

A case–control study of dietary and erythrocyte membrane fatty acids in asthma

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Summary

Background Epidemiological evidence suggests that increased dietary ω -6 and reduced ω -3 fatty acid intake, may have contributed to the rising prevalence of asthma, but these hypotheses have not been tested in studies comparing both dietary intake and objective measures of polyunsaturated fatty acids.

Objective To assess whether a higher intake of ω -6 or a lower intake of ω -3 fatty acids increases the risk of asthma, by measuring dietary fatty acid intake by a food frequency questionnaire (FFQ) and erythrocyte membrane fatty acids, as an objective biomarker of intake.

Methods We have compared individual fatty acid intake estimated by FFQ and by mass spectrometry of fasting erythrocyte cell membranes in 89 cases of asthma and 89 community-matched controls.

Results The odds of asthma were increased in relation to intake of the ω -3 fatty acids eicosapentaenoic acid (odds ratio (OR) for difference between the 25th and 75th centiles of intake = 1.89, 95% CI 1.15–3.11) and docosahexaenoic acid (OR = 2.11, 95% CI 1.19–3.74). There was no evidence of any difference in erythrocyte membrane levels of ω -3 fatty acids, while the odds of asthma were reduced in relation to linoleic acid (ω -6) membrane levels (OR = 0.45, 95% CI 0.21–0.95).

Conclusion These findings suggest that dietary ω -3 fatty acids do not play a major role in protecting against asthma, and that higher levels of erythrocyte membrane linoleic acid are associated with a lower risk of asthma.

Keywords asthma, diet, fatty acids

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Introduction

Asthma is one of the commonest chronic diseases in developed countries, and a major cause of morbidity. Over recent decades, there has been a dramatic increase in the incidence and prevalence of asthma [1] and although the factors responsible have not been identified, dietary changes are amongst those implicated [2]. In particular, it has been suggested that increasing dietary intake of ω -6 polyunsaturated fatty acids [3], and particularly linoleic acid [4] (a major dietary source of ω -6 fatty acid) may have adverse effects on asthma [5], while dietary ω -3 fatty acids may reduce asthma risk [6, 7]. All of these effects may be mediated by effects of polyunsaturated fatty acids on eicosanoid production [8] and inflammatory cell membrane fluidity [9], both of which may influence the pathogenesis of asthma [10, 11].

To date, however, the majority of the evidence of the effect of dietary fatty acid intake on asthma has come from epidemiological studies that have measured intake using food frequency questionnaires (FFQs), which are a relatively

imprecise measure of nutrient intake. Of the two studies to use an objective measure of serum fatty acids, one is a small study of plasma fatty acids in 17 children which demonstrated higher levels of linoleic acid and lower proportions of arachidonic acid (AA) in children with atopic asthma compared to controls [12]; the other is a case–control study in adults which demonstrated no difference in serum fatty acid levels, but did not take fasting samples [13]. In the only primary prevention study to date in this area, a diet high in ω -3 fatty acids was given to newborn babies considered at risk of asthma and allergy, and interim data at 18 months of age demonstrated a reduction in 'wheeze ever' and 'wheeze lasting more than a week', but no benefit for other secondary outcome measures relating to wheeze or for the prevalence of asthma in this group [14].

We have, therefore, compared fatty acid levels measured using a specially adapted and validated FFQ [15], and mass spectrometry of fasting erythrocyte cell membranes, in cases with asthma and matched, non-asthmatic controls.

Methods

From January 2000 to March 2001, 426 cases with physician-diagnosed asthma using regular inhaled corticosteroids were

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identified from our departmental asthma volunteer database and from local primary care registers, and 510 non-asthmatic controls, individually matched by primary care register, sex and age, were contacted and invited to participate. To minimize bias due to seasonal variation, we tried to study matched cases and controls as closely as possible in time, avoiding the Christmas and New Year period. Cases and controls were informed that the researchers were interested in 'asthma and food'. We excluded cases who were using leukotriene antagonists or oral corticosteroids, and exclusion criteria for both cases and controls were a diagnosis of hypertension, cardiovascular disease, other metabolic or inflammatory disorders, usage of dietary oil supplements, cholesterol-lowering or non-steroidal anti-inflammatory drugs, pregnancy, current breastfeeding or a smoking history of more than 10 pack-years.

