Plasma fatty acids in asthmatic children of Northern Greece

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Submitted: 3 May 2008
Accepted: 6 November 2008

Arch Med Sci 2009; 5, 1: 51-56

Abstract

Introduction: Asthma has genetic determinants and environmental influences. One of the latter is diet. The aim of this study was to determine whether the composition of plasma fatty acids, as a measure of dietary intake, differed in Greek children with and without asthma; also, to correlate plasma fatty acids with lung function.

Material and methods: The study included 2 groups of children. Group A consisted of 228 children with asthma and Control group B of 48 healthy children without asthma or atopy. Parents of the participating children completed a food frequency questionnaire. Total plasma fatty acids were determined by gas chromatography.

Results: According to our results total plasma fatty acids levels were similar in both groups. There were no significant differences in total saturated, monounsaturated, and polyunsaturated omega-3 and omega-6 fatty acids levels. Children of the control group were found to have higher levels of steatic acid (18:00) and α-linolenic acid (18:3ω3) and the difference was statistically significant ($p < 0.01$ and $p = 0.022$) respectively. In order to investigate a possible relationship between lung function and plasma fatty acid levels, we divided asthmatic children into two subgroups, according to spirometry results. Levels of omega-3 fatty acids 20:5 (eicosapentaenoic acid – EPA), 22:5 (docosapentaenoic acid – DPA) and 22:6 (docosahexaenoic acid – DHA) were found higher in asthmatic children with normal lung function ($p < 0.01$).

Conclusions: Our results indicate that there are differences in the consumption of some whole foods in the population of children we studied and that asthmatic children consumed less frequently fresh fruit and raw vegetables.

Key words: asthma, omega-3 fatty acids, omega-6 fatty acids, children.

Introduction

Asthma is a chronic inflammatory disorder of the airways leading to airways hyperresponsiveness and associated symptoms. Until recently, most studies have reported that the prevalence of asthma has increased despite the progress that has been made in the treatment options [1]. Although asthma has genetic determinants, these alone cannot account for this increase. Instead, these rapid increases are most likely the result of changes in environmental influences with consequent changes in gene-environment interactions, which increase the expression of genetic susceptibility [2, 3].

Among the environmental factors that have changed is diet. The number of studies providing evidence on the relation between dietary factors and asthma has increased significantly in the past 10 years. Reviews of the literature on nutrition and asthma have identified fruits, vitamin C, vitamin E, and β-carotene as likely to be protective against asthma [2-7]. Various fatty acids and oily fish may play a role, but further evidence is needed [7-9]. Most of the studies included in these reviews have been on adults. Only a few studies have been published on various dietary components and asthma (symptoms) in children [10-16].

Research on fatty acid effects has focused on two main areas: intake of omega-3 (n)-3 polyunsaturated fatty acids from fish oils, and of omega-6 and trans-fatty acids [17, 18]. Omega-3 fatty acids are considered essential fatty acids, which means that they are essential to human health but cannot be manufactured by the body. For this reason, omega-3 fatty acids must be obtained from food such as fish and certain plant oils. It is important to maintain an appropriate balance of omega-3 and omega-6 (another essential fatty acid) in the diet as these two substances work together to promote health. Extensive research indicates that omega-3 fatty acids reduce inflammation, improve lung function in adults with asthma and help prevent certain chronic diseases. Omega-6 fatty acids have the opposite effect: they tend to increase inflammation and worsen respiratory function. So, intake of omega-3 (n)-3 polyunsaturated fatty acids from fish oils is potentially beneficial, and intake of omega-6 and trans-fatty acids may be detrimental to asthma [17, 18].

In the typical Western diet, 20-25-fold more omega-6 polyunsaturated fatty acids (PUFA) than omega-3 PUFA are consumed. This results in the release of proinflammatory arachidonic acid metabolites. Eicosapentaenoic acid and docosahexaenoic acid are omega-3 PUFA derived from fish oil. They competitively inhibit omega-6 PUFA arachidonic acid (AA) metabolism, thus reducing the generation of pro-inflammatory 4-series leukotrienes (LTs) and 2-series prostaglandins (PGs) and the production of cytokines from inflammatory cells. These data are consistent with the proposed pathway by which dietary intake of omega-3 PUFA modulates lung disease [19].

