

ORIGINAL PAPER

Maternal fish intake during pregnancy and atopy and asthma in infancy

I. Romieu*, M. Torrent[†], R. Garcia-Esteban[‡], C. Ferrer[‡], N. Ribas-Fitó[‡], J. M. Antó^{‡,§} and J. Sunyer^{‡,§}*National Institute of Public Health, Cuernavaca, Mexico, [†]Area de Salud de Menorca, IB-SALUT, Menorca, Spain, [‡]Centra de Recerca en Epidemiologia Ambiental (CREAL), Institut Municipal d'Investigacio Medica, Barcelona, Spain and [§]Universitat Pompeu Fabra, Barcelona, SpainClinical and
Experimental
Allergy

Summary

Background There is growing evidence that n-3 fatty acids have anti-inflammatory properties and may modulate immune response. Dietary intake of these nutrients during pregnancy could play a role in the risk of asthma and atopy in the offspring.**Methods** Using data from a cohort of women (n = 462) enrolled during pregnancy and whose offspring were followed up to 6 years, we evaluated the impact of fish consumption during pregnancy on the incidence of atopy and asthma. Dietary intake was assessed by food frequency questionnaire (42 items) applied by an interviewer.**Results** Thirty-four percent of infants had a medical diagnosis of eczema at age 1 year, 14.3% of the children were atopic [based on skin prick test (SPT) at 6 years], and 5.7% had atopic wheeze at age 6 years. After adjusting for potential confounding factors, fish intake during pregnancy was protective against the risk of eczema at age 1 year, a positive SPT for house dust mite at age 6 years and atopic wheeze at age 6 years [odds ratio (OR) = 0.73 95% confidence interval (CI) 0.55–0.98, OR = 0.68, 95% CI 0.46–1.01 and OR = 0.55, 95% CI 0.31–0.96, respectively]. For an increase in fish intake from once per week to 2.5 times per week, the risk of eczema at age 1 year decreased by 37%, and the risk of positive SPT at age 6 years by 35%. Stratification by breastfeeding showed that fish intake was significantly related to a decrease risk in persistent wheeze among non-breastfed children (*P* for interaction < 0.05). No protective effect was observed among breastfed children.**Conclusion** Our data suggest a protective effect of fish intake during pregnancy on the risk of atopy-related outcomes.**Keywords** asthma, atopy, children, fish intake, n-3 PUFA, pregnancy

Submitted 31 July 2006; revised 22 January 2007; accepted 23 January 2007

Correspondence:

Dr Isabelle Romieu, Instituto Nacional de Salud Publica, 655 Avenida Universidad, Col. Santa Maria Ahuacatitlán, 62508, Cuernavaca, Morelos, México.
E-mail: iromieu@correo.insp.mx

Introduction

There is growing evidence that ω -3 polyunsaturated fatty acids (PUFAs) have anti-inflammatory properties and may modulate immune responses [1]. Increase in atopy and asthma observed in western countries could be related to a decreased intake of these nutrients while the intake of n-6 PUFAs from vegetables oil and prepared food has increased [1, 2]. Fish, particularly oily fish, is a major source of long chain n-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and could therefore play a role to modulate the risk of atopy and asthma [3].

PUFAs are important constituents of cells where they play a role assuring the correct environment for membrane protein function, maintenance of membrane

fluidity and regulating gene expression and cellular function [4]. Increase intake of n-3 PUFAs can decrease inflammatory reactions by changing the contents of lipid membranes and other substrates which are in turn the substrates for eicosanoid inflammatory mediators [5].

The substitution in the membrane of n-3 PUFAs [α linolenic acid (18:3n-3) and EPA (20:5n-3)] for n-6 fatty acids [linoleic acid (LA; 18:2n-6)] leads to the production of fewer and less potent inflammatory mediators, prostaglandin E₃ (PGE₃) instead of PGE₂, and leukotriene 5 (LTB₅) instead of leukotriene 4 (LTB₄). Higher concentrations of EPA were found in diets low in LA [6]. In addition, n-3 PUFAs can modulate T cell function directly through effects on cell membrane fluidity and consequent cell signalling and gene transcription [7] and reduce the

capacity of antigen presenting cells to present Ag to T cells [8].

