



Fish, Fruit and Other Food

## Fish and seafood consumption during pregnancy and the risk of asthma and allergic rhinitis in childhood: a pooled analysis of 18 European and US birth cohorts

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## Abstract

**Background:** It has been suggested that prenatal exposure to n-3 long-chain fatty acids protects against asthma and other allergy-related diseases later in childhood. The extent to which fish intake in pregnancy protects against child asthma and rhinitis symptoms remains unclear. We aimed to assess whether fish and seafood consumption in pregnancy is associated with childhood wheeze, asthma and allergic rhinitis.

**Methods:** We pooled individual data from 60 774 mother-child pairs participating in 18 European and US birth cohort studies. Information on wheeze, asthma and allergic rhinitis prevalence was collected using validated questionnaires. The time periods of interest were: infancy (0-2 years), preschool age (3-4 years), and school age (5-8 years). We used multivariable generalized models to assess associations of fish and seafood (other than fish) consumption during pregnancy with child respiratory outcomes in cohort-specific analyses, with subsequent random-effects meta-analyses.

**Results:** The median fish consumption during pregnancy ranged from 0.44 times/week in The Netherlands to 4.46 times/week in Spain. Maternal fish intake during pregnancy was not associated with offspring wheeze symptoms in any age group nor with the risk of child asthma [adjusted meta-analysis relative risk (RR) per 1-time/week = 1.01, 95% confidence interval 0.97-1.05] and allergic rhinitis at school age (RR = 1.01, 0.99-1.03). These results were consistently found in further analyses by type of fish and seafood consumption and in sensitivity analyses.

**Conclusion:** We found no evidence supporting a protective association of fish and seafood consumption during pregnancy with offspring symptoms of wheeze, asthma and allergic rhinitis from infancy to mid childhood.

**Key words:** Wheezing, asthma, allergic rhinitis, children, fish, seafood, pregnancy

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**Key Messages**

- Findings from previous studies on the association of fish intake during pregnancy with offspring asthma and other allergy-related diseases are inconsistent.
- Heterogeneity between previous findings might be partly explained by small sample sizes and differences in diet assessment and health outcome definitions.
- We studied the association of fish and seafood consumption in pregnancy with child asthma and rhinitis outcomes in a combined analysis of 18 European and US birth cohorts with harmonized exposure and outcome variables according to a common protocol.
- Our results do not provide evidence of a beneficial association between fish and seafood consumption in pregnancy and development of asthma and allergic rhinitis symptoms in children up to 8 years of age.

**Introduction**

Asthma and allergic rhinitis are two of the most common chronic diseases in childhood, and their prevalence rates have increased over the past decades.<sup>1</sup> Emerging evidence suggests that a nutritional stressor or stimulus applied in intrauterine and early postnatal life can affect the development and maturation of respiratory and immune systems.<sup>2</sup> In this context, there has been a strong debate about the role of fish intake during pregnancy in the prevention of child asthma and other allergic diseases. Fish, and especially oily fish, is a rich source of n-3 long-chain fatty acids, which have been suggested to decrease the risk of allergic disease by exerting anti-inflammatory properties and modulating immune responses.<sup>3</sup> However, it is also a major source of human exposure to harmful environmental contaminants, such as methylmercury, polychlorinated biphenyls and dioxins.<sup>4</sup> Findings from birth cohort studies on the association of fish intake during pregnancy with offspring allergy-related disease symptoms are inconsistent, with reports of beneficial (i.e. lower incidence),<sup>5–8</sup> null<sup>9–16</sup> or even harmful effects.<sup>17</sup> Likewise, prospective studies assessing n-3 fatty acid intake<sup>18</sup> or biomarker levels in pregnancy<sup>19–25</sup> and trials of n-3 long-chain fatty acid supplementation in pregnancy<sup>18,26,27</sup> have yielded divergent results.

Most previous studies relied on relatively small sample sizes with a short duration of follow-up, and findings from one study were not replicated across populations with different fish consumption patterns. In the present analysis, we addressed these limitations by harmonizing and pooling for the first time individual data from a large network of cohorts in Europe and the USA, involving 60 774 pregnant women and their children with repeated follow-ups until the age of 8 years, to assess the strength and consistency of the association of fish and seafood consumption during pregnancy with the risk of childhood wheeze, asthma and allergic rhinitis.