Fatty acid consumption is variable. Western European countries consume mainly saturated fats. Greece (among 56 countries) has been found to have the highest consumption of olive oil and monounsaturated fatty acids [20]. There is evidence that the prevalence of asthma has increased in Greece as in most parts of the world. However, the majority of Greek children suffer from mild to moderate forms of the disease [21].

The aim of this study was to determine whether the composition of plasma fatty acids, as a measure of dietary intake, differed between Greek children with and without asthma (atopic and non-atopic) and to correlate plasma fatty acids with lung function.

Material and methods

Subjects

Two hundred and twenty-eight children with asthma, 148 boys and 80 girls, ranging in age from 5 to 15 years, were included in the study. All children had been diagnosed as having asthma for at least one year and were attending the asthma outpatient clinic of our department regularly. They were enrolled in the study during a stable state of their disease. Those reporting asthma exacerbations in the past 30 days, and requiring reliever therapy at the time of enrolment, were excluded. Also, children with significant underlying disease, those taking nutritional supplements and those taking oral corticosteroids were excluded from the study. Forty-eight healthy children, 32 boys and 16 girls, aged 5-15 years, having no history of asthma and other atopic diseases were recruited as controls.

Anthropometric data

Body weight and height were measured with children wearing light clothes and no shoes. Body mass index was computed as body weight divided by height squared.

Pulmonary function

Pulmonary function testing was performed in 204 asthmatic children able to cooperate and in all children of the control group. Curves for FEV1 and flow volume were obtained with a spirometer. FEV1 data were presented as percentages (100%) after adjusting for age, sex and height.

Atopy

Skin prick tests were performed in children with asthma in order to define atopic status. Atopy was defined as a mean wheal size of 3 mm to at least one of 12 allergens applied with skin prick to the forearm (house dust, *Dermatophagoides pteronyssinus*, *D. farinae*, cat hair and dander, dog hair and dander, mixed feather, rye-grass, timothy grass, ragweed, olive, *Aspergillus fumigatus* and *Alternaria tenius*).

Food frequency questionnaire

Parents of the participating children were asked to complete a food frequency questionnaire. Among the food items investigated, animal fats (bread and butter, milk, cheese, liver), vegetable fats (margarine, olives), and food containing omega-3 fatty acids (bluefish) were included. The questionnaire assessed weekly consumption using a five-level scale: never, less than once a week, 1-2 times per week, 3-4 times per week, 5-7 times per week. The questionnaire also included a section addressed to mothers investigating the type of fat they used in cooking.

Blood analysis

Venous samples (10 ml) were collected with EDTA (1 g/l) as anticoagulant in the fasting state (14 h). Full blood counts were performed by an automated full blood count analyzer. Plasma samples were then stored at –70°C until extraction. Total plasma fatty acids were determined by gas chromatography [22].

Statistical analysis

The data were coded, entered into a database, and analysed by SPSS. Differences between groups were determined by Student's *t*-test and Mann-Whitney test for continuous variables and by χ^2 test for categorical variables.

Results

Characteristics of the participants are summarised in Table I. There were no significant differences detected in male/female ratio or age between the groups. In both the asthmatic and the control group 23.68 and 16.7% of the children respectively had BMI above the 90th percentile for age and sex. This difference was not statistically significant. The majority of asthmatic children (134/228, 58.77%) were defined as atopic according to the results of skin prick testing. The most common aero-allergen was *Dermatophagoides pteronyssinus*.

The food frequency questionnaire was completed by all the children's parents. Frequent consumption of foods such as fruits, raw and cooked vegetables and dairy products was reported. In order to analyze data, we redefined categories as once a week or less, 1-4 times per week, 5-7 times per week. Almost all children reported daily consumption of milk. On the other hand, there were no children reporting frequent consumption of bluefish; categories for fish consumption were redefined as less than once every 2 weeks and at least once every 2 weeks. Results of the food frequency questionnaire are displayed in Table II.

Children in the control group were found to consume fresh fruits and raw vegetables more frequently than asthmatic children (p = 0.019 and p = 0.025 respectively). Also, the control group reported more frequent consumption of dairy products – mainly white cheese (p < 0.005). There were no differences detected in the frequency of consumption of red meat, poultry, precooked (fast food) meals and bluefish.

