Diet and asthma: vitamins and methyl donors

Yueh-Ying Han, Josh Blatter*, John M Brehm, Erick Forno, Augusto A Litonjua, Juan C Celedón*

Diet changes can partly explain the high burden of asthma in industrialised nations. Findings from experimental studies have stimulated many observational studies of the association between vitamins (A, C, D, and E) or nutrients acting as methyl donors (folate, vitamin B_{12}, and choline) and asthma. However, observational studies are susceptible to several sources of bias; well conducted randomised controlled trials (RCTs) are the gold standard to establish whether diet has an effect on asthma. Evidence from observational studies and a few RCTs strongly justifies ongoing and future RCTs in three areas: vitamin D for the prevention or treatment of asthma, choline supplementation as adjuvant treatment for asthma, and vitamin E to prevent the detrimental effects of air pollution in patients with asthma. At present, insufficient evidence exists to recommend supplementation with any vitamin or nutrient acting as a methyl donor to prevent or treat asthma.

Lancet Respir Med 2013; 1: 813–22

Published Online July 31, 2013 http://dx.doi.org/10.1016/S2213-2600(13)70126-7

*Authors contributed equally to the manuscript

Division of Pediatric Pulmonary Medicine, Allergy, and Immunology, Children's Hospital of Pittsburgh of UPMC, Department of Pediatrics, University of Pittsburgh, Pittsburgh, PA, USA (Y-Y Han PhD, J Blatter MD, J M Brehm MD, E Forno MD, Prof J C Celedon MD)**; and Channing Division of Network Medicine and Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA** (A A Litonjua MD)

Correspondence to: Prof Juan C Celedón, Children's Hospital of Pittsburgh of UPMC, 4401 Penn Avenue, Suite 9130, Rangos Building, Pittsburgh, PA 15224, USA **juan.celedon@chp.edu**

Introduction

Asthma is a major public health problem in the USA[1] and worldwide.[2] Whereas the prevalence of asthma in industrialised nations has reached a relative plateau (after a sharp rise from the 1960s to the 1990s), asthma prevalence has continued to increase in many non-industrialised countries.[1,2] The causes of this global asthma epidemic are largely unidentified; they are probably multifactorial and might include changes in diet—eg, decreased consumption of fruits and vegetables and increased intake of refined grains, red meats, and saturated fats.[3]

In this Review we examine evidence of a link between two groups of dietary components that have been extensively studied in connection with asthma: vitamins (A, C, D, and E) and nutrients acting as methyl donors (folate, vitamin B_{12}, and choline). In view of the fact that atopy (allergic sensitisation) is common in individuals with asthma, particularly children, we also review findings for atopy or atopic diseases (eg, atopic dermatitis) where applicable.

Vitamins with antioxidant properties and asthma

Vitamin A

Oxidative stress, resulting from an imbalance between reactive oxidant species and antioxidants, can lead to tissue damage, airway inflammation, abnormal immune responses,[4,5] and increased asthma severity.[6] Antioxidant defences include endogenous antioxidant enzymes and exogenous agents from the diet. Serum concentrations of antioxidants have been positively associated with forced expiratory volume in 1 s (FEV_1) in people with and without asthma.[7,8]

Key messages

- Inadequate evidence exists to recommend nutritional supplementation with any specific vitamin or methyl donor to prevent or treat asthma
- Evidence supports undertaking of randomised controlled trials for: vitamin D to prevent or treat asthma; choline as adjuvant treatment for asthma; and vitamin E to ameliorate the negative effects of air pollution on asthma

Vitamin A contributes to key biological processes in human beings, including growth, vision, epithelial differentiation, reproduction, and immune responses.[9] The two dietary sources of this vitamin are pre-formed vitamin A (retinol) and pro-vitamin A (carotenoids).[10] Dietary intake of retinol comes from animal sources (eg, whole milk, liver, and eggs) and fortified foods. Orange–yellow fruits and vegetables (eg, carrots) are the main dietary sources of carotenoids, including α-carotene, β-carotene, and β-cryptoxanthins.

Oxidative stress might exacerbate asthma by increasing the release of pro-inflammatory cytokines, airway inflammation, and airway responsiveness.[4,5,11] Vitamin A might improve prevention or treatment of asthma by downregulation of oxidative stress[12] or via direct effects on the immune system.[13] For example, vitamin A downregulates T-helper (Th)2 (pro-allergic) immune responses by increasing proliferation of T-regulatory cells[14,15] and inhibiting Th17 (pro-inflammatory) cells.[16] However, vitamin A also enhances Th2 immune responses (eg, interleukin 4 expression).[17] Carotenoids might promote balanced Th1 or Th2 immune responses,[18] and lycopene supplementation reduces allergic airway inflammation,[19] decreases concentrations of circulating interleukin 4 and interleukin 5,[19] and increases interferon-γ expression[20] in murine models.

In 2009, Allen and colleagues[21] undertook a systematic review and (when methodologically appropriate) a meta-analysis of 21 observational studies of dietary intake or serum concentration of vitamin A (or its components) and asthma outcomes in children and adults (not including studies of prenatal diet or prenatal antioxidant levels). Self-reported dietary intake of vitamin A or its components (retinol and carotenoids) was inversely associated with asthma (difference for comparison of cases *vs* controls–182 μg per day, 95% CI –288·4 to –75·3) and asthma severity (difference for comparison of severe *vs* mild disease –344 μg per day, 95% CI –575·2 to –112·6). However, dietary intake of vitamin A was not significantly associated with wheeze or airway responsiveness.[21]

In 2010, Nurmatov and colleagues[22] examined pre-natal and post-natal dietary intake or serum concentration of

vitamin A and its components as part of a systematic review and meta-analysis of observational studies of diet or nutrients for the primary prevention of asthma and allergy in children younger than 17 years.[22] For vitamin A and its components, evidence from 17 studies (including three birth cohorts) was deemed methodologically weak but possibly supportive of an effect on asthma but not on wheeze. For carotenoids, findings from two birth cohort studies showed no significant association between maternal dietary intake during pregnancy and wheeze or asthma in the child by the age of 2 years (when a confident diagnosis of asthma is not possible).[23–25]

Since the publication of these two systematic reviews,[21,22] investigators of a longitudinal study of 17 528 Norwegian adults (aged 19–55 years) noted that daily consumption of cod liver oil (containing vitamin A [1000 μg/5 mL] and vitamin D [400 IU/5 mL]) was significantly associated with increased odds of incident asthma over a follow-up of 11 years (adjusted odds ratio [OR] 1·6, 95% CI 1·3–2·0).[26] Limitations of this study include potential selection bias (about 26% of participants had missing data for cod liver oil intake), exclusion of children, and possible residual confounding by cigarette smoking or vitamin D intake.

