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Maternal dietary antioxidant intake in pregnancy and childhood respiratory and atopic outcomes: birth cohort study

Annabelle Bédard[1], Kate Northstone[2], John W. Holloway[3], A. John Henderson[2,4] and Seif O. Shaheen[1,4]

Affiliations: [1]Centre for Primary Care and Public Health, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK. [2]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK. [3]Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK. [4]These authors are joint senior authors.

Correspondence: Seif O. Shaheen, Centre for Primary Care and Public Health, Blizard Institute, Barts and The London School of Medicine and Dentistry, 58 Turner Street, London, E1 2AB, UK.
E-mail: s.shaheen@qmul.ac.uk

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ABSTRACT Evidence for a possible protective effect of maternal dietary antioxidant intake during pregnancy on childhood asthma and other atopic outcomes is conflicting, and associations with childhood lung function have been little studied.

In the Avon Longitudinal Study of Parents and Children, we analysed associations between maternal intake of fruits, vegetables, vitamins C and E, carotene, zinc, and selenium in pregnancy and current doctor-diagnosed asthma, atopy and lung function in 8915 children at age 7–9 years. Potential modification of associations by maternal smoking and common maternal antioxidant gene polymorphisms was explored to strengthen causal inference.

After controlling for confounders, positive associations were observed between maternal intake of zinc and childhood forced expiratory volume in 1 s and forced vital capacity (difference in age-, height- and sex-adjusted SD units per quartile increase in maternal dietary zinc intake β 0.05 (95% CI 0.01–0.08); p_{trend}=0.01 and 0.05 (95% CI 0.02–0.09); p_{trend}=0.005, respectively). Weak evidence was found for an interaction between maternal zinc intake and maternal glutathione *S*-transferase *GSTM1* genotype on childhood forced vital capacity ($p_{interaction}$=0.05); association among the *GSTM1* null group β 0.11 (95% CI 0.05–0.17); p_{trend}=0.001.

Our results suggest that a higher maternal intake of zinc during pregnancy may be associated with better lung function in the offspring.

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Introduction

A declining dietary intake of antioxidants has been proposed as a possible explanation for the large increase in the prevalence of asthma and atopy seen in the West in recent decades [1], and this has led to interest in the role of maternal antioxidant dietary intake in pregnancy in the aetiology of childhood asthma and atopic diseases. Although some studies have suggested a possible protective effect of maternal intake of vitamin E, zinc, fruits and vegetables during pregnancy [2–4], the evidence overall is conflicting. A recent meta-analysis concluded that, while there is some evidence for a protective effect of maternal intake of zinc and vitamin E on childhood wheeze, evidence regarding asthma and other atopic outcomes is inconclusive [5]. Evidence regarding childhood lung function is limited to one study [2].

A concern with all observational studies, and particularly in nutritional epidemiology, is that findings may be confounded [6]. One way to strengthen causal inference is to demonstrate biologically plausible interactions. Researchers have hypothesised that a diet low in antioxidants may increase susceptibility to oxidant injury and airway inflammation [7]. Maternal smoking during pregnancy has been associated with adverse respiratory outcomes in children [8, 9] and in the Avon Longitudinal Study of Parents and Children (ALSPAC), maternal smoking during pregnancy was associated with reduced mid-expiratory flows in childhood [10]. A recent randomised clinical trial suggested that vitamin C supplementation in pregnant, smoking females may reduce the deleterious effect of maternal smoking on infant pulmonary function [11]. However, to the best of our knowledge, no observational study has investigated potential interactions between maternal dietary antioxidant intake and maternal smoking during pregnancy on respiratory and atopic outcomes in later childhood. Similarly, interactions between maternal diet and common antioxidant gene polymorphisms have not been explored, although a few studies conducted in children have investigated possible interactions between common glutathione *S*-transferase (GST) gene polymorphisms and antioxidant intake on atopic and respiratory outcomes [12–14].

The aim of this study was to investigate the associations between maternal intake of dietary antioxidants in pregnancy and childhood respiratory and atopic outcomes (including lung function), and to explore whether these associations were modified by maternal smoking during pregnancy and common maternal antioxidant gene polymorphisms, which could potentially strengthen causal inference.

Methods

Participants

The ALSPAC is a population-based birth cohort that recruited 14 541 predominantly White pregnant females resident in Avon, UK with expected dates of delivery from April 1, 1991 to December 31, 1992. These pregnancies resulted in 13 613 singletons who were alive at 1 year of age. 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 [15, 16] and further information can be found at: http://www.alspac.bris.ac.uk, which contains details of all the data that are available (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Ethics approval was obtained from the ALSPAC Ethics and Law Committee (IRB 00003312) and the Local NHS Research Ethics Committees

Outcome assessment

Children were defined as having current doctor-diagnosed asthma at 7.5 years (primary outcome) if mothers responded positively to the question "Has a doctor *ever* actually *said* that your study child has asthma?" and positively to one or both of the questions "Has your child had any of the following in the past 12 months: wheezing with whistling; asthma?". Parental reports of a doctor's diagnosis of asthma agree well with a general practitioner-recorded diagnosis in ALSPAC [17]. Atopy at 7 years was defined as a positive reaction (maximum diameter of any detectable weal) to *Dermatophagoides pteronyssinus*, cat or grass (after subtracting positive saline reactions from histamine and allergen weals, and excluding children unreactive to 1% histamine). Lung function was measured by spirometry (Vitalograph 2120; Vitalograph, Maids Moreton, UK) at age 8.5 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 forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and maximal mid-expiratory flow (*i.e.* forced expiratory flow at 25–75% of FVC ($FEF_{25–75}$)). Lung function measurements were transformed to age-, height- and sex-adjusted SD units [18]. The tests adhered to American Thoracic Society (ATS) criteria for standardisation and reproducibility of flow–volume measurement [19], with the exception of ATS recommendations for duration of expiration, since many young children cannot sustain exhalation for 6 s to establish FVC [20]. We therefore used no volume change over >1 s to define the plateau phase of the flow–volume curve as the end-of-test criterion in those unable to blow >6 s. Lung function at 15 years was also considered as a secondary outcome of interest in *post hoc* analyses (see Statistical analyses section).

Exposures of interest

Data on maternal diet in pregnancy were collected by a food frequency questionnaire (FFQ) sent out at 32 weeks gestation to mothers, covering all the main foods consumed in Britain [21]. The questionnaire included questions about the weekly frequency of consumption of 43 food groups and food items, with the possibility for respondents to tick one of the following options: never or rarely, once in 2 weeks, 1–3 times a week, 4–7 times a week, or more than once a day. One question on the weekly frequency of fresh fruit consumption and six questions on the weekly frequency of vegetables (peas, sweetcorn, broad beans; cabbage, brussels sprouts, kale and other green leafy vegetables; other green vegetables; carrots; other root vegetables; salad) were used to estimate weekly intake of fruits and vegetables, respectively, using standard portions [22]. The FFQ was used to estimate daily nutrient intakes for each female, by multiplying the daily frequency of consumption of a food by the nutrient content [23] of a standard portion [22] of that food and summing this for all the foods consumed. Daily intakes of vitamins C and E, zinc, selenium, and carotene were estimated in this way. To ensure consistency, all dietary exposure variables were categorised in quartiles. A maternal dietary antioxidant score was derived for each mother by adding the intake quartile for each of the five antioxidant nutrients, thus ranging from 5 to 20. Information on the child's intake of antioxidants at 3 years, and maternal and paternal antioxidant intake at 4 years post-partum, was collected using a similar FFQ.