The majority of mothers (> 75%) reported that they used only olive oil in cooking. Less than 25% of mothers reported use of olive oil in combination with some other seed oil (more commonly sunflower oil). Use of margarine was rare among mothers other than those of north or central European origin.

Total plasma fatty acid levels were similar in asthmatic patients (atopic and non-atopic) and the control group (Table III). There were no

Table I. Characteristics of participants of the study

	Asthma group *n* = 228	Control group *n* = 48	*p*
Boys/girls	1.85	2	NS
Mean age [± SE]	8.8 ±0.23	9.1 ±0.36	NS
BMI ≥ 90 ct (n%)	23.68	16.7	NS
Atopy [n%]	58.77*	0**	< 0.0001

**by skin prick testing, ** by history, NS – not statistically significant*

Table II. Frequency of consumption of specific food items according to the food frequency questionnaire (asthmatic patients and control group)

Type of food		Asthma group *n* (%)	Control group *n* (%)	*p*
Fruits	1	29 (12.70%)	3 (6.30%)	0.019
	2	96 (42.10%)	13 (27.10%)	
	3	103 (45.20%)	32 (66.70%)	
Raw vegetables	1	40 (17.55%)	4 (8.3%)	0.025
	2	105 (46.00%)	16 (33.3%)	
	3	83 (36.45%)	28 (58.3%)	
Cooked vegetables	1	102 (44.75%)	22 (45.8%)	NS
	2	124 (54.38%)	25 (52.1%)	
	3	2 (0.87%)	1 (2.1%)	
Red meat	1	12 (5.27%)	5 (10.4%)	NS
	2	214 (93.86%)	40 (83.3%)	
	3	2 (0.87%)	3 (6.3%)	
Poultry	1	113 (49.56%)	33 (66.7%)	NS
	2	114 (50%)	15 (31.3%)	
	3	1 (0.44%)	0 (0%)	
Dairy products	1	22 (9.65%)	0 (0%)	< 0.005
	2	61 (26.75%)	6 (13.3%)	
	3	145 (63.6%)	39 (86.7%)	
Precooked meals	1	106 (46.5%)	25 (53.2%)	NS
	2	101 (44.3%)	17 (36.2%)	
	3	21 (9.2%)	5 (10.6%)	
Bluefish	4	168 (73.7%)	41 (85.4%)	NS
	5	60 (26.3%)	7 (14.6%)	

1 – once a week or less, 2 – 1-4 times a week, 3 – 5-7 times per week, 4 – less than once every 2 weeks, 5 – at least once every 2 weeks, NS – not statistically significant

Table III. Levels (mmol/l) of plasma fatty acids in asthmatic, atopic and non-atopic patients, and controls. Values are presented as median, with first and third quartiles in parentheses

