Long-term air pollution exposure and lung function in 15 year-old adolescents living in an urban and rural area in Germany: the GINIplus and LISAplus cohorts

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ABSTRACT

Introduction: The impact of outdoor air pollution exposure on long-term lung development and potential periods of increased lung susceptibility remain unknown. This study assessed associations between early-life and current residential exposure to air pollution and lung function at 15-years of age in two German birth cohorts.

Methods: Fifteen year-old participants living in an urban and rural area in Germany underwent spirometry before and after bronchodilation (N=2266). Annual average (long-term) exposure to nitrogen dioxide (NO_2), particles with aerodynamic diameters less than 2.5 $\mu g/m^3$ ($PM_{2.5}$) mass and less than 10 $\mu g/m^3$ (PM_{10}) mass, $PM_{2.5}$ absorbance and ozone were estimated to each participant's birth-, ten- and 15-year home address using land-use regression and kriging (ozone only) modelling. Associations between lung function variables and long-term pollutant concentrations were assessed using linear regression models adjusted for host and environmental covariates and recent short-term air pollution exposures.

Results: Long-term air pollution concentrations assessed to the birth-, ten- and 15-year home addresses were not associated with lung function variables, before and after bronchodilation, in the complete or study area specific populations. However, several lung function variables were negatively associated with long-term NO_2 concentrations among asthmatics. For example, NO_2 estimated to the 15-year home address was associated with the ratio of forced expiratory volume in one second to forced vital capacity (FEV_1/FVC) and the mean flow rate between 25% and 75% of FVC (-3.5%, 95% confidence interval [-6.0, -1.0] and -297.4 ml/s [-592.6, -2.1] per 5.9 $\mu g/m^3$ increase in NO_2, respectively). Nearly all effect estimates for the associations between the short-term $PM_{2.5}$ mass, PM_{10} mass and ozone

concentrations and the lung function variables were negative in the complete population.

Conclusions: Early-life and current long-term air pollution exposures and lung function at the age of 15 years were not associated in the complete study population. Asthmatics may represent a vulnerable group.

KEYWORDS

air pollution, asthma, birth cohort, FEV_1, FVC, lung function

ABBREVIATIONS

FEF_{25}	forced expiratory flow rates at 25% of exhaled FVC
FEF_{25-75}	mean flow rate between 25% and 75% of FVC
FEF_{50}	forced expiratory flow rates at 50% of exhaled FVC
FEF_{75}	forced expiratory flow rates at 75% of exhaled FVC
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
GINIplus	German Infant study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy development
LISAplus	influence of Life style factors on the development of the Immune System and Allergies in East and West Germany plus the influence of traffic emissions and genetics
LUR	land-use regression
NO_2	nitrogen dioxide
PEF	peak expiratory flow
$PM_{2.5}$	particulate matter with aerodynamic diameters < 2.5 μm
PM_{10}	particulate matter with aerodynamic diameters < 10 μm
TRAP	traffic-related air pollution

1.0 INTRODUCTION

Lung function in early adolescence is an important marker of respiratory health and early indicator of later-life cardiorespiratory morbidity and mortality (Sin et al. 2005). Several studies have confirmed that short-term variations in air pollutants can lead to acute changes in lung function, but the long-term chronic effects of air pollution exposure are less well understood (Künzli and Tager 2000). A review conducted in 2008 concluded that there is strong support for an association between air pollution exposure and reduced lung function in children and adolescents (Götschi et al. 2008). However, the majority of the considered studies were cross-sectional and several of them assessed long-term exposure to outdoor air pollution on an aggregate (community) level, and were thus limited in their ability to capture within-city air pollution variability which is largely attributable to local sources, such as traffic. A more recent review which focused specifically on traffic-related air pollution (TRAP) concluded that the evidence was "suggestive but not sufficient" to infer causality due to the limited and methodologically heterogeneous literature (Tager et al. 2010).

Current knowledge gaps concern potential periods of increased susceptibility during lung development and the relative importance of early-life versus current exposures. The results among the three studies that have examined these research gaps using individual-level TRAP estimates are inconsistent. Both early exposure (during the first year of life) and lifetime exposure to nitrogen dioxide (NO_2) and particulate matter with aerodynamic diameters < 2.5 μm ($PM_{2.5}$) and < 10 μm (PM_{10}) were negatively associated with lung function expiratory flow variables among 2307 Norwegian nine and ten year-olds (Oftedal et al. 2008). In another study, lung function at eight years was associated with traffic PM_{10} only during the first year of life, but not with later exposures, among 1924 Swedish children (Schultz et al.

