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Association of early life and acute pollen exposure with lung function and exhaled nitric oxide (FeNO). A prospective study up to adolescence in the GINIplus and LISA cohort

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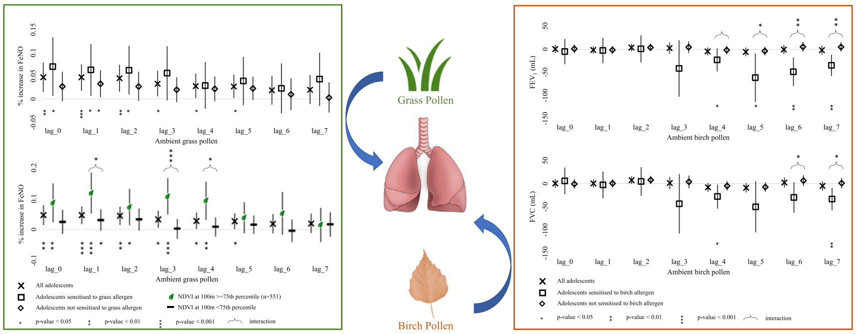
HIGHLIGHTS

- Exposure to grass pollen in infancy is associated with reduced lung capacity at 15.
- Increased acute grass pollen exposure resulted in higher airway inflammation levels.
- Associations between pollen and airway inflammation are stronger in greener areas.
- Birch pollen is associated with reduced lung function only in sensitised adolescents.

GRAPHICAL ABSTRACT



Abbreviations: ATS/ERS, American Thoracic Society and European Respiratory Society; FEF_{25-75}, the mean flow rate between 25% and 75% of the forced vital capacity; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GA2LEN, Global Allergy and Asthma European Network; GINIplus, German Infant study on the influence of Nutrition Intervention plus air pollution and genetics on allergy development; IQR, inter quartile range; lag 0, day of testing; lag 1, day prior to testing; lag 2, two days prior to testing; lag 3, three days prior to testing; lag 4, four days prior to testing; lag 5, five days prior to testing; lag 6, six days prior to testing; lag 7, seven days prior to testing; LISA, life-style related factors on the development of the Immune System and Allergies in East and West Germany; NDVI, Normalised Difference Vegetation Index.

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ABSTRACT

Background: Pollen exposure has both acute and chronic detrimental effects on allergic asthma, but little is known about its wider effects on respiratory health. This is increasingly important knowledge as ambient pollen levels are changing with the changing global climate.

Objective: To assess associations of pollen exposure with lung function and fractional exhaled nitric oxide (FeNO) at age 15 in two prospective German birth cohorts, GINIplus and LISA.

Methods: Background city-specific pollen exposure was measured in infancy (during the first three months of life), and contemporary (on the day of and 7 days prior to lung function measurement). Greenness levels within circular buffers (100–3000 m) around the birth and 15-year home addresses were calculated using the satellite-derived Normalized Difference Vegetation Index. Regression models were used to assess the associations of grass and birch pollen with lung function and FeNO, and the modifying effects of residential greenness were explored.

Results: Cumulative early life exposure to grass pollen was associated with reduced lung function in adolescence (FEV_1: −4.9 mL 95%CI: −9.2, −0.6 and FVC: −5.2 mL 95%CI: −9.8, −0.5 per doubling of pollen count). Acute grass pollen exposure was associated with increased airway inflammation in all children, with higher FeNO increases in children living in green areas. In contrast acute birch pollen exposure was associated with reduced lung function only in children sensitised to birch allergens.

Conclusion: This study provides suggestive evidence that early pollen exposure has a negative effect on later lung function, which is in turn influenced by acute pollen exposures.

1. Introduction

The ongoing changes to our climate are impacting wind pollinated plants. Pollen seasons are starting earlier (Damialis et al., 2019), while increases in CO_2 levels have been shown to increase both pollen production (Albertine et al., 2014) and, in some cases, pollen allergenicity (Singer et al., 2005). The effect on human health and wellbeing of allergenic pollen cannot be understated. Allergic disease is a recognised global epidemic (Platts-Mills, 2015) carrying a considerable economic burden (Linneberg et al., 2016). Increases in the levels of acute ambient pollen concentrations have been associated with reduced lung function (Gruzieva et al., 2015) and increased markers of airway inflammation (Baraldi et al., 1999; Roberts et al., 2004; Vahlkvist et al., 2006) in a small number of studies. Exposure to high levels of pollen in infancy was suggested as a risk factor for allergic respiratory diseases in later childhood (Erbas et al., 2013; Harley et al., 2009; Lowe et al., 2012). Our prior study (Lambert et al., 2019) observed that higher levels of ambient grass pollen in the first seven days of life were associated with reduced lung function at 12 years among children from Melbourne, Australia.

Childhood lung function has been seen to be a good predictor of lung function in adulthood (Bui et al., 2018). The identification of factors that can affect how lungs develop is therefore critical. Poor lung function has been associated with a wide range of adverse health outcomes (Zaigham et al., 2016; Yeh et al., 2014; Gulsvik et al., 2012). Elevated levels of airway inflammation biomarkers such as fractional exhaled nitric oxide (FeNO) is a signal of respiratory system inflammation (Montuschi, 2002). As chronic airway inflammation can lead to pathological remodelling, altering airway wall structures and function (Fehrenbach et al., 2017), identification of factors effecting it are likewise critical.

