

Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis

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ABSTRACT

Background: Epidemiological studies suggest that dietary intake of vitamins A, C and E may be associated with the occurrence of asthma. A systematic review and meta-analysis was conducted in accordance with MOOSE guidelines to determine whether vitamins A, C and E, measured as dietary intakes or serum levels, are associated with asthma.

Methods: MEDLINE, EMBASE, CINAHL, CAB abstracts and AMED (up to November 2007), conference proceedings and bibliographies of papers were searched to identify studies of asthma, wheeze or airway responsiveness in relation to intakes and serum concentrations of vitamins A, C and E. Pooled odds ratios (OR) or mean differences (MD) with 95% confidence intervals (CI) were estimated using random effects models.

Results: A total of 40 studies were included. Dietary vitamin A intake was significantly lower in people with asthma than in those without asthma (MD $-182 \mu\text{g/day}$, 95% CI -288 to -75 ; 3 studies) and in people with severe asthma than in those with mild asthma (MD $-344 \mu\text{g/day}$; 2 studies). Lower quantile dietary intakes (OR 1.12, 95% CI 1.04 to 1.21; 9 studies) and serum levels of vitamin C were also associated with an increased odds of asthma. Vitamin E intake was generally unrelated to asthma status but was significantly lower in severe asthma than in mild asthma (MD $-1.20 \mu\text{g/day}$, 95% CI -2.3 to -0.1 ; 2 studies).

Conclusions: Relatively low dietary intakes of vitamins A and C are associated with statistically significant increased odds of asthma and wheeze. Vitamin E intake does not appear to be related to asthma status.

Diet has been widely implicated in the aetiology of cardiovascular disease^{1,2} and other disorders, but the role of diet in the aetiology of respiratory diseases is less clearly defined. Over the past 15 years many observational epidemiological studies have reported associations between diet and asthma, and particularly for the antioxidant vitamins A, C and E. However, randomised controlled trials of vitamin supplementation in asthma thus far have been inconclusive.³⁻⁶ In view of this apparent inconsistency between the observational and experimental data,⁷ we have attempted to determine the magnitude of the overall associations of these vitamins estimated by the observational studies by carrying out a systematic review and meta-analysis to provide pooled quantitative estimates of the likely magnitude of the effect of dietary intake and blood levels of antioxidant vitamins on a range of measures of asthma and asthma severity.

METHODS

Systematic review methods

A comprehensive search of the MEDLINE (1950 to November 2007), EMBASE (1980 to November 2007), CINAHL (1982 to November 2007) and AMED (1985 to November 2007) was conducted to identify all published comparative epidemiological studies assessing the relation between antioxidant vitamins and asthma status. Search terms were selected under guidance from the Centre for Reviews and Dissemination and the Airways Group Specialist search terms.⁸ Reference lists from identified relevant studies and previous reviews were scanned and checked for further studies. Abstracts were identified through searching CAB abstracts database (1973 to November 2007) and American Thoracic Society conferences (2003-2007). No language restrictions were applied.

The exposures of interest were measures of dietary intake or of objective levels of vitamins in body fluids of the antioxidant vitamins A, C and E. The primary outcome measure used was asthma (physician-diagnosed, self-reported physician-diagnosed or defined as exercise-induced bronchospasm/constriction); secondary outcomes included wheeze, airway reactivity and asthma severity. Studies using spirometry or symptoms of cough and breathlessness as their main outcomes were excluded as these outcomes are likely to be relatively non-specific to asthma.

Two authors (SA and JL-B) reviewed the titles and then the abstracts, excluding irrelevant papers after each stage. Disagreements were resolved by discussion. The full text of the remaining papers was sought and the following exclusion criteria applied: reviews, duplicated studies, those without relevant outcomes and those in which no quantitative results or p values were presented, inability to source the text. Included studies were assessed for methodological quality using the Newcastle-Ottawa Scale for Quality Assessment (NOS).⁹ The median score of 6 was used to distinguish moderate and high quality studies from poorer quality studies.

Statistical analysis

Data were extracted independently by two authors (SA and JL-B). Dichotomous outcomes are presented as odds ratios (OR) with 95% confidence intervals (CI) and continuous outcomes are presented as mean differences (MD) with 95% CI. Conversions of biochemical units of measurement for the exposures were performed where necessary to allow for comparisons of units across studies.¹⁰

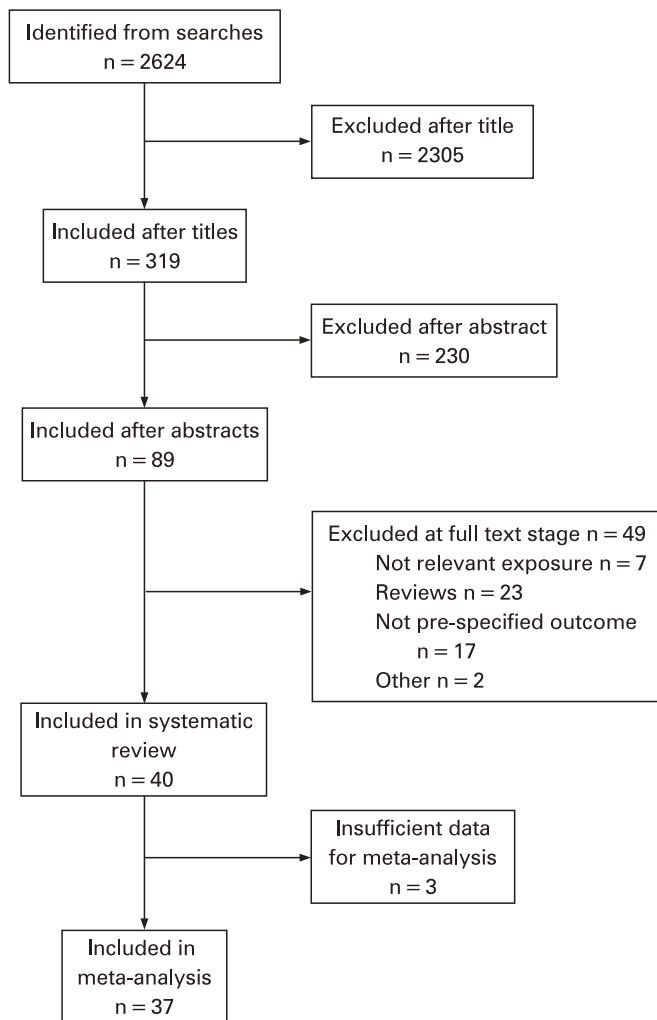


Figure 1 Flow chart for identifying studies.

Where exposure was expressed in quantiles, vitamin levels are expressed as high or low, typically representing a comparison of the lowest with the highest exposure categories.