2012). Finally, a meta-analysis of five European birth cohorts, including 5921 children aged six to eight years, found that small decreases in lung function variables were associated with current but not early exposure to nitrogen oxides, $PM_{2.5}$ absorbance and $PM_{2.5}$ mass (Gehring et al. 2013). These studies also differ in their results regarding the role played by asthma, with asthmatics identified as a vulnerable subgroup in the first two studies (Oftedal et al. 2008; Schultz et al. 2012) but not the third (Gehring et al. 2013). Other studies that have reported associations among non-asthmatics (e.g. Gauderman et al. 2007; Gauderman et al. 2015) are also important as they suggest that air pollution may adversely affect lung function among otherwise healthy subjects. Additional studies with individual-level exposure assessments available at several time points are needed to better understand these findings.

Recently, the 15-year follow-ups of the "German Infant study on the influence of Nutrition Intervention plus environmental and genetic influences on allergy development" (GINIplus) and "influence of Life style factors on the development of the Immune System and Allergies in East and West Germany plus the influence of traffic emissions and genetics" (LISAplus) birth cohorts were completed. These large birth cohorts have long-term, detailed and frequent follow-ups, and environmental exposures have been assessed at several home addresses during each participant's lifetime. A previous analysis of air pollution effects on $FEV_{0.5}$ at six years in these cohorts (as part of a meta-analysis) yielded some negative but non-significant effect estimates (Gehring et al. 2013). There is a need to analyze the recently available lung function data to determine 1) whether the relative importance of early versus current TRAP exposure can be clarified, 2) whether TRAP-induced persistent long-term changes in lung development (up to 15 years of age) exist and 3) what role asthma may play in this relationship. Thus, the current study aimed to assess potential associations between early-life and current individual-level TRAP exposures and lung function at 15 years of age among birth cohort participants living in an urban and a rural area in Germany, and whether current asthma modified any observed associations.

2.0 MATERIALS AND METHODS

2.1 Study population

GINIplus is a German prospective birth cohort of 5991 children born at full-term and normal weight recruited between 1995 and 1998 in the cities of Munich and Wesel. Children with at least one atopic parent or sibling were allocated to an intervention study arm which investigated the effect of different hydrolyzed formulas consumed during the first four months of life on allergy development (N = 2252). All children whose parents did not give consent for the randomized clinical trial or who did not have a family history of allergic diseases were allocated to the observation study arm (N = 3739). Here, we used data from the entire GINIplus cohort, regardless of the two study arms. LISAplus is a population-based prospective birth cohort of 3094 children born at full-term and normal weight recruited between 1997 and 1999 in the same two areas as described above as well as in the German cities of Leipzig and Bad Honnef (original study size was 3097 but three participants withdrew their consent to participate). Detailed descriptions of the cohorts' recruitment and follow-up strategies have been published (Heinrich et al. 2002; von Berg et al. 2010; Zutavern et al. 2006). Both studies were approved by the local Ethics Committees and written consent was obtained from all parents of participants.

The present analysis is restricted to children living in the city of Munich and adjacent regions of Upper Bavaria and Swabia (a predominantly urban area; hereon referred to as GINI/LISA South) and in the city of Wesel and the adjacent regions of Münster and Düsseldorf (a predominantly rural area; hereon referred to as GINI/LISA North). As the GINIplus and LISAplus birth cohorts have very similar study designs, data were pooled and are presented for the complete study population and per geographical area (GINI/LISA South and GINI/LISA North) as separate air pollution land-use regression (LUR) models were developed for each area. The Leipzig and Bad Honnef study areas were excluded as the

159 required air pollution and lung function data were not available.

160

161 *2.2 Lung function measurements*

162 Measurements were performed in line with the ATS/ERS recommendations (Miller et al. 2005), before
163 and after bronchodilation with salbutamol. Post-bronchodilation measurements are considered as they
164 allow an examination of whether any observed associations with air pollutants may be reversible,
165 thereby indicating a functional mechanism (i.e. increased muscle tone of the airways). A detailed
166 description of the methodology and quality control steps is provided in the Supplementary Information.
167 For each participant, spirometric indices were taken from the test with the largest sum of the forced
168 expiratory volume in one second (FEV_1) and the forced vital capacity (FVC). The ratio of these two
169 measures was calculated (FEV_1/FVC), as was the peak expiratory flow (PEF) and forced expiratory
170 flow variables indicative of small airways and airway narrowing (forced expiratory flow rates at 25%
171 (FEF_{25}), 50% (FEF_{50}) and 75% (FEF_{75}) of exhaled FVC and mean flow rate between 25% and 75% of
172 FVC (FEF_{25-75})).