Residential greenness (i.e., degree of vegetation) may be of benefit to human health through the promotion of exercise and socialising, the reduction of harmful exposures such as noise, heat, pollution, and increases in microbiome diversity (Hartig et al., 2014; Markevych et al., 2017). On the other hand, it can be indicative of point sources of pollination - areas of higher residential greenness may have a higher local pollen concentration than the ambient concentration measured (Lambert et al., 2019). The Normalised Difference Vegetation Index (NDVI) provides a measure of greenness around the home that is of increasing interest to children's health (Hsieh et al., 2019). A recent study provided some evidence that children whose homes are in greener areas have better lung function (Fuertes et al., 2020). Residential greenness has been shown to modify the association between early life pollen exposure and lung function in Melbourne (Lambert et al., 2019).

Using two well established German birth cohorts, we have explored the associations in two exposure windows, early life and adolescence, of two types of pollen with lung function and FeNO. Grass (*Poaceae*) pollen is the leading aeroallergen worldwide, with a wide distribution of grass species and highly allergenic pollen (Damialis et al., 2019). Birch (Betula spp.) pollen is a well-established allergen in Germany (Werchan et al., 2018). This study is the first to concurrently examine the associations between cumulative pollen concentrations in infancy on subsequent lung function in adolescents, as well as acute exposure at the time of lung function measurement. To our knowledge, this is the only study to explore the potential modification of these associations by residential greenness in the northern hemisphere.

2. Methods

2.1. GINIplus/LISA

Participants of two prospective, population based German birth cohorts, GINIplus (Berg et al., 2010) and LISA (Heinrich et al., 2002), with 15 year follow up were analysed in this study. Details of the studies is provided elsewhere (von Berg et al., 2016). Briefly, GINIplus recruited 5991 neonates between 1995 and 1998 from Munich, Wesel and surrounding areas. LISA recruited 3094 newborns between 1997 and 1999 from Munich, Wesel, Leipzig, Bad Honnef and surrounding areas. The inclusion criteria were the same for both cohorts: German families with a healthy, full-term normal birth weight newborn. Ethical approval was obtained by the local medical ethical committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia). Written informed consent was obtained from all participating families. Data from the LISA and GINIplus birth cohorts were pooled and are presented for the complete study population considering study group and study centre as covariates in the analyses. Residential addresses at birth and 15 years to assess greenness were only available for Munich and Wesel; thus, the population was restricted to these study areas (Fig. S1).

2.2. Lung function

Lung function measurements were performed by spirometry at 15 years follow-ups in line with American Thoracic Society and European Respiratory Society (ATS/ERS) recommendations (Miller et al., 2005). Flow-volume curves were obtained using a pneumotachograph-type spirometer in both study centres (EasyOne

Worldspirometer, ndd, Zurich, Switzerland). We focused our analyses and results on four spirometry parameters taken pre-bronchodilation (forced expiratory volume in 1 s (FEV_1) and the forced vital capacity (FVC), the ratio of these measures (FEV_1/FVC) and the mean flow rate between 25% and 75% of FVC ($FEF_{25–75}$)) that represent lung volume and airway function.

2.3. FeNO

FeNO was determined in line with the current ATS/ERS recommendations (ATS/ERS, 2005) during a controlled expiration over 6 s using the handheld device, NIOX MINO (Aerocrine) (Maestrelli et al., 2007). Full details have been reported previously (Liu et al., 2014). FeNO was natural-log transformed to obtain a normal distribution.

2.4. Pollen

Daily ambient pollen concentrations were obtained from 7 day recording volumetric spore traps (Hirst, 1952) located at rooftop height (approx. 20 m above the ground) at hospitals in Munich and Bochum, near Wesel. Two types of pollen were recorded and investigated, birch (*Betula*) and grass (*Poaceae*).

Daily 24-hour average counts were used for the exposure measure of acute ambient pollen concentration on the day of lung function measurement (lag 0), the day before measurement (lag 1), two days prior (lag 2), and so on up to seven days prior (lag 7). In order to increase comparability between the pollen types, effects are presented per inter quartile range (IQR) increase (100 pollen grains/m^3 for birch and 15 pollen grains/m^3 for grass).

Pollen exposure during early life was examined separately as the cumulative count of the pollen concentration for each pollen type over the first seven days (7 day) and three months (3 month) of life (Lambert et al., 2019). The cumulative pollen data were analysed after a log base 2 transformation with an offset of 1 applied, allowing the coefficients to be interpreted as the effect of doubling pollen exposure.

2.5. Greenness

Greenness was assessed using NDVI, derived from Landsat 7 ETM satellite images at birth and 8 OLI satellite images at 15 years, at a 30 by 30-meter pixel resolution. Residential greenness was defined as the mean of NDVI values in circular 100, 300, 500, 1000 and 3000 m buffers around each participant's home address. NDVI values are between −1 and 1, with higher values indicating an increased density of green biomass (Weier and Herring, 2000).

2.6. Other variables

Demographic, health and lifestyle information on the study participants was collected using self-administered questionnaires completed by the parents at birth, and parents and children at 15 years follow-up.