Atopic sensitization is likely to occur *in utero* [9, 10]; therefore fish intake during pregnancy is particularly relevant. Fish intake during pregnancy (measured retrospectively at school age) has been related to a decreased risk of atopy to food allergens in the offspring in non-atopic mother only [11] while maternal oily fish consumption during pregnancy (also retrospectively assessed) appears to modulate the risk of early childhood asthma only in children born of a mother with an history of asthma [12]. Lower levels of γ -linolenic acid have been found in serum phospholipids of infants with eczema than in controls [13]. Prospective studies show disturbances in both the n-6 and n-3 fatty acid composition of serum and milk from atopic and non-atopic mothers [14–16]; however to date, there are no prospective data on the impact of dietary intake during pregnancy on allergy and asthma in the offspring.

To determine the impact of n-3 PUFAs intake (measured through fish consumption) during pregnancy on the incidence of atopy and asthma in early infancy, we used data from a large cohort of women enrolled during pregnancy and whose offsprings were followed up to 6.5 years of age.

Methods

Methods have been described previously [17]. Briefly, all women presenting for antenatal care in Menorca, Spain, over a 12-month period starting in mid 1997 were recruited (n = 507). Four hundred and eighty-two (95%) children were subsequently enrolled and from those 462 (97%) provided complete outcome data after 6.5 years of follow-up.

At baseline women filled a general purpose questionnaire. Three months after delivery a face to face food frequency questionnaire (FFQ) referred to the pregnancy period was applied by an interviewer. At birth, cord blood samples were obtained on 405 (83%) children and at 4 years blood sample were obtained on 360 (75%) children. Mothers were interviewed at 6, 14 months and 2 years after delivery regarding the type and duration of exclusive breastfeeding. Number of asthmatic parents, maternal smoking, parity, education and social class came from a questionnaire administered to the pregnant mothers [18]. Gestational age, birth weight and maternal body mass index (BMI) during pregnancy (first trimester), and BMI before pregnancy were extracted from clinical records and child BMI at 6.5 years was measured using standardized methods.

During the follow-up, parents answered a questionnaire on a yearly basis (with interviewer) and report all medical events over the preceding 12 months. One or more episodes of wheezing over 12 months constituted wheez-

ing during a given year. Outcomes of interest included: medical diagnosis of eczema at age 1 year, atopy at age 4 years based on IgE levels in serum, specific IgE levels to house dust mite (HDM), atopy at age 6 years based on skin prick test (SPT) response, skin test positivity to HDM, persistent wheeze at age 6 years (wheezing at 6 years and in any preceding years) and atopic wheeze at age 6 years (defined as atopy and wheeze at age 6 years). The study was approved by the corresponding ethical committees and written informed consent was obtained from the parents of all children [17].

Dietary assessment

All mothers completed the FFQ including 35 items, some of which were further specified [for example fish intake was further divided into intake of fatty fish (such as salmon, tuna, herring, sardines), non-fatty fish (hake, sole, gilthead bream) and mixed fatty and non-fatty fishes], giving in fact a 42 items FFQ. The questionnaire also included additional information on the type of oil used (for salad and for cooking) and changes in diet in the recent months. This questionnaire was based on the validated EPIC-Norfolk FFQ [19], translated into Spanish and shortened regrouping some foods into food groups. The number of times participant consumed specific foods during pregnancy were classified into never, times per year, month, week or day depending on the frequencies reported assuming a standard portion size for each items.

Skin prick test

Atopy of children at age 6 years and maternal atopy were measured using prick test [18]. A weal of 3 mm or greater in the presence of a positive histamine control and a negative uncoated control constituted a positive skin test. A positive skin test to at least one allergen [Der p 1 (HDM), Fel d 1, grass pollen, olive tree (*olea*), mixed gramineae, parietaria] was considered indicative of atopy.

Laboratory analysis

Specific IgE levels against Der p 1, Fel d 1 and mixed grass pollens were determined in the same laboratory using Uni cap (Pharmacia, Uppsala, Sweden) in the 4-year serum samples.

Statistical analysis

Complete data on dietary intake and type of fish consumed was available on 479 mothers. Twenty-one mothers (4.4%) reported never eating fish. In this group the prevalence of atopy and asthma was high (61.9% and 28.6% compared with 35% and 6% in the rest of our sample). These women were excluded from the analysis

