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Asthma, allergy, and responses to methyl donor supplements and nutrients

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After a brief period of stabilization, recent data have shown that the prevalence of asthma and allergic diseases continues to increase. Atopic diseases are major public health problems resulting in significant disability and resource use globally. Although environmental factors influence the development of atopic disease, dietary changes might partially explain the high burden of atopic disease. Potential mechanisms through which diet is suspected to effect asthma and allergy susceptibility are through epigenetic changes, including DNA methylation. Dietary methyl donors are important in the one-carbon metabolic pathway that is essential for DNA methylation. Findings from both observational studies and interventional trials of dietary methyl donor supplementation on the development and treatment of asthma and allergy have produced mixed results. Although issues related to the differences in study design partially explain the heterogeneous results, 2 other issues have been largely overlooked in these studies. First, these nutrients affect one of many pathways and occur in many of the same foods. Second, it is now becoming clear that the human intestinal microbiome is involved in the metabolism and production of the B vitamins and other methyl donor nutrients. Future studies will need to account for both the interrelationships between these nutrients and the effects of the microbiome. (J Allergy Clin Immunol 2014;133:1246-54.)

Key words: *Allergy, asthma, betaine, choline, vitamin B2, vitamin B6, vitamin B12, folate, methyl donor, microbiome*

Abbreviations used
LINE-1: Long interspersed element-1
MTHFR: Methylenetetrahydrofolate reductase
SAM: S-adenosyl-methionine

Asthma and allergic diseases, including allergic rhinitis and atopic dermatitis, continue to be major public health problems resulting in significant disability and resource use globally.[1,2] Although the trend in asthma prevalence in the United States had initially stabilized, since 2003, the prevalence of asthma and allergic disease in the United States and abroad has been increasing.[3-7] There is substantial evidence for the genetic predisposition to complex diseases, such as asthma and allergy, with multiple genetic loci demonstrating reproducible associations with these diseases, but genetics alone is unable to explain the overall burden of atopic disease.

Although the cause of the increasing prevalence is not definitively known, several factors have been proposed to account for this increase, including environmental causes.[8,9] Environmental exposures, such as tobacco smoke, have been shown to influence the incidence of both asthma and allergy in children[10-12] and adults.[13,14] Other environmental exposures, including traffic-related air pollution, have also shown modest associations with atopic diseases in children.[15-17] However, even the contribution of these environmental exposures to asthma and allergy susceptibility is unlikely to fully explain the increasing prevalence of asthma and allergic diseases worldwide.

Epidemiologic data suggest that changes in dietary patterns might also partially explain the increasing prevalence of asthma and allergy in industrialized countries.[18-20] However, the role of certain dietary nutrients in the development of atopic diseases has been conflicting in part because of the fact that the role of diet in the development of atopy is complex, difficult to measure, and limited by a dearth of interventional trials investigating the effect of diet on atopic disease susceptibility.

METHYL DONORS

One of the proposed mechanisms by which diet is suspected to affect asthma and allergy susceptibility is through ***epigenetic*** mechanisms, including DNA ***methylation***, which results in heritable changes in gene expression without alteration of the original DNA sequence.[21] Methylation of CpG islands within regulatory regions of DNA is an important mechanism for the control of gene expression and has been shown to be transmissible across generations.[22] DNA methylation results from the transfer of methyl groups to the cytosine in the CpG dinucleotide, resulting in a reversible modification that can alter both chromosome stability and suppress gene transcription. Environmental exposures, including air pollution and dietary factors, have been shown to

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Terms in boldface and italics are defined in the glossary on page 1247.

influence the epigenome.[23] Furthermore, recent evidence from the ***Normative Aging Study*** suggests that prior allergen sensitization is associated with increased methylation of the retrotransposon-derived element Alu, which is a proxy for global DNA methylation ($\beta = 0.32$ for sensitized vs nonsensitized patients, $P = .003$), even after adjustment for smoking status and air pollutants.[24]