Maternal smoking during pregnancy

Maternal smoking habits during the 3 months before pregnancy and at several time-points during pregnancy were recorded using self-reported questionnaires on an ordinal categorical scale (never, passive smoking only, 1–9 cigarettes per day, 10–19 cigarettes per day or $\geqslant$20 cigarettes per day). The highest category reported at any time during pre-pregnancy or pregnancy was used in the analysis.

Genotypes of interest

Maternal DNA was a mixture of samples extracted from blood collected during pregnancy and from lymphoblastoid cell lines. The majority of the children's DNA samples were extracted from cord blood or venous blood collected at age 7 years, with a small number extracted from venous blood collected at 43–61 months. The *GSTT1* and *GSTM1* gene deletion genotyping was performed using a real-time PCR method described previously [24]. Two single nucleotide polymorphisms (SNPs) were typed in mothers and children by LGC Genomics (formerly KBiosciences, Hoddesdon, UK), using a competitive allele-specific PCR system (KASPar): a SNP in *GSTP1* (G313A, Ile105Val, rs1695) and a SNP in *GPX4* (glutathione peroxidase 4; rs713041, at position 718). The GST gene polymorphisms are common and we have previously investigated their role (and interactions) in childhood asthma in ALSPAC [25, 26]. We have also reported interactions between pre-natal selenium status and childhood *GPX4* genotype on childhood asthma (glutathione peroxidase 4 is a selenium-dependent enzyme) [27].

Potential confounders

We selected potential confounding factors that are known (from existing literature) to be associated with one or more of the outcomes of interest [28]. These included maternal age at delivery, sex of child, multiple pregnancy, season of birth, maternal history of atopic diseases (hay fever, asthma, eczema, allergies, or attacks of wheezing with whistling on the chest or attacks of breathlessness in the past 2 years), parity, highest educational qualification, housing tenure, financial difficulties, ethnicity, breastfeeding duration and maternal factors during pregnancy (smoking status, anxiety score (Crown–Crisp Experiential Index) [29], paracetamol use, antibiotic use, infections (urinary infection, influenza, rubella, thrush, genital herpes, other), supplement use and total energy intake ($kJ \cdot day^{-1}$)).

Statistical analyses

Logistic regression and linear regression were used to analyse associations between dietary exposure variables and binary and continuous outcomes, respectively. Dietary exposure variables were analysed in quartiles, first as a categorical variable using the lowest quartile as reference to allow for a nonlinear pattern of association and second as a continuous variable to test for linear trend (*i.e.* per increasing quartile effect). For all regression analyses, two stages of adjustment were used. In Model 1, we adjusted for total energy intake only. In Model 2, we adjusted additionally for all potential confounders listed previously.

Sensitivity analyses

When evidence for associations persisted after adjustment for potential confounders, we conducted a number of additional analyses: 1) additional adjustment for potential mediators (*i.e.* gestational age at delivery [30, 31], birthweight [32, 33], maternal pre-pregnancy body mass index (BMI) and weight gain during pregnancy [34–36], and child's BMI at age 7 years [37, 38]; see supplementary figure S1 for directed acyclic graph); 2) additional adjustment for maternal dietary intake of total polyunsaturated fatty

acids [5]; 3) mutual adjustment for maternal dietary intake of antioxidants that were found to be associated with the same childhood outcome; 4) exclusion of mothers taking supplements in pregnancy (vitamins/zinc); and 5) exclusion of mothers with implausible energy intakes (<2500 or >25 000 kJ·day^{-1} [39]).

Further investigation of confounding

We also used two approaches to further investigate potential confounding of associations with pre-natal exposures: 1) we controlled additionally for child's intake of the same exposure at 3 years of age and 2) we used a parental comparison approach to investigate potential unmeasured confounding by genetic or shared environmental or lifestyle factors (further details in supplementary material) [40, 41].

To correct for potential loss to follow-up bias, we used inverse probability weighting and assigned to each female a weight that is the inverse of the probability of her selection for given values of covariates (further details in supplementary material) [42].

Exploration of interactions

To explore potential modification of dietary associations by maternal smoking we stratified by maternal smoking history (dichotomised) and tested for interaction. Maternal smoking during pregnancy has been found to be associated with reduced childhood $FEF_{25–75}$ in ALSPAC [10]. To explore potential modification of this association by maternal dietary antioxidant exposures, we stratified by antioxidant intake (above *versus* below median) and tested for interaction. Distributions of allele frequencies for each polymorphism in mothers and children were formally tested for deviation from Hardy–Weinberg equilibrium using a likelihood ratio test. To investigate whether associations between dietary exposures and childhood outcomes were modified by maternal antioxidant genotype, we stratified by maternal *GSTM1* and *GSTT1* null genotypes, and by *GSTP1* genotype. We also stratified the associations between maternal dietary selenium intake and outcomes by maternal *GPX4* genotype (since glutathione peroxidase 4 is a selenium-dependent enzyme). All statistical analyses were carried out using Stata version 12.1 (StataCorp, College Station, TX, USA).

Results

Of the 13 972 singletons and twins alive at 1 year of age, information on maternal diet during pregnancy was available for 12 078, of whom there was information on at least one of the outcomes of interest for 8915 children (supplementary figure S2). Characteristics of the 8915 mother–child pairs who were included in the analyses and those of the 3163 mother–child pairs with information on maternal diet who were excluded because of incomplete outcome data are compared in table 1.

After controlling for energy intake only, maternal intakes of fruits and vitamin C during pregnancy were negatively associated with childhood asthma. However, these associations attenuated towards the null after further adjustment for potential confounders (table 2). No other association was found between other dietary antioxidant exposures and childhood asthma or atopy (table 2). After controlling for energy intake and all other potential confounders, there was weak evidence for a positive association between maternal intake of vegetables and childhood FEV_1 and $FEF_{25–75}$ (table 3). Positive associations were observed between maternal intake of zinc and childhood FEV_1 and FVC, with evidence of a dose–response relationship. There was weaker evidence for positive associations between maternal carotene intake and childhood FEV_1 and FVC, and between maternal selenium intake and childhood FVC (table 3). Positive associations were observed between the maternal antioxidant score and childhood FEV_1 and FVC, with evidence of a dose–response relationship (table 3). If zinc intake was omitted from the antioxidant score, the latter was no longer significantly associated with childhood lung function (data not shown).