No primary randomised controlled trial (RCT) of vitamin A for the prevention or treatment of asthma has been done. However, Checkley and colleagues[27] did a secondary analysis in subgroups of Nepalese adults and children participating in two RCTs of vitamin A supplementation (administered for 16 months) to prevent mortality in children younger than 6 years, and vitamin A or β-carotene supplementation during pregnancy to prevent pregnancy-related mortality. In this analysis, neither vitamin A nor β-carotene supplementation (to pre-school children [n=3879] or pregnant women [n=1551]) was significantly associated with asthma or wheeze (at adolescence or young adulthood in the first trial, and at school age in the second trial). Although the investigators had previously reported that vitamin A supplementation during pregnancy leads to increased lung function (FEV_1 and forced vital capacity [FVC]) in school-aged Nepalese children,[28] they recorded no significant results for FEV_1 or FVC (a marker of airflow obstruction and asthma severity[29]) in their new analysis. This study was limited by potential selection bias due to loss of follow-up (ranging from about 18% to about 40%), variability in the dose given to pre-school children, and possible confounding by additional vitamin A supplementation received as part of a nationwide programme.

Higher intake of tomatoes, carrots, and leafy vegetables (which are rich in carotenoids such as α-carotene, β-carotene, lutein, and zeaxanthin) has been associated with lower prevalence of asthma in women.[30] Of the pro-vitamin A carotenoids, β-carotene has been most intensely studied because of its protective properties against the effects of free radicals and oxidative damage. A meta-analysis of five observational studies showed that high dietary intake of β-carotene was not significantly associated with asthma or FEV_1.[31] However, this meta-analysis was limited by sample size and residual confounding. Results of two RCTs showed that modification of dietary intake of carotenoids or lycopene through a diet rich in fruits and vegetables[32] or (lycopene-rich) tomato extract and tomato juice[33] improved indicators of asthma control, including lung function measures and time to disease exacerbations in adults with asthma. Larger RCTs (including both children and adults) with longer follow-up are needed to further validate these findings.

Vitamin C

Vitamin C (ascorbic acid), a potent water-soluble antioxidant found in various fruits and vegetables, has been associated with lower risks of cardiovascular disease, stroke, and cancer.[34] An anti-inflammatory role for vitamin C is further supported by the finding that vitamin C supplementation reduces C-reactive protein (CRP) in individuals with raised CRP concentrations.[35] Vitamin C could have beneficial effects on asthma through its immune-modulatory (eg, prostaglandin inhibition), antioxidant, or anti-inflammatory properties. In experimental models, vitamin C has been linked to decreased airway responsiveness, and reduced perivascular and peribronchiolar infiltration by inflammatory cells.[36,37] In support of this hypothesis, results of a cross-sectional study of Turkish children showed reduced plasma concentrations of vitamin C (and other non-enzymatic antioxidants such as vitamin E and carotenoids) in patients with asthma.[38] Similarly, an increase of 1 standard deviation in serum concentrations of vitamin C and other non-enzymatic antioxidants (eg, β-carotene and selenium) was associated with a reduction in asthma prevalence in a study of US children.[39]

In their systematic review and meta-analysis of 32 observational studies in children and adults, Allen and colleagues[21] reported that low dietary intake of vitamin C (variably defined across studies as lowest tertile or quintile, or as a low intake level [eg, <56 or <60 mg per day]) was significantly associated with slightly increased odds of asthma (OR 1·12, 95% CI 1·04–1·20), with generally consistent results for wheeze and airway responsiveness, but null findings for asthma severity.[21] Conversely, Nurmatov and colleagues[22] reported methodologically weak and unsupportive evidence of an effect of vitamin C on the development of asthma or atopy in their review of 14 studies (including three birth cohorts).[22] The discrepant conclusions of these two meta-analyses are probably connected to major differences in the criteria for study inclusion—eg, Allen and colleagues' study did not include birth cohorts. After publication of these reviews, investigators of a cross-sectional study of 1964 Greek children aged 1–3 years reported that dietary intake of vitamin C was significantly and inversely associated with wheeze, but not with asthma.[40]

In 2009, a Cochrane review of nine RCTs (aggregate sample size of 330 participants) of vitamin C supplementation and asthma outcomes (symptoms, lung function measures, total IgE, or use of inhaled steroids) concluded that insufficient evidence exists to recommend a role for vitamin C in the treatment of asthma.[41] No primary RCT of vitamin C supplementation to prevent asthma has been done. However, investigators of a secondary analysis of an RCT of vitamin C (1000 mg) and vitamin E (400 IU RRR α-tocopherol) supplementation to prevent pre-eclampsia in pregnant women recorded no significant effects on asthma or respiratory symptoms in the offspring of participants at age 2 years[42] Longer follow-up of children participating in this study could further understanding of the association between vitamin C and childhood asthma.

Vitamin E

Vitamin E comprises several fat-soluble components with distinct antioxidant properties.[10] Vitamin E occurs naturally in eight chemical forms—α, β, γ, and δ-tocopherol, and α, β, γ, and δ-tocotrienol—of which α-tocopherol has the greatest bioavailability. Dietary sources of vitamin E include nuts, seeds, green vegetables, and vegetable oils. Vitamin E might help to prevent or treat asthma by downregulating oxidative stress, airway inflammation, and Th2-immune responses (eg, reducing production of interleukin 4 by human peripheral blood mononuclear cells).[13,43] The antioxidant and anti-inflammatory properties of vitamin E are supported by findings from an unblinded trial, which showed that administration of a vitamin E isoform (d-α-tocopherol) for 16 weeks reduced F_2 isoprostanes (a marker of oxidative stress) in bronchoalveolar lavage fluid and airway responsiveness to methacholine (but not to specific allergens) in adults with mild atopic asthma.[44] Notably, plasma γ-tocopherol is inversely associated with total IgE and allergic sensitisation in adults.[45,46]

In their review and meta-analysis of 24 observational studies of vitamin E in children and adults, Allen and colleagues[21] reported a significant association between low self-reported dietary intake and physician-diagnosed asthma (difference for comparison of cases and controls –1·91 mg per day, 95% CI –2·5 to –1·3 mg per day) but not with self-reported asthma, wheeze, or airway responsiveness. Lower dietary intake (but not serum concentration) of vitamin E was also significantly associated with increased asthma severity.[21] From a review of 15 observational studies (including three birth cohorts), Nurmatov and colleagues[22] concluded that the evidence linking vitamin E intake during pregnancy to wheeze or asthma in childhood was methodologically weak but sufficiently supportive of a potential effect, warranting follow-up in clinical trials.

Pearson and colleagues[47] showed no effect of vitamin E supplementation (500 mg per day) for 6 weeks on airway responsiveness in an RCT in 72 British adults with asthma. Consistent with this lack of effect of vitamin E on asthma morbidity, investigators of a second RCT reported that an antioxidant supplement (containing β-carotene, vitamin C, and vitamin E) had no effect on plasma F_2 isoprostanes, exhaled nitric oxide, or immune responses of peripheral blood mononuclear cells in 54 adults with allergy (defined as a doctor-diagnosed allergic rhinitis or asthma, and sensitisation to at least one allergen).[48] By contrast with these negative results, four RCTs have reported that vitamin E-containing antioxidants reduce ozone-induced bronchoconstriction in patients with[49,50] and without[51,52] asthma, which suggests a potential protective effect of vitamin E against the detrimental effects of ozone. Although no primary RCT of vitamin E supplementation to prevent asthma has been done, a secondary analysis of an RCT of vitamin C and vitamin E supplementation to prevent pre-eclampsia in pregnant women showed no effect on respiratory function in the offspring at age 2 years.[42]

A question that remains outstanding is whether vitamin E obtained from foods has different effects from vitamin E obtained from supplements. A pilot study confirmed the feasibility of a food-based RCT to increase vitamin E intake to 15 mg per day in pregnant women;[53] However, whether this method of increasing vitamin E intake would yield better results than the use of supplements is unknown.

