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Pollen exposure at birth and adolescent lung function, and modification by residential greenness.

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Statement of Contribution

Shyamali C. Dharmage, Adrian J. Lowe, Michael J. Abramson, Paul S Thomas, Catherine M Bennett and Caroline J. Lodge designed, obtained funding and conducted the Melbourne Atopic Cohort Study. Katrina A Lambert, Bircan Erbas and Luke A Prendergast led the analysis of data. All authors contributed to interpreting of the data, drafting the manuscript, to the intellectual content and revising of the final draft of the manuscript. The final version of the manuscript was approved by all the authors.

Conflict of Interest

Katrina Lambert is funded by a La Trobe University PhD Postgraduate Scholarship and a Research Funding Area: Building Healthy Communities top-up scholarship. Caroline Lodge, Adrian J Lowe and Shyamali C Dharmage are supported by NHMRC fellowships. Michael Abramson holds investigator initiated research grants from Pfizer and Boehringer-Ingelheim for unrelated research. He has also received assistance with conference attendance from Sanofi. All authors declare no conflict of interest.

ABSTRACT

Background: Exposure to high levels of pollen in infancy is a risk factor for allergic respiratory diseases in later childhood, but effects on lung function are not fully understood. We aim to examine associations between grass pollen exposure in the first months of life and lung function at 12 and 18 years, and explore potential modification.

Methods: Using the Melbourne Atopy Cohort Study, a birth cohort of children with a family history of allergic diseases, we modelled the association between cumulative grass pollen exposure up to 3 months after birth, on FEV_1, FVC and FEV_1/FVC ratio at 12 and 18 years. We also assessed modifying effects of residential greenness levels (derived from satellite imagery), asthma and early life sensitization to rye grass.

Results: Grass pollen exposure in the first seven days was associated with a reduction in FEV_1 (-15.5mL; 95%CI: -27.6, -3.3 per doubling of pollen count) and FVC (-20.8mL; -35.4, -6.1) at 12 years, but not at 18 years. Increase in cumulative grass pollen exposure up to 3 months were negatively associated with FVC at 12 and 18. Higher residential greenness increased the reduction in FVC associated with exposure pollen in the first three months of life

Conclusion: Early exposure to grass pollen was associated with decreased lung function in children and adolescents. Targeted interventions for pollen avoidance strategies that take into account local topography could be implemented alongside other clinical interventions such as immunotherapy.

Keywords: aeroallergen; greenness; pollen; spirometry

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are urgent public health problems with high rates of morbidity and mortality [1]. Reduced lung function at age 7 has been shown to predict COPD and asthma-COPD overlap syndrome at age 45 [2]. Environmental factors are important in modifying the development of lung function, but to date most studies have only looked at air pollutants [3,4]. The potential effect of pollen exposure on lung function has received limited attention. Previous work has mainly assessed the association between pollen exposure and lung function in short term studies over a single pollen season [5-7]. These studies found a correlation between increasing pollen levels and increased levels of fractional expired nitric oxide (FeNO), but no association with forced expired volume in one second (FEV_1). A more recent cross-sectional study of a Swedish cohort [8] found exposure to grass pollen during the preceding day to be associated with a reduction in FEV_1 at age 8. They found the negative effect of grass pollen on lung function was stronger in children sensitized to pollen allergens.

Studies including our own have shown that exposure to grass pollen in the first 3 months of life was associated with allergy and asthma [9,10]. We have also shown that exposure to grass pollen during the first 3 months of life was associated with hay fever, and exposure by 6 months with asthma later in childhood [9]. Harvey and colleagues reported increased odds of wheeze at age 2 years in infants exposed to cypress, pine and alder pollen in the first 3 months of life. This birth cohort recruited mothers from a Mexican background in an agricultural region of California [10]. However, no study has yet examined the effect on lung function of grass pollen exposure at birth or in the first 3 months of life in children or adolescents.