Asthma was defined based on the Global Allergy and Asthma European Network (GA2LEN) definition (Carlsen et al., 2006). Children were considered as currently having asthma if their parents responded positively to at least two of the three following questions: (1) Has a doctor diagnosed asthma in your child? (2) Has your child taken asthma medication during the last 12 months? (3) Has your child had wheezing or whistling in the chest in the last 12 months?

Parents were asked to report their child's birthweight in grams and mode of birth (Caesarean or natural) at birth in LISA and at 1 and 15 years in GINIplus. Early lung infections were defined as a doctor's diagnosis of pneumonia or obstructive bronchitis within the first three years of life. Indoor second-hand smoke exposure during early life was determined by parental report of the child being exposed to second-hand smoke at home at 4 months in GINIplus and at birth or six months in LISA. Parental education was classified into three categories based on the highest number of school years of either parent (high: >10 years; medium: 10 years; low: <10 years) and parental atopy was positive if the mother or father reported doctor diagnosis of asthma, eczema or hay fever ever. Indoor second-hand smoke exposure at 15 years was positive if the adolescent reported indoor second-hand smoke exposure at least once a week (Luzak et al., 2017).

Serum IgE levels were measured using the standardized CAP-RAST FEIA method (ThermoFischer, Freiburg, Germany) to determine sensitisation status of children to general food allergen mixture (FX5: egg white, codfish, cow milk, wheat flour, peanut, and soybean) and general inhalant allergen mixture (SX1: cat, dog, mugwort, birch, timothy, rye, *Cladosporium herbarum*, and *Dermatophagoides pteronyssinus*). A child was considered sensitised to a substance if the specific IgE level was 0.35 kU/L or higher. If the screening test was positive (≥0.35kU/L), the single allergens, including birch (T3) and grass (G6) allergens, were tested.

The total 25(OH)D concentrations in serum were measured by Roche's vitamin D total test on the fully automated Modular system (E170, Roche Diagnostics, Mannheim, Germany). Serum 25(OH)D concentrations were adjusted for the date of blood sampling by fitting a generalised additive model with the residuals then added to the overall mean of the vitamin D concentrations to account for seasonal variability (Flexeder et al., 2017).

Peak height and peak weight velocities was calculated using nonlinear random effects models (Flexeder et al., 2016) and represents the maximum of the first derivative of the individual height or weight gain curves obtained between birth and two years of age. Height and weight measurements were obtained during the children's preventive medical check-ups to monitor growth.

2.7. Statistical analysis

Associations of pollen exposure with lung function and FeNO (natural log) outcomes were assessed by linear regression analysis. Base models were minimally adjusted for study (GINIplus intervention; GINIplus observation; LISA), area, age, sex, height and weight at 15 years. Further adjustment for covariates considered ever asthma; sensitisation; early life factors of birthweight, mode of birth, peak height and weight velocities, early lung infections and indoor second-hand smoke exposure; parental education; parental atopy; seasonally adjusted vitamin D; and indoor second-hand smoke exposure at time of lung function testing. The models were then reduced by automated model selection using the R “glmulti” (Calcagno and de Mazancourt, 2010) package with the Akaike Information Criterion (AIC).

To assess effect modification by residential greenness, in addition to continuous NDVI, we dichotomised NDVI into “High residential greenness” (defined 75th percentile or greater) and “Low residential greenness”. The level of 75th percentile was selected a priori. Potential effect modification by sensitisation was also investigated. Statistical analyses were performed using R version 3.5.1.

3. Results

3.1. Characteristics

A total of 2334 adolescents living in the Munich ($n = 1335$) and Wesel ($n = 999$) areas underwent lung function testing and FeNO measurements as part of the 15-year GINIplus/LISA follow-up (Table 1). Approximately half the children were male (51%) and 7% had ever been diagnosed with asthma, while 43% were sensitised to at least one of the inhalant allergens.

3.2. Ambient pollen concentrations and residential greenness

Daily ambient pollen concentration remained relatively low for most of period during the periods of data collection (median of 0), with a

Table 1
Descriptive characteristics of the 2334 adolescent participants of this study.

		n (%)
Study	GINIplus observation	893 (38.26)
	GINIplus intervention	911 (39.03)
	LISA	530 (22.71)
Area	Munich	1335 (57.2)
	Wesel	999 (42.8)
Age (years)	Mean (SD)	15.3 (0.3)
Sex	Male	1199 (51.37)
	Female	1135 (48.63)
Height (cm)	Mean (SD)	171.5 (8.3)
Weight (kg)	Mean (SD)	61.9 (11.9)
Asthma ever	No	2156 (93.41)
	Yes	152 (6.59)
Birth weight (g)	Mean (SD)	3467.7 (454.8)
Sensitisation to inhalant allergens[a]	No	1182 (50.64)
	Yes	1005 (43.06)
	NA	147 (6.30)
Sensitisation to grasses[a]	No	1478 (63.3)
	Yes	709 (30.4)
	NA	147 (6.3)
Sensitisation to birch[a]	No	1666 (71.4)
	Yes	521 (22.3)
	NA	147 (6.3)
Sensitisation to food allergens[a]	No	1931 (82.73)
	Yes	256 (10.97)
	NA	147 (6.30)
Seasonally adjusted vitamin D (nmol/L)	Mean (SD)	67.3 (24.7)
Early lung infections	No	1453 (67.52)
	Yes	699 (32.48)
Peak height velocity	Mean (SD)	43.6 (5.1)
Second-hand smoke exposure during early life	No	1850 (80.26)
	Yes	455 (19.74)
Second-hand smoke exposure at 15 years old	No	1723 (79.15)
	Yes	454 (20.85)
Highest parental education	Low (<10 yrs)	142 (6.1)
	Medium (=10 yrs)	621 (26.66)
	High (>10 yrs)	1566 (67.24)
Parental history of atopic disease	No	879 (37.68)
	Yes	1454 (62.32)