Vitamin D

Vitamin D is a nutrient and hormone that is key to the metabolism of calcium and phosphorus. Vitamin D is primarily acquired from sun exposure, with diet (eg, oily fish and dairy products) and supplements as secondary sources. Vitamin D status is assessed by measurement of the major circulating form of vitamin D—namely, serum or plasma 25-hydroxyvitamin D_3 (25[OH]D).[54] The terms vitamin D deficiency (a serum concentration of 25[OH]D <20 ng/mL) and vitamin D insufficiency (20–29 ng/mL) are commonly used, but an expert panel in the USA proposed a single threshold (≥20 ng/mL) as sufficient for musculoskeletal health.[55] This threshold is controversial, however, because no consensus has been reached on vitamin D sufficiency for non-musculoskeletal (eg, respiratory) health.[56] Irrespective of the threshold used, reduced serum or plasma vitamin D concentrations are commonly detected in children and adults in the USA, particularly in subgroups at high risk for asthma or asthma morbidity—eg, African Americans, Puerto Ricans, and obese people.[57,58]

Although the effects (if any) of vitamin D on asthma and asthma morbidity are not fully understood, they might include regulation of gene expression or immune responses (eg, upregulation of regulatory T cells[59]), affecting lung development or function, preventing weight gain,[60] attenuating or preventing viral illness (a common cause of asthma exacerbations), and enhancing steroid responsiveness.[58] Of these potential effects, the

most extensively studied are those on viral illnesses and steroid responsiveness. Findings from observational studies and RCTs suggest that vitamin D has a more consistent and stronger effect against viral respiratory illnesses in children[61–63] than in adults.[64] Results from observational and experimental studies[65–70] also suggest that vitamin D insufficiency or deficiency is associated with decreased steroid responsiveness. Consistent with findings for viral illnesses, the effects of vitamin D on steroid responsiveness might be stronger in children than in adults.[71]

In 2011, Paul and colleagues[58] reviewed the findings of ten observational studies[72–81] (including seven birth cohorts[75–81]) of dietary intake or serum concentration of vitamin D and asthma, concluding that evidence for a causal link is insufficient. Since then, investigators of six observational studies (two cross-sectional[82,83] and four longitudinal,[84–87] including two birth cohorts[84,85]) in children[83–86] or adults[60,82] have reported an inverse association[82,83] or no association[84–87] between serum or cord blood vitamin D concentration and asthma. Of the six published birth cohort studies that followed up at least 750 children each, one yielded negative results for asthma or wheeze at age 6 years,[85] whereas five reported an inverse association between vitamin D status during pregnancy (assessed as maternal dietary intake, or maternal serum or cord blood vitamin D concentration) and wheeze[76–78,80] or asthma[79] between the ages of 1·3 and 5 years. All these birth cohort studies had substantial loss of follow-up (ranging from around 24% to around 53%), which could have led to selection bias.

Three population-based studies (two cross-sectional[57,88] and one longitudinal[70]) showed an association between reduced serum vitamin D concentrations and severe disease exacerbations or core measures (eg, hospitalisations) of severe exacerbations in Costa Rican, North American, and Puerto Rican children with asthma. In the most recent of these studies,[57] vitamin D insufficiency or deficiency (a serum 25[OH]D concentration <30 ng/mL) was associated with increased odds of one or more severe asthma exacerbations in the previous year in Puerto Rican children, even after adjustment for household income, African racial ancestry, time spent outdoors, and other covariates. Notably, this association was stronger in non-atopic children (OR 6·2, 95% CI 2·1–21·2, p<0·01) than in atopic children (2·0, 95% 1·0–4·1, p=0·04), suggesting that vitamin D affects the risk of severe asthma exacerbations through mechanisms other than regulation of allergic immune responses—eg, modulation of viral illnesses or corticosteroid responses. Consistent with findings for severe exacerbations in children with (predominantly) mild to moderate asthma, reduced vitamin D concentrations are associated with increased airway smooth muscle mass, decreased lung function, and worse disease control in children with severe, therapy-resistant asthma.[89]

Results of ongoing RCTs of vitamin D supplementation during pregnancy to prevent asthma in childhood (NCT00920621, NCT00856947) or (as add-on therapy) treatment failure in adulthood (NCT01248065) have not yet been published.[58] In view of the present evidence, a clinical trial of vitamin D supplementation to prevent severe asthma exacerbations in school-aged children is both justifiable and necessary.

Nutrients acting as methyl donors

DNA methylation, a type of epigenetic regulation, is a mechanism underlying some gene–environment interactions on complex diseases such as asthma.[90] In particular, changes in DNA methylation can affect the pathogenesis of asthma by increasing or decreasing the expression of disease-susceptibility genes.

DNA methylation is catalysed by enzymes that transfer methyl groups (methyl-transferases) from the methylating agent S-adenosylmethionine to cytosine. In human beings, methyl donors for DNA methylation are mostly derived from dietary methyl groups. Folate-dependent and choline-dependent pathways interact at the point that homocysteine is converted to methionine for derivation of S-adenosylmethionine.[91] In the first pathway, tetrahydrofolates and vitamin B_{12} are cofactors required by methionine synthase. In the second pathway, the methylation of homocysteine to form methionine is catalysed by betaine homocysteine methyltransferase.[91–93] Both transmethylation metabolic pathways closely interconnect with methyltetrahydrofolate, B_{12}, choline, and methionine. Methyl donors affect DNA methylation and, potentially, immune responses (figure).

Folate, a type of water-soluble vitamin B and a cofactor (as tetrahydrofolates) in the transfer of one-carbon moieties, has a key role in aminoacid metabolism, purine and pyrimidine synthesis, and formation of S-adenosylmethionine. Dark leafy vegetables, fortified cereals and bread are major dietary sources of folate. Folate deficiency is associated with neural tube defects in embryos, macrocytic anaemia, cardiovascular diseases, and cancer.[94] Because of its known preventive effects against neural tube defects, folate supplements are routinely prescribed to women who are pregnant or planning to conceive.[95] Vitamin B_{12} (cobalamin) is necessary for proper formation of red blood cells, neurological function, and DNA synthesis, and is found in meat (especially liver), poultry, fish and shellfish, eggs, milk, and milk products. Vitamin B_{12} deficiency is associated with neural tube defects, cardiovascular diseases, cognitive decline, macrocytic anaemia, and osteoporosis.[96] Choline is a lipotropic agent involved in several biological functions (eg, neurotransmitter production, signalling lipids, and components of structural membranes), and as a methyl group donor.[97] Dietary sources of choline include meat, liver, eggs, poultry, fish and shellfish, peanuts, and cauliflower.

