

Original article

A differing pattern of association between dietary fish and allergen-specific subgroups of atopy

Background: We examined the role of fish intake in the development of atopic disease with particular reference to the possibility of differential effects on allergen-specific subgroups of sensitization.

Methods: The exposure of interest was parental report of fish intake by children aged 8 years at the 1997 Childhood Allergy and Respiratory Health Study ($n = 499$). The outcomes of interest were subgroups of atopy: house dust mite (HDM)-pure sensitization [a positive skin-prick test (SPT) ≥ 2 mm to Der p or Der f only], ryegrass-pure sensitization (a positive SPT ≥ 2 mm to ryegrass only); asthma and hay fever by allergen-specific sensitization.

Results: A significant association between fish intake and ryegrass-pure [adjusted odds ratio (AOR) 0.37 (0.15–0.90)] but not HDM-pure sensitization [AOR 0.87 (0.36–2.13)] was found. Fish consumption significantly decreased the risk for ryegrass-pure sensitization in comparison with HDM-pure sensitization [AOR 0.20 (0.05–0.79)].

Conclusions: We have demonstrated a differential effect of fish intake for sensitization to different aeroallergens. This may be due to the different timing of allergen exposure during early life. Further investigation of the causes of atopic disease should take into account allergen-specific subgroups.

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There has been a suggestion that a number of environmental factors are linked to child atopic disease. The role of polyunsaturated fatty acids (PUFA) intake in particular foods rich in ω -3 PUFA such as fish in atopic disease is under investigation. Several observational studies found a significant inverse association between fish intake and atopic disease (1–3). According to the fatty acid hypothesis, increased ratio of ω -6/ ω -3 PUFA may shift the immune responses towards the T helper 2 (Th2) subtype through synthesis of prostanoids. More specifically, ω -3 PUFA exhibit their immunomodulatory effect through competitive inhibition with ω -6 PUFA, and increasing dietary ω -3 PUFA results in the reduction of precursors of inflammatory mediators such as arachidonic acid from linoleic acid (4). ω -3 fatty acids can also inhibit the oxygenation of arachidonic acid by cyclooxygenase (4). Thus, foods rich in ω -3 fatty acids such as fish may downregulate Th2 responses and associated atopic disease.

Despite this evidence, other observational studies (5, 6) failed to show any beneficial association between fish intake and asthma. Moreover, fish oil supplementation

did not significantly improve asthma symptoms in several randomized controlled trials (RCTs) (7, 8). Part of this controversy may be due to conceptual difficulties to define atopic disease, including asthma, and its phenotype heterogeneity (9). Absence of a precise definition makes it difficult to diagnose asthma and to assess the diagnostic value of objective measures of the disease (10). More difficult is the delineation of phenotype subgroups within the broad spectrum of atopic disease.

A recent novel analysis by this group using mutually exclusive subgroups demonstrated that environmental factors may exert different influences on allergen-specific subgroups within atopy (11) with a large family size strongly associated with reduced sensitization to ryegrass allergens but not to house dust mite (HDM) allergens. In addition, a differing pattern of allergen-specific sensitization for disease phenotypes was found. Asthma was strongly associated with HDM sensitization and hay fever was more associated with ryegrass sensitization (11).

We postulated that similar to the sibling effect detailed above, fish intake may be another childhood influence which could exhibit a differential protective effect on

allergen sensitization. Here, we investigate this issue, taking into account atopic phenotype.

Methods

Comprehensive baseline infant data was collected as part of the Tasmanian Infant Health Survey (TIHS) in 1988–89 (12). Data on childhood fish consumption, allergic sensitization and atopic disease were utilized from the Childhood Allergy and Respiratory Health Survey, a follow-up study which took place in 1997 (13). Both these studies received ethical approval from the Human Ethics Committee of the University of Tasmania.