[a] Serum IgE level ≥ 0.35kU/L at age 15. Food allergen mixture (FX5: egg white, codfish, cow milk, wheat flour, peanut, and soybean); Inhalant allergen mixture (SX1: cat, dog, mugwort, birch, timothy, rye, *Cladosporium herbarum*, and *Dermatophagoides pteronyssinus*).

Table 3
Mean residential greenness at birth and age 15 in the two areas.

	Munich Mean (SD)	Wesel Mean (SD)	All Mean (SD)
Birth			
100 m	0.477 (0.143)	0.522 (0.108)	0.495 (0.132)
300 m	0.482 (0.115)	0.526 (0.082)	0.500 (0.105)
500 m	0.489 (0.107)	0.533 (0.075)	0.506 (0.098)
1000 m	0.498 (0.103)	0.546 (0.069)	0.517 (0.094)
3000 m	0.524 (0.088)	0.581 (0.050)	0.546 (0.080)
15 years			
100 m	0.518 (0.118)	0.562 (0.113)	0.537 (0.118)
300 m	0.518 (0.095)	0.567 (0.080)	0.540 (0.092)
500 m	0.523 (0.091)	0.576 (0.075)	0.546 (0.088)
1000 m	0.532 (0.089)	0.586 (0.068)	0.556 (0.085)
3000 m	0.558 (0.084)	0.618 (0.059)	0.585 (0.080)

short but intense birch pollen season and a longer grass pollen season (Table 2). Maximal pollen counts were higher in Wesel than Munich, with ambient birch pollen exceeding 1000 grains/m^3 on 26 days during the study periods in Wesel vs 11 days in Munich. Mean NDVI was higher in Wesel than Munich (Table 3).

3.3. Early life pollen exposure and lung function at 15 years

Cumulative grass pollen exposure over the first three months of life was associated with a small but significant reduction in FEV$_1$ (β: −4.1 mL 95%CI: −8.0, −0.3 per doubling of pollen count) and FVC at 15 years (β: −4.3 mL 95%CI: −8.5, −0.0) which remained significant after further adjustment for potential confounding factors (β: −4.9 mL 95%CI: −9.2, −0.6 and β: −5.2 mL 95%CI: −9.8, −0.5 respectively) (Table 4). Seven-day cumulative grass pollen exposure was associated with a reduction in FVC (β: −6.7 mL 95%CI: −13.1, −0.2 per doubling of pollen count) but not FEV$_1$. Early life pollen exposure was not associated with FEF$_{25-75}$ or the FEV$_1$/FVC ratio (Table S1). Early life exposure to birch pollen was not associated with any lung function measures.

3.4. Pollen exposure at 15 years and lung function

Among those sensitised to birch pollen, increased exposure to ambient birch pollen four to seven days prior was associated with decreased FEV$_1$ and FVC (Fig. 1). There was no association seen among those not sensitised to birch. The association was more consistent for FEV$_1$ with significant interactions between sensitisation to birch and birch pollen exposure at lags 4, 5, 6 and 7. An increase of 100 grains/m^3 of birch

Table 2
Daily ambient pollen concentrations of birch and grass pollen, in the two areas during the exposure windows of early life and 15 years.

		Min	25th %ile	50th %ile	75th %ile	Max	Mean (SD)	Length pollen season[a] Mean(SD)
Birch								
Wesel	Early life window (1996–1999)	0	0	0	2	10,000	66 (578)	37 (8.5)
	15 year window (2011–2014)	0	0	0	0	5820	86.7 (457.8)	39.8 (3.5)
Munich	Early life window (1996–1999)	0	0	0	0	2300	28 (136.2)	44.5 (14.7)
	15 year window (2011–2014)	0	0	0	0	2892	38 (209.7)	40.3 (11.9)
Grass								
Wesel	Early life window (1996–1999)	0	0	0	5	249	7 (19.1)	100.3 (7.0)
	15 year window (2011–2014)	0	0	0	1	159	6 (17.5)	81.7 (19.3)
Munich	Early life window (1996–1999)	0	0	0	8	136	7 (16.5)	111.7 (9.9)
	15 year window (2011–2014)	0	0	0	4	132	5 (12.7)	101 (10.2)

[a] Days. From first day ≥5 grains/m^3 to last day ≥5 grains/m^3 in each calendar year.

Table 4
The association between early life pollen exposure and lung function measures.

