Dietary intake of vitamin A, lung function and incident asthma in childhood

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Abstract

Background Longitudinal epidemiological data are scarce on the relationship between dietary intake of vitamin A and respiratory outcomes in childhood. We investigated whether a higher intake of preformed vitamin A or pro-vitamin β-carotene in mid-childhood is associated with higher lung function and with asthma risk in adolescence.

Methods In the Avon Longitudinal Study of Parents and Children, dietary intakes of preformed vitamin A and β-carotene equivalents were estimated by food frequency questionnaire at 7 years of age. Post-bronchodilator forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and forced expiratory flow at 25–75% of FVC (FEF25–75%) were measured at 15.5 years and transformed to z-scores. Incident asthma was defined by new cases of doctor-diagnosed asthma at age 11 or 14 years.

Results In multivariable adjusted models, a higher intake of preformed vitamin A was associated with higher lung function and a lower risk of incident asthma: comparing top versus bottom quartiles of intake, regression coefficients for FEV1 and FEF25–75% were 0.21 (95% CI 0.05–0.38; ptrend=0.008) and 0.18 (95% CI 0.03–0.32; ptrend=0.02), respectively; odds ratios for FEV1/FVC below the lower limit of normal and incident asthma were 0.49 (95% CI 0.27–0.90; ptrend=0.04) and 0.68 (95% CI 0.47–0.99; ptrend=0.07), respectively. In contrast, there was no evidence for association with β-carotene. We also found some evidence for modification of the associations between preformed vitamin A intake and lung function by BCMO1, NCOR2 and SCGB1A1 gene polymorphisms.

Conclusion A higher intake of preformed vitamin A, but not β-carotene, in mid-childhood is associated with higher subsequent lung function and lower risk of fixed airflow limitation and incident asthma.

Introduction

Vitamin A is a versatile vitamin involved in multiple biological processes including lung development through regulating the expression of several hundred genes [1]. As an essential micronutrient, vitamin A must be obtained from the diet either as preformed vitamin A, comprising mainly retinyl esters in animal foods, or as pro-vitamin A, comprising mainly β-carotene in plant foods [1]. In contrast to preformed vitamin A, β-carotene is converted to retinoids, with different bioconversion abilities partly explained by genetic variability [2].

Animal data suggest that retinoic acid, the ultimate metabolite of vitamin A, plays a crucial role early in life in alveolar development [3], maintenance and regeneration [4], and thus influences elastic recoil [3]; studies also suggest a key role in airway development [5], although evidence for an influence of vitamin A deficiency on airway hyperresponsiveness is conflicting [6, 7]. However, in humans the relationship

https://doi.org/10.1183/13993003.04407-2020
between dietary vitamin A and respiratory outcomes is not clear, especially in ranges of intake that do not cause severe hypovitaminosis. Follow-up of a trial in a vitamin A-deficient area showed that vitamin A supplementation in early life (peri-pregnancy) improved offspring lung function [8], with no impact on the subsequent risk of asthma [9]. In a birth cohort study in Norway, excess vitamin A intake in pregnancy was associated with a higher risk of childhood asthma [10], while some case–control studies in children have suggested an inverse association between dietary or serum vitamin A and asthma [11]. In adults, an inverse association between serum retinol and subsequent airway obstruction was reported [12], whereas more recently a positive association was found between vitamin A intake and asthma [13].

Maximal attainment of lung function as a young adult through optimal growth is important, as lung function in early adulthood is a powerful predictor of subsequent comorbidities and mortality [14, 15]. Prenatal and early postnatal time windows are critical time windows for lung development [16], and tracking of lung function from infancy, through childhood, to adulthood has been clearly demonstrated [17–19]. However, reports that alveolarisation continues throughout childhood [20, 21] suggest that catch-up of alveolar growth, at least, is possible beyond infancy. Moreover, recent epidemiological studies have also shown that accelerated growth in forced expiratory volume in 1 s (FEV₁) occurs in a proportion of children [18, 19].

We know little about environmental influences on catch-up growth [22], and longitudinal epidemiological data on the link between diet in childhood and later respiratory outcomes are scarce [23–26]; in particular, such evidence for dietary intake of vitamin A in childhood is, to the best of our knowledge, absent.

In this study, we have explored the relationships of preformed and pro-vitamin A intake at 7 years of age with lung function and incident asthma up to adolescence. To strengthen causal inference we have also explored whether these associations were modified by polymorphisms linked to bioconversion of β-carotene or metabolism of vitamin A, and by a polymorphism in the gene encoding club cell secretory protein; serum levels of the latter are increased by vitamin A supplementation [27] and positively associated with lung function growth in childhood [28].

Methods

Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort that recruited predominantly White pregnant women resident in Avon, UK (14,541 pregnancies) with expected dates of delivery from 1 April 1991 to 31 December 1992. The cohort has been followed since birth with annual questionnaires and, since age 7 years, with objective measures in annual research clinics. The study protocol has been described previously [29, 30] and further information can be found at www.alspac.bris.ac.uk, which contains details of all the data that are available (www.bristol.ac.uk/alspac/researchers/our-data). Ethics approval was obtained from the ALSPAC Ethics and Law Committee (IRB 00003312) and the Local National Health Service Research Ethics Committees. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004).