The Tasmanian Infant Health Survey

The TIHS was a cohort study of infants born from 1988 to 1995 in Tasmania, Australia. This birth cohort study operated from hospitals in Tasmania, Australia, where approximately 93% of births in Tasmania occurred (14). The sample of eligible infants represented approximately one-fifth of live births in the state from 1988 to 1995.

Infants born were scored to assess the risk of sudden infant death syndrome (SIDS) using a perinatal score model based on maternal age, birth weight, infant sex, intention to bottle feed, season of birth and duration of second stage of labor (14). Infants exceeding a scoring cut-off were eligible for inclusion. Multiple births were automatically included. The eligibility criteria and study methods are discussed in more detail elsewhere (12).

Data were obtained on three occasions. Interviews were conducted in hospital on the fourth day of the infant's life, at home during the fifth postnatal week, and by telephone at approximately 12 weeks postnatally. In 1988–89, 609 (88% of eligible) infants born in northern Tasmania had a home interview at 1 month of age.

The 1997 Childhood Allergy and Respiratory Health Study (CARHS)

In 1997, 596 TIHS participants born in 1988 or 1989 were identified in the northern region of Tasmania (excluding offshore islands) through school records (13). Of these, 499 (84%) agreed to participate in the CARHS. Both infant home visit and hospital interview data were available for 456 CARHS participants.

The parental interview and child assessment were conducted in the Launceston General Hospital. The parental questionnaire included questions on asthma, wheeze, hay fever and eczema from the International Study of Asthma and Allergies in Childhood (ISAAC) (15), questions on the home environment and other factors including fish intake. The cutaneous reaction to exposure to aeroallergens was done by skin-prick testing (SPT). The tested allergens included the house dust mites *Dermatophagoides pteronyssinus* (Der p) and *D. farinae* (Der f), cat, dog, *Alternaria* (type of mould), ryegrass, cow's milk, egg, and peanut (Hollister-Stier purified allergen extracts supplied by Bayer, Sydney, Australia) and positive (histamine 10 mg/ml and 1 mg/ml) and negative (glycerine) controls. SPTs were carried out on 498 children. The mean age at the time of SPT was 8.71 (SD 0.59) years. The study methods are discussed in more detail elsewhere (11).

Definitions

A positive weal allergen reaction of ≥ 2 mm to any aeroallergen at 15 min was defined as atopy (16). The disease outcomes of interest were categories of atopic sensitization (sensitization to ryegrass,

HDM and other aeroallergens) and the asthma and hay fever phenotypes. The pattern of overlapping sensitization to aeroallergens is illustrated in Fig. 1. A positive SPT to either Der p or Der f regardless of sensitization to other aeroallergens was classified as any HDM sensitization (segments a, c, d, e; Fig. 1), and a positive SPT to ryegrass regardless of sensitization to other aeroallergens as any ryegrass sensitization (segments b, c, d, f; Fig. 1).

The construction of response variables for the pure categories of atopic sensitization was adapted from the previous study (11). 'No sensitization' was coded as '0' and used as the baseline reference group. Each specific mutually exclusive category was coded '1'. 'A positive SPT to either Der p or Der f but a negative SPT to ryegrass and other aeroallergens' was termed HDM-pure sensitization (segment a, Fig. 1). A positive SPT to ryegrass but a negative SPT to HDM and other aeroallergens was termed ryegrass-pure sensitization (segment b, Fig. 1). Mixed sensitization was defined as a positive SPT to Der p or Der f and also ryegrass regardless of sensitization to other aeroallergens (segments c, d; Fig. 1). Segment g in Fig. 1 is sensitization to cat, dog or *Alternaria* only (11). Subjects without a given outcome were excluded from the analyses involving that outcome.