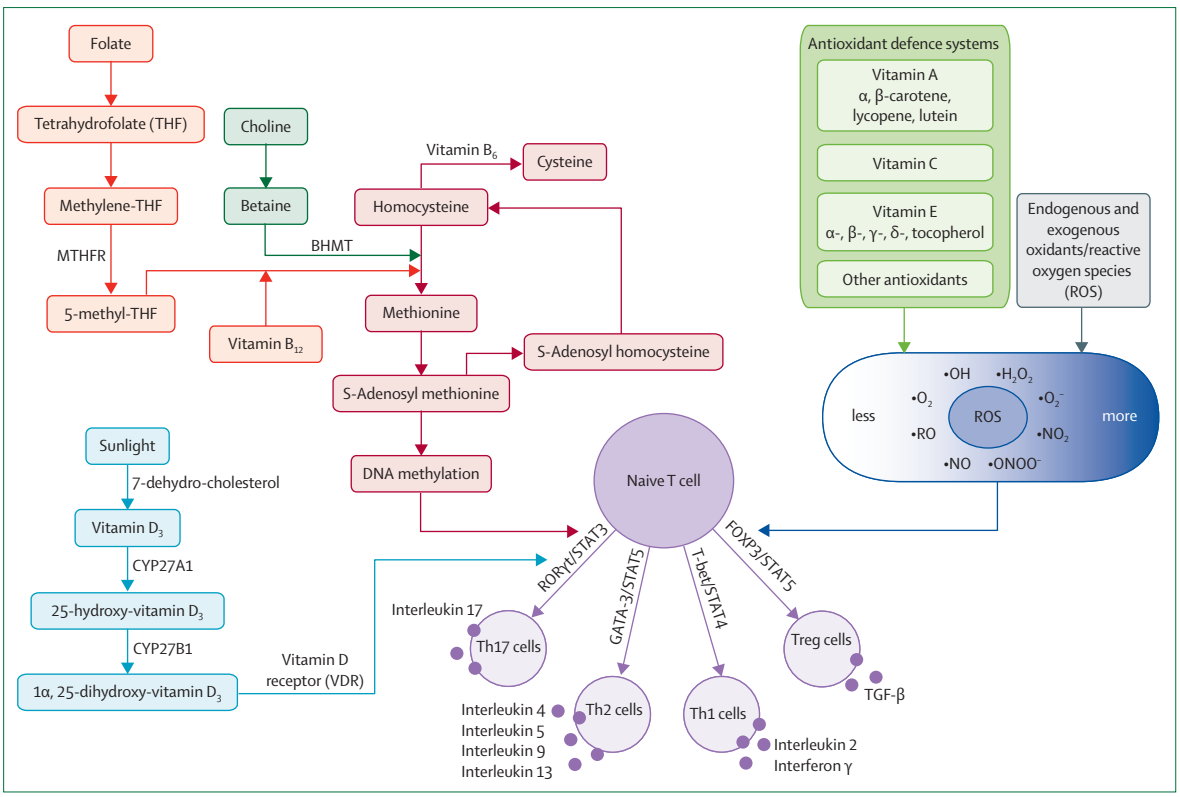

Figure: **Potential mechanisms of action of methyl donors and vitamins (A, C, E, and D) on Th1 and Th2 immune responses**
BHMT=betaine-homocysteine methyl-transferase. RORγt=retinoic acid-related orphan receptor γt. GATA-3=GATA binding protein 3. T-bet=T-box transcription factor. FOXP3=forkhead box P3. STAT=signal transducer and activator of transcription. TGF=transforming growth factor. MTHFR=methylenetetrahydrofolate reductase. Th=T helper cells. Treg=regulatory T cells.

Choline deficiency is associated with neurological disorders, cardiovascular diseases, and inflammation.[98]

Nutrients acting as methyl donors could contribute to asthma risk by affecting DNA methylation and, ultimately, gene expression. In a mouse model, a maternal diet rich in methyl donors (including folic acid, vitamin B_{12}, and choline) during pregnancy resulted in increased severity of allergic airway disease[99] in the F1 (offspring) generation and (to a lesser extent) the F2 generation. This dietary effect is probably mediated by dysregulation of lymphocyte maturation and enhancing Th2 immune responses.[99] This effect, in turn, is probably caused by DNA methylation of genes that are crucial to Th1 and Th2 immune balance (eg, *Runx3*, which is implicated in negative regulation of allergic airway disease in mice).[99] Although these findings have generated substantial interest, their relevance to human beings is unclear because of the high doses of methyl donors used in the dietary intervention and scarcity of data supporting the effects of individual methyl donors (eg, folic acid).

To our knowledge, no animal models of the effects of exclusive folate intake or exclusive vitamin B_{12} intake on experimental asthma (allergic airway disease) have been published. Intranasal or oral administration of choline, however, has been shown to reduce the number of eosinophils and reactive oxidant species in bronchoalveolar lavage fluid in a murine model of allergic airway disease.[100] Together with findings in adults[101,102] and adolescents[102] with[101] and without[102] asthma, this suggests that choline might attenuate (and not enhance) allergic inflammation in general and airway inflammation in particular.

Results from experimental studies and the recommendation of folic acid supplementation in women who are pregnant or planning to conceive have triggered observational studies in children and adults. Because of their longitudinal design, birth cohort studies can best examine whether folate status or folic acid supplementation during pregnancy is associated with the development of asthma or atopy. However, not all birth cohort studies on this topic have gathered data for either maternal dietary intake or folate level in early pregnancy.[23,103–112] Of these 11 studies, seven showed no significant association between maternal folic acid intake (through diet and supplements) and asthma;[23,103,105,107,108,110,111] three of these negative studies had a short duration of follow-up,[23,103,111] (limiting their assessment of asthma, which is difficult before the age of 6 years) and the rest lost a large proportion of children to follow-up. Although

the results of four birth cohort studies[104,106,111,112] showed a positive association between folate status during pregnancy and asthma or atopy, they were limited by short duration of follow-up, non-comprehensive phenotypic assessment, or inconsistent results for asthma and atopy. The authors of the largest of these positive studies (n=32 077) noted that folic acid supplementation during pregnancy was associated with slightly increased risk of wheeze (adjusted relative risk 1·06, 95% CI 1·03–1·10) and lower respiratory tract infections in infancy.[104] Only three birth cohort studies have assessed biomarkers of folate.[110–112] In two of these studies,[110,111] maternal folate status during pregnancy (assessed by measurement of plasma[111] or intracellular[110] folic acid concentration) was not associated with childhood asthma. In the third study,[112] maternal plasma folate concentration in the second trimester of pregnancy was significantly and linearly associated with increased odds of asthma at age 3 years. However, none of these studies has separately measured folate species (vitamers). Whether folate status affects disease severity or control in people who already have asthma is unclear.[113]

Two cross-sectional studies in adults yielded negative findings for dietary intake or circulating concentration of vitamin B_{12} and asthma or atopy.[114,115] Additionally, investigators of two birth cohort studies reported no significant association between maternal dietary intake or circulating B_{12} concentration during pregnancy and wheeze in young children, yielding conflicting findings for eczema or atopic dermatitis.[103,111] We are not aware of published birth cohort studies of choline status during pregnancy and asthma or atopy.

No RCTs have been done on methyl donor supplementation during pregnancy to prevent asthma. This absence of research is understandable for folate because of weak and conflicting evidence from observational studies, and ethical concerns about withholding or reducing folic acid supplementation during conception or pregnancy because of its documented beneficial effects. Although no RCTs of folate or vitamin B_{12} supplementation to treat asthma have been published, Mehta and colleagues[101] did a 6-month RCT to examine the effects of choline supplementation (1500 mg, twice per day) on indicators of disease severity or control in 76 Indian adults with asthma. In this trial, choline supplementation led to reductions in symptoms, medication use, concentrations of cytokine (interleukin 4, interleukin 5, and tumour necross factor-α), and airway responsiveness.