Meta-analyses were performed to calculate weighted effect measures across studies using random effects models. Heterogeneity between the studies was assessed using I^2 ,¹¹ and if levels of heterogeneity ($I^2 > 75\%$) existed, then meta-analyses were not performed. Subgroup analyses relating to methods of asthma diagnosis, adult versus children and methodological quality of the studies were conducted to explore reasons for moderate levels of heterogeneity ($I^2 > 50\%$). Analysis was performed using Review Manager 4.2.9 (Version 4.2 for Windows, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2003); p values < 0.05 were considered statistically significant. The systematic review was carried out in accordance with the MOOSE guidelines.¹²

RESULTS

Overview of included studies

From an initial 2624 papers and abstracts identified from the literature searches, 89 had potentially eligible abstracts and 40 met the selection criteria (table 1, fig 1). Twenty-three of these assessed asthma as an outcome,^{13–35} 9 assessed wheeze,^{36–44} 5 assessed both asthma and wheeze,^{45–49} 2 assessed airway reactivity^{50–51} and 1 assessed both asthma and airway reactivity.⁵² Twenty-six

of the papers studied the effects of measures of dietary intake;^{16–19 23 25 27 29 31 32 34–38 40–45 47 49–52} 23 studied biochemical levels in serum or plasma^{13–15 17 18 20 21 23 24 26–28 30 31 33 34 36 37 39 42 46 48 50} and 1 assessed the effects of antioxidant levels in sputum.²² Further information about the individual studies is presented in the online supplement.

Methodological quality of the studies

The NOS scores for the 40 studies ranged from 3 to 9, with a median of 6; 26 of the 40 studies (65%) were scored as being of moderate or high level (≥ 6) methodological quality.

Dietary intake and serum levels of vitamin A

Overall, 21 papers reported the association of vitamin A or its components on one or more of the outcome measures,^{15–18 24 25 27–29 31 35 36 38–40 48 51 52} of which 11 papers assessed exposure to vitamin A,^{15–17 19 20 24 34 36 39 43 45} 10 assessed β -carotene,^{17 18 25 31 36 38 39 48 51 52} 2 assessed α -carotene,^{17 18} 6 assessed retinol^{25 28 36 40 51 52} and 4 assessed carotene.^{29 31 35 40} The results for β -carotene, α -carotene, retinol and carotene are presented in the online supplement.

Asthma as the outcome

Self-reported dietary intake was significantly reduced in asthma by 182 $\mu\text{g}/\text{day}$ (95% CI -288.42 to -75.25 ; $I^2 = 0\%$; 3 studies; fig 2). Pooled serum levels were also reduced in asthma (4 studies; all high quality and ascertained asthma using physician diagnosis), though not to the point of statistical significance (by 0.17 $\mu\text{mol}/\text{l}$ (95% CI -0.36 to 0.02); $I^2 = 59\%$; fig 2). However, the direction of the association seemed to differ between adults and children, with pooled serum levels being significantly reduced in the two studies in children (MD -0.25 $\mu\text{mol}/\text{l}$, 95% CI -0.40 to -0.10 ; fig 2).

Asthma severity as the outcome

Cases of severe asthma had significantly lower dietary intakes of vitamin A (MD -344 $\mu\text{g}/\text{day}$ (95% CI -575.17 to -112.64 ; $I^2 = 0\%$; 2 studies; fig 3) than cases of mild disease. Results of the four studies assessing serum levels showed extreme heterogeneity ($I^2 = 80\%$) so a pooled analysis was not carried out; however, a subgroup analysis suggested that physician-diagnosed cases of severe asthma (all studies of lower quality and conducted in adults) had significantly lower serum levels of vitamin A (MD -0.48 $\mu\text{mol}/\text{l}$, 95% CI -0.66 to -0.30 ; $I^2 = 0\%$; 3 studies; fig 3) than cases of mild disease. No relation was seen for self-reported severity of asthma (all conducted in children and of higher quality) (MD -0.02 , 95% CI -0.17 to 0.13; 1 study; fig 3).

Wheeze as the outcome

Although no association was seen between low dietary intakes of vitamin A and wheeze (OR 0.58, 95% CI 0.29 to 1.16; 1 study), low serum levels were significantly associated with a 24% decreased odds of wheeze (95% CI 0.63 to 0.92; 1 study).

Airway reactivity as the outcome

No significant associations were seen for total dietary intake of vitamin A (MD 0.02, 95% CI -0.08 to 0.12 per doubling intake).⁴³

Dietary intake and serum levels of vitamin C

A total of 32 papers reported the association with vitamin C.^{13 14 16 17 19 21–23 25 27–30 32–43 45–49 51 52}

Table 1 Characteristics of the included studies

Study	Design	Exposure	Dietary component measured [§]	Methodological quality score*	Geographical area	Population
Aderelle ¹³	Case-control	Plasma	C	8	Nigeria	Children
Anetor ¹⁴	Case-control	Serum	C	6	Nigeria	Adult
Arora ¹⁵	Case-control	Serum	A	8	India	Children
Baker ^{34,†}	Case-control	Serum, dietary	A, C, E	8	England	Adult
Bodner ³⁶	Nested case-control	Plasma, dietary	A, C, E	8	Scotland	Adult
Burns ⁴⁹	Cross-sectional	Dietary	C, E	5	USA and Canada	Children
Cook ³⁷	Cross-sectional	Plasma, dietary	C	4	England and Wales	Children
De Luis ¹⁶	Case-control	Dietary	A, C, E	7	Spain	Adult
Fogarty ^{50,‡}	Cross-sectional	Serum, dietary	E	6	England	Adult
Ford ¹⁷	Cross-sectional	Serum, dietary	A, C, E	4	USA	Adult
Garcia-Larsen ⁴³	Cross-sectional	Dietary	A, C, E	5	Chile	Adult
Gibson ^{18,†}	Case-control	Serum	A	7	Australia	Adult
Grievink ³⁸	Cross-sectional	Dietary	A, C, E	5	Netherlands	Adult
Hijazi ⁴⁵	Case-control	Dietary	A, C, E	7	Saudi Arabia	Children
Huang ¹⁹	Cross-sectional	Dietary	A, C, E	5	Taiwan	Children
Kalayci ^{20,‡}	Case-control	Serum	A, C, E	7	Turkey	Children
Kelly ²¹	Case-control	Plasma	C, E	6	Sweden	Adult
Kelly ³⁹	Cross-sectional	Serum	A, C, E	7	Scotland	Adult
Kongerud ²²	Case-control	Sputum	C, E	3	USA	Adult
Mainous ⁴⁶	Cross-sectional	Serum	C	4	USA	Adult
Misso ²³	Case-control	Serum, dietary	C	8	Australia	Adult
Mizuno ²⁴	Case-control	Serum	C, E	7	Japan	Children
Murray ^{40,†}	Case-control	Dietary	A, C, E	7	England	Children
Nagel ^{25,*}	Case-control	Dietary	A, C, E	7	Germany	Adult
Olusi ^{26,‡}	Case-control	Serum	C	5	Nigeria	Not specified
Omenaas ⁴¹	Cross-sectional	Dietary	C	5	Norway	Adult
Patel ³⁵	Population-based case-control	Dietary	A, C, E	8	England	Adult
Picado ²⁷	Case-control	Serum, dietary	A, C, E	7	Spain	Adult
Powell ^{28,†}	Cross-sectional	Serum	A, C, E	6	England	Children
Romieu ⁴⁷	Cross-sectional	Dietary	C	5	USA	Children
Rubin ⁴⁸	Cross-sectional	Serum	A, C, E	6	USA	Children
Salam ⁴⁴	Cohort	Dietary	A, C, E	7	USA	Children
Schwartz ⁴²	Cross-sectional	Serum, dietary	A, C	6	USA	Any age
Shaheen ²⁹	Case-control	Dietary	A, C, E	5	England	Adult
Shidfar ³⁰	Case-control	Serum	C	7	Iran	Adult
Soutar ⁵¹	Case-control	Dietary	A, C, E	8	Scotland	Adult
Tan-Un ³¹	Case-control	Serum, dietary	A, E	6	China	Adult
Troisi ³²	Cohort	Dietary	A, C, E	7	USA	Adult
Vural ³³	Case-control	Serum	C	4	Turkey	Adult
Woods ⁵²	Cross-sectional	Dietary	A, C, E	5	Australia	Adult