Exposure assessment

We used dietary information collected by food frequency questionnaire (FFQ) at 81 months (~7 years) of age, which was completed by the child’s mother or the main carer. The FFQ included questions about usual consumption of up to 56 food groups and 12 drinks, with five frequency options ranging from “never or rarely” to “more than once a day” and daily consumption of specific types of bread, fat spreads/oils and milk [31]. Standard portion sizes based on typical consumption patterns in Britain [32] were adapted for the age of children and used to estimate the daily intake of each food group. Total energy and nutrient intakes were calculated by multiplying estimated food intake (g·day⁻¹) by their estimated nutrient content from UK food composition tables [33, 34] and summing this across all the foods consumed. Accordingly, daily intakes of vitamin A were estimated separately as the intakes of preformed vitamin A and pro-vitamin A carotenes in the form of β-carotene equivalents (sum of β-carotene and half the amounts of α-carotene and α- and β-cryptoxanthins). The major sources of preformed vitamin A were, on average, fat spreads (24.2%), milk (21.6%), cold meats (8.8%), cheese (7.6%), yoghurt (6.1%), liver and liver pate (4.7%), eggs (4.1%), and school meals (4.3%). The major sources of carotene were, on average, carrots (52.1%), other vegetables (10.4%), school meals (8.8%), squash and cordial soft drinks (7.0%), fat spreads (3.2%), fruit (2.3%), and milk (2.2%). We estimated total vitamin A intake by adding intakes of β-carotene equivalents (divided by 12) and preformed vitamin A to give retinol activity equivalents (RAEs) [2].
**Outcome assessment**

Lung function was assessed by spirometry (Vitalograph 2120; Vitalograph, Maids Moreton, UK) at 15.5 years, after withholding short-acting bronchodilators for at least 6 h and long-acting bronchodilators and theophyllines for at least 24 h. The best of three reproducible flow–volume curves was used to measure FEV₁, forced vital capacity (FVC) and forced expiratory flow at 25–75% of FVC (FEF₂₅₋₇₅%) indicating maximal mid-expiratory flow, before and 15 min after administration of 400 mg salbutamol. These lung function measurements were transformed to z-scores based on the Global Lung Function Initiative (GLI) curves. Accordingly, a standardised measure of an observed lung function measurement was mapped onto the distribution of the population from which the GLI reference values are derived, adjusting for age, height and ethnicity, and separately by sex [35, 36]. The GLI reference values were generated using the GLI R macro (https://github.com/ihlytras/spiro) [37]. The tests adhered to American Thoracic Society criteria for standardisation and reproducibility of flow–volume measurement [38]. We used post-bronchodilator lung function measures as our primary outcomes because, for FEV₁ and FEF₂₅₋₇₅%, these are likely to more closely reflect growth and calibre of the airways, rather than airway tone.

Our second primary outcome of interest was incident asthma. At 91 months (∼7.5 years), 128 months (∼11 years) and 166 months (∼14 years) of age, we defined current doctor-diagnosed asthma if mothers responded positively to the question “Has a doctor ever actually said that your study child has asthma?”, and to at least one of the concurrent following questions which asked if the child had had wheezing, wheezing and whistling in the chest, asthma or asthma medication in the last 12 months. Among those children who were not identified as having current doctor-diagnosed asthma at 7.5 years, we defined those with current doctor-diagnosed asthma at 11 or 14 years as cases of incident asthma. The parental reports of a doctor’s diagnosis of asthma in ALSPAC agreed well with a general practitioner-recorded diagnosis (sensitivity 88.5% and specificity 95.7%) [39].

**Genotyping and single nucleotide polymorphism selection**

We considered single nucleotide polymorphisms (SNPs) that were associated with bioavailability or metabolism of vitamin A in the literature and selected those that could plausibly interact with dietary vitamin A intake and/or have been associated with lung function. We excluded SNPs that were in linkage disequilibrium ($r^2$$>$0.80 using the LDmatrix tool for the British population; https://ldlink.nci.nih.gov) and those with minor allele frequency <0.2 so as not to limit power for stratified analyses. The first SNP of interest (rs3741240) was in the SCGB1A1 gene, which encodes for the club cell secretory protein CC16 (secretoglobin family 1A member 1). This SNP was shown in a genome-wide association study to have the strongest correlation with serum levels of CC16 [40]. CC16 is an airway epithelial biomarker; serum levels are increased by vitamin A supplementation [27] and are positively associated with lung function growth in childhood [28]. We also included rs12708369 in the NCOR2 (nuclear receptor corepressor 2) gene, which is found in the retinoic acid signalling pathway and has been associated with FVC in ALSPAC [41]. Among SNPs that were more likely to interact directly with dietary intake and bioavailability [42, 43], we selected five SNPs in the BCMO1 (β-carotene 15,15′-monooxygenase 1) locus: rs6564851, rs11645428 and rs6420424 (upstream) [44], and rs7501331 and rs12934922 in the coding region [45]. These SNPs have been associated with efficiency of conversion of β-carotene to the intermediate forms of vitamin A and correlated with fasting plasma concentrations of β-carotene, among which rs6564851 had the strongest association [44]. We hypothesised that effects of a higher intake of preformed vitamin A would be greater in poor carotene converters and that effects of a higher intake of carotene would be greater in efficient carotene converters (further details of selected SNPs are given in supplementary table E1). Genotypes were imputed (IMPUTE2) using the Haplotype Reference Consortium genomes reference panel (1.1) and imputation quality was capped (in addition to minor allele frequency) at an imputation information metric score (INFO) >0.95 (further details are given in the supplementary material).