Understanding the role of greenness surrounding a participants' home may also be important in pollen exposure pathways. The Normalised Difference Vegetation Index (NDVI) provides a broad measure of vegetation around the home that is of increasing interest [11,12]. Assessing the modifying effects of residential greenness may be important in better understanding the role of grass pollen exposure in temperate regions.

Melbourne experiences high ambient levels of rye grass pollen in spring, making it a useful setting to assess the risk of grass pollen exposure in the first months of life on subsequent lung function in adolescents. Using the Melbourne Atopy Cohort Study (MACS), an established birth cohort of children with a family history of allergic disease, we examined the associations between persistent exposure to cumulative grass pollen in the first seven days, and again at three months of life, on subsequent lung function at 12 and 18 years. Secondary aims were to assess whether these associations were modified by local residential greenness and whether asthma in childhood or sensitization to rye grass mediated these associations.

METHODS

Study design and population

The Melbourne Atopy Cohort Study (MACS) commenced as a randomized controlled trial (RCT) of 620 infants, with at least one first-degree relative with a history of allergic disease (self-reported asthma, eczema, hay fever or severe food allergy). Infants were recruited between 1990 and 1994 from Melbourne, Australia [13]. The initial RCT investigated the effect of three types of formula (cows' milk, soya or partially hydrolyzed whey formula) on risk of allergic disease [14]. It has since been analyzed as an observational birth cohort study for nonrandomized exposures. Clinical assessments of MACS participants were performed at ages 12 and 18 years. Ethics approval was granted by the Mercy Maternity and Royal Children's Hospitals and, to conduct of this analysis, from La Trobe University Ethics Committees. Written informed consent was obtained from all mothers, and from all participants at the 18-year follow-up.

Data collection and measurements

Demographic data

Baseline demographic details were obtained by questionnaire during pregnancy. Following birth, standardized telephone questionnaires were administered by a research nurse trained in allergy. These questionnaires were administered every 4 weeks from birth to 64 weeks, at 78 weeks and at 2 years, documenting any episodes of illness since the previous interview. An annual follow up was then conducted until the children were 7 years old. Further data collection occurred between 2002 and 2006 (mean age 12 years) and 2009 and 2013 (age 18 years). The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was administered at 12 and 18 years. Anthropometric measurements were obtained at 12 and 18 years (height to nearest 0.1 cm and weight to the nearest 0.1 kg).

Pollen Exposure

Daily, 24-hour average pollen concentrations (grains/m^3), grass pollen counts collected from a single Burkard volumetric trap, located on the roof of the University of Melbourne, were collected from the 1st of October 1991 until the 30th of December 1993.

Residential greenness

Local residential greenness was calculated by the Normalized Difference Vegetation Index (NDVI) derived from the publically available Landsat 5 Thematic Mapper Surface Reflectance images (https://earthexplorer.usgs.gov/), with one image taken every 16-days at a 30 by 30-meter pixel resolution. Buffers of 100, 500 and 1000 around the residential home address at birth were calculated using QGIS 2.18.15 (Open Source Geospatial Foundation). Each participant was matched to a suitable cloud-free (<10% cloud cover) image as close to the date of birth as possible, with 77% of images matched within one month of birth. NDVIs were computed by linear interpolation for participants without a suitable cloud-free image in this time period, taking the mean of the NDVIs for the closest day/month one year prior and one year after their date of birth.

Lung function

Pre-bronchodilator spirometry was measured at both 12 and 18 years, while post bronchodilator spirometry was only performed during the 18 year follow up. Spirometry was performed according to then current American Thoracic Society (ATS) (1994) or ATS/European Respiratory Society (2005) guidelines [15]. Participants were advised to abstain from short-acting bronchodilators for 4 hours and long-acting bronchodilators for 12 hours before spirometry. The Global Lung Initiative reference values were used to calculate z-scores for sensitivity analysis.