Discussion

Several nutrients or vitamins with antioxidant or anti-inflammatory effects, acting additively or synergistically, could have beneficial effects on asthma. For example, whereas increased intake of fruits and vegetables decreases the risk of asthma,[30,32] a so-called westernised diet—eg, rich in meats, sodium, and refined carbohydrates—increased the risk of frequent wheezing.[116] Thus, well designed observational studies and RCTs of particular diets or dietary patterns would be justifiable and of great interest, particularly as a diet rich in fruits and vegetables and low in saturated fats or carbohydrates has known benefits to overall health. However, such a diet is not always easy to adhere to because of practical reasons, including cultural beliefs and financial limitations. Specific circumstances (such as area of residence, work habits, or fear of being outdoors because of safety concerns [eg, exposure to violence]) could limit sun exposure, which is a main determinant of circulating vitamin D concentrations. Thus, supplementation of beneficial vitamins or nutrients could be a relatively inexpensive and practical alternative for some populations with asthma or at risk of developing asthma.

All observational studies (cross-sectional and longitudinal) of vitamins or nutrients acting as methyl donors are susceptible to bias due to differential entry or retention of participants according to both the exposure and outcome of interest (selection bias), confounding by environmental or lifestyle factors (eg, socioeconomic status, physical activity, intake of other nutrients), and false-positive results due to multiple testing. Additionally, cross-sectional studies are subject to reverse causation (ie, if the disease leads to increased or decreased concentrations of a particular vitamin or nutrient) and recall bias. Furthermore, most observational studies do not have adequate statistical power to test for interactions between the vitamin or nutrient of interest and other dietary components because of the sample sizes usually used in these studies. Despite these limitations, observational studies are often helpful in deciding whether to undertake an RCT and to prioritise the need for a particular RCT. Although RCTs remain the gold standard to determine whether a vitamin or nutrient affects asthma, small or poorly conducted trials might be subject to false negatives, residual confounding (eg, if the two groups being compared are not balanced with regard to potential confounders), or bias due to differential adherence to treatment or insufficient dosing or duration of the intervention of interest.

The figure shows some of the potential mechanisms of action of methyl donors and vitamins (A, C, E, and D) on immune responses. Tables 1 and 2 summarise evidence linking vitamins or nutrients acting as methyl donors to asthma or asthma morbidity. Of the four vitamins of interest, data from observational studies support ongoing or future RCTs of vitamin D supplementation for the prevention or treatment of asthma (eg, to prevent severe disease exacerbations). Findings for vitamins A, C, and E best support a large RCT of vitamin E supplementation to ameliorate the detrimental effects of air pollution on disease morbidity in patients with asthma. For vitamins A, C, and E, additional follow-up of children in ongoing birth cohort studies could help to prioritise the need for RCTs of primary prevention.

	Potential mechanisms of action	Observational studies	RCTs
Vitamin A	Downregulation of oxidative stress and Th2 (allergic) immune responses	Findings from >20 studies (including three birth cohorts) provide weak evidence for an inverse association between dietary intake and asthma (but not wheeze). Prenatal or postnatal vitamin A supplementation was not significantly associated with asthma, wheeze, or FEV_1/FVC in children. Intake of vitamin A and D (as cod liver oil) was associated with incident asthma in a study of adults	No primary RCT of vitamin A to prevent or treat asthma in children or adults has been done
Vitamin C	Downregulation of oxidative stress and Th2 (allergic) immune responses	Findings from more than 30 studies of postnatal dietary intake suggest an inverse association between vitamin C intake and asthma in children and adults, with generally consistent results for wheeze and airway responsiveness but not asthma severity. When studies of prenatal dietary intake are reviewed along with those of postnatal intake, weak and inconsistent evidence exists of an association between vitamin C intake and asthma or atopy in children	No primary RCT of vitamin C supplementation during pregnancy to prevent asthma has been done. Although nine RCTs have shown no significant evidence for an effect of vitamin C supplementation on indicators of asthma severity or control, none has had adequate statistical power to detect weak to moderate effects of vitamin C on asthma morbidity
Vitamin D	Enhanced steroid responsiveness, antiviral properties, upregulation of T-regulatory cells, prevention of gains in adiposity, effects on lung development or function	Results from six of seven birth cohort studies with sample size >750 mother–child pairs support an inverse association between maternal vitamin D status during pregnancy and asthma or wheeze in childhood. Longer follow-up is necessary to properly assess an association with asthma in all six positive studies. Findings from three studies (one longitudinal) suggest that vitamin D insufficiency or deficiency is associated with increased risk of severe asthma exacerbations	RCTs of vitamin D supplementation during pregnancy to prevent asthma have not been done. Similarly, results of RCTs of vitamin D supplementation to reduce asthma morbidity in children or adults have not been published
Vitamin E	Downregulation of oxidative stress, airway inflammation, and Th2 (allergic) immune responses	Findings from >20 studies of postnatal dietary intake suggest an inverse association between vitamin E intake and physician-diagnosed asthma in children and adults, with inconsistent results for wheeze, self-reported asthma, and airway responsiveness. When studies of prenatal dietary intake are reviewed along with those of postnatal intake, weak evidence exists of an inverse association between vitamin E intake and asthma or atopy in children	No primary RCT of vitamin E supplementation during pregnancy to prevent asthma has been done. Although two RCTs showed no significant effects of vitamin E supplementation on airway responsiveness or inflammatory and immune markers in adults with asthma, other findings support a protective effect of vitamin E supplementation against ozone-induced bronchoconstriction

RCTs=randomised controlled trials. FEV_1=forced expiratory volume in 1 second. FVC=forced vital capacity. Th2=type 2 helper T cell.

Table 1: **Collective findings of studies and trials of potential effects of vitamins on asthma**

	Potential mechanisms of action	Observational studies	RCTs
Folate	Differential DNA methylation of genes affecting Th1/Th2 immune responses, ultimately enhancing Th2 (allergic) immune responses and airway inflammation	Findings from 20 observational studies (including 11 birth cohorts) do not support moderate or strong effects of folate on asthma or atopy. Two studies with major methodological weaknesses provide insufficient evidence to assess a potential association between folate and asthma severity or control	No primary RCT of folic acid supplementation to prevent or treat asthma in children or adults has been done
Vitamin B_{12}	If any, similar to those listed for folate	Four studies (including two birth cohorts) showed no significant association with wheeze or asthma, with conflicting results for eczema or atopic dermatitis from the two birth cohort studies	No primary RCT of vitamin B_{12} supplementation to prevent or treat asthma has been done
Choline	Downregulation of oxidative stress, eosinophilia, and allergic airway inflammation	None, to our knowledge	No primary RCT of choline supplementation during pregnancy to prevent asthma has been done. Findings from an RCT in adults with asthma showed that choline supplementation reduces symptoms, medication use, airway responsiveness, and circulating concentrations of Th2 cytokines

RCTs=randomised controlled trials. Th=T-helper cells.