**Statistical analysis**

Among 8135 children with plausible data on vitamin A intake at 7 years (excluding children with implausible total energy intake: <15000 or >140000 kJ·week⁻¹), 2985–3121 participants had data on post-bronchodilator lung function measures at 15.5 years (depending on the specific measure) and data on incident asthma were complete for 4540 participants (supplementary figure E1). We employed linear regression to examine associations between intakes of preformed vitamin A or carotene (in quartiles) and lung function measures. Logistic regression models were used to test associations with incident asthma and with airflow limitation, defined as an FEV₁/FVC ratio below the lower limit of normal (LLN), representing the lower 5% of study population z-scores. Linear trend was tested by including median intake of quartiles as a pseudo-continuous variable in the models. We selected known potential confounding factors from the existing literature [46] and by using a directed acyclic graph approach [47] (supplementary figure E2). Details of multivariable models and covariates are explained in the supplementary material.
We carried out stratified analyses, a priori, to explore potential modification of dietary associations by maternal and paternal history of atopy (yes/no), maternal smoking when the child was 7 years of age (yes/no), and SGRB1A1, NCOR2 and five BCMO1 genotypes (supplementary table E1). Potential interactions were assessed by testing cross-product terms of these factors with quartiles (median values) as a continuous factor in regression models. We also carried out several sensitivity analyses, a priori, that are explained in the supplementary material in detail, including further adjustment for other potential confounders, restricted cubic spline analysis to examine the dose–response relationship and inverse probability weighting to correct for potential loss-to-follow-up bias [48]. All statistical analyses were performed using Stata version 14.2 (StataCorp, College Station, TX, USA).

Results

We estimated median (interquartile range) intakes of vitamin A as 429 (332–538) μg·day⁻¹ for preformed vitamin A, 1744 (1464–2309) μg·day⁻¹ for carotene (β-carotene equivalent) and 590 (470–723) μg·day⁻¹ RAE for total vitamin A (comprising a 26.9% contribution by carotene). For comparison with recommended dietary allowances [49], see supplementary figure E3. Table 1 shows that children with higher intakes of preformed vitamin A were more likely to be male, have a smoking mother and have a younger sibling, while less likely to have an older sibling. Mothers of children who had higher intakes of carotene were more educated (supplementary table E2). Children with higher intakes of either preformed vitamin A or carotene had a generally more health-conscious dietary pattern reflected in higher intakes of vitamins C, D and E, and zinc as well as ω-3 from fish, and had higher maternal intakes of vitamin A during pregnancy (table 1 and supplementary table E2).

Lung function

Higher intake of preformed vitamin A was associated with a higher FEV₁ and FEF₂₅₋₇₅%. There was also weak evidence of association with FVC, but not with FEV₁/FVC, analysed as a continuous variable (table 2). However, when we analysed the dichotomous outcome of airflow limitation (defined as FEV₁/FVC < LLN), higher intake was inversely associated (OR comparing top versus bottom quartile in model 2: 0.49, 95% CI 0.27–0.90; \( p_{\text{trend}}=0.04 \)). We did not find any evidence of association between carotene intake and lung function measures overall (table 2).

There was no evidence of interaction with maternal atopy, paternal atopy or maternal smoking (data not shown). We also tested relationships with pre-bronchodilator lung function measures, and found the same pattern of associations between preformed vitamin A intake and FEV₁ and FEF₂₅₋₇₅%, although slightly weaker (supplementary table E3).

In stratified analysis by SGRB1A1 genotype (rs3741240), there were positive associations between preformed vitamin A intake and FEF₂₅₋₇₅% and FEV₁/FVC only in homozygous carriers of the G allele (\( P_{\text{interaction}}=0.02 \)), and the suggestion of a similar pattern with FEV₁ (\( P_{\text{interaction}}=0.07 \)), while no evidence of effect modification was observed for β-carotene intake (table 3). When we stratified by NCOR2 genotype (rs12708369), preformed vitamin A intake was positively associated with FEV₁ and FVC in carriers of the C allele, but negatively associated in those homozygous for the T allele (\( P_{\text{interaction}}=0.02 \) and \( \leq 0.01 \), respectively) (table 4). A similar pattern of associations was observed between carotene intake and FEF₂₅₋₇₅% in those homozygous for the C and T allele, respectively (\( P_{\text{interaction}}=0.009 \)).

We also found evidence of effect modification by two of the SNPs in the coding region of BCMO1: in carriers of the T allele of rs7501331 (low converters of β-carotene), but not in those homozygous for the C allele, higher intake of preformed vitamin A was associated with higher FEV₁ and FVC (\( P_{\text{interaction}}=0.03 \) and \( 0.01 \), respectively), whereas in carriers of the A allele of rs12934922 (high converters of β-carotene) higher intake of β-carotene was associated with higher FEV₁ (\( P_{\text{interaction}}=0.01 \)) (supplementary table E4). We did not find any other convincing evidence of interaction with the other four BCMO1 SNPs (data not shown).

