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Serum folate metabolites, asthma, and lung function in a nationwide U.S. study

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Capsule Summary

In a U.S. nationwide study, a high serum 5-MTHF to UMFA ratio is associated with decreased odds of asthma, increased FEV₁ in children and adults, and increased FVC in adults.

Key Words

Folate, folic acid, asthma, lung function, NHANES

To the Editor:

Folate is a major dietary source of the methyl donors needed to form S-adenosylmethionine (SAM) from homocysteine. Unlike folate from natural dietary sources, folic acid from fortified food or supplements requires dihydrofolate reductase (DHFR) to be reduced to tetrahydrofolate (THF), which can then be metabolized to 5-methyl-tetrahydrofolate (5-MTHF), the main circulating folate form involved in DNA methylation (**Figure E1**). Unmetabolized folic acid (UMFA) in serum may occur when consuming excess folic acid (~300 µg) that saturates DHFR in the body¹.

There is inconsistent evidence of adverse effects of folic acid supplementation on asthma or allergy in children, and little is known about folate and asthma in adults². We examined the relation between serum total folate and folate metabolites (5-MTHF and UMFA) and current asthma or lung function among children and adults who participated in the National Health and Nutrition Examination Survey (NHANES). We also investigated whether 5-MTHF to UMFA ratio (5-MTHF/UMFA) is associated with asthma or lung function in this population.

NHANES is a cross-sectional nationwide survey of a representative sample of the U.S. population. Ethnic minorities, low-income whites, and individuals 80 years and older are over-sampled. NHANES was approved by the institutional review board of the CDC. Informed consent was obtained from all study participants. Data from subjects who participated in NHANES 2011-2016 and who did not report being pregnant were included in the current analysis.

Different folate forms in serum (total folate, 5-MTHF, UMFA and 5-MTHF/UMFA) were measured by isotope-dilution high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), as described in the NHANES laboratory procedure manual (https://wwwn.cdc.gov/nchs/data/nhanes/2007-2008/labmethods/FOLFMS_E_MET.PDF).

Current asthma was defined by a positive answer to both of the following questions: “Has a doctor or other health professional ever told you that you have asthma?”, and “Do you still have asthma?”. Spirometry was available for subjects aged 6 to 79 years who participated in NHANES 2011-2012. Eligible participants performed spirometry following American Thoracic Society recommendations. Percent (%) predicted FEV₁, FVC and FEV₁/FVC were calculated using Global Lung Initiative 2012 equations that account for age, sex, race/ethnicity, and height³.

Sampling weights, stratification, and clusters provided by the NHANES were incorporated into the analysis. Wald chi-square tests and *t*-tests were used for bivariate analyses. The Spearman rank correlation coefficient (*r*) was used to examine the correlation between folate metabolites. Multivariable logistic regression was used for the analysis of quartiles of serum levels of folate forms and current asthma. All multivariable models were adjusted for age, gender, race/ethnicity, annual household income, body mass index (BMI, in adults) or BMI z-score (in children), family history of asthma, season of sampling, serum cotinine, and (in adults) pack-years of cigarette smoking. Because of significant linear trends in the bivariate analyses of quartiles of folate forms and lung function measures, folate forms were treated as ordinal (continuous) variables for the multivariable linear regression analysis of lung function. All statistical analyses were conducted using the SAS SURVEY procedure and SAS 9.4 software.

The current analysis included 5,933 children (aged 6 to 19 years) and 12,739 adults (aged 20 to 79 years)(**Figure E2**). Of these participants, 1,642 children and 3,502 adults from NHANES 2011-2012 had spirometry data and were included in the analysis of lung function measures. A comparison of the characteristics of subjects who were and were not included in this analysis are shown in **Table E1**.

Compared to children without current asthma, those with asthma were more likely to be non-Hispanic black and to have a family history of asthma, a higher BMI z-score, a lower 5-MTHF/UMFA, and lower FEV₁ and FEV₁/FVC (**Table E2**). Compared to adults without current asthma, those with asthma were more likely to be female and non-Hispanic white or black, and to have a family history of asthma, an annual household income <\$20,000, higher BMI and pack-years of cigarette smoking, and lower lung function measures (**Table E2**).