Dietary methyl donors, including folate and choline, are necessary for the one-carbon metabolic pathway that produces S-adenosyl-methionine (SAM), which is the universal methyl donor that is essential for the DNA methylation process to occur (Fig 1). Vitamin B12 and betaine, which are additional agents derived from the diet, are also necessary for methionine synthesis, which is another important step in DNA methylation. Therefore differential intake of these nutrients might lead to differences in DNA methylation and ultimately alter gene expression. In an agouti mouse model, mothers receiving a diet supplemented with methyl donors resulted in hypermethylation of the ***metastable epiallele*** in the progeny, resulting in a brown coat color, lower incidence of obesity, and increased longevity.[25] In a mouse model of allergic airway disease, *in utero* supplementation of a diet that is high in methyl donors (including folic acid, choline, and vitamin B12) resulted in increased atopy (IgE and eosinophils) and airway responsiveness in the exposed progeny.[26] In addition, *in utero* exposure to a high methyl donor diet was associated with increased methylation of the Runt-related transcription factor (*Runx3*) gene in the progeny, which resulted in decreased expression of the gene. *Runx3* is known to negatively regulate allergic airway disease, suggesting that the methylation changes induced by a diet high in methyl donors might result in increased atopic diseases.[26] Given the emerging evidence from animal models demonstrating a role for dietary methyl donors in the development of atopic diseases, dietary methyl donors have been investigated in human subjects as a possible cause of the increasing prevalence of atopic disease.

In human subjects dietary methyl groups, such as folate, vitamin B12, and choline, are a source of methyl donors for DNA methylation (Table I). Methyl donors, especially folate, have been extensively studied in the setting of asthma and, to a lesser degree, in the setting of allergy. However, the data for the association of dietary methyl donors with the development of asthma and allergy in human subjects remain unclear. In this review we examine the existing evidence for the role of methyl donors in the development and treatment of asthma and allergy. We have limited this review to articles that have examined specific nutrients rather than foods or diets that contain the nutrient. Food intake and dietary patterns and asthma and allergies have recently been reviewed, and the readers are directed to those reviews.[18,20,27,28]

Folate

Folate is a water-soluble B vitamin that is naturally present in a variety of foods and available in nutritional supplements. It is naturally occurring in the diet as folate, and folic acid is a fully oxidized monoglutamate form that is used as a dietary supplement.[29] Folic acid consists of a p-aminobenzoic molecule linked to a pteridine ring and a single molecule of glutamic acid. Dietary folates are polyglutamates, which include additional glutamate residues.[30] Folate is found naturally in foods, including dark leafy vegetables, fruits, nuts, beans, poultry, meat, eggs, seafood, and dairy products. In addition, it is found (in folic acid form) in fortified food products, including breads, cereals, and grain products. However, dietary supplements, including multivitamins, are the

GLOSSARY

EPIGENETIC: Epigenetic changes are those that are heritable but not encoded by DNA sequence changes. Epigenetic changes include chromatin folding, attachment to the nuclear matrix, and histone modifications, including methylation and acetylation.

IL-4, IL-5, TNF-α: IL-4 has a myriad of allergy-promoting effects, including increasing IgE production. Epigenetic changes, such as hypomethylation of the IL-4 promoter in animal models by using diesel exhaust particles, are associated with increased IgE levels. The inhibition of histone deacetylases upregulates IL-5 production, and asthmatic patients have increased levels of histone acetyltransferase that can be improved after inhaled glucocorticoids. Similarly, TNF-α–induced expression of eotaxin occurs through histone acetylation.

METABOLOMICS: A rapid high-throughput characterization of metabolites found in an organism that might reflect genotype-environment interactions. There are at least 2900 metabolites detectable in the human body, and these can be specific to certain body fluids, such as urine, serum, and cerebrospinal fluid.

METASTABLE EPIALLELE: Metastable epialleles are gene alleles that are variably expressed in genetically identical subjects because of epigenetic changes that occur during development (eg, in the fetus) and that are vulnerable to environmental influences.

METHYLATION: DNA methylation normally occurs at CpG dinucleotides and prevents transcription factors from binding to DNA. Methylation also recruits methyl-CpG binding proteins that repress chromatin remodeling. Therefore hypomethylated DNA is thus more readily transcribed. Methylation can also occur on histones (eg, on lysine residues). Although this modification does not directly affect chromatin folding, it provides a binding surface for a variety of histone-modifying enzymes that can alter chromatin to active and inactive states. These enzymes include histone acetyltransferases, deacetylases, methylases, and ATP-dependent chromatin remodeling enzymes.

MICROBIOME: The human microbiome is the collection of microbes, including bacteria and viruses, that are found in the human body. These bacteria are important in both health and disease. These bacteria are involved in our ability to digest food, produce vitamins, and affect our immune system. The community of microbes present in a subject can increase susceptibility to disease. Therefore there is great interest in studying the effect of the microbiome in terms of its relationship to health and disease.