Asthma

We identified 390 (8.6%) cases of incident asthma at 11 or 14 years. There was weak evidence of an inverse association between preformed vitamin A intake and incident asthma (table 5). When stratified by paternal history of atopy, we found evidence of lower risk of asthma with higher intakes of preformed vitamin A in children without a paternal history of atopy (OR comparing top versus bottom quartile: 0.52, 95% CI 0.28–0.97; \( P_{\text{trend}}=0.03 \)), but not in those with it (OR 1.36, 95% CI 0.73–2.55; \( P_{\text{trend}}=0.19 \)) (\( P_{\text{interaction}}=0.02 \)). We did not find any evidence of association between carotene intake and incident asthma overall (table 5) nor any other evidence of interaction with non-genetic factors (data not shown).
| TABLE 1 | Participant characteristics according to quartiles of preformed vitamin A intake at 7 years of age |
|----------------|---------------------------------------------------------------|
| Quartiles of preformed vitamin A intake p-value |
| Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 |
| Participants | 1359 (25.2) | 1343 (24.9) | 1350 (25.1) | 1332 (24.7) |
| Preformed vitamin A intake μg·day⁻¹ | 261±58.4 | 382±27.6 | 480±31.3 | 680±173.6 |
| Male | 638 (46.9) | 627 (46.7) | 663 (49.1) | 731 (54.9) |
| Older siblings | 742 (54.6) | 720 (53.6) | 686 (50.8) | 639 (48.0) |
| Younger siblings | 624 (45.9) | 674 (50.2) | 717 (53.1) | 782 (58.7) |
| Total energy intake kJ·day⁻¹ | 6214±1291 | 7136±1189 | 7890±1235 | 9139±1728 |
| BMI kg·m⁻² | 16.2±2.1 | 16.0±1.9 | 16.2±2.0 | 16.2±1.9 |
| Health-conscious dietary pattern score | −0.27±0.89 | −0.08±0.88 | 0.07±0.94 | 0.33±1.05 |
| Season of dietary information collection | |
| Winter | 339 (24.9) | 354 (26.4) | 347 (25.7) | 339 (25.5) |
| Spring | 424 (31.2) | 393 (29.3) | 398 (29.5) | 370 (27.8) |
| Summer | 366 (26.9) | 372 (27.6) | 381 (28.2) | 395 (29.7) |
| Autumn | 207 (15.2) | 214 (15.9) | 214 (15.9) | 213 (16.0) |
| Missing | 23 (1.7) | 9 (0.7) | 10 (0.7) | 15 (1.1) |
| History of food allergy | 262 (19.4) | 219 (16.3) | 221 (16.4) | 234 (17.6) |
| Any supplement use | 450 (33.1) | 440 (32.8) | 453 (33.6) | 461 (34.6) |
| Protein intake g·day⁻¹ | 52.8±12.2 | 61.1±11.5 | 66.7±11.6 | 77.2±15.8 |
| Vitamin C intake mg·day⁻¹ | 68.2±31.4 | 72.9±32.5 | 78.2±33.0 | 85.3±35.8 |
| Vitamin D intake mg·day⁻¹ | 2.14±0.7 | 2.69±0.8 | 3.01±0.9 | 3.45±1.1 |
| Vitamin E intake mg·day⁻¹ | 7.49±2.6 | 9.23±2.9 | 10.32±3.3 | 11.83±4.0 |
| Zinc intake mg·day⁻¹ | 5.19±1.3 | 5.99±1.3 | 6.57±1.3 | 7.66±1.8 |
| VLC n-3 PUFA intake from fish mg·day⁻¹ | 65.9±73.3 | 77.9±84.6 | 83.8±91.0 | 98.7±99.2 |
| Parental factors | |
| Maternal age at pregnancy years | 29.5±4.4 | 29.4±4.4 | 29.5±4.4 | 29.1±4.5 |
| Maternal education | 281 (20.7) | 267 (19.9) | 237 (17.6) | 236 (17.7) |
| O-level | 464 (34.1) | 450 (33.5) | 476 (35.3) | 451 (33.9) |
| A-level or degree | 593 (43.6) | 608 (45.3) | 624 (46.2) | 619 (46.3) |
| Missing | 21 (1.5) | 18 (1.3) | 13 (1.0) | 26 (2.0) |
| Housing tenure during pregnancy | |
| Mortgaged/owned | 1156 (85.1) | 1136 (84.6) | 1151 (85.3) | 1071 (80.4) |
| Council rented | 74 (5.4) | 71 (5.3) | 74 (5.3) | 107 (8.0) |
| Non-council rented | 74 (5.4) | 71 (5.3) | 68 (5.0) | 91 (6.8) |
| Missing | 55 (4.0) | 65 (4.8) | 57 (4.2) | 63 (4.7) |
| Financial difficulty during pregnancy | 1156 (85.3) | 1136 (84.5) | 1153 (85.9) | 1105 (83.3) |
| Maternal ethnicity | 200 (14.8) | 195 (14.7) | 189 (14.1) | 222 (16.7) |
| White | 1307 (96.2) | 1297 (96.6) | 1324 (98.1) | 1292 (97.0) |
| Non-White | 25 (1.8) | 27 (2.0) | 11 (0.8) | 14 (1.1) |
| Missing | 27 (2.0) | 19 (1.4) | 15 (1.1) | 26 (2.0) |
| Maternal history of atopy | 713 (52.5) | 712 (53.0) | 700 (51.9) | 652 (48.9) |
| No | 594 (43.7) | 579 (43.1) | 613 (45.4) | 622 (46.7) |
| Yes | 52 (3.8) | 52 (3.9) | 37 (2.7) | 58 (4.4) |
| Paternal history of atopy | 584 (43.0) | 601 (44.8) | 553 (41.0) | 582 (43.7) |
| No | 414 (30.5) | 385 (28.7) | 470 (34.8) | 388 (29.1) |
| Yes | 361 (26.6) | 357 (26.6) | 327 (24.2) | 362 (27.2) |
| Maternal smoking | 1085 (79.8) | 1102 (82.1) | 1100 (81.5) | 1020 (76.6) |
| No | 219 (16.1) | 194 (14.4) | 201 (14.9) | 257 (19.3) |
| Yes | 55 (4.0) | 47 (3.5) | 49 (3.6) | 55 (4.1) |
| Preformed vitamin A intake at 32 weeks of gestation μg·day⁻¹ | 302±249 | 335±276 | 357±319 | 414±379 |