	FEV_1 (mL)			FVC (mL)		
	Estimate	2.50%	97.50%	Estimate	2.50%	97.50%
Minimally adjusted models						
7 day cumulative (Birch)	−2.5	−8.1	3.2	−1.2	−7.3	4.9
3 months cumulative (Birch)	−0.1	−3.2	3.0	0.9	−2.4	4.3
7 day cumulative (Grass)	−4.7	−10.6	1.2	**−6.7**	**−13.1**	**−0.2**
3 months cumulative (Grass)	**−4.1**	**−8.0**	**−0.3**	**−4.3**	**−8.5**	**−0.0**
Further adjusted models						
7 day cumulative (Birch)	−3.5	−9.3	2.3	−2.2	−8.5	4.1
3 months cumulative (Birch)	−1.8	−5.3	1.6	−0.2	−3.9	3.5
7 day cumulative (Grass)	−3.8	−10.0	2.3	**−6.8**	**−13.5**	**−0.2**
3 months cumulative (Grass)	**−4.9**	**−9.2**	**−0.6**	**−5.2**	**−9.8**	**−0.5**

Minimally adjusted models are adjusted for study, area, sex and exact age, height and weight at the 15 year follow up.
Further adjustment for 7 day models: asthma ever; indoor second hand smoke at 15; seasonally adjusted vitamin D levels; mother's education.
Further adjustment for 3 month models: asthma ever; sensitisation to inhalant allergens; early life respiratory infection; peak height velocity; indoor second hand smoke at 15; seasonally adjusted vitamin D levels; mother's education.
Log_2 scale; results are interpretable as per doubling of the pollen count.
Bold data indicates p-value < 0.05.

pollen one week prior to lung function testing was associated with a decrease in FEV_1 of 35.1 mL (95%CI: −58.1, −12.2). Daily ambient birch pollen was not associated with FeNO levels and showed associations with the FEV_1/FVC ratio and $FEF_{25–75}$ only at lag 6 in sensitised individuals (Table S2).

Daily ambient grass pollen was associated with slight increases in FeNO levels on the day of lung function testing and up to five days prior (Fig. 2). The largest associations were seen on the day of testing (0.05% 95%CI: 0.02, 0.08 per 15 grains/m^3) and the day prior (0.05% 95%CI: 0.02, 0.08). Associations between grass pollen and FEV_1/FVC ratio and $FEF_{25–75}$ were seen at lags three to six, with the largest effect seen on those sensitised at lag 3 (FEV_1/FVC ratio − 0.64% (95%CI: −1.17, −0.11) and $FEF_{25–75}$ -120.44 (95%CI: −196.54, −44.35)).

Grass pollen levels were associated with a decrease in FEV_1 at lag 4 (−20.2 mL 95%CI: −40.2, −0.2), and lag 3 among those sensitised to grass pollen (−48.2 mL 95%CI: −85.1, −11.2) (Table S2). When grass and birch pollen levels were included in the same model neither direction nor significance of these associations changed (data not shown).

3.5. Effect modification by residential greenness

The association between acute grass pollen exposure at 15 years of age and FeNO was modified by NDVI in the 100 m buffer. Living in areas of higher residential greenness increased the association between daily ambient grass pollen and increased FeNO (Fig. S2). The day prior to lung function testing a 15 grains/m^3 increase in daily grass pollen was associated with an increase in FeNO of 0.12% (95%CI: 0.05, 0.18) in those living in the highest quartile of residential greenness. In those not living in the highest quartile of residential greenness a 0.03% (−0.00, 0.07) increase was seen (p value for interaction: 0.022) (Fig. 3). At lag 3, grass pollen was associated with an increase in FeNO of 0.11% (95%CI: 0.05, 0.17) in those living in the highest quartile of residential greenness, with no association in those not (p-value for