Eligible, consenting participants completed a self-administered, semi-quantitative FFQ (DietQ, Tinuviel Software, Warrington, UK), adapted and validated to assess intake of the main dietary fatty acids [15]: linoleic acid, AA, palmitic acid, stearic acid and oleic acid, along with the ω -3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Pearson correlation coefficients for the unadjusted fatty acids intake of the FFQ, measured against 7 day weighed records, were 0.44, 0.71, 0.51, 0.61, 0.29, 0.59 and 0.55, respectively. All participants were asked to refrain from eating or drinking anything except water from 22:00 hours before a morning appointment at which a fasting venous blood sample was taken. Within 2 h, erythrocytes were separated by centrifugation and washed with normal saline at 4°C, and the two mixtures were then added to 0.2 mL of packed erythrocytes for preparation of the methyl esters: 0.25 mL of methanol containing 1 g/L margaric acid (which acted as the internal standard) with 20 g/L of butylated hydroxytoluene; followed by 2 mL of chloroform/methanol (1/2; v/v) before storing samples at -70°C [16]. All chemicals were Analar grade or higher, obtained from Sigma-Aldrich Co. Ltd (Dorset, UK). Fatty acid methyl esters were then prepared by transesterification [17] using acetyl chloride and, after dissolving in benzene, were analysed by gas chromatograph mass spectrometry as previously described [18]. Relative percentage levels of the same individual fatty acids were then quantified by comparing peak area ratios with those from external standard mixtures treated the same as the specimens.

Subjects were tested for allergen skin sensitivity to *Dermaphagoides pteronyssinus*, cat fur and mixed grass pollen with histamine (1 mg/mL histamine in 50% glycerol) and saline controls (Bencard, Worthing, UK). Weal reactions were read at 15 min, taking the average of the largest measurable diameter, and the diameter perpendicular to this. Participants were asked to refrain from taking antihistamine medication for at least 24 h beforehand. Allergen skin sensitization was defined as a weal reaction to allergen of at least 3 mm greater than that of the saline control. Those subjects with sensitization to any one or more of the three allergens tested were defined as atopic.

We compared absolute intake of dietary fatty acids measured by FFR and percentage levels of erythrocyte membrane fatty acids between cases and controls with conditional logistic regression, adjusting for potential confounding by season at time of study (as defined by winter, spring, summer,

autumn), body mass index (BMI), and dietary energy intake in megajoules. To provide a consistent and dimensionless measure of risk across the various ranges of fatty acid intakes, we have presented our results in terms of odds ratios (ORs) for asthma in the 75th percentile relative to the 25th centile of dietary intake and percentage erythrocyte membrane levels for each fatty acid studied. We compared dietary and erythrocyte membrane levels of linoleic and arachidonic ω -6 fatty acids, and of eicosapentaenoic and docosahexaenoic ω -3 fatty acids as primary hypotheses. We determined at the outset of the study that a sample size of 80 matched case-control pairs would provide at least 90% power to detect a 0.5 standard deviation (SD) difference in each polyunsaturated fatty acid level between cases and controls.

Results

Of the 426 potential cases and 510 controls approached, 195 (46%) and 156 (31%), respectively, agreed to participate, and 96 (49%) cases and 100 (64%) controls met the entry criteria. We were unable to recruit a suitably matched control for seven cases, so 89 matched pairs were included in the final analyses.

The mean (SD) age of the cases and controls was, respectively, 42.8 (9.7) and 43.0 (9.9) years, and 74% (66 matched pairs) were female. Cases and controls were similar in terms of BMI, smoking status and social class (Table 1), intakes of macronutrients and other important micronutrients (Table 2). Atopy was more prevalent in cases, with 72 (81%) of the cases and 21 (24%) of controls having a positive skin prick test to one or more allergens.