Data are presented as n (%) or mean±SD, unless otherwise specified. BMI: body mass index; VLC n-3 PUFA: very-long-chain ω-3 polyunsaturated fatty acid. #: children included in incident asthma or lung function analysis (n=5384).
There was an inverse association between preformed vitamin A intake and incident asthma in individuals with an upstream *BCMO1* SNP genotype associated with poor conversion of carotene (rs6564851_GG), but also in individuals with a coding region *BCMO1* SNP genotype associated with high conversion (rs7501331_CC). However, there was no evidence of statistically significant interaction by genotype (table 6). In contrast, there was a positive association between carotene intake and incident asthma in individuals with an upstream *BCMO1* SNP genotype associated with high conversion (rs6564851_TT), but also in individuals with another coding region *BCMO1* SNP genotype associated with low conversion (rs12934922_TT) (table 6). We found a similar pattern of associations when stratified by other *BCMO1* SNPs (supplementary table E5).

**Sensitivity analyses**
We did not find evidence of any nonlinear associations, except between preformed vitamin A and FEV₁ (p_nonlinearity=0.04) using the restricted cubic spline analysis. The associations between intake of preformed vitamin A and FEV₁, FEF₂₅–₇₅% and incident asthma did not materially change after further adjustment for dietary patterns (“health-conscious”, “junk” and “traditional”, separately), any history of food allergy, breastfeeding, urban/rural locality, physical activity, body mass index (imputed for 8.7–12.1% missing data), atopy measured by skin prick test and maternal intake of vitamin A in pregnancy, as well as other dietary factors including intakes of vitamins C, D and E, zinc, protein, and n-3 fatty acids from fish (supplementary tables E6 and E8). The null associations for carotene intake also remained the same after these further adjustments (supplementary tables E7 and E8). When we tested energy-adjusted intakes using...

| Quartiles of vitamin A intake | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 |
|-------------------------------|------------|------------|------------|------------|
| **Preformed vitamin A**       | median (IQR) mg·day⁻¹ | 276 (224–305) | 382 (359–407) | 477 (452–506) | 637 (581–721) |
| FEV₁                          |            |            |            |            |
| Model 1                       | 0.00       | −0.01 (−0.15–0.13) | −0.01 (−0.16–0.13) | 0.20 (0.03–0.36) |
| Model 2                       | 0.00       | −0.02 (−0.16–0.12) | −0.01 (−0.15–0.14) | 0.21 (0.05–0.38) |
| FVC                           |            |            |            |            |
| Model 1                       | 0.00       | −0.01 (−0.14–0.13) | −0.03 (−0.17–0.11) | 0.14 (−0.02–0.30) |
| Model 2                       | 0.00       | −0.01 (−0.14–0.13) | −0.02 (−0.16–0.12) | 0.15 (−0.01–0.31) |
| FEF₂₅–₇₅%                     |            |            |            |            |
| Model 1                       | 0.00       | 0.02 (−0.10–0.14) | 0.06 (−0.06–0.19) | 0.07 (−0.07–0.21) |
| Model 2                       | 0.00       | 0.01 (−0.11–0.13) | 0.05 (−0.07–0.18) | 0.08 (−0.06–0.22) |
| β-carotene equivalent         | median (IQR) mg·day⁻¹ | 956 (646–1328) | 1607 (1538–1671) | 1945 (1827–2105) | 3268 (2670–3616) |

IQR: interquartile range; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF₂₅–₇₅%: forced expiratory flow at 25–75% of FVC. Multivariable model 1: sex and total energy intake. Multivariable model 2: further adjusted for maternal education, housing tenure at birth, financial difficulty during pregnancy, maternal ethnicity, maternal history of atopic disease, paternal history of atopic disease, maternal smoking, older sibling, younger sibling, supplement use and season when the food frequency questionnaire was completed. *²*: linear trend was tested by treating the median values of quartiles as a continuous variable.
the residual method, preformed vitamin A was almost similarly associated with FEV\textsubscript{1} and FEF\textsubscript{25–75%}, (multivariable adjusted regression coefficients per SD: 0.07, 95% CI 0.02–0.12 and 0.07, 95% CI 0.02–0.11, respectively) and incident asthma (multivariable adjusted OR per SD: 0.85, 95% CI 0.75–0.97), the associations with FVC and FEV\textsubscript{1}/FVC did not change either, and no association was observed for carotene intake at 7 years of age, 25.5% and 55.9% did not have data on incident asthma or lung function at 15.5 years, respectively. However, findings were similar when we applied inverse probability weighting to correct for selection bias due to loss to follow-ups (data not shown).
TABLE 4 Linear regression coefficients (95% CI) for post-bronchodilator lung function measures (z-scores) according to quartiles of intakes of preformed vitamin A and β-carotene equivalent, stratified by NCO2R (nuclear receptor corepressor 2)° genotype (rs12708369)

