2008-2009, a follow-up of the 18- to 19-year-old offspring of originally enrolled mothers was undertaken. At that time, 517 (97%) mother-child dyads were still alive and living in Denmark. An overview of the original trial and the follow-up is presented in Fig 1.

The study was approved by the local ethics committee (M-ÅA 20060182) and the Danish Data Protection Agency (journal no. 2006-41-6257), and all participants provided written consent. The Aarhus Trial is registered under NCT01353807.

Register-based outcomes

We assessed cases of asthma medication use from the national prescription register, which holds information on all prescriptions filled in Danish pharmacies written by doctors from all levels of the health care sector.[18] This allows for complete follow-up of all subjects remaining within the country over their lifetime. We were able to follow-up 522 (98%) of the 533 subjects born to women in the trial; the remaining 11 subjects were lost to follow-up because of emigration. We used Anatomical Therapeutic Chemical classification system codes R03A, R03B, R03C, and R03D for asthma and R01AC, R01AD, R01AX, R06A, S01GA, and S01GX for allergic rhinitis. We defined cases of asthma medication by using a modified validated definition[19] of those who had filled 2 or more prescriptions for β_2-agonists or steroids or 1 or more prescriptions for leukotriene receptor antagonists. Patients with allergic rhinitis were defined as those having filled 2 or more allergic rhinitis prescriptions, including eye drops, nasal decongestants, and oral antihistamines. The capture time was from the start of the register in 1995 until the end of 2014.

As in our previous report,[11] we also identified patients with a discharge diagnosis of asthma from the national patient register. Follow-up was extended by 7 years relative to our previous report through the end of 2013. Briefly, we used International Classification of Diseases, Eighth Revision, and International Classification of Diseases, Tenth Revision, codes 493.00, 493.01, 493.02, 493.08, 493.09, J45.0, J45.1, J45.2, J45.8, and J45.9. Hospital diagnoses of allergic rhinitis were not included as an outcome because of so few cases (n = 9 [1%]).

Clinical outcomes and biomarkers

We performed lung function tests and obtained blood samples from 243 (46%) subjects who participated in a follow-up clinical examination at age 18 to 19 years in 2008-2009. Levels of serum total IgE, specific IgE against 12 common inhalant allergens, and eosinophil cationic protein were quantified by using a fluoroimmunoassay with ImmunoCAP (Phadia Laboratory Systems AB, Uppsala, Sweden). Allergic sensitization was defined as a positive test result for specific IgE (≥0.35 kU_A/L) to at least 1 of the 12 allergens. A cutoff of 1.00 kU_A/L or greater was also examined. Furthermore, based on sensitization status (≥0.35 kU_A/L), we examined phenotypes of allergic asthma (asthma medication use with sensitization) and nonallergic asthma (asthma medication use without sensitization). FEV_1 and forced vital capacity values were measured with a daily calibrated Vitalograph spirometer (Vitalograph, Ennis, Ireland). From these measurements, we calculated the FEV_1/forced vital capacity ratio and FEV_1 as a percentage of predicted value (based on values from Qaunjer et al[20]) as continuous variables.

Self-reported outcomes

In 2008, at approximately 18 years of age, 382 (72%) offspring completed a self-administered Web-based questionnaire with 4 questions on asthma and hay fever that followed the International Studies on Asthma and Allergies in Childhood core questionnaire.[21] Based on answers to this questionnaire, we identified subjects reporting "ever doctor diagnosed asthma," "ever doctor diagnosed hay fever," "current asthma medication use," and "current hay fever symptoms."

Analytic strategy

We decided *a priori* to base the evaluation of the effects of fish oil on the comparison between the fish oil group and the olive oil group because these groups consisted of a double-blind administration of oil capsules. Therefore our main analyses included 396 mother-offspring pairs, 262 from the fish oil group and 134 from the olive oil group. This decision is supported by data collected postpartum and discussed in detail elsewhere,[11] showing that randomization between the 2 blind arms worked well, that olive oil was provided in isocaloric amounts to fish oil, and that the dose of olive oil could reasonably be assumed to be inert in relation to the outcome under study. In contrast, the no oil group was unblinded, was informed of the objectives of the study and the potential health benefits of fish consumption, and had comparable effects on gestation length as seen in the active intervention arm in the original trial.[14] Therefore we considered the no oil arm to represent the effect of a passive intervention consisting of nutritional advice. Comparisons of outcomes between the no oil arm and the active intervention (fish oil) and control (olive oil) arms are presented in Tables E1 and E2 in this article's Online Repository at www.jacionline.org.

Cumulative incidence curves were generated by using the Kaplan-Meier method. In primary intention-to-treat analyses Cox proportional hazards regression analysis was used to estimate the effect of fish oil relative to that of olive oil expressed by hazard ratios (HRs) and 95% CIs; age was used as the