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# Dietary intake of α-linolenic acid and low ratio of *n*-6:*n*-3 PUFA are associated with decreased exhaled NO and improved asthma control

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## Abstract

As recently described, adherence to the Mediterranean diet is associated with improved asthma control. However, evidence of how specific nutrients such as fatty acids and antioxidants may affect this relationship remains largely unknown. We aimed to examine the association between dietary intake of fatty acids and antioxidants and asthma control. A cross-sectional study was developed in 174 asthmatics, mean age of 40 (SD 15) years. Dietary intake was obtained by a FFQ, and nutritional content was calculated using Food Processor Plus™ software (ESHA Research, Inc., Salem, OR, USA). Good asthma control was defined by the combination of forced expiratory volume during the first second, exhaled NO (eNO) and Asthma Control Questionnaire (ACQ) score (control: forced expiratory volume in the first second ≥80 %; eNO ≤35 ppb; ACQ <1·0, scale 0–6 score). Multiple linear and logistic regression models were performed to analyse the associations between nutrients and asthma outcomes, adjusting for confounders. A high *n*-6:*n*-3 PUFA ratio predicted high eNO, whereas high intakes of *n*-3 PUFA, α-linolenic acid (ALA) and SFA were associated with low eNO. Odds for controlled asthma improved along with an increased intake of *n*-3 PUFA (OR 0·14, 95 % CI 0·04, 0·45; *P* for trend=0·001), SFA (OR 0·36, 95 % CI 0·13, 0·97; *P* for trend=0·047) and ALA (OR 0·18, 95 % CI 0·06, 0·58; *P* for trend=0·005). A high *n*-6:*n*-3 PUFA ratio increased the odds for uncontrolled asthma (OR 3·69, 95 % CI 1·37, 9·94; *P* for trend=0·009), after adjusting for energy intake, sex, age, education and use of inhaled corticosteroids. Higher intakes of *n*-3 PUFA, ALA and SFA were associated with good asthma control, while the risk for uncontrolled asthma increased with a higher *n*-6:*n*-3 PUFA ratio. The present results introduce a protective effect of ALA in asthma control, independent of marine *n*-3 fatty acids, and provide a rationale to dietary intervention studies in asthma.

**Key words: Asthma: Airway inflammation: Diet: Fatty acids: α-Linolenic acid: Antioxidants**

The increase in asthma prevalence in the last decades has been suggested to be related to environment and lifestyle changes, from which diet and physical activity (PA) appear as obvious[1]. The following two dietary hypotheses have been proposed: (1) an increase in the consumption of vegetable oils and margarines and a decline in fat of animal origin and fish consumption shifting the *n*-6:*n*-3 ratio of dietary PUFA from 1:1 to 15–17:1[2]; (2) a decrease in the intake of fresh fruit, vegetables and whole cereals leading to a reduction in dietary antioxidants[3]. Several non-experimental studies have provided evidence supporting the lipid[2] and the antioxidant[3] hypotheses; however, the same has not been reported in intervention trials. Potential benefits of marine *n*-3 PUFA, namely EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), in inflammatory modulation and asthma have been proposed, whereas the link with the precursor α-linolenic acid (ALA; 18 : 3*n*-3) is still scarce.

We have recently reported that high adherence to a Mediterranean dietary pattern was associated with improved asthma control[4]. This was particularly relevant as asthma control definition incorporated symptoms, lung function and airway inflammation[5]. Among Mediterranean diet food items, nuts

**Abbreviations:** ACQ, Asthma Control Questionnaire; ALA, α-linolenic acid; eNO, exhaled NO; FEV1, forced expiratory volume in the first second; PA, physical activity.

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(high in ALA) and fresh fruit emerged as positively associated with lung function and asthma control, respectively. However, few data exist on the associations of individual fatty acids, micronutrients and asthma control.

In the present study, we aimed to investigate the association between several types of fatty acids, antioxidant micronutrients and asthma control, measured by symptoms, lung function and airway inflammation, and we hypothesised that *n*-3 PUFA and antioxidant micronutrients provided from the diet could be associated with improved asthma control in asthmatic patients.

## Materials and methods

### Participants and study design

A total of 219 consecutive patients, older than 16 years old, attending an outpatient Asthma and Allergy clinic at a University Hospital, with a medical diagnosis of asthma, were invited to participate in a cross-sectional study. Exclusion criteria were food allergy, changing of dietary patterns in the last 12 months, pregnancy, presence of diseases which involved specific nutritional therapy and dietary planning, acute illness in the last 4 weeks or inability to comply with the measurement instruments. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human patients were approved by the Institutional ethics committee. Written informed consent was obtained from all patients before inclusion.

