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n6/n3 Hypothesis and Allergies: Biologically Plausible, but not Confirmed*

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Abstract: The dietary intake of certain fatty acids might contribute to the development of atopic diseases like allergic rhinitis and asthma. We investigated the association between the ratio of n-6 and n-3 fatty acids in serum phospholipids and hay fever or allergic sensitisation in adults. Data from a population based cross-sectional study on respiratory health including measurement of fatty acids in serum phospholipids of 740 adults between 20 and 64 years of age were analysed.

We could not find any significant association between n6/n3-ratio of fatty acids in serum phospholipids and hay fever or allergic sensitisation neither in the total population nor in the sub-population stratified by sex.

Since no previous study on fatty acid intake confirmed the n6/n3-ratio hypothesis and this study did not find any association between the n6/n3-ratio in serum-phospholipids and hay fever or allergic sensitization, we conclude that the n6/n3- ratio hypothesis is not confirmed although biological plausible.

Key words: hay fever, allergic sensitisation, diet, ECRHS

Introduction

In the last three decades western countries have witnessed increase in the prevalence of atopic diseases [1, 2]. Besides other environmental factors speculations on the contribution of the western diet to this increase have been made, in particular changes in the consumption of n-6 and n-3 polyunsaturated fatty acids. The increase in atopic diseases goes along with large changes in the consumption of fatty acids. While the dietary intake of vegetable oil-based products rich in the n-6 polyunsaturated fatty acid linoleic acid rose over the last decades, the intake of saturated and polyunsaturated n-3 fatty acids decreased [3]. Linoleic acid is a precursor of arachidonic acid which can be converted to the proinflammatory eicosanoids leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2). PGE_2 may shift the balance of T-helper cells from type 1 to type 2 and may thus lead to an enhanced production of immunoglobulin E (IgE) from B-cells [4, 5]. In contrast, eicosanoids derived from the n-3 fatty acid eicosapentaenoic acid (EPA) can down-regulate the production of PGE_2.

It is hypothesised that the rise of the n6/n3-ratio in the diet results in an enhanced production of arachidonic-derived eicosanoids, while eicosanoids derived from eicosapentaenoic acid are produced in smaller quantities [6].

Although this hypothesis is popular - found in monographs [7] - and is investigated in some studies, supporting empirical evidence is still lacking. Since no substantial association between the ratio of ingested n-6 and n-3 fatty acids and atopic diseases was found in several studies [8-10], some authors concluded that data on dietary intake of fatty acids alone might be insufficient due to possible modification of the association by metabolism and genetic determinants.

Therefore we used the fatty acid composition of serum phospholipids as a marker of intake and subsequent metabolism to investigate the association between n6/n3-ratio, hay fever and allergic sensitisation in adults.

Methods

Study Subjects

Present study draws sample from one of the two surveys conducted in Germany as part of the European Community Respiratory Health Survey (ECRHS). This survey was conducted in adults aged 20-64 years in Erfurt 1991-92. Study design and population sampling are described in detail elsewhere [11, 12]. In brief, a total of 1282 participants answered the main questionnaire and from 1258 participants blood samples were drawn for analysis of specific and total IgE. Also, a serum sample was stored at -80 °C for later analyses. Additionally, a subset of the participants was asked to

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participate in a dietary survey, where they filled in three-day weighted records on their diet [8]. From this subset, unthawed serum samples were still available for 740 participants - 313 women and 427 men. For the present study, these samples were used for fatty acid assessment in their serum phospholipids.

Questionnaire Data and Blood Tests

Participants who answered "Yes" to the question: "Do you have nasal allergies, including hay fever?" were categorised as participants who suffered from hay fever. Allergic sensitisation to common aeroallergens (house dust mite *Dermatophagoides pteronyssinus*, grass pollen, cat, *Cladosporium* and birch pollen, a local allergen in northern Europe) was assessed by specific serum IgE concentrations, using the Pharmacia CAP System. A subject with at least one specific IgE $\geq$ 0.7 kU/l was categorised as sensitised.

Because only 18 participants answered "yes" to the question "Have you ever had asthma", we did not analyse asthma as an outcome variable.

Ethics committee of the Medical School, Erfurt approved the study.

Analysis of Fatty Acids

Serum was kept frozen at -80 °C until analysis in 2003 and was never thawed before the measurement of fatty acids. Serum lipids were extracted with hexane/ isopropanol (3:2). Phospholipids were isolated by thin layer chromatography and fatty acid methyl esters were obtained by acid catalysed trans-esterification in methanol. Methyl esters were extracted into hexane and frozen until analysis in a gas chromatograph [13]. In total, 36 fatty acids were measured and the results were expressed as percentage of the total fatty acids in serum phospholipids.