*Methodological quality of studies based on the Newcastle-Ottawa Quality Assessment Scale. †Non-parametric data presented in article. ‡Excluded from meta-analysis due to insufficient data. §Constituent of vitamin A or E measured.

Asthma as the outcome

Low levels of dietary vitamin C intake were associated with a significant increase in the relative odds of asthma (OR 1.12, 95% CI 1.04 to 1.20; $I^2 = 0\%$; 9 studies; fig 4). Meta-analysis of studies reporting serum vitamin C levels and the odds of asthma was not carried out because of extreme levels of heterogeneity ($I^2 = 94\%$; 2 studies; both studies were of moderate to high quality and used self-reported asthma diagnosis); however, individual studies showed that low levels of serum vitamin C were associated with increased odds of asthma (OR 1.01, 95% CI 1.00 to 1.02 per 1 $\mu\text{mol/l}$ increase;³⁵ OR 1.22, 95% CI 1.11 to 1.33).⁴⁸

The mean dietary intake of vitamin C was not significantly different in people with asthma compared with those without asthma (MD -8.62 mg/day, 95% CI -19.77 to 2.53 ; $I^2 = 19\%$; 5 studies). Pooled analyses of mean serum levels of vitamin C and asthma status could not be performed owing to high levels of heterogeneity ($I^2 = 100\%$; 9 studies), primarily relating to one

study¹⁴ in which extremely high levels of vitamin C were seen in the control group (mean 272.54 $\mu\text{mol/l}$). A sensitivity analysis restricted to high-quality studies (NOS ≥ 8) excluded this finding¹⁴ and revealed that people with asthma had mean serum levels that were significantly lower (by 16.8 $\mu\text{mol/l}$, 95% CI -21.5 to -12.1 ; $I^2 = 0\%$; 2 studies), while non-significant reductions were seen in moderate and lower quality studies (fig 5). Subgroup analyses based on age (adult: $I^2 = 99.5\%$, 6 studies; children: $I^2 = 84\%$, 2 studies) and method of asthma ascertainment (physician-diagnosed: $I^2 = 88\%$, 6 studies; self-reported: 1 study; unknown method: 1 study) indicated that these factors did not explain any of the observed heterogeneity.

Asthma severity as the outcome

Similar dietary intake (MD -16.13 mg/day, 95% CI -41.02 to 8.76 , $I^2 = 0\%$; 2 studies) and serum levels (MD -5.41 $\mu\text{mol/l}$, 95% CI -14.17 to 3.35 ; $I^2 = 67\%$; 4 studies) of vitamin C were seen between severe and non-severe asthma.

Figure 2 Dietary intake ($\mu\text{g/day}$) and serum levels ($\mu\text{mol/l}$) of vitamin A and asthma. Mean differences relate to the difference in mean dietary intakes or serum levels of vitamin A. Squares are the difference in means between people with asthma and those without asthma, and bars represent 95% confidence intervals (CI). Negative mean differences indicate lower levels seen in subjects with asthma than in those without asthma. Arrows on the end of bars indicate that the limits are beyond the scale presented.