| Quartiles of vitamin A intake | p_trend-value | p_interaction-value |
|------------------------------|---------------|---------------------|
| Preformed vitamin A          |               |                     |
| FEV1                         |               |                     |
| Genotype TT                  | 0.00          | −0.41 (−0.81−0.02)  |
| Genotype CT                  | 0.00          | −0.03 (−0.26−0.19)  |
| Genotype CC                  | 0.00          | 0.01 (−0.23−0.26)   |
| FVC                          |               |                     |
| Genotype TT                  | 0.00          | −0.23 (−0.61−0.15)  |
| Genotype CT                  | 0.00          | −0.05 (−0.26−0.16)  |
| Genotype CC                  | 0.00          | 0.06 (−0.18−0.29)   |
| FEV1/FVC                     |               |                     |
| Genotype TT                  | 0.00          | −0.24 (−0.60−0.13)  |
| Genotype CT                  | 0.00          | 0.10 (−0.08−0.29)   |
| Genotype CC                  | 0.00          | −0.04 (−0.26−0.17)  |
| FEF25-75%                    |               |                     |
| Genotype TT                  | 0.00          | −0.34 (−0.68−0.00)  |
| Genotype CT                  | 0.00          | −0.09 (−0.09−0.28)  |
| Genotype CC                  | 0.00          | −0.05 (−0.27−0.18)  |
| β-carotene equivalent        |               |                     |
| FEV1                         |               |                     |
| Genotype TT                  | 0.00          | −0.44 (−0.82−0.05)  |
| Genotype CT                  | 0.00          | 0.09 (−0.12−0.31)   |
| Genotype CC                  | 0.00          | 0.15 (−0.09−0.38)   |
| FVC                          |               |                     |
| Genotype TT                  | 0.00          | −0.54 (−0.91−0.17)  |
| Genotype CT                  | 0.00          | 0.09 (−0.11−0.30)   |
| Genotype CC                  | 0.00          | 0.03 (−0.19−0.26)   |
| FEV1/FVC                     |               |                     |
| Genotype TT                  | 0.00          | 0.18 (−0.17−0.53)   |
| Genotype CT                  | 0.00          | 0.05 (−0.13−0.23)   |
| Genotype CC                  | 0.00          | 0.14 (−0.06−0.35)   |
| FEF25.75%                    |               |                     |
| Genotype TT                  | 0.00          | −0.04 (−0.37−0.29)  |
| Genotype CT                  | 0.00          | 0.00 (−0.18−0.19)   |
| Genotype CC                  | 0.00          | 0.22 (0.01−0.44)    |

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF25–75%: forced expiratory flow at 25–75% of FVC. Multivariable model: sex, total energy intake, maternal education, housing tenure at birth, financial difficulty during pregnancy, maternal ethnicity, maternal history of atopic disease, paternal history of atopic disease, maternal smoking, older sibling, younger sibling, supplement use and season when the food frequency questionnaire was completed. °: in TT, CT and TT groups, sample sizes were 380, 1227 and 948 for FEV1, and 397, 1287 and 985 for both FVC and FEF25–75%, respectively. †: linear trend was tested by treating the median values of quartiles as a continuous variable.

Discussion

In ALSPAC children overall we found that higher intake of preformed vitamin A, but not β-carotene, in mid-childhood was associated with a higher subsequent FEV1 and FEF25–75%. There was also weak evidence for an inverse association between intake of preformed vitamin A and incident asthma. To the best of our knowledge, these are novel findings, which were robust to various sensitivity analyses.

The difference in FEV1 between the top and bottom quartiles of preformed vitamin A intake was clinically important and comparable to the mean difference in z-scores of pre-bronchodilator FEV1 according to asthma status (0.24, 95% CI 0.11–0.37). Associations between preformed vitamin A intake and lung function were stronger for FEV1 and FEF25–75% than for FVC, suggesting a stronger influence on airway than alveolar development. Furthermore, the stronger associations with post- than pre-bronchodilator measures suggest that higher intake may promote growth and calibre, rather than tone, of large and small airways. The weak inverse association with asthma may therefore also reflect a beneficial effect on airway growth. Moreover, the strong inverse association with fixed airflow limitation (FEV1/FVC < LLN) may have implications for the development of later chronic obstructive pulmonary disease.
### Table 5

Odds ratios (95% CI) for incident asthma at 11 or 14 years according to quartiles of intakes of preformed vitamin A and β-carotene equivalent, adjusted for potential confounders

| Quartiles of vitamin A intake | Preformed vitamin A | β-carotene equivalent |
|------------------------------|---------------------|-----------------------|
|                              | Cases/non-cases     | Model 1               | Cases/non-cases     | Model 1               |
|                              | Quartile 1          | Quartile 2            | Quartile 3          | Quartile 4            | Quartile 1          | Quartile 2          | Quartile 3          | Quartile 4          |
|                              | 108/1026            | 90/1063               | 99/1037             | 93/1024               | 95/1023              | 76/1044             | 111/1061            | 108/1022            |
| Preformed vitamin A          | 1.00                | 0.76 (0.56–1.02)      | 0.81 (0.59–1.11)    | 0.70 (0.49–1.01)      | 1.00                 | 0.78 (0.57–1.07)    | 1.12 (0.83–1.51)    | 1.12 (0.82–1.54)    |
| Model 2                      | 1.00                | 0.77 (0.57–1.04)      | 0.81 (0.59–1.10)    | 0.68 (0.47–0.99)      | 0.07                 | 0.80 (0.58–1.10)    | 1.15 (0.84–1.56)    | 1.16 (0.85–1.60)    |

Data for case/non-cases are presented as n/n. Multivariable model 1: sex and total energy intake. Multivariable model 2: further adjusted for maternal education, housing tenure at birth, financial difficulty during pregnancy, maternal ethnicity, maternal history of atopic disease, paternal history of atopic disease, maternal smoking, older sibling, younger sibling, supplement use and season when the food frequency questionnaire was completed.

