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Potential role of folate status on pulmonary function in pediatric asthma

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ABSTRACT

Objective: The aim of this study was to explore the relationship between plasma folate status and measures of airway disease (as reflected by spirometry) in children with asthma on a traditional Mediterranean diet. We hypothesized that folate deficiency is associated with lung function impairment in this group of children.
Methods: This cross-sectional study included 64 children with mild asthma (52% boys, mean age 8 ± 2 y) residing in Athens, Greece. Clinical assessments included spirometry and fractional exhaled nitric oxide (FeNO). Plasma 5-methyltetrahydrofolate (5-MTHF) was analyzed using high performance-liquid chromatography assay and examined in participants using quartiles (Q1–Q4).
Results: Of the 64 children, 45.3% were folate deficient (5-MTHF <10 nmol/L). A positive relationship was observed between 5-MTHF and forced vital capacity (FVC; β = 0.79, 95% confidence interval (CI), 0.14–1.44; adjusted $P = 0.019$), forced expiratory volume in 1s (FEV_1) (β = 0.78; 95% CI, 0.01–1.55; adjusted $P = 0.046$), and peak expiratory flow (PEF; β = 1.64; 95% CI, 0.14–3.15; adjusted $P = 0.033$) in girls only, adjusting for body mass index and regular exercise. Girls with low plasma folate concentrations (Q1), compared with girls with high concentrations (Q4) had 8.64% lower FVC (β = −8.64; 95% CI, −16.18 to −1.09; adjusted $P = 0.027$), 10.35% FEV_1 (β = −10.35; 95% CI, −18.82 to −1.89; adjusted $P = 0.019$), and 18.72% PEF (β = −18.72; 95% CI, −36.30 to −1.14; adjusted $P = 0.038$).
Conclusion: The findings of this study highlighted the potential negative effects of folate deficiency on pulmonary function in girls with asthma, the importance of monitoring folate status in children with asthma, and early prevention strategies.

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Introduction

Asthma has been described as an umbrella of several discrete disease entities of multifactorial etiology caused by complex interactions between genetic, environmental, and behavioral factors including dietary changes, and is characterized by chronic airway inflammation and obstruction [1]. In children, asthma is apparent around 6 y of age and in about 60% of cases will persist into adulthood [2]. Over the life course, asthma remains a substantial burden and a cause of morbidity, impaired school and work performance due to absenteeism, poor mental health and well-being, and an increased economic cost for hospital admissions, emergency visits for medical care, and pharmaceutical expenses [3]. Therefore, adequate treatment is paramount to establish control early during the disease life cycle to improve quality of life and lung function, reduce morbidity, severity, and disease progression into adulthood, coupled with potential cost savings for the family and the health care system. Secular trends in increasing asthma prevalence have implicated adoption of a Western lifestyle including dietary

BE and CI were equal senior authors and were responsible for the supervision of the study. MMP, CI, BE were responsible for the conceptualization and design. MMP, BE, KL, CK, and DT were responsible for the data curation, formal analysis, investigation, methodology, clinical tests, and laboratory analysis. MMP was responsible for writing of the original and final drafts. CI, BE, CK, DT, and KL were responsible for the review and editing of the drafts. The authors had no conflicts of interest to declare.

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changes as a contributing factor to the alarming increase in children [4]. Studies have shown that low plasma levels of diet-derived antioxidant vitamins C, D, and E, β-carotene, glutathione, glutamine (a metabolite of protein degradation), and folate are associated with increased airway oxidative stress, diminished lung function, and symptoms in children with asthma [5–9]. Furthermore, evidence from systematic reviews and meta-analyses of observational studies examining the effect of prenatal and childhood exposure to dietary antioxidants and asthma in children suggest that a higher intake of antioxidant vitamins, zinc, and selenium during pregnancy, infancy, and childhood reduced the likelihood of asthma and wheezing in children ≤18 y, despite substantial risk for bias due to inconsistencies among study designs, sample size, exposure, and asthma assessment tools [10–12]. Although food allergy is a risk factor for asthma development and both conditions can co-exist [13], studies assessing folate and risk for food allergy have reported mixed findings. For example, a nested case–control study of 1394 children participating in the Boston Birth Cohort found that serum folate (as represented by 5-methyltetrahydofolate [5-MTHF]) and unmetabolized folic acid (UMFA) concentrations in early or later childhood were not associated with increased risk for food allergy. However, high concentrations of UMFA at birth were associated with increased risk for food allergy [14]. However, to our knowledge, no clinical trials have been conducted investigating the association of folate status and asthma during childhood and this deserves further investigation.

Folate is a water-soluble B vitamin that is naturally found in leafy vegetables (such as spinach, beans, asparagus, and broccoli), legumes, egg yolks, liver, yeast, whole wheat bread, and some citrus fruits (oranges) and in fortified products (cereals, dairy). It is essential for normal cell growth, replication, erythropoiesis, DNA synthesis, and brain development, and therefore critically important during periods of rapid growth from childhood to adolescence [15,16] (Fig. 1). A deficiency in folate occurs when the staple diet consists of unfortified wheat, corn, or rice, and low intake of legumes and folate-rich vegetables and fruit, as well as poor absorption of ingested folate, alteration of folate metabolism due

