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Dietary antioxidant intake in school age and lung function development up to adolescence

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"Take home" message: Dietary antioxidant intake in school age may influence lung function development as measured by FEV_1 up to adolescence among children with asthma. In contrast, no association was observed among children without asthma.

Key words: antioxidants, asthma, diet, lung function, total antioxidant capacity

Short title: Dietary antioxidant intake and lung function

ABSTRACT

Dietary antioxidant intake has been hypothesized to influence lung function. The association between total antioxidant capacity (TAC) of the diet at age 8 years and lung function development up to 16 years in 2307 participants from the Swedish population-based birth cohort BAMSE was investigated.

Information on TAC was obtained from a food frequency questionnaire at 8 years. Lung function was measured by spirometry at 8 and 16 years, impulse oscillometry (IOS) and fractional exhaled nitric oxide (FE_{NO}) at 16 years. Low lung function was defined as forced expiratory volume in one second (FEV_1) z-score below the 25th percentile. Longitudinal associations between TAC and lung function were analyzed by mixed effect models adjusted for potential confounders. Stratification by asthma at 8 years was performed to examine effect modification.

The median TAC intake was 10067 μmol TE/g, with boys having a lower mean compared to girls (9963 vs 10819 μmol TE/g). In analyses of lung function change between 8 and 16 years, there were no statistically significant associations between TAC in tertiles and spirometry results for the total study population. Among children with asthma at 8 years (prevalence 7%), higher TAC was associated with higher mean FEV_1 (0.46 SD, 95% CI:0.11;0.80) and decreased odds of low lung function at 16 years (OR 0.28, 95% CI:0.12;0.65). There were no associations between TAC and forced vital capacity or IOS/FE_{NO} results.

High dietary antioxidant intake in school age may be associated with improved lung function development from school age to adolescence among children with asthma.

INTRODUCTION

In recent years, the importance of a full growth to maximal lung function in childhood has been reinforced by accumulating evidence that lung function deficits established by school age may track into adult life (1, 2). Thus, achieving optimal lung function is an important goal in the prevention of chronic respiratory diseases and subsequent mortality, and a major public health objective (3). However, less is known about factors that might influence lung function trajectories (4, 5).

The association between dietary factors with antioxidant and anti-inflammatory properties and risk of asthma and other chronic respiratory diseases in the general population has been previously investigated (6-8). Prospective studies examining the association between maternal diet during pregnancy and the occurrence of asthma and other allergic diseases in the offspring have contributed information on the role of dietary exposures early in life (9). Analyses from the Swedish BAMSE birth cohort also show that a high intake of dietary antioxidants at age 8 years was associated with a reduced risk of subsequent development of IgE sensitization to inhalant allergens and allergic asthma (10). A recent prospective study from Japan found a significant inverse association between fruit intake and the onset of respiratory allergic symptoms in schoolchildren (11).

Epidemiological studies on the association between dietary antioxidants and lung function show conflicting results (12-14). Most studies have been cross-sectional, but a prospective study in middle-aged adults from three participating countries of the European Community Respiratory Health Survey indicated that a higher intake of fruits and tomatoes was associated with a slower decline in lung function 10 years later (15). A recent case-control study in Puerto Rican children indicated that a diet with frequent consumption of vegetables and grains and low consumption of dairy products and sweets was associated with higher lung function, as measured by forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) (16).

Longitudinal studies on childhood diet and subsequent lung function development are still lacking. Thus, it remains unclear if diet in school age influences lung function. The aim of this study was to investigate the association between dietary antioxidant intake at age 8 years and lung function development between 8 and 16 years. In order to estimate the cumulative action of the antioxidants present in foods, total antioxidant capacity (TAC) of the diet was used (10).

METHODS

Study population and study design

The study was conducted within the population-based birth cohort BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology), to which 4089 children (born 1994-1996) from predefined areas of Stockholm County, Sweden have been followed repeatedly from infancy (4, 17). In brief, baseline information was collected through parental questionnaires when the children were on average two months old and follow-up questionnaires eliciting information on symptoms of allergic diseases and selected exposures were answered by the parents when the children were 1, 2, 4 and 8 years and by the adolescents themselves at 16 years. At ages 8 and 16 years, participants were invited to clinical examinations, which included anthropometric measurements, lung function testing and blood sampling using standardized methods. Sera have been analyzed for specific IgE to common inhalant and food allergens. Drop-out rates remained low at all ages, and at 16 years 78% (n=3180) completed the questionnaire and 62% (n=2547) attended the clinical examination. The BAMSE study and respective follow-ups were approved by the Regional Ethical Review Board, Karolinska Institutet, Stockholm, Sweden, and written informed consent was obtained from parents at 8 years and study participants at 16 years.

Dietary assessment

Diet was assessed at 8 years, using a food frequency questionnaire (FFQ). The FFQ was most often filled out by a parent (57%) or by a parent together with the child (40%) and included questions about 98 foods and beverages commonly consumed in Sweden. Children (n = 2614) were asked how often, on average, they had consumed each type of food or beverage during the past 12 months. There were 10 pre-specified response categories ranging from 'never' to '≥3 times/day'. Calculation of the TAC of the FFQ items has been previously described (10). Briefly, individual TAC estimates were obtained by combining the information on frequency of consumption of specific food items with information from a database of common foods analyzed with the oxygen radical absorbance capacity (ORAC) method (18) on the average ORAC content [μmol Trolox equivalents/day (TE/day)] of age-specific portion sizes. ORAC values were further energy-adjusted using the residuals method (19). There were 35 food items (including 20 fruits

and vegetables) with available ORAC values, while there was no available information on TAC from dietary supplements.

Lung function testing

Details of lung function testing have been described elsewhere (4). Shortly, lung function was measured by spirometry at 8 years (n=1832) using a 2200 Pulmonary Function Laboratory (SensorMedics, Anaheim, California, USA) and by impulse oscillometry (IOS) (n=2452) followed by spirometry at 16 years (n=2056) using a Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, California, USA). The same spirometry test protocol was used at both time-points. All participants performed repeated maximal expiratory flow volume (MEFV) measurements. The highest values of FEV_1 and FVC were extracted and used for analysis, provided that the subject's effort was accepted as being maximal by the test leader, the MEFV curve passed visual quality inspection and the two highest FEV_1 and FVC readings were reproducible according to American Thoracic Society/ European Respiratory Society (ATS/ERS) criteria (20). FEV_1/FVC ratios were calculated and expressed as percentages. Standard deviation (SD) scores (z-scores) for FEV_1, FVC, and FEV_1/FVC were computed accounting for age, sex, height and ethnicity (21). Regarding IOS measurements, the mean value of resistance at 5 and 20 Hz (R_5, R_{20}), frequency dependence of resistance (R_{5-20}), and the square root of the area of reactance ($AX^{0.5}$) were used for analyses. Measurements of fractional exhaled nitric oxide (FE_{NO}, expressed as parts per billion, ppb) were performed at 16 years (n=2087) at an expiratory flow of 50 mL/s, using an online chemiluminescent (CLD88) analyser (Eco Medics AG, Duernten, Switzerland). Details of lung function measurements, as well as asthma and other definitions are described in the online supplement.