173

174 According to ATS/ERS standards, a bronchodilator response was considered positive when either FEV_1
175 and/or FVC changed by more than 12% and 200 ml (Pellegrino et al. 2005). During the lung function
176 testing, body weight and height were measured by trained research staff using calibrated equipment. To
177 allow international comparisons, standard deviation scores (z-scores) for FEV_1, FVC, FEV_1/FVC,
178 FEF_{75} and FEF_{25-75}, derived using reference equations for spirometry from the Global Lung Function
179 Initiative (www.lungfunction.org), were calculated (Quanjer et al. 2012). These z-scores are sex, age,
180 height and ethnicity specific. A z-score lower than -1.64 indicates lung function below the lower limit
181 of normal.

182

183 *2.3 Long-term air pollution exposure assessment*

184 Long-term (annual average) concentrations of NO_2, PM_{10} mass, $PM_{2.5}$ mass and $PM_{2.5}$ absorbance were
185 estimated to each participant's home address at birth, ten-years and at the time of lung function
186 measurement (15-years) using LUR models originally derived as part of the "European Study of
187 Cohorts for Air Pollution Effects" (www.escapeproject.eu; Beelen et al. 2013; Cyrys et al. 2012;
188 Eeftens et al. 2012a, 2012b). Briefly, between October 2008 and November 2009, three two-week air
189 pollution monitoring campaigns were performed at twenty (for PM_{10} mass, $PM_{2.5}$ mass and $PM_{2.5}$
190 absorbance) and forty (for NO_2) sites in both the GINI/LISA South and GINI/LISA North study areas.
191 Site-specific annual averages were calculated using the average of these three measurement periods and
192 then were adjusted for temporal variation using data from a centrally located background reference site
193 which operated continuously throughout the measurement year. Next, area-specific LUR models were
194 developed, which relate these site-specific measured annual average concentrations to predictor
195 variables derived from Geographic Information Systems through a supervised stepwise multivariable
196 linear regression (model explained variance (R^2) of the LUR models ranged from 0.78 for $PM_{2.5}$ mass
197 in GINI/LISA South to 0.97 for $PM_{2.5}$ absorbance in GINI/LISA North). The area-specific LUR models
198 were used to estimate exposures to the addresses of participants. Ozone estimates from the "Air
199 Pollution Modeling for Support to Policy on Health and Environmental Risk in Europe" project
200 (www.apmosphere.org) were also available. For this latter pollutant, a one square kilometer resolution
201 concentration map was developed across 15 European Union States using universal kriging modeling
202 and several European-wide datasets on monitored air pollution, land cover, altitude, transport networks,
203 meteorology and population (Beelen et al. 2009, $R^2 = 0.53$). Unlike for the other pollutants, the ozone
204 estimates were not derived using any specific traffic components and thus represent background air
205 pollution rather than TRAP. Data from this map were used to assign ozone concentrations to the birth-,
206 ten- and 15-year home addresses.

2.4 Short-term air pollution exposure assessment

Hourly concentrations of NO_2, PM_{10} mass, $PM_{2.5}$ mass and ozone were used to control for potential short-term air pollution exposure effects on lung function. As no data on short-term $PM_{2.5}$ absorbance concentrations were available, short-term $PM_{2.5}$ mass concentrations were used as a surrogate for this pollutant instead. For GINI/LISA South, these data were obtained from a background monitoring site located in a Munich suburban area (Johanneskirchen) that is approximately nine kilometers northeast of the centre of Munich. For GINI/LISA North, these data were obtained from one monitoring site located in the suburban area of Wesel-Feldmark, which is approximately two kilometers northeast of the centre of Wesel. For NO_2, PM_{10} mass and $PM_{2.5}$ mass, the average of the daily concentrations of the seven days prior to lung function testing was calculated. As lag effects of short-term air pollutants on lung function have been reported for up to five days (Ward and Ayres 2004), a seven day time frame was chosen to capture all potential lag effects, as was done previously in a large multi-centre study (Gehring et al. 2013). For ozone, the maximum eight hour average concentration between 0:00 and 24:00 the day before lung function testing was calculated, as recent high exposures to ozone may be most relevant. The influence of using the average calculated for the day of testing as well as for the three days before testing was examined in sensitivity analyses for this pollutant.