Table 2: **Collective findings of studies and trials of potential effects of nutrients acting as methyl donors on asthma**

Recommendations for fortification of foods or supplementation of folic acid to women of childbearing age were established after the peak in the asthma epidemic in industrialised countries. This fact, combined with evidence from observational studies, suggests that any effect of folate status on development of asthma is likely to be small, and should thus be assessed in the context of ongoing large birth cohort studies, preferably with biomarkers in addition to dietary information. At present, adequate observational studies of folate status and asthma morbidity do not exist. Whereas little evidence exists for a role of vitamin B_{12} in the pathogenesis of asthma or asthma morbidity, data from a single RCT support large RCTs of choline supplementation to treat asthma in patients with established disease. In view of existing data, birth cohort studies of choline status and asthma are also justified.

In summary, there is insufficient evidence to recommend the use of any vitamin supplement or nutrient acting as methyl donor for the prevention or

Search strategy and selection criteria

We searched PubMed for articles published in English from January, 1990, to May, 2013, on vitamins, methyl donors, and asthma. We selected highly relevant articles with the terms "asthma" or "atopy" and "vitamin A" or carotenoids (including carotenes ["α-carotene" or "β-carotene" or "lycopene"] and xanthophylls ["lutein" or "zeaxanthin"]), "vitamin C" or "ascorbic acid", "vitamin D" or "calcitriol", "vitamin E" or "tocopherol", "folate", "vitamin B_{12}", and "choline".

treatment of asthma. Ongoing RCTs should help confirm or refute promising findings for vitamin D and choline in the prevention or treatment of asthma.

Contributors

Y-YH and JB contributed to the study concept, literature search, data collection, and manuscript writing. JMB, EF, and AAL contributed to the study concept and manuscript writing. JCC contributed to the study concept, literature search, and manuscript writing.

Conflicts of interest

JCC has consulted for Genentech in 2011 on an issue unrelated to the content of this manuscript. The other authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by grant HL079966 from the US National Institute of Health and an endowment from the Heinz Foundation. Neither funding agency had any role in the preparation of this manuscript.

References

1 Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, health care use, and mortality: United States, 2005–2009. *Natl Health Stat Report* 2011; **32:** 1–14.

2 Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006; **368:** 733–43.

3 Seaton A, Godden DJ, Brown K. Increase in asthma: a more toxic environment or a more susceptible population? *Thorax* 1994; **49:** 171–74.

4 Caramori G, Papi A. Oxidants and asthma. *Thorax* 2004; **59:** 170–73.

5 Riedl MA, Nel AE. Importance of oxidative stress in the pathogenesis and treatment of asthma. *Curr Opin Allergy Clin Immunol* 2008; **8:** 49–56.

6 Katsoulis K, Kontakiotis T, Leonardopoulos I, Kotsovili A, Legakis IN, Patakas D. Serum total antioxidant status in severe exacerbation of asthma: correlation with the severity of the disease. *J Asthma* 2003; **40:** 847–54.

7 Schunemann HJ, Grant BJ, Freudenheim JL, et al. The relation of serum levels of antioxidant vitamins C and E, retinol and carotenoids with pulmonary function in the general population. *Am J Respir Crit Care Med* 2001; **163:** 1246–55.

8 Ochs-Balcom HM, Grant BJ, Muti P, et al. Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. *Eur J Clin Nutr* 2006; **60:** 991–99.

9 Tanumihardjo SA. Vitamin A: biomarkers of nutrition for development. *Am J Clin Nutr* 2011; **94:** 658S–65S.

10 Litonjua AA. Fat-soluble vitamins and atopic disease: what is the evidence? *Proc Nutr Soc* 2012; **71:** 67–74.

11 Fitzpatrick AM, Teague WG, Holguin F, Yeh M, Brown LA. Airway glutathione homeostasis is altered in children with severe asthma: evidence for oxidant stress. *J Allergy Clin Immunol* 2009; **123:** 146–52 e8.

12 Paiva SA, Russell RM. Beta-carotene and other carotenoids as antioxidants. *J Am Coll Nutr* 1999; **18:** 426–33.

13 Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol* 2008; **8:** 685–98.

14 Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med* 2007; **204:** 1765–74.

15 Sun CM, Hall JA, Blank RB, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 2007; **204:** 1775–85.

16 Mucida D, Park Y, Kim G, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007; **317:** 256–60.

17 Lovett-Racke AE, Racke MK. Retinoic acid promotes the development of Th2-like human myelin basic protein-reactive T cells. *Cell Immunol* 2002; **215:** 54–60.

18 Chew BP, Park JS. Carotenoid action on the immune response. *J Nutr* 2004; **134:** 257S–261S.

19 Hazlewood LC, Wood LG, Hansbro PM, Foster PS. Dietary lycopene supplementation suppresses Th2 responses and lung eosinophilia in a mouse model of allergic asthma. *J Nutr Biochem* 2011; **22:** 95–100.

20 Lee CM, Chang JH, Moon DO, et al. Lycopene suppresses ovalbumin-induced airway inflammation in a murine model of asthma. *Biochem Biophys Res Comm* 2008; **374:** 248–52.

21 Allen S, Britton JR, Leonardi-Bee JA. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. *Thorax* 2009; **64:** 610–19.

22 Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: systematic review and meta-analysis. *J Allergy Clin Immunol* 2011; **127:** 724–33 e1–30.

23 Litonjua AA, Rifas-Shiman SL, Ly NP, et al. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 y of age. *Am J Clin Nutr* 2006; **84:** 903–11.

24 Martindale S, McNeill G, Devereux G, Campbell D, Russell G, Seaton A. Antioxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. *Am J Respir Crit Care Med* 2005; **171:** 121–28.

25 Ly NP, Gold DR, Weiss ST, Celedon JC. Recurrent wheeze in early childhood and asthma among children at risk for atopy. *Pediatrics* 2006; **117:** e1132–28.

26 Mai XM, Langhammer A, Chen Y, Camargo CA Jr. Cod liver oil intake and incidence of asthma in Norwegian adults—the HUNT study. *Thorax* 2013; **68:** 25–30.

27 Checkley W, West KP Jr, Wise RA, et al. Supplementation with vitamin A early in life and subsequent risk of asthma. *Eur Respir J* 2011; **38:** 1310–19.

28 Checkley W, West KP Jr, Wise RA, et al. Maternal vitamin A supplementation and lung function in offspring. *N Engl J Med* 2010; **362:** 1784–94.

29 Ramsey CD, Celedon JC, Sredl DL, Weiss ST, Cloutier MM. Predictors of disease severity in children with asthma in Hartford, Connecticut. *Pediatr Pulmonol* 2005; **39:** 268–75.

30 Romieu I, Varraso R, Avenel V, Leynaert B, Kauffmann F, Clavel-Chapelon F. Fruit and vegetable intakes and asthma in the E3N study. *Thorax* 2006; **61:** 209–15.

31 Gao J, Gao X, Li W, Zhu Y, Thompson PJ. Observational studies on the effect of dietary antioxidants on asthma: a meta-analysis. *Respirology* 2008; **13:** 528–36.

32 Wood LG, Garg ML, Smart JM, Scott HA, Barker D, Gibson PG. Manipulating antioxidant intake in asthma: a randomised controlled trial. *Am J Clin Nutr* 2012; **96:** 534–43.