because of the likelihood that their disease had modified their fish intake. Therefore our analysis included 458 mothers and their offspring. We assigned proportional weight to each frequency of consumption in order to obtain a weekly intake for all items provided in the FFQ. The following weights were assigned: never = 0, once per year = 1/52, once per month = 1/4, once per week = 1 and once per day = 7. The fish intake score was log transformed to normalize its distribution. To assess the role of fish intake during pregnancy on the risk of eczema, atopy, specific IgE levels and allergen sensitization, a persistent wheeze at 6 years (wheezing at 6 years and at least one previous year) and atopic wheeze at age 6 years we used logistic regression. Multivariate models were used to adjust for confounding variables and to test for interactions [20]. The following variables were considered as potential confounding factors: gender, maternal age, maternal and paternal atopy, maternal and paternal asthma, maternal and paternal social class [21], maternal smoking during pregnancy, maternal BMI before pregnancy (as proxy for caloric intake), gender, gestational age, birth weight, parity, breastfeeding, ownership of pets, BMI at age 6.5 years, dichlorodiphenyldichloroethylene in cord blood, and the child's fish consumption at age 4 years (using the same scoring system than for the mother's fish intake). All variables significantly related with the outcome of interest in the bivariate models ($P < 0.2$) were included in the multivariate model, and only retained if they had a statistically significant association (< 0.1) or modified the coefficient of fish intake by a 5%. The shape of the dose-response relationships were assessed using Generalized Additive Models (GAM). GAMs are flexible statistical methods that may be used to identify and characterize non-linear regression effects [22]. We observed a linear relationship between the fish intake score in the log scale and the different outcome of interest.

Because blood samples were not obtained on all children and that some missing variables were present on the yearly questionnaires, the sample size varies slightly between outcomes. General characteristics of infants with and without blood samples at birth and age 4 were similar. Women who did not breastfeed were more likely to be from lower social class and to have a low-weight baby (< 2500 g). Those variables were taken into account in regression models stratified by breastfeeding if justified (as described above). Analyses were conducted using Stata software, version 8.2 (StataCorp., College Station, TX, USA). Statistical tests of hypotheses were two-tailed with significance set at $P < 0.05$.

Results

Thirty-four percent of infants had a medical diagnosis of eczema as reported by the parents at 1 year of age. Atopy was present in 12.6% of the children at age 4 years and in

14.3% of children at age 6 years. Positive prick test sensitivity to HDM was present in 12.8% of the children. Close to 6% of the mother reported a medical diagnosis of asthma and 35% were atopic. 20.6% reported smoking during pregnancy. Most of the infants (93.2%) weighed more than 2.500 kg at birth. Eighty-three percent of the women breastfed.

Fish intake at least once a week was reported by 85.8% of the women among whom 39.7% reported fish intake up to once per week, 38.2% more than once to twice per week and 22.1% more than twice per week. The overall mean fish intake in our population corresponded to a frequency of 1.5 times per week. Women's interviews were conducted in different seasons of the year; however we did not observe differences in the reporting of fish consumption by season (test Kruskal-Wallis $P = 0.95$).

Table 1 presents the association of the characteristics of the mother, father and infant and fish intake score during

Table 1. Fish dietary intake score* of the mother during pregnancy according to population characteristics†

	Geometric			P-value
	N	Mean	(95% CI)	
Maternal age				
≤ 26	130	1.200	(1.056–1.363)	0.295
26–30	169	1.322	(1.182–1.479)	
> 30	156	1.441	(1.283–1.620)	
Maternal atopy (prick test)				
No	287	1.350	(1.238–1.472)	0.245
Yes	154	1.277	(1.135–1.437)	
Maternal asthma				
No	428	1.357	(1.265–1.456)	0.017
Yes	27	0.915	(0.692–1.209)	
Maternal social class				
Non-manual	206	1.469	(1.328–1.625)	0.219
Manual	148	1.241	(1.102–1.398)	
Housewife	87	1.198	(1.025–1.399)	
Maternal smoking during pregnancy				
Never	206	1.345	(1.215–1.489)	0.909
Former	156	1.314	(1.169–1.477)	
Current	94	1.300	(1.118–1.512)	
Paternal atopy (prick test)				
No	108	1.361	(1.170–1.582)	0.594
Yes	94	1.201	(1.022–1.412)	
Paternal asthma				
No	424	1.338	(1.247–1.436)	0.139
Yes	29	1.192	(0.910–1.562)	
Paternal social class				
Non-manual	161	1.356	(1.207–1.522)	0.840
Manual	283	1.291	(1.183–1.409)	
Gender				
Females	219	1.302	(1.179–1.437)	0.501
Males	237	1.347	(1.225–1.481)	
Gestational age				
≥ 37 weeks	436	1.328	(1.238–1.424)	0.493
< 37 weeks	20	1.264	(0.912–1.752)	