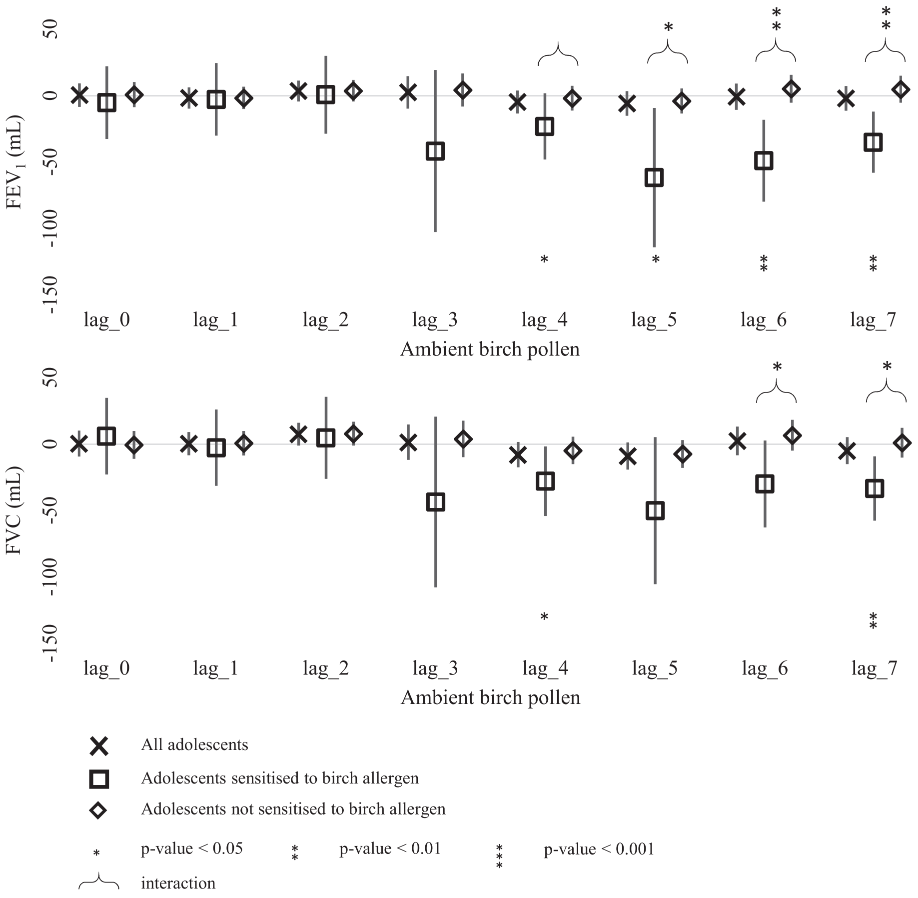

Fig. 1. Exposure to birch pollen and FEV_1 and FVC in adolescents.

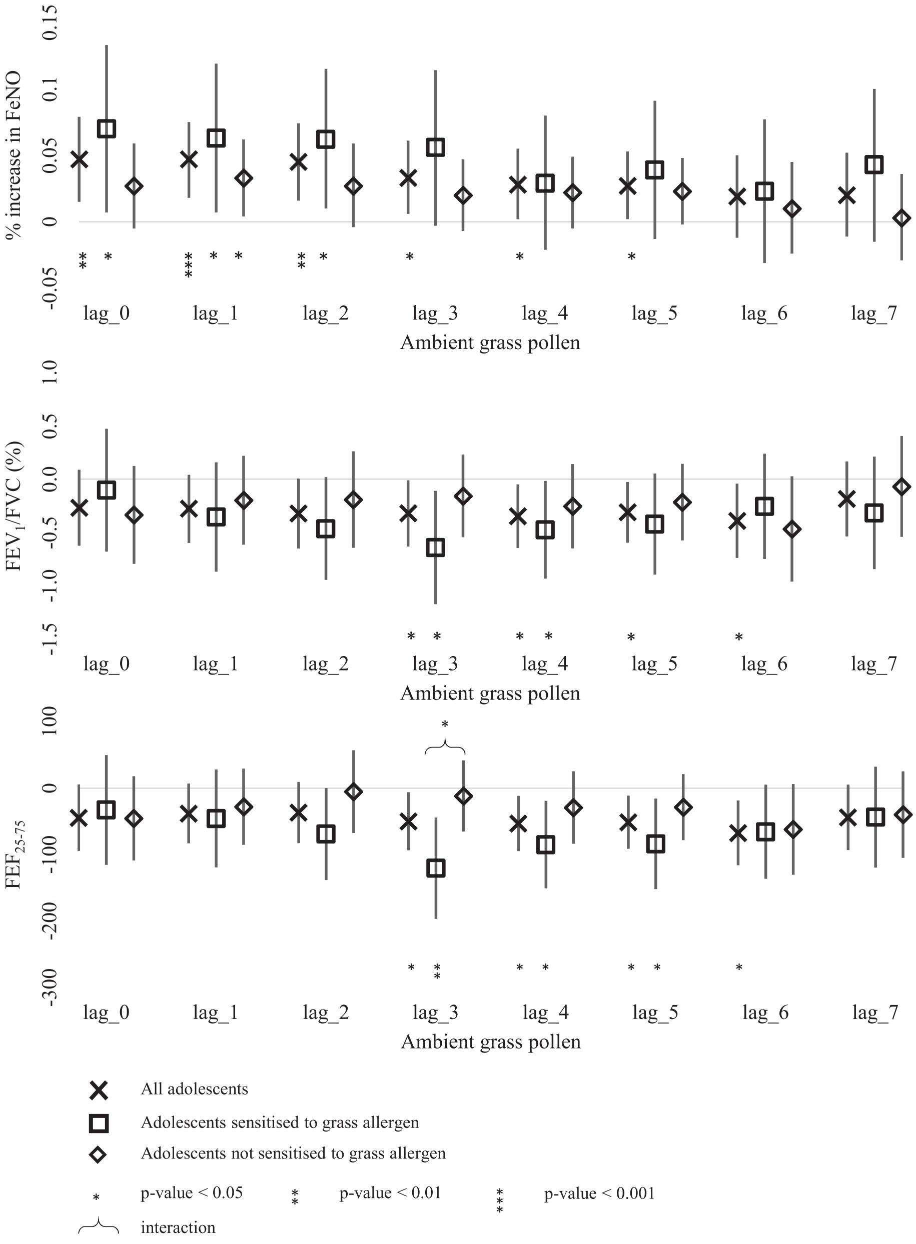

Fig. 2. Exposure to grass pollen and FeNO, FEV_1/FVC and FEF_{25-75} in adolescents.

interaction: 0.001). This result appears to be driven by adolescents from the Wesel rather than Munich area (Table S3). Residential greenness did not modify the associations between pollen and FEV_1 nor pollen and FVC.

4. Discussion

Cumulative exposure to grass, but not birch, pollen in the first three months of life was associated with decreased FEV_1 and FVC at age 15 with no significant association observed for the ratio. These indices characterize a restrictive pattern where lung capacity is reduced compared to normal levels without a relative change between FEV_1 and FVC. This pattern suggests a global reduction in lung function affecting both airways and lung parenchyma associated with increased grass pollen exposure in infancy. Understanding effects of exposures during this time period is critical. The most substantial structural developments of the lungs occur in utero and during the first year after birth (Burri, 2006; Copland and Post, 2004).