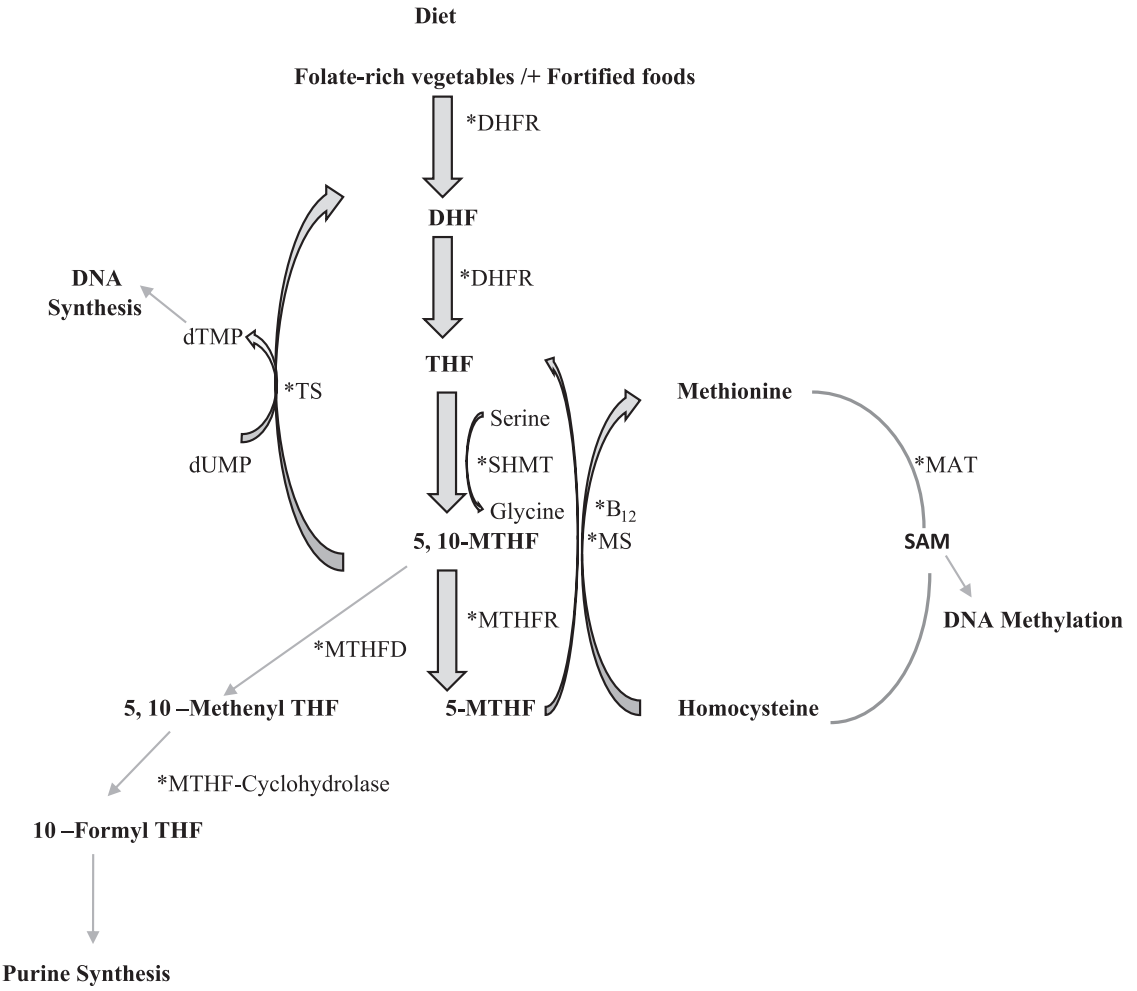


Fig. 1. Folate mechanism. Folate in foods is reduced by the enzyme DHFR to form DHF and by the action of the same enzyme to form THF. Then, THF is converted to 5, 10-MTHF by SHMT via donation of a methyl and hydroxy group from serine, which results in the production of the amino acid glycine in the process. 5,10-MTHF is synthesized to 5-MTHF by MTHFR. In addition, 5-MTHF can be reverted back to THF by MS, which also generates methionine from homocysteine in the presence of vitamin B_{12}. Methionine is converted to SAM by MAT, which is reverted back to homocysteine. 5, 10-MTHF is a crucial intermediate because it can be recycled back to DHF by TS, undergo further conversion by MTHFD to 5,10-methenyl THF and to 10-formyl THF, a precursor for nucleic acid synthesis by the action of methenyl THF cyclohydrolase. DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine; monophosphatedUMP, deoxyuridine monophosphate; MAT, methionine adenosyltransferase; MS, methionine synthase; 5-MTHF, methylenetetrahydrofolate; MTHF, cyclohydrolase–methenyl; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, methylenetetrahydrofolate reductase; SAM, *S*-adenosyl-methionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate.

to genetic defects such as MTHF reductase polymorphism or drug interactions [16,17].

With respect to the therapeutic effect of folate in childhood allergy, few observational studies have been published reporting mixed results [9,18–21]. Additional intervention studies performed in a variety of child populations are required to address the gaps in the literature on the preventive/therapeutic potential of folate in respiratory disease.

Given the dearth of studies investigating folate concentrations in children with asthma, the objective of this study was to explore the relationship between plasma folate and measures of airway disease (as reflected by spirometry) in a cohort of school children with asthma. We hypothesized that folate deficiency is associated with lung function impairment in this population of children.

Materials and methods

This cross-sectional study was a subanalysis of baseline data from the Mediterranean diet cohort study that investigated the efficacy of the traditional Greek Mediterranean diet enriched with oily fish on asthma symptoms and pulmonary function [22] in a sample of 64 school-aged children (5–12 y) with doctor-diagnosed "mild asthma," which was well-controlled asthma (as defined by GINA guidelines, 2019) [23]. Children were recruited from a single-centered pediatric asthma clinic located in the city of Athens, Greece. In brief, children were excluded if they suffered from severe or chronic asthma [24], food allergies (especially fish or seafood), gastroesophageal reflux disease, cystic fibrosis, or congenital respiratory disease; if they were taking oral glucocorticoid medication or high-dose multivitamin supplements. Details of the study design and data collection have been published elsewhere [22,25]. A description of sample size calculation is provided in Supplement 1.

For the purpose of this study, the exposure was folate concentrations as measured by plasma 5-MTHF and the outcomes were markers of pulmonary function forced expiratory volume in 1s (FEV_1), forced vital capacity (FVC), ratio of FEV_1/FVC, peak expiratory flow (PEF), forced expiratory flow at 25–75% of the pulmonary volume ($FEF_{25-75\%}$) and fractional exhaled nitric oxide (FeNO). The study was conducted according to the guidelines laid down in the 1964 Helsinki Declaration and all procedures involving subjects were approved by the Human Ethics Committee of La Trobe University. Written informed consent was obtained from all families for experimentation with pediatric participants.

Clinical Assessments

Pulmonary function tests

Pulmonary function was measured using spirometry (FEV_1, FVC, FEV_1/FVC, PEF, $FEF_{25-75\%}$) and bronchial inflammation by FeNO, which were performed by trained technicians according to the European Respiratory Society (ERS)/American Thoracic Society (ATS) protocol [26,27]. A portable hand-held device MIR SPIROBANK !! (Medical International Research, New Berlin, WI, USA) was used which is a validated tool recommended by the ATS for the assessment of pulmonary function in children (≥5 y of age). Spirometry was performed pre- and postbronchodilator administration. Normal lung function was considered to be values of FEV_1, FVC, and PEF ≥80% predicted, FEV_1/FVC 75%–85% and $FEF_{25-75\%}$ ≥65% as well as a *Z*-score cutoff of −1.64 for symptomatic patients [28]. Given that the children had well-controlled mild asthma (day symptoms and need for reliever medication ≤2 weekly, night symptoms ≤1 monthly and no limitation of daily activities and no exacerbation risk) [1], and that the aim of the present study was not to diagnose asthma based on evidence of reversible airway limitation [26], we used spirometry values prebronchodilator administration. Following ERS guidelines, FeNO <20 ppb is indicative of no airway inflammation in pediatrics [27]. An extensive description of clinical assessments is available in Supplement 1.