NORMATIVE AGING STUDY: The Normative Aging Study (NAS) is a longitudinal study that has looked at the effects of aging on a number of variables, including cardiovascular disease, obesity, depression, and pollution. The NAS was instituted by the Veterans Administration in 1963 to follow 2280 male subjects with no known chronic medical issues. Participants are examined every 3 to 5 years.

PLATELET-ACTIVATING FACTOR: Platelet-activating factor is a mediator of anaphylaxis. Blood levels of platelet-activating factor are increased after anaphylaxis, and these levels correlate with the severity of the anaphylactic reaction.

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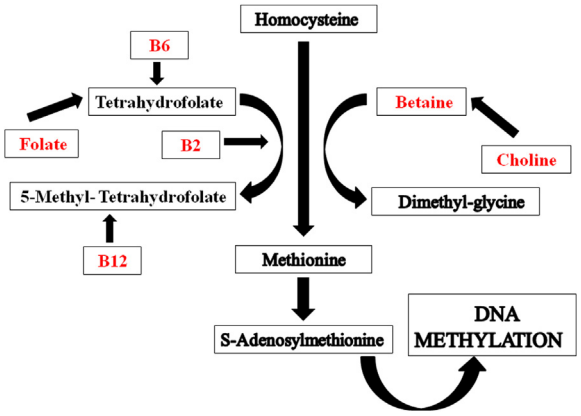

FIG 1. Simplified diagram of the one-carbon metabolic pathway. The figure demonstrates where dietary methyl donors, identified in *red*, are involved in the one-carbon metabolic pathway and how they influence DNA methylation. DNA methylation depends on the availability of methyl groups from S-adenosyl-methionine, which is derived from methionine. This figure highlights the connection between folate, choline, methionine, betaine, and vitamins B2, B6, and B12 on DNA methylation. The pathways intersect at the formation of methionine from homocysteine.

major source of folic acid in human subjects. Unlike naturally occurring folate, which is hydrolyzed in the intestines and reduced to tetrahydrofolate on absorption, folic acid requires reduction and methylation in the liver through dihydrofolate reductase. It should be noted that controversy exists regarding how best to measure folate status in human subjects,[31-33] but this is beyond the scope of the current review.

Folate is necessary for many biologic processes, including purine and pyrimidine synthesis and amino acid production. Folate is also needed for the methylation of deoxyuridylate to thymidylate in the formation of DNA, which is required for normal cell division. Folate in the form of 5-methyl-tetrahydrofolate provides the methyl groups necessary for the formation of SAM, which is the universal methyl donor required for DNA methylation. In addition to its role in DNA methylation, folate can have antioxidant effects, which is relevant to asthma and allergies.[34,35] The use of folic acid supplements before and during the first trimester of pregnancy reduces the risk of neural tube defects and other congenital anomalies and therefore is recommended as part of public health guidelines worldwide.[36-39] Because asthma and allergies develop in early childhood, prenatal exposures are thought to play a role in the development of these disorders, and folate intake has been studied as a potential factor.

The association between folate and asthma was recently extensively reviewed by Blatter et al.[40] With regard to the question of prenatal folate intake, folic acid supplementation, or maternal folate status and the development of asthma and allergies in children, a few studies showed increased risks of early wheeze and lower respiratory tract illnesses,[41] asthma,[42,43] and eczema (but not asthma).[44] However, the bulk of birth cohort studies have shown no effect of folate intake or folic acid supplement use at various points in pregnancy[45-51] and asthma or allergies. We agree with Blatter et al[40] that given the weight of the evidence against a moderate to strong effect of maternal folate status on asthma and allergies, there is no reason to change the current recommendations regarding prenatal folic acid supplementation.