A positive response to the ISAAC question, 'Has your child had wheezing or whistling in the chest in the last 12 months?' (15) was defined as asthma. This question has a sensitivity of 0.81 and a specificity of 0.85 for the physician diagnosis of asthma in childhood (17). The allergen-specific categories of asthma were constructed as follows. Absence of asthma was termed 'no asthma' and used as the baseline reference group. The comparison groups were: asthma without sensitization, asthma with concomitant ryegrass-pure sensitization, asthma with concomitant HDM-pure sensitization, asthma with concomitant mixed sensitization. The allergen-specific categories of hay fever were constructed similarly. Of 499 children,

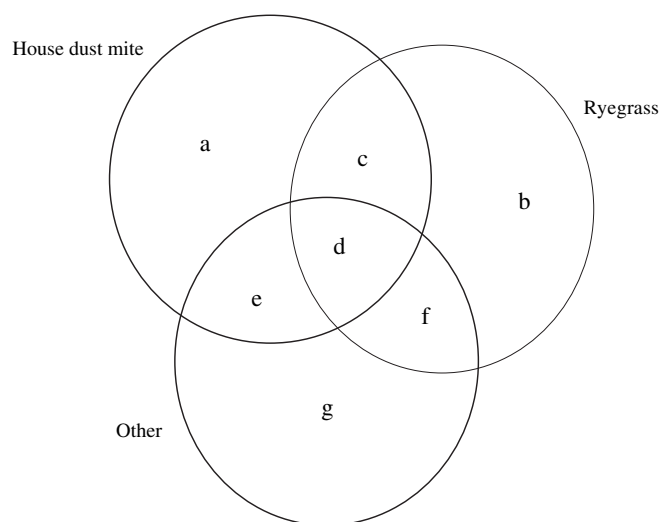


Figure 1. The overlapping pattern of allergen specific sensitization, the 1997 Childhood Asthma and Respiratory Health Study. (a) sensitization to HDM only ($n = 69$); (b) sensitization to ryegrass only ($n = 34$); (c) sensitization to HDM and ryegrass ($n = 29$); (d) sensitization to HDM, ryegrass and other aeroallergens ($n = 44$); (e) sensitization to HDM and other aeroallergens ($n = 15$); (f) sensitization to ryegrass and other aeroallergens ($n = 9$); (g) sensitization cat, dog, or *Alternaria* only ($n = 6$) (non-atopic $n = 292$).

498 had data on atopic sensitization, 497 on asthma and hay fever (Table 1).

The exposure of interest was child fish intake at age 8 years assessed by parental response to 'how often does your child eat fish?' in a self-completed questionnaire with 'never', 'once a week or less', and 'more than once a week' as possible answers. A large proportion of children were in the second group (80.8%, 399/494) with relatively few in the third group (6.5%, 32/494). As the strength

of association between the fish intake categories and the pure categories of atopic sensitization were similar (Table 2), the exposure variable was included in the subsequent analyses as binary with the baseline group of 'never' responses.

Statistical methods

Odds ratios (OR) and 95% confidence intervals for associations between fish intake and each atopic outcome (cross-sectional data) were derived from logistic regression models as previously used in past work on fish intake and development of atopic disease (1, 2, 6). To identify possible confounders, first, the exposure–outcome associations were stratified by possible confounders which included the components of the perinatal scoring system for cohort entry and factors suggested by literature, and pooled Mantel–Haenszel estimates were obtained (18). Secondly, the magnitude and significance of the association between each factor and fish intake or each atopic outcome were estimated separately by logistic regression (Table 2). Comparison of ryegrass-pure sensitization with HDM-pure sensitization was performed by a logistic model with binary outcome of '1' for ryegrass-pure and '0' for HDM-pure sensitization (11). If the *P*-value associated with the Wald test for a variable was ≤ 0.05 , then the association was said to vary significantly by the outcome. The change-in-estimate strategy was used to control for confounding: covariates that changed the OR of difference in the effect of fish intake for ryegrass-pure compared with HDM-pure sensitization, the main outcome of interest, by $> 10\%$ were entered into the multivariate model (19). The statistical significance of interaction terms between the dependent variables was tested by likelihood ratio tests. None of the interaction terms were significant at

Table 1. Prevalence of selected study variables, the 1997 Childhood Asthma and Respiratory Health Study