Type of fatty acid	Atopic 134/228	Non-atopic 94/228	Controls 48	*p*
Myristic, 14:0	0.110 (0.075-0.150)	0.111 (0.083-0.132)	0.130 (0.106-0.160)	NS
Palmitic, 16:0	2.560 (2.35-2.86)	2.590 (2.28-2.79)	2.560 (2.31-2.90)	NS
Stearic, 18:0	1.230 (1.07-1.49)	1.200 (1.08-1.34)	1.420 (1.35-1.58)	< 0.01
Lignoceric, 24:0	0.020 (0.015-0.027)	0.022 (0.018-0.027)	0.021 (0.016-0.025)	NS
Total saturated fatty acids (SFA)	4.180 (3.50-4.44)	3.930 (3.55-4.25)	4.240 (3.71-4.61)	NS
Palmitoleic, 16:1ω7	0.163 (0.128-0.209)	0.181 (0.137-0.232)	0.145 (0.109-0.236)	NS
Oleic, 18:1ω9	2.037 (1.678-2.323)	2.058 (1.745-2.319)	1.870 (1.528-2.163)	NS
Vaccenic, 18:1ω7	0.120 (0.104-0.136)	0.125 (0.097-0.141)	0.011 (0.089-0.136)	NS
Eicosenoic, 20:1	0.013 (0.010-0.017)	0.014 (0.010-0.018)	0.011 (0.010-0.017)	NS
Total monounsaturated fatty acids (MUFA)	2.340 (1.95-2.69)	2.350 (2.05-2.69)	2.200 (1.77-2.57)	NS
Linoleic, 18:2	3.222 (2.898-3.692)	3.279 (2.945-3.731)	3.303 (2.997-3.688)	NS
γ-Linolenic, 18:3ω-6	0.039 (0.027-0.056)	0.039 (0.032-0.057)	0.038 (0.029-0.053)	NS
Dihomo-γ-linolenic, 20:3	0.149 (0.099-0.183)	0.164 (0.113-0.188)	0.152 (0.119-0.185)	NS
Arachidonic, 20:4	0.598 (0.490-0.678)	0.616 (0.496-0.705)	0.617 (0.515-0.715)	NS
Total ω-6 polyunsaturated fatty acids (PUFA)	4.079 (3.579-4.60)	4.174 (3.678-4.575)	4.118 (3.685-4.525)	NS
α-Linolenic, 18:3ω-3	0.021 (0.017-0.027)	0.021 (0.014-0.026)	0.025 (0.021-0.031)	0.022
Eicosapentaenoic (EPA), 20:5	0.021 (0.016-0.028)	0.024 (0.017-0.032)	0.022 (0.017-0.026)	NS
Docosapentaenoic (DPA), 22:5	0.026 (0.018-0.030)	0.028 (0.023-0.033)	0.025 (0.021-0.029)	NS
Docosahexaenoic (DHA), 22:6	0.104 (0.073-0.131)	0.113 (0.084-0.140)	0.109 (0.090-0.132)	NS
Total ω-3 polyunsaturated fatty acids (PUFA)	0.174 (0.140-0.215)	0.184 (0.153-0.233)	0.184 (0.154-0.208)	NS
TOTAL	10.73 (9.67-11.70)	10.81 (9.62-11.51)	10.62 (9.41-11.69)	NS
ω-3:ω-6	0.044	0.045	0.044	NS

NS – not statistically significant

significant differences in total saturated, monounsaturated, and polyunsaturated omega-3 and omega-6 fatty acid levels. Monounsaturated fatty acids exceeded 20% of total fatty acids in most children. Saturated fatty acid and polyunsaturated fatty acid proportions were almost equal. In most cases they did not exceed 40% of total plasma fatty acids. The results are presented in Table III as median values and the 1st and 3rd quartiles are given in parentheses. Children of the control group were found to have higher levels of stearic acid (18:0) and α-linolenic acid (18:3ω3) and the difference was statistically significant (p < 0.01 and p = 0.022 respectively).

In order to investigate a possible relationship between lung function and plasma fatty acid levels, we divided asthmatic children into two groups, according to spirometry results: children with normal lung function (FEV1 > 80%) and children with impaired lung function (FEV1 < 80%). Results are presented in Table IV. Levels of omega-3 fatty acids 20:5 (eicosapentaenoic acid – EPA), 22:5 (docosapentaenoic acid – DPA) and 22:6 (docosahexaenoic acid – DHA) were found to be higher in asthmatic children with normal lung function (p < 0.01).

Discussion

The most important finding in this study was the fact that asthmatic children who had normal lung function also had high levels of omega-3 fatty acids, which are thought to be beneficial. This was

Table IV. Levels (mmol/l) of plasma fatty acids in children with normal and impaired lung function. Values are presented as median, with first and third quartiles in parentheses

Type of fatty acid	FEV1 < 80% *n* = 60	FEV1 ≥ 80% *n* = 144	*p*
α-Linolenic 18:3ω-3	0.022 (0.015-0.026)	0.022 (0.015-0.027)	NS
Eicosapentaenoic (EPA), 20:5	0.020 (0.012-0.024)	0.026 (0.018-0.033)	< 0.01
Docosapentaenoic (DPA), 22:5	0.022 (0.015-0.030)	0.027 (0.022-0.030)	< 0.01
Docosahexaenoic (DHA), 22:6	0.083 (0.063-0.140)	0.110 (0.080-0.150)	< 0.01
Total ω-3 polyunsaturated fatty acids (PUFA)	0.15 (0.11-0.14)	0.18 (0.14-0.23)	NS
Total ω-6 polyunsaturated fatty acids (PUFA)	4.05 (3.47-4.49)	4.23 (3.65-4.58)	NS
ω-3:ω-6	0.040	0.047	NS

NS – not statistically significant

attributed to the Mediterranean diet consumed by these children, which appears to meet current dietary recommendations and also to be associated with lower severity of cardiovascular diseases and cancer [23-25]. Most of the studies conducted in children and adults assessing diet and asthma were performed in populations on a typical "Western" type diet [10, 26-31]. As far as we are aware, this is the first study to investigate the dietary habits and the levels of plasma fatty acids in relation to the lung function of asthmatic children in Greece.