### Nutritional intake

Dietary intake was obtained by a self-administered, semi-quantitative FFQ, validated for Portuguese adults[6]. The FFQ is an 86-item questionnaire that assessed usual dietary intake over the previous 12 months, including usual food groups and beverages. Food intake was estimated by multiplying the frequency of consumption (about nine possibilities from 'never or less than 1 time/month', to '6 or more times/d') by the weight of the standard portion size of the food item. A seasonal variation factor was considered for foods in which production and consumption are not regular over the year (mean of 3 months). Nutritional intake was calculated using an adapted Portuguese version of the software Food Processor Plus® (ESHA Research, Inc., Salem, OR, USA), nutritional analysis software that converts food intake into total energy and nutrients, based on food composition tables available from the US Department of Agriculture and national data from typical Portuguese foods. Dietary intake of different types of fatty acids (*n*-3 and *n*-6 PUFA, SFA and MUFA) and micronutrients involved in antioxidant status and potentially relevant for asthma (vitamins E, C, carotene, retinol, Mg and Zn)[1] were selected as primary independent variables of interest. Although Se plays a role as a cofactor of glutathione peroxidase and as the potential suppressor of asthma inflammation, it was not considered in the nutritional analysis. Considering the wide variation in the content of the major Se food sources (depending on the geographic origin and soil levels), food composition data for Se measure by FFQ are considered unreliable[7].

### Anthropometry and physical activity assessment

BMI was calculated after body weight and height measurements with the subject lightly clothed and barefooted, using a mechanical balance with a stadiometer (Seca model 700®; Seca Headquarter, Hamburg, Germany). Weight and height were determined to the nearest 0·1 kg and 0·5 cm, respectively. BMI was calculated as the weight (kg) divided by the square of the height ($m^2$).

PA was measured using the International Physical Activity Questionnaire – short version[8]. The short 7 d self-administered version is a seven-item questionnaire that provides information about the frequency and duration of four domains: sedentary activity, time spent walking, and moderate- and vigorous-intensity PA. PA within domains was estimated by weighting the reported frequency (events/week) by duration (min/event) and by a metabolic equivalent level assigned to each activity (walking = 3·3; moderate-intensity PA = 4·0 and vigorous-intensity PA = 8·0). A combined total PA was computed as the sum of the activity domain scores (total PA = walking + moderate-intensity PA + vigorous-intensity PA) and reported as a continuous measure (total PA score = total metabolic equivalent-min/week).

### Asthma control and quality of life: definitions and assessment

Asthma control was defined by combining the results of lung function, exhaled NO (eNO) and the Asthma Control Questionnaire (ACQ) score[5]. Subjects were classified as having 'controlled' asthma if simultaneously they had forced expiratory volume in the first second (FEV1) ≥80 % predicted[9], eNO ≤35 ppb[10] and ACQ score below 1·00[11]. If any of these features were not present, subjects were classified as 'non-controlled'. Lung function was measured by the determination of FEV1 using PIKO-1® (Ferraris Respiratory Europe Limited, Hertford, Herts, UK)[12]. Patients were asked to perform a set of three technically acceptable manoeuvres, and the highest FEV1 measurement was registered and expressed as percentage predicted, as recommended by the American Thoracic Society.

eNO was measured with the NIOX® system (Aerocrine, Stockholm, Sweden), using the online technique recommended by the American Thoracic Society[13], at a flow rate of 50 ml/s.

The seven-item ACQ was designed to assess clinical asthma control during the previous week. A seven-point scale (0 = no impairment, 6 = maximum impairment) was used, and the score was calculated as the mean of the seven items, ranging from 0 (totally controlled) to 6 (severely uncontrolled)[14].

Asthma quality of life was measured by the asthma life quality test, developed by the American College of Allergy, Asthma and Immunology, and validated in Portuguese[15]. The self-administered asthma life quality test includes twenty questions of dichotomous answer (yes/no) assessing six domains: activity and sleep; symptoms; triggers; unscheduled health care use; medication; psychological. Total score was calculated as the sum of affirmative responses, ranging from 0 to 20 (lower values indicate better asthma quality of life).

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**Table 1.** Characteristics of participants according to asthma control*
(Mean values, standard deviations, number of subjects and percentages)