Other variables

Childhood diagnosis of asthma: Report of doctor diagnosed asthma in the previous 12 months collected from parents during the telephone interviews conducted when children were aged 6 or 7 years. Upper respiratory tract infection (URTI) before 5 weeks: report of an URTI collected from parents' report during telephone interviews conducted when children were 4 to 5 weeks old. Early sensitization to rye: a positive reaction to skin prick testing (SPT) during the 2 year follow up. A positive SPT was defined as a wheal of at least 3 mm diameter [13]. Maternal education was assessed by questionnaire at baseline and used as a surrogate measure of socio-economic status.

Statistical methods

We used linear regression to model the associations between grass pollen exposure and lung function outcomes. Grass pollen exposure during early life was examined as the cumulative count of grass pollen exposure over the first seven days (7 day) and three months (3 month) of life. The cumulative pollen data were analyzed after a log base 2 transformation with an offset of 1 was applied. An estimate of 1 can therefore be interpreted as an increase of 1 mL per doubling of the cumulative pollen count (from 10 to 20 grains/m^3 or from 25 to 50 grains/m^3) for FEV_1 and FVC, and for the FEV_1/FVC ratio an increase of 1% per doubling of the cumulative pollen count. Non-linearity was assessed by Generalized Additive Models (GAMs) [16]. Where non-linearity was detected, fractional polynomials were added to the linear regression to allow a flexible curve to be fitted to the dose-response data [17]. Figures were generated to describe the change in lung function related to change in early life pollen exposure, as fractional polynomials are difficult to interpret from a table. Natural cut-points in the 3 month cumulative pollen data of 20, 320 and 2950 grains /m^3 were used, corresponding to the 10th, 50th and 75th percentiles (Supplementary Figure 1). At both 12 and 18 years we used pre-bronchodilator forced expiratory volume during the first second (FEV_1), forced vital capacity (FVC) and FEV_1/ FVC ratio in mL. Bronchodilator response, >12% and 200ml increase from pre- to post- bronchodilator lung function [18], was analyzed by logistic regression at 18 years.

All regression models were adjusted *a priori* for age (years), height (cm), sex, maternal education and the presence of an URTI before 5 weeks. We dichotomized NDVI to assess effect modification, with "High residential greenness" defined as NDVI at birth in the 75th percentile or greater. The level of 75th percentile was selected *a priori*. We considered a p value ≤ 0.10 as significant for the interaction terms and only present strata-specific results for significant interactions. Sensitivity analysis was conducted by removing all observations with imputed NDVIs without a cloud-free image. Interactions were also assessed at the 65th and 70th percentiles. We tested for effect modification by asthma diagnosis at 6/7 years and sensitization to rye grass at 2 years.

Statistical analyses were performed using Stata release 14.1 (College Station, TX, USA).

RESULTS

Of the 620 original MACS participants, 486 (78%) had lung function measurements at age 12 or age 18 years. Grass pollen data were available for 320 of these (66%). There was no significant difference between the groups of participants with or without grass pollen data in terms of sex, URTI before 5 weeks, early sensitization or childhood asthma (Table 1). Those with pollen data are significantly younger as pollen data were not available at the start of recruitment.

The association between exposure to grass pollen and lung function outcomes

Cumulative grass pollen exposure over the first seven days of life varied from zero to 1878 grains/m^3 with a median of 17 grains/m^3. Exposure over the first three months was similarly varied (median: 320 grains/m^3, range: 7-7349).

Cumulative grass pollen exposure over the first seven days was associated with a significantly reduced FVC (β: -20.8mL 95%CI: -35.4, -6.1 per doubling of pollen count) and FEV_1 (β: -15.5mL 95%CI: -27.6, -3.3) at age 12 in adjusted models (Table 2). Cumulative grass pollen exposure over the first three months of life revealed no association with FEV_1 or FVC, however it was associated with a small increase in the FEV_1/FVC ratio at age 18 years (β: 0.4% 95%CI: 0.1, 0.7).