Statistical analyses

Differences between children who were included and excluded from the study population were analysed by the chi-square and the t-test, for categorical and continuous variables respectively. The distribution of selected exposure characteristics by tertiles (T1, T2, T3) of TAC (linear relationship not assumed) was compared by the chi-square test (categorical covariates) and analysis of variance (ANOVA) (continuous covariates). Multivariate linear regression on the mean was used to analyze associations between dietary TAC in tertiles at age 8 years and lung

function parameters at ages 8 and 16 years. Tests for trends were performed by assigning the median value of dietary TAC within each tertile and tested as a continuous variable in the model. Analyses were stratified by gender and potential interactions with gender were tested by the Wald test using an interaction term between TAC and gender in the statistical model. Covariates were identified from previous literature (22) and included maternal age<26 years (yes or no), older siblings (≥1 older sibling at birth, yes or no), socioeconomic status (categorized on the basis of parents' occupation as manual and non-manual workers), parental allergic disease (any maternal or paternal history of asthma or hay fever, yes or no), maternal smoking during pregnancy (yes or no) and parental smoking in infancy (yes or no). Additional adjustment for educational level, energy intake, dietary vitamin D and fish intake, supplement use, obesity, physical activity and active smoking at 16 years did not influence the results and was not included in the final models. In order to assess effect modification, stratified analysis by asthma status at 8 years was conducted based on *a priori* determination. In stratified analysis, the two top tertiles were combined due to small numbers. Sensitivity analysis, using other asthma definitions and symptoms, adjusting for inhaled steroid use, as well as excluding supplement users and children who reported avoidance of fruits or vegetables due to allergic symptoms, was conducted.

Associations between TAC in tertiles at 8 years and spirometry results (main outcome) up to 16 years were further analyzed longitudinally by mixed-effects linear regression with a random intercept, an unstructured correlation matrix and restricted maximum likelihood estimation. An interaction term between TAC and the time indicator variable was incorporated into the model to estimate age-specific associations at 8 and 16 years and changes in lung function between 8 and 16 years. For low lung function (binary variable), defined as FEV_1 z-score below the 25^{th} percentile (Q1) due to small numbers, logistic regression analysis was used. IOS and FE_{NO} results were analyzed on the median using quantile regression, due to non-normally distributed data.

Participants who answered the questionnaire with baseline information and follow-up questionnaires at 8 and 16 years, and had a FFQ with a mean energy intake within ±3 log SD, as well as anthropometric and lung function measurements at 8 and/or 16 years were included in the present study. In total, 2307 participants fulfilled these criteria (figure S1).

All analyses were performed using the statistical software STATA V.13 (StataCorp, College Station LP, TX, USA).

RESULTS

Descriptive results on exposure and outcomes

The children included in the study population (n=2307) were comparable to the excluded children (n=1782) with regard to distribution of selected characteristics (table S1). At 8 years, the median TAC intake was 10067 μmol TE/g, which corresponds approximately to two servings of apples per day (10), with boys having an 8% lower mean TAC intake compared to girls (9963 vs 10819 μmol TE/g, $p<0.001$). Additionally, children with older siblings and children who came from a household with university education level at baseline had a significantly higher TAC intake than those without older siblings and from a household with lower education, whereas children with higher TAC intake tended to use less inhaled steroids (**Table 1**).

Distribution of anthropometric and lung function characteristics among children in the 8- and 16-year examination is shown in Tables S2 and S3.

Associations between dietary TAC at 8 years and lung function at 8 and 16 years

In linear regression analyses, associations between dietary TAC in tertiles at 8 years and spirometry results at 8 and 16 years were not statistically significant, although higher mean FEV_1 and FVC were observed for boys (table S4). Tests for trend or interaction with gender were not statistically significant.

Figure 1 presents the results from the mixed-effect model analyses of the longitudinal association between dietary TAC at 8 years and lung function up to 16 years. In analyses of lung function change between 8 and 16 years, there were no associations of TAC and spirometry results for the total study population. Consistent with the linear regression results, higher mean FEV_1 and FVC were observed for boys, but associations were not significant.

Associations between dietary TAC at 8 years and lung function at 16 years by asthma status

To assess possible effect modification, we stratified our analysis by asthma at 8 years. Asthma prevalence in the study population was 7% (n=163) at 8 years;106 out of 163 children with asthma (65%) had also IgE sensitization to inhalant and/or food allergens; 134 (82%) had used inhaled steroids occasionally or regularly and 105 (64%) had used bronchodilators in the past 12 months.

Children with asthma had 7% lower mean dietary TAC compared to children without asthma (9708 vs 10450 μmol TE/g, $p<0.01$). Among children with asthma at 8 years, higher TAC intake (second and third tertiles combined) at 8 years was associated with higher mean FEV_1 at 16 years (200.0 ml, 95% CI:38.3;361.6 vs -7.3 ml, 95% CI:-57.2;42.6 among children without asthma, p for interaction 0.018) (Table S5). This association remained comparable among children with asthma and IgE sensitization, as well as after adjustment for inhaled steroid use and exclusion of children who reported avoidance of fruits or vegetables due to allergic symptoms (data not shown), and supplement users (Table S6).

In the longitudinal model, higher TAC intake at 8 years was associated with increased FEV_1 at 16 years (0.46 SD, 95% CI: 0.11;0.80) among children with asthma (**Figure 2**). Regarding change in lung function between 8 and 16 years, there is some evidence for increased mean FEV_1 among children with asthma and higher TAC intake, but results were not statistically significant. In sensitivity analysis using other asthma definitions and symptoms, results were consistent (table S7). There were no associations among children without asthma, or between TAC intake and FVC.

Low lung function at 16 years (defined as Q_1 FEV_1 z-score) was observed in 36% (46/128) of children with asthma and in 24% (369/1534) of children without asthma. In multivariate logistic regression analysis, higher TAC intake at 8 years was associated with lower odds of low lung function at 16 years among children with asthma (OR 0.28, 95% CI:0.12;0.65), while no association was observed among children without asthma (OR 0.96, 95% CI:0.74;1.25, p for interaction between TAC and asthma 0.008) (**Table 2**).

Finally, higher dietary TAC at 8 years was not associated with any of the measured indices in analyses of lung function using IOS or FE_{NO} (Table S8 and **Table 3**).