### Table 6

Adjusted odds ratios (aORs) (95% CI) for incident asthma at 11 or 14 years according to quartiles of intakes of preformed vitamin A and β-carotene equivalent, stratified by *BCMO1* (β-carotene 15,15′-monooxygenase 1) genotypes

| Quartiles of vitamin A intake | Preformed vitamin A | β-carotene equivalent |
|------------------------------|---------------------|-----------------------|
|                              | Upstream *BCMO1*: rs6564851 | BCMO1 coding region: rs7501331 |
|                              | TT¶: cases/non-cases | CT: cases/non-cases | TT: cases/non-cases |
|                              | aOR (95% CI)        | aOR (95% CI)       |
| Total genotypes              | 16/189              | 25/285              | 5/51               |
| TT¶: cases/non-cases        | 1.00                | 1.00                | 1.00               |
| TG: cases/non-cases         | 41/389              | 36/421              | 26/221             |
| aOR (95% CI)                | 1.00                | 0.87 (0.54–1.43)    | 2.26 (1.12–4.52)   |
| GG: cases/non-cases         | 53/463              | 36/303              | 6/51               |
| aOR (95% CI)                | 1.00                | 0.43 (0.21–0.85)    | 0.62 (0.31–1.23)   |

Data for case/non-cases are presented as n/n. Adjusted odds ratio (multivariable model) for sex, total energy intake, maternal education, housing tenure at birth, financial difficulty during pregnancy, maternal ethnicity, maternal history of atopic disease, paternal history of atopic disease, maternal smoking, older sibling, younger sibling, supplement use and season when the food frequency questionnaire was completed.

### Table 6

Adjusted odds ratios (aORs) (95% CI) for incident asthma at 11 or 14 years according to quartiles of intakes of preformed vitamin A and β-carotene equivalent, stratified by *BCMO1* (β-carotene 15,15′-monooxygenase 1) genotypes

| Quartiles of vitamin A intake | Preformed vitamin A | β-carotene equivalent |
|------------------------------|---------------------|-----------------------|
|                              | Upstream *BCMO1*: rs6564851 | BCMO1 coding region: rs12934922 |
|                              | TT¶: cases/non-cases | AA¶: cases/non-cases | TT: cases/non-cases |
|                              | aOR (95% CI)        | aOR (95% CI)        |
| Total genotypes              | 7/192               | 25/244               | 13/157             |
| TT¶: cases/non-cases        | 1.00                | 1.00                | 1.00               |
| TG: cases/non-cases         | 45/375              | 34/304              | 8/172              |
| aOR (95% CI)                | 1.00                | 0.72 (0.45–1.17)    | 0.62 (0.24–1.58)   |
| GG: cases/non-cases         | 53/466              | 34/303              | 28/252             |
| aOR (95% CI)                | 1.00                | 0.49 (0.31–0.78)    | 0.61 (0.01–5.73)   |

Data for case/non-cases are presented as n/n. Adjusted odds ratio (multivariable model) for sex, total energy intake, maternal education, housing tenure at birth, financial difficulty during pregnancy, maternal ethnicity, maternal history of atopic disease, paternal history of atopic disease, maternal smoking, older sibling, younger sibling, supplement use and season when the food frequency questionnaire was completed. Linearity was tested by treating the median values of quartiles as a continuous variable; homozygous alleles linked to a more efficient conversion of carotene pro-vitamin A.
Vitamin A is the most multifunctional vitamin in the human body and the only one with a storage system buffering against dietary insufficiency, which underlines its evolutionary importance [1]. Overt vitamin A deficiency is mostly a problem in poorly nourished populations, due to the lower consumption of animal foods. While in the developed world it is estimated that >20% of the population may not meet the recommended intake due to modern societal habits [1], the estimated level of total vitamin A intake in this study was higher than the recommended dietary allowance for children 4–8 years of age (400 µg·day⁻¹ RAE) [49]. An intestinal negative feedback loop restricts β-carotene absorption and cleavage in response to vitamin A status [42]. Therefore, the lack of association between β-carotene and lung function outcomes might be explained by its limited contribution to vitamin A status in this population, which is in line with other Western societies (<30%) [2].

Previous findings on the link between serum concentration of retinol or β-carotene and lung function were in adults and were conflicting [12, 50]. However, the serum concentration of vitamin A biomarkers reflects a combined effect of dietary intake, bioavailability and metabolism. Vitamin A is mainly stored in the liver which tightly regulates the circulatory level of retinol; the latter does not decline until the liver is almost depleted [1]. Nevertheless, our findings suggest that, even in a Western population of children without overt vitamin A deficiency, higher intakes of vitamin A may beneficially influence lung growth and hence optimal lung function attainment.