Unlike the first seven days, cumulative grass pollen exposure over the first three months of life showed a non-linear association with lung function (Figure S2). Comparing cut-points of the three month pollen data to the reference category of 0-19 grains/m^3 of grass pollen showed a significant, but non-linear reduction in FEV_1 and FVC at 12 years (Table 2). All grass pollen measurements were associated with a slight increase in the FEV_1/FVC ratio at age 18 years. Grass pollen was not associated with bronchodilator responsiveness at age 18 years (Table S1). The pattern of associations is consistent when GLI z-scores are used (Table S2).

Effect modification by satellite measures of residential greenness

The association between cumulative pollen exposure in the first three months of life and lung function in the high NDVI strata is clearly non-linear (p-values < 0.01; Figure 1). The pattern is similar for both FEV_1 and FVC at both time points. The initial drop and then levelling out seen here can also be seen in the associations with the cut-off values in Tables S3 and S4. Sensitivity analysis (Table S5) showed no major differences in the effect sizes. The result was robust to changes in the definition of high NDVI from 75th percentile to 70th percentile (Table S6). However, the reference category of 0 – 19 grains/m^3 of pollen represents a small number of children with good lung function (Table S7). Altering the reference category to the larger and more stable segment of the population represented by 20-320 grains/m^3 of cumulative pollen exposure in the first three months of life (Table S8 and Table S9), reveals a significant interaction ($p<0.01$) in the low pollen group.

Neither childhood asthma nor early sensitization to rye grass pollen modified the association between cumulative grass pollen exposures and lung function (data not shown).

DISCUSSION

Our study is the first to show an association between exposure to ambient grass pollen in the first 3 months of life and lung function later in childhood and adolescence. The association was evident at 12 years of age, with cumulative grass pollen exposure over the first seven days of life associated with significant reductions in FEV_1 and FVC. Higher pollen counts over the first 3 months of life were associated with a reduction in FVC at 12 and 18 years. Exposure to ambient grass pollen in the first seven days and 3 months of life was also associated with a slight increase in the FEV_1/FVC ratio at age 18, due to reduction in FVC but preserved FEV_1.

This study adds to a growing body of literature that suggests pollen exposure in early life is an important early life risk factor for allergic respiratory diseases later in childhood and adolescence. However, most studies showing such associations have only used month or season of birth as a surrogate measure of pollen exposure [19-28], with fewer studies using actual ambient pollen counts [9,10,29].

Exposure to pollen in early life is but one of several factors potentially contributing to the complex set of determinants of an individual's lung function throughout life [30]. Several of these factors temporally coincide with the cyclic nature of the pollen seasons. Early life respiratory tract infections [31] and low maternal vitamin D levels [32] also exhibit strong seasonal variation and have been associated with lung function in childhood and adolescence. Residual confounding by other seasonal effects complicate the interpretation of findings of studies using season of birth, and this may explain why some studies concluded that birth during the pollen season was associated with a decreased risk of atopic diseases [19,26,27] rather than an increase [20-22,24,28].

We have attempted to mitigate the effect of residual confounding by using actual pollen exposure rather than season and included URTI within 5 weeks after birth as a proxy for the rates of circulating respiratory viruses in early life. Neither of these altered the associations with pollen. Data was not available on maternal vitamin D levels so residual confounding may remain.

Studies using ambient pollen concentrations have shown that exposure was associated with increased odds of wheeze at 2 years of age [10], asthma and allergic rhinitis at 6/7 years of age [9], and hospitalization for asthma within the first year of life [29]. However even here, there is debate as to whether it is pollen exposure in late pregnancy or early life that is more important.

While the changes to lung function associated with early life exposure to grass pollen that we observed in this study were relatively small compared to the effects of early tobacco smoke exposure [33], they were similar to the effect sizes seen with exposure to traffic-related air pollution during the first year of life [34]. Changes in adolescent lung function carry life long-term implications for lung development and overall respiratory morbidity.