DISCUSSION

In our study of 2307 children from a population-based birth cohort, higher TAC intake at 8 years was associated with increased FEV_1 and decreased odds of low lung function at 16 years among children with asthma. We observed no statistically significant associations between TAC and lung function among children without asthma, or between TAC and other than spirometry measurements.

To our knowledge, this is the first prospective study investigating the association between dietary antioxidant intake in early school age and lung function development from school age to adolescence. Fresh fruits and vegetables are dietary sources rich in antioxidants, such as vitamins and minerals, β-carotene, flavonoids, isoflavonoids and polyphenolic compounds (23). Respiratory airways are highly susceptible to oxidative damage and antioxidants may protect the airways against oxidants from both endogenous (activated inflammatory cells) and exogenous (indoor and outdoor air pollution, smoke exposure) sources (8). Previous cross-sectional studies in adults (12-14, 24-26) and children (16, 27), and prospective studies in adults (13, 28, 29) have indicated that higher intake of dietary antioxidants may be associated with better lung function. However, responses to antioxidants might be modified by life stage, genetic susceptibility and environmental sources of oxidative stress (7).

In our study, asthma was an effect modifier in the association between TAC and lung function. Oxidative stress plays a major role in the pathophysiology of asthma, due to chronic activation of airway inflammatory cells and a high intake of antioxidants has been reported to be protective against asthma risk and severity (23, 30). Moreover, changes in gut microbiome modulated by dietary intake have recently been linked to alterations in immune responses and lung disease (31). Our results are consistent with a previous prospective study showing that fruit and vegetable intake had a beneficial effect on inflammatory response and lung function in asthmatic children (32). Moreover, a lower mean TAC intake was observed in children with asthma in our study. This is in line with previous studies showing that children with asthma have lower levels of antioxidants in the serum (32, 33). Thus, additional antioxidants may have greater impact on children with asthma since they have higher demands. In a recent study on diet and allergic symptoms in children, the protective effect observed from higher intake of fruits and vegetables in children aged 6-7 years was less or not observed in children 13-14 years old (34).

In our study, we did not observe significant gender differences in the association between TAC and lung function, although boys had a lower mean TAC intake compared to girls. Gender differences have previously been described among adults, and it was suggested that oxidative stress may be associated with airflow limitation in males, but not in females, due to lower serum antioxidant levels and mediation via hormonal mechanisms (35, 36).

A major strength of our study is the population-based longitudinal design and the large sample size with limited loss to follow-up. In contrast to most previous studies that have focused on fruits, vegetables and individual antioxidants (11, 25, 27-29), we used TAC, which reflects the sum of dietary antioxidant intake and takes synergistic and antagonistic effects between compounds into account (10). Nevertheless, associations with specific nutrients may be diluted using the TAC approach. Additionally, TAC was available only at 8 years and potential dietary changes from 8 to 16 years were not taken into account. Of the 98 food items in the FFQ, 35 had available TAC values, including the most important dietary antioxidant sources, such as fruits, vegetables, whole grains, nuts and chocolate (18). In contrast, dietary supplements were not included in the calculation of TAC. However, we were able to adjust for use of dietary supplements and several other confounding factors. Moreover, we excluded children who used supplements to control for potential misclassification of exposure and children who reported avoidance of fruits or vegetables due to allergic symptoms to control for potential reverse causality (37), but these exclusions did not affect the observed associations. Lung function was measured with standardized protocols at 8 and 16 years, although lung function measurements post-bronchodilator were not available. This repeated assessment is a major strength of our study, which enabled us to study TAC in relation to change in lung function. Additionally, IOS, a method measuring respiratory mechanics in contrast to airway caliber measured by spirometry, has not been described in relation to TAC.

Misclassification of exposure may be present since TAC values were not available for all food items. Despite this, a FFQ similar to the one used in our study was found to have reasonable validity in adults (38). The FFQ enquired on usual diet the past 12 months and some misclassification due to difficulty to recall past diet cannot be entirely ruled out. However, information on diet was reported before the assessment of the outcome and misclassification of exposure is likely to be non-differential.

In conclusion, results from this longitudinal study indicate that antioxidant intake may be associated with lung function development among children with asthma. The antioxidant intake in the highest TAC tertile in this study corresponds to current recommendations for the general population to consume five servings of fruits and vegetables per day (39). Together with previous studies (40), our findings emphasize the importance of dietary recommendations for asthma patients. Given the high prevalence of asthma among children and adolescents, our findings may have important public health implications.

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Conflict of Interest

The authors have no potential conflicts of interest to disclose.

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Table 1. Distribution of selected characteristics in the study population in relation to the total antioxidant capacity of the diet (N=2307)

Selected variables	Tertiles of the TAC of the diet			
	T1	**T2**	**T3**	
	n=750	**n=779**	**n=778**	
ORAC, µmol TE/day, median (min-max)	6946 (1768-8615)	10009 (8615-11477)	13530 (11477-33097)	
▪ ***Categorical variables***	**n(%)**	**n(%)**	**n(%)**	***p*-value**
Boys	433 (57.7)	375 (48.1)	343 (44.1)	<0.001
Maternal age <26 years	62 (8.3)	52 (6.7)	45 (5.8)	0.153
Parental allergic disease	251 (33.7)	252 (32.5)	223 (29.1)	0.142
High socioeconomic status	631 (85.4)	658 (85.1)	667 (86.7)	0.625
University education	393 (52.4)	423 (54.4)	457 (58.7)	0.038
Maternal smoking during pregnancy	94 (12.5)	87 (11.2)	90 (11.6)	0.701
Parental smoking during infancy	161 (21.5)	150 (19.4)	160 (20.7)	0.572
Older siblings	316 (42.1)	373 (47.9)	391 (50.3)	0.005
▫ ***Age 8 years***				
Overweight and obesity	129 (17.2)	157 (20.2)	170 (21.9)	0.070
Physical activity >2 times/w	119 (15.9)	113 (14.5)	112 (14.4)	0.646
Asthma[1]	65 (8.8)	54 (7.0)	44 (5.7)	0.064
Inhaled steroid use in the past 12 m	74 (9.9)	58 (7.5)	51 (6.6)	0.046
Inhalant IgE sensitisation	186 (26.3)	210 (28.7)	170 (23.2)	0.058
Food IgE sensitisation	146 (20.6)	156 (21.3)	137 (18.7)	0.455
Allergy to fruits and vegetables[2]	88 (12.8)	67 (9.3)	66 (9.3)	0.046
Use of multivitamins	315 (42.5)	316 (41.1)	334 (43.6)	0.622
Fish intake ≥2 times/w	269 (36.0)	293 (37.7)	321 (41.5)	0.079
▫ ***Age 16 years***				
Overweight and obesity	128 (19.1)	103 (14.5)	111 (15.6)	0.056