The effect of maternal folate intake on DNA methylation and the development of atopic disease in offspring is a topic of recent interest.[52] In a study of 24 pregnant women, maternal folic acid intake during pregnancy and cord blood folate levels were correlated with cord blood long interspersed element-1 (LINE-1) methylation, a measure of global DNA methylation.[53] Furthermore, among 107 pregnant Korean women, serum folate levels were significantly associated with percentage methylation of the placenta.[54] Additionally, periconceptional use of folic acid supplements was associated with higher methylation of insulin-like growth factor II *(IGF2)* in young children.[55] However, in a prospective cohort study of 534 pregnant women participating in Project Viva, there was no significant association found between maternal dietary intake of folate, as assessed by using a questionnaire, in both the first and second trimesters of pregnancy with cord blood LINE-1 methylation.[56] The discrepancy in these results might in part be due to relatively small sample sizes in the studies and in the assessment of DNA methylation. Studies using newer and more comprehensive methods[57,58] of assessing DNA methylation (using genome-wide techniques) are underway, and use of these methods together with folate intake assessment can better answer the question of whether DNA methylation of specific regions in the genome might modify the risk of asthma and allergies.

Postnatal studies of the association of folate and asthma have also resulted in mixed findings. Two studies have shown an increased risk with folate. In a small study of 138 at-risk children, those with higher folate levels in early childhood (<6 years old) had increased risks of sensitization in later childhood to foods and aeroallergens compared with children who had lower levels.[59] However, there were no associations of increased folate levels with asthma or wheezing. In a large cross-sectional study of 1601 Australian adults, dietary intake of folate was significantly associated with asthma but not with markers of airway hyperresponsiveness or atopy.[60] Three large cross-sectional studies have found a protective effect. In a study of 6784 Danish adults, higher serum folate levels were found to be associated with lower risk for an asthma diagnosis, but no associations were found with lung function or atopy.[61] On the other hand, in the US National Health and Nutrition Examination Survey cohort (both children and adults), higher serum folate levels were associated with decreased risks for wheeze, total serum IgE, and atopy, but no association was noted for physician-diagnosed asthma.[62] In a study of 1030 English adults, dietary folate intake was associated with a lower risk for physician-diagnosed asthma.[63] Finally, 4 studies have found no associations between either serum folate levels[34,64,65] or folate intake[66] with asthma or allergies.

As can be seen above, results of postnatal studies of folate and asthma and allergy have been inconsistent. Furthermore, randomized controlled trials investigating folic acid supplementation on asthma and allergy susceptibility are lacking. We agree with the conclusion of Blatter et al[40] that there are insufficient data to exclude an effect of folate on either asthma and allergy development or modification of disease course. Reasons for the heterogeneous results of these studies might include differences in study design and analytic approach, the difficulty of folate status measurements, timing of folate supplementation, confounding because of other exposures, sample size, age, differences in the phenotypic assessment of allergy and atopy, or differences in genetic predisposition. In addition to these study design considerations, Fig 1 shows that other methyl donor nutrients are

TABLE I. Methyl donor sources, function, and mechanisms of action

Methyl donor	Dietary sources*	Function	Mechanism of action in methylation reactions
Folate	Green leafy vegetables, fruits, nuts, beans, grains, fortified cereals, eggs, dairy	Purine and pyrimidine production, amino acid production, cell division	Production of SAM
Choline	Soybeans, egg, peanuts, cauliflower, lentils, flax seeds	Precursor to phosphatidylcholine and sphingomyelin; aids in movement of lipids into and out of cells	Production of SAM
Vitamin B12	Fish, meat, poultry, eggs, dairy products	Red blood cell formation, DNA synthesis, neurologic function	Methionine synthesis
Vitamin B2 (riboflavin)	Eggs, meat, milk, cheese	Energy production, antioxidant, red blood cell production	Methionine production
Vitamin B6 (pyridoxine)	Fish, beef liver, fruits, vegetables, grains	Homocysteine metabolism, metabolism of fats and protein, nervous system development	Folate metabolism
Betaine	Beets, broccoli, spinach, seafood, grains	Homocysteine metabolism, immune modulation	Methionine production

*Dietary sources other than nutritional supplements or fortified foods.

involved in the pathway leading to DNA methylation. Thus perturbations in folate intake might not be sufficient to derail the pathway unless there are also changes to the levels of the other methyl donor nutrients. Although some of the reports reviewed above[47,50,51,60,61,63,66] investigated other nutrients or foods, these were studied individually, and no consideration was given to the relationships between each of these nutrients. Although studies of diet patterns[67,68] address this issue to a certain extent, pathway- or systems-based analyses[69] might help dissect the complex interrelationships between individual nutrients.