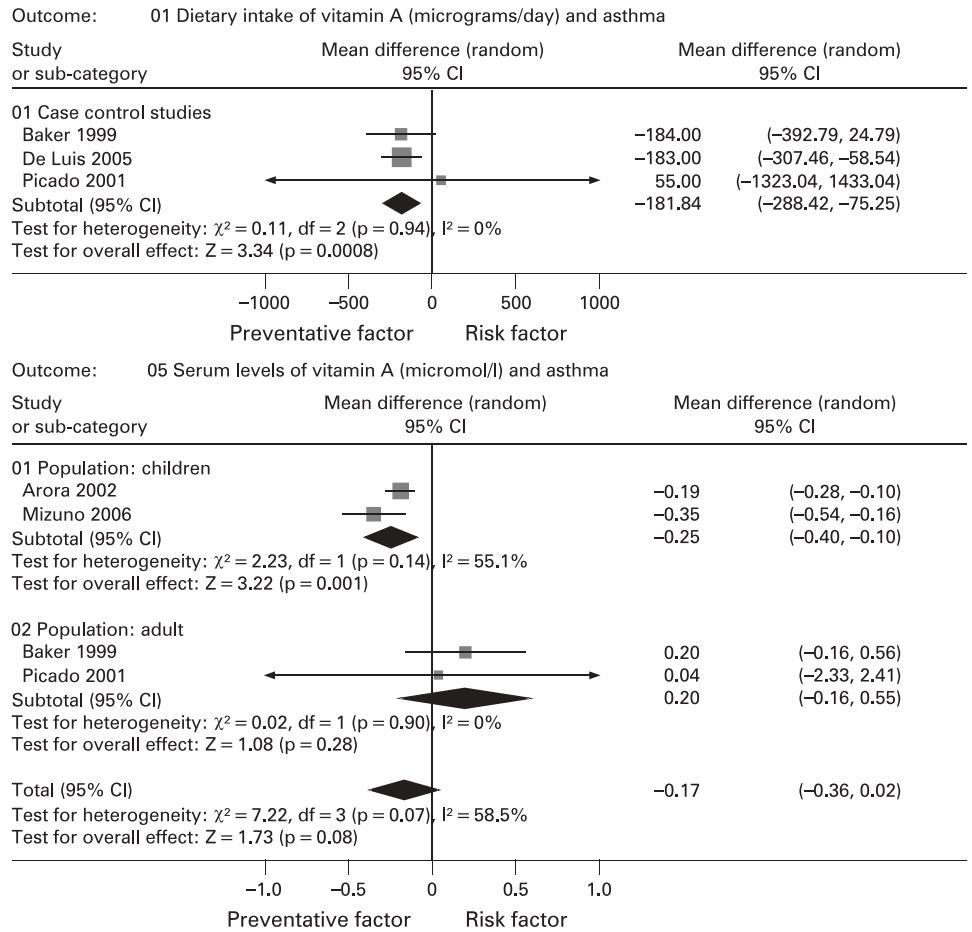


Figure 3 Dietary intake ($\mu\text{g/day}$) and serum levels ($\mu\text{mol/l}$) of vitamin A and asthma severity. Mean differences relate to the difference in mean dietary intakes or serum levels of vitamin A. Squares are difference in means between people with severe asthma and those without severe asthma, and bars represent 95% confidence intervals (CI). Negative mean differences indicate that lower levels are seen in people with severe asthma than in those with mild asthma. Arrows on the end of bars indicate that the limits are beyond the scale presented.

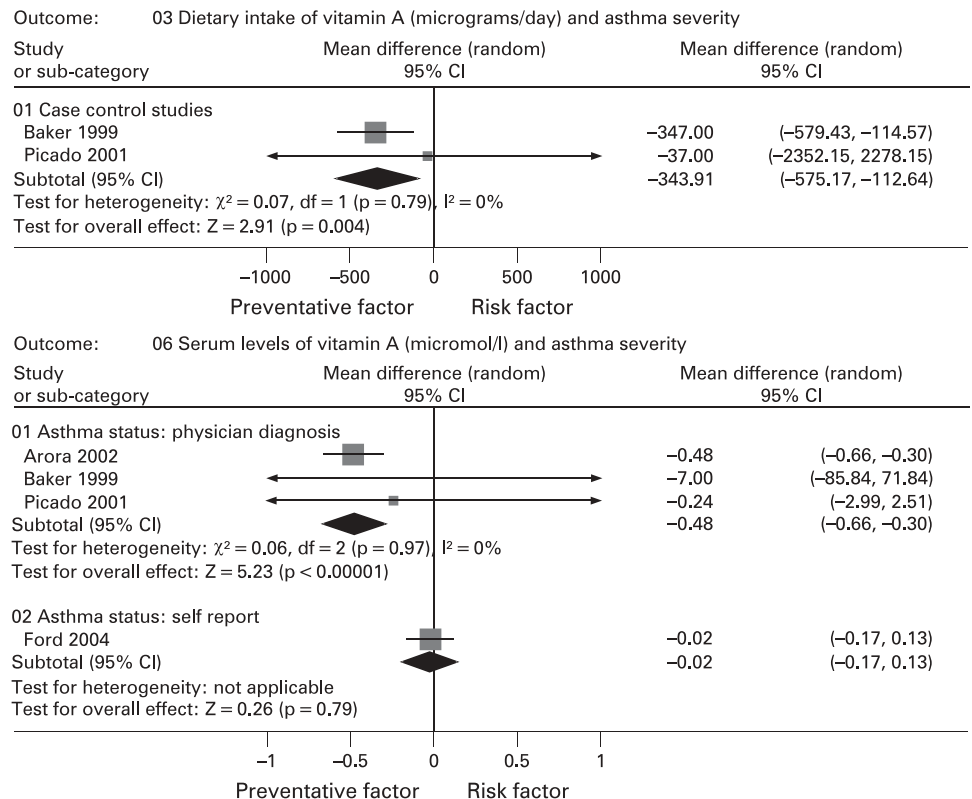


Figure 4 Dietary intakes (mg/day) of vitamin C and asthma. Odds ratios relate the ratio of odds of low dietary levels of vitamin C in people with asthma compared with those without, and bars represent 95% confidence intervals (CI). Odds ratios <1 indicate that lower dietary levels of vitamin C are seen in people with asthma than in those without asthma. Arrows on the end of bars indicate the limits are beyond the scale presented.

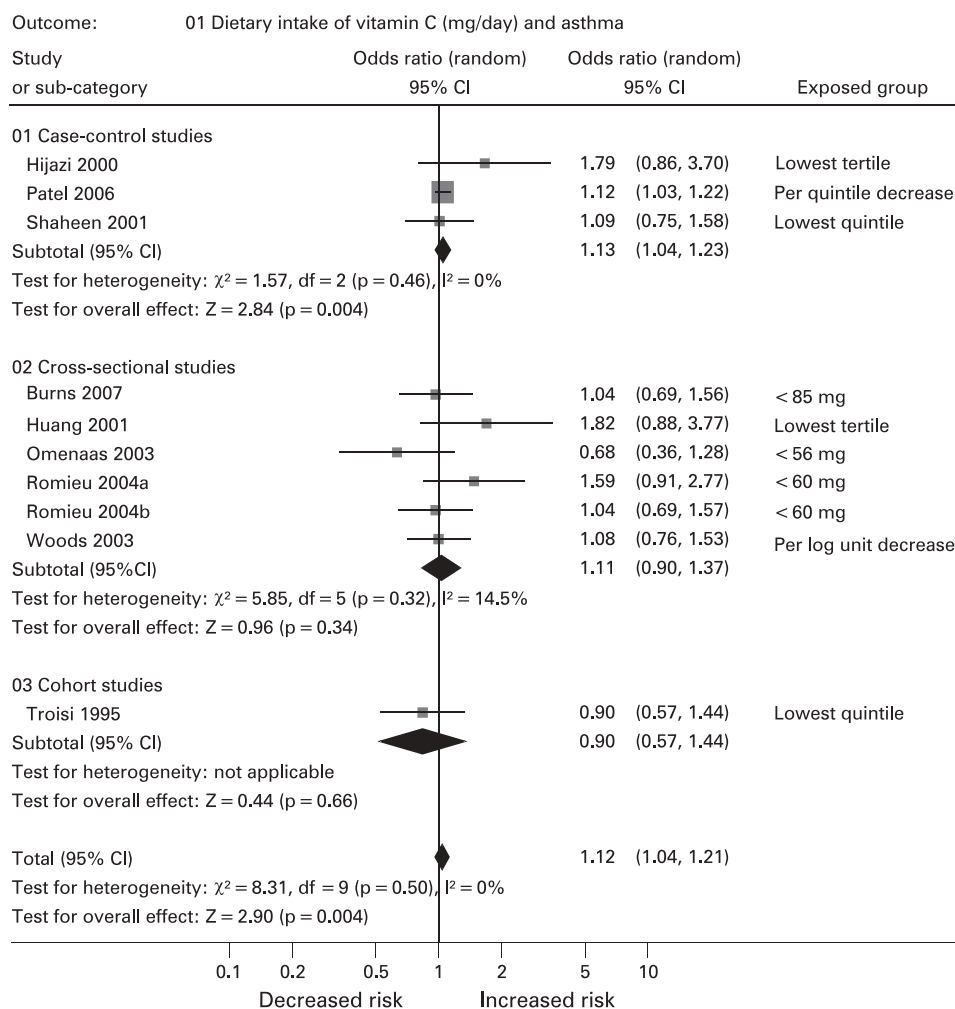


Figure 5 Serum levels ($\mu\text{mol/l}$) of vitamin C and asthma. Mean differences relate to the difference in mean serum levels of vitamin C. Squares are differences in mean between people with asthma and those without asthma and bars represent 95% confidence intervals (CI). Negative mean differences indicate that lower levels are seen in people with asthma than in those without asthma. Arrows on the end of bars indicate the limits are beyond the scale presented.