In children, total folate was positively and highly correlated with 5-MTHF ($r=0.98$), while the correlation between total folate and UMFA or 5-MTHF/UMFA was low to moderate ($r=0.26$ to 0.37). Similar correlations were observed in adults (**Figure E3**).

Compared to children with a 5-MTHF/UMFA level in the first (lowest) quartile, those whose ratio was in the second quartile had 28% decreased odds of current asthma, but there was no significant association between a 5-MTHF/UMFA in the third or fourth quartile and asthma (**Figure 1** and **Table E3**). Compared to adults with a 5-MTHF/UMFA level in the first quartile, those whose ratio was in the fourth (highest) quartile had 22% decreased odds of current asthma, with a trend for linearity approaching statistical significance ($P=0.05$, **Figure 1** and **Table E3**).

Total folate, 5-MTHF, and UMFA were not associated with current asthma in either children or adults (**Table E3**). Similar findings were obtained after the analysis was additionally adjusted for alcohol consumption (**Table E4**).

In children, each quartile increment in 5-MTHF/UMFA was associated with a 0.60% increment in %predicted FEV₁ and increased 5-MTHF and UMFA levels were associated with decreased FEV₁/FVC (**Table 1** and **Figure E4**). In adults, total folate, 5-MTHF, and 5-MTHF/UMFA were each positively associated with % predicted FEV₁ and FVC, with each quartile increment in 5-MTHF/UMFA having the strongest estimated effect on FEV₁ and FVC.

Total serum folate has been positively associated with current wheeze but not with doctor-diagnosed asthma among U.S. children and adults⁴. On the contrary, a 10 ng/ml decrement in serum folate was associated with asthma in Peruvian children⁵. Similarly, another study showed that adults with a total folate level in the first quartile had 37%-43% higher odds of doctor-diagnosed asthma than those with a total folate level in the fourth quartile⁶. Our study differs from prior reports in that we examined not only total folate but also folate metabolites in serum.

As for lung function, a 10 ng/ml decrement in total folate was associated with a 0.28 decrement in FEV₁/FVC z-score in a prior study of asthmatic children⁵. In contrast, total folate was not associated with lung function in a study of Danish adults⁶. In the current analysis, total folate, 5-MTHF, and 5-MTHF/UMFA were positively associated with lung function measures in adults and 5-MTHF/UMFA was positively associated with FEV₁ in children. The magnitude of these changes was small, and thus an overall healthy diet may have greater effects on (and be more

relevant to) lung function than a single vitamin such as folate. The observed negative association between 5-MTHF or UMFA and FEV₁/FVC in children requires further investigation.

Independent of 5-MTHF, a higher plasma UMFA has been associated with decreased natural killer cell cytotoxicity in healthy adults taking high doses of folic acid supplements⁷. A recent study reported that a high UMFA level in cord blood was associated with development of food allergy in young children⁸. UMFA may affect folate-dependent metabolic pathways by depleting DHFR and accumulating DHF, which reduces 5-MTHF generation and could affect DNA methylation⁹. By examining the 5-MTHF/UMFA ratio, we were able to account for the intracellular balance of 5-MTHF and UMFA in association with asthma or lung function.

We acknowledge several limitations. First, we cannot determine a temporal relationship between folate metabolites and asthma or lung function in this cross-sectional study. Second, we lacked data on some potential confounders such as allergic sensitization. Third, we have no data on genetic variants that may influence folate metabolism. Fourth, serum folate levels may not reflect long-term folate status, best assessed by repeated measures of serum folate within an individual over time. Lastly, we could not test for interaction between total folate or folate metabolites and other types of B vitamins or nutrients.

In summary, we show that a high 5-MTHF/UMFA is inversely associated with current asthma in adults and positively associated with lung function in children and adults. Our study supports future longitudinal studies of folate metabolites, asthma, and lung function.