**Mechanism**

We found evidence for effect modification of the association between preformed vitamin A intake and lung function by a SCGB1A1 polymorphism that has been shown to regulate circulating levels of CC16 [40]. Lower concentrations of CC16, an anti-inflammatory pneumoprotein produced by club cells in the airways, have been associated with lung function deficits [28, 51], increased airway resistance and hyperresponsiveness attributed to airways remodelling [51]. Furthermore, vitamin A treatment increased circulating CC16 in humans [27]. Thus, we speculate that an increase in CC16 might mediate the positive association between vitamin A intake and lung function. The interactions we found with CC16 support this hypothesis: higher vitamin A intake was associated with better lung function only in children with a genetic tendency to produce more CC16 (GG genotype) [40], suggesting that this genotype might carry a greater potential for upregulation by vitamin A.

The associations between preformed vitamin A intake and lung function measures were also modified by an NCOX2 polymorphism. NCOX2 is in the retinoic acid signalling pathway and the variant is in a strong transcriptional enhancer element in lung fibroblasts [41]. The positive associations we found in carriers of the C allele, the variant associated with higher FVC in children [41], suggest that vitamin A might also have a role in lung growth through the regulation of fibroblasts. The negative associations seen in those homozygous for the T allele suggest a “flip-flop” gene–nutrient interaction [52].

Regarding BCMO1 polymorphisms which influence vitamin A bioavailability, we hypothesised that children with genetically lower efficiency of β-carotene conversion may benefit more from a higher intake of preformed vitamin A. This was supported by the interactions with polymorphisms in the upstream BCMO1 for both incident asthma and lung function. In contrast, when we stratified by a polymorphism in the coding region, an inverse association with incident asthma was paradoxically in high converters. Another unexpected finding was the positive association between carotene intake and incident asthma, when stratified by BCMO1 genotypes. Given the contradictory and paradoxical nature of some of these gene–nutrient interactions, they should be interpreted with caution.

**Strengths and limitations**

Strengths of the ALSpac birth cohort include its population-based prospective design, large size, rich information on diet and potential confounders, and availability of the various genotype data. The post-bronchodilator assessment of lung function enabled us to better assess airway growth by eliminating reversible airflow limitation. We controlled for numerous potential confounders in the analyses and performed various sensitivity analyses; however, the possibility of unmeasured or residual confounding cannot be ruled out. A sizeable proportion of eligible children at 7 years were not included in our analyses, but our inverse probability weighting analysis showed that this is unlikely to have biased our findings, as generally expected in longitudinal studies [53]. While misclassification of the dietary exposures was inevitable, the prospective nature of the study makes them more likely to have been non-differential with respect to the outcomes, which would tend to bias effect estimates towards the null. Some other limitations of the FFQ include fewer items relevant to carotene intake compared with preformed vitamin A, and some important sources such as broccoli and sweet potato were not included. Given the semiquantitative nature of the FFQs, our estimated “absolute” intakes should be regarded as approximate. In view of the multiple
analyses carried out, our main findings require replication. Given the a priori nature of the hypotheses, however, and the correlation between lung function measures, it did not seem appropriate to correct for multiple testing. However, findings with borderline statistical significance, such as the association with airflow limitation, should be interpreted cautiously. Finally, the generalisability of our findings to other populations, particularly those with an overt vitamin A deficiency, warrants further research.

Conclusions
A higher intake of preformed vitamin A, but not β-carotene, in mid-childhood was associated with higher subsequent lung function and lower risk of fixed airflow limitation and incident asthma.

Acknowledgements: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole Avon Longitudinal Study of Parents and Children team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. We would also like to thank Annabelle Bédard (Université Paris-Saclay, Paris, France) for her assistance at the beginning of this project and Hossein Tabatabaeian (National University of Singapore, Singapore) for his consultation on the genetic aspects of this study. This paper is dedicated to the memory of our late colleague John Henderson, who led the programme of respiratory follow-up in ALSPAC and without whom this study would not have been possible.

Author contributions: M. Talaei and S.O. Shaheen conceived the study. M. Talaei performed the statistical analyses. M. Talaei drafted the manuscript with S.O. Shaheen. P.M. Emmett advised on dietary and nutritional aspects. R. Granell and O. Mahmoud advised on asthma and lung function. D.A. Hughes advised on genetic aspects. S. Guerra advised on CC16. All authors assisted in interpreting the data and critically edited the manuscript. All authors have seen and approved the final version of the manuscript. S.O. Shaheen had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest: M. Talaei has nothing to disclose. D.A. Hughes has nothing to disclose. O. Mahmoud has nothing to disclose. P.M. Emmett reports grants from Nestle Nutrition, personal fees for consultancy from the European Food Safety Authority, outside the submitted work. R. Granell has nothing to disclose. S. Guerra has nothing to disclose. S.O. Shaheen has nothing to disclose.

Support statement: This project and M. Talaei were funded by the Rosetrees Trust and The Bloom Foundation (grant M771). D.A. Hughes is supported by a Wellcome Investigator Award (number 202802/Z/16/Z). The UK Medical Research Council and the Wellcome Trust (grant 217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors, and R. Granell and P.M. Emmett will serve as guarantors for the contents of this paper. Genome-wide association study data were generated by Sample Logistics and Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe. A comprehensive list of grant funding is available on the ALSPAC website (www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). Funding information for this article has been deposited with the Crossref Funder Registry.

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