Table 1. Continued

	Geometric			P-value
	N	Mean	(95% CI)	
Birth weight				
>2500 g	425	1.333	(1.242–1.431)	0.182
≤2500 g	31	1.218	(0.937–1.583)	
Parity, firstborn				
No	234	1.357	(1.234–1.493)	0.634
Yes	222	1.292	(1.171–1.425)	
Breastfeeding				
No	76	1.285	(1.087–1.519)	0.427
Yes	380	1.333	(1.237–1.437)	
Duration of breastfeeding				
Never	76	1.285	(1.087–1.519)	0.448
≤3 months	121	1.300	(1.139–1.485)	
3–6 months	120	1.450	(1.269–1.656)	
>6 months	139	1.268	(1.120–1.435)	
Owned pets at age 1				
No	414	1.317	(1.226–1.415)	0.901
Yes	32	1.396	(1.078–1.807)	
BMI at 6.5 years				
Healthy weight	277	1.338	(1.230–1.455)	0.149
At risk of overweight	70	1.227	(1.038–1.450)	
Overweight	55	1.578	(1.307–1.906)	
pp-DDE cord blood				
≤0.7	123	1.410	(1.232–1.614)	0.397
0.7–1.45	131	1.280	(1.123–1.459)	
≥1.45	129	1.205	(1.056–1.375)	

*Times per week log transformed.

†Some missing information in questionnaires.

CI, confidence interval; BMI, body mass index; DDE, dichlorodiphenyldichloroethylene.

pregnancy. Only maternal asthma was significantly related to fish intake. Women with a medical diagnosis of asthma ate significantly less fish than women without a medical diagnosis of asthma ($P=0.017$). Most of the women reported eating non-fatty fish (59.4%), 36.8% reported eating both non-fatty and fatty fish, only 3.7% of the women reported eating only fatty fish.

Table 2 presents the dietary fish intake score according to the different outcomes of interest. Fish intake during pregnancy was lower for infants with a diagnosis of eczema at 1 year ($P=0.050$). No effect of fish intake was observed on IgE levels at age 4 years; however at age 6 years, fish intake during pregnancy was associated with sensitivity to HDM ($P=0.048$) and to atopic wheeze ($P=0.028$). Sensitivity to other allergens than HDM at age 6 years was very low ($n=3$ for cat, $n=1$ for gramineae, $n=9$ for olive tree and $n=1$ for parietaria).

Fish intake during pregnancy was related to eczema at age 1 year, atopy (positivity to any SPT), sensitivity to HDM and atopic wheeze at age 6 years with a dose-response relation in the log scale. After adjusting for potential confounding factors these associations remained

Table 2. Fish dietary intake score* of the mother during pregnancy by health outcomes†

	Geometric			P-value
	N	Mean	(95% CI)	
Eczema at 1 year				
No	255	1.390	(1.269–1.523)	0.050
Yes	132	1.183	(1.043–1.343)	
Unknown	69	1.377	(1.156–1.641)	
Specific IgE to any‡ at 4 years				
No	291	1.359	(1.254–1.474)	0.767
Yes	42	1.314	(1.062–1.625)	
Specific IgE to HDM				
<0.35 kU/L	293	1.353	(1.249–1.467)	0.991
≥0.35 kU/L	40	1.355	(1.090–1.685)	
Specific SPT to any‡ at 6 years				
<3 mm	342	1.391	(1.290–1.499)	0.096
≥3 mm	57	1.173	(0.975–1.410)	
SPT HDM				
<3 mm	348	1.395	(1.295–1.503)	0.048
≥3 mm	51	1.127	(0.928–1.369)	
Persistent wheeze at 6 years				
No	415	1.344	(1.254–1.441)	0.522
Yes	27	1.227	(0.934–1.611)	
Atopic wheeze at 6 years				
No wheeze no atopy	313	1.383	(1.277–1.497)	0.028
Wheeze and atopy	19	0.942	(0.683–1.301)	

*Times per week log transformed.

†Some missing information on outcomes. For eczema: unknown = 69; data on IgE available on 335 children; data on atopy at age 6 years available on 401 children, data on persistent wheeze available on 444 children; data on atopic wheeze do not include children with atopy no wheeze = 50 and no atopy and wheeze = 17.

‡Allergen tested included: house dust mite [HDM (Der p 1), cat, grass pollen, olive tree (*olea*), mixed gramineae and parietaria].