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Table 1– Serum total folate and folate metabolites and lung function measures, NHANES 2011-2012

	Children (n=1,642)	Adults (n=3,502)
Serum levels of folate forms	β (95% CI), P-value	
Total folate		
% predicted FEV ₁	-0.11 (-0.97, 0.75)	0.58 (0.10, 1.07)*
% predicted FVC	0.26 (-0.56, 1.09)	0.55 (0.23, 0.88)[†]
% predicted FEV ₁ /FVC	-0.38 (-0.76, 0.01)	0.08 (-0.27, 0.43)
5-Methyl-tetrahydrofolate, 5-MTHF		
% predicted FEV ₁	-0.15 (-1.06, 0.76)	0.61 (0.10, 1.11)*
% predicted FVC	0.25 (-0.61, 1.12)	0.55 (0.17, 0.92)[†]
% predicted FEV ₁ /FVC	-0.41 (-0.78, -0.04)*	0.12 (-0.21, 0.45)
Unmetabolized folic acid, UMFA		
% predicted FEV ₁	-0.66 (-1.52, 0.20)	-0.44 (-1.08, 0.20)
% predicted FVC	-0.17 (-1.06, 0.73)	-0.28 (-0.83, 0.27)
% predicted FEV ₁ /FVC	-0.52 (-0.88, -0.15)[†]	-0.18 (-0.59, 0.22)
5-MTHF to UMFA ratio		
% predicted FEV ₁	0.60 (0.05, 1.15)[†]	0.77 (0.10, 1.45)[†]
% predicted FVC	0.33 (-0.34, 1.00)	0.64 (0.06, 1.23)[†]
% predicted FEV ₁ /FVC	0.30 (-0.18, 0.78)	0.17 (-0.10, 0.44)

Models were adjusted for household income, BMI z-score (in children) or BMI (in adults), family history of asthma, season of sampling, current asthma status, serum cotinine, and (in adults) pack-years of cigarette smoking.

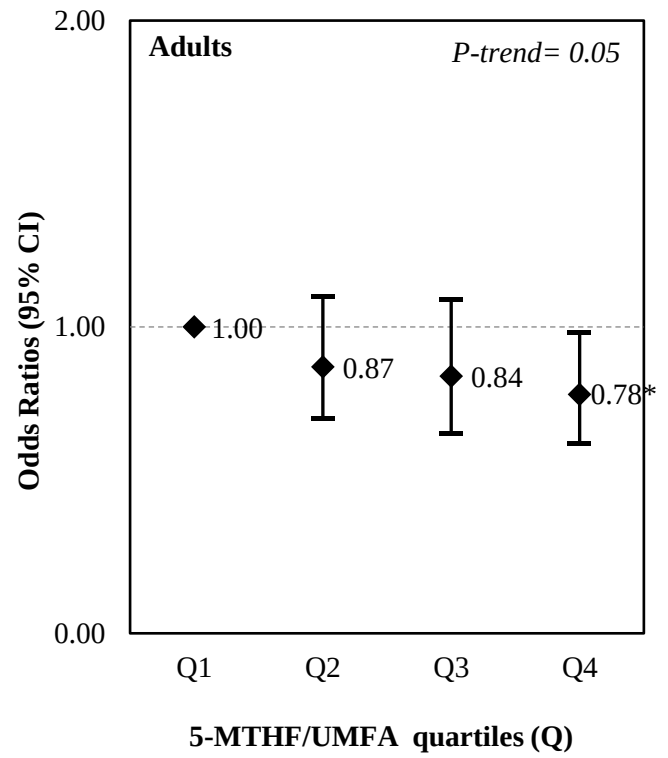
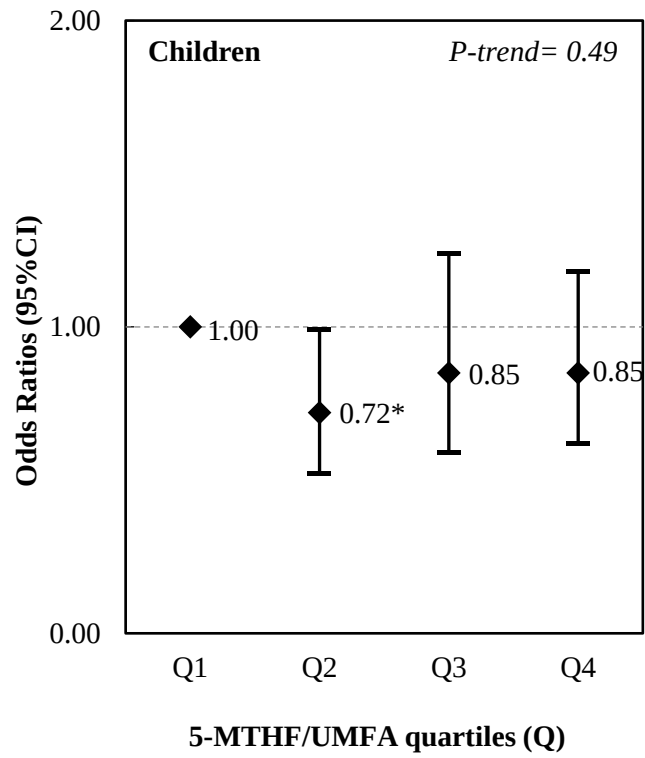
*P<0.05; [†]P<0.01;