Folate status determination

Using standard technique, peripheral blood samples (4 mL) were collected from children with a butterfly needle attached to a syringe and distributed to vials after a 2-h fast. Plasma determination of 5-MTHF was measured by automated fluorescence polarization immunoassay (IMx, Abbott Laboratories, Chicago,IL, USA) via high-performance liquid chromatography assay (HPLC) [29].

According to World Health Organization (WHO) recommendations, serum or erythrocyte concentrations of folate provide an accurate estimate of the population's status [16]. Folate deficiency for all ages is considered to be serum folate concentration <10 nmol/L (4 ng/mL) [16], using 5-MTHF acid, the predominant folate species in serum and erythrocytes as a proxy [30]. For the purpose of this study, folate status was examined in children using quartiles (Q1–Q4).

Statistical analysis

Continuous variables were assessed for normality using Shapiro–Wilks test, and are described as means ± SD and categorical variables as frequencies and percentages. Comparisons between boys and girls were assessed using *t* test and in the case of skewness by non-parametric Mann–Whitney, and $\chi 2$ tests for categorical variables. We explored associations between folate and outcomes using linear regression models adjusting for potential confounders that are known asthma risk factors such as body mass index (BMI) and physical activity [4]. These variables were retained in the adjusted models if they changed the estimated effect size of the primary exposure by ≥10% or were statistically significant at the 5% level. Separate regression models were fitted with folate as the continuous and dichotomous variable (deficient <10 nmol/L [4 ng/mL] and normal ≥10 nmol/L) using the total sample and stratified by sex. Furthermore, folate was divided into quartiles based on 25th, 50th, 75th percentiles and the minimum and maximum values (Q1: 6.20–8.44, Q2: 8.45–10.29, Q3: 10.30–12.54, Q4: 12.55–22.90 nmol/L). The regression procedure was repeated using the highest folate quartile (Q4) as the reference value, which is consistent with prior studies [19,20]. The effect of folate was expressed as unstandardized β coefficient with corresponding 95% confidence interval (CI). Statistical significance was considered $P < 0.05$. All analyses were conducted using the SPSS version 20 (IBM, Armonk, NY, USA).

Results

Demographic and clinical characteristics for the total sample and by sex are presented in Table 1. Of the 64 children (age range: 5–12 y) with mild asthma, 52% were boys (33), and average age was 7.9 ± 2.2 y (mean ± SD). Pulmonary function tests showed that 97% (62) of participants had no impairment of lung function (% predicted FVC, FEV_1, and PEF ≥80%) or indication of eosinophilic airway inflammation with 75% (48) of children having (FeNO <20 ppb), reflecting good adherence to asthma therapy. At time of lung function measures, none of the children were taking folate supplements. Analysis of plasma folate concentrations as measured by 5-MTHF showed that serum folate levels ranged from 6.2 to 22.9 nmol/L. Approximately 45.3% (29) of the children were deficient in folate (<10 nmol/L), 21.9% (14) borderline (plasma folate range 10–10.9 nmol/L), but no sex differences were observed. Overall, mean plasma folate concentrations for the total sample were low at 11.1 ± 3.8 nmol/L.

For children deficient in folate (<10 nmol/L), the value of %PEF was significantly lower in girls than in boys (mean ± SD girls vs boys: 85.8 ± 14.8 vs 98.3 ± 14.6; $P = 0.03$). The same trend was observed for FVC, FEV_1, FEV_1/FVC, and $FEF_{25-\%}$ but higher for FeNO, although they did not attain significance.

Regression model

For the total sample, linear regression of folate levels as the continuous variable showed no association of 5-MTHF (continuous) on spirometry or FeNO in the unadjusted model and adjusted for confounding factors BMI and regular exercise (Supplementary Table 1).

The same outcome was observed when folate status as the dichotomous variable (deficient: 5-MTHF <10 nmol/L; normal: ≥10 nmol/L) and as quartiles were entered into the regression model (Supplementary Tables 2 and 3).

To assess sex differences, we stratified the data according to boys and girls and refitted the regression models. The unadjusted regression model revealed a statistically significant association in girls only for 5-MTHF (continuous) and %PEF (β = 1.56; 95% CI, 0.10–3.00; $P = 0.037$), but borderline for %FVC (β = 0.72; 95% CI, 0.00–1.44; $P = 0.05$) and $\%FEV_1$ (β = 0.78; 95% CI, −0.34 to 1.59; $P = 0.06$). However, when controlling for BMI and regular exercise, a stronger effect of 5-MTHF was observed in girls for %FVC (β = 0.79; 95% CI, 0.14–1.44; adjusted $P = 0.019$), $\%FEV_1$

Table 1
Demographic and clinical characteristics for total sample and per sex

Variable	Demographic and clinical characteristics			
	Total (N = 64) Mean ± SD	Boys (n = 33) Mean ± SD	Girls (n = 31) Mean ± SD	*P*-value*
Age (y)	7.9 ± 2.2	7.9 ± 2.3	8.1 ± 2.2	0.73†
BMI (*Z*-scores)‡	−0.24 (−0.79 to 0.56)	−0.25 (−0.85 to 0.59)	−0.09 (−0.74 to 0.47)	0.97†
%pred FVC (L)	95.5 ± 9.9	95.7 ± 10.9	95.2 ± 9.1	0.84
%pred FEV_1	98.2 ± 9.7	99 ± 9.3	97.3 ± 10.2	0.48
%pred FEV_1/FVC	102.2 ± 6.3	103 ± 6.3	101.3 ± 6.3	0.27
%pred PEF	93.9 ± 18.9	96.9 ± 18.9	90.6 ± 18.5	0.18
%pred $FEF_{25-75\%}$	102.2 ± 19.7	104.4 ± 17.9	99.7 ± 21.4	0.34
FeNO (ppb)	13.9 ± 13.7	12.8 ± 14.2	15.1 ± 13.4	0.32†
5-MTHF (nmol/L)	11.1 ± 3.8	10.7 ± 3.1	11.6 ± 4.5	0.75†
Exercise (<3 times/wk)	50% (32/64)	45.5% (15/33)	54.8% (17/31)	0.45§
Folate <10 nmol/L (deficient) % (n)	45.3% (29/64)	45.5% (15/33)	45.2% (14/31)	0.98§
Folate quartiles % (n)				
Quartile 1 (6.20–8.44)	25% (16/64)	21.2% (7/33)	29% (9/31)	0.36§
Quartile 2 (8.45–10.29)	21.9% (14/64)	27.3% (9/64)	16.1% (5/31)	
Quartile 3 (10.30–12.54)	28.1% (18/64)	33.3% (11/64)	22.6% (7/31)	
Quartile 4 (12.55–22.90)	25% (16/64)	18.2% (6/64)	32.3% (10/31)	
Medication (%)‖ Yes	82.8% (53/64)	78.8% (26/33)	87.1% (27/31)	0.38§
No	17.2% (11/64)	21.2% (7/33)	12.9% (4/31)	