| | Controlled asthma (*n* 40) | | Non-controlled asthma (*n* 134) | | |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | *P* |
| Demographic | | | | | |
| Age (years) | 42·9 | 13·4 | 39·7 | 15·6 | 0·206† |
| Sex (*n*) | | | | | 0·445‡ |
| Female | 31 | | 111 | | |
| Male | 9 | | 23 | | |
| Education | | | | | 0·723‡ |
| ≤4 years | | | | | |
| *n* | 14 | | 55 | | |
| % | 35 | | 41 | | |
| 5–9 years | | | | | |
| *n* | 10 | | 34 | | |
| % | 25 | | 25 | | |
| ≥ 10 years | | | | | |
| *n* | 16 | | 45 | | |
| % | 40 | | 34 | | |
| BMI (kg/m$^2$) | 26·8 | 4·5 | 27·3 | 5·3 | 0·620† |
| Physical activity (MET-min/week) | | | | | 0·328‖ |
| Median | 1405 | | 1844 | | |
| Range | 0–8739 | | 0–9492 | | |
| Present smoker | | | | | 0·119‡ |
| *n* | 6 | | 7 | | |
| % | 15 | | 5 | | |
| Clinical | | | | | |
| Atopic | | | | | 0·138‡ |
| *n* | 24 | | 97 | | |
| % | 65 | | 77 | | |
| Allergic rhinitis | | | | | 0·753‡ |
| *n* | 26 | | 90 | | |
| % | 65 | | 68 | | |
| Present ICS | | | | | 0·007‡** |
| *n* | 22 | | 103 | | |
| % | 55 | | 77 | | |
| Exhaled NO (ppb) | | | | | <0·001‖** |
| Mean | 19·5 | | 33·0 | | |
| 95 % CI | 16·8–22·6 | | 28·8–37·9 | | |
| FEV1 | 103·8 | 22·3 | 82·7 | 22·3 | <0·001†** |
| ALQ score | 10·1 | 3·8 | 11·7 | 4·2 | 0·027†** |
| ACQ score | 0·4 | 0·3 | 1·5 | 1·0 | <0·001†** |
| Nutritional intake | | | | | |
| Total energy intake (kJ/d) | 14 121 | 4091·95 | 13 459·92 | 5133·76 | 0·457† |
| Protein (% TEV) | 16·3 | 2·9 | 15·8 | 3·7 | 0·433† |
| Total carbohydrates (% TEV) | 35·6 | 6·7 | 35·0 | 7·6 | 0·667† |
| Sugars§ | 15·0 | 5·8 | 14·5 | 5·2 | 0·638† |
| Total fat (% TEV) | 46·2 | 8·7 | 43·7 | 9·6 | 0·148† |
| PUFA | 6·2 | 1·4 | 6·3 | 1·6 | 0·752† |
| *n*-6 | 4·9 | 1·4 | 5·1 | 1·5 | 0·614† |
| *n*-3 | 0·7 | 0·1 | 0·6 | 0·2 | 0·090† |
| ALA (g) | 2·09 | 0·73 | 1·80 | 0·77 | 0·032† |
| EPA (g) | | | | | 0·016‖ |
| Median | 0·12 | | 0·09 | | |
| Range | 0·00–0·40 | | 0·00–1·70 | | |
| DHA (g) | | | | | 0·021‖ |
| Median | 0·25 | | 0·21 | | |
| Range | 0·10–1·00 | | 3·90–0·00 | | |
| EPA + DHA (g) | | | | | 0·020‖ |
| Median | 0·36 | | 0·31 | | |
| Range | 1·43–0·13 | | 5·58–0·01 | | |
| MUFA | 16·7 | 3·9 | 15·8 | 3·7 | 0·183† |
| SFA | | | | | 0·081‖ |
| Median | 20·3 | | 16·7 | | |
| Range | 9·0–32·0 | | 8·0–36·0 | | |
| *Trans*-FA | 0·6 | 0·3 | 0·6 | 0·3 | 0·832† |
| *n*-6:*n*-3 ratio | | | | | 0·017‖** |
| Median | 7·2 | | 8·2 | | |
| Range | 3·5–15·2 | | 3·5–24·2 | | |

**Table 1.** *Continued*

| | Controlled asthma (*n* 40) | | Non-controlled asthma (*n* 134) | | |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | *P* |
| MUFA:SFA ratio | | | | | 0·325‖ |
| Median | 0·86 | | 0·90 | | |
| Range | 0·6–1·6 | | 0·5–1·8 | | |
| Ethanol (% TEV) | | | | | 0·015‖** |
| Median | 1·4 | | 3·7 | | |
| Range | 0·0–24·8 | | 0·0–37·0 | | |
| Cholesterol (mg) | 644·0 | 209·0 | 590·0 | 245·0 | 0·211† |
| Total dietary fibre (g) | 28·5 | 9·2 | 26·0 | 12·5 | 0·254† |
| Carotene (μg RAE) | | | | | 0·360‖ |
| Median | 478·3 | | 430·4 | | |
| Range | 113·5–1871·6 | | 52·7–2501·5 | | |
| Retinol (μg RAE) | 1483·5 | 4384·1 | 1402·0 | 3884·0 | 0·212‖ |
| Vitamin E (mg α-TE) | 10·3 | 14·4 | 8·5 | 37·0 | 0·074‖ |
| Median | 10·3 | | 8·5 | | |
| Range | 5·2–19·5 | | 2·4–39·4 | | |
| Vitamin C (mg) | 143·7 | 207·8 | 131·0 | 136·1 | 0·338‖ |
| Mg (mg) | 416·9 | 103·4 | 386·3 | 151·5 | 0·233† |
| Zn (mg) | 20·4 | 7·8 | 18·7 | 8·3 | 0·259† |

MET, metabolic equivalent; ICS, inhaled corticosteroid; exhaled NO, fraction of exhaled NO; ppb, parts per billion; FEV1, forced expiratory volume in the first second; ALQ, asthma life quality test; ACQ, Asthma Control Questionnaire; TEV, total energy value; ALA, α-linolenic acid; FA, fatty acid; RAE, retinol A equivalents; TE, tocopherol equivalents.
* Macro- and micronutrients are presented as unadjusted variables.
† *t* test.
‡ $\chi^2$ test.
§ Sugars refer to all monosaccharides and disaccharides added to foods by the manufacturer, cooking or consumer, plus sugars naturally present in honey, syrups and fruit juices.
‖ Mann–Whitney *U* test
** $P<0·05$.