Food consumption was assessed using a food frequency questionnaire. Among the different methods available to measure food consumption, the food frequency questionnaire is considered to be the most appropriate for epidemiological studies and has been validated in a large number and variety of studies and populations [32]. Our questionnaire was short and simple. It was limited to a number of broad food groups and not to portion sizes. Our data enabled us to rank children according to their consumption frequencies of a number of food groups, but cannot be used to calculate total energy or nutrient intakes.

Our results indicate that there are differences in the consumption of some whole foods in the population of children we studied. We have found that asthmatic children consumed less frequently fresh fruit and raw vegetables. Previous studies have already confirmed that low intake of oranges and other vitamin C containing fruits during winter increases the risk of wheezing symptoms in children and adults [6, 10, 11, 14, 33, 34].

Daily consumption of full cream milk, milk products and butter has been associated with low rates of asthma and/or wheeze in adults and children [4, 31]. The vast majority of children in our study consumed full cow's milk once or twice per day. However, there was detected a significant difference in the frequency of consumption of dairy products, mainly white cheese. Children of the control group consumed dairy products more frequently than asthmatic children.

A number of studies have indicated that margarine consumption is associated with allergic sensitization and diseases in children, as it may contain several-fold higher amounts of n-6 fatty acids than butter [10, 35]. In our study, the food frequency questionnaire indicated that margarine was not an important food element in the diet of children. As expected, the most important vegetable-derived fat in the diet of the children was olive oil [20]. This finding indicates that the population of our study, though mainly urban, has not yet adapted a "westernized" diet.

A low consumption of oily fish has been found to be associated with an increased risk of asthma, although the addition of fish oil to the diet and the supplementation of eicosapentaenoic acid have failed to improve asthma [29, 36]. There were no children in our study reporting frequent consumption of fish.

There were no differences detected in total plasma fatty acids and total saturated, monounsaturated, and polyunsaturated omega-3 and omega-6 fatty acid levels between asthmatic and non-asthmatic children. Also there were no differences detected in total plasma fatty acids and total saturated, monounsaturated, and polyunsaturated omega-3 and omega-6 fatty acid levels between atopic and non-atopic asthmatic children.

Children of the control group were found to have higher levels of stearic acid (18:00) and α-linolenic acid (18:3ω3) and the difference was statistically significant ($p < 0.01$ and $p = 0.022$ respectively).

Levels of n-3 fatty acids 20:5 (eicosapentaenoic acid – EPA), 22:5 (docosapentaenoic acid – DPA) and 22:6 (docosahexaenoic acid – DHA) were found to be higher in asthmatic children with normal lung function ($p < 0.01$).

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) competitively inhibit n-6 PUFA arachidonic (AA) metabolism, thus reducing the generation of proinflammatory four-series LTs and two-series prostanoids (PGs and thromboxanes) and the production of cytokines from inflammatory cells. Moreover, there is accumulating evidence that fatty acids can also modulate signalling molecules and transcription factors. Omega-3 PUFA therefore seem to interfere with early inflammatory signal transduction processes and are thus capable of blunting hyperinflammation [37].

In a recent study published by Woods RK and colleagues bronchial reactivity was negatively correlated with levels of the n-3 fatty acid 22:6 [38].

In conclusion, in this study a potential hypothesis requiring investigation relates to the possible asthma-related benefits associated with actively and markedly decreasing levels of omega-6 fatty acid intake concurrent with increasing the intake of omega-3 fatty acids.

Epidemiological studies with robust design and novel approaches in the field of dietary omega-3 and omega-6 fatty acids and childhood asthma can potentially lead to the generation of many new hypotheses with clinical and public health implications.

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