Using the FFQ estimates of dietary intake, asthmatics had higher levels of the ω -3 fatty acids, DHA and EPA, and these differences were statistically significant after adjustment for

Table 1. Demographic data of cases and controls

	Cases (n = 89)	Controls (n = 89)
Mean age (SD)	42.8 (9.7)	43.0 (9.9)
Number of males (%)	23 (26)	23 (26)
Mean BMI (kg/m ²)	25.5 (5.2)	25.4 (4.7)
(SD) n = 86*, 81*		
Atopy (%)	72 (81)	21 (24)
Smoking status: n (%)		
Current	0	1 (1)
Median total pack years (IQR)	6.25 (2.3–9.0)	7.5 (3.0–9.0)
Mean inhaled corticosteroid:		
mmg (SD)		
Beclomethasone n = 63*	560 (411)	
Budesonide n = 18	461 (355)	
Fluticasone n = 6*	600 (540)	
Social class†: n (%)		
I	10 (11)	13 (15)
II	35 (39)	35 (39)
III	41 (46)	36 (41)
IV	2 (2)	4 (5)
V	1 (1)	1 (1)

*Missing data. †I, professional; II, intermediate; III, skilled; IV, semi-skilled manual; V, unskilled manual.

SD, standard deviation; BMI, body mass index; IQR, interquartile range.

season of data collection, BMI and energy intake (Table 3). There was no difference between cases and controls in levels of the intake of ω -6 fatty acids (AA or linoleic acid), nor in total ω -3 or ω -6 fatty acids, total polyunsaturated fatty acids, or the ratio of ω -3 to ω -6 fatty acid intake.

In the comparisons of the same fatty acids in erythrocyte cell membranes, there was no difference between cases and controls in the percentage of ω -3 DHA (Table 4), while levels of EPA were undetectable in all but two of the cases and two of the controls. Levels of ω -6 linoleic and AA acids were both lower in asthma cases than controls, significantly so for linoleic acid before and after adjustment for potential confounders.

There was also no difference between cases and controls in dietary intakes, or erythrocyte membrane levels of each of the other fatty acids measured in this study, including palmitic, stearic and oleic acids, after adjustment for potential confounders.

Conclusions

This study was designed to use estimates of both dietary intake and objective markers of fatty acids in cell membranes to test the prior hypotheses that a higher intake of ω -6 polyunsaturated fatty acids may increase the risk of mild to moderate asthma, and that conversely ω -3 acids may reduce

it. These data conflict with the hypothesis that ω -3 fatty acids are protective in asthma, and demonstrate that increased self-reported dietary EPA and DHA intake were associated with a greater risk of asthma. Furthermore, higher erythrocyte membrane levels of linoleic acid were associated with a decreased risk of asthma.

In line with evidence that dietary fatty acids may also be involved in the pathogenesis of chronic obstructive airways disease (COPD) [19], we excluded subjects with a smoking history of more than 10 pack years to avoid bias arising from potential diagnostic misclassification. We are unable to exclude the possibility of selection bias in our study, although we tried to minimize the possibility of this by selecting controls from the same primary care populations, and hence communities as the cases with asthma. Nonetheless, only 46% of cases and 31% of controls invited to participate in the study responded, and we have no data as to the characteristics of those individuals who failed to respond, and so the potential for selection bias in this study exists. A higher percentage of women participated than men, which may limit the generalizability of our findings. It was not apparent that the observed relationships were different in men and women in our study, but our study was not powered to look for these interactions.

Our findings for dietary intake were derived from a FFQ purposely designed and validated to obtain details on specific individual fatty acids in the UK diet [15] with correlation coefficients ranging from 0.29 to 0.71 for the fatty acids measured which compare favourably with FFQ's elsewhere [20]. Nonetheless, the limitations of FFRs in introducing measurement error have recently been highlighted [21]. It seems most likely that the errors introduced in assessing dietary intake were random errors, which would have reduced the ORs observed towards unity. Differential recall of diet in asthmatics compared to controls might have introduced recall bias but was minimized in this study by not informing the participants which aspect of diet the study was assessing, and by additionally using an objective marker of dietary intake, erythrocyte membrane fatty acid levels. While the exposures measured are current, dietary behaviours tend to be relatively constant, and so current diet may be considered a marker for previous dietary habits. We excluded confounding by age, sex, social class and local region of residence in our study by matching our cases and controls for age (in 10-year bands), sex, and for primary care physician; the latter ensured that cases and controls lived in a similar district and achieved good matching by social class (see Table 1). There was no