Variable	Overall	Among those with asthma	Among those with hay fever
	% (n/N)	% (n/N)	% (n/N)
Any fish consumption in 1997	87.3 (431/494)	83.7 (128/153)	86.4 (108/125)
Non-atopic	58.6 (292/498)	43.1 (66/153)	35.2 (44/125)
Any ryegrass sensitization	23.3 (116/498)	37.3 (57/153)	51.2 (64/125)
Ryegrass-pure sensitization	6.8 (34/498)	5.9 (9/153)	11.2 (14/125)
Any house dust mite sensitization	31.5 (157/498)	47.1 (72/153)	49.6 (62/125)
House dust mite-pure sensitization	13.9 (69/498)	13.7 (21/153)	10.4 (13/125)
Mixed sensitization	14.7 (73/498)	29.4 (45/153)	36.8 (46/125)
Cat, dog, or alternaria sensitization	14.7 (74/498)	30.1 (46/153)	28.8 (36/125)
Asthma	30.6 (153/497)	–	56.9 (70/123)
Hay fever	25.2 (125/497)	46.1 (70/152)	–

Table 2. Associations between fish consumption, the pure categories of atopic sensitization (the Childhood Asthma and Respiratory Health Study), and selected factors (the Tasmanian Infant Health Survey and Childhood Asthma and Respiratory Health Study)

Factor	Any fish intake in 1997 (yes vs no)		Ryegrass-pure sensitization		House dust mite-pure sensitization	
	Odds ratio (95% CI)	<i>P</i> -value	Odds ratio (95% CI)	<i>P</i> -value	Odds ratio (95% CI)	<i>P</i> -value
Any fish intake in 1997						
No fish (referent)	–	–	1.00	–	1.00	–
Once a week or less	–	–	0.26 (0.11–0.58)	0.001	1.07 (0.45–2.55)	0.88
More than once a week	–	–	0.31 (0.06–1.53)	0.15	0.96 (0.25–3.72)	0.96
Family history of asthma at birth*	1.23 (0.70–2.15)	0.47	1.76 (0.86–3.60)	0.12	1.87 (1.09–3.18)	0.02
Duration of second stage of labour (≤ 4 min, per minute increase)	1.42 (1.01–2.01)	0.05	0.94 (0.59–1.50)	0.79	0.93 (0.66–1.32)	0.70
Multiple birth*	2.37 (0.92–6.10)	0.08	2.00 (0.84–4.72)	0.12	1.23 (0.59–2.55)	0.58
Child's sex (male vs female)	0.90 (0.50–1.64)	0.74	0.58 (0.28–1.20)	0.14	1.14 (0.63–2.07)	0.66
Maternal age (per year increase)	1.02 (0.96–1.09)	0.47	1.01 (0.93–1.09)	0.84	1.03 (0.97–1.09)	0.37
Season of birth (May–July vs rest of the year)	0.87 (0.51–1.48)	0.60	1.13 (0.55–2.32)	0.75	1.10 (0.64–1.87)	0.73
Low birth weight (< 2500 g vs ≥ 2500 g)	1.40 (0.68–2.86)	0.36	0.81 (0.32–2.05)	0.66	0.88 (0.45–1.71)	0.71
Carpet in infant's bedroom*	1.34 (0.57–3.17)	0.50	1.91 (0.43–8.41)	0.39	1.30 (0.52–3.25)	0.58
Child feather quilt use*	0.96 (0.57–1.64)	0.89	0.80 (0.39–1.63)	0.54	0.38 (0.22–0.66)	0.001
Sheepskin use in infancy*	0.72 (0.39–1.35)	0.31	0.66 (0.24–1.79)	0.41	1.36 (0.74–2.52)	0.32
Plastic mattress liner in infancy*	1.49 (0.85–2.61)	0.16	1.06 (0.51–2.21)	0.88	2.10 (1.18–3.75)	0.01
At-birth intention to bottle-feed*	0.63 (0.37–1.08)	0.09	0.94 (0.45–1.98)	0.88	1.33 (0.78–2.26)	0.29
Any bottle-feeding at 1 month*	0.44 (0.23–0.86)	0.02	1.89 (0.82–4.37)	0.14	2.52 (1.31–4.86)	0.01
Solids introduced at 12 weeks*	1.17 (0.63–2.18)	0.61	0.88 (0.39–1.99)	0.76	1.30 (0.73–2.31)	0.38
Mother smoked during Pregnancy*	0.59 (0.34–1.01)	0.05	0.88 (0.43–1.79)	0.72	1.05 (0.62–1.77)	0.87
Cigarette smoking in the same room with the infant*	1.17 (0.66–2.06)	0.59	1.00 (0.48–2.09)	0.99	0.85 (0.49–1.48)	0.58
Domestic gas used for cooking or heating in infancy*	0.47 (0.15–1.49)	0.20	1.04 (0.13–8.63)	0.97	1.03 (0.21–4.96)	0.97
Lower respiratory tract infection at 12 weeks*	4.48 (1.06–18.90)	0.04	0.55 (0.16–1.88)	0.34	0.88 (0.40–1.93)	0.75
Number of siblings in 1997 (per sibling increase)	0.78 (0.23–2.65)	0.69	0.58 (0.39–0.86)	0.01	1.01 (0.82–1.25)	0.93