Asthma	71 (10.1)	51 (6.9)	62 (8.4)	0.091
Inhaled steroid use in the past 12 m	82 (11.3)	53 (7.1)	52 (6.8)	0.003
Inhalant IgE sensitisation	314 (47.9)	311 (44.7)	285 (40.7)	0.029
Food IgE sensitisation	108 (16.5)	85 (12.2)	87 (12.4)	0.039
Active smoking	76 (10.4)	86 (11.4)	92 (12.1)	0.597
▪ ***Continuous variables***	**mean (SD)**	**mean (SD)**	**mean (SD)**	***p*-value**
Energy intake (Kcal)	1911.7 (467.0)	1915.5 (451.5)	1889.1 (464.7)	0.474

Total antioxidant capacity (TAC) intake (μmol Trolox equivalents/day) as measured with oxygen radical absorbance capacity (ORAC) assay, energy-adjusted to 1900 kcal/day, presented in tertiles (T1, T2, T3).
SD: standard deviation.

P-values were calculated from the chi-square test for categorical variables and ANOVA for continuous variables.

[1] Asthma was defined based on the parental questionnaire at age 8 years as more than 3 episodes of wheeze in the last 12 months AND/OR at least 1 episode of wheeze in the last 12 months, in combination with inhaled steroids occasionally or regularly.

[2] Allergic symptoms related to fruits and/or vegetables or avoidance of any fruit or vegetable due to allergic symptoms.

Table 2. Association between TAC (tertiles 2 and 3 combined vs reference tertile 1) at 8 years and lowest quartile (Q1) of FEV_1 at age 16 years stratified by asthma at 8 years (N=415)

	No asthma at 8 years			**Asthma at 8 years**		
	n	**OR**	**95%CI**	**n**	**OR**	**95%CI**
Q1:FEV1, z-score *p-value for interaction with asthma = 0.008*						
T1	118		reference	24		reference
T2 and T3	251	0.96	0.74-1.25	22	0.28	0.12-0.65

Logistic regression adjusted for sex, height and age at examination, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years. OR: odds ratio, CI: confidence interval, T: TAC tertile.

Table 3. Association between TAC (tertiles 2 and 3 combined vs reference tertile 1) at 8 years IOS and FE_{NO} results at 16 years stratified by asthma at 8 years

	No asthma at 8 years			Asthma at 8 years		
	n	**β**	**95% CI**	**n**	**β**	**95% CI**
IOS results						
R_5, $Pa \cdot L^{-1} \cdot s$	1800	-5.1	-13.8-3.6	137	4.9	-31.0-40.9
R_{20}, $Pa \cdot L^{-1} \cdot s$	1800	-0.2	-7.7-7.2	137	-9.5	-37.7-18.6
R_{5-20}, $Pa \cdot L^{-1} \cdot s$	1800	-1.5	-6.3-3.4	137	-0.7	-21.9-20.5
$AX^{0.5}$, $(Pa \cdot L^{-1})^{0.5}$	1799	0.2	-0.3-0.7	137	0.5	-1.7-2.7
Additional parameters						
FE_{NO}, ppb	1512	0.6	-0.4-1.7	117	-4.6	-15.5-6.4

IOS and FE_{NO} data were analyzed by linear regression on the median, adjusted for sex, height and age at examination, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

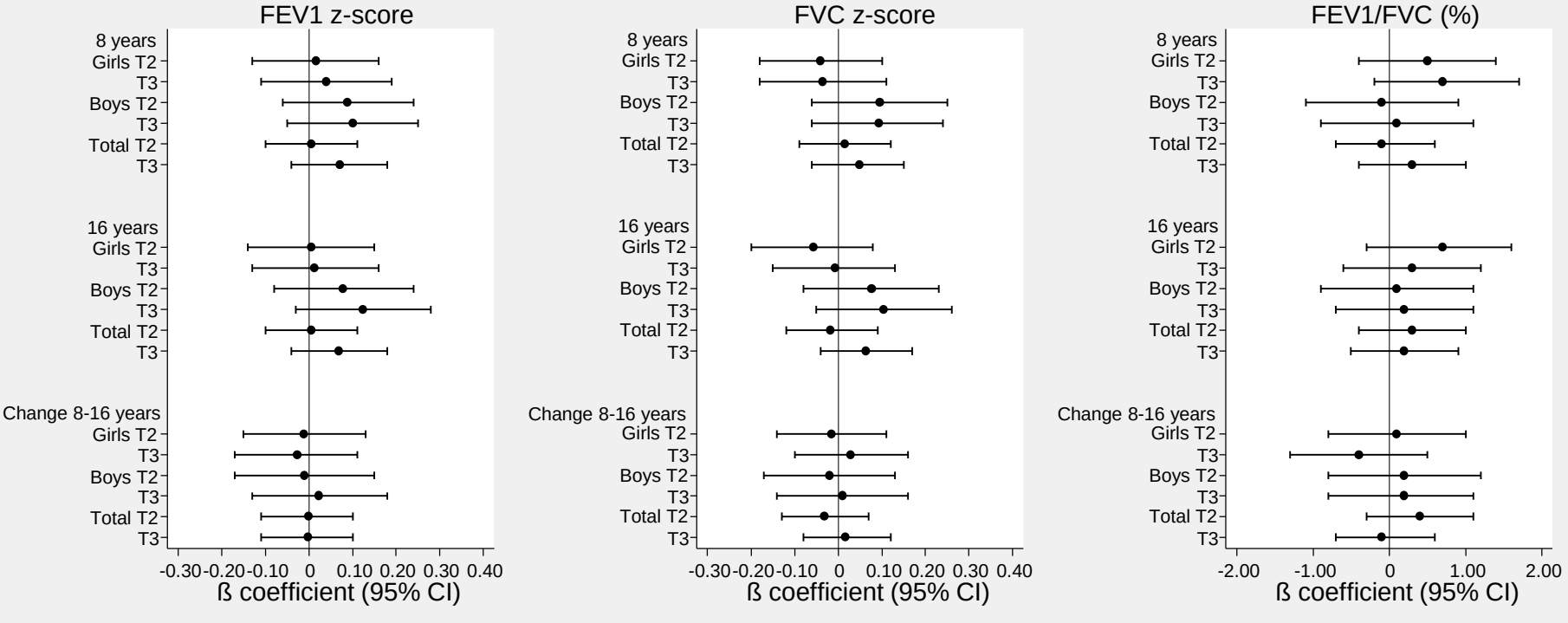

Figure 1. Associations between TAC in tertiles (T1 reference, T2, T3) at 8 years and adjusted spirometry results at 8 and 16 years

β-coefficients and 95% confidence intervals (CI) were estimated using mixed effect models (n=2115 subjects with 3306 observations), adjusted for maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.
Totals additionally adjusted for sex.