Table 2. Table of macro- and micronutrient intakes estimated by food frequency questionnaire

	Cases: mean (SD)	Controls: mean (SD)
Total energy (MJ)	8.0 (2.2)	7.7 (1.8)
Alcohol (g/day)	12.0 (14.3)	10.3 (12.7)
Carbohydrate (g/day)	231.6 (62.7)	229.0 (53.5)
Fat (g/day)	69.0 (26.8)	67.1 (27.4)
Protein (g/day)	80.1 (21.2)	76.7 (17.1)
Starch (g/day)	117.1 (36.7)	118.7 (33.5)
Sugar (g/day)	113.3 (36.7)	109.1 (33.4)
Calcium (mg/day)	992.4 (255.6)	980.6 (260.0)
Folate (mmg/day)	255.5 (79.3)	257.9 (70.5)
Iron (mg/day)	11.6 (3.2)	11.4 (3.2)
Magnesium (mg/day)	322.6 (113.3)	320.4 (101.6)
Sodium (mg/day)	2477.5 (711.1)	2308.1 (597.1)
Vitamin C (mg/day)	103.0 (52.6)	101.6 (48.6)

SD, standard deviation.

Table 3. FFQ estimated dietary fatty acid intake in asthma cases and controls

	Absolute fatty acid intake (mg)		OR for the 75th vs. 25th percentile of intake (unadjusted)	OR for the 75th vs. 25th percentile of intake (adjusted*)
	Mean (SD)			
	Asthma (<i>n</i> = 89)	Control (<i>n</i> = 89)		
Linoleic acid	10.10 (5.48)	10.43 (6.65)	0.92 (0.63–1.35)	0.79 (0.38–1.61)
Arachidonic acid	0.13 (0.08)	0.11 (0.05)	1.42 (0.99–2.03)	1.53 (0.92–2.55)
Total ω -6 fatty acids	10.33 (5.50)	10.61 (6.66)	0.93 (0.64–1.36)	0.80 (0.39–1.64)
Eicosapentaenoic acid	0.13 (0.13)	0.10 (0.09)	1.38 (1.00–1.90)	1.89 (1.15–3.11)
Docosahexaenoic acid	0.17 (0.15)	0.13 (0.10)	1.37 (0.97–1.92)	2.11 (1.19–3.74)
Total ω -3 fatty acids	1.56 (0.64)	1.53 (0.77)	1.04 (0.74–1.45)	1.18 (0.63–2.19)

*Adjusted for season of data collection, body mass index and energy intake. FFQ, food frequency questionnaire; SD, standard deviation; OR, odds ratio.

Table 4. Fasting erythrocyte membrane fatty acid levels in asthma cases and controls

	Fatty acid percentage Mean (SD)		OR for the 75th vs. 25th percentile of intake (unadjusted)	OR for the 75th vs. 25th percentile of intake (adjusted*)
	Asthma (<i>n</i> = 89)	Control (<i>n</i> = 89)		
Linoleic acid	7.4 (2.5)	8.6 (2.6)	0.47 (0.29–0.76)	0.45 (0.21–0.95)
Arachidonic acid	15.4 (3.4)	16.3 (3.9)	0.70 (0.47–1.06)	0.76 (0.45–1.26)
Docosahexaenoic acid	5.8 (3.6)	6.3 (4.7)	0.84 (0.56–1.25)	1.31 (0.70–2.45)

*Adjusted for season of data collection, body mass intake and energy intake. SD, standard deviation; OR, odds ratio.

evidence that either group was eating significantly healthier diets than the other when other micro and macronutrients were analysed (Table 2). It is possible in fact that our design resulted in overmatching of our cases and controls, since social class is strongly associated with diet. This would have resulted in ORs that underestimated the true difference between asthmatics and controls, but seems unlikely to explain our findings which reveal significant differences but in directions contrary to the hypothesized effects. It proved logistically difficult to recruit and study matched cases and controls at exactly the same time, and though we succeeded in studying 45% of pairs within 3 months of each other, we presented our findings adjusted for the season of data collection. This made little qualitative difference to our findings, and it is therefore unlikely that the study results were explained by seasonal dietary variation. We also recognize that we have analysed the effects of eight individual fatty acids and the significance levels of our results need to be interpreted conservatively, but nevertheless, the size and direction of observed effects are in direct contrast with existing hypotheses relating ω -3 fatty acids in asthma.