The significant associations observed between maternal zinc intake and the maternal antioxidant score during pregnancy and childhood FEV_1 and FVC remained unattenuated in all the sensitivity analyses (see Statistical analyses section), whereas associations with the other dietary exposures weakened (data not shown). The significant associations observed between maternal zinc intake and the maternal antioxidant score and childhood FEV_1 and FVC also remained unattenuated after adjusting for child dietary zinc intake and antioxidant score, respectively, at age 3 years. In subsets of the cohort with complete data for paternal (respectively, maternal) zinc intake after pregnancy, no association was found between paternal (respectively, maternal) zinc intake or antioxidant score after pregnancy and childhood lung function (data not shown). The inverse probability weighting analysis did not alter the main results (data not shown). *Post hoc* analyses of the associations between maternal zinc intake and childhood FEV_1 and FVC at 15 years (n=3669) showed similar findings to those observed at 8 years (difference in age-, height- and sex-adjusted SD units per quartile increase in maternal dietary zinc intake β 0.06 (95% CI 0.01–0.11); p_{trend}=0.01 and 0.06 (95% CI 0.01–0.10); p_{trend}=0.02, respectively). However, no association was found between the maternal antioxidant score and childhood FEV_1 and FVC at 15 years (data not shown).

TABLE 1 Characteristics of mothers and offspring who were included and excluded in the analyses[#]

	Included	Excluded	p-value[¶]
Subjects n	8915	3163	
Maternal intake in pregnancy			
Vitamin C mg·day^{-1}	82±35	74±36	<0.001
Vitamin E mg·day^{-1}	8.7±4.1	8.0±4.1	<0.001
Zinc mg·day^{-1}	8.3±2.4	7.8±2.4	<0.001
Selenium µg·day^{-1}	72.2±27.9	66.1±27.2	<0.001
Carotene µg·day^{-1}	2170±1176	2018±1175	<0.001
Fruits g·week^{-1}	671±390	557±392	<0.001
Vegetables g·week^{-1}	949±474	888±505	<0.001
Mother's age years	28.9±4.6	26.5±5.1	<0.001
Parity			
0	45.5	42.8	<0.001
1	36.1	34.1	
⩾2	18.5	23.0	
Sex of child			
Male	51.1	52.2	0.28
Female	48.9	47.8	
Multiple pregnancy			
Singleton	97.6	97.1	0.14
Twin	2.4	2.9	
Season of birth			
Winter	16.2	15.8	0.65
Spring	26.9	26.7	
Summer	30.1	31.3	
Autumn	26.7	26.2	
Breastfeeding duration			
Never	21.2	35.4	<0.001
<3 months	31.5	32.9	
3– <6 months	13.8	10.4	
⩾6 months	33.5	21.3	
Mother's educational level			
Certificate of Secondary Education	15.4	32.7	<0.001
Vocational	9.0	12.2	
Ordinary level	35.4	32.6	
Advanced level	25.1	15.6	
Degree	15.1	6.8	
Maternal ethnicity			
White	98.1	95.5	<0.001
Non-White	1.9	4.5	
Housing tenure			
Owned/mortgaged	83.7	62.5	<0.001
Council rented	9.4	24.0	
Noncouncil rented	6.9	13.5	
Financial difficulties			
Yes	17.1	22.9	<0.001
Maternal history of atopic diseases			
Yes	68.3	68.9	0.62
Maternal anxiety score in pregnancy			
0–9	21.3	16.9	<0.001
10–14	25.7	21.6	
15–19	25.9	24.6	
⩾20	27.2	36.9	
Maximum maternal tobacco exposure			
None	26.5	17.5	<0.001
Passive only	46.0	36.1	
1–9 cigarettes per day	8.0	9.5	
10–19 cigarettes per day	11.3	19.9	
⩾20 cigarettes per day	8.2	17.1	
Maternal paracetamol use during pregnancy			
Yes	62.4	64.6	0.03

Continued

TABLE 1 Continued

	Included	Excluded	p-value[¶]
Maternal antibiotic use during pregnancy			
Yes	16.1	14.5	0.04
Maternal vitamin/zinc supplement use during pregnancy			
Yes	21.6	20.0	0.06
Maternal infections in pregnancy			
Yes	45.8	46.9	0.27
Total energy intake kJ·day^{-1}	7260±1966	7162±2153	0.02
Maternal pre-pregnancy BMI			
<18.50 kg·m^{-2}	4.3	6.4	<0.001
18.50–24.99 kg·m^{-2}	75.4	72.8	
25.00–29.99 kg·m^{-2}	15.1	14.8	
⩾30.00 kg·m^{-2}	5.2	6.0	
Birthweight			
<2500 g	4.3	5.7	<0.001
2500–2999 g	13.8	15.2	
3000–3499 g	35.4	36.6	
3500–3999 g	33.2	30.8	
⩾4000 g	13.3	11.7	
Gestational age weeks	39.5±1.8	39.4±1.8	0.03
Child's BMI at 7 years			
<15.00 kg·m^{-2}	28.1	29.6	0.51
15.00–17.49 kg·m^{-2}	52.5	45.5	
17.50–20.49 kg·m^{-2}	15.2	19.3	
⩾20.50 kg·m^{-2}	4.2	5.7	
Maternal weight gain during pregnancy			
Quartile 1	25.3	28.4	<0.001
Quartile 2	24.8	24.4	
Quartile 3	25.6	22.0	
Quartile 4	24.4	25.2	

Data are presented as mean±SD or %, unless otherwise stated. BMI: body mass index. [#]: n=12 078; [¶]: Chi-squared tests were used for categorical variables, and t-tests and Wilcoxon tests were used for nonskewed- and skewed-distributed continuous variables, respectively.

When we stratified maternal dietary associations by maternal smoking, there was no evidence of effect modification by smoking on any childhood outcome (table 4). Conversely, when we stratified the association between maternal smoking during pregnancy and childhood $FEF_{25–75}$ (β per smoking category increase in the whole cohort 0.05 (95% CI −0.07–−0.02); p_{trend}=0.0001) by maternal intake (above and below median) of dietary antioxidants, associations between maternal smoking and childhood $FEF_{25–75}$ were stronger for mothers with below median intakes of fruits, vitamin C, vitamin E and the maternal antioxidant score, although there was no statistical evidence of interaction (supplementary table S1).