Satellite measures of residential greenness can be used to identify point sources of pollen not detected by a central monitor [35]. A central trap only collects data on wind borne pollen which may have travelled large distances, depending on wind direction, speed and local topography. Combining the ambient pollen count from the trap and the satellite measures of residential greenness surrounding the participants' home may more accurately reflect pollen exposure. When satellite measures of residential greenness at birth were considered as an effect modifier, NDVI modified the associations between cumulative grass pollen exposure at 3 months and FEV_1 and FVC at both 12 and 18 years. In children with high greenness exposures (NDVI>75%le), low exposure to pollen (0-19 grains) in the first three months of life was associated with increased FEV_1 and FVC. Greenness has been shown to have both positive and negative effects on atopic respiratory diseases [12], and the underlying biological mechanisms are not yet clear [36]. Point source pollination and the natural filtering of far travelling pollen of residential green space could be contributing factors. The amount of greenness may also impact on the more direct exposure to pollens in terms of behavior [37] with access to parks and increased walkability positively correlated to greenness and time spent outside [38].

Strengths and Limitations

A major strength of our study was the access to daily counts of grass pollen collected through the year. Although this was not available for the entire MACS cohort, we were able to use actual counts in the majority, permitting accurate assessment of any effect due to grass pollen. While there is still potential for residual confounding by a range of factors that temporally coincide with high pollen exposure, effects seen in proxy measures such as birth during the pollen season or month of birth are more susceptible to such. However, only one centrally located Burkard trap was available to collect daily levels of pollen, which will have resulted in some misclassification of the actual pollen exposure for individual children due to spatial variability. However, we focused on grass pollen as the prominent allergenic species in this region and the majority of pollen comes from distant grasslands and is distributed over the city of Melbourne [9]. As such, during peak grass pollen seasons, all individuals are likely to receive similar exposures.

We had to impute NDVI values for approximately 5% of our sample with pollen data. The sensitivity analysis removing these observations also showed similar associations. Nevertheless, missing NDVI data are a potential limitation of our analysis. A further limitation arises from the presentation of the pre-bronchodilation lung function results. Presumably, the deficit in FEV_1 at 12 years is related to active asthma causing obstruction, but we cannot be sure of this, as we did not administer a bronchodilator. By 18 years, there has been dramatic expansion of the airways, and also a decline in the prevalence of asthma. As such, it is less likely for an impairment to be detectable. It is also possible that as participants age, the influence of early life factors is likely to wane.

Conclusion

Grass pollen exposure in the first months of life is associated with lower lung function later in childhood and adolescence. In children with high greenness exposures, low pollen exposure in the first three months of life was associated with increased FEV_1 and FVC. This effect is possibly capturing behavioral differences in the way local populations interact with their environments which is likely to be related to the degree of greenness. Further research is needed to replicate these findings in a larger cohort of children with varying family history of allergic disease and deepen understanding of the mechanisms by which early life pollen exposure could affect lung function. Exposure to grass pollen at critical time periods during early life may be associated with some form of structural change in the lung parenchyma. If replicable, these findings would support the development of targeted interventions for pollen avoidance strategies that take into account local topography that could be implemented alongside other clinical interventions such as immunotherapy.

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Table 1: Sample characteristics of the MACS participants with lung function data, with or without grass pollen data

Childhood factors	**Pollen data available**		
	No (N=160)	Yes (N=326)	p
Male sex %(n)	50 (80)	51 (166)	0.923
URTI before 5 weeks	5 (8)	2.8 (9)	0.292
Sensitisation 2yrs	11 (17)	6.9 (22)	0.152
Childhood asthma	45 (64)	40 (122)	0.305
Age (in years)			
Age 12	12.75 ± 1.65	10.94 ± 1.66*	<0.001
Age 18	19.14 ± 1.02	17.41 ± 1.07*	<0.001
Lung Function			
Age 12	(n=115)	(n=250)	
FEV_1 (mL)	2696 ± 724	2219 ± 551*	<0.001
FVC (mL)	2916 ± 726	2433 ± 594*	<0.001
FEV_1/FVC	0.92 ± 0.07	0.91 ± 0.07	0.326
Age 18	(n=134)	(n=275)	
FEV_1 (mL)	3847 ± 846	3821 ± 762	0.769
FVC (mL)	4594 ± 1026	4519 ± 948	0.478
FEV_1/FVC	0.84 ± 0.08	0.85 ± 0.07	0.307

Data presented as % and (number) or mean ± SD.