Studies investigating the association of genetic variants in genes in the folate metabolic pathway with asthma and allergy have been limited to date. The vast majority of studies investigating the role of genetic associations have been performed on genetic variants in the methylenetetrahydrofolate reductase *(MTHFR)* gene because of the association of its common variant, C677T, with reduced enzymatic function of the gene.[70] The results of studies investigating genetic associations of the C677T *MTHFR* genotype with asthma and atopy have been variable. In a cross-sectional study of 1671 subjects from Denmark, the *MTHFR* genotype was associated with specific IgE levels.[71] However, in a population-based birth cohort that included genotypic information on 7356 mothers and 5364 children from the Avon Longitudinal Study of Parents and Children, there was no genetic association of the *MTHFR* C677T genotype with atopy, allergy, or asthma in the mothers or children.[46] In addition, a smaller population-based study of 1189 adults from Denmark also showed no association of the *MTHFR* polymorphism with allergy (IgE and skin tests) or asthma.[72] Interestingly, in a population-based prospective cohort study of 2001 children that investigated the interaction between *MTHFR* genetic variants and methyl donor levels on asthma and eczema, a significant interaction of the *MTHFR* C677T mutation and higher folate levels with an increased risk of eczema was identified.[73] Although there might be a difference in genetic predisposition that underlies the association of methyl donors with asthma and allergy disease susceptibility, these results suggest that there might also be complex gene-environment or gene-gene interactions that have yet to be fully elucidated. Furthermore, genetic studies investigating other genes in this pathway might help elucidate the genetic predisposition to allergy and asthma.

An additional reason for the heterogeneous effects of folate might be differences in intestinal microbiota in the study populations. It has been shown that bacteria commonly found in human large intestines can produce folate,[74] and although folate is mainly absorbed in the small intestines, absorption also occurs in the large intestines.[75] Several strains of bacteria from the genus *Bifidobacteria* have been shown to produce folate both *in vitro*[76] and *in vivo*.[77] Bacteria from the genus *Lactobacillus* generally need folate for growth, but one strain, *Lactobacillus plantarum*, has been shown to be a folate producer.[78] It is easy to see how folate production by intestinal microbiota can modify the effects of folate ingested in the diet, and this has already been considered in studies of diet and colon cancer risk.[78,79] Although there is keen interest in the gut ***microbiome*** and asthma and allergy risk,[80,81] these studies are in their infancy, and future studies in cohorts that are collecting both diet and microbiome data will be needed to investigate this link.

Choline

Choline is a trimethylated water-soluble B vitamin that is important in many biologic functions.[82] Although it is synthesized in small quantities by the liver, choline is present in many foods and is a component of nutritional supplements and multivitamins. Choline is naturally found in many food sources, including soybeans, egg, peanuts, cauliflower, lentils, and flax seeds.[83] Choline is a precursor of phosphatidylcholine and sphingomyelin, which are phospholipids that are components of biologic membranes and are involved in maintaining cell structure. Choline has also been shown to aid in the movement of lipids in and out of cells.[84,85] In addition, it is a precursor to ***platelet-activating factor*** and acetylcholine. Choline can be oxidized into betaine, which provides the methyl groups needed to resynthesize methionine from homocysteine, thereby providing methionine for methylation reactions.[86] This pathway is an alternative to one that uses the cofactor 5-methyltetrahydrofolate (Fig 1).

Animal models have demonstrated the anti-inflammatory properties of choline supplementation in patients with inflammatory arthritis.[87] In addition, an *in vitro* model showed that choline deficiency resulted in the loss of membrane phosphatidylcholine and the induction of apoptosis.[88] Mice fed a choline-deficient diet showed altered antioxidant properties and an increase in oxidative stress.[89] Furthermore, dietary choline supplementation resulted in decreased reactive oxygen species and a suppressed inflammatory response to ovalbumin challenge in a murine model of allergic airway disease.[90]

Human data for the association of choline with the development of asthma and allergy is limited (Table II). A small study of

TABLE II. Results of studies investigating the role of nonfolate methyl donors in the primary prevention or treatment of asthma and allergy