### *Statistical analysis*

Descriptive statistics are expressed as means and standard deviations, and proportions (%), whereas PA and several nutrient data are presented as medians and ranges given the non-normal distribution. eNO was logarithmically transformed to attain normal distribution and is presented as geometric means and 95% CI. Atopic status, defined by positive skin prick tests, medical diagnosis of allergic rhinitis, present use of inhaled corticosteroid, education (≤4, 5 to 9 and ≥10 years) and smoking status (non-smoker, past smoker and present smoker) were also recorded.

Nutritional variables were adjusted for total energy intake using the nutrient residual model[16]. In this model, energy-adjusted nutrient intakes are computed as the residuals from the regression analysis, with total energy intake as independent variables and absolute intakes as dependent variables. The associations between nutritional intake and asthma outcomes were performed using linear regression, multiple linear regression and unconditional logistic regression models. Linear regression was initially fitted to analyse the associations between nutrient intake (independent variables) and asthma outcomes (dependent variables). Multiple linear regression models adjusted for confounders were performed separately for eNO, FEV1, asthma life quality and ACQ scores (categorical confounder variables were transformed into dummy variables). Logistic regression models were also performed to analyse the associations between nutritional intake and asthma control level. Energy-adjusted nutrients were categorised into tertiles. OR were calculated by reference with the lowest tertile.

Sex, education, age, energy intake, BMI, PA score, smoking, atopy, rhinitis and inhaled corticosteroid were analysed as potential confounders. Only the variables that were significantly associated with each of the asthma outcomes in the univariate analysis were considered in the final regression and logistic models. Considering that smoking status and PA were not significantly associated with eNO, FEV1, asthma life quality or ACQ scores, and that their inclusion as confounders did not influence the effects, these variables were therefore not included in the final models. Considering the biological plausibility related to dietary intake, sex, age and total energy intake, these were considered in all models. A 0·05 level of significance and 95% CI were considered. Data analysis was performed using the statistical package SPSS®, version 17.0 (SPSS Inc., Chicago, IL, USA).

## Results

From the 219 patients invited, forty-five were excluded (twenty-one did not fulfil the inclusion criteria, nine had dietary changes in the last 12 months, eight had incomplete data records, four were considered as energy intake outliers and three refused to participate). Energy intake outliers were previously excluded from the study and were defined as having energy intake values above the arithmetic mean (SD 2), and implausibly low intakes (<2092 kJ (<500 kcal) for women and <3347·2 kJ (<800 kcal) for men). The characteristics of

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**Table 2.** Associations between nutrient intake and airway inflammation, lung function, asthma quality of life and Asthma Control Questionnaire score (ACQ)

(β Coefficients and 95 % confidence intervals)