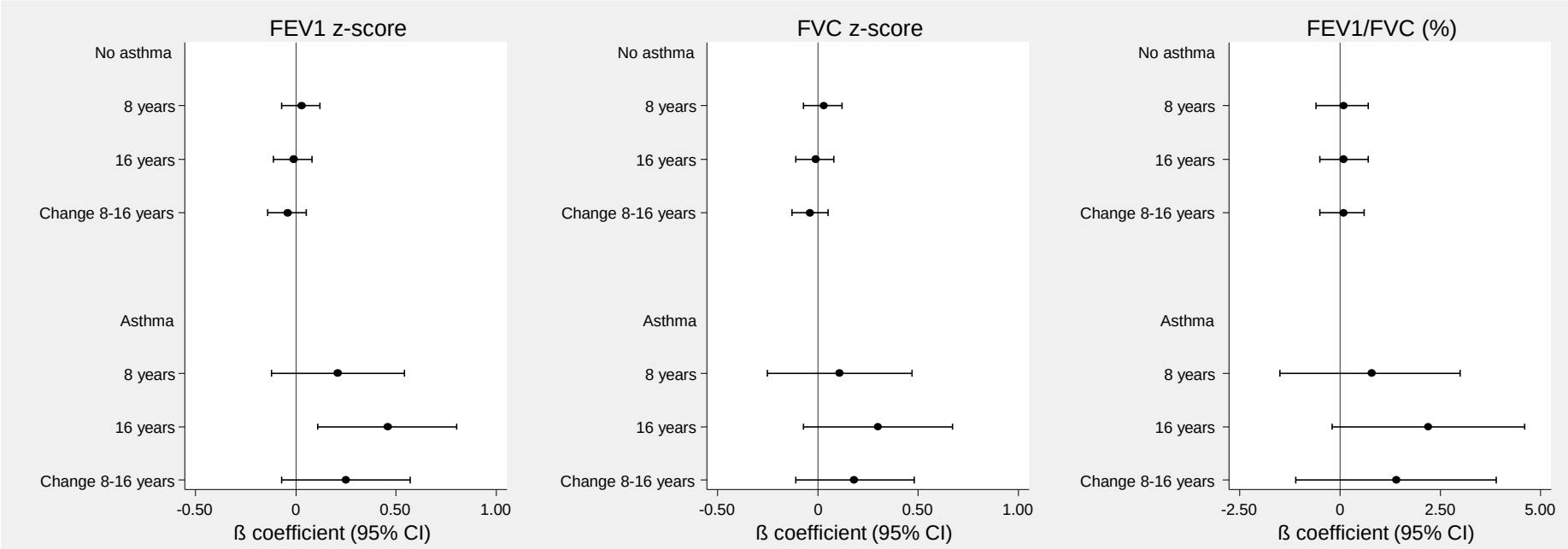

Figure 2. Associations between TAC (tertiles 2 and 3 combined vs reference tertile 1) at 8 years and adjusted spirometry results at 8 and 16 years stratified by asthma at 8 years

β-coefficients and 95% confidence intervals (CI) were estimated using mixed effect models (n=1948 subjects without asthma with 3027 observations and n=154 subjects with asthma with 258 observations), adjusted for sex, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

Supplementary Material

Dietary antioxidant intake in school age and lung function development up to adolescence

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Methods

Lung function measurements

Lung function was measured by spirometry at 8 years (n=1832) using a 2200 Pulmonary Function Laboratory (SensorMedics, Anaheim, California, USA) and by impulse oscillometry (IOS) (n=2452) followed by spirometry at 16 years (n=2056) using a Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, California, USA). The same spirometry test protocol was used at both time-points, including calibration and quality criteria. All participants performed at least three maximal expiratory flow volume (MEFV) recordings in the sitting position, wearing a nose clip. The highest values of forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) were extracted and used for analysis, provided that the subject's effort was accepted as being maximal by the test leader, the MEFV curve passed visual quality inspection and the two highest FEV1 and FVC readings were reproducible according to American Thoracic Society/ European Respiratory Society (ATS/ERS) criteria (1).

IOS is a noninvasive effort-independent method, which measures respiratory system impedance by superimposing external pressure impulses generated from a loudspeaker, producing recordable waveforms. Measurement was performed during tidal breathing with the lips tightly sealed around the

mouthpiece and supporting cheeks with their hands. Signals free from artefacts that lasted for at least 20 seconds were saved for analysis. At least two recordings were performed per subject. Quality control was performed at the time for examination by visual inspection of the waveforms, and given that coherence, which is a measure of testing reliability, was >0.80 at 10 Hertz (Hz), the mean value of resistance at 5 and 20 Hz (R_5, R_{20}), frequency dependence of resistance (R_{5-20}) and the square root of the area of reactance ($AX^{0.5}$) were used for analysis. The IOS software extracts resistance (R) and reactance (X) in the range of 3-35 Hz from the impedance. Resistance at a low frequency (e.g. R_5) is thought to represent properties of both proximal and distal airways, while resistance at higher frequency (e.g. R_{20}) represents proximal airway resistance. Frequency dependence of resistance i.e. R_{5-20}, may thus represent distal (small) airway resistance (2). The IOS test was performed before spirometry according to clinical standards. The IOS system was calibrated each day using a 3l precision syringe and a reference resistance (0.20 kPa/l/s).
Fractional exhaled nitric oxide (FE_{NO}, expressed as parts per billion, ppb) is a non-invasive test and a biomarker denoting eosinophilic (Th2 driven) lower airway inflammation. Measurements of FE_{NO} (n=2087) were performed at 16 years at an expiratory flow of 50 mL/s ($FE_{NO}50$), using an online chemiluminescent (CLD88) analyser (Eco Medics AG, Duernten, Switzerland), according to ERS/ATS guidelines (3).

Asthma definitions

Asthma was defined based on the parental questionnaire at age 8 years as more than 3 episodes of wheeze in the last 12 months AND/OR at least 1 episode of wheeze in the last 12 months, in combination with inhaled steroids occasionally or regularly. *Inhalant IgE sensitisation* was defined as positive phadiatop test (IgE-value ≥ 0.35 kU/l). *Food IgE sensitisation* was defined as positive fx5 test (IgE-value ≥ 0.35 kU/l).