33 Wood LG, Garg ML, Powell H, Gibson PG. Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: proof of concept. *Free Radic Res* 2008; **42:** 94–102.

34 Padayatty SJ, Katz A, Wang Y, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J Am Coll Nutr* 2003; **22:** 18–35.

35 Block G, Jensen CD, Dalvi TB, et al. Vitamin C treatment reduces elevated C-reactive protein. *Free Radic Biol Med* 2009; **46:** 70–77.

36 McKeever TM, Britton J. Pulmonary perspective: diet and asthma. *Am J Respir Crit Care Med* 2004; **170:** 725–29.

37 Jeong YJ, Kim JH, Kang JS, Lee WJ, Hwang YI. Mega-dose vitamin C attenuated lung inflammation in mouse asthma model. *Anat Cell Biol* 2010; **43:** 294–302.

38 Sackesen C, Ercan H, Dizdar E, et al. A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. *J Allergy Clin Immunol* 2008; **122:** 78–85.

39 Rubin RN, Navon L, Cassano PA. Relationship of serum antioxidants to asthma prevalence in youth. *Am J Respir Crit Care Med* 2004; **169:** 393–98.

40 Emmanouil E, Manios Y, Grammatikaki E, et al. Association of nutrient intake and wheeze or asthma in a Greek pre-school population. *Pediatr Allergy Immunol* 2010; **21:** 90–95.

41 Kaur B, Rowe BH, Arnold E. Vitamin C supplementation for asthma. *Cochrane Database Syst Rev* 2009; **1:** CD000993.

42 Greenough A, Shaheen SO, Shennan A, Seed PT, Poston L. Respiratory outcomes in early childhood following antenatal vitamin C and E supplementation. *Thorax* 2010; **65:** 998–1003.

43 Li-Weber M, Giaisi M, Treiber MK, Krammer PH. Vitamin E inhibits IL-4 gene expression in peripheral blood T cells. *Eur J Immunol* 2002; **32:** 2401–08.

44 Hoskins A, Roberts JL 2nd, Milne G, Choi L, Dworski R. Natural-source d-alpha-tocopheryl acetate inhibits oxidant stress and modulates atopic asthma in humans in vivo. *Allergy* 2012; **67:** 676–82.

45 Kompauer I, Heinrich J, Wolfram G, Linseisen J. Association of carotenoids, tocopherols and vitamin C in plasma with allergic rhinitis and allergic sensitisation in adults. *Pub Health Nutr* 2006; **9:** 472–79.

46 Fogarty A, Lewis S, Weiss S, Britton J. Dietary vitamin E, IgE concentrations, and atopy. *Lancet*. 2000; **356:** 1573–74.

47 Pearson PJ, Lewis SA, Britton J, Fogarty A. Vitamin E supplements in asthma: a parallel group randomised placebo controlled trial. *Thorax* 2004; **59:** 652–56.

48 Dunstan JA, Breckler L, Hale J, et al. Supplementation with vitamins C, E, beta-carotene and selenium has no effect on anti-oxidant status and immune responses in allergic adults: a randomized controlled trial. *Clin Exp Allergy* 2007; **37:** 180–87.

49 Romieu I, Sienra-Monge JJ, Ramirez-Aguilar M, et al. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *Am J Respir Crit care Med* 2002; **166:** 703–09.

50 Trenga CA, Koenig JQ, Williams PV. Dietary antioxidants and ozone-induced bronchial hyperresponsiveness in adults with asthma. *Arch Environ Health* 2001; **56:** 242–49.

51 Romieu I, Meneses F, Ramirez M, et al. Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. *Am J Respir Crit Care Med* 1998; **158:** 226–32.

52 Grievink L, Zijlstra AG, Ke X, Brunekreef B. Double-blind intervention trial on modulation of ozone effects on pulmonary function by antioxidant supplements. *Am J Epidemiol* 1999; **149:** 306–14.

53 Clark J, Craig L, McNeill G, Smith N, Norrie J, Devereux G. A novel dietary intervention to optimize vitamin E intake of pregnant women to 15 mg/day. *J Acad Nutr Diet* 2012; **112:** 297–301.

54 Hollis BW. Assessment and interpretation of circulating 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in the clinical environment. *Endocrinol Metab Clin North Am* 2010; **39:** 271–86.

55 Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2010; **96:** 53–58.

56 Holick MF. The IOM D-lemma. *Public Health Nutr* 2011; **14:** 939–41.

57 Brehm JM, Acosta-Perez E, Klei L, et al. Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *Am J Respir Crit Care Med* 2012; **186:** 140–46.

58 Paul G, Brehm JM, Alcorn JF, Holguin F, Aujla SJ, Celedon JC. Vitamin D and asthma. *Am J Respir Crit Care Med* 2012; **185:** 124–32.

59 Chambers ES, Nanzer AM, Richards DF, et al. Serum 25-dihydroxyvitamin D levels correlate with CD4(+)Foxp3(+) T-cell numbers in moderate/severe asthma. *J Allergy Clin Immunol* 2012; **130:** 542–44.

60 Mai XM, Chen Y, Camargo CA Jr, Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study. *Am J Epidemiol* 2012; **175:** 1029–36.

61 Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr* 2010; **91:** 1255–60.

62 Jartti T, Ruuskanen O, Mansbach JM, Vuorinen T, Camargo CA Jr. Low serum 25-hydroxyvitamin D levels are associated with increased risk of viral coinfections in wheezing children. *J Allergy Clin Immunol* 2010; **126:** 1074–76, 6 e1–4.

63 Camargo CA Jr, Ganmaa D, Frazier AL, et al. Randomized trial of vitamin D supplementation and risk of acute respiratory infection in Mongolia. *Pediatrics* 2012; **130:** e561–7.

64 Murdoch DR, Slow S, Chambers ST, et al. Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. *JAMA* 2012; **308:** 1333–39.

65 Xystrakis E, Kusumakar S, Boswell S, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest* 2006; **116:** 146–55.

66 Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *J Allergy Clin Immunol* 2010; **125:** 995–1000.

67 Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. *Am J Respir Crit Care Med* 2010; **181:** 699–704.

68 Forno E, Lescher R, Strunk R, et al. Decreased response to inhaled steroids in overweight and obese asthmatic children. *J Allergy Clin Immunol* 2011; **127:** 741–49.

69 Wu AC, Tantisira K, Li L, Fuhlbrigge AL, Weiss ST, Litonjua A. Effect of vitamin D and inhaled corticosteroid treatment on lung function in children. *Am J Respir Crit Care Med* 2012; **186:** 508–13.

70 Brehm JM, Schuemann B, Fuhlbrigge AL, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010; **126:** 52–58 e5.

71 Goleva E, Searing DA, Jackson LP, Richers BN, Leung DY. Steroid requirements and immune associations with vitamin D are stronger in children than adults with asthma. *J Allergy Clin Immunol* 2012; **129:** 1243–51.

72 Hypponen E, Sovio U, Wjst M, et al. Infant vitamin d supplementation and allergic conditions in adulthood: northern Finland birth cohort 1966. *Ann N Y Acad Sci* 2004; **1037:** 84–95.

73 Devereux G, Wilson A, Avenell A, McNeill G, Fraser WD. A case-control study of vitamin D status and asthma in adults. *Allergy* 2010; **65:** 666–67.