Figure Legends**Figure 1- Serum 5-MTHF to UMFA ratio and current asthma in children and adults**

Footnote: 5-MTHF= 5-methyl-tetrahydrofolate; UMFA= unmetabolized folic acid.

Models were adjusted for age, gender, race/ethnicity, annual household income, BMI z-score (children) or BMI (adults), family history of asthma, serum cotinine, pack-years of smoking (in adults), and season of sampling.

*P<0.05



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Table E1- Characteristics of NHANES 2011-2016 participants who were and were not included in the current analysis

Characteristics	Children (6-19 years)		Adults (20-79 years)	
	Included (n=5,933)	Excluded (n=508)	Included (n=12,739)	Excluded (n=1,551)
Age (years)	12.7 ± 0.1	13.3 ± 0.3 [†]	46.3 ± 0.3	45.8 ± 0.6
Male gender	3037 (51.7)	244 (48.4)	6133 (48.4)	857 (55.5) [†]
Race/ethnicity				
Non-Hispanic white	1545 (54.0)	79 (39.8) [†]	4705 (66.1)	453 (56.8) [†]
Non-Hispanic black	1503 (13.6)	120 (15.7)	2856 (11.0)	353 (12.5)
Mexican American/Other Hispanic	2005 (23.6)	194 (30.8)	3166 (14.7)	482 (20.7)
Others	880 (8.9)	115 (13.6)	2012 (8.3)	263 (10.0)
Have health insurance coverage	5302 (90.8)	449 (90.0)	9957 (82.7)	1170 (78.2) [†]
Annual household income < \$20,000	1262 (15.1)	32 (12.1)	2618 (13.8)	213 (17.0) [†]
Family history of asthma	1993 (33.8)	106 (25.5) [†]	2702 (21.2)	229 (20.2)
Body mass index (kg/m ²)	22.1 ± 0.1	21.9 ± 0.3	29.2 ± 0.1	29.2 ± 0.1
BMI z-score	0.7 ± 0.03	0.6 ± 0.1	-	-
Serum cotinine level (ng/ml)	7.4 ± 1.0	10.6 ± 4.7	56.4 ± 2.6	70.0 ± 4.2 [†]
Pack-years of cigarette smoking	-	-	7.2 ± 0.3	6.5 ± 0.6
Serum folate metabolites (nmol/L)				
Total folate	50.8 ± 0.7	50.5 ± 1.4	44.2 ± 0.5	44.3 ± 3.2
5-Methyl-tetrahydrofolate, 5-MTHF	47.3 ± 0.6	46.6 ± 1.1	40.8 ± 0.5	41.4 ± 3.0
Unmetabolized folic acid, UMFA	2.0 ± 0.1	2.4 ± 0.4	2.0 ± 0.1	1.5 ± 0.2 [†]
5-MTHF to UMFA ratio	60.5 ± 0.9	62.8 ± 2.3	54.2 ± 1.0	55.3 ± 1.5
Folate deficiency (total folate <10 nmol/L)	7 (0.1)	2 (0.4)	118 (0.8)	14 (0.9)

Results are shown as mean ± standard error (SE) for continuous variables, and as N (%) for binary variables.