CI, confidence interval, SPT, skin prick test.

similar (Table 3). For an increase in fish intake from once per week to 2.5 times per week the risk of eczema at age 1 year decreased by 37% [odds ratio (OR) = 0.73, 95% confidence interval (CI) (0.55–0.98), the risk of atopy at age 6 years (positive SPT) decreased by 35.1% (OR = 0.74, 95% CI 0.50–1.09), and the risk of sensitivity to HDM at age 6 years decreased by 47.1% (OR = 0.68, 95% CI 0.46–1.01)]. Figure 1 illustrates the relation between the probability of HDM sensitivity at age 6 years and the weekly frequency of maternal fish intake during pregnancy (in log scale). For example, a change from twice per month (0.5 times per week) to once per week, reduced the risk of HDM sensitivity by 32% (OR = 0.76, 95% CI 0.55–0.98) and from once a week to 2.5 times per week by 47%. Fish intake had also a protective effect on atopic wheeze at age 6 years. An increase in fish intake from once per week to 2.5 times per week, reduced the risk of atopic wheeze by 81.8% (OR = 0.55, 95% CI 0.31–0.96) (Table 3). Further adjustment by the child's fish intake at

Table 3. OR (95% CI) per unit increase* for fish consumption

Outcome	Crude			Adjusted**†		
	OR	(95% CI)	P-value	OR	(95% CI)	P-value
Eczema at 1 year ¹	0.76	(0.58–1.00)	0.050	0.73	(0.55–0.98)	0.036
Specific IgE to any at 4 years ²	0.93	(0.59–1.47)	0.767	0.93	(0.59–1.47)	0.768
Specific IgE to HDM at 4 years ²	1.00	(0.62–1.61)	0.991	1.00	(0.62–1.62)	0.984
Specific SPT to any at 6 years ³	0.73	(0.51–1.06)	0.096	0.74	(0.50–1.09)	0.123
Specific SPT to HDM at 6 years ³	0.69	(0.47–1.00)	0.048	0.68	(0.46–1.01)	0.058
Persistent wheeze at 6 years ⁴	0.85	(0.51–1.41)	0.522	0.87	(0.51–1.49)	0.615
Atopic wheeze at 6 years ⁵	0.57	(0.35–0.94)	0.028	0.55	(0.31–0.96)	0.034

*Times per week log transformed.

†All models were adjusted for maternal asthma.

Also adjusted for: (1) type of fish, and smoking during pregnancy; (2) birth weight; (3) gender, maternal atopy and maternal social class; (4) gender, gestational age, and maternal social class; (5) gender and maternal social class.

OR, oddsratio; CI, confidence interval; HDM, house dust mite; SPT, skin prick test.

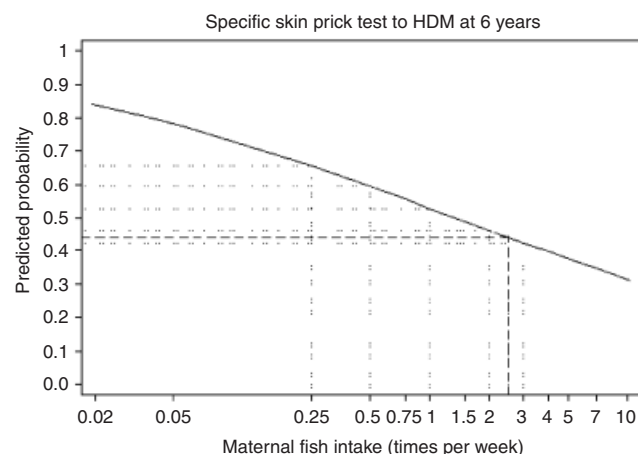


Fig. 1. Predicted probability of skin prick test sensitivity to house dust mite (HDM) at 6 years of age associated with weekly frequency of fish intake (log scale). Based on Fig. 1, the following changes in risk can be calculated; for example for a change from once a month (0.25 per week) to once a week reduces the risk by 72%; from twice per month (0.5 per week) to once per week by 32% and a change from once a week to 2.5 times per week by 47%.

age 4 years did not modify the results regarding specific IgE at age 4 years or specific SPT at age 6 years. Neither mother or father atopy or asthma status appeared to modify this association. (Data not shown). We repeated the analyses excluding women who reported fish intake greater than once per day and results remained similar.

Because breastfeeding has been related to the risk of atopy and that breast milk has a high content in n-3 PUFAs hypothesized to decrease the risk of atopic diseases, we stratified our analysis by breastfeeding. While the effect of fish intake score for eczema and atopy was not modified by breastfeeding, we observed that the fish intake during pregnancy was inversely related to the risk of persistent wheeze (P for interaction <0.05) and atopic wheeze at age 6 years (P for interaction <0.10) only among non-breastfed children. No protective effect was

observed among breastfed children. For an increase in fish intake from once per week to 2.5 times per week, the risk of persistent wheeze and atopic wheeze at age 6 years decreased approximately by 90% (OR = 0.10, 95% CI 0.02–0.69 and OR = 0.10, 95% CI 0.01–0.88, respectively). However because of the small number of subjects in these categories, results should be interpreted with caution.