As n-6 fatty acids C18:2 n-6 (linoleic acid), C18:3 n-6 (γ-linolenic acid), C20:2 n-6, C20:3 n-6 (dihomo-γ-linolenic acid), C20:4 n-6 (arachidonic acid), C22:2 n-6, C22:4 n-6 and C22:5 n-6 were summarised. C18:3 n-3 (α-linolenic acid), C18:4 n-3, C20:3 n-3, C20:5 n-3 (eicosapentaenoic acid), C22:5 n-3 and C22:6 n-3 (docosahexaenoic acid) were summarised as n-3 fatty acids. Dividing the sum of n-6 over the sum of n-3 fatty acids assessed n6/n3-ratio.

Statistical Analysis

We applied logistic regression models to assess the association of allergic sensitisation or hay fever with the n6/n3-ratio of fatty acids in serum phospholipids. Odds ratios (OR) were adjusted for sex, age group, body mass index, education, smoking status and total energy intake. Additionally we conducted a sex-stratified analysis. We did not adjust for family history of atopy because this confounder showed no effect on the point estimates and many participants were not aware of the atopic status of their parents, who were born long before 1950. Linearity of the association between atopy and the n6/n3-ratio was tested with S plus version 6. SAS version 8.2 was used for all other calculations (SAS Institute, Cary, NC).

Results

Table 1 shows the basic characteristics of the study population included in this analysis. No statistically significant sex differences were observed in the prevalence of hay fever or allergic sensitisation (at least one specific IgE $\geq$ 0.7 kU/l). Neither the sum of n-6 fatty acids, nor the sum of n-3 fatty acids, nor the ratio of n-6 fatty acids/ n-3 fatty acids in serum phospholipids differed significantly between men and women.

The proportion of all n-6 fatty acids varied between 22.2% and 39.8% of total fatty acids and of all n-3 fatty acids between 2.9% and 13.9%. The range of the n6/n3-ratio was 2.02 to 10.55, cut-points of the quartiles were $\leq$ 4.85, $\leq$ 5.79 and $\leq$ 6.78 for the total study population, $\leq$ 4.84, $\leq$ 5.72 and $\leq$ 6.77 for women and $\leq$ 4.89, $\leq$ 5.83 and $\leq$ 6.79 for men.

No significant association between the n6/n3-ratio in serum phospholipids and hay fever or allergic sensitisation was observed, neither in the total analysed population, nor after stratification by sex (Table 2). In addition, there were no associations between the sum of all n-6 respectively all n-3 fatty acids and hay fever or allergic sensitisation (data not shown).

Discussion

In contrast to the hypothesis of Black and Sharpe [6] on positive association between n6/n3-ratio and atopic diseases, we did not find any association between the n6/n3-ratio of fatty acids in serum phospholipids and hay fever or allergic sensitisation in this group of adults.

Moreover, our data even indicated an inverse association in most cases, although not reaching statistical significance.

In a recently published study, Woods et al. also did not find any association between n6/n3-ratio in serum phospholipids and allergic sensitisation, assessed by skin prick test [9].

A few epidemiologic studies investigated the association between fatty acid intake and atopic diseases. A German EPIC substudy found non-significant inverse relationship between the n6/n3-ratio of dietary fatty acids, assessed by a food frequency questionnaire, and hay fever in adults [10]. Analysing dietary data of this study, Trak-Fellermeier et al. also did not find any association between n6/n3-ratio of dietary fatty acids and hay fever or allergic sensitisation in adults [8].

Other studies on association between fatty acid intake and hay fever and allergic sensitisation did not use the n6/n3-ratio, but assessed either fish [14, 15], margarine [16, 17], or n-6 or n-3 polyunsaturated fatty acid intake [18]. Although high fish intake is expected to decrease the n6/n3-ratio, a study conducted on teenagers in Taiwan failed to find association between fish intake and allergic rhinitis [14]. Wakai et al. also did not see a relationship between n-3 polyunsaturated fatty acid intake and allergic rhinitis in women, but they found positive association between n-6 polyunsaturated fatty acid intake and allergic rhinitis [18]. On the other hand, a Norwegian study reported a negative association between introduction of fish in the diet before the age of 12 months and allergic rhinitis

Table 1. Characteristics of the study population.

	Total (n = 739)	Women (n = 312)	Men (n = 427)
Hay fever* (%)	10.3	11.9	9.1
Allergic sensitisation † (%)	22.9	22.8	23.0
Age (years) ‡	41.5 ± 12.3	40.0 ± 12.0	42.7 ± 12.3
Body mass index ‡	25.5 ± 4.0	24,9 ± 4.4	25,9 ± 3.7
Energy intake (kcal/d) ‡	2225 ± 620	1837 ± 436	2509 ± 579
Smoking status (%)			
Never smokers	40.2	52.6	31.2
Exsmokers	27.7	19.5	33.7
Current smokers	32.1	27.9	35.1
Educational level (%)			
High	46.0	44.0	47.4
Medium	32.4	34.1	31.2
Low	21.6	21.9	21.4
Fatty acids in serum-phospholipids			
Sum n-6 § (%)	34.47 ± 2.35	34.66 ± 2.06	34.33 ± 2.54
Sum n-3 ‖ (%)	6.22 ± 1.43	6.25 ± 1.43	6.19 ± 1.44
N6/n3 ratio¶	5.84 ± 1.42	5.85 ± 1.46	5.84 ± 1.39