*Available only in NHANES 2011-2012

[†]P<0.05 for comparison of subjects who were and were not included in the current analysis, within each age group.

Table E2- Characteristics of participants by asthma status and age group, NHANES 2011-2016

Characteristics	Children (6-19 years)		Adults (20-79 years)	
	Controls (n=5,215)	Asthma (n=718)	Controls (n=11,632)	Asthma (n=1,107)
Age (years)	12.7 ± 0.1	12.8 ± 0.1	46.3 ± 0.4	46.5 ± 0.7
Male gender	2651 (52.0)	386 (49.9)	5763 (49.8)	370 (33.7) [‡]
Race/ethnicity				
Non-Hispanic white	1363 (54.2)	182 (53.0) [‡]	4228 (65.7)	477 (70.2) [‡]
Non-Hispanic black	1247 (12.7)	256 (19.9)	2557 (10.4)	299 (13.0)
Mexican American/Other Hispanic	1809 (24.2)	196 (19.2)	2956 (15.1)	210 (10.3)
Others	796 (9.0)	84 (7.9)	1891 (8.5)	121 (6.4)
Have health insurance coverage	4627 (90.5)	675 (92.8)	9037 (82.4)	920 (85.8)
Annual household income < \$20,000	1093 (14.8)	549 (17.6)	2296 (13.1)	322 (20.4) [‡]
Family history of asthma	1505 (29.1)	488 (68.8) [‡]	2179 (18.8)	523 (45.5) [‡]
Body mass index (kg/m ²)	21.9 ± 0.1	23.2 ± 0.4 [‡]	29.0 ± 0.1	31.1 ± 0.4 [‡]
BMI z-score	0.7 ± 0.03	0.9 ± 0.1 [‡]	-	-
Serum cotinine level (ng/ml)	7.6 ± 1.1	5.5 ± 1.2	55.8 ± 2.6	62.9 ± 5.7
Pack-years of cigarette smoking	-	-	7.0 ± 0.3	8.6 ± 0.8 [†]
Serum folate metabolites (nmol/L)				
Total folate	50.9 ± 0.6	49.7 ± 1.2	44.0 ± 0.5	46.4 ± 2.4
5-Methyl-tetrahydrofolate, 5-MTHF	47.5 ± 0.6	46.0 ± 1.2	40.6 ± 0.5	42.8 ± 2.3
Unmetabolized folic acid, UMFA	1.9 ± 0.1	2.2 ± 0.3	2.0 ± 0.1	2.1 ± 0.2
5-MTHF to UMFA ratio	61.0 ± 0.9	56.5 ± 1.7 [†]	54.3 ± 1.0	53.1 ± 4.0
Folate deficiency (total folate <10 nmol/L)	6 (0.1)	1 (0.2)	102 (0.8)	16 (1.0)
Lung function measures [*]				
Percent predicted FEV ₁	104.0 ± 0.7	99.3 ± 1.3 [‡]	97.7 ± 0.4	89.7 ± 1.7 [‡]
Percent predicted FVC	105.6 ± 0.7	105.0 ± 1.4	101.3 ± 0.3	98.3 ± 1.3 [†]
Percent predicted FEV ₁ /FVC	98.1 ± 0.2	94.1 ± 0.5 [‡]	96.1 ± 0.2	90.8 ± 1.2 [‡]

Results are shown as mean ± standard error (SE) for continuous variables, and as N (%) for binary variables. BMI z-scores were calculated based on the 2000 U.S. CDC growth charts

*Available only in NHANES 2011-2012

[†]P<0.05 or [‡]P<0.01 for comparison of subjects with asthma vs. controls.