Discussion

Our results suggest that fish intake during pregnancy may provide a protective effect on the risk of eczema and atopy in the offspring. We estimated that for an increase of fish intake from once per week to 2.5 times per week, the risk of eczema at age 1 year decreased by 37%, the risk of atopy at age 6 years by 35%, and of atopic wheeze at age 6 years by 82%. n-3 PUFAs, important nutrients of both fish and breast milk, are likely to play a protective role in the incidence of atopic-related outcomes.

To our knowledge this is the first longitudinal study to assess prospectively in a general population of pregnant women the impact of maternal diet during pregnancy on the incidence of atopic diseases up to age 6 years. Previous longitudinal studies have evaluated the association of the content of n-3 PUFAs in colostrums and breast milk on the risk of atopic disease in the offspring. A cohort study conducted in atopic and non-atopic mothers report lower levels of n-3 PUFAs in the breast milk of mother with atopic offspring at 18 months of life [23]. The Melbourne atopy cohort study which involves children born into families where at least one first-degree relative had an atopic disease reported higher levels of n-3 PUFAs in the colostrums of mother of atopic offspring at 6 and 24 months [16]. Other prospective studies also showed disturbances in both the n-6 and n-3 fatty acid composition of serum and milk from atopic and non-atopic mothers [14, 15]. However, no consistent pattern has been observed.

Recently, a cross-sectional study conducted among Italian children (median age 5 years) suggest that consumption of fish during pregnancy (assessed retrospectively) decreased the risk of SPT positivity mostly to food allergens in children of non-allergic mothers [11]. In the Californian Children Health Study maternal oil fish intake also assessed retrospectively through telephone interview was inversely related to asthma diagnosis among children born to mother with a history of asthma. No effect was observed among children of non-asthmatic mothers [12]. Atopy in children was not evaluated. In both studies, the large time span between pregnancy and the interview and the fact that dietary assessment was conducted after disease diagnosis might have led to exposure misclassification and potential recall bias. Fish intake in our population was close to that reported in an Italian population [11] in which 75% of the mothers reported eating fish once a week or more but larger than that reported in a US population [18] where few mothers ate fish on a daily or weekly basis. Kull et al. [24] recently reported that regular fish intake during the first year of life among infant without a family history of allergic disease was associated with a reduced risk for allergic disease by age 4 years and for sensitization. No data on maternal fish intake during pregnancy was available; however it is likely that mother's diet influences her infant dietary intake. This is in accordance with our results. In our study, we did not have information on fish intake during the first year of life but had dietary information at age 4 years. Including this information in our model did not modify our results.

The role of n-3 PUFAs in the incidence of atopy is not well understood. Two reports of randomized-controlled trial suggest that dietary n-3 PUFAs supplements during pregnancy [25] or in early postnatal period [26] could have immunomodulatory properties and/or associated clinical effects on atopy and asthma in offspring. n-3 PUFA supplementation during pregnancy from 20-week gestation until delivery among women with allergic diseases decreased the level of T-helper type 2 (Th2) cytokines (IL-13) in umbilical cord and expression of Th2 cytokine by neonatal mononuclear cells [25] as well as neonatal lipid peroxidation [27]. n-3 supplementation study during pregnancy in atopic mothers have shown a lower risk for sensitization to food allergen at 1 year as well as lower severity score of atopic dermatitis in the offspring of the supplemented group [25]. In addition, the breast milk of supplemented mothers was richer in DHA and correlated positively with IgA suggesting a role in of n-3 PUFAs in mucosal immune function [28]. Dietary supplementation with n-3 PUFAs in infants with family history of asthma resulted in fewer symptoms at 18 months and lower total IgE serum levels, but no difference in medical diagnosis of asthma or atopy [26].

In our study, the protective effect of fish consumption on atopic diseases was observed in offspring of both

atopic and non-atopic mother and breastfeeding did not modify the association of fish intake with atopy in children. However, the impact of fish intake on persistent wheeze and atopic wheeze was only present in children without breastfeeding suggesting that n-3 PUFAs or other components related to fish intake transferred during fetal life could decrease the risk of persistent wheeze. Several compounds of breastfeeding may act to protect the infant from developing asthma such as the transmission of IgA, cytokines-like INF γ , or through the protection against viral diseases in addition to the composition of PUFAs [29]. Therefore, the impact of n-3 PUFAs might be more easily detected among non-breastfed infants.