Methyl donor	Potential mechanism of action	Observational studies*	RCTs
Choline	Animal models demonstrate anti-inflammatory and anti-oxidant properties of choline	No observational studies	No primary prevention studies
			RCT of asthmatic patients showed no improvement in asthma and allergy symptoms or lung function (n = 23).[91]
			RCT of asthmatic patients showed choline supplementation resulted in decreased asthma symptom scores, increased symptom-free days, and decreased medication use.[93]
			RCT of asthmatic patients showed choline supplementation resulted in improved quality of life, decreased airway responsiveness, and decreased inflammatory markers.[92]
Vitamin B12	A cofactor for folate; provides methyl groups for synthesis of methionine and S-adenosyl-methionine	Maternal serum vitamin B12 was associated with increased atopic dermatitis in a birth cohort (n = 8742).[44]	No RCTs
		Japanese birth cohort showed maternal vitamin B12 intake during pregnancy was not associated with wheeze or eczema (n = 763).[50]	
		Serum vitamin B12 level and dietary intake was not associated with asthma or atopy in an adult cohort study (n = 6784).[61]	
Vitamin B2	Important in methionine synthesis and its role as a methyl donor	Japanese birth cohort showed maternal vitamin B2 intake during pregnancy was not associated with wheeze or eczema (n = 763).[50]	No RCTs
		Riboflavin intake was associated with reduced risk of allergy (n = 195).[103]	
		Adult cohort study showed no association with vitamin B2 with prevalence of allergy (n = 3872).[104]	
		Cross-sectional study of adult subjects with and without atopy showed no association of vitamin B2 with atopy (n = 1671).[71]	
Vitamin B6	Important in methionine synthesis and its role as a methyl donor	Japanese birth cohort showed maternal vitamin B6 intake during pregnancy was not associated with wheeze or eczema (n = 763).[50]	RCT of asthmatic patients showed that pyridoxine supplementation was not associated with asthma symptoms or lung function (n = 31).[107]
		Cross-sectional study of adult subjects with and without atopy showed no association of vitamin B2 with atopy (n = 1671).[71]	RCT of adult asthmatic patients showed vitamin B6 levels were associated with decreased asthma symptoms and exacerbation rates (n = 15).[108]
			RCT of childhood asthmatic patients treated with pyridoxine showed significant improvement in use of bronchodilators and cortisone use (n = 76).[109]
Betaine	Important in methionine synthesis and its role as a methyl donor; involved in global methylation	No observational studies	No RCTs

RCT, Randomized controlled trial.
*Observational studies include both birth cohort and cross-sectional studies.

23 asthmatic patients with documented aspirin sensitivity treated with choline magnesium trisalicylate demonstrated the safety of choline supplementation but did not result in improvement in pulmonary function, nasal congestion, or rhinorrhea.[91] However, studies of the treatment of asthmatic patients with tricholine citrate demonstrated symptomatic improvement in their asthma, with decreased symptom scores, increased symptom-free days, and decreased rescue medication use.[92,93] Furthermore, subjects treated with high-dose choline were also noted to have improvements in lung function[92] and had decreased airway

responsiveness.[93] In a treatment trial of 76 asthmatic patients treated with choline or standard pharmacotherapy, choline supplementation resulted in improved quality of life, decreased asthma medication use, improvements in airway responsiveness, and significantly reduced markers of inflammation, including ***IL-4***, ***IL-5***, and ***TNF-α***.[94] Interestingly, in a case-control study investigating the metabolomic signature of the serum of asthmatic patients compared with nonasthmatic control subjects, choline levels were significantly less in asthmatic subjects.[95] In addition, this metabolomic profile was associated with decreased lung function, including FEV_1 percent predicted,[95] demonstrating a possible role for choline in the pathogenesis of asthma.

More studies are needed to elucidate the effects of choline in the development of asthma and allergies. As with folate, there appears to be a role for the intestinal microbiome in mediating the effects of dietary choline. Through ***metabolomics*** studies, the role of gut microbiome metabolism of dietary choline in atherosclerosis has been elucidated. Gut microbiome metabolism of choline and phosphatidylcholine lead to the formation of trimethylamine N-oxide,[96,97] which was recently shown to increase the risk of major cardiovascular events, such as death, myocardial infarction, or stroke.[98] The role of trimethylamine N-oxide in patients with asthma and allergies is unknown; however, the metabolomic study[95] cited above linking lower choline levels in serum with asthma suggests that this line of research should be investigated further.