BMI, body mass index; $FEF_{25-75\%}$, forced expiratory flow at 25–75% of the pulmonary volume; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity; PEF, peak expiratory flow; %pred, % predicted; 5-MTHF, 5-methyltetrahydrofolate
FEV1/FVC- ratio of forced expiratory volume in 1s and forced vital capacity.
**P* values estimated using *t* test.
†Mann–Whitney test.
‡BMI *Z*-score presented as median, 25th and 75th percentiles.
§χ^2 test.
‖Corticosteroids and antileukotriene therapy.

Table 2
Adjusted linear regression model for 5-MTHF (continuous) vs pulmonary function tests per sex adjusted for BMI and regular exercise

Parameter / Covariates	Boys (n = 33)			Girls (n = 31)			
	β	95% CI	Adj *P*-value	β	95% CI	Adj *P*-value	$P_{interaction}$
% pred FVC							
5-MTHF	−0.73	−1.98 to 0.52	0.24	0.79	0.14 to 1.44	0.019	0.029
BMI	0.55	−0.41 to 1.51	0.25	1.18	0.38 to 1.97	0.00	
Regular exercise	7.70	0.02 to 15.38	0.05	−2.93	−8.65 to 2.79	0.30	
% pred FEV_1							
5-MTHF	−0.69	−1.77 to 0.38	0.19	0.78	0.01 to 1.55	**0.046**	**0.034**
BMI	0.52	−0.30 to 1.34	0.21	0.92	−0.01 to 1.86	0.05	
Regular exercise	5.86	−0.73 to 12.45	0.08	−6.14	−12.90 to 0.63	0.07	
% pred FEV_1/FVC							
5-MTHF	0.09	−0.69 to 0.87	0.81	−0.07	−0.61 to 0.46	0.77	0.69
BMI	−0.12	−0.72 to 0.47	0.67	−0.36	−1.01 to 0.30	0.28	
Regular exercise	−2.4	−7.16,2.40	0.32	−2.71	−7.45 to 2.02	0.25	
% pred PEF							
5-MTHF	−1.11	−3.25 to 1.04	0.29	1.64	0.14 to 3.15	**0.033**	**0.031**
BMI	−1.59	−3.24 to 0.06	0.06	0.92	−0.92 to 2.76	0.31	
Regular exercise	3.59	−9.58 to 16.77	0.58	−0.01	−13.28 to 13.27	0.99	
% pred $FEF_{25-75\%}$							
5-MTHF	−1.08	−3.24 to 1.08	0.31	0.93	−0.87 to 2.72	0.30	0.19
BMI	0.99	−0.67 to 2.65	0.23	0.04	−2.16 to 2.23	0.97	
Regular exercise	6.46	−6.81 to 19.72	0.33	−11.26	−27.12 to 4.60	0.16	
FeNO							
5-MTHF	0.85	−0.88 to 2.59	0.32	−0.21	−1.37 to 0.95	0.71	0.29
BMI*	−0.64	−1.97 to 0.69	0.33	−0.35	−1.77 to 1.07	0.61	
Regular exercise	−1.82	−12.46 to 8.81	0.73	−5.70	−15.96 to 4.55	0.26	

BMI, body mass index; $FEF_{25-75\%}$, forced expiratory flow at 25–75% of the pulmonary volume; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity; PEF, peak expiratory flow; %pred, % predicted; 5-MTHF, 5-methyltetrahydrofolate
5-MTHF as the continuous variable
Values expressed as unstandardized β coefficient and 95% CI
Significant *P* values indicated in **bold**
*Adusted *P* value from linear regression model adjusted for BMI and regular exercise.

(β = 0.78; 95% CI, 0.01–1.55; adjusted *P* = 0.046), and %PEF (β = 1.64; 95% CI, 0.14–3.15; adjusted *P* = 0.033; Table 2). The same trend was obtained in the interaction analysis. No effect was observed in boys.

When repeating the regression models (unadjusted and adjusted) with folate levels (deficient/normal) as the dichotomous independent variable no associations were observed for either sex (Supplementary Table 4).

Table 3
Regression analysis showing sex differences in the association between folate quartiles and pulmonary function tests from the regression analysis