When the study population was restricted to mother–child pairs with complete data on maternal genotype, the main findings described above were similar (results not shown). Maternal and child genotype frequencies did not deviate significantly from Hardy–Weinberg equilibrium. When we stratified associations between maternal intake of fruits and vegetables during pregnancy and childhood outcomes by maternal GST gene polymorphisms, there was weak evidence for an interaction between vegetables intake and *GSTM1* genotype on FVC ($p_{interaction}$=0.07), with a positive association only if mothers were *GSTM1* null (table 5). When we investigated interactions between maternal intake of other antioxidants and maternal GST gene polymorphisms on childhood outcomes, weak evidence was found for an interaction between zinc and *GSTM1* on childhood FVC (β 0.11 (95% CI 0.05–0.17); p_{trend}=0.001 and 0.02 (95% CI −0.05–0.08); p_{trend}=0.57, respectively, for the null and non-null maternal *GSTM1* genotype groups; $p_{interaction}$=0.05). No interaction was found between maternal intake of other antioxidant nutrients or the antioxidant score and GST gene polymorphisms on any childhood outcome (data not shown). No interaction was found between maternal intake of selenium during pregnancy and maternal *GPX4* genotype on childhood outcomes (supplementary table S2). As a *post hoc* analysis, we studied the associations between maternal zinc intake and childhood FVC, stratified by combinations of maternal and child *GSTM1* genotypes. We observed positive associations if mothers were *GSTM1* null, regardless of the

TABLE 2 Associations between maternal dietary antioxidant intake and childhood asthma and atopy

	Asthma[#]		Atopy[¶]	
	Model 1[+]	Model 2[§]	Model 1[+]	Model 2[§]
Total fruits				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.80 (0.61–1.05)	0.87 (0.66–1.15)	0.89 (0.68–1.16)	0.84 (0.63–1.10)
Quartile 3	0.73 (0.57–0.94)	0.86 (0.66–1.12)	1.06 (0.82–1.36)	0.92 (0.70–1.20)
Quartile 4	0.68 (0.52–0.88)	0.82 (0.62–1.10)	1.06 (0.82–1.37)	0.85 (0.65–1.13)
Per quartile	0.90 (0.83–0.97)	0.95 (0.88–1.04)	1.06 (0.99–1.13)	0.98 (0.91–1.06)
p_{trend}	0.004	0.26	0.12	0.58
Total vegetables				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.94 (0.77–1.15)	0.96 (0.79–1.18)	0.98 (0.82–1.18)	0.94 (0.78–1.14)
Quartile 3	0.90 (0.75–1.09)	0.93 (0.77–1.14)	0.96 (0.81–1.15)	0.90 (0.75–1.08)
Quartile 4	0.84 (0.69–1.02)	0.88 (0.71–1.08)	1.07 (0.89–1.28)	0.96 (0.79–1.15)
Per quartile	0.95 (0.89–1.01)	0.96 (0.90–1.02)	1.02 (0.96–1.08)	0.98 (0.93–1.04)
p_{trend}	0.08	0.22	0.52	0.57
Vitamin C				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.83 (0.68–1.02)	0.90 (0.74–1.11)	1.03 (0.85–1.24)	0.94 (0.78–1.14)
Quartile 3	0.86 (0.70–1.04)	0.98 (0.79–1.20)	1.06 (0.88–1.28)	0.93 (0.76–1.13)
Quartile 4	0.77 (0.63–0.95)	0.89 (0.71–1.11)	1.20 (0.99–1.44)	0.98 (0.80–1.20)
Per quartile	0.93 (0.87–0.99)	0.97 (0.91–1.05)	1.06 (1.00–1.13)	1.00 (0.93–1.06)
p_{trend}	0.03	0.46	0.05	0.93
Vitamin E				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.83 (0.67–1.02)	0.90 (0.73–1.12)	1.03 (0.86–1.25)	0.97 (0.80–1.17)
Quartile 3	1.06 (0.87–1.31)	1.18 (0.95–1.46)	1.10 (0.91–1.32)	1.00 (0.82–1.22)
Quartile 4	0.88 (0.70–1.10)	0.99 (0.78–1.26)	1.12 (0.91–1.37)	0.99 (0.80–1.22)
Per quartile	0.99 (0.92–1.06)	1.03 (0.95–1.11)	1.04 (0.98–1.11)	1.00 (0.94–1.07)
p_{trend}	0.80	0.46	0.23	0.98
Zinc				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	1.05 (0.85–1.30)	1.15 (0.92–1.43)	1.00 (0.82–1.21)	0.91 (0.75–1.11)
Quartile 3	1.01 (0.80–1.27)	1.15 (0.90–1.47)	1.13 (0.92–1.40)	0.98 (0.78–1.22)
Quartile 4	0.97 (0.73–1.30)	1.15 (0.85–1.57)	1.07 (0.82–1.38)	0.85 (0.64–1.12)
Per quartile	0.99 (0.90–1.08)	1.04 (0.94–1.15)	1.04 (0.95–1.13)	0.96 (0.88–1.05)
p_{trend}	0.78	0.42	0.39	0.41
Selenium				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.95 (0.77–1.17)	1.04 (0.84–1.29)	1.09 (0.90–1.31)	1.00 (0.83–1.22)
Quartile 3	0.95 (0.77–1.18)	1.04 (0.83–1.31)	1.14 (0.94–1.39)	0.99 (0.80–1.21)
Quartile 4	0.90 (0.71–1.15)	1.03 (0.79–1.35)	1.07 (0.86–1.33)	0.87 (0.68–1.10)
Per quartile	0.97 (0.90–1.05)	1.01 (0.93–1.10)	1.02 (0.95–1.10)	0.95 (0.89–1.03)
p_{trend}	0.44	0.83	0.53	0.23
Carotene				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.95 (0.78–1.15)	0.94 (0.77–1.15)	0.91 (0.76–1.09)	0.91 (0.75–1.09)
Quartile 3	0.91 (0.74–1.10)	0.94 (0.77–1.16)	1.03 (0.87–1.23)	1.01 (0.84–1.21)
Quartile 4	0.89 (0.73–1.09)	0.93 (0.75–1.14)	1.00 (0.84–1.20)	0.93 (0.77–1.12)
Per quartile	0.96 (0.90–1.02)	0.98 (0.91–1.05)	1.01 (0.96–1.07)	0.99 (0.93–1.05)
p_{trend}	0.23	0.51	0.67	0.68
Antioxidant score				
Quartile 1	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Quartile 2	0.98 (0.79–1.21)	1.09 (0.88–1.36)	1.04 (0.85–1.27)	0.94 (0.76–1.15)
Quartile 3	0.91 (0.72–1.15)	1.06 (0.83–1.37)	1.20 (0.96–1.48)	1.00 (0.80–1.26)
Quartile 4	0.81 (0.62–1.05)	0.98 (0.74–1.31)	1.19 (0.94–1.51)	0.92 (0.71–1.20)
Per quartile	0.93 (0.86–1.01)	0.98 (0.90–1.08)	1.07 (0.99–1.15)	0.98 (0.91–1.07)
p_{trend}	0.07	0.72	0.08	0.70

Data are presented as odds ratio (95% CI), unless otherwise stated. [#]: n=7677; [¶]: n=6117; [+]: model controlling for energy intake only; [§]: model controlling for energy intake, smoking, infections, supplements, antibiotics and paracetamol use during pregnancy, and maternal educational level, housing tenure, financial difficulties, ethnicity, age, parity, history of atopic diseases, anxiety, sex of child, season of birth, multiple pregnancy and breastfeeding duration.