**Methods****Study population**

European birth cohort studies participating in our previous analysis on fish intake in pregnancy and birth outcomes<sup>28</sup> were invited to participate if information on symptoms of asthma and rhinitis in childhood was available. From the 19 potentially eligible cohorts, 16 cohorts provided relevant data for the present analysis: ABCD (The Netherlands); DNBC (Denmark); FLEHS I (Belgium); GASPII (Italy); Generation R study (The Netherlands); Generation XXI study (Portugal); HUMIS (Norway); INMA (Spain); KOALA (The Netherlands); Lifeways Cross Generation (Ireland); LucKi (The Netherlands); NINFEA (Italy); PELAGIE (France); PIAMA (The Netherlands); RHEA (Greece); and SWS (UK). In addition, the Project Viva cohort from Massachusetts and the Bologna Birth Cohort agreed to take part in the current analysis. All participating cohorts covered deliveries from 1996 to 2011. In all cohorts, participants' parents or legal guardians provided written informed consent, and ethical approval was obtained from the local authorized institutional review boards. A data transfer agreement document was signed by each cohort study, and anonymized datasets were transferred to the University of Crete for analysis.

Overall, the study population included 60 774 mother-child pairs with information on fish intake during pregnancy, selected confounding variables and at least one of the health outcomes studied. Characteristics of the participating cohorts are shown in the eTable 1 and eFigure 1 (available as [Supplementary data](#) at *IJE* online).

**Fish and seafood consumption during pregnancy**

The exposure of interest was the frequency (times/week) of total fish, fatty fish, lean fish and seafood (other than fish) intake during pregnancy derived from cohort-specific food

frequency questionnaires or questionnaires specifically designed to assess fish intake during pregnancy (eTable 1, available as [Supplementary data](#)). Salmon, herring, mackerel, trout, sardines, Greenland halibut, anchovy, gurnard and tuna were classified as fatty fishes, whereas cod, pollock, plaice, flounder, garfish and similar species were classified as lean fishes. To align our analysis with the recent advice from the United States Food and Drug Administration and Environmental Protection Agency<sup>29</sup> on fish intake during pregnancy, and examine a potential dose-response relationship, we categorized total fish intake into the following categories; low:  $\leq 1$  time/week, moderate:  $> 1$  but  $\leq 3$  times/week and high:  $> 3$  times/week.

### Symptoms of asthma and rhinitis in childhood

Participating cohorts provided information on wheeze, asthma and allergic rhinitis occurrence, mostly obtained by questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC),<sup>30</sup> up to a maximum follow-up of 8 years. Cohort-specific information on outcome collection is shown in eTable 2 (available as [Supplementary data](#)). We defined wheeze in infancy as the presence of any episode of wheezing or whistling in the chest during the first 2 years of life, and wheeze in preschool and school-age children was defined as the presence of wheezing or whistling in the chest in the past 12 months reported at the ages of 3-4 years and 5-8 years, respectively, depending on the cohort-specific time points of assessment. Persistent wheeze at preschool and school age was defined as presence of wheeze both in infancy and the respective time period examined.<sup>31</sup> We defined asthma at preschool and school age as satisfying at least two of the three following criteria for each time period: (i) ever-reported doctor diagnosis of asthma; (ii) presence of wheezing or whistling in the chest in the past 12 months; or (iii) asthma medication in the past 12 months at the ages of 3-4 years and 5-8 years, respectively.<sup>32</sup> Allergic rhinitis at school age was defined as ever-reported diagnosis of allergic rhinitis or hay fever at the age of 5-8 years. If participants among the cohorts had repeatedly collected data within the same period, we used data collected at the oldest age.

### Covariates

Important covariates or effect modifiers were defined as similarly as possible among the cohorts. Information on maternal pre-pregnancy body mass index (BMI; in  $\text{kg}/\text{m}^2$ ), maternal smoking during pregnancy (yes/no), gestational weight gain (in kg), maternal age at delivery (in years), birthweight (in grams), gestational age (in weeks) and child

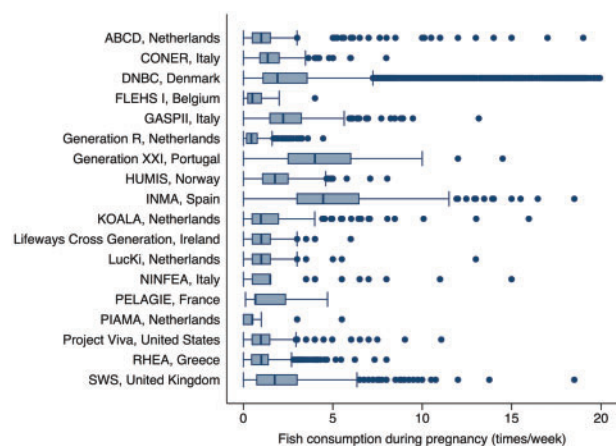
sex (male or female) was collected by means of interviews or self-administered questionnaires, *ad hoc* measurements, birth records or medical registries. Information on parity (primiparous or multiparous), breastfeeding (yes/no), maternal education (cohort-specific definitions of low, medium or high), attendance at nursery school during infancy (yes/no), exposure to mould or dampness at home during infancy (yes/no) and history of parent asthma or hay fever (yes/no) was obtained through interviews or self-administered questionnaires.