	Boys		Girls	
Parameter	Unadjusted β (95% CI);*P*	Adjusted β (95% CI); *P*-value*	Unadjusted β (95% CI); *P*-value	Adjusted β (95% CI); *P*-value*
%FVC				
Q1	5.81 (−6.75 to 18.37); *P* = 0.35	6.89 (−5.07 to 18.86); *P* = 0.25	**−8.68 (−16.75 to −0.61); *P* = 0.036**	**−8.64 (−16.18 to −1.09); *P* = 0.027**
Q2	2.67 (−9.23 to 14.57); *P* = 0.65	1.28 (−10.32 to 12.87); *P* = 0.82	−1.90 (−11.52 to 7.72); *P* = 0.69	−4.01 (−13.0 to 4.98); *P* = 0.37
Q3	7.30 (−4.16 to 18.76); *P* = 0.20	8.34 (−2.61 to 19.29); *P* = 0.13	−8.19 (−16.84 to 0.47); *P* = 0.06	−8.15 (−16.24 to 0.05); *P* = 0.05
Q4	Reference			
%FEV_1				
Q1	4.38 (−6.30 to 15.07); *P* = 0.41	5.26 (−5.17 to 15.69); *P* = 0.31	**−11.23 (−19.88 to −2.59); *P* = 0.013**	**−10.35 (−18.82,−1.89); *P* = 0.019**
Q2	5.11 (−5.01 to 15.23); *P* = 0.31	4.49 (−5.62 to 14.61); *P* = 0.37	−0.50 (−10.80 to 9.80); *P* = 0.92	−1.67 (−11.76,8.41); *P* = 0.74
Q3	7.12 (−2.63 to 16.87); *P* = 0.15	8.03 (−1.52 to 17.58); *P* = 0.09	**−10.04 (−19.31 to −0.77); *P* = 0.035**	−9.11 (−18.19,−0.034); *P* = 0.05
Q4	Reference			
%FEV_1/FVC				
Q1	−1.98 (−9.27 to 5.31); *P* = 0.58	−2.30 (−9.64 to 5.03); *P* = 0.52	−1.91 (−8.01 to 4.19); *P* = 0.53	−1.15 (−7.31 to 5.00); *P* = 0.70
Q2	2.61 (−4.29 to 9.52); *P* = 0.45	3.39 (−3.71 to 10.50); *P* = 0.34	2.20 (−5.07 to 9.47); *P* = 0.54	3.33 (−4.01,10.66); *P* = 0.36
Q3	−0.29 (−6.94 to 6.36); *P* = 0.93	−0.55 (−7.26 to 6.16); *P* = 0.87	−1.37 (−7.91 to 5.17); *P* = 0.67	−0.56 (−7.17 to 6.04); *P* = 0.86
Q4	Reference			
%PEF				
Q1	12.83 (−8.87 to 34.54); *P* = 0.24	11.37 (−9.47,32.21); *P* = 0.27	**−17.92 (−34.69,1.15); *P* = 0.037**	**−18.72 (−36.30 to −1.14); *P* = 0.038**
Q2	10.17 (−10.40,30.73); *P* = 0.32	6.13 (−14.07,26.32); *P* = 0.54	−9.50 (−29.49 to 10.49); *P* = 0.34	−11.36 (−32.30,9.58); *P* = 0.27
Q3	0.92 (−18.88 to 20.73); *P* = 0.92	−1.20 (−20.27 to 17.87); *P* = 0.89	−14.84 (−32.83 to 3.14); *P* = 0.10	−15.69 (−34.54 to 3.15); *P* = 0.10
Q4	Reference			
%$FEF_{25-75\%}$				
Q1	2.40 (−17.56 to 22.37); *P* = 0.81	3.55 (−16.66 to 23.76); *P* = 0.72	−15.30 (−34.89 to 4.29); *P* = 0.12	−12.95 (−33.27 to 7.38); *P* = 0.20
Q2	17.38 (−1.53,36.30); *P* = 0.07	18.79 (−0.79 to 38.37); *P* = 0.06	−0.30 (−23.65 23.05); *P* = 0.98	1.07 (−23.15 to 25.28); *P* = 0.93
Q3	6.10 (−12.11,24.32); *P* = 0.49	7.55 (−10.94 to 26.04); *P* = 0.41	−18.0 (−39.02 to 2.99); *P* = 0.09	−15.51 (−37.31 to 6.28); *P* = 0.15
Q4	Reference			
FeNO				
Q1	−11.29 (27.67,5.10); *P* = 0.17	−11.94 (−28.80 to 4.92); *P* = 0.16	−2.42 (−15.62 to 10.78); *P* = 0.71	−0.99 (−14.73,12.76); *P* = 0.88
Q2	−5.56 (−21.08 to 9.97); *P* = 0.47	−6.34 (−22.68 to 10.00); *P* = 0.43	−2.40 (−18.14 to 13.34); *P* = 0.76	−1.22 (−17.59,15.15); *P* = 0.87
Q3	−3.90 (−18.86,11.03); *P* = 0.59	−4.74 (−20.16 to 10.69); *P* = 0.53	−4.34 (−18.50 to 9.82); *P* = 0.53	−2.81 (−17.55 to 11.92; *P* = 0.69
Q4	Reference			

$FEF_{25-75\%}$, forced expiratory flow at 25–75% of the pulmonary volume; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity; PEF, peak expiratory flow; %pred, % predicted.
n (male) = 33; n (female) = 31
P value shown for each folate quartile group
Significant *P* values indicated in **bold**
Values shown as unstandardized β coefficient and 95% CI
Q1: Folate Quartile 1 6.20–8.44 nmol/L; Q2: Quartile 2 8.45–10.29 nmol/L; Q3–Quartile 3 10.30–12.54 nmol/L; Q4–Quartile 4 12.50–22.90 nmol/L
*Regression model adjusted for BMI and regular exercise.

Furthermore, addition of folate quartiles into the adjusted model revealed significant associations for girls in the lowest folate quartile (Q1) and %FVC (adjusted P = 0.027), %FEV_1 (adjusted P = 0.019), and %PEF (adjusted P = 0.038) as compared with those in the higher folate quartile (Q4) (Table 3). More specifically, in the adjusted model, girls with low plasma concentrations (Q1) had 8.64% lower FVC (β = −8.64, 95% CI, −16.18 to −1.090; adjusted P = 0.027), 10.35% FEV_1 (β = −10.35; 95% CI, −18.82 to −1.89; adjusted P = 0.019) and 18.72% PEF (β = −18.72, 95% CI, −36.30 to −1.14; adjusted P = 0.038) as compared with girls with high folate concentrations (Q4). No association was observed for boys or for FeNO.

Discussion

There is paucity of data on the role of folate and lung function in the pediatric population. Novel to this study, our findings support the hypothesis that low folate consumption is problematic in pediatric patients with asthma, even in children on a Mediterranean diet. Two main points were highlighted in this study. First, the majority of children in the present study (67.2%) were low in plasma folate concentrations (5-MTHF <11 nmol/L). Of these children, 45.3% were deficient (<10 nmol/L) according to WHO standards [16]. In our recent publication, dietary data revealed that Greek children with asthma have low intake of fruit, vegetables, legumes, and cereals, all important dietary sources of folate [22]. This is consistent with previous studies investigating food group and micronutrient intake in pediatric patients with asthma [19] as well as with the general Hellenic pediatric population [31,32]. One other factor contributing to suboptimal folate levels is the current global economic crisis, which has resulted in the decline in purchase and consumption of healthy foods such as fruit, vegetables, and fish, due to increase in cost as compared with unhealthy food choices [33–35]. Furthermore, food fortification policies are not mandatory in all countries of the European Union including Greece [36] and populations rely on food fortification of staple foods for adequate folate intake [37,38]. Alternatively, high consumption of fruits and vegetables, which are rich in antioxidants (folate, vitamins C, E, β-carotene) and bioactive molecules (flavonoids, polyphenols), were associated with improved lung function (↑FEV_1 and ↑FVC) in children, and lower odds of asthma and wheeze [39,40].