TABLE 3 Associations between maternal dietary antioxidant intake and childhood lung function

	FEV_1		FVC		FEF_{25-75}	
	Model 1[#]	Model 2[¶]	Model 1[#]	Model 2[¶]	Model 1[#]	Model 2[¶]
Subjects n	6062		6157		6157	
Total fruits						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	−0.02 (−0.13–0.09)	−0.03 (−0.14–0.08)	−0.01 (−0.11–0.08)	−0.04 (−0.15–0.07)	−0.01 (−0.12–0.10)	−0.03 (−0.14–0.08)
Quartile 3	0.04 (−0.06–0.15)	0.02 (−0.09–0.12)	−0.01 (−0.10–0.07)	−0.03 (−0.14–0.08)	0.07 (−0.04–0.17)	0.04 (−0.07–0.14)
Quartile 4	0.06 (−0.05–0.17)	0.03 (−0.09–0.14)	0.04 (−0.05–0.13)	0.02 (−0.10–0.13)	0.05 (−0.06–0.15)	0.01 (−0.10–0.13)
Per quartile	0.03 (0.00–0.06)	0.02 (−0.01–0.05)	0.02 (0.00–0.05)	0.02 (−0.01–0.05)	0.02 (−0.01–0.05)	0.01 (−0.02–0.04)
p_{trend}	0.04	0.25	0.09	0.26	0.12	0.41
Total vegetables						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	0.06 (−0.01–0.14)	0.05 (−0.03–0.13)	0.02 (−0.05–0.10)	0.02 (−0.06–0.09)	0.08 (0.00–0.15)	0.06 (−0.02–0.14)
Quartile 3	0.02 (−0.05–0.09)	0.01 (−0.07–0.08)	−0.01 (−0.08–0.06)	−0.02 (−0.09–0.05)	0.06 (−0.01–0.13)	0.05 (−0.03–0.12)
Quartile 4	0.10 (0.03–0.17)	0.08 (0.01–0.16)	0.07 (0.00–0.15)	0.06 (−0.01–0.14)	0.10 (0.03–0.18)	0.09 (0.01–0.16)
Per quartile	0.03 (0.00–0.05)	0.02 (0.00–0.05)	0.02 (0.00–0.04)	0.01 (−0.01–0.04)	0.03 (0.01–0.05)	0.02 (0.00–0.05)
p_{trend}	0.03	0.09	0.12	0.22	0.01	0.05
Vitamin C						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	0.05 (−0.02–0.13)	0.04 (−0.04–0.12)	0.06 (−0.01–0.14)	0.05 (−0.02–0.13)	0.02 (−0.05–0.10)	0.01 (−0.07–0.09)
Quartile 3	0.05 (−0.03–0.12)	0.02 (−0.05–0.10)	0.05 (−0.02–0.12)	0.03 (−0.04–0.11)	0.02 (−0.05–0.10)	0.00 (−0.07–0.08)
Quartile 4	0.06 (−0.02–0.14)	0.04 (−0.05–0.12)	0.05 (−0.02–0.13)	0.04 (−0.05–0.12)	0.05 (−0.02–0.13)	0.03 (−0.05–0.12)
Per quartile	0.02 (−0.01–0.04)	0.01 (−0.02–0.03)	0.01 (−0.01–0.04)	0.01 (−0.02–0.03)	0.02 (−0.01–0.04)	0.01 (−0.02–0.04)
p_{trend}	0.17	0.53	0.30	0.62	0.19	0.45
Vitamin E						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	−0.03 (−0.10–0.05)	−0.03 (−0.11–0.04)	0.00 (−0.07–0.08)	0.01 (−0.07–0.08)	−0.02 (−0.09–0.06)	−0.04 (−0.11–0.04)
Quartile 3	−0.02 (−0.10–0.06)	−0.03 (−0.11–0.05)	0.02 (−0.06–0.10)	0.02 (−0.06–0.10)	−0.02 (−0.10–0.05)	−0.05 (−0.13–0.03)
Quartile 4	0.01 (−0.07–0.10)	−0.01 (−0.09–0.08)	0.04 (−0.04–0.13)	0.04 (−0.04–0.13)	0.00 (−0.08–0.09)	−0.03 (−0.12–0.05)
Per quartile	0.01 (−0.02–0.03)	0.00 (−0.03–0.03)	0.01 (−0.01–0.04)	0.01 (−0.01–0.04)	0.00 (−0.02–0.03)	−0.01 (−0.04–0.02)
p_{trend}	0.63	0.98	0.26	0.28	0.90	0.48
Zinc						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	0.05 (−0.03–0.13)	0.04 (−0.04–0.12)	0.04 (−0.04–0.12)	0.03 (−0.05–0.11)	0.05 (−0.03–0.13)	0.03 (−0.05–0.11)
Quartile 3	0.11 (0.02–0.20)	0.08 (−0.01–0.17)	0.13 (0.04–0.21)	0.11 (0.02–0.20)	0.06 (−0.03–0.15)	0.02 (−0.07–0.11)
Quartile 4	0.18 (0.07–0.29)	0.14 (0.03–0.25)	0.16 (0.05–0.26)	0.14 (0.03–0.25)	0.13 (0.02–0.23)	0.07 (−0.04–0.18)
Per quartile	0.06 (0.03–0.09)	0.05 (0.01–0.08)	0.06 (0.02–0.09)	0.05 (0.02–0.09)	0.04 (0.00–0.07)	0.02 (−0.02–0.06)
p_{trend}	0.001	0.01	0.001	0.005	0.03	0.28
Selenium						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	0.05 (−0.03–0.13)	0.03 (−0.05–0.11)	0.05 (−0.03–0.13)	0.05 (−0.03–0.13)	0.04 (−0.04–0.12)	0.00 (−0.07–0.08)
Quartile 3	0.06 (−0.02–0.14)	0.04 (−0.04–0.13)	0.05 (−0.03–0.13)	0.05 (−0.04–0.18)	0.06 (−0.02–0.14)	0.02 (−0.07–0.10)
Quartile 4	0.09 (0.00–0.18)	0.06 (−0.03–0.16)	0.11 (0.02–0.20)	0.10 (0.01–0.20)	0.05 (−0.04–0.14)	−0.01 (−0.10–0.09)
Per quartile	0.03 (0.00–0.06)	0.02 (−0.01–0.05)	0.03 (0.00–0.06)	0.03 (0.00–0.06)	0.02 (−0.01–0.05)	0.00 (−0.03–0.03)
p_{trend}	0.05	0.22	0.03	0.06	0.25	0.95
Carotene						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	0.01 (−0.07–0.08)	−0.01 (−0.09–0.06)	0.02 (−0.05–0.09)	0.01 (−0.06–0.09)	−0.02 (−0.09–0.05)	−0.05 (−0.12–0.02)
Quartile 3	0.05 (−0.02–0.12)	0.03 (−0.05–0.10)	0.02 (−0.05–0.10)	0.01 (−0.06–0.09)	0.03 (−0.04–0.11)	0.00 (−0.07–0.07)
Quartile 4	0.10 (0.03–0.18)	0.08 (0.01–0.16)	0.08 (0.01–0.16)	0.08 (0.00–0.15)	0.07 (0.00–0.15)	0.04 (−0.03–0.12)
Per quartile	0.04 (0.01–0.06)	0.03 (0.00–0.05)	0.03 (0.00–0.05)	0.02 (0.00–0.05)	0.03 (0.00–0.05)	0.02 (−0.01–0.04)
p_{trend}	0.004	0.02	0.03	0.05	0.03	0.17
Antioxidant score						
Quartile 1	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)	0.00 (reference)
Quartile 2	−0.01 (−0.09–0.07)	−0.03 (−0.11–0.06)	0.01 (−0.07–0.09)	0.02 (−0.06–0.10)	−0.01 (−0.09–0.07)	−0.04 (−0.13–0.04)
Quartile 3	0.03 (−0.06–0.12)	0.01 (−0.09–0.10)	0.03 (−0.05–0.12)	0.04 (−0.06–0.13)	0.04 (−0.05–0.12)	−0.01 (−0.10–0.09)
Quartile 4	0.12 (0.02–0.22)	0.08 (−0.02–0.19)	0.13 (0.03–0.22)	0.12 (0.02–0.23)	0.07 (−0.03–0.17)	0.01 (−0.10–0.12)
Per quartile	0.04 (0.01–0.08)	0.03 (0.00–0.07)	0.04 (0.01–0.07)	0.04 (0.01–0.07)	0.03 (0.00–0.06)	0.01 (−0.02–0.04)
p_{trend}	0.004	0.04	0.005	0.01	0.06	0.52