### Statistical analysis

We used multivariable generalized linear models for binary outcomes (modified Poisson<sup>33</sup>) to estimate relative risks (RRs) and their 95% confidence intervals (CIs) for the associations of maternal fish and seafood consumption during pregnancy with offspring wheeze, asthma and allergic rhinitis in childhood. Selection of suitable covariates for model adjustment was based on previous knowledge and a directed acyclic graph (DAG) approach (eFigure 2, available as [Supplementary data](#) at *IJE* online). The set of variables identified by the DAG were maternal education, maternal smoking during pregnancy, parity, breastfeeding and parent asthma or hay fever (maternal allergy for PELAGIE). This list of variables plus maternal age and child sex, which are considered important determinants of child health outcomes, were the covariates included in multivariable models. For the cohort Generation XXI, the adjusted models did not include parent history of asthma or hay fever, owing to a large proportion of missing information on this variable. Further adjustment for maternal pre-pregnancy BMI, attendance at nursery school during infancy and exposure to mould or dampness at home during infancy did not materially change estimates for fish intake but reduced substantially the sample size; thus, we did not include these factors in our final models.<sup>34</sup> Additionally, we did not include birthweight or gestational age in our main models, as we assumed that they are potential mediators in the pathway between fish intake during pregnancy and childhood respiratory health outcomes.<sup>28,35</sup>

We analysed associations by undertaking cohort-specific analyses with subsequent random-effects meta-analysis. We assessed heterogeneity between cohort-specific effect estimates with the  $I^2$  statistic and  $\chi^2$  test from Cochran's Q.<sup>36</sup> We also did pooled analysis including all participating cohorts by using mixed models with a random cohort intercept.

We performed several sensitivity analyses. First, we assessed the robustness of the results by repeating the meta-analyses while excluding one cohort at a time. Second, we examined the potential effect of the geographical location



**Figure 1.** Frequency of fish consumption during pregnancy (times/week) in participating cohorts. The line within the box marks the median; the boundaries of the box indicate the 25th and 75th percentiles; horizontal bars denote the variability outside the upper and lower quartiles (i.e., within 1.5 IQR of the lower and upper quartiles); and circles represent outliers.

of participating cohorts, based on the United Nations' classification (Southern Europe, Western Europe, Northern Europe and Northern America), by conducting meta-regression analyses. Third, we made further adjustment for gestational weight gain, used as a proxy for energy intake during pregnancy, for cohorts with available information. Fourth, we included birthweight and gestational age in the multivariable models to account for potential mediating effect of fetal growth. Finally, we assessed whether the effect estimates for wheeze in infancy and asthma in childhood varied by parent asthma or hay fever (yes vs no), and child sex (boy vs girl) by introducing interaction terms (one at a time), and conducting stratified analyses.<sup>34</sup> We performed analyses with STATA version 13 (StataCorp) and R version R3.1 (R Foundation).