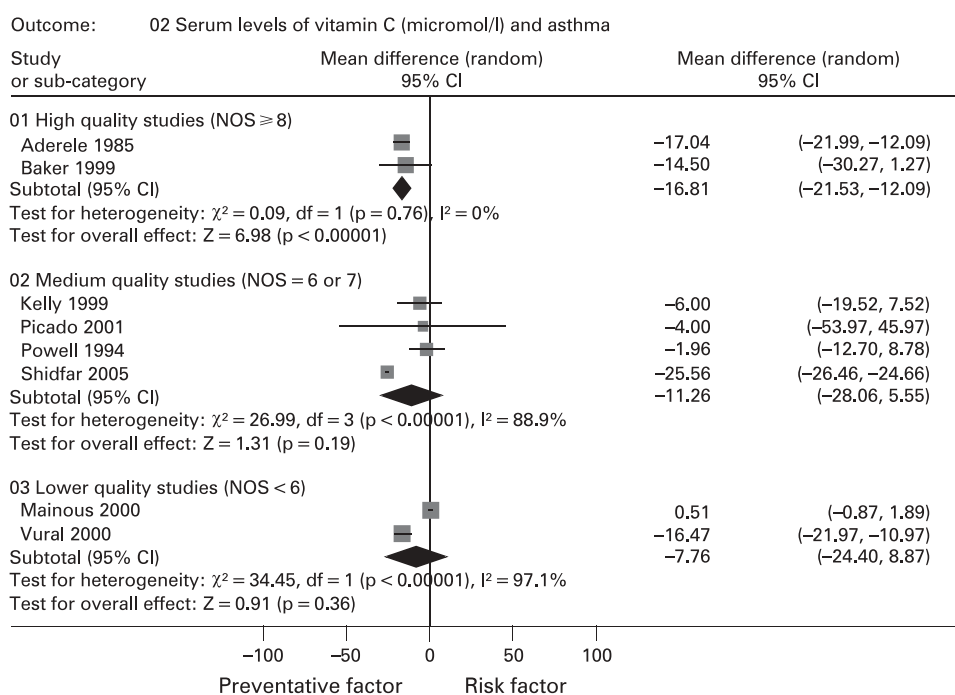
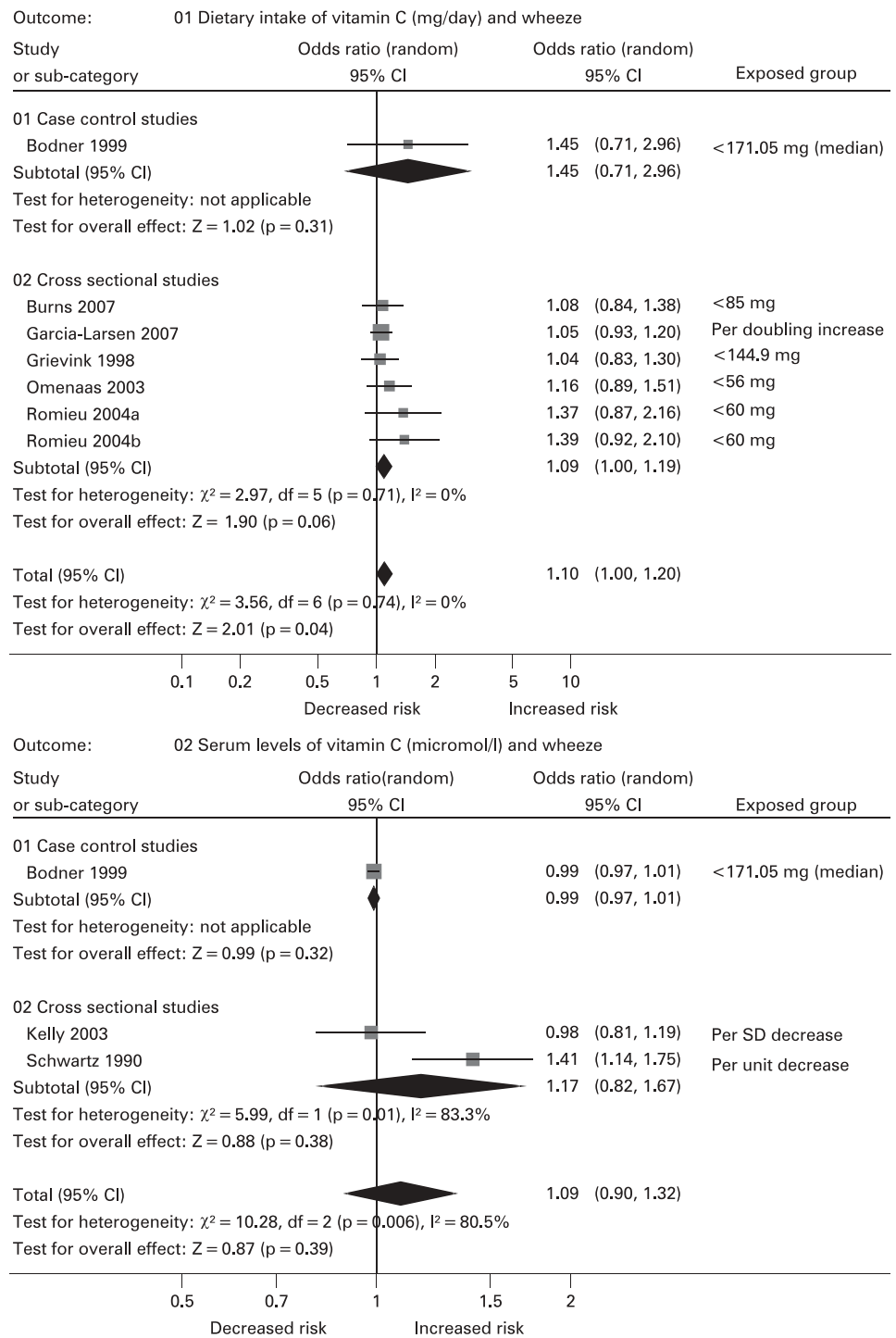


Figure 6 Dietary intake (mg/day) and serum levels ($\mu\text{mol/l}$) of vitamin C and wheeze. Odds ratios relate the ratio of odds of low dietary intake or serum levels of vitamin C in people with wheeze compared with those without, and bars represent 95% confidence intervals (CI). Odds ratios <1 indicate that lower levels of vitamin C are seen in people with wheeze than in those without wheeze. Arrows on the end of bars indicate the limits are beyond the scale presented.