The time window of exposure is becoming a key aspect in the study of diseases involving systems with a long developmental length such as the immunological and respiratory system [17]. It is possible that the immunomodulating benefits of n-3 PUFAs may be greater during critical stages of early immune development before allergic responses are established. Allergen-specific responses are already evident at birth and allergic disease is often manifest within the first month of life suggesting that the processes that lead to allergic diseases can be initiated very early in immune development [1]. The attenuated immunologic responses observed in neonates delivered from women supplemented with fish oil support this hypothesis [7, 25, 30].

Some limitations of our study need to be addressed. We obtained quantitative data on fish intake (fatty fish or non-fatty fish) but could not separately assess the impact of the type of fish because of the small number of subjects reporting intake of fatty fish only. However, similar protective effects were observed when data were stratified by type of fish intake (intake of non-fatty fish vs. intake of both non-fatty and fatty fish) suggesting that intake of non-fatty fish also played a protective role. Other sources of n-3 PUFAs from vegetables oil were not taken into account. However, the majority of our population reported using olive oil. We also created a score for fruit and vegetable intake during pregnancy and did not observe association of these scores with atopy or wheeze in our population. We cannot exclude that fish consumption might be a marker of specific life style. Adjusting for variables such as social class, education, smoking during pregnancy did not modify the association. It is therefore unlikely given the association observed that an unknown risk factor could explain our findings.

The diagnosis of eczema was based on the parent report of medical diagnosis on the 1-year questionnaire. Although this diagnosis was not validated it is unlikely that parents would erroneously report a medical diagnosis of eczema in their child and the proportion of eczema in our population is in accordance with previous report from Spain [31]. However, some infants with mild eczema may not have been diagnosed. The likelihood that this under

diagnosis be correlated to the mother's fish intake is very low and therefore misclassification would lead to an underestimation of the association. In addition, our diagnosis of atopy at age 6 years was based on the objective measurement of SPT to several allergens including Der p 1, cat, grass pollen, olive tree, mixed gramineae, parietaria. Most of the atopic children had a positive test for HDM (89.5%). Positive tests to other allergen were scarce and could not be studied separately. Persistent wheeze at age 6 years was based on the prospective evaluation of symptoms since birth reported by the mother. Wheezing at age 6 years is less associated with low-respiratory illnesses than at an earlier age and therefore more likely to be a marker of asthma. Persistent wheeze at age 6 years is highly correlated with wheezing after age 6 years and persistent wheezers have significantly lower lung function than never wheezers [32]. Given the small number of children with medical asthma diagnosis and complete nutritional data, we were not able to fully explore the association of medical diagnosis of asthma with fish intake during pregnancy, but our results suggest an association of fish intake with persistent wheezing in non-breastfed children.

A recent review on nutrition and allergic disease conclude that the beneficial effect of n-3 PUFAs on asthma and allergy is biologically plausible and that recent supplementation studies during pregnancy and early life are promising [33]. Our data on fish intake during pregnancy collected in a prospective manner suggest that a component of fish, most likely n-3 PUFAs might modulate the risk of atopy in children. The protective effect was observed independently of the atopic or asthmatic status of the mother. Further studies are needed to better understand the mechanisms of this protective effect and the most relevant window of exposure. Ongoing follow-up of our cohort will allow determining if this protective effect persists in older children.

Acknowledgements

Supported by the Instituto de Salud Carlos III red de Grupos Infancia y Media Ambiente (G03/176) and by the Fundacio 'La Caixa' (00/077-00) and the Instituto de Salud Carlos III, red de Centros de Investigacion en Epidemiologia y Salud Publica (C03/09). Dr Romieu was supported in part by the National Center for Environmental Health – Centers for Disease Control and Prevention, Atlanta, GA, USA, the GA²LEN project (EU contract FOODCT-2004-506378) and Ministry of Education and Science, Spain, SAB2004-0192.