We are unable to explain the discrepancy between the effect of dietary ω -6 fatty acids and the lack of effect of erythrocyte fatty acids on risk of asthma. This may be a consequence of the measurement error involved or due to differential metabolism of fatty acids, either due to intrinsic differences due to asthma or extrinsic differences such as exposure to medications such as inhaled corticosteroid. Similarly, the difference in the erythrocyte fatty acid membrane fatty acid linoleic acid levels between cases and controls may be due to the effects of asthma therapy with inhaled corticosteroids. However, animal studies have shown that glucocorticoids prevent AA release from phospholipids [22] and, therefore, any corticosteroid effect would be expected to potentially increase rather than decrease linoleic acid levels in asthmatic subjects. Furthermore, cases taking oral corticosteroids were excluded from the study, and the low to moderate doses of inhaled corticosteroids used by study participants are unlikely to have a significant effect.

Although ecological studies have previously reported associations between asthma symptoms and dietary intake of fats and fatty food [23, 24], other epidemiological studies have been less consistent in their findings. Cross-sectional studies have shown positive associations between asthma and total fat intake [25], foods rich in animal fats [26], and saturated fats [27], and a protective effect of fish consumption [6, 7] and monounsaturated fat [27]. In contrast, no association was found between use of animal or vegetable oils in cooking and asthma in a case-control study of 12-year-old children, although a positive association was seen with food

obtained from fast food outlets [28]. In the only prospective study to date assessing the relationship between dietary fats and adult-onset asthma, no consistent association was seen between any class of fats or linoleic acid and doctor diagnosed asthma [29]. However, in an 18-month interim report from a primary prevention study of dietary ω -3 fatty acid supplementation in 'high risk' newborn infants, those with a diet comprising an ω -3 to ω -6 ratio of 1–5 had a 9.8% reduction of 'wheeze ever' and 7.8% reduction of 'wheeze for more than a week' compared to those with a normal diet, although there was no difference in asthma diagnosis between groups [14]. None of these studies has involved biological measures of individual fatty acid levels. There are only two studies to date which include objective measures. The first compared serum fatty acids in cases and controls and found no difference in 13 fatty acids studied including those presented in our data [13], although the samples taken were not fasting, which questions the comparability of these data with ours. The second, another small study demonstrated high plasma linoleic acid and lower AA in 17 children with asthma compared to controls [12]. It is important to emphasize that our data is in an adult population with established asthma, and care should be taken in extrapolating our observations to other age groups. In addition, we do not have data on the duration of asthma, a factor that theoretically may also influence fatty acid metabolism.

The observation that erythrocyte membrane linoleic acid has an apparent protective effect on risk of asthma was unexpected, although may simply reflect increased consumption of eicosanoid precursors due to the increased inflammation associated with asthma [30]. It has been hypothesized that ω -3 fatty acids may have a protective role in asthma, due to their ability to competitively antagonize the metabolism of AA to the pro-inflammatory eicosanoids. However, in this study, cases were found to have a higher dietary intake of the ω -3 fatty acids EPA and DHA compared to controls, and one possible explanation for this is that the recent high profile of a potential protective effect of fish on asthma may have led to a higher intake in those with the disease ie reverse causality. The majority of intervention studies of fatty acids in asthma have concentrated on fish oil supplements, which comprise predominantly ω -3 fatty acids. A recent Cochrane review concluded, however, that there was little evidence of clinical benefit in asthma from ω -3 oil supplementation in the available data [31]. Nonetheless, it is plausible that ω -3 fatty acids may influence the risk of asthma earlier in life. Although unable to assess early life exposures to fatty acids, our study suggests that ω -3 fatty acids do not have a major protective role in the pathogenesis or epidemiology of asthma.

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