*Binary variable (yes vs no).

$P \leq 0.05$ level. The allergen-specific categories of asthma and hay fever were analyzed by multinomial logistic regression. We made no adjustment for multiple testing but report (18) all analyses undertaken to allow readers to make formal adjustments if they desire. All analyses were conducted using STATA 8 (20).

Results

Table 1 shows the prevalence of exposure and outcome variables in the study sample. The majority of children (87.3%) ate fish. Overall, 41% (206/498) of children were sensitized to at least one allergen. HDM sensitization was more prevalent than ryegrass sensitization. Fifteen percent of children were sensitized to both ryegrass and HDM (mixed sensitization). Prevalence of sensitization to cat, dog or *Alternaria* was 15% (Fig. 1). Asthma was slightly more prevalent than hay fever.

When examining the exposure–factor and factor–outcome associations, bottle-feeding at 1 month and maternal smoking during pregnancy were negatively associated with fish intake in childhood. Family history of asthma, plastic mattress liner use in infancy, and any bottle-feeding at 1 month were positively associated, and feather quilt use in childhood was negatively associated with HDM-pure sensitization (Table 2).

We then examined the associations between fish intake and allergen-specific sensitization (Table 3). Overall, fish intake was not associated with atopy [OR 0.69 (0.40–1.17)]. On univariate analyses, a protective effect of fish for any ryegrass and ryegrass-pure sensitization was found. Fish intake was not associated with any of the categories of HDM sensitization. On multivariate analysis, the association with the ryegrass-pure sensitization remained significant. Fish consumption significantly decreased the risk for ryegrass-pure sensitization in comparison with HDM-pure sensitization by 80% [adjusted odds ratio (AOR) 0.20 (0.05–0.79)]. Fish intake was not significantly associated with the mixed sensitization [AOR 0.68 (0.30–1.53)].

Mixed sensitization was strongly associated with both asthma [OR 5.48 (3.17–9.46)] and hay fever [OR 9.97 (5.59–17.77)] suggesting that simultaneous sensitization to both ryegrass and HDM rather than sensitization to one of these allergens is a stronger risk factor for asthma

and hay fever. Asthma was not significantly associated with either HDM-pure [OR 1.49 (0.83–2.67)] or ryegrass-pure sensitization [OR 1.28 (0.57–2.88)]. Hay fever was not significantly associated with HDM-pure sensitization [OR 1.31 (0.66–2.59)] but strongly related to ryegrass-pure sensitization [OR 4.15 (1.94–8.89)].