Sensitivity analyses (Table S7): *Any wheeze* was defined as at least 1 episode of wheeze in the last 12 months. *Doctor's diagnosis of asthma* was defined as doctor's diagnosis of asthma ever in life up to the date of questionnaire 8 or 16. Current asthma was defined as doctor's diagnosis of asthma ever combined with wheeze in the last 12 months at 8 or 16 years. Finally, *according to GALEN/MEDALL* (4), *asthma* was defined as at least TWO of the following three criteria at 8 years:

1. Symptoms of wheeze in the last 12 months prior to the date of questionnaire 8
2. Ever doctor's diagnosis of asthma
3. Asthma medicine occasionally or regularly last 12 months

Other definitions

Parental allergic disease: Mother and/or father with physician-diagnosed asthma and asthma medication and/or physician-diagnosed hay fever in combination with furred pets allergy and/or pollen allergy at the time of baseline questionnaire.
Maternal smoking during pregnancy: The mother smoked at least one cigarette per day in any point of time during the pregnancy.
Parental smoking during infancy: Any of the parents smoked at least one cigarette/day at the time of questionnaire 0.
Socioeconomic status: Socioeconomic status at birth for the household according to dominance order in 2 classes (low: blue collar vs. high: white collar worker).
Education: Education level of the household at baseline in 3 levels (1: elementary school, 2: high school, 3: university).

Statistical analyses

IOS results were not transformed to z-scores, due to lack of reference values, but adjusted for height and age. IOS and FE_{NO} were analyzed on the median using quantile regression, due to non-normally distributed data.
Mixed-effect models for longitudinal data take the correlation between repeated measurements on the same individual into account.
Children with missing data on one of the exposures, outcomes or covariates were not included in that specific analysis, except for the mixed-effect models analyses which require data on the outcome from at least one time-point (see exact number in each table).

Results

Figure S1. Flow chart of the inclusion to the study population. FFQ: food frequency questionnaire, SD: standard deviation

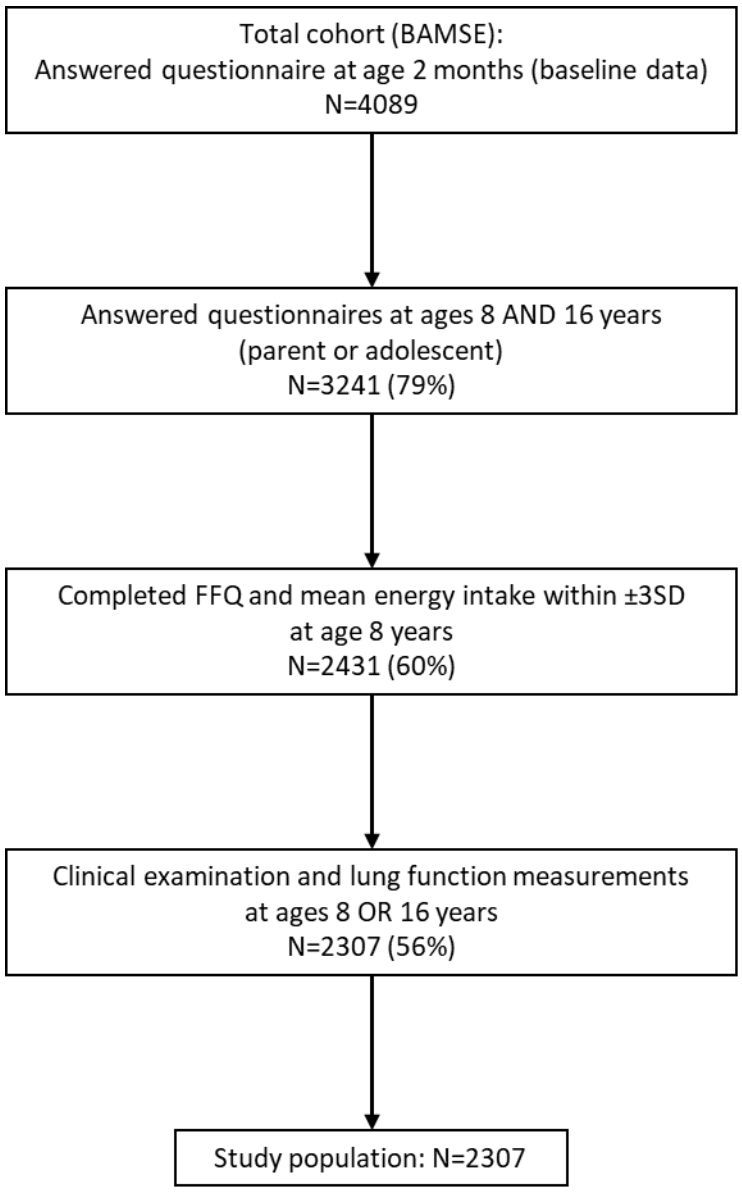

Table S1. Distribution of selected characteristics among children included and excluded from the study population

Selected variables	**Children included in the study population N=2307**		**Children excluded from the study population N=1782**		
▪ ***Categorical variables***	***n***	***%***	***n***	***%***	***p-value***[2]
Boys	1151	49.9	914	51.3	0.375
Parental allergic disease	726	31.7	474	27.0	0.001
Maternal smoking during pregnancy	271	11.8	256	14.4	0.013
Parental smoking during infancy	471	20.5	384	21.7	0.356
High socioeconomic status	1956	85.8	1367	78.7	<0.001
University education	1273	55.2	888	50.0	<0.001
Either parent born out of Sweden	485	21.1	233	21.1	0.989
Caesarean section[1]	271	12.0	236	13.5	0.153
Exclusive breastfeeding ≥4 months	1846	81.4	1270	76.9	<0.001
Older siblings	1080	46.8	900	50.5	0.018
▪ ***Continuous variables***	***Mean***	***SD***	***Mean***	***SD***	***p-value***[3]
Maternal age (y)	31.0	4.5	30.3	4.5	<0.001
Birth weight[1] **(g)**	3538	548	3531	557	0.695
Gestational age[1] **(w)**	39.5	1.8	39.5	1.8	0.625

95% CI: 95% confidence interval, SD: standard deviation. Numbers may not add up to total due to missing.

[1] Variables obtained from the medical birth register, [2] p-values obtained from the chi-square test, [3] p-values obtained from the *t*-test.