Another important finding highlighted in the present study was that there was a dose–response effect of plasma folate on dynamic volumes (FVC and FEV_1) and PEF in girls only. Stratification of plasma folate concentrations into quartiles showed that girls with low folate concentrations had reduced spirometric measurements (as reflected by %FVC, %FEV_1, and %PEF) compared to girls with high folate concentrations to compared to girls with high folate concentrations. Girls in the lowest folate quartile (Q1) had 8.64% lower FVC, 10.35% FEV_1, and 18.72% PEF than those in the highest folate quartile (Q4). No association was observed in boys or for FeNO. This is interesting because according to ATS/ERS standards,

variability in FEV_1 by 10% (in this case a decrease in FEV_1 by 10.35% in girls) indicates deterioration in lung function, which is clinically significant [23]. Therefore, it is possible that strategies targeting normalization of low serum folate concentrations in female pediatric asthma patients mediated by increased intake of fruits and vegetables could improve lung function comparable to pharmacotherapy. The potential efficacy of dietary folate as a therapeutic adjunct to conventional asthma care remains to be elucidated in future research.

Our findings are novel and extend to the limited database [19,21,41]. Lin et al., in a prospective cohort study of 150 children (5–17 y of age) with persistent asthma, of whom 92% were black, reported no significant difference in lung function parameter (FEV_1/FVC) across folate quartiles [21]. Regarding airway inflammation, unlike our study, a bell-shaped relationship was observed between folate quartiles and FeNO with low folate levels being associated with low FeNO and low-moderate levels with increased inflammation. Interestingly, the same pattern was observed for serum immunoglobulin E levels, an indicator of atopy. Thus, suggesting that folate may act specifically on allergic airway inflammation as compared with lung function and that there might be a threshold at which folate exerts a prophylactic or adverse effect on airway inflammation [42].

On the other hand, Han et al., in a recent cross-sectional study, included data from 5933 children (6–19 y of age) participating in the National Health and Nutrition Examination Survey (NHANES), demonstrated that a high ratio of serum 5-MTHF to UMFA was positively associated with FEV_1 in children [41]. Specifically, each quartile increment in the ratio of serum 5-MTHF to UMFA level was associated with a 0.60% increase in FEV_1 (β = 0.60; 95% CI, 0.05–1.15). However, no associations were observed between total folate, 5-MTHF, or UMFA and FEV_1 or FVC in children.

Nicholson et al., in a case–control study of 884 Peruvian children 9–19 y of age (n = 413 with asthma) found that a 10-ng/mL decrease in plasma folate levels was associated with 0.28 decrease in prebronchodilator FEV_1/FVC *Z*-score (β = −0.28; 95% CI, −0.52 to −0.05) in children with asthma after adjusting for age, sex, BMI, and socioeconomic status [19]. With respect to FEV_1, a significant association was observed in the unadjusted analysis (β = −0.29; 95% CI, −0.57 to −0.02) which became non-significant after adjustment. No association was reported for FVC or for children without asthma. As aforementioned, variability between the findings of our study and of the above could be due to age and population differences, genetic variability and dietary habits, adjustment for confounding factors, timing/season of folate assessment, thresholds used for folate levels, asthma severity, differences in lung function parameters measured, lack of data regarding folate supplementation. and dietary intake. It is worth mentioning that common to both of the latter studies, sex differences exploring the effects of folate on lung function tests were not assessed, which could have contributed to the diversity among results. One more discrepancy, previous studies including ours measured total folate levels in plasma and not UMFA. It is possible that UMFA might influence the one-carbon folate metabolic pathways differently from folate-derived from dietary sources. Nevertheless, in the study by Han et al., no association was found between 5-MTHF, FVC, and FEV_1 in children, which is in agreement with our findings for the total sample. However, data on sex differences for the association between 5-MTHF and spirometry in children was not provided by the authors, and remains to be explored. From a statistical point of view, our study included a small sample, therefore our results could be significant due to chance. Larger well-designed studies are recommended to replicate our findings. Additionally, Nicholson defined folate deficiency at 20 ng/mL and clinically, deficiency for all ages is considered at <4 ng/mL, based on homocysteine levels as the metabolic indicator [16]. However, consistent with our observations, Nicholson noted no association between serum folate concentrations and FeNO in the adjusted analysis for children with and without asthma.

Nevertheless, collectively, the findings of these studies posit that dietary folate might play a crucial role in the development and perpetuation of asthma. In our study, the prophylactic association between normal folate status and improved lung function might be explained by the role of folate in epigenetics [43]. The proposed mechanism by which folate is suspected to affect asthma susceptibility is by acting as a methyl donor promoting DNA methylation thus suppressing the expression of key genes involving T-cell differentiation and regulation [43]. Feasibly, normal serum folate levels, could prevent T-helper (Th)2 pro-allergic immune response that lead to airway and systemic inflammation and as a result improve lung function and at low serum levels as is in the case of folate deficiency, initiate and sustain a Th2 inflammatory response and proliferation of inflammatory cytokines, features of asthma development (as was reflected by low measurements of %FVC, $\%FEV_1$, and %PEF in girls). Another potential avenue is that there might be a window of exposure at which folate confers protective effects against asthma development and progression in children [44]. Li et al., in a meta-analysis exploring the effect of maternal folate encompassing both dietary intake and supplementation in childhood asthma, documented a linear relationship between increased folate in pregnancy and greater asthma risk in offspring [45], whereas another not [46], while exposure in later childhood [9,18] and adulthood [47,48], protective.

One more interesting observation in the present study was sex differences observed in pulmonary function. In particular, lower PEF a measure of airflow through bronchi, and thus the degree of airway obstruction was significantly lower in girls deficient in folate (<10 mol/L) than in boys. The same pattern was found for FEV_1, FVC, FEV_1/FVC, and $FEF_{25-75\%}$, although it did not reach significance. This is in line with another study reporting higher PEF measures in boys compared with girls [49]. Furthermore, increased blood eosinophils is a biomarker of asthma-associated airway inflammation [23]. Higher counts have been noted in girls with asthma than boys signifying more severe asthma [50], and are worth further investigation in our sample of children.

It is well-recognized that anatomic and hormone differences between the sexes influence respiratory function [51]. Reduced airway diameter and lung volume in females result in lower PEF and vital capacity compared with males [51]. From this perspective, Fukagawa et al. observed that transmethylation and methylation rates of homocysteine were higher in women than in men most likely due to hormonal control mediated by estrogen [52]. Feasibly, hormonal differences due to early maturity in girls could be one of the reasons explaining the significant associations we observed between folate and spirometry in girls only. From a nutritional point of view, although we did not assess food intake data, a previous study has documented lower dietary intakes of folate in Greek girls ages 9 to 13 y compared with boys [32]. Therefore, in combination with low folate intake, a deficiency of methyl groups impair one-carbon metabolism resulting in DNA instability, along with accumulation of homocysteine could consequently lead to increased production of reactive oxygen species that favor the expression of proinflammatory genes and vascular inflammation [53]. It is this state of chronic oxidative stress that polarizes the Th2 immune response associated with asthma [54], which was measured by decreased values of PEF in girls. Therefore, it is possible that high folate levels could prevent inflammation by reducing oxidative stress in airways. On the other hand, we did not find

associations between folate quartiles and FeNO in children. Given that serum folate was measured at one time point only and that the majority of children were taking asthma medication and had well-controlled asthma, it is possible that these factors contributed to no statistical associations due to potential masking effects.