| | Exhaled NO (ppb) | | FEV1 (% predicted) | | AQL score | | ACQ score | |
|---|---|---|---|---|---|---|---|---|
| | Confounder-adjusted† | | Confounder-adjusted‡ | | Confounder-adjusted§ | | Confounder-adjusted‖ | |
| | β¶ | 95 % CI | β | 95 % CI | β | 95 % CI | β | 95 % CI |
| Fatty acids | | | | | | | | |
| *n*-6 PUFA (g) | −0·024 | −0·035, 0·083 | 0·786 | −1·164, 2·734 | 0·035 | −0·248, 0·318 | −0·037 | −0·110, 0·036 |
| *n*-3 PUFA (g) | −0·502* | −0·928, −0·075 | 9·690 | −4·373, 23·753 | −1·163 | −3·222, 0·896 | −0·260 | −0·792, 0·272 |
| ALA (g) | −0·357* | −0·608, −0·105 | 3·180 | −5·086, 11·446 | −0·998 | −0·202, 0·207 | −0·283 | −0·593, 0·027 |
| EPA (g) | −0·003 | −0·753, 0·747 | 16·056 | −8·850, 40·962 | −0·422 | −4·082, 3·239 | −0·232 | −1·176, 0·711 |
| DHA (g) | −0·005 | −0·343, 0·332 | 6·477 | −4·752, 17·706 | −0·182 | −1·831, 1·467 | −0·100 | −0·525, 0·325 |
| EPA + DHA (g) | −0·003 | −0·236, 0·230 | 4·632 | −3·111, 12·376 | −0·127 | −1·265, 1·010 | −0·070 | −0·363, 0·223 |
| *n*-6:*n*-3 ratio | 0·053* | 0·017, 0·089 | −0·072 | −1·278, 1·134 | 0·072 | −0·104, 0·248 | −0·002 | −0·047, 0·044 |
| MUFA | −0·022 | −0·050, −0·007 | −0·222 | −0·663, 1·108 | 0·028 | −0·158, 0·103 | −0·007 | −0·041, 0·026 |
| SFA | −0·021* | −0·038, −0·004 | −0·032 | −0·580, 0·515 | −0·051 | −0·131, 0·029 | −0·009 | −0·030, 0·011 |
| MUFA:SFA ratio | 0·637* | 0·075, 1·199 | 3·417 | −14·765, 21·600 | 1·902 | −0·724, 4·529 | 0·355 | −0·321, 1·030 |
| Antioxidant micronutrients | | | | | | | | |
| Carotene (μg RAE) | 0·001 | −0·000, 0·001 | 0·017 | −0·009, 0·042 | 0·001 | −0·005 0·003 | −0·001 | −0·002, 0·000 |
| Retinol (μg RAE) | −0·001 | −0·001, 0·000 | 0·007 | −0·006, 0·021 | −0·002 | −0·004, 0·000 | 0·000 | −0·001, 0·000 |
| Vitamin E (mg α-TE) | −0·074 | −0·166, 0·019 | 2·055 | −0·841, 4·950 | 0·086 | −0·340, 0·519 | −0·051 | −0·161, 0·058 |
| Vitamin C (mg) | 0·002 | −0·002, 0·005 | 0·061 | −0·056, 0·177 | 0·008 | −0·009, 0·025 | −0·001 | −0·006, 0·003 |
| Mg (mg) | −0·001 | −0·004, 0·003 | 0·106 | −0·009, 0·222 | 0·010 | −0·007, 0·027 | −0·003 | −0·007, 0·002 |
| Zn (mg) | 0·006 | −0·068, 0·080 | 0·159 | −2·224, 2·543 | −0·095 | −0·444, 0·255 | −0·008 | −0·098, 0·082 |

ppb, Parts per billion; FEV1, forced expiratory volume in the first second; AQL, asthma quality of life; ALA, α-linolenic acid.

* $P < 0·05$.

† Linear regression; multiple linear regression adjusted for: energy intake, sex, age, BMI, education, rhinitis and atopic status.

‡ Linear regression; multiple linear regression adjusted for: energy intake, sex, age, rhinitis and education.

§ Linear regression; multiple linear regression adjusted for: energy intake, sex, age, BMI, education and inhaled corticosteroid (ICS).

‖ Linear regression; multiple linear regression adjusted for: energy intake, sex, age, education and ICS.

¶ Representing the adjusted ratio of geometric means.