Vitamin B12

Vitamin B12 is a water-soluble vitamin that plays a key role in one-carbon metabolism. Vitamin B12 is naturally found in several foods, including fish, meat, poultry, eggs, and dairy products. Vitamin B12 is also found in fortified foods, including cereals and grains, as well as in dietary supplements.[29] Vitamin B12 exists in several forms and contains the mineral cobalt, resulting in the name "cobalamins." Methylcobalamin and 5-deoxyadenosylcobalamin are the forms of vitamin B12 that are important in human metabolism.[99] Vitamin B12 is required for red blood cell formation, DNA synthesis, and proper neurologic function.[100] Folate metabolism is influenced by vitamin B12 levels.[101] Vitamin B12 acts as a cofactor for folate and provides methyl groups for the synthesis of methionine and its derivate, SAM, which is a universal methyl donor for multiple substrates: DNA, RNA, proteins, lipids, and hormones. Vitamin B12 deficiency results in decreased methylation of deoxyuridylic acid to deoxythymidylic acid, leading to an excessive incorporation of uracil into DNA, as well as DNA hypomethylation.[102] Animal models have shown that increased vitamin B12 levels are associated with an increased risk of allergic airway disease that is mediated through epigenetic mechanisms, including changes in DNA methylation.[26]

The results of studies investigating vitamin B12 intake and the development of atopy in human subjects have been variable. In a single birth cohort maternal serum vitamin B12 concentrations during pregnancy were significantly associated with an overall increased prevalence of atopic dermatitis in children.[44] However, a study of 763 asthmatic children in Japan showed that maternal vitamin B12 intake during pregnancy was not associated with the development of wheeze or eczema.[50] In addition, serum levels and dietary intake of vitamin B12 were not associated with asthma and atopy in a large adult cohort study.[61] These studies have been limited by small sample sizes and a lack of interventional studies. Therefore the role of vitamin B12 in the development of asthma and allergy remains unclear.

Vitamins B2 and B6

Vitamin B2, or riboflavin, is required for several cellular processes. There are a variety of dietary sources of vitamin B2, including eggs, meat, milk, and cheese. In addition, it is found in many fortified foods, including cereals and rice, as well as in nutritional supplements, including multivitamins. Vitamin B6 is a water-soluble vitamin that occurs naturally in many foods. Vitamin B6 is the generic name for 6 compounds with vitamin B6 activity: pyridoxine, pyridoxal, pyridoxamine, and their respective 5′-phosphate esters. The vitamin B6 compounds occur naturally in fish, beef liver, fruits, vegetables, and grains.[29] In the United States most adults obtain the majority of their vitamin B6 from fortified foods, including cereals, beef, and poultry. Furthermore, vitamin B6 is found in multivitamins and supplements containing other B complex vitamins. Vitamins B2 and B6 perform a wide variety of biologic functions, including assisting in the metabolic pathway of one-carbon compounds (Fig 1).

Although the data for the role of vitamins B2 and B6 in asthma pathogenesis are limited, several small studies have investigated the role of vitamins B2 and B6 on the risk of atopy. In a study investigating maternal vitamin B intake during pregnancy and the subsequent risk of wheeze and eczema in infants, Miyake et al[50] demonstrated no evidence of association of maternal vitamin B2 or B6 intake with the subsequent risk of wheeze or eczema up to 2 years of age in Japanese children. In a small cross-sectional study evaluating the prevalence of allergy in schoolchildren in Vietnam, vitamin B2 intake was found to be associated with a reduced risk of allergy ($P = .038$).[103] Using the European Respiratory Health Survey data to determine the role of dietary intake on the prevalence of allergic sensitization, Heinrich et al[104] showed no association of vitamin B2 intake and the prevalence of allergy. Finally, in a cross-sectional population-based study of 1671 subjects with and without atopy in Denmark, dietary intake of vitamins B2 and B6 was not significantly associated with atopy.[71] However, there was noted to be at least a borderline interaction between *MTHFR* genotype (C677T) and the dietary intake of vitamins B2 and B6 with a decreased risk of atopy.[71]

Although there are data to suggest that vitamin B6 levels are associated with homocysteine metabolism[105] and that increased dietary intake of vitamin B6 results in decreased plasma homocysteine levels,[106] its role in asthma and allergy is not yet clear. A few studies have investigated these vitamins in patients with established asthma. In a small study of 31 patients with asthma participating in a double-blind trial, vitamin B6 supplementation in those with steroid-dependent asthma was not associated with improvement in lung function, asthma symptom scores, or 24-hour urinary 5-hydroxyindoleacetic acid levels.[107] However, in a different study of 15 adult asthmatic patients, erythrocyte pyridoxal phosphate concentrations were significantly lower in asthmatic patients compared with those seen in nonasthmatic control subjects.[108] Furthermore, supplementation with vitamin B6 in these subjects was associated with decreased asthma symptoms and decreased exacerbation rates.[108] In a double-blind study of 76 asthmatic children, supplementation with vitamin B6 was associated with improvement in asthma symptoms, resulting in a reduction in the dosage of asthma-treatment medications.[109]