74 Freishtat RJ, Iqbal SF, Pillai DK, et al. High prevalence of vitamin D deficiency among inner-city African American youth with asthma in Washington, DC. *J Pediatr* 2010; **156:** 948–52.

75 Gale CR, Robinson SM, Harvey NC, et al. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2008; **62:** 68–77.

76 Devereux G, Litonjua AA, Turner SW, et al. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007; **85:** 853–59.

77 Camargo CA Jr, Rifas-Shiman SL, Litonjua AA, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. *Am J Clin Nutr* 2007; **85:** 788–95.

78 Miyake Y, Sasaki S, Tanaka K, Hirota Y. Dairy food, calcium and vitamin D intake in pregnancy, and wheeze and eczema in infants. *Eur Respir J* 2010; **35:** 1228–34.

79 Erkkola M, Kaila M, Nwaru BI, et al. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. *Clin Exp Allergy* 2009; **39:** 875–82.

80 Camargo CA Jr, Ingham T, Wickens K, et al. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics* 2011; **127:** e180–7.

81 Hollams EM, Hart PH, Holt BJ, et al. Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. *Eur Respir J* 2011; **38:** 1320–27.

82 Carroll KN, Gebretsadik T, Larkin EK, et al. Relationship of maternal vitamin D level with maternal and infant respiratory disease. *Am J Obstetr Gynecol* 2011; **205**: 215 e1–7.

83 Bener A, Ehlayel MS, Tulic MK, Hamid Q. Vitamin D deficiency as a strong predictor of asthma in children. *Int Arch Allergy Immunol* 2012; **157:** 168–75.

84 Rothers J, Wright AL, Stern DA, Halonen M, Camargo CA Jr. Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen sensitization in children from Tucson, Arizona. *J Allergy Clin Immunol* 2011; **128:** 1093–99 e1–5.

85 Pike KC, Inskip HM, Robinson S, et al. Maternal late-pregnancy serum 25-hydroxyvitamin D in relation to childhood wheeze and atopic outcomes. *Thorax* 2012; **67:** 950–56.

86 Tolppanen AM, Sayers A, Granell R, Fraser WD, Henderson J, Lawlor DA. Prospective association of 25-Hydroxyvitamin D3 and D2 with childhood lung function, asthma, wheezing, and flexural dermatitis. *Epidemiology* 2013; **24:** 310–19.

87 Mai XM, Langhammer A, Camargo CA Jr, Chen Y. Serum 25-hydroxyvitamin D levels and incident asthma in adults: the HUNT Study. *Am J Epidemiol* 2012; **176:** 1169–76.

88 Brehm JM, Celedon JC, Soto-Quiros ME, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009; **179:** 765–71.

89 Gupta A, Sjoukes A, Richards D, et al. Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. *Am J Respir Crit Care Med* 2011; **184:** 1342–49.

90 Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. *Am J Respir Crit Care Med* 2008; **177:** 567–73.

91 Niculescu MD, Zeisel SH. Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. *J Nutr* 2002; **132:** 2333–5S.

92 Zeisel SH. Importance of methyl donors during reproduction. *Am J Clin Nutr* 2009; **89:** 673–7S.

93 Bailey LB, Gregory JF 3rd. Folate metabolism and requirements. *J Nutr* 1999; **129:** 779–82.

94 Stover PJ. Physiology of folate and vitamin B12 in health and disease. *Nutr Rev* 2004; **62:** S3–12.

95 Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. *Nutrients* 2011; **3:** 370–84.

96 O'Leary F, Samman S. Vitamin B12 in health and disease. *Nutrients* 2010; **2:** 299–316.

97 Blusztajn JK. Choline, a vital amine. *Science* 1998; **281:** 794–95.

98 Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. *Nutrition Rev* 2009; **67:** 615–23.

99 Hollingsworth JW, Maruoka S, Boon K, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. *J Clin Invest* 2008; **118:** 3462–69.

100 Mehta AK, Arora N, Gaur SN, Singh BP. Choline supplementation reduces oxidative stress in mouse model of allergic airway disease. *Eur J Clin Invest* 2009; **39:** 934–41.

101 Mehta AK, Singh BP, Arora N, Gaur SN. Choline attenuates immune inflammation and suppresses oxidative stress in patients with asthma. *Immunobiology* 2010; **215:** 527–34.

102 Detopoulou P, Panagiotakos DB, Antonopoulou S, Pitsavos C, Stefanadis C. Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. *Am J Clin Nutr* 2008; **87:** 424–30.

103 Miyake Y, Sasaki S, Tanaka K, Hirota Y. Maternal B vitamin intake during pregnancy and wheeze and eczema in Japanese infants aged 16–24 months: the Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol* 2011; **22:** 69–74.

104 Haberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. *Arch Dis Child* 2009; **94:** 180–84.

105 Nwaru BI, Erkkola M, Ahonen S, et al. Intake of antioxidants during pregnancy and the risk of allergies and asthma in the offspring. *Eur J Clin Nutr* 2011; **65:** 937–43.

106 Whitrow MJ, Moore VM, Rumbold AR, Davies MJ. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. *Am J Epidem* 2009; **170:** 1486–93.

107 Martinussen MP, Risnes KR, Jacobsen GW, Bracken MB. Folic acid supplementation in early pregnancy and asthma in children aged 6 years. *Am J Obstet Gynecol* 2012; **206:** 72 e1–7.

108 Granell R, Heron J, Lewis S, Davey Smith G, Sterne JA, Henderson J. The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort. *Clin Exper Allergy* 2008; **38:** 320–28.

109 Bekkers MB, Elstgeest LE, Scholtens S, et al. Maternal use of folic acid supplements during pregnancy, and childhood respiratory health and atopy. *Eur Respir J* 2012; **39:** 1468–74.

110 Magdelijns FJ, Mommers M, Penders J, Smits L, Thijs C. Folic acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. *Pediatrics* 2011; **128:** e135–44.

111 Kiefte-de Jong JC, Timmermans S, Jaddoe VW, et al. High circulating folate and vitamin B-12 concentrations in women during pregnancy are associated with increased prevalence of atopic dermatitis in their offspring. *J Nutr* 2012; **142:** 731–38.

112 Haberg SE, London SJ, Nafstad P, et al. Maternal folate levels in pregnancy and asthma in children at age 3 years. *J Allergy Clin Immunol* 2011; **127:** 262–64, 4 e1.

113 Lin JH, Matsui W, Aloe C, et al. Relationships between folate and inflammatory features of asthma. *J Allergy Clin Immunol* 2012; **131:** 918–20.

114 Husemoen LL, Toft U, Fenger M, Jorgensen T, Johansen N, Linneberg A. The association between atopy and factors influencing folate metabolism: is low folate status causally related to the development of atopy? *Int J Epidemiol* 2006; **35:** 954–61.

115 Thuesen BH, Husemoen LL, Ovesen L, et al. Atopy, asthma, and lung function in relation to folate and vitamin B(12) in adults. *Allergy* 2010; **65:** 1446–54.

116 Tromp, II, Kiefte-de Jong JC, de Vries JH, et al. Dietary patterns and respiratory symptoms in pre-school children: the Generation R Study. *Eur Respir J* 2012; **40:** 681–89.