Wheeze as the outcome

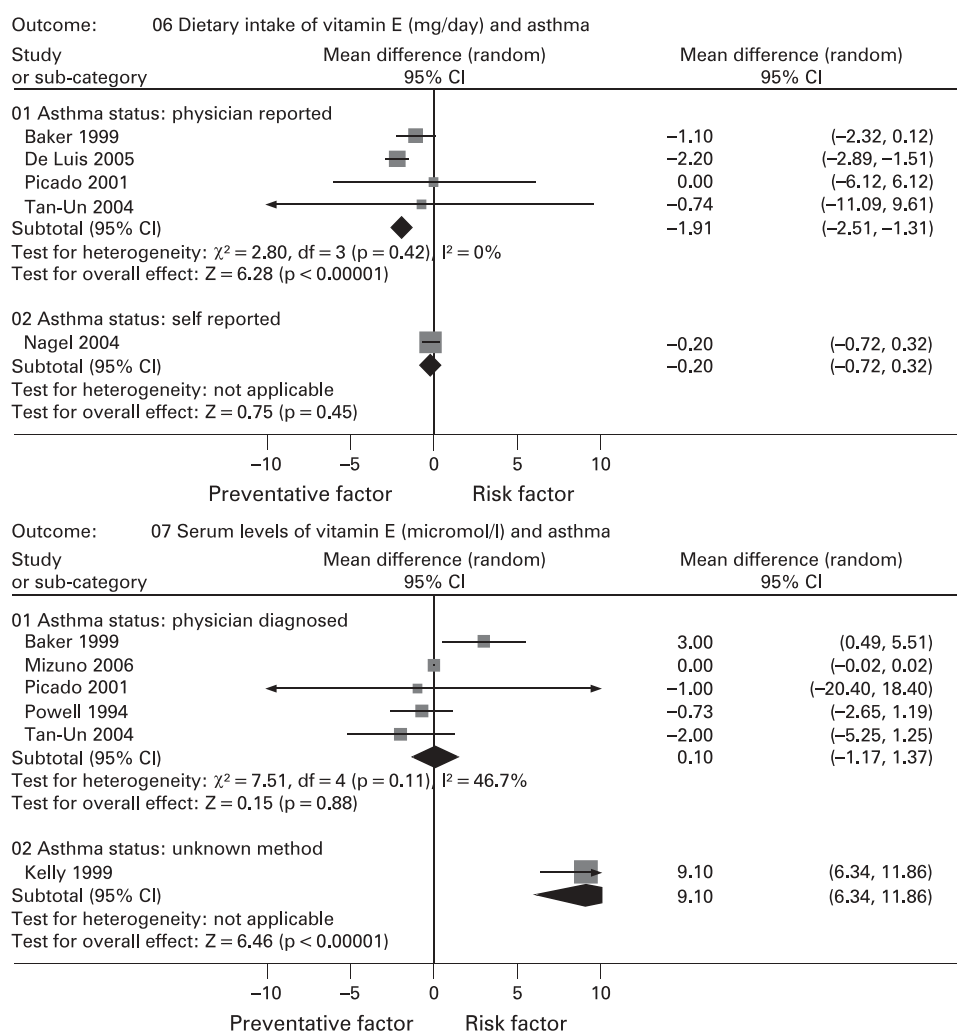
An increased odds of wheeze was associated with lower dietary intakes of vitamin C (OR 1.10, 95% CI 1.00 to 1.20; p = 0.04 $I^2 = 0\%$; 6 studies; fig 6). Extreme levels of heterogeneity were seen between the studies that assessed serum levels of vitamin C and the odds of wheeze (3 studies; $I^2 = 81\%$), therefore pooled analyses were not performed. All three studies were of moderate to high quality and assessed wheeze using self-reported questionnaire; however, a subgroup analysis based on age found that no effect was present in adults (OR 0.99, 95% CI 0.97 to 1.01; $I^2 = 0\%$; 2 studies), but a significant 41% increase in the odds of wheeze was seen in the study that included participants

of all ages (95% CI 1.14 to 1.75).⁴² Additionally, mean dietary intake (MD 4.70 mg/day, 95% CI -26.6 to 36.0; 1 study) and mean serum vitamin C (MD 0.06 $\mu\text{mol/l}$, 95% CI -1.00 to 1.12; $I^2 = 0\%$; 2 studies) were similar in people with and without wheeze.

Airway reactivity as the outcome

Three heterogeneous studies conducted in adults^{43 51 52} reported the relation between dietary intake of vitamin C and airway reactivity as measured using methacholine challenge. Two studies found evidence of a significant increase in airway reactivity with lower levels of vitamin C (OR 7.14; 95% CI 1.92

Figure 7 Dietary intakes (mg/day) and serum levels ($\mu\text{mol/l}$) of vitamin E and asthma. Mean differences relate to the difference in mean dietary intake or serum levels of vitamin E. Squares are differences in mean between people with asthma and those without asthma, and bars represent 95% confidence intervals (CI). Negative mean differences indicate that lower levels are seen in people with asthma than in those without asthma. Arrows on the end of bars indicate the limits are beyond the scale presented.



to 25.0;⁵¹ MD -0.12, 95% CI -0.22 to -0.03 per doubling in intake),⁴³ but no association was seen in the other study (OR 1.04, 95% CI 0.76 to 1.43).⁵²

Dietary intake and serum levels of vitamin E

Twenty-four studies reported the association with vitamin E levels,^{16 17 21 22 24 25 27-29 31 32 34-36 38-40 43-45 48 49 51 52} of which 4 assessed α -tocopherol in particular.^{21 28 31 36}

Asthma as the outcome

Pooled analyses could not be performed to assess the relation between mean dietary intake ($I^2 = 81\%$; 5 studies) or mean serum levels ($I^2 = 90\%$; 6 studies) of vitamin E and asthma due to extreme heterogeneity. All of the studies that measured mean dietary intake of vitamin E were of moderate to high quality and conducted in adults, and therefore only subgroup analyses based on ascertainment of asthma status were conducted. Significantly lower mean levels of dietary vitamin E were associated with asthma in studies which used physician diagnoses (MD -1.91 mg/day, 95% CI -2.51 to -1.31; $I^2 = 0\%$; 4 studies; fig 7), however no effect was seen for self reported asthma status (MD -0.20 mg/day; 95% CI -0.72 to 0.32; 1 study; fig 7). All of the studies that measured serum levels of vitamin E were of high quality, so subgroup analyses based on age and ascertainment of asthma were conducted. Age did not explain the observed heterogeneity (adults: MD 3.12,

95% CI -2.53 to 8.76, $I^2 = 89\%$, 4 studies; children: MD 0.00, 95% CI -0.02 to 0.02, $I^2 = 0\%$; 2 studies), and no significant difference in mean serum vitamin E levels were seen with asthma in studies which used physician diagnoses (MD 0.10 $\mu\text{mol/l}$, 95% CI -1.17 to 1.37; $I^2 = 47\%$; 5 studies; fig 7). However, significantly increased levels of vitamin E were seen in the study which did not define asthma status (MD 9.10 $\mu\text{mol/l}$, 95% CI 6.34 to 11.86; 1 study; fig 7).