References

- 1 Prescott SL, Calder PC. N-3 polyunsaturated fatty acids and allergic disease. *Curr Opin Clin Nutr Metab Care* 2004; **7**:123–9.
- 2 Calder PC. Abnormal fatty acid profiles occur in atopic dermatitis but what do they mean? *Clin Exp Allergy* 2006; **36**:138–41.
- 3 Jones PJH, Kubow S. Lipids, sterols and their metabolites. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, eds. *Modern nutrition in health and disease*, 10th Edn. Baltimore, MD: Lippincott Williams and Wilkins, 2006; 92–135.
- 4 Calder PC, Deckelbaum RJ. CD36: taste the difference? *Curr Opin Clin Nutr Metab Care* 2006; **9**:77–8.
- 5 Schwartz J, Weiss ST. Dietary factors and their relation to respiratory symptoms. The Second National Health and Nutrition Examination Survey. *Am J Epidemiol* 1990; **132**:67–76.
- 6 James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 2000; **71**:343S–8S.
- 7 Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA. Fatty acids and lymphocyte functions. *Br J Nutr* 2002; **87**:S31–48.
- 8 Hughes DA, Pinder AC. N-3 polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes and inhibit antigen-presentation in vitro. *Clin Exp Immunol* 1997; **110**:516–23.
- 9 Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996; **7**:109–16.
- 10 Prescott SL, Macaubas C, Holt BJ *et al*. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; **160**:4730–7.
- 11 Calvani M, Alessandri C, Sopo SM *et al*. Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. *Pediatr Allergy Immunol* 2006; **17**:94–102.
- 12 Salam MT, Li YF, Langholz B, Gilliland FD. Maternal fish consumption during pregnancy and risk of early childhood asthma. *J Asthma* 2005; **42**:513–8.
- 13 Laitinen K, Sallinen J, Linderborg K, Isolauri E. Serum, cheek cell and breast milk fatty acid compositions in infants with atopic and non-atopic eczema. *Clin Exp Allergy* 2006; **36**:166–73.
- 14 Yu G, Bjorksten B. Serum levels of phospholipid fatty acids in mothers and their babies in relation to allergic disease. *Eur J Pediatr* 1998; **157**:298–303.
- 15 Duchon K. Are human milk polyunsaturated fatty acids (PUFA) related to atopy in the mother and her child? *Allergy* 2001; **56**:587–92.
- 16 Stoney RM, Woods RK, Hosking CS, Hill DJ, Abramson MJ, Thien FC. Maternal breast milk long-chain n-3 fatty acids are associated with increased risk of atopy in breastfed infants. *Clin Exp Allergy* 2004; **34**:194–200.
- 17 Sunyer J, Torrent M, Munoz-Ortiz L *et al*. Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children. *Environ Health Perspect* 2005; **113**:1787–90.
- 18 Polk S, Sunyer J, Munoz-Ortiz L *et al*. A prospective study of Fel d1 and Der p1 exposure in infancy and childhood wheezing. *Am J Respir Crit Care Med* 2004; **170**:273–8.
- 19 Bingham SA, Welch AA, McTaggart A *et al*. Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr* 2001; **4**:847–58.
- 20 Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986; **73**:132–22.

- 21 Basagana X, Torrent M, Atkinson W *et al.* Domestic aeroallergen levels in Barcelona and Menorca (Spain). *Pediatr Allergy Immunol* 2002; 13:412–17.
- 22 Hastie T, Tibshirani R. *Generalized additive models*. New York: Chapman & Hall, 1990.
- 23 Duchon K, Casas R, Fageras-Bottcher M, Yu G, Bjorksten B. Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. *Pediatr Allergy Immunol* 2000; 11:29–39.
- 24 Kull I, Bergström A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. *Allergy* 2006; 61:1009–15.
- 25 Dunstan JA, Mori TA, Barden A *et al.* Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003; 112:1178–84.
- 26 Marks GB, Mihrshahi S, Kemp AS *et al.* Prevention of asthma during the first 5 years of life: a randomized controlled trial. *J Allergy Clin Immunol* 2006; 118:53–61.
- 27 Barden A, Mori TA, Dunstan JA *et al.* Fish oil supplementation in pregnancy lowers F2-isoprostanes in neonates at high risk of atopy. *Free Radical Res* 2004; 38:233–9.
- 28 Dunstan JA, Roper J, Mitoulas L, Hartmann PE, Simmer K, Prescott SL. The effect of supplementation with fish oil during pregnancy on breast milk immunoglobulin A, soluble CD14, cytokine levels and fatty acid composition. *Clin Exp Allergy* 2004; 34:1237–42.
- 29 Friedman NJ, Zeiger RS. The role of breast-feeding in the development of allergies and asthma. *J Allergy Clin Immunol* 2005; 115:1238–48.
- 30 Calder PC, Kew S. The immune system: a target for functional foods? *Br J Nutr* 2002; 88:S165–77.
- 31 Sunyer J, Jarvis D, Pekkanen J *et al.* Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol* 2004; 114:1033–9.
- 32 Morgan WJ, Stern DA, Sherrill DL *et al.* Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. *Am J Respir Crit Care Med* 2005; 172:1253–8.
- 33 Tricon S, Willers S, Smit HA *et al.* Nutrition and allergic disease. *Clin Exp Allergy Rev* 2006; 6:11788.