Overall, fish intake was not significantly associated with a reduced risk of asthma [OR 0.65 (0.37–1.12)] or hay fever [OR 0.89 (0.49–1.62)]. Clearer associations were revealed when these outcomes were broken down into allergen-specific subgroups. Fish intake was significantly inversely associated only with asthma linked to ryegrass-pure sensitization [AOR 0.20 (0.04–0.90)]. A similar pattern was found for hay fever linked to ryegrass-pure sensitization [OR 0.25 (0.08–0.78)] (Table 4).

Further examination of the confounding factors

The following factors were further examined for their potential confounding effect: components of the perinatal scoring system (maternal age, birth weight, infant's sex, intention to bottle-feed, season of birth, duration of second stage of labour, multiple birth), the composite perinatal score, any bottle-feeding at 1 month of age, antenatal exposure to smoking, domestic gas used for cooking or heating in infancy, infant and child exposure to active cigarette smoking in the same room, mould observed in the infant's bedroom by the research interviewer, history of an upper respiratory tract infection by 1 month of age, history of a lower respiratory tract infection by 12 weeks of age, introduction of solids at 12 weeks, breastfeeding without solids or infant formula at 12 weeks, carpet in infant's bedroom, family history of asthma at birth, child feather quilt use, and child feather pillow use.

For atopic sensitization, the main outcome of interest was the magnitude of the effect of fish intake for ryegrass-pure compared with HDM-pure sensitization. When adding the factors one at a time into the multivariate model, antenatal exposure to smoking decreased the AOR for the difference in effect by 11% [AOR 0.16 ($P = 0.01$)] and lower respiratory tract infection by 12 weeks of age increased the AOR by 15% [AOR 0.23 ($P = 0.04$)]. The other factors did not change the differ-

Table 3. Fish consumption and likelihood of atopic sensitization, the 1997 Childhood Asthma and Respiratory Health Study

Atopy subgroup	Prevalence of atopic subgroup among children who did not eat fish in 1997 % (n/N)	Prevalence of atopic subgroup among children who ate fish in 1997 % (n/N)	Odds ratio (95% CI)	P-value	Adjusted odds ratio* (95% CI)	P-value
Any atopy	49.2 (31/63)	40.0 (172/430)	0.69 (0.40–1.17)	0.17	0.74 (0.42–1.32)	0.31
Any ryegrass sensitization	34.9 (22/63)	21.6 (93/430)	0.51 (0.29–0.91)	0.02	0.60 (0.32–1.11)	0.10
Any HDM sensitization	31.8 (20/63)	31.2 (134/430)	0.97 (0.55–1.72)	0.93	0.96 (0.52–1.77)	0.89
Ryegrass pure sensitization	17.5 (11/63)	5.4 (23/430)	0.26 (0.12–0.58)	0.001	0.37 (0.15–0.90)	0.03
HDM pure sensitization	11.1 (7/63)	14.0 (60/430)	1.06 (0.45–2.52)	0.89	0.87 (0.36–2.13)	0.77

*Adjusted for sheepskin and plastic mattress use in infancy, child's sex, and number of siblings in 1997.

Table 4. Fish intake and likelihood of disease phenotypes by categories of atopic sensitization, the 1997 Childhood Asthma and Respiratory Health Study