Although definitive clinical trials are lacking, a review of the current literature only supports a limited effect of vitamins B2 and B6 on atopic disease susceptibility and disease treatment because of the discrepancy of the results obtained to date. Some of the variability in the results of these studies might be due to study design, sample size, analytic approach, the difficulty of B vitamin status assessment, and age.

Betaine

Betaine is a water-soluble molecule that is involved in a variety of biologic processes, including DNA methylation. Betaine is found in a variety of food sources, including beets, broccoli, spinach, seafood, and grains. In addition, it is found in small quantities in nutritional supplements and multivitamins.[110] It is essential in one-carbon metabolism, playing an essential role in the transition from homocysteine to methionine. Studies investigating the role of betaine in the development of asthma and allergy are lacking. Furthermore, studies investigating the role of betaine in DNA methylation in human subjects are limited. In a large cohort study of 534 pregnant women in Project Viva, there was no significant association found between maternal dietary intake of betaine in both the first and second trimesters of pregnancy with cord blood LINE-1 methylation.[56] However, periconceptional intake of betaine was inversely associated with cord blood methylation, but the effect of betaine on methylation was attenuated after adjustment for cadmium intake.[56] Given its importance in one-carbon metabolism, studies investigating the role of dietary betaine intake in the development of asthma and allergy are warranted.

SUMMARY AND FUTURE DIRECTIONS

Dietary changes are a proposed mechanism underlying the increasing prevalence of asthma and allergic disease worldwide. Several observational studies and a few small interventional trials have attempted to assess the role of dietary methyl donors in the development and treatment of asthma and allergy. Because of the interest in the early origins of asthma and allergic diseases and because of recommendations for folic acid supplementation in pregnancy for the prevention of neural tube defects, folate intake has been investigated as a potentially modifiable exposure in pregnancy. However, the results of the studies investigating prenatal folate intake or folate status published to date have been mixed, limiting any definitive conclusions on the role of prenatal folate with regard to its association with asthma and allergy. Studies investigating postnatal folate intake or status in childhood or adulthood have also been mixed, and studies of other dietary methyl donors have been limited.

Although more studies are needed to allow for more definitive conclusions regarding the effect of these nutrients on asthma and allergies, appropriate attention to study design is essential for future investigations. In addition, 2 other issues need to be considered in either analyzing existing data sets or designing new studies: broader approaches, such as pathway or systems analyses, and incorporation of the effects of the intestinal microbiome. Few of the studies reviewed here have investigated more than 1 nutrient. Given that these nutrients are intricately related in pathways (Fig 1), future studies need to include analyses of multiple nutrients. Furthermore, given the complexities of the one-carbon metabolic pathway, analyses, including a systems-based approach or pathway analyses, might further our understanding of the role of dietary methyl donors in the development of atopic disease.[69]

Finally, the role of the microbiome in health is gaining wider recognition. The intestinal microbiome has been shown to be actively involved in the production and metabolism of these nutrients. As reviewed, several strains of the genus *Bifidobacteria* have the ability to produce folate. The microbiome also has a role in either the production or metabolism of other B vitamins (reviewed by LeBlanc et al[74]). B vitamin production by intestinal bacteria is mediating or modifying the effects of ingested nutrients and might partly explain the heterogeneity in the results of the studies investigating these nutrients to date. The design of future studies will need to incorporate analyses of the intestinal microbiome to fully understand the effects of these nutrients on human health in general and in the development of asthma and allergies in particular. For example, in the ongoing Vitamin D Antenatal Asthma Reduction Trial (U01 HL091528 and ClinicalTrials.gov no. NCT00920621), we are collecting both prenatal and postnatal dietary information (including vitamins other than vitamin D) and stool samples (R01HL108818) to enable such studies. Integration of these types of data sets (ie, dietary data and microbiome data) will lead to novel insights into the biologic basis of health and disease.[111,112]

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