**Table 3.** Association between nutrient intake and asthma control
(Odds ratios and 95 % confidence intervals)

| | Energy-adjusted† | | | Confounder-adjusted‡ | | |
|---|---|---|---|---|---|---|
| | OR | 95 % CI | *P* for trend | OR | 95 % CI | *P* for trend |
| Fatty acids | | | | | | |
| *n*-6 PUFA (g/d) | | | | | | |
| < 5·19 | 1·00 | Reference | | 1·00 | Reference | |
| 5·19–7·12 | 0·58 | 0·25, 1·35 | | 0·53 | 0·22, 1·30 | |
| > 7·12 | 1·25 | 0·49, 3·18 | 0·659 | 1·23 | 0·45, 3·35 | 0·707 |
| *n*-3 PUFA (g/d) | | | | | | |
| < 0·73 | 1·00 | Reference | | 1·00 | Reference | |
| 0·73–0·94 | 0·21* | 0·07, 0·67 | | 0·18* | 0·05, 0·62 | |
| > 0·94 | 0·14* | 0·04, 0·44 | 0·001* | 0·14* | 0·04, 0·45 | 0·001* |
| ALA (g/d) | | | | | | |
| < 1·54 | 1·00 | Reference | | 1·00 | Reference | |
| 1·54–1·96 | 0·21* | 0·07, 0·61 | | 0·19* | 0·06, 0·59 | |
| > 1·96 | 0·23* | 0·08, 0·67 | 0·010* | 0·18* | 0·06, 0·58 | 0·006* |
| EPA (g/d) | | | | | | |
| < 0·07 | 1·00 | Reference | | 1·00 | Reference | |
| 0·07–0·14 | 0·85 | 0·32, 2·24 | | 0·80 | 0·29, 2·21 | |
| > 0·14 | 0·34 | 0·14, 0·83 | 0·496 | 0·37 | 0·15, 0·99 | 0·689 |
| DHA (g/d) | | | | | | |
| < 0·16 | 1·00 | Reference | | 1·00 | Reference | |
| 0·16–0·30 | 0·82 | 0·32, 2·07 | | 0·82 | 0·31, 2·19 | |
| > 0·30 | 0·48 | 0·20, 1·17 | 0·086 | 0·55 | 0·22, 1·40 | 0·189 |
| EPA + DHA (g/d) | | | | | | |
| < 0·23 | 1·00 | Reference | | 1·00 | Reference | |
| 0·23–0·43 | 0·78 | 0·30, 2·02 | | 0·82 | 0·30, 2·21 | |
| > 0·43 | 0·21 | 0·18, 1·03 | 0·045 | 0·49 | 0·19, 1·23 | 0·107 |
| *n*-6:*n*-3 ratio | | | | | | |
| < 6·45 | 1·00 | Reference | | 1·00 | Reference | |
| 6·45–8·11 | 2·18 | 0·95, 4·99 | | 2·24 | 0·93, 5·36 | |
| > 8·11 | 4·14* | 1·59, 10·74 | 0·004* | 3·69* | 1·37, 9·94 | 0·009* |
| MUFA (g/d) | | | | | | |
| < 16·12 | 1·00 | Reference | | 1·00 | Reference | |
| 16·12–20·06 | 0·58 | 0·23, 1·47 | | 0·50 | 0·19, 1·35 | |
| > 20·06 | 0·44 | 0·18, 1·10 | 0·083 | 0·47 | 0·17, 1·27 | 0·154 |
| SFA (g/d) | | | | | | |
| < 16·84 | 1·00 | Reference | | 1·00 | Reference | |
| 16·84–23·90 | 0·46 | 0·18, 1·19 | | 0·51 | 0·19, 1·34 | |
| > 23·90 | 0·39* | 0·15, 0·98 | 0·056 | 0·36* | 0·13, 0·97 | 0·047* |
| MUFA:SFA ratio | | | | | | |
| < 0·81 | 1·00 | Reference | | 1·00 | Reference | |
| 0·81–1·0 | 1·45 | 0·62, 2·39 | | 1·44 | 0·59, 3·52 | |
| > 1·0 | 1·69 | 0·71, 4·01 | 0·203 | 1·89 | 0·75, 4·78 | 0·072 |
| Antioxidant micronutrients | | | | | | |
| Carotene (μg RAE/d) | | | | | | |
| < 152 | 1·00 | Reference | | 1·00 | Reference | |
| 152–238 | 1·00 | 0·41, 2·46 | | 1·10 | 0·43, 2·77 | |
| > 238 | 0·69 | 0·29, 1·61 | 0·310 | 0·76 | 0·31, 1·87 | 0·412 |
| Retinol (μg RAE/d) | | | | | | |
| < 95 | 1·00 | Reference | | 1·00 | Reference | |
| 95–849 | 0·33* | 0·13, 0·83 | | 0·32* | 0·12, 0·87 | |
| > 849 | 0·55 | 0·21, 1·46 | 0·334 | 0·54 | 0·19, 1·50 | 0·355 |
| Vitamin E (mg α-TE/d) | | | | | | |
| < 3·22 | 1·00 | Reference | | 1·00 | Reference | |
| 3·22–4·02 | 0·89 | 0·35, 2·29 | | 0·84 | 0·31, 2·26 | |
| > 4·02 | 0·43 | 0·18, 1·03 | 0·049* | 0·43 | 0·17, 1·10 | 0·079 |
| Vitamin C (mg/d) | | | | | | |
| < 52 | 1·00 | Reference | | 1·00 | Reference | |
| 52–66 | 0·83 | 0·35, 1·94 | | 0·84 | 0·35, 2·04 | |
| > 66 | 1·11 | 0·46, 2·69 | 0·740 | 1·17 | 0·46, 3·02 | 0·667 |
| Mg (mg/d) | | | | | | |
| < 149 | 1·00 | Reference | | 1·00 | Reference | |
| 149–175 | 0·66 | 0·26, 1·63 | | 0·72 | 0·28, 1·85 | |
| > 175 | 0·55 | 0·22, 1·33 | 0·184 | 0·63 | 0·25, 1·61 | 0·338 |
| Zn (mg/d) | | | | | | |
| < 5·73 | 1·00 | Reference | | 1·00 | Reference | |
| 5·73–7·26 | 0·84 | 0·35, 2·01 | | 0·97 | 0·38, 2·45 | |
| > 7·26 | 0·80 | 0·33, 1·92 | 0·606 | 0·79 | 0·31, 2·01 | 0·657 |

ALA, α-linolenic acid, RAE, retinol A equivalents.
* $P<0·05$.
† Logistic regression adjusted for energy intake.
‡ Logistic regression adjusted for energy intake, sex, age, education and inhaled corticosteroid.

excluded patients, regarding age, education, smoking status and asthma severity, were similar to the 174 patients (81 %) included in the analysis.