Data are presented as β (difference in age-, height- and sex-adjusted SD units) (95% CI), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25-75}: forced expiratory flow at 25–75% of FVC. [#]: model controlling for energy intake only; [¶]: model controlling for energy intake, smoking, infections, supplements, antibiotics and paracetamol use during pregnancy, and maternal educational level, housing tenure, financial difficulties, ethnicity, age, parity, history of atopic diseases, anxiety, sex of child, season of birth, multiple pregnancy and breastfeeding duration.

TABLE 4 Associations between maternal dietary antioxidant intake and childhood outcomes stratified by maternal smoking during pregnancy

	Asthma		Atopy		FEV_1		FVC		$FEF_{25–75}$	
	OR[#] (95% CI)	p_{trend}	OR[#] (95% CI)	p_{trend}	β[#] (95% CI)	p_{trend}	β[#] (95% CI)	p_{trend}	β[#] (95% CI)	p_{trend}
Subjects n	7677		6117		6062		6157		6157	
Total fruits intake										
Non/passive smokers	0.99 (0.89–1.09)	0.81	1.02 (0.93–1.12)	0.61	0.02 (−0.02–0.05)	0.41	0.02 (−0.02–0.05)	0.35	0.01 (−0.02–0.05)	0.43
Active smokers	0.90 (0.78–1.04)	0.16	0.87 (0.76–1.01)	0.07	0.02 (−0.04–0.08)	0.49	0.02 (−0.04–0.08)	0.45	0.00 (−0.06–0.06)	1.00
$p_{interaction}$[¶]	0.59		0.14		0.60		0.90		0.93	
Total vegetables intake										
Non/passive smokers	1.00 (0.92–1.08)	0.91	0.99 (0.93–1.06)	0.85	0.02 (−0.01–0.05)	0.15	0.02 (−0.01–0.05)	0.16	0.02 (−0.01–0.04)	0.23
Active smokers	0.88 (0.78–1.00)	0.05	0.94 (0.83–1.07)	0.34	0.02 (−0.03–0.07)	0.36	0.00 (−0.05–0.05)	0.96	0.04 (0.00–0.09)	0.07
$p_{interaction}$[¶]	0.18		0.70		0.96		0.35		0.25	
Vitamin C intake										
Non/passive smokers	1.00 (0.92–1.09)	0.99	1.01 (0.94–1.09)	0.75	0.00 (−0.03–0.03)	0.81	0.01 (−0.02–0.04)	0.53	0.00 (−0.03–0.03)	0.86
Active smokers	0.92 (0.81–1.05)	0.23	0.93 (0.82–1.06)	0.30	0.02 (−0.03–0.07)	0.49	0.00 (−0.05–0.05)	0.95	0.02 (−0.03–0.07)	0.41
$p_{interaction}$[¶]	0.54		0.57		0.55		0.65		0.30	
Vitamin E intake										
Non/passive smokers	1.06 (0.97–1.16)	0.19	0.97 (0.90–1.05)	0.44	0.00 (−0.03–0.03)	0.96	0.02 (−0.01–0.05)	0.25	−0.01 (−0.05–0.02)	0.38
Active smokers	0.96 (0.84–1.11)	0.60	1.12 (0.98–1.28)	0.11	0.00 (−0.06–0.05)	0.91	0.00 (−0.05–0.05)	0.95	0.00 (−0.05–0.06)	0.94
$p_{interaction}$[¶]	0.39		0.09		0.83		0.51		0.53	
Zinc intake										
Non/passive smokers	1.06 (0.94–1.19)	0.36	0.96 (0.86–1.06)	0.39	0.04 (0.00–0.08)	0.05	0.05 (0.01–0.09)	0.02	0.01 (−0.03–0.05)	0.58
Active smokers	1.01 (0.85–1.21)	0.90	1.01 (0.85–1.21)	0.90	0.05 (−0.02–0.12)	0.15	0.05 (−0.02–0.12)	0.14	0.04 (−0.03–0.11)	0.30
$p_{interaction}$[¶]	0.92		0.61		0.74		0.72		0.76	
Selenium intake										
Non/passive smokers	0.99 (0.90–1.10)	0.87	0.93 (0.86–1.02)	0.12	0.03 (−0.01–0.06)	0.13	0.03 (0.00–0.07)	0.07	0.01 (−0.03–0.04)	0.76
Active smokers	1.05 (0.90–1.22)	0.56	1.03 (0.89–1.19)	0.72	0.00 (−0.06–0.06)	0.93	0.03 (−0.03–0.08)	0.40	−0.01 (−0.07–0.04)	0.64
$p_{interaction}$[¶]	0.50		0.31		0.32		0.52		0.67	
Carotene intake										
Non/passive smokers	0.98 (0.91–1.07)	0.70	0.98 (0.91–1.05)	0.53	0.02 (0.00–0.05)	0.10	0.02 (−0.01–0.05)	0.12	0.01 (−0.02–0.04)	0.52
Active smokers	0.97 (0.85–1.10)	0.59	1.02 (0.90–1.15)	0.78	0.04 (−0.01–0.09)	0.11	0.03 (−0.02–0.08)	0.25	0.04 (−0.01–0.08)	0.15
$p_{interaction}$[¶]	0.83		0.47		0.75		0.85		0.32	
Antioxidant score										
Non/passive smokers	1.01 (0.91–1.13)	0.85	0.96 (0.87–1.05)	0.35	0.03 (−0.01–0.07)	0.18	0.04 (0.00–0.08)	0.03	0.00 (−0.04–0.04)	0.93
Active smokers	0.94 (0.80–1.11)	0.44	1.08 (0.92–1.27)	0.37	0.05 (−0.01–0.11)	0.13	0.04 (−0.03–0.10)	0.23	0.04 (−0.02–0.10)	0.22
$p_{interaction}$[¶]	0.70		0.25		0.86		0.64		0.35	

β: difference in age-, height- and sex-adjusted SD units; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75}$: forced expiratory flow at 25–75% of FVC. [#]: per category/quartile of dietary intake, controlling for energy intake, infections, supplements, antibiotics and paracetamol use during pregnancy, and maternal educational level, housing tenure, financial difficulties, ethnicity, age, parity, history of atopic diseases, anxiety, sex of child, season of birth, multiple pregnancy and breastfeeding duration; [¶]: treating smoking as a binary variable and dietary exposures as continuous variables.

child's *GSTM1* genotype (table 6). *Post hoc* analysis did not show evidence of an interaction between maternal zinc intake and maternal *GSTM1* on childhood FVC at 15 years ($p_{interaction}$=0.16).