Table S2. Distribution of anthropometric and lung function characteristics among children in the 8-year (n=2307) and 16-year (n=2094) examination

	8 years						16 years					
	Girls			Boys			Girls			Boys		
	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
Anthropometric characteristics												
Age, y	1156	8.3	0.5	1151	8.4	0.5	1056	16.7	0.4	1038	16.7	0.4
Height, m	1156	1.32	0.06	1151	1.33	0.06	1054	1.67	0.06	1038	1.80	0.07
Weight, kg	1156	30.0	5.5	1151	30.4	5.3	1056	60.8	9.1	1038	70.4	11.7
Lung function characteristics												
FEV_1, ml	869	1735.2	256.9	839	1824.6	279.5	900	3484.3	435.4	775	4494.5	652.9
FEV_1, z-score	868	0.48	0.95	839	0.37	0.92	898	-0.03	0.91	775	-0.03	0.98
FVC, ml	869	1990.9	294.8	839	2149.0	341.0	900	4040.2	513.7	775	5389.6	775.9
FVC, z-score	869	0.63	0.91	839	0.57	0.91	898	0.17	0.89	775	0.17	0.96
FEV_1/FVC, %	869	87.3	5.3	839	85.2	5.9	900	86.5	6.1	775	83.7	6.7
FEV_1/FVC, z-score	869	-0.34	0.89	839	-0.35	0.90	898	-0.37	0.94	775	-0.32	0.98
	n	median	range	n	median	range	n	median	IQR	n	median	IQR
R_5, $Pa \cdot L^{-1} \cdot s$							1004	395	105	993	320	90
R_{20}, $Pa \cdot L^{-1} \cdot s$							1004	375	90	993	305	75
R_{5-20}, $Pa \cdot L^{-1} \cdot s$							1004	20.0	55.0	993	15.0	50.0
$AX^{0.5}$, $(Pa \cdot L^{-1})^{0.5}$							1004	16.4	5.8	992	12.7	5.2
FE_{NO}, ppb							836	14.1	10.5	842	17.6	13.8
Blood eosinophils (10^9 cells/L)							984	0.1	0.1	979	0.2	0.1
Blood neutrophils (10^9 cells/L)							984	3.5	1.6	979	3.0	1.4

SD: standard deviation, IQR: interquartile range, FEV1: forced expiratory volume in 1 sec, FVC: forced vital capacity, R5: mean value of resistance at 5 Hz, R20: mean value of resistance at 20 Hz, R5-20: mean value of resistance between 5 Hz and 20 Hz, AX0.5: square root of the area under the reactance curve, FE_{NO}: fractional exhaled nitric oxide at an expiratory flow of 50 mL/s

Table S3. Distribution of lung function characteristics in girls (n=1156) and boys (n=1151) by tertiles of the TAC of the diet (T1, T2, T3)

	Girls			**Boys**		
	Tertiles of the TAC of the diet					
	T1	**T2**	**T3**	**T1**	**T2**	**T3**
	n=386	**n=385**	**n=385**	**n=384**	**n=384**	**n=383**
	Spirometry at 8 years					
	n=300	**n=292**	**n=277**	**n=290**	**n=280**	**n=269**
	mean (SD)	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**
FEV_1, ml	1728 (242)	1732 (258)	1746 (271)	1820 (278)	1836 (290)	1818 (270)
FEV_1, z-score	0.45 (0.89)	0.46 (0.98)	0.52 (0.98)	0.28 (0.94)	0.41 (0.94)	0.43 (0.87)
FVC, ml	1989 (269)	1985 (296)	2000 (320)	2149 (344)	2164 (355)	2133 (323)
FVC, z-score	0.64 (0.85)	0.61 (0.94)	0.65 (0.93)	0.49 (0.93)	0.62 (0.94)	0.60 (0.86)
FEV_1/FVC, %	87.0 (5.4)	87.4 (5.2)	87.6 (5.5)	85.0 (6.1)	85.1 (5.9)	85.5 (5.7)
FEV_1/FVC, z-score	-0.39 (0.89)	-0.34 (0.85)	-0.30 (0.92)	-0.37 (0.93)	-0.36 (0.90)	-0.32 (0.88)
	Spirometry at 16 years					
	n=291	**n=301**	**n=308**	**n=257**	**n=256**	**n=262**
	mean (SD)	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**
FEV_1, ml	3495 (468)	3479 (421)	3480 (418)	4503 (709)	4523 (616)	4458 (630)
FEV_1, z-score	-0.04 (0.96)	-0.04 (0.87)	-0.02 (0.90)	-0.10 (1.04)	-0.01 (0.95)	0.02 (0.93)
FVC, ml	4068 (533)	4017 (511)	4036 (499)	5416 (815)	5433 (762)	5321 (747)
FVC, z-score	0.19 (0.91)	0.13 (0.86)	0.18 (0.91)	0.12 (0.99)	0.20 (0.96)	0.20 (0.94)
FEV_1/FVC, %	86.1 (6.3)	86.8 (5.8)	86.5 (6.3)	83.4 (7.0)	83.6 (6.8)	84.0 (6.2)
FEV_1/FVC, z-score	-0.42 (0.95)	-0.32 (0.90)	-0.36 (0.98)	-0.35 (1.02)	-0.32 (0.99)	-0.29 (0.92)
	IOS at 16 years					
	n=324	**n=339**	**n=341**	**n=331**	**n=328**	**n=334**
	median (IQR)	**median (IQR)**	**median (IQR)**	**median (IQR)**	**median (IQR)**	**median (IQR)**
R_5, $Pa \cdot L^{-1} \cdot s$	395 (100)	400 (110)	395 (105)	325 (95)	322.5 (90)	320 (90)
R_{20}, $Pa \cdot L^{-1} \cdot s$	375 (85)	370 (100)	375 (80)	310 (75)	305 (75)	305 (70)
R_{5-20}, $Pa \cdot L^{-1} \cdot s$	20.0 (52.5)	20.0 (60.0)	20.0 (55.0)	15.0 (50.0)	15.0 (50.0)	15.0 (40.0)
$AX^{0.5}$, $(Pa \cdot L^{-1})^{0.5}$	16.1 (5.8)	16.7 (6.0)	16.6 (6.0)	12.6 (5.4)	12.5 (5.0)	12.6 (4.8)
	Additional parameters at 16 years					

	n=273	**n=283**	**n=280**	**n=273**	**n=282**	**n=287**
FE_{NO} (ppb)	13.6 (10.6)	13.8 (11.9)	14.8 (9.5)	17.8 (15.2)	17.6 (14.7)	17.3 (12.9)
	n=321	**n=332**	**n=331**	**n=320**	**n=328**	**n=331**
Blood eosinophils (10^9 cells/L)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)
Blood neutrophils (10^9 cells/L)	3.5 (1.6)	3.6 (1.6)	3.5 (1.6)	3.0 (1.3)	2.9 (1.5)	3.0 (1.4)

Table S4. Associations between TAC in tertiles (T1 reference, T2, T3) at 8 years and spirometry results at 8 and 16 years by sex