According to asthma control definition, 23 and 77 % of the subjects were classified, respectively, as having controlled and non-controlled asthma (Table 1). Considering the energy contribution of macronutrients, no significant differences between these two groups were observed for total carbohydrates, total fat and SFA intake. However, controlled patients had a significantly lower *n*-6:*n*-3 PUFA ratio intake compared with non-controlled patients ($P=0{\cdot}017$) and had a significantly higher intake of ALA ($P=0{\cdot}022$), EPA ($P=0{\cdot}016$), DHA ($P=0{\cdot}021$) and EPA + DHA ($P=0{\cdot}020$); dietary intakes of *n*-3 PUFA ($P=0{\cdot}090$), SFA ($P=0{\cdot}081$) and vitamin E ($P=0{\cdot}074$) were higher in controlled asthmatics, but these differences were not statistically significant.

The associations of the dietary intake of fatty acids and antioxidant micronutrients with markers of asthma adjusted for energy intake, sex, age, BMI, education, atopy, rhinitis and inhaled corticosteroids are presented in Table 2. Higher *n*-6:*n*-3 PUFA and MUFA:SFA ratios were associated with higher eNO, whereas higher intakes of *n*-3 PUFA and SFA were associated with lower eNO. Higher ALA intake was associated with lower eNO, even after adjustment for marine *n*-3 PUFA ($R$ −0·356, 95 % CI −0·609, −0·105; $P=0{\cdot}006$). No significant associations were found for EPA + DHA and asthma outcomes. Energy-adjusted MUFA and Mg were associated with eNO and FEV1, respectively; however, after adjusting for confounders, these associations were no longer significant.

The OR for asthma control accordingly with the dietary intake of fatty acids and antioxidant micronutrients are given in Table 3. Intake of *n*-3 PUFA between 0·73 and 0·94 g/d and above 0·94 g/d reduced the odds of non-controlled asthma (second tertile: OR 0·18, 95 % CI 0·05, 0·62; third tertile: OR 0·14; 95 % CI 0·04, 0·45; $P$ for trend=0·001), while *n*-6:*n*-3 PUFA above 8·45 had the opposite effect (third tertile: OR 3·69, 95 % CI 1·37, 9·94; $P$ for trend=0·009), after adjusting for energy intake, sex, age, education and inhaled corticosteroids. Dietary intake of ALA between 1·54 and 1·96 and above 1·96 g/d reduced the odds of non-controlled asthma (second tertile: OR 0·19, 95 % CI 0·06, 0·59; third tertile: OR 0·18, 95 % CI 0·06, 0·58; $P$ for trend=0·006), after adjusting for confounders. After adjusting also for alternate *n*-3 PUFA, the protective effect of ALA in asthma control still remained significant (second tertile: OR 0·19, 95 % CI 0·06, 0·60; third tertile: OR 0·18, 95 % CI 0·06, 0·58; $P$ for trend=0·005), independent of EPA + DHA. No significant association was observed for EPA + DHA and asthma control.

The higher intake of SFA (>23·9 g/d) also decreased the probability of having non-controlled asthma (OR 0·36, 95 % CI 0·13, 0·97; $P$ for trend=0·047), after controlling for confounders. Considering micronutrients, dietary intake of retinol between 95 and 849 μg retinol A equivalents/d decreased the odds of having non-controlled asthma; however, the trend according to the retinol intake category was not significant ($P$ for trend=0·355). A protective trend was also observed for energy-adjusted vitamin E and asthma control (OR 0·43, 95 % CI 0·18, 1·03; $P=0{\cdot}049$); however, this association was no longer significant after the final adjustment.

## Discussion

In the present study, higher dietary intakes of *n*-3 PUFA and SFA were associated with a decreased levels of eNO and improved likelihood of asthma being under control, while a high ratio of *n*-6:*n*-3 PUFA had the opposite effect. In addition, higher dietary intake of ALA was associated with lower eNO and reduced the likelihood of non-controlled asthma, independent of marine *n*-3 PUFA. No significant associations between the dietary intake of EPA + DHA and antioxidant vitamins and minerals and asthma outcomes were observed.

The present results are limited by the cross-sectional design of the study which leaves open any possible cause–effect relationship and the role of other factors. Nevertheless, an inverse causal relationship is not probable and we assessed established lifestyle factors that could have an important role in asthma and that influence nutrient intake, such as total energy intake, PA and BMI, and the association between nutrients and asthma outcomes was extensively adjusted for confounders.

To the best of our knowledge, this is the first study exploring the association between different types of dietary fatty acids and antioxidant nutrient intake, and asthma control. Moreover, we assess the dietary intake of vegetable (ALA) and marine (EPA + DHA) *n*-3 PUFA, and report for the first time the protective effect of ALA in asthma control. The score we used to assess control, which included different dimensions of the disease such as inflammation, lung function and symptoms, has been shown to explain 77 % of the variability of asthma control[5]. Another important strength of the present study was the FFQ that we used, since it has been validated for Portuguese adults[6], and it has been shown to provide reliable estimates for *n*-3 PUFA and SFA[6].