Discussion

In this large, population-based, birth cohort study, we found that a higher maternal zinc intake during pregnancy was associated, in a dose–response fashion, with higher FEV_1 and FVC in the offspring, after controlling for potential confounders. To the best of our knowledge this is a novel finding. Only one other birth cohort study has investigated the relation between maternal diet in pregnancy and childhood lung function, and did not report any association between maternal zinc intake and lung function in the offspring at age 5 years [2], but the sample size was much smaller than ours. We also found weak evidence for an interaction between maternal zinc intake during pregnancy and maternal *GSTM1* genotype on childhood FVC. Interactions between maternal intake of antioxidants and antioxidant genotype on childhood lung function and other respiratory and atopic outcomes have not previously been investigated. The graded nature of the associations between maternal zinc intake and lung function is in keeping with a causal effect on lung growth and development, and persistence of the association from childhood to adolescence strengthens causal inference further. While we also found positive associations between the maternal antioxidant score during pregnancy (derived from five antioxidant nutrients) and childhood lung function, these were largely explained by maternal zinc intake.

TABLE 5 Associations between maternal fruits and vegetables intake and childhood outcomes stratified by maternal glutathione *S*-transferase gene polymorphisms

	Asthma		Atopy		FEV_1		FVC		$FEF_{25–75}$	
	OR# (95% CI)	p_{trend}	OR# (95% CI)	p_{trend}	β# (95% CI)	p_{trend}	β# (95% CI)	p_{trend}	β# (95% CI)	p_{trend}
Subjects	4953		3911		4011		4080		4080	
Total fruits intake										
GSTT1										
Non-null (n=4376)	0.91 (0.81–1.02)	0.10	1.01 (0.90–1.12)	0.92	0.01 (−0.03–0.06)	0.52	0.02 (−0.02–0.06)	0.39	0.00 (−0.04–0.04)	0.95
Null (n=870)	1.00 (0.77–1.30)	0.99	0.92 (0.71–1.18)	0.49	0.04 (−0.06–0.14)	0.42	0.04 (−0.06–0.13)	0.48	0.04 (−0.06–0.14)	0.46
$p_{interaction}$	0.71		0.47		0.44		0.50		0.48	
GSTM1										
Non-null (n=2476)	0.84 (0.72–0.98)	0.03	1.01 (0.87–1.17)	0.93	0.01 (−0.05–0.06)	0.77	0.03 (−0.03–0.08)	0.30	−0.03 (−0.09–0.03)	0.29
Null (n=2799)	0.94 (0.82–1.09)	0.44	0.98 (0.85–1.12)	0.73	0.04 (−0.02–0.09)	0.20	0.02 (−0.03–0.08)	0.46	0.04 (−0.01–0.10)	0.13
$p_{interaction}$	0.12		0.54		0.53		0.87		0.26	
GSTP1, rs1695										
A:A (n=2289)	0.96 (0.81–1.13)	0.61	0.99 (0.85–1.16)	0.94	0.01 (−0.05–0.07)	0.66	0.01 (−0.05–0.07)	0.75	0.02 (−0.04–0.08)	0.47
G:A (n=2529)	0.91 (0.78–1.06)	0.23	0.98 (0.85–1.13)	0.76	0.02 (−0.04–0.08)	0.55	0.02 (−0.04–0.08)	0.46	0.00 (−0.06–0.06)	0.97
G:G (n=670)	0.83 (0.61–1.13)	0.25	0.80 (0.59–1.10)	0.17	0.00 (−0.11–0.12)	0.94	0.01 (−0.10–0.12)	0.83	0.01 (−0.11–0.13)	0.85
$p_{interaction}$	0.60		0.94		0.59		0.87		0.31	
Total vegetables intake										
GSTT1										
Non-null (n=4376)	0.91 (0.83–1.00)	0.05	1.01 (0.93–1.10)	0.85	0.04 (0.00–0.07)	0.03	0.05 (0.01–0.07)	0.006	0.02 (−0.01–0.05)	0.21
Null (n=870)	1.00 (0.80–1.25)	0.98	0.99 (0.80–1.23)	0.95	0.01 (−0.07–0.09)	0.84	−0.03 (−0.11–0.05)	0.46	0.04 (−0.04–0.12)	0.30
$p_{interaction}$	0.25		0.98		0.81		0.22		0.68	
GSTM1										
Non-null (n=2476)	0.90 (0.79–1.02)	0.09	0.96 (0.86–1.08)	0.54	0.01 (−0.03–0.06)	0.54	0.01 (−0.03–0.05)	0.59	0.03 (−0.01–0.07)	0.19
Null (n=2799)	0.94 (0.84–1.06)	0.32	1.05 (0.95–1.17)	0.33	0.06 (0.01–0.10)	0.01	0.06 (0.02–0.10)	0.004	0.02 (−0.02–0.06)	0.41
$p_{interaction}$	0.52		0.15		0.24		0.07		0.41	
GSTP1, rs1695										
A:A (n=2289)	0.98 (0.86–1.12)	0.76	1.02 (0.90–1.15)	0.77	0.06 (0.01–0.11)	0.01	0.06 (0.02–0.11)	0.008	0.04 (−0.01–0.08)	0.12
G:A (n=2529)	0.98 (0.87–1.10)	0.72	1.06 (0.94–1.18)	0.33	0.02 (−0.03–0.06)	0.41	0.02 (−0.02–0.07)	0.27	0.00 (−0.04–0.05)	0.88
G:G (n=670)	0.89 (0.69–1.15)	0.37	0.81 (0.64–1.02)	0.08	0.01 (−0.07–0.10)	0.78	−0.01 (−0.10–0.07)	0.76	0.06 (−0.03–0.15)	0.21
$p_{interaction}$	0.42		0.19		0.35		0.18		0.82	

β: difference in age-, height- and sex-adjusted SD units; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75}$: forced expiratory flow at 25–75% of FVC. #: per category/quartile of fruits/vegetables intake, controlling for energy intake, smoking, infections, supplements, antibiotics and paracetamol use during pregnancy, and maternal educational level, housing tenure, financial difficulties, ethnicity, age, parity, history of atopic diseases, anxiety, sex of child, season of birth, multiple pregnancy and breastfeeding duration.