## Results

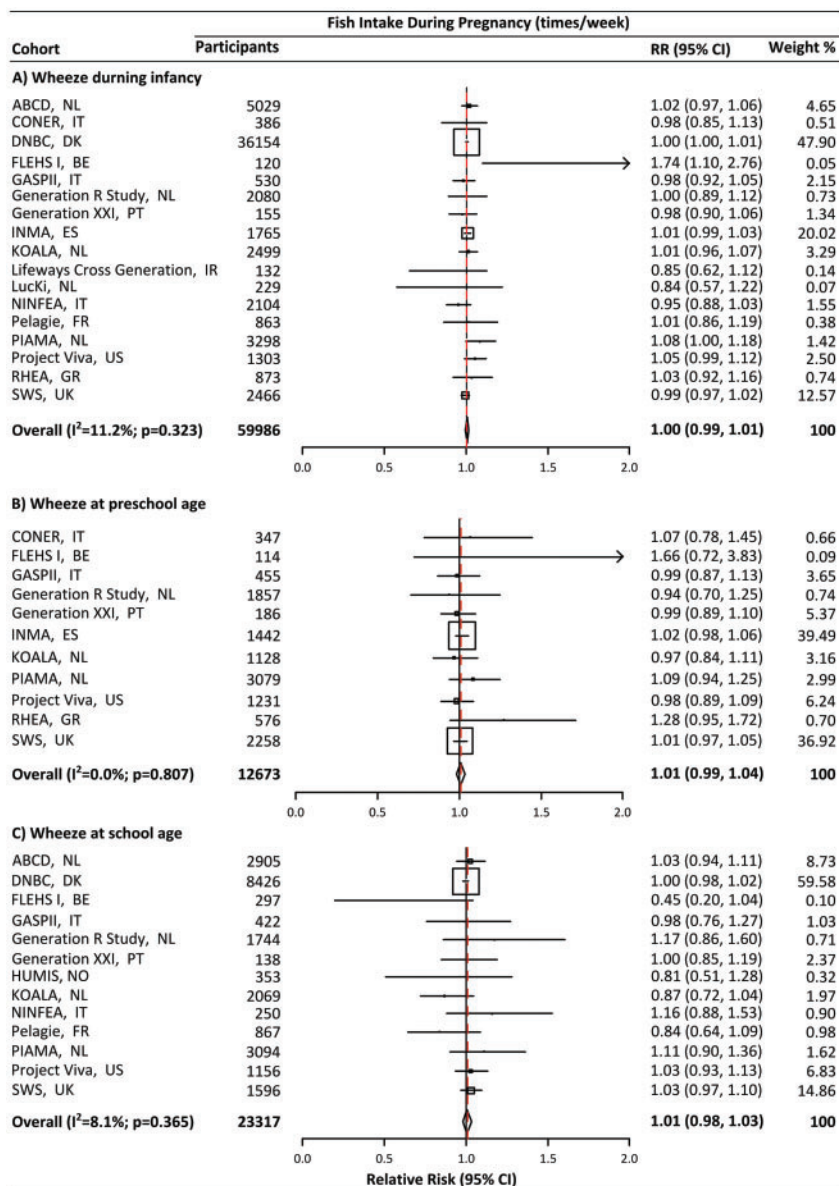
Participants' characteristics are shown in eTables 3 to 6 (available as [Supplementary data](#) at *IJE* online). Mothers had a median age of 30.4 years, were predominantly non-smokers, gave birth mainly to normal weight neonates and most of them breastfed their children. The ratio of boys to girls was 1.04 (eTable 3). The median fish consumption during pregnancy varied between cohorts and ranged from 0.44 times/week in the Generation R Study to 4.46 times/week in the INMA study (Figure 1; eTable 4). The highest median seafood intake was also reported in the INMA study (0.92 times/week) (eTable 4). We observed no differences in fish consumption levels between mothers with and without asthma or hay fever across cohorts (eTable 5).

In total, 17 518 (29.2%), 1949 (15.4%), and 3050 (13.1%) children had wheeze in infancy (0-2 years), at preschool (3-4 years) and school age (5-8 years), respectively (eTable 6); 921 (9.2%) and 2479 (10.5%) children had asthma at preschool and school age, respectively, and 1914 (5.4%) children had allergic rhinitis at school age (eTable 6).

Maternal fish intake during pregnancy was not associated with offspring wheeze symptoms in any age group (Figure 2 and Table 1). Also, no association was observed for persistent wheeze at preschool or school age (eTable 7, available as [Supplementary data](#) at *IJE* online). When we examined the risk of asthma at either preschool or school age, we observed no association with fish intake during pregnancy [adjusted meta-analysis RR per 1-time/week 1.02 (95% CI 0.97-1.07) and 1.01 (0.97-1.05), respectively; Figure 3 and Table 2]. Similarly, fish intake was not associated with the diagnosis of allergic rhinitis among school-age children (Table 2).

Further analysis by type of fish and seafood (other than fish) consumption did not modify the observed associations (Tables 1 and 2). Crude and adjusted estimates did not differ substantially (data not shown). Heterogeneity between cohort-specific risk estimates was low for most exposure-outcome associations (Tables 1 and 2; eTable 7), and the combined effect estimates from random-effect meta-analyses were broadly similar to those from the pooled analysis (eTables 8 and 9, available as [Supplementary data](#) at *IJE* online).

In sensitivity analyses, omitting one cohort at a time did not materially change the results (eTables 10 and 11, available as [Supplementary data](#) at *IJE* online). The risk estimates also remained similar when we took into account the geographical location of participating cohorts in meta-regression analyses, and when additional adjustment was made for gestational weight gain or birthweight and gestational age in multivariable models (data not shown). In stratified analyses, we found that fish intake in pregnancy was associated with a lower risk of asthma in preschool children with no history of parental asthma or hay fever [RR per 1 time/week 0.91 (95% CI 0.86-0.96)], but not in those of parents having either of these conditions [1.04 (0.98-1.10)]; *P* for interaction = 0.03; eTable 12, available as [Supplementary data](#) at *IJE* online). However, this interaction with parental history of asthma or hay fever was not observed for associations with wheeze or with asthma and allergic rhinitis at school age. Fish intake during pregnancy tended to be more protective against asthma in school-age boys [RR per 1 time/week 0.99 (0.99-1.00)] but not in girls [1.01 (0.99-1.03)]; *P* for interaction = 0.03, but associations were small in magnitude and not clinically relevant (eTable 12).