* defined as answering "yes" to the question "Do you have nasal allergies, including hay fever?"
† defined as at least one specific IgE ≥ 0.7 kU/l
‡ mean ± SD
§ sum n-6 fatty acids = (C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:2 n-6 + C22:4 n-6 + C22:5 n-6)/ total fatty acids
‖ sum n-3 fatty acids = (C18:3 n-3 + C18:4 n-3 + C20:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3)/ total fatty acids
¶ n6/n3-ratio = sum n-6 fatty acids/ sum n-3 fatty acids

Table 2. Association between n6/n3-ratio in serum phospholipids and atopic outcomes in adults.

	OR (95% CI)		
Atopic outcome	2nd Quartile*	3rd Quartile*	4th Quartile*
Total (n = 737)			
Hay fever †§	0.57 (0.28-1.16)	0.63 (0.32-1.26)	0.67 (0.34-1.34)
Allergic Sensitisation ‡§	0.79 (0.47-1.32)	0.90 (0.54-1.49)	0.84 (0.50-1.40)
Women (n = 311)			
Hay fever ‖	1.12 (0.41-3.05)	0.47 (0.14-1.53)	0.80 (0.27-2.37)
Allergic Sensitisation ‖	1.20 (0.50-2.88)	0.72 (0.29-1.81)	1.46 (0.62-3.43)
Men (n = 426)			
Hay fever ‖	0.46 (0.16-1.31)	0.80 (0.33-1.96)	0.73 (0.29-1.84)
Allergic Sensitisation ‖	0.64 (0.33-1.22)	0.86 (0.46-1.62)	0.52 (0.26-1.02)

* 1st quartile was set as the reference category
† defined as answering "yes" to the question "Do you have nasal allergies, including hay fever?"
‡ defined as at least one specific IgE ≥ 0.7 kU/l
§ adjusted for sex, age group (20-29, 30-39, 40-49, 50-59, ≥60 years), education (low, middle, high), smoking status (never, ex, current), body mass index (continuous) and total energy intake (kcal/d, continuous)
‖ adjusted for age group (20-29, 30-39, 40-49, 50-59, ≥60 years), education (low, middle, high), smoking status (never, ex, current), body mass index (continuous) and total energy intake (kcal/d, continuous)

at the age of 4 years [15]. Presumably high amounts of fish should to be eaten in very early ages to see the effects, which is not common in most other western countries.

A study on children in Leipzig, eastern Germany, reported a significantly higher frequency of hay fever in children, whose margarine consumption increased from 1989 to 1995/96, compared to children who ate equal or less margarine [16]. In a sex-stratified analysis Bolte et al. observed a positive association between consumption of margarine as spread and allergic sensitisation and rhinitis symptoms during the last 12 months in boys, but not in girls living in the state of Sachsen-Anhalt, eastern Germany [17].

Presumably the assessment of fatty acid intake by using a single food is too crude to draw conclusions on the role of the n6/n3-ratio for the development of hay fever or allergic sensitisation, where the food may be a marker for certain lifestyle. Because the positive associations between certain foods, namely fish and margarine were only seen in children, they might have an influence only in early years of life.

Summarising the results of our study and the results of other papers, there is no support for Black and Sharpe's hypothesis [6] that a high n6/n3-ratio indicates an increased risk for the development of atopic diseases.

We measured fatty acid concentrations in serum phospholipids as a biomarker for fatty acid intake within the recent past [19, 20] and for metabolism, because fatty acid concentration in serum phospholipids better reflects fatty acid availability in the body. We do not know the extent to which ingested fatty acids get metabolised in the body. There are some factors, which influence measured fatty acid biomarker level: for example genetic polymorphisms of elongase and desaturase enzymes or nutritional status [19].

It might be possible that long storage of serum samples has led to oxidation of the long chain polyunsaturated fatty acids. But to us it seems unlikely that predominantly n-3 or n-6 polyunsaturated fatty acids were damaged. Zeleniuch-Jacquotte et al. reported that storage of serum samples up to 12 years at -80 °C protected fatty acids in phospholipids against oxidation very well [21].

We used self-reported hay fever as outcome variable, which was not confirmed by a physician. Because allergic sensitisation, measured by analysis of specific IgE in serum showed similar associations with the n6/n3 fatty acid ratio in serum phospholipids, we do not think that self-reported diagnosis caused a strong bias.

Our study results should be interpreted with caution because of the cross-sectional nature of our study design.

Since no previous study on fatty acid intake confirmed the n6/n3-ratio hypothesis and this study did not find any association between the n6/n3-ratio in serum-phospholipids and hay fever or allergic sensitization in adults, we conclude that the n6/n3- ratio hypothesis is not justifiable although biological plausible.

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