*p<0.05

Table 2: The association between grass pollen exposure and lung function measures

		n	FEV_1 (mL)	FVC (mL)	FEV_1/FVC (%)
Age 12	**n = 250**				
7 day	Cumulative count		**-15.1 (-27.3, -2.9)***	**-20.5 (-35.2, -5.8)****	0.1 (-0.2, 0.4)
3 month	Cumulative count		-9.3 (-22.4, 3.8)	-15.0 (-30.8, 0.8)	0.1 (-0.2, 0.4)
	0 – 19 grains/m^3	26	**Ref**	**Ref**	**Ref**
	20-320 grains/m^3	99	-99.8 (-205.1, 5.50)	**-161.4(-280.0, -41.9)****	1.0 (-1.0, 3.7)
	321-2950 grains/m^3	62	-64.0 (-175.0, 46.9)	**-146.1(-272.2, -20.2)***	2.2 (-0.1, 4.4)
	>2950 grains/m^3	63	-74.8 (-174.3, -24.8)	**-117.7 (-230.7, -4.7)***	0.3 (-1.6, 2.2)
Age 18	**n = 275**				
7 day	Cumulative count		-0.4 (-19.3, 18.4)	-20.0 (-41.7, 1.7)	**0.4 (0.1, 0.6)****
3 month	Cumulative count		4.2 (-15.6, 24.1)	-17.8 (-40.6, 5.1)	**0.4 (0.1, 0.7)****
	0 – 19 grains/m^3	29	**Ref**	**Ref**	**Ref**
	20-320 grains/m^3	111	-98.2 (-242.0, 45.7)	-153.5 (-315.7, 8.7)	1.0 (-1.1, 3.1)
	321-2950 grains/m^3	64	-29.9 (-183.3, 123.6)	**-179.1 (-352.2, -6.1)***	**2.8 (0.6, 5.1)***
	>2950 grains/m^3	71	-48.3 (-184.1, 87.5)	**-155.4 (-308.5, -2.2)***	**2.0 (0.01, 4.0)***

Adjusted for age, height, sex, URTI before 5 weeks, mother's education

*p<0.05 **p<0.01

7 day: Cumulative pollen count over the first 7 days of life. Effect per doubling of the pollen count.