	8 years				16 years			
	Girls (n=869)		Boys (n=839)		Girls (n=900)		Boys (n=775)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
FEV1, ml								
T1	reference		reference		reference		reference	
T2	5.9	-24.3-36.0	25.5	-6.1-57.1	-11.0	-71.7-49.7	42.5	-49.5-134.4
T3	14.3	-16.6-45.3	31.6	-0.1-63.4	-5.1	-65.8-55.7	60.7	-31.3-152.7
p for trend		0.477		0.131		0.888		0.231
FVC, ml								
T1	reference		reference		reference		reference	
T2	-1.8	-35.7-32.1	24.8	-12.5-62.1	-35.9	-105.9-34.0	37.4	-66.3-141.2
T3	7.3	-27.5-42.2	28.3	-9.2-65.8	-13.9	-83.9-56.1	50.6	-53.3-154.4
p for trend		0.601		0.172		0.918		0.296
FEV1/FVC, %								
T1	reference		reference		reference		reference	
T2	0.4	-0.5-1.3	0.2	-0.7-1.2	0.5	-0.5-1.5	0.3	-0.9-1.5
T3	0.5	-0.4-1.4	0.3	-0.7-1.3	0.2	-0.8-1.2	0.3	-0.8-1.5
p for trend		0.534		0.867		0.849		0.712

Spirometry data were analyzed by linear regression on the mean, adjusted for height and age at examination, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

Table S5. Associations between TAC (tertiles 2 and 3 combined vs reference tertile 1) at 8 years and adjusted spirometry results at 8 and 16 years stratified by asthma at 8 years

		FEV1, ml		FVC, ml		FEV1/FVC, %	
	n	β	95% CI	β	95% CI	β	95% CI
8 years							
No asthma	1526	8.3	-11.7-28.3	7.4	-15.6-30.3	0.1	-0.5-0.7
Asthma	134	31.4	-41.9-104.8	33.1	-57.6-123.7	0.4	-1.9-2.6
p for interaction		0.525		0.524		0.870	
16 years							
No asthma	1501	-7.3	-57.2-42.6	-6.4	-62.9-50.2	0.1	-0.6-0.8
Asthma	124	200.0	38.3-361.6	85.9	-126.2-297.9	2.5	-0.2-5.1
p for interaction		0.018		0.353		0.070	

Linear regression on the mean adjusted for sex, height and age at examination, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

Table S6. Associations between TAC (tertiles 2 and 3 combined vs reference tertile 1) at 8 years and adjusted spirometry results at 8 and 16 years for the total population and excluding supplement users, stratified by asthma at 8 years

		FEV1, z-score		FVC, z-score		FEV1/FVC, %	
	n	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
Total population							
No asthma	1948						
8 years		0.03	-0.07-0.12	0.03	-0.07-0.12	0.1	-0.6-0.7
16 years		-0.01	-0.11-0.08	-0.01	-0.11-0.08	0.1	-0.5-0.7
Change		-0.04	-0.14-0.05	-0.04	-0.13-0.05	0.1	-0.5-0.6
Asthma	154						
8 years		0.21	-0.12-0.54	0.11	-0.25-0.47	0.8	-1.5-3.0
16 years		0.46	0.11-0.80	0.30	-0.07-0.67	2.2	-0.2-4.6
Change		0.25	-0.07-0.57	0.18	-0.11-0.48	1.4	-1.1-3.9
Excluding supplement users							
No asthma	1145						
8 years		0.11	-0.01-0.24	0.08	-0.04-0.20	0.3	-0.5-1.1
16 years		0.05	-0.08-0.18	0.04	-0.09-0.16	0.2	-0.6-1.1
Change		-0.06	-0.19-0.06	-0.05	-0.17-0.07	-0.1	-0.8-0.7
Asthma	85						
8 years		0.35	-0.10-0.81	0.08	-0.42-0.59	2.6	-0.6-5.7
16 years		0.42	-0.02-0.86	0.07	-0.43-0.56	3.9	0.9-6.9
Change		0.07	-0.38-0.52	-0.02	-0.46-0.42	1.4	-2.1-4.8

β-coefficients and 95% confidence intervals (CI) were estimated using mixed effect models, adjusted for sex, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

Table S7. Associations between TAC (tertiles 2 and 3 combined vs reference tertile 1) at 8 years and FEV_1 at 8 and 16 years among children with asthma or wheeze at 8 years

FEV_1, z-score	**MeDALL asthma**		**Doctor's diagnosis of asthma up to 8 years**		**Current asthma**		**Any wheeze**	
	Diff.	**95% CI**	**Diff.**	**95% CI**	**Diff.**	**95% CI**	**Diff.**	**95% CI**
	n=232		**n=149**		**n=138**		**n=241**	
8 years	0.07	-0.18-0.33	-0.05	-0.36-0.25	0.16	-0.16-0.48	0.13	-0.13-0.40
16 years	0.27	0.00-0.54	0.30	-0.02-0.61	0.50	0.16-0.84	0.33	0.06-0.60
Overall	0.16	-0.06-0.39	0.11	-0.16-0.37	0.31	0.03-0.59	0.22	-0.01-0.46
Change	0.20	-0.07-0.46	0.35	0.03-0.67	0.34	0.00-0.68	0.20	-0.06-0.46

β-coefficients and 95% confidence intervals (CI) were estimated using mixed effect models, adjusted for sex, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

Interaction terms: MeDALL asthma p=0.284, time-specific p=0.088, Doctor's diagnosis of asthma up to 8 years p=0.737, time-specific p=0.020, Current asthma p=0.086, time-specific p=0.024, Any wheeze p=0.153, time-specific p=0.068

Table S8. Associations between TAC (in tertiles, T1 reference) at 8 years and IOS and FE_{NO} results at 16 years

	T1	T2				T3			
		Girls		Boys		Girls		Boys	
	β	β	95% CI	β	95% CI	β	95% CI	β	95% CI
IOS results (n=1004 girls and n=993 boys)									
R_5, $Pa \cdot L^{-1} \cdot s$	0	0.5	-13.9-14.9	-6.7	-19.1-5.7	-10.3	-24.8-4.3	-10.8	-23.2-1.7
R_{20}, $Pa \cdot L^{-1} \cdot s$	0	-5.0	-18.4-8.4	-0.4	-10.1-9.4	-6.9	-20.4-6.7	-7.4	-17.2-2.3
R_{5-20}, $Pa \cdot L^{-1} \cdot s$	0	-3.0	-10.9-5.0	1.2	-5.8-8.1	-5.7	-13.8-2.3	-4.5	-11.4-2.5
$AX^{0.5}$, $(Pa \cdot L^{-1})^{0.5}$	0	0.3	-0.5-1.1	0.2	-0.5-0.8	0.1	-0.7-1.0	0.1	-0.6-0.7
Additional parameters (n=836 girls and n=842 boys)									
FE_{NO}, ppb	0	0.02	-1.5-1.5	-0.3	-2.4-1.7	1.2	-0.4-2.7	0.4	-1.6-2.5

IOS and FE_{NO} data were analyzed by linear regression on the median, adjusted for height and age at examination, maternal smoking during pregnancy, parental smoking during infancy, parental allergic disease, socioeconomic status, older siblings and maternal age < 26 years.

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