Disease phenotype	Atopy subgroup	Prevalence of disease phenotype by atopic subgroups among children who did not eat fish in 1997	Prevalence of disease phenotype by atopic subgroups among children who ate fish in 1997	Odds ratio (95% CI)	P value	Adjusted* odds ratio (95% CI)	P value
		% (n/N)	% (n/N)				
No asthma (referent category)	—	63.5 (40/63)	72.5 (301/429)	1.00	—	1.00	—
Asthma	No sensitization	15.9 (10/63)	13.1 (56/429)	0.71 (0.33–1.50)	0.37	0.86 (0.37–2.00)	0.73
	Ryegrass-pure sensitization	6.4 (4/63)	1.2 (5/429)	0.16 (0.04–0.61)	0.01	0.20 (0.04–0.90)	0.04
	HDM-pure sensitization	1.6 (1/63)	4.7 (20/429)	2.52 (0.33–19.35)	0.37	2.88 (0.37–22.49)	0.31
	Mixed sensitization	12.7 (8/63)	8.6 (37/429)	0.58 (0.25–1.35)	0.21	0.47 (0.20–1.13)	0.09
No hay fever (referent category)	—	74.2 (46/62)	76.5 (329/430)	1.00	—	1.00	—
Hay fever	No sensitization	3.2 (2/62)	9.8 (42/430)	2.93 (0.69–12.54)	0.15	2.75 (0.63–11.95)	0.18
	Ryegrass-pure sensitization	8.1 (5/62)	2.1 (9/430)	0.25 (0.08–0.78)	0.02	0.50 (0.13–1.99)	0.33
	HDM-pure sensitization	3.2 (2/62)	2.6 (11/430)	0.77 (0.17–3.58)	0.74	0.59 (0.12–2.87)	0.51
	Mixed sensitization	11.3 (7/62)	9.1 (39/430)	0.78 (0.33–1.85)	0.57	0.74 (0.29–1.90)	0.53

*Adjusted for any bottle-feeding at 1 month and any maternal smoking during pregnancy.

ence in effect by >10% (data not shown). The AOR range was 0.16 ($P = 0.01$) to 0.23 ($P = 0.04$). On average, fish intake lowered the risk for ryegrass-pure sensitization compared with HDM-pure sensitization more than four-fold after various possible confounders were taken into account. Thus, differential effect of dietary fish in reducing ryegrass-pure but not HDM-pure sensitization was of substantial magnitude.

Discussion

This report examines the effect of fish consumption on the development of atopy and asthma and demonstrates that fish consumption may exert a differential effect on allergen-specific sensitization (a significant protective association for ryegrass-pure but not HDM-pure sensitization) and provides further evidence that environmental factors may influence sensitization to different allergens differently (11). Concerning the protective effects of any fish consumption on the subsets of atopic disease (asthma and allergic rhinitis) associated with ryegrass sensitization, because of subset analysis the numbers are small and larger cohorts are required to confirm this observation.

The main strength of the study was the development and use of clearly delineated categories of atopy, asthma and hay fever. This allowed for a more precise assessment of the effect of fish consumption on atopic disease. The study had a large capacity to control for confounding. Fish intake was measured by a parental report of how often the child ate fish which is inferior to a semi-quantitative food frequency questionnaire (21). The type of fish consumed can also be of significance. A recent study found a significant trend (in univariate analysis) for inverse association between physician-diagnosed asthma and 'oily fish', but not between the disease and 'shellfish', 'other seafood', or 'all fish' (22). Hodge et al. (1) found a significantly reduced risk of current asthma among

children who ate oily fish, but not among children who ate non-oily fish. The strength of the causal relationship is also lowered by the fact that exposure was only a binary variable and dose-response could not be calculated. Clearly, the type of fish, the amount (dose) and duration of fish consumption are likely to be important factors. Furthermore, the ratio of ω -6 to ω -3 PUFA rather than the total amount of PUFA is important (23). These problems in the measurement of fish intake here would most likely be non-differential, reducing the study's ability to detect an association with the atopic outcomes. However, exposure measures on fish intake would be unlikely to vary by allergen-specific subgroups, particularly within asthma. We found no association with either atopy or asthma overall. The small sample size did not allow an examination of combined or mutually exclusive disease phenotypes, e.g. asthma and hay fever combined, asthma without hay fever. The cohort entry criteria were designed to recruit infants at a higher risk of SIDS. Thus, the cohort is not representative of all live births in Tasmania at that time. However, adjustment for the components of the perinatal scoring system did not alter the findings and there is no reason to believe that the study population was substantially different from the general population with regard to the development of allergy and atopy. Although many of the controlled factors were from prospective data, the data on child fish consumption were cross-sectional. In general, cross-sectional data does not allow a clear inference of causality to be drawn.