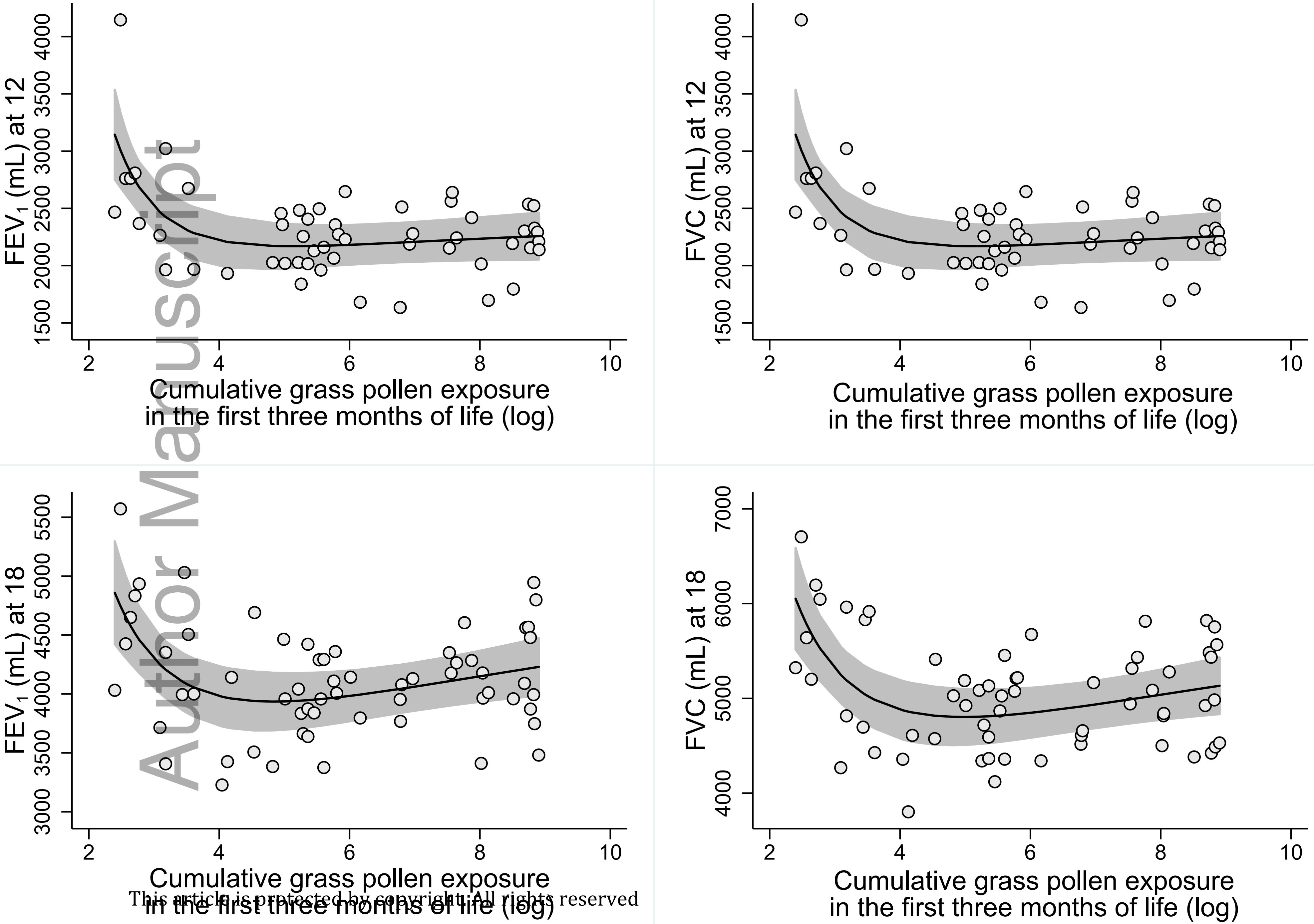
3 month: Cumulative pollen count over the first 3 months of life. Effect per doubling of the pollen count.

Pollen exposure at birth and adolescent lung function, and modification by residential greenness

Katrina A Lambert, Caroline Lodge, Adrian J Lowe, Luke A Prendergast, Paul S Thomas, Catherine M Bennett, Michael J Abramson, Shyamali C Dharmage, Bircan Erbas

- Early life exposure to pollen was associated with reduced lung function at 12 years in a high-risk birth cohort.
- Associations with a non-linear reduction in forced vital capacity (FVC) were also apparent at 18 years.
- Exposure to high residential greenness modified the association at 18 years.

FEV_1: Forced expiratory volume during the first second; FVC: forced vital capacity


FEV$_1$ (mL) at 12
1500
2000
2500
3000
3500
4000
2
4
6
8
10
Cumulative grass pollen exposure
in the first three months of life (log)
FVC (mL) at 12
1500
2000
2500
3000
3500
4000
2
4
6
8
10
Cumulative grass pollen exposure
in the first three months of life (log)
FEV$_1$ (mL) at 18
3000
3500
4000
4500
5000
5500
2
4
6
8
10
Cumulative grass pollen exposure
in the first three months of life (log)
FVC (mL) at 18
4000
5000
6000
7000
2
4
6
8
10
Cumulative grass pollen exposure
in the first three months of life (log)