**Figure 2.** Adjusted associations of fish consumption during pregnancy (times/week) with offspring wheeze in infancy, preschool age, and school age. Wheeze in infancy was defined as presence of any episode of wheezing or whistling in the chest during the first 2 years of life. Wheeze at preschool age was defined as presence of wheezing or whistling in the chest in the past 12 months at the age of 3-4 years. Wheeze at school age was defined as presence of wheezing or whistling in the chest in the past 12 months at the age of 5-8 years. RRs (95% CIs) by cohort were obtained by using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal education, breastfeeding, smoking during pregnancy, parity, parent asthma or hay fever, and child sex. Combined estimates were obtained by using a random-effects meta-analysis. The names of the cohorts and number of participants per each cohort are shown on the left. Cohort-specific RRs (95% CIs), and their weights in meta-analysis are shown on the right. Squares represent the point estimate of each cohort, while the size of the square is proportional to the weight with which each cohort contributed to the overall RR; horizontal lines denote 95% CIs; and diamonds represent overall estimates. RR= relative risk.

## Discussion

In this study of more than 60 000 mother child pairs from several locations in Europe and the USA with repeated follow-up data from birth up to 8 years, we found no protective association between fish and seafood consumption during pregnancy and risk of childhood wheeze, asthma

and allergic rhinitis. The present study is a major extension of previous collaborative work examining fish intake during pregnancy and child health.<sup>28,37</sup>

Fetal life is a critical time period for airway and immune development, during which dietary exposures could have irreversible long-term influences on the development of

**Table 1.** Adjusted combined associations<sup>a</sup> of fish and seafood (other than fish) consumption in pregnancy with childhood wheeze

		Wheeze										
		Infancy <sup>b</sup>			School age <sup>d</sup>							
		Preschool age <sup>c</sup>			School age <sup>d</sup>							
		Cohorts	Participants	RR (95% CI)	I <sup>2</sup> (P heter) <sup>e</sup>	Cohorts	Participants	RR (95% CI)	I <sup>2</sup> (P heter) <sup>e</sup>			
Fish consumption (times/week)	17	59986	1.00 (0.99-1.01)	11.2 (0.32)	11	12673	1.01 (0.99-1.04)	0.0 (0.81)	13	23317	1.01 (0.98-1.03)	8.1 (0.37)
Fish consumption categories <sup>f</sup>												
> 1 but ≤ 3 times/week	17	25144	0.98 (0.94-1.03)	17.7 (0.25)	11	3308	0.98 (0.88-1.10)	0.0 (0.65)	11	8318	1.01 (0.93-1.10)	0.0 (0.59)
> 3 times/week	14	13311	0.96 (0.89-1.03)	16.8 (0.27)	8	2039	0.98 (0.84-1.14)	0.0 (0.44)	9	3382	1.05 (0.95-1.17)	0.0 (0.69)
Type of fish consumption (times/week)												
Fatty fish	11	46492	1.00 (0.99-1.01)	0.0 (0.79)	8	7623	1.00 (0.96-1.04)	0.0 (0.78)	7	14156	0.99 (0.96-1.02)	0.0 (0.88)
Lean fish	11	46492	1.00 (0.99-1.01)	0.0 (0.76)	8	7623	1.04 (0.99-1.10)	25.9 (0.22)	7	14156	1.01 (0.98-1.03)	0.0 (0.44)
Seafood consumption (times/week)	14	49579	1.04 (0.99-1.10)	56.8 (0.01)	9	7737	1.03 (0.97-1.10)	0.0 (0.82)	10	15572	1.06 (0.94-1.20)	23.1 (0.23)

<sup>a</sup>Relative risks (95% CIs) were estimated by random-effects meta-analysis by cohort. Generalized linear models for binary outcomes (modified Poisson) were adjusted for maternal age, maternal education, breastfeeding, smoking during pregnancy, parity, parent asthma or hay fever and child sex.