Table E3 – Serum folate metabolites and current asthma, by age group

	Children (n=5,933)	Adults (n=12,739)
Serum folate metabolites	Odds ratio (95% confidence interval),	
Total folate (nmol/L)		
Quartile 1	1.0 (reference)	1.0 (reference)
Quartile 2	1.08 (0.73, 1.62)	0.82 (0.63, 1.09)
Quartile 3	1.13 (0.80, 1.60)	1.08 (0.84, 1.40)
Quartile 4	1.16 (0.83, 1.63)	0.92 (0.72, 1.19)
5-Methyl-tetrahydrofolate, 5-MTHF (nmol/L)		
Quartile 1	1.0 (reference)	1.0 (reference)
Quartile 2	1.21 (0.79, 1.85)	0.85 (0.65, 1.12)
Quartile 3	1.09 (0.76, 1.56)	1.04 (0.82, 1.31)
Quartile 4	1.15 (0.84, 1.59)	0.90 (0.69, 1.17)
Unmetabolized folic acid, UMFA (nmol/L)		
Quartile 1	1.0 (reference)	1.0 (reference)
Quartile 2	1.12 (0.82, 1.52)	0.96 (0.73, 1.28)
Quartile 3	1.20 (0.91, 1.57)	1.22 (0.94, 1.58)
Quartile 4	1.16 (0.84, 1.60)	1.17 (0.90, 1.52)
5-MTHF to UMFA ratio		
Quartile 1	1.0 (reference)	1.0 (reference)
Quartile 2	0.72 (0.52, 0.99)*	0.87 (0.70, 1.10)
Quartile 3	0.85 (0.59, 1.24)	0.84 (0.65, 1.09)
Quartile 4	0.85 (0.62, 1.18)	0.78 (0.62, 0.98)*[†]

All models were adjusted for age, gender, race/ethnicity, annual household income, BMI z-score (children) or BMI (adults), family history of asthma, season of sampling, serum cotinine, and (in adults) pack-years of cigarette smoking.

*P<0.05; [†]P of trend = 0.05

Table E4 – Serum folate metabolites and current asthma in adults, adjusting for alcohol consumption

Serum folate metabolites	Odds ratio (95% Confidence interval)
Total folate (nmol/L)	
Quartile 1	1.0 (reference)
Quartile 2	0.85 (0.62, 1.15)
Quartile 3	1.24 (0.92, 1.66)
Quartile 4	0.94 (0.70, 1.27)
5-Methyl-tetrahydrofolate, 5-MTHF (nmol/L)	
Quartile 1	1.0 (reference)
Quartile 2	0.86 (0.64, 1.17)
Quartile 3	1.17 (0.90, 1.52)
Quartile 4	0.92 (0.68, 1.24)
Unmetabolized folic acid, UMFA (nmol/L)	
Quartile 1	1.0 (reference)
Quartile 2	0.96 (0.69, 1.34)
Quartile 3	1.31 (0.98, 1.75)
Quartile 4	1.22 (0.89, 1.67)
5-MTHF to UMFA ratio	
Quartile 1	1.0 (reference)
Quartile 2	0.88 (0.70, 1.13)
Quartile 3	0.82 (0.65, 1.08)
Quartile 4	0.78 (0.61, 1.00)^{*†}

All models were adjusted for age, gender, race/ethnicity, annual household income, BMI z-score (children) or BMI (adults), family history of asthma, season of sampling, serum cotinine, and (in adults) pack-years of cigarette smoking.

^{*}P=0.05; [†]P of trend = 0.05

Online Repository Figure Legends**Figure E1 - Folate and folic acid metabolic pathway**

Footnote: DHFR= dihydrofolate reductase; SHMT=serine hydroxy-methyltransferase; 5-FCL=5-formyltetrahydrofolate-cyclo-ligase; MTHFR=methylenetetrahydrofolate reductase

Figure E2 - Flowchart for selection of the participants included in the current analysis.**Figure E3– Spearman rank correlation coefficient (r) between serum total folate and folate metabolites**

Footnote: 5-MTHF=5-methyl-tetrahydrofolate; UMFA=unmetabolized folic acid. Two adults with extremely high serum total folate levels were excluded from the figures for presentation

Figure E4 - Percent predicted lung function measures by quartiles of 5-MTHF to UMFA ratio

Footnote: 5-MTHF=5-methyl-tetrahydrofolate; UMFA=unmetabolized folic acid.

Models adjusting for household income, BMI z-score (in children) and BMI (in adults), family history of asthma, serum level of cotinine, seasons of sampling, pack-year smoking (in adults) and current asthma status.