In the present study, a higher dietary intake of *n*-3 PUFA (>0·94 g/d) and ALA (>1·96 g/d) reduced the odds of non-controlled asthma. Considering that the prevalence of non-controlled asthma in the present study was high, the OR may be biased towards overestimating the risk. Nevertheless, even though we could admit an overestimation of the protective effect, the reverse result should not be expected.

ALA is the major plant-based *n*-3 PUFA and exerts main effects through conversion to EPA and DHA, when dietary intake of marine PUFA is low[17,18]. Long-chain *n*-3 PUFA decreases the production of inflammatory mediators, competitively inhibiting the metabolism of arachidonic acid (generating less active prostenoids and leukotrienes), suppressing IgE production, and thereby potentially acting to reduce airway inflammation and bronchoconstriction in asthma[18,19]. However, results were inconclusive. A systematic review from Cochrane of the clinical effects of *n*-3 PUFA fish oil supplementation in established asthma suggests that the results are not consistent and that there is little evidence to recommend such supplementation in order to improve asthma control[20]. Reconciling the data from experimental and observational studies is difficult, most probably, due to

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different methods of assessment of dietary intake and different definitions of asthma. Taken the data into account from previous cross-sectional studies, it seems that dietary or serum *n*-3 PUFA levels are directly associated with lung function, at least in asthmatics[21–23] and atopy[24], and are protective for the risk of asthma or atopy. Recently, in a large population-based study, asthma risk was doubled in subjects who had never eaten fish during childhood and a minimum of weekly fish intake in adulthood was protective against asthma symptoms[25]. In a small study, fish oil supplementation failed to provide any benefit in eNO, lung function or asthma control in asthmatic women[26]. In the present study, dietary intake of *n*-3 PUFA and ALA was associated with improved asthma control and lower eNO, independent of EPA + DHA. Higher intake of ALA (and also not EPA or DHA) was previously associated with a decreased risk of allergic sensitisation and allergic rhinitis in adults[27]. However, the link between ALA and asthma is still poorly addressed. In the present study, dietary intake of EPA and DHA is very similar between controlled and non-controlled subjects, and we have found no significant associations between EPA or DHA and asthma outcomes. There is evidence suggesting that ALA, EPA and DHA might have heterogeneous and potentially independent effects on inflammation, gene expression and chronic diseases; therefore, a better understanding of the individual role of *n*-3 PUFA in inflammatory diseases, such as asthma, is needed[17,18]. It has been suggested that higher margarine intake rich in *n*-6 PUFA is associated with an increased risk of asthma[28,29] and hay fever[30] in adulthood, and eczema and allergic sensitisation in children[31]. Dietary intake of *n*-6 PUFA was similar among controlled and non-controlled subjects, and therefore, no significant associations for *n*-6 PUFA and asthma outcomes were observed. Nevertheless, the ratio of *n*-6:*n*-3 PUFA above 8·45, which was more than tripled the odds of non-controlled asthma, was associated with increased levels of eNO.

In the present study, we analysed the total SFA intake, irrespective of the specific types. However, different types of SFA could have different effects. Foods high in SFA, such as butter[32], whole milk[32,33] and non-pasteurised farm milk[34–36], have been consistently associated with a reduced risk of asthma. For milk, it is not clear whether associations should be attributed to SFA, vitamin A or even to microbial agents (in the case of whole non-pasteurised milk or farm-related co-exposures)[34–36]. Therefore, the present results on SFA could also be a proxy of a dietary pattern high in milk and dairy products. Several epidemiological studies have reported beneficial associations for higher intake of nutrients that may be relevant in the redox mechanisms, such as vitamin C[21,37,38], vitamin E[37,39], carotenoids[40,41], Se[7] and Mg[42]. However, these findings are not conclusive[43] and intervention studies with single nutrient supplementation have been disappointing[44–47]. Inverse associations with asthma have also been observed for foods rich in these micronutrients, such as fresh fruit[7,33,48,49] and vegetables[48], and nuts[4,50,51], and additional benefits may arise from the synergistic effects between nutrients in foods and specific dietary patterns. Nuts contain a high proportion of ALA, fibre, vitamins, minerals and many bioactive compounds that may modulate redox status, and inflammatory and immune responses[30,52]. We have previously reported that intake of nuts is positively associated with lung function, and high adherence to an overall healthy dietary pattern, such as the Mediterranean diet, is associated with an improved asthma control in adults, independent of other lifestyle factors[4].

In summary, the present results provide additional support for the benefits of adequate dietary advice and give a rationale to nutritional intervention studies in asthmatics. Healthy eating in asthma, providing foods high in ALA, such as nuts, and an adequate balance between *n*-6 and *n*-3 PUFA, may reduce disease severity and improve asthma control, independent of other lifestyle factors[4].

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