By contrast, acute birch pollen exposure was associated with an obstructive pattern in sensitised children. In this pattern, the lung capacity (FVC) was mostly unaffected, but the volume of air blown out quickly (FEV_1) was reduced, a result of airway narrowing. Grass pollen was associated with an immediate increased FeNO in all adolescents, not just those sensitised. This raises the possibility of grass pollen and sub-pollens acting as an irritant as well as allergenic exposure on airway inflammation (Davies et al., 1997; Nosbaum et al., 2009), although further mechanistic studies need to be conducted. This increase was seen up to lag 5 but was strongest when the increased exposure was between lag 0 and lag 3. Living in areas of higher residential greenness significantly increased this association, possibly due to localised sources of grass pollen. The associations seen between grass pollen and lung function measures were obstructive in nature and strongest at lags 3 and 4. This three-day lag is of particular interest, with both reduction in the FEV_1/FVC ratio suggesting an obstruction and increase in FeNO indicating airway inflammation. Reductions in the ratio and increases in airway inflammation is typical of asthmatic exacerbations. A recent meta-analysis

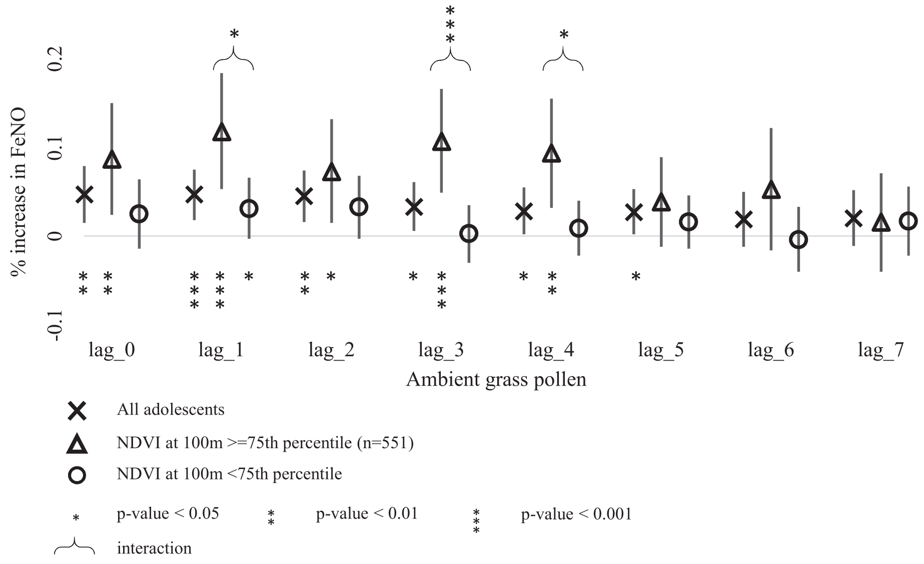

Fig. 3. Exposure to grass pollen and FeNO, with modification by high residential greenness.

showed that increased levels of grass pollen at this lag are associated with increased emergency department presentations for childhood asthma (Erbas et al., 2018).

Comparable studies focusing on airway inflammation and lung function are scarce. Our findings are in agreement with a London study of asthmatic children (age 6 to 16) sensitised to Timothy grass (*Phleum pratense*) (Roberts et al., 2004), which found a positive correlation between increased FeNO and increased concentration of ambient grass pollen on the day of measurement and up to one week prior and no association with FEV$_1$. A Swedish study (Gruzieva et al., 2015) with a more comparable general population found no association between grass pollen and lung function at age 16, while in a younger age group (8 years old) increased exposure to grass pollen at lag 1 was associated with a reduced FEV$_1$ and FVC. This study did not investigate FeNO.

Acute birch pollen exposure appears to have a more delayed effect on lung function than grass pollen, being associated with an effect on sensitised children nearly a week after elevated levels. Our study is the first with a timeframe sufficient to detect this association. A previous study in Copenhagen, Denmark (Vahlkvist et al., 2006) followed 11 asthmatic children aged 5 to 15 years, sensitised to birch pollen, through the birch pollen season. They reported no association between ambient pollen counts and FEV$_1$, however, they only investigated lag 0,1 and 2. The null finding reported by Gruzieva et al. (2015) is based on a mixed sample of sensitised and non-sensitised children.

Cumulative grass pollen exposure over the first seven days of life was associated with a smaller reduction in lung capacity than that found in Melbourne, Australia (Lambert et al., 2019). This is likely an artefact of the shorter grass pollen season in Wesel and Munich compared to Melbourne. Further, the Melbourne sample was much smaller and only included children with a family history of allergic disease – although reducing our sample similarly did not significantly increase the magnitude of the reduction.

We were unable to replicate the result from the Melbourne study which showed a non-linear pattern such that the highest lung function values were seen in those with low exposure to pollen and high residential greenness. However, we did find a novel result: those with high residential greenness close to the residence were more susceptible to the effects of grass pollen on FeNO. The mechanism for this effect requires further exploration (Hartig et al., 2014). Likely explanations include the increase in point sources of pollination and/or behavioural modification, with children in greener areas more likely to spend time outside being physically active (Grigsby-Toussaint et al., 2011) and thus in the path of wind-borne pollen on high pollen days.