No relation was seen between the odds of asthma and lower dietary intakes of vitamin E (OR 1.23, 95% CI 0.90 to 1.69; $I^2 = 74\%$; 6 studies) or serum levels of vitamin E (OR 1.04, 95% CI 0.91 to 1.19; 1 study). All of the studies of the relation between dietary intake of vitamin E and the odds of asthma (6 studies) used self-reported ascertainment of asthma. A subgroup analysis of these studies showed a non-significant trend towards an increased odds of asthma with lower levels of dietary vitamin E (adults: OR 1.25, 95% CI 0.93 to 1.67; $p = 0.13$; $I^2 = 53\%$; 4 studies); however, studies were too heterogeneous for comparisons in children ($I^2 = 91\%$; 2 studies). A subgroup analysis based on quality of the studies did not explain any of the observed heterogeneity between the 6 studies (moderate to high quality: $I^2 = 84\%$, 3 studies; low quality: $I^2 = 63\%$, 3 studies).

Asthma severity as the outcome

Significantly lower dietary intake was seen in those with severe asthma (MD -1.20 $\mu\text{g/day}$, 95% CI -2.29 to -0.11; $I^2 = 0\%$; 2

studies) but there was no difference in mean serum levels (MD $-0.34 \mu\text{mol/l}$, 95% CI -3.79 to 2.94 ; $I^2 = 31.5\%$; 3 studies) compared with those with less severe disease.

Wheeze as the outcome

Similar levels of dietary intakes of vitamin E were seen in people with wheeze and those without (MD $0.80 \mu\text{g/day}$, 95% CI -0.23 to 1.83 ; 1 study). No relation was seen between the odds of wheeze and lower serum levels (OR 1.08 , 95% CI 0.88 to 1.30 ; 1 study) or lower levels of dietary intake (OR 0.90 , 95% CI 0.68 to 1.20 ; $I^2 = 66\%$; 5 studies).

Airway reactivity as the outcome

No association was seen between the risk of airway reactivity and lower dietary intakes of vitamin E (OR 1.06 , 95% CI 0.48 to 2.38 ; $I^2 = 39\%$; 2 studies; MD -0.08 , 95% CI -0.26 to 0.09 per doubling intake; 1 study).

DISCUSSION

This systematic review and meta-analysis of the evidence on antioxidant vitamin intake and asthma status shows a consistent negative association between overall dietary vitamin A intake and the odds of asthma and severe asthma, although findings for wheeze were less consistent. The mean deficit of $182 \mu\text{g}$ vitamin A per day in people with asthma relates to 26% and 30% of the latest recommended daily intakes of vitamin A for men and women, respectively.⁵⁴ Additionally, most of the studies assessing the association found mean levels of vitamin A in people with asthma were below the recommended daily intakes. Vitamin A intake was lower in people with severe asthma than in those with less severe asthma by the equivalent of about half the recommended daily amount (50% in men and 57% in women). The results for the specific types of vitamin A (retinol, α -carotene, total carotenoids and carotene) did not generally reach statistical significance at the 5% level; however, there was a tendency for increased serum β -carotene levels to be associated with reduced odds of asthma.

For vitamin C we found evidence of an increase of approximately 12% in the odds of asthma associated with lower levels of intake of vitamin C. Results from individual studies also suggested that mean serum levels tended to be lower in people with asthma. These findings were generally supported by the results for wheeze and airway reactivity; however, associations with asthma severity were not conclusive. Measures of vitamin E were, however, generally unrelated to asthma status, although significantly lower mean dietary intakes (by approximately 2 mg/day , about 20% of the daily recommended intake of vitamin E) were seen in studies of people with physician-diagnosed asthma.

These findings contrast with those of a recent meta-analysis of published studies⁵⁵ which found no significant association between dietary antioxidant intake and asthma or lung function. However, this meta-analysis searched only for studies in adults published in English language journals and listed in only one electronic database, and the meta-analyses were based on a combined outcome of asthma or wheeze. The present study was conducted with higher methodological rigor, in accordance with the MOOSE guidelines,¹² used more comprehensive sources and search strategies and, where possible, validated search terms.^{8 56 57}

One concern relating to our analysis is that the methods used to ascertain levels of the antioxidant vitamins (either through dietary intake, plasma and serum or sputum levels) were

inconsistent over the range of the studies included. Even within a particular type of ascertainment such as food frequency questionnaires, substantial variation existed between the methods used and the periods covered by the questionnaire, some assessing dietary intake over the last 12 months and some over 6 months. In addition, due to the design of the majority of studies included in the review, the levels of antioxidant vitamins were assessed after the onset of asthma. The use of both food frequency questionnaires and of biological levels to estimate dietary intake have recognised strengths and weaknesses, but a major strength of questionnaires is that they measure typical dietary patterns over several months and, while the quantitative estimates of intake they provide are relatively imprecise, their ranking of intake is more representative⁵⁸ and therefore suitable for combination in the meta-analysis of quantile effects. Biochemical levels offer the advantage of objectivity but often reflect only relatively recent intake and may also be subject to homeostatic or other metabolic or excretory influences that distort the relation between biological level and dietary intake. For example, levels of vitamin C in peripheral blood show relatively little variation between intakes above the minimum recommended daily intake⁵⁹ and are therefore less representative of between-subject differences in intake than questionnaire measures. Thus, while dietary and serum measures are correlated for vitamin A,⁶⁰ vitamin C⁶¹ and vitamin E⁶² and, in particular, strong correlations have been reported for antioxidant vitamins in people with asthma,⁶¹ these methodological differences are still likely to cause some discrepancy in the observed relations between diet and disease.

Most of the studies we analysed did not report adjusted results, so the results from our analyses are primarily based on crude estimates which were either extracted or estimated from these studies and so are subject to the unadjusted effects of confounding factors such as age,^{26 63} socioeconomic status,^{13 35} smoking,^{36 64} body mass index,⁴⁷ the effects of correlated intakes of other antioxidant vitamins and, in particular for vitamin E, the effects of total cholesterol and lipid levels. The most common factors adjusted for in the included studies were age (23 studies), sex (18 studies), smoking (active or passive, 16 studies) and social class (12 studies); only 6 studies adjusted for the effects of other antioxidant vitamins in their analyses.