Strengths and limitations

The findings of the present study should be viewed in light of the foregoing considerations. A strength of the present study was the homogenous population with respect to ethnicity, diet, and environmental factors. Notably, we explored the association of folate on a range of ventilatory parameters, whereas previous studies reported details for FEV_1, FVC, and FEV_1/FVC only [19,21]. Another strong point was that none of the children were taking folate supplements, since increased oral intake is associated with high serum folate levels [15].

A few shortcomings in the present study deserve acknowledgment. Our findings were derived from a small sample of Mediterranean schoolchildren with asthma, therefore these findings cannot be extrapolated to the general population. Additionally, the small study sample makes interpretation of strata-analysis difficult. Nevertheless, our findings may be broadly relevant to pediatric populations at high risk for asthma morbidity. Another potential weakness is that we lacked data on genetic variants. Furthermore, a single measurement of serum folate undertaken in children does not reflect long-term folate status. Due to laboratory limitations, we could not assess for interactions between folate and other vitamins or nutrients. A topic of interest worth pursuing would be the association between folate and spirometry postbronchodilator administration. Future studies could be strengthened by including repeated measurements of folate, a broader range of inflammatory biomarkers such as plasma immunoglobulin E, Th2, homocysteine, inflammatory eicosanoid concentrations as well as folate-metabolism gene polymorphisms (*MTHFR*). This study was cross-sectional and limits our ability to conclude that there is a causal effect between folate status and lung function in children with asthma. Nevertheless, findings on sex differences are novel and address gaps in knowledge of biological and behavioral effects on lung function in childhood.

Conclusion

The findings of this study highlighted the potential negative effects of folate deficiency on pulmonary function in girls with asthma, the importance of monitoring folate status in children with asthma, and early prevention strategies such as adequate folate in the diet and supplementation when needed. Future well-designed, robust clinical trials are recommended to assess the clinical efficacy of folate intake in the management of pediatric asthma as well as elucidate the molecular mechanisms involved.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2021.111267.

References

[1] Papadopoulos N, Arakawa H, Carlsen KH, Custovic A, Gern J, Lemanske R, et al. International consensus on (ICON) pediatric asthma. Allergy 2012;67:976–97.
[2] Trivedi M, Denton E. Asthma in children and adults—what are the differences and what can they tell us about asthma? Front Pediatr 2019;7:256.
[3] World Health Organization. Asthma. Geneva, Switzerland: Author; 2017.
[4] Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet 2015;386:1075–85.
[5] Holguin F. Oxidative stress in airway diseases. Ann Am Thorac Soc 2013;10: S150–7.
[6] McGowan EC, Matsui EC, Keet C. Higher serum 5-methyltetrahydrofolate (5-MTHF) levels are associated with lower risk of wheeze in the National Health and Nutrition Examination Survey (NHANES). J Allergy Clin Immunol 2015;135:AB228.
[7] Sackesen C, Ercan H, Dizdar E, Soyer O, Gumus P, Tosun BN, et al. A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. J Allergy Clin Immunol 2008;122:78–85.
[8] Igde M, Baran P, Oksuz BG, Topcuoglu S, Karatekin G. Association between the oxidative status, vitamin D levels and respiratory function in asthmatic children. Niger J Clin Prac 2018;21:63–8.
[9] Matsui EC, Matsui W. Higher serum folate levels are associated with a lower risk of atopy and wheeze. J Allergy Clin Immunol 2009;123. 1253—9.e2.
[10] Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: systematic review and meta-analysis. J Allergy Clinical Immunol 2011;127. 724—33.e1—30.
[11] Patelarou E, Giourgouli G, Lykeridou A, Vrioni E, Fotos N, Siamaga E, et al. Association between biomarker-quantified antioxidant status during pregnancy and infancy and allergic disease during early childhood: a systematic review. Nutr Revs 2011;69:627–41.
[12] Garcia-Larsen V, Del Giacco SR, Moreira A, Bonini M, Charles D, Reeves T, et al. Asthma and dietary intake: an overview of systematic reviews. Allergy 2016;71:433–42.
[13] Emons JAM, Gerth van Wijk R. Food allergy and asthma: is there a link? Curr Treat Options Allergy 2018;5:436–44.
[14] McGowan EC, Hong X, Selhub J, Paul L, Wood RA, Matsui EC, et al. Association between folate metabolites and the development of food allergy in children. J Allergy Clin Immunol Pract 2020;8. 132—40.e5.
[15] Bailey LB, Stover PJ, McNulty H, Fenech MF, 3rd Gregory JF, Mills JL, et al. Biomarkers of nutrition for development-folate review. J Nutrition 2015;145:1636s–80s.
[16] World Health Organization. Serum and red blood cell folate concentrations for assessing folate status in populations. Geneva, Switzerland: Author; 2012.
[17] Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 2014;44:480–8.
[18] Okupa AY, Lemanske RF Jr., Jackson DJ, Evans MD, Wood RA, Matsui EC. Early-life folate levels are associated with incident allergic sensitization. J Allergy Clin Immunol 2013;131. 226—8.e1-2.
[19] Nicholson A, Pollard SL, Lima JJ, Romero KM, Tarazona-Meza C, Malpartida-Guzman G, et al. Serum folate concentrations, asthma, atopy, and asthma control in Peruvian children. Respir Med 2017;133:29–35.
[20] Blatter J, Brehm JM, Sordillo J, Forno E, Boutaoui N, Acosta-Perez E, et al. Folate deficiency, atopy, and severe asthma exacerbations in Puerto Rican children. Ann Am Thorac Soc 2016;13:223–30.
[21] Lin JH, Matsui W, Aloe C, Peng RD, Diette GB, Breysse PN, et al. Relationships between folate and inflammatory features of asthma. J Allergy Clin Immunol 2013;131:918–20.
[22] Papamichael MM, Katsardis C, Lambert K, Tsoukalas D, Koutsilieris M, Erbas B, et al. Efficacy of a Mediterranean diet supplemented with fatty fish in ameliorating inflammation in paediatric asthma: a randomised controlled trial. J Hum Nutr Diet 2019;32:185–97.
[23] Global Initiative for Asthma. Pocket guide for Asthma management and prevention (for adults and children older than 5 years). In: Asthma Global Initiative for Asthma, editor. Available at: https://ginasthma.org/pocket-guide-for-asthma-management-and-prevention/. Accessed August 25, 2020.
[24] Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343–73.
[25] Papamichael MM, Katsardis C, Tsoukalas D, Erbas B, Itsiopoulos C. A clinical trial of Mediterranean diet enriched with fatty fish in pediatric asthma: study protocol. J Pharmacy Pharmacol 2018;6:225–39.
[26] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319–38.
[27] Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011;184:602–15.
[28] Katsardis CV, Alexandraki S, Paraskakis E. Spirometry in children 6-16 years old. In: Katsardis Ch, Koumbourlis A, Anthracopoulos M, Paraskakis E, editors. Paediatric pulmonary function testing indications and interpretation, New York, NY: NOVA Biomedical; 2015. p. 15–42.