Other observational studies (1–3) have also found inverse associations between fish intake and asthma. Failure of several RCTs to show improvements in clinical symptoms in asthmatics treated with fish oil (7, 8) has been suggested to be due to the possibility that (a) fish oil may exert its beneficial effect by reducing the risk of allergen sensitization rather than having a direct effect on asthma, (b) asthma cannot be reversed once it has

developed, (c) the time of supplementation was too short, or (d) the dose was too low (24). The findings here indicate that the allergen-specific atopic subgroups of studied asthmatics are also important.

Our findings indicate that different allergen sensitization patterns within the broad categories of atopy, hay fever or asthma across the studies may have contributed to the inconsistent findings on fish intake and atopic disease (1, 2, 5, 6). We also speculate that the differing pattern of association of fish consumption and allergen-specific subgroups of atopic disease may be partly attributed to the exposure timing. The concept has been suggested by studies on early-childhood respiratory illnesses and allergic susceptibility (25, 26). Von Mutius (27) demonstrated that even changes as profound as those occurring in the eastern part of Germany do not affect the inception of childhood asthma if they happen after the third birthday. The same study also found that the prevalence of hay fever and atopy have increased between 1991–92 and 1995–96. Their explanation of the findings was that factors operating very early in life are important for the acquisition of asthma, whereas the development of atopic sensitization and hay fever is affected by environmental factors occurring beyond infancy in addition to early-life factors. Similarly, the number of younger siblings was more strongly inversely associated with hay fever than asthma (28) reflecting a longer window of opportunity for the sibling effect on hay fever. Here, as in other studies (29, 30), the hay fever phenotype was more closely linked to ryegrass than HDM sensitization.

We suggest that the differing pattern of association of fish consumption and allergen-sensitisation may be partly attributed to the exposure timing. Mechanisms that contribute to downregulation of IgE responses to environmental allergens are most likely to exert their major effects at, or around the time of, initial exposure to the allergens rather than when T-cell memory is established and T-cell response is shifted to a permanent Th1 or Th2 pattern (31). Exposure and sensitization to HDM is likely to occur constantly very early in life, even at 1 month of age (32) while exposure to ryegrass usually occurs intermittently throughout childhood and teenage years (33, 34). A recent study (35) found a significant age-related proliferative response to ryegrass allergens, but not to HDM, and an increased age-related Th2 deviation among ryegrass-sensitive but not HDM-sensitive atopic subjects. Thus there may be a greater influence of later childhood factors in the development of ryegrass sensitization than HDM sensitization. Consistent with this, fish intake in this study cohort was uncommon in the first 3 months of life (0.4%). However, by the age of 6 years, 94% (460/489) of children ate fish. This timing of allergen exposure together with the later age of fish consumption

may allow a larger window of opportunity for PUFA immunomodulation for ryegrass than HDM. Thus, as the time of consolidation of Th2 responses to different aeroallergens varies with T-cell memory for HDM allergens being established in early childhood and for ryegrass allergens throughout childhood (35), the 'usual' introduction of fish into diet might be too late to protect against HDM-sensitization developed in early life. A parallel-group RCT involving high-risk newborns found no effect of ω -3-rich fish oil supplements on overall sensitization to aeroallergens and HDM sensitization at 18 months (36). However, at this stage the prevalence of sensitisation to ryegrass was less than 5% and long-term follow-up with a further examination of ryegrass sensitization is in progress.

We have previously reported that the sibling effect on atopy varies by allergen-specific sensitization status (11). Here, a similar difference was observed for another putative protective factor – fish intake. Interestingly, the inverse associations between fish and any atopy or asthma were weak and non-significant overall. These inverse associations only were strong and significant for sensitization to ryegrass only and asthma associated with ryegrass-pure sensitization. Further investigation of the causes of atopic disease should include the examination of allergen-specific phenotypes when considering the influence of environmental factors.

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