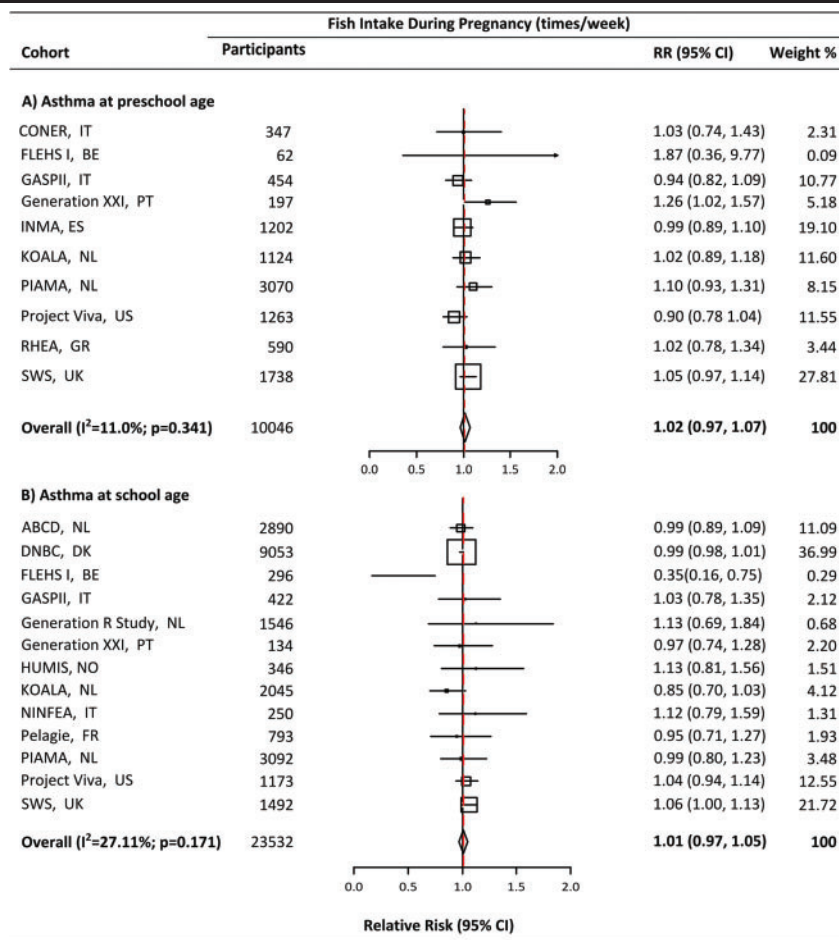
<sup>b</sup>Wheeze in infancy was defined as presence of any episode of wheezing or whistling in the chest during the first 2 years of life.

<sup>c</sup>Wheeze at preschool age (3-4 years) was defined as presence of wheezing or whistling in the chest in the past 12 months.

<sup>d</sup>Wheeze at school age (5-8 years) was defined as presence of wheezing or whistling in the chest in the past 12 months at the age of 5-8 years.

<sup>e</sup>I<sup>2</sup> statistic and P-value for heterogeneity estimated by the  $\chi^2$  test from Cochran's Q.

<sup>f</sup>Reference category: ≤ 1 time/week.



**Figure 3.** Adjusted associations of fish consumption during pregnancy (times/week) with offspring asthma at preschool age and school age. Asthma at preschool age (3-4 years) and school age (5-8 years) was defined as satisfying at least two of the three following criteria for each time period: (1) ever-reported diagnosis of asthma, (2) presence of wheezing or whistling in the chest in the past 12 months, or (3) asthma medication in the past 12 months. RRs (95% CIs) by cohort were obtained by using generalized linear models for binary outcomes (modified Poisson) adjusted for maternal age, maternal education, breastfeeding, smoking during pregnancy, parity, parent asthma or hay fever, and child sex. Combined estimates were obtained by using random-effects meta-analysis. The names of the cohorts and number of participants per each cohort are shown on the left. Cohort-specific RRs (95% CIs), and their weights in meta-analysis are shown on the right. Squares represent the point estimate of each cohort, while the size of the square is proportional to the weight with which each cohort contributed to the overall RR; horizontal lines denote 95% CIs; and diamonds represent overall estimates. RR= relative risk.

asthma and other allergy-related diseases.<sup>38</sup> The n-3 long-chain fatty acids contained within fish can be transferred across the placenta and have been proposed to exert a protective effect through involvement in several immune regulatory pathways. Animal and *in vitro* studies have shown n-3 fatty acids to alter the expression of inflammatory genes by modifying transcription factor activation, inhibit T cell signalling through alteration of membrane lipid rafts and decrease the production of proinflammatory eicosanoids, cytokines and reactive oxygen species, as well as to give rise to a family of anti-inflammatory mediators termed resolvins (reviewed in Calder *et al.*<sup>39</sup>). A beneficial effect of n-3 long-chain fatty supplementation in fetal or early life on childhood allergic disease symptoms has been shown in many, but not all, randomized trials.<sup>18,26,27,40</sup> A relation between supplementation dose and effect becomes more

apparent in the very low end of the exposure spectrum of the n-3 long-chain fatty acids. Although fish and seafood is the major source of preformed n-3 long-chain fatty acids in the diet in most populations worldwide, maternal status is also determined by endogenous conversion from dietary alpha-linolenic acid from plant sources. A minority of the population has a genetically determined low endogenous conversion rate, and hence a higher dependence of preformed n-3 long-chain fatty acid intake from the diet.<sup>41</sup> Therefore, our results do not exclude the possibility of a protective effect of fish intake against asthma and allergy in vulnerable subgroups.