It could be speculated that exposure to pollen in early life may be related to changes in DNA methylation and thereby affect the individual's disease risk. It has been shown that cord blood DNA methylation is associated with lung function in childhood, which might be associated with asthma and COPD development later in life (den Dekker et al., 2019). Another study (Clifford et al., 2017) found changes in bronchial epithelial DNA methylation following the exposure to Diesel exhaust or allergen, or a combination of both. Furthermore, different DNA methylation profiles in CD4+ T-cells clearly differentiated between seasonal allergic rhinitis patients and healthy controls during and outside the pollen season (Nestor et al., 2014). Therefore, a gene-environment interaction through the life span could explain, at least partially, the observed effects. Immunotherapy maybe necessary at an early point time in those genetically susceptible individuals. Targeting therapies that are likely to alter DNA methylation could be considered in future studies.

The present study has several strengths. It is the first study to explore the associations between ambient pollen concentration during early life and at time of lung function measure in the same population. The two large German based birth cohorts created power to explore multiple associations including interactions between sensitisation statuses and residential greenness levels. We were also able to explore associations in a wide range of lung function measurements (FEV$_1$, FVC, FEV$_1$/FVC and FEF$_{25–75}$) as well as marker of airway inflammation (FeNO); providing a more holistic look at the potential effects of pollen on the respiratory system.

However, our study is not without limitations. Some selection bias may exist in this study due to the initial recruitment of only German-speaking parents and loss to follow up. Participants who completed the 15 year follow up have higher parental education level and lower second-hand smoke exposure than the source cohorts. Second, ambient pollen concentrations were collected by two traps, one for the Munich area and one for the Wesel area, with children assigned the exposure measured on the closest trap. While the Munich trap was located in the centre of Munich, for logistics reasons the Wesel trap was located 46 km to the south east in Bochum. While this is within the range of high correlation between pollen traps (Osborne et al., 2017), as wind born pollen travels great distances, the possibility of exposure misclassification exists. Further, while we examined concentration of the pollen grains in the atmosphere, the amount of major allergen (Bet v 1/Ph p 5) within the pollen may be regionally and seasonal dependent (Karatzas et al., 2018). In addition, NDVI detects general vegetation thus providing a relatively crude measure that does not distinguish between allergenic and specific vegetation. However, it is a standardized global measure available at multiple time points, allowing direct comparison between this study and others. Lastly, our study of the acute effects of pollen was cross-sectional, so causality cannot be inferred. A panel study with repeated measurements of lung function and FeNO might have served as a more robust design.

5. Conclusion

Early life exposure to grass pollen was associated with significant reductions in lung function at age 15. While further studies are required to determine the mechanism behind this association, our results suggest that grass pollen avoidance strategies for infants may be of benefit. Grass pollen in the days before testing was further associated with increased FeNO, which was higher in children living in green areas. Birch pollen exposure had a delayed association with reduced lung function in sensitised children. Pollen-sensitised individuals and those with asthma should be made aware of available pollen forecasts and the possible influence of pollen in the days after exposure.

CRediT authorship contribution statement

Katrina A. Lambert: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Iana Markevych:** Software, Writing - review & editing, Validation. **Bo-Yi Yang:** Software, Writing - review & editing. **Carl-Peter Bauer:** Investigation, Resources, Writing - review & editing. **Dietrich Berdel:** Investigation, Resources, Writing - review & editing. **Andrea von Berg:** Investigation, Resources, Writing - review & editing. **Karl-Christian Bergmann:** Investigation, Resources, Writing - review & editing. **Caroline Lodge:** Supervision, Writing - review & editing. **Sibylle Koletzko:** Investigation, Resources, Writing - review & editing. **Luke A. Prendergast:** Supervision, Writing - review & editing. **Tamara Schikowski:** Investigation, Resources, Writing - review & editing. **Holger Schulz:** Investigation, Resources, Writing - review & editing. **Matthias Werchan:** Investigation, Resources, Writing - review & editing. **Joachim Heinrich:** Funding acquisition, Resources, Visualization, Writing - review & editing. **Marie Standl:** Project administration, Resources, Supervision, Writing - review & editing. **Bircan Erbas:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The LISA Study group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Munich (Heinrich J, Schnappinger M, Brüske I, Ferland M, Schulz H, Zeller C, Standl M, Thiering E, Tiesler C, Flexeder C); Department of Pediatrics, Municipal Hospital “St. Georg”, Leipzig (Borte M, Diez U, Dorn C, Braun E); Marien Hospital Wesel, Department of Pediatrics, Wesel (von Berg A, Berdel D, Stiers G, Maas B); Pediatric Practice, Bad Honnef (Schaaf B); Helmholtz Centre of Environmental Research – UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (Lehmann I, Bauer M, Röder S, Schilde M, Nowak M, Herberth G, Müller J); Technical University Munich, Department of Pediatrics, Munich (Hoffmann U, Paschke M, Marra S); Clinical Research Group Molecular Dermatology, Department of Dermatology and Allergy, Technische Universität München (TUM), Munich (Ollert M, J. Grosch).

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LISA

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143006.

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