A surprising observation was the lack of interaction between maternal intake of antioxidants and maternal smoking on childhood outcomes. We hypothesised that a higher intake of antioxidants might be particularly beneficial if the fetus was exposed to tobacco smoke, a source of oxidative stress, but no such effect modification was seen. To the best of our knowledge, this has not been investigated before. However, when we examined the detrimental effect of maternal smoking on $FEF_{25–75}$, we found that effect estimates were generally larger if mothers had below average intakes of antioxidants, especially vitamin C and vitamin E, than if their intakes were above average. While these differences were not statistically significant on formal testing for interaction, they are in keeping with a trial in pregnant smokers which showed that the detrimental effect of smoking on infant lung function was reduced by vitamin C supplementation in pregnancy [11]. One possible explanation for why we did not see a statistically significant interaction between antioxidant intake and smoking is that the estimated maximum vitamin C intake from food alone in ALSPAC pregnant females was 256 $mg \cdot day^{-1}$, which is much lower than the 500 mg daily intake of vitamin C taken by mothers in the trial [11], although ~20% of ALSPAC females were also taking vitamin supplements.

While some studies have suggested a possible protective effect of maternal intake of vitamin E, zinc, fruits and vegetables during pregnancy on childhood asthma and atopy [2–4], we found no evidence to support this, nor were the other antioxidant nutrients associated with these outcomes, which is concordant with two recent systematic reviews and meta-analyses [5, 43]. Given the size of our study, we therefore believe that the new totality of evidence (including ALSPAC) indicates that there is unlikely to be a causal relationship between dietary antioxidant intake in pregnancy and risk of childhood asthma and atopy.

Mechanisms

A plausible explanation for the associations we observed between maternal zinc intake during pregnancy and childhood lung function, and especially FVC, could be that pre-natal zinc status influences growth

TABLE 6 Associations between maternal zinc intake and childhood forced vital capacity (FVC) stratified by combinations of maternal and child glutathione *S*-transferase *GSTM1* genotypes

GSTM1		n	FVC[#]	
Mother	**Child**		**β[¶] (95% CI)**	**p_{trend}**
Non-null	Non-null	956	0.04 (−0.12–0.05)	0.41
Non-null	Null	452	0.04 (−0.11–0.18)	0.60
Null	Non-null	439	0.13 (−0.01–0.27)	0.07
Null	Null	1167	0.15 (0.07–0.23)	0.0002

β: difference in age-, height- and sex-adjusted SD units. [#]: n=3014; [¶]: per quartile of zinc intake, controlling for energy intake, smoking, infections, supplements, antibiotics and paracetamol use during pregnancy, and maternal educational level, housing tenure, financial difficulties, ethnicity, age, parity, history of atopic diseases, anxiety, sex of child, season of birth, multiple pregnancy and breastfeeding duration.

and development of fetal lungs. In support of this hypothesis, zinc deficiency has been associated with impaired fetal lung growth in rats [44]. According to the FFQ completed in pregnancy, the main sources of dietary zinc were red meat and poultry in ALSPAC pregnant females. Although zinc is generally considered to be an antioxidant, it can serve such a function only indirectly and the term "pro-antioxidant" is more appropriate [45]. While the interaction between maternal zinc intake and maternal *GSTM1* genotype on childhood FVC is in keeping with a pro-antioxidant effect of zinc on pre-natal lung growth (stronger association if the mother was *GSTM1* null and therefore had compromised enzymatic antioxidant defences), the lack of effect modification by maternal smoking and the lack of effect modification by maternal *GSTM1* genotype on FVC in adolescence do not support such an interpretation. However, zinc influences growth through multiple, complex pathways [46] and effects on fetal lung growth may not involve its pro-antioxidant properties.

Strengths and limitations

Strengths of the ALSPAC birth cohort include its population-based prospective design, rich information on numerous potential confounders and detailed phenotypic outcome measurements. ALSPAC's size gave us greater statistical power than previous, smaller birth cohorts that have investigated this research question. Another major strength of the ALSPAC birth cohort is that maternal DNA was collected, enabling maternal genotyping and exploration of interactions with pre-natal exposures, which is not possible in most other birth cohort studies.

Although the FFQ that we used had not been formally calibrated against other instruments such as diet diaries, it was based on the one used by YARNELL *et al.* [47], which has been validated against weighed dietary records, and modified in the light of a more recent weighed dietary survey [21]. While there will have been some misclassification of dietary exposures, this is likely to be nondifferential with respect to the outcomes of interest and would be expected to bias effect estimates towards the null; in other words, the magnitude of associations may have been underestimated and small or modest effects may have been missed. The possibility of uncontrolled or residual confounding cannot be ruled out. However, we think that confounding of the main findings by lifestyle or other aspects of maternal diet in pregnancy is unlikely, as we controlled for numerous potential confounders in the analyses, including post-natal zinc intake. The null findings for maternal and paternal zinc intakes after pregnancy make confounding by unmeasured familial behaviours linked to zinc intake and offspring lung function a less likely explanation for the main findings.

As with any longitudinal study, we cannot rule out the possibility that exclusion of mother–child pairs without complete information might have biased our findings. However, it could be argued that, for our results to be totally spurious for maternal zinc intake and childhood lung function in those included in our analysis (and for the associations to be truly null in the population as a whole), associations in the excluded mother–child pairs would have to be at least of equal magnitude in the opposite direction, which seems unlikely. Furthermore, loss to follow-up bias has been shown to only slightly modify associations in longitudinal studies, including in ALSPAC [48], and the results of our inverse probability weighting analysis confirmed that loss to follow-up is unlikely to have biased our results. In view of the multiple analyses carried out, we cannot exclude the possibility that the main findings occurred by chance; hence, they should be interpreted with caution and require replication in another birth cohort study. Given the *a priori* nature of the general hypothesis being tested and the fact that some outcomes of interest were highly correlated, it did not seem appropriate to correct for multiple testing.

Conclusions

We conclude that a higher maternal intake of zinc during pregnancy may improve lung function, and especially FVC, in the offspring, but further studies are needed to confirm these results. A Mendelian randomisation approach could be used to strengthen causal inference. If the association with pre-natal zinc status is causal, this may have greater implications in developing countries where zinc deficiency is a bigger problem today than it is in the West [49]. In contrast, we found no evidence that maternal dietary antioxidant intake in pregnancy is associated with risk of childhood asthma or atopy, suggesting that intervening in pregnancy to increase antioxidant intake would be unlikely to succeed as a strategy to prevent these conditions.

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Author contributions: A. Bédard and S.O. Shaheen conceived the study and drafted the manuscript. All authors were involved in the analysis strategy, K. Northstone gave advice on the dietary data, and A. Bédard performed the statistical analyses. A.J. Henderson was responsible for all clinical respiratory and allergy data collection. J.W. Holloway was responsible for generation of the genotyping data. All authors participated in the interpretation of the findings, reviewed the manuscript and revised it critically before submission. All authors have seen and approved the final version of the manuscript.

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