Several birth cohort studies have examined the association of prenatal fish intake with asthma and rhinitis symptoms in childhood and have produced mixed results. High fish intake during pregnancy was associated with a



**Table 2.** Adjusted combined associations<sup>a</sup> of fish and seafood (other than fish) consumption in pregnancy with asthma and allergic rhinitis in childhood

	Asthma <sup>b</sup>						Allergic rhinitis					
	Preschool age			School age			School age <sup>c</sup>			School age <sup>c</sup>		
	Cohorts	Participants	RR (95% CI) I <sup>2</sup> (P heter) <sup>d</sup>	Cohorts	Participants	RR (95% CI) I <sup>2</sup> (P heter) <sup>d</sup>	Cohorts	Participants	RR (95% CI) I <sup>2</sup> (P heter) <sup>d</sup>	Cohorts	Participants	RR (95% CI) I <sup>2</sup> (P heter) <sup>d</sup>
Fish consumption (times/week)	10	10046	1.02 (0.97-1.07) (0.34)	13	23532	1.01 (0.97-1.05) (0.17)	10	35789	1.01 (0.99-1.03) (0.44)	10	35789	1.01 (0.99-1.03) (0.44)
Fish consumption categories <sup>e</sup>												
> 1 but ≤ 3 times/week	8	2717	0.93 (0.78-1.10) (0.52)	11	8472	1.02 (0.92-1.12) (0.46)	10	14777	0.99 (0.84-1.16) (0.26)	10	14777	0.99 (0.84-1.16) (0.26)
> 3 times/week	7	1643	1.00 (0.68-1.45) (0.15)	9	3585	1.09 (0.97-1.23) (0.61)	7	7827	0.99 (0.77-1.26) (0.23)	7	7827	0.99 (0.77-1.26) (0.23)
Type of fish consumption (times/week)												
Fatty fish	8	6914	0.96 (0.88-1.06) (0.33)	7	14661	1.00 (0.97-1.03) (0.63)	6	26853	1.02 (0.98-1.05) (0.83)	6	26853	1.02 (0.98-1.05) (0.83)
Lean fish	8	6914	1.06 (0.99-1.14) (0.81)	7	14661	1.04 (0.96-1.12) (0.17)	6	26853	0.99 (0.86-1.14) (0.09)	6	26853	0.99 (0.86-1.14) (0.09)
Seafood consumption (times/week)	9	6976	1.03 (0.92-1.15) (0.51)	10	16002	1.07 (0.91-1.25) (0.11)	7	27101	1.04 (0.87-1.24) (0.13)	7	27101	1.04 (0.87-1.24) (0.13)

<sup>a</sup>Relative risks (95% CIs) were estimated by random-effects meta-analysis by cohort. Generalized linear models for binary outcomes (modified Poisson) were adjusted for maternal age, maternal education, breastfeeding, smoking during pregnancy, parity, parent asthma or hay fever and child sex.

<sup>b</sup>Asthma at preschool age (3-4 years) and school age (5-8 years) was defined as satisfying at least two of the three following criteria for each time period: (i) ever-reported diagnosis of asthma; (ii) presence of wheezing or whistling in the chest in the past 12 months; or (iii) asthma medication in the past 12 months.

<sup>c</sup>Allergic rhinitis at school age (5-8 years) was defined as ever-reported diagnosis of allergic rhinitis or hay fever.

<sup>d</sup>I<sup>2</sup>-statistic and P-value for heterogeneity estimated by the  $\chi^2$  test from Cochran's Q.

<sup>e</sup>Reference category: ≤ 1 time/week.

lower risk of wheeze up to the age of 6.5 years in the Infancia y Medio Ambiente-Environment and Childhood Project-Menorca cohort,<sup>5,7</sup> and the Danish National Birth Cohort study showed a protective effect of fish intake on ever asthma at the age of 7 years, by using hospital admission and prescription registry data.<sup>6</sup> Other prospective studies conducted in The Netherlands,<sup>12</sup> Finland,<sup>11,16</sup> Norway,<sup>14</sup> Spain and Greece,<sup>13</sup> France<sup>15</sup> and Japan<sup>9,10</sup> did not find an association of prenatal fish consumption with wheeze or asthma occurrence in childhood. Only few previous studies have assessed symptoms of childhood allergic rhinitis in association with fish intake in pregnancy; one UK study found that higher maternal intake of oily fish was protective against hay fever at age 5 years,<sup>8</sup> whereas the Danish National Birth Cohort study<sup>6</sup> and a Finnish study<sup>11</sup> found no association with allergic rhinitis up to the age of 7 years. Heterogeneity in previous findings may arise from differences in sample sizes, exposure assessment, health outcome definition and adjustment for confounding variables. In the present study, we had a large sample size and harmonized information on exposure variables, potential confounders and child asthma and rhinitis symptoms. We examined child outcomes at different time points from early infancy to mid childhood, and found little evidence for an association with fish intake during pregnancy.