[29] Sobczynska-Malefora A, Harrington DJ, Voong K, Shearer MJ. Plasma and red cell reference intervals of 5-methyltetrahydrofolate of healthy adults in whom biochemical functional deficiencies of folate and vitamin B 12 had been excluded. Adv Hematol 2014;2014:465623.
[30] Yetley EA, Pfeiffer CM, Phinney KW, Fazili Z, Lacher DA, Bailey RL, et al. Biomarkers of folate status in NHANES: a roundtable summary. Am J Clin Nutr 2011;94:303s–12s.
[31] Manios Y, Moschonis G, Grammatikaki E, Mavrogianni C, van den Heuvel EG, Bos R, et al. Food group and micronutrient intake adequacy among children, adults and elderly women in Greece. Nutrients 2015;7:1841–58.
[32] Manios Y, Moschonis G, Mavrogianni C, Bos R, Singh-Povel C. Micronutrient intakes among children and adults in Greece: the role of age, sex and socioeconomic status. Nutrients 2014;6:4073–92.
[33] Filippidis FT, Schoretsaniti S, Dimitrakaki C, Vardavas CI, Behrakis P, Connolly GN, et al. Trends in cardiovascular risk factors in Greece before and during the financial crisis: the impact of social disparities. Eur J Public Health 2014;24:974–9.
[34] Jones NR, Conklin AI, Suhrcke M, Monsivais P. The growing price gap between more and less healthy foods: analysis of a novel longitudinal UK dataset. PLoS One 2014;9:e109343.
[35] Miller V, Yusuf S, Chow CK, Dehghan M, Corsi DJ, Lock K, et al. Availability, affordability, and consumption of fruits and vegetables in 18 countries across income levels: findings from the Prospective Urban Rural Epidemiology (PURE) study. Lancet Glob Health 2016;4:e695–703.
[36] Food Fortification Initiative. Europe: Food Fortification Initiative; 2019. Available at: http://ffinetwork.org/regional_activity/europe.php. Accessed August 25, 2020.
[37] Slagman A, Harriss L, Campbell S, Muller R, McDermott R. Folic acid deficiency declined substantially after introduction of the mandatory fortification programme in Queensland, Australia: a secondary health data analysis. Public Health Nutr 2019;22:3426–34.
[38] Pfeiffer CM, Sternberg MR, Zhang M, Fazili Z, Storandt RJ, Crider KS, et al. Folate status in the US population 20 y after the introduction of folic acid fortification. Am J Clin Nutr 2019;110:1088–97.
[39] Han YY, Forno E, Alvarez M, Colon-Semidey A, Acosta-Perez E, Canino G, et al. Diet, lung function, and asthma exacerbations in Puerto Rican children. Pediatr Allergy Immunol Pulmonol 2017;30:202–9.
[40] Hosseini B, Berthon BS, Wark P, Wood LG. Effects of fruit and vegetable consumption on risk of asthma, wheezing and immune responses: a systematic review and meta-analysis. Nutrients 2017;9:341.
[41] Han YY, Forno E, Rosser F, Celedón JC. Serum folate metabolites, asthma, and lung function in a nationwide US study. J Allergy Clin Immunol 2020;146. 220−2.e8.
[42] Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 2008;118:3462–9.
[43] Kabesch M, Michel S, Tost J. Epigenetic mechanisms and the relationship to childhood asthma. Eur Respir J 2010;36:950–61.
[44] McStay CL, Prescott SL, Bower C, Palmer DJ. Maternal folic acid supplementation during pregnancy and childhood allergic disease outcomes: a question of timing? Nutrients 2017;9.
[45] Li W, Xu B, Cao Y, Shao Y, Wu W, Zhou J, et al. Association of maternal folate intake during pregnancy with infant asthma risk. Sci Rep 2019;9:8347.
[46] Crider KS, Cordero AM, Qi YP, Mulinare J, Dowling NF, Berry RJ. Prenatal folic acid and risk of asthma in children: a systematic review and meta-analysis. Am J Clin Nutr 2013;98:1272–81.
[47] Patel BD, Welch AA, Bingham SA, Luben RN, Day NE, Khaw KT, et al. Dietary antioxidants and asthma in adults. Thorax 2006;61:388–93.
[48] Thuesen BH, Husemoen LL, Ovesen L, Jorgensen T, Fenger M, Gilderson G, et al. Atopy, asthma, and lung function in relation to folate and vitamin B(12) in adults. Allergy 2010;65:1446–54.
[49] Fabiana Andrade, da Silva Dias de S, Renato da Costa Teixeira, Araújo DA, Barbosa TR. Sousa Fabianne de Jesus Dias de, Cruz RV. Lung function and functional capacity in school age children. J Fisioterapia em Movimento 2017;30:77–84.
[50] Rubner F, Jackson D, Tisler C, Rajamanickam J, Gern J, Iemanske RJ. Relationships among eosinophils, asthma, and sex in a high risk birth cohort. J Allergy Clin Immunol 2015:AB228.
[51] LoMauro A, Aliverti A. Sex differences in respiratory function. Breathe 2018;14:131–40.
[52] Fukagawa NK, Martin JM, Wurthmann A, Prue AH, Ebenstein D, O'Rourke B. Sex-related differences in methionine metabolism and plasma homocysteine concentrations. Am J Clin Nutr 2000;72:22–9.
[53] Fu Y, Wang X, Kong W. Hyperhomocysteinaemia and vascular injury: advances in mechanisms and drug targets. Brit J Pharmacol 2018;175:1173–89.
[54] King MR, Ismail AS, Davis LS, Karp DR. Oxidative stress promotes polarization of human T cell differentiation toward a T helper 2 phenotype. J Immunol 2006;176:2765–72.