Overall, our findings from this systematic review and meta-analysis indicate that low levels of vitamin C intake and—to a lesser extent—vitamin A are consistently associated with asthma risk to a degree that, if causal, would be sufficient to be clinically relevant. These findings are plausible, given the recognised anti-inflammatory and antioxidant actions of these vitamins.⁵⁰ However, these conclusions have not been supported by the limited data available from randomised clinical trials of dietary supplementation with vitamin C for which a recent Cochrane review concluded that there is no appreciable effect.³ One explanation for this discrepancy would be that the observational data are systematically flawed by biases leading to spurious results from meta-analyses,⁶⁵ and particularly publication bias. We attempted to minimise the effects of publication bias by performing comprehensive searches that included “grey” literature; however, we were unable to formally test for publication bias due to the small numbers of studies included in the meta-analyses. Another possibility is that the observed relation between nutrient intake and disease is not causal but arises from correlation with other causally-related nutrients or non-dietary exposures. Alternatively, the observation may reflect a causal association arising from dietary intakes during early life that tend to track into adulthood, or from more

sustained periods of intake than have been tested in randomised trials to date. Reverse causation is also a potential explanation, particularly in severe asthma,³⁴ but there is no evidence that this occurs in the milder disease that predominated in the studies analysed.

The epidemiological evidence thus suggests that vitamins A and C are linked to asthma. Epidemiological studies and meta-analyses are useful for identifying association between exposures and diseases⁶⁵ but cannot reliably establish causation. Further investigations are necessary to account for the observed associations using well-designed randomised controlled trials of vitamin supplementation in asthma. Trials of vitamin C supplementation to date have been disappointing; whether the effect of vitamin A will prove more important to clinical management or whether the observed associations with diet are due to confounding effects will only be resolved by further clinical trials.

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SUPPLEMENTARY MATERIAL

Additional information on Results

A variety of methods were used to ascertain asthma status. Physician diagnosis of asthma was used in 16 studies [13-16, 18, 20, 22-24, 26-28, 30, 31, 33, 34]. Twelve studies established asthma status using a self report questionnaire [17, 19, 25, 29, 32, 35, 45-49, 52]. In these studies the questionnaires all asked whether the participant had been diagnosed by a doctor/physician as having asthma, with the exception of one study [29], in which the question 'are you currently taking medication for asthma?' was asked. Although the ascertainment of asthma status varied across the papers, the questions used were mainly based on, or similar to, those used in the ISAAC questionnaire [53]. Only one paper did not fully describe how they defined asthma [21]. Wheezing was established in all papers through self-reported questionnaire, and used similar questions also typically based on, or similar to, the ISAAC questions [53]. Eleven studies assessed wheezing in the last 12 months [35, 37, 38, 41-48] and one study wheezing since the age of 15 years [36]. The remaining 3 studies either assessed current wheeze [39, 49] or defined wheeze as at least 3 previous episodes [40]. Four studies used assessments of airway hyper-responsiveness measured in all cases using methacholine challenge [43, 50-52].

Twenty-five studies included only adults [14, 16-18, 21-23, 25, 27, 29-33, 35, 36, 38, 39, 41, 43, 46, 50-52], 13 only children [13, 15, 19, 20, 24, 28, 37, 40, 44, 45, 47-49], one included both [42] and one did not specify the age of its subjects [26]. The majority of studies were conducted in the US or the UK (Table 1). Two studies used a cohort design [32, 44], and the rest used either cross-sectional surveys [17, 19, 28,

37-39, 41-43, 46-50, 52] or case-control designs [13-16, 18, 20-27, 29-31, 33-36, 40, 45, 51].

β-carotene, α-carotene, retinol and carotene studies

Asthma as the outcome

Five studies [17, 18, 25, 48, 52] assessed the effect of β-carotene. No significant effect of mean levels of intake of β-carotene was seen (MD -100μg/day, 95% CI -204.73 to 3.26; I²=0%; 2 studies), or of low levels (OR 0.96, 95% CI 0.67 to 1.39; 1 study), though other individual studies suggested that mean serum levels of β-carotene were significantly lower in asthma (MD -0.08μmol/l, 95% CI -0.15 to -0.01) [17], and that lower serum levels were associated with a significantly increased risk of asthma (OR 1.12, 95% CI 1.04 to 1.21) [48].

The effect of dietary and serum levels of α-carotene on asthma was studied in only one report [18], which found no evidence of a relation (dietary: MD -80μg/day, 95% CI -1665.93 to 1505.93; serum: MD 0.00μmol/l, 95%CI -45.29 to 45.29). Individual results from three studies [18, 25, 28] found no evidence of association for the effect of retinol on asthma (OR 1.30, 95% CI 0.91 to 1.85; mean dietary intake: MD 0.00μg/day, 95% CI -45.83 to 45.83; serum levels: MD -0.13μmol/l, 95% CI -0.29 to 0.03). Results suggested that intake of total carotenoids (MD -0.02 μg/day, 95% CI -1189 to 1189; I²=0%; 2 studies [18, 31] and serum levels (MD -18.00μg/l, 95% CI -552.09 to 516.09; 1 study [18] were similar in asthma and in controls.

Asthma severity as the outcome

Data from one study [17] found no relation between levels of α -carotene and asthma severity (MD $-0.02\mu\text{mol/l}$, 95% CI -0.05 to 0.01). No studies reported the relation between β -carotene or retinol and asthma severity.

Wheeze as the outcome

Three studies [36, 38, 39] assessed the relation between β -carotene and wheeze; no associations were seen between wheeze and low dietary intake levels (OR 1.22, 95% CI 0.94 to 1.56; $I^2=12\%$, 2 studies) or low serum levels (OR 0.66, 95% CI 0.27 to 1.59; $I^2=67\%$, 2 studies). Data from one case-control study [40] found similar dietary intake levels of carotene (MD $419.70\mu\text{g/day}$, 95% CI -130.59 to 969.99) and retinol (MD $0.00\mu\text{g/day}$, 95% CI -47.25 to 47.25) in participants who reported wheeze and those who did not. Data from one case control study [36] found no relationship between low serum levels of retinol and the risk of wheeze (OR 1.30, 95% CI 0.98 to 1.79).

Airway reactivity as the outcome

No significant associations were seen for total dietary intake of vitamin A (MD 0.02, 95% CI -0.08 to 0.12 per doubling intake [43]) or for the odds of airway reactivity for lower dietary intakes of retinol (OR 1.05, 95% CI 0.78 to 1.41; $I^2=0\%$, 2 studies [18, 51]) or lower dietary intakes of β -carotene (OR 1.56, 95% CI 0.46 to 5.26; $I^2=73\%$, 2 studies [18, 51]).