Fish is generally considered an integral component of a healthy diet. However, it is a complex nutritional exposure. Fish is a good source of beneficial nutrients, including n-3 long-chain fatty acids, but is also a common route of exposure to persistent organic pollutants, such as polychlorinated biphenyls and dioxins.<sup>4</sup> There is emerging evidence suggesting that prenatal exposure to these chemicals can exert adverse immunomodulatory effects and increase the risk of developing allergic disease symptoms in childhood.<sup>42,43</sup> Hence, it is possible that pollutants bio-accumulating in fish counterbalance the effects of beneficial nutrients, moving the association between fish intake and child respiratory health towards the null. We did not have the possibility to collect information on amounts of nutrients and environmental chemicals contained in fish. Although we collected information on different fish types (fatty fish, lean fish, shellfish), we did not have enough data to distinguish between consumption of big and small species and fish origin, which would be relevant with respect to toxicant exposure. Further analyses using biomarker information on both nutrients and toxicants in fish are required to examine their interplay, as well as to disentangle potential opposite effect on child outcomes.

Genetic predisposition plays a major role in asthma.<sup>44,45</sup> A recent systematic review concluded that family history of allergy should be considered as an

important effect modifier when studying the association of diet with the development of childhood asthma or allergies.<sup>34</sup> It has been proposed that atopic heredity is associated with abnormalities in fatty acid metabolism,<sup>46</sup> and this might affect body utilization of the fatty acids contained in fish. Previous studies examining a potential modifying effect of maternal history of asthma or atopy on prenatal fish intake have produced inconsistent results.<sup>47,48</sup> In our study, we found that, among children of parents with no history of asthma or hay fever, prenatal fish intake was associated with lower asthma risk at preschool age, but not later in childhood or with wheeze or allergic rhinitis. Thus we treat this result with caution, as we cannot rule out the possibility that the observed association is due to chance. Further prospective studies incorporating genetic analysis are needed to evaluate whether genetic predisposition can modify the effect of fish intake during pregnancy on child allergy symptoms.

As in any observational study, there is the possibility of unmeasured residual confounding accounting for at least part of the observed findings. We had no data available to adjust for energy intake or dietary patterns during pregnancy; however, adjustment for gestational weight gain, as a good proxy for total energy intake in pregnancy,<sup>49,50</sup> did not appreciably change the effect estimates. Moreover, although we examined breastfeeding as a potential confounder, other differences in child diet or lifestyle factors might have influenced the observed findings. However, the absence of important heterogeneity across cohorts and the similar effect estimates observed in the meta-analysis and the pooled analysis provide evidence in support of more robust and generalized conclusions.

Similar to most studies assessing the health effects of diet, we used self-reported dietary information, and we cannot rule out the possibility of exposure measurement error, especially in the absence of biomarkers of fish intake. However, most cohorts assessed fish intake using food frequency questionnaires that were developed and validated for use in pregnancy. Additionally, previous analyses in many participating cohorts have shown fish intake, as assessed by questionnaires, to positively correlate with exposure to n-3 long-chain fatty acids or environmental pollutants commonly found in fish.<sup>51-57</sup> Any measurement error in the assessment of fish intake itself is likely to be non-differential with respect to the outcomes of interest, given that fish intake levels did not differ between mothers with and without asthma or hay fever across the cohorts, and dietary data were collected before child respiratory outcomes were known. Child asthma and allergic rhinitis symptoms were ascertained by symptom-based questionnaires, which could lead to outcome misclassification.

However, most participating cohorts used questionnaires adapted from the ISAAC study.<sup>30</sup> To enhance asthma outcome accuracy, we used a definition developed by a panel of experts within the Mechanisms of the Development of Allergy consortium<sup>32</sup> that requires at least two conditions to be met. We assessed several asthma-related phenotypes, raising concern about multiple testing. However, an application of correction to take into account multiple comparisons would be inappropriate given that we studied outcomes that are highly correlated.<sup>58</sup>

In conclusion, this large multicohort study found no evidence supporting a protective association between fish intake during pregnancy and offspring symptoms of wheeze, asthma and allergic rhinitis from infancy to mid childhood.

## Supplementary Data

Supplementary data are available at *IJE* online.

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## Author Contributions

N.S., T.R. and L.C. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: L.C. Acquisition of data: all authors. Statistical analysis: T.R. and N.S. Interpretation of data: all authors. Drafting of the manuscript: N.S. and L.C. Critical revision of the manuscript for important intellectual content: all authors. Study supervision: L.C.

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