2.5 Statistical Analysis

Differences between the current study population and the original birth cohorts were assessed using the Chi-square and Student's t-test for categorical and continuous variables, respectively. Influential outcome, exposure and covariate combinations were examined using scatterplots. Pooled and area-specific cross-sectional associations between the air pollutants and lung function variables were analyzed using linear regression. The lung function variables and air pollutants were entered as continuous variables without transformation. Homoscedasticity of the residuals as well as whether they

were normally distributed was examined. Results are expressed as change in lung function variable per interquartile range increase of each long-term air pollutant (5.9 $\mu g/m^3$ for NO_2, 4.7 $\mu g/m^3$ for PM_{10} mass, 4.1 $\mu g/m^3$ for $PM_{2.5}$ mass, 0.40 10^{-5} $m^{-1}$for $PM_{2.5}$ absorbance and 5.8 $\mu g/m^3$ for ozone in the complete population for the 15-year addresses). Analyses were conducted using the statistical program R, version 3.0.1 (R Core Team 2012).

Crude models were adjusted for age, height and weight at the time of lung function testing and sex (pooled models also adjusted for study area). The main models were additionally adjusted for birth weight, breast feeding up to six months, parental atopy, maternal smoking during pregnancy, secondhand smoke exposure in the home during early life (one to four years), personal smoking at the age of 15 years (smoking cigarettes daily/several times per week/once per week/less than once a week versus never smoking), current asthma (defined according to GA^{2}LEN (Carlsen et al. 2006; Håland et al. 2006)) or a bronchodilator response during the lung function testing, parental education (defined using three categories based on the highest number of years of education of either parent; low < ten years, medium = ten years, high > ten years), cohort (GINIplus intervention group, GINIplus observational group, LISAplus) and the respective short-term air pollution exposures, as previously defined. Additional adjustments for secondhand smoke exposure in the home between six and 15 years (not included in main models as information is missing for 8.2% of participants), as well as use of natural gas for cooking, mold/ dampness in the home or the presence of furry pets in the home, all during the first year of life, daycare attendance during the first two years of life, older siblings and respiratory infections the week before testing were also considered.

Given a possible association between air pollution and asthma (Guarnieri and Balmes 2014; Tager et al. 2010) and that asthmatics have reduced lung function, asthma may be a potential mediator in the

relationship between air pollution exposure and lung function. Sensitivity analyses were thus conducted in which 1) current asthma (defined according to GA^{2}LEN (Carlsen et al. 2006; Håland et al. 2006)) was excluded as a covariate from the models 2) an interaction term between current asthma and the air pollution concentration was included and 3) associations were stratified by current asthma.

As previous studies have reported sex-specific differences in associations between air pollution exposure and lung function (Frye et al. 2003; Gauderman et al. 2007; Oftedal et al. 2008; Schultz et al. 2012), effect medication by sex was also assessed using an interaction term in the model between sex and the air pollution concentration and also by subsequently stratifying the models by sex. Further sensitivity analyses included stratification by whether the participant had moved since birth and exclusion of potential air pollutant outliers, defined as more than four standard deviations away from mean (six long-term NO_2 estimates). Finally, to examine potential non-linear effects, models were run after natural log (ln) transformation of the lung function variables and generalized additive models were considered.

3.0 RESULTS

3.1 Study population

In total, 2266 adolescents had available information on lung function pre-bronchodilation and air pollutants assessed to the 15-year home address (1274 and 992 in GINI/LISA South and North, respectively; flow chart of study population and geographical distribution of the study participants are provided in the Supplementary Information, Figure S1 and Figure S2, respectively). Characteristics for the complete and area-specific populations are presented in Table 1. Compared to the original cohorts, adolescents included in the current study were more likely to be female, to have been breastfed, to have participated in the intervention component of GINIplus and to have parents with atopy and high education. Furthermore, participants were less likely to have been exposed to tobacco smoke *in utero*, at home during the first four years of life and between six and 15 years of age.

3.2 Distribution of lung function variables

The mean and standard deviation of the lung function variables, measured before and after bronchodilation, in the complete and study area specific populations are presented in Table 2 (distributions of the standard deviation scores (z-scores; Quanjer et al. 2012) are provided in the Supplementary Information, Table S1). As expected, mean lung function variables were slightly higher after bronchodilation (except for FVC). The lung function variables tended to be greater in GINI/LISA South than GINI/LISA North. Mean lung function variables before and after bronchodilation were higher among boys, with the exception of FEV_1/FVC, which was higher among girls (not shown). However, the means of the z-scores for FEV_1 (before bronchodilation only), FVC and FEF_{25-75} were higher among girls. The means of the z-scores for the lung function variables before bronchodilation were slightly negative.

293 *3.3 Distribution of air pollutants*

294 Summary statistics of the distributions of the long-term air pollutants estimated to the 15-year home
295 addresses and the short-term air pollution estimates are provided in Table 3 for the complete and study
296 area specific populations. The long-term median NO_2, PM_{10} mass and $PM_{2.5}$ mass concentrations were
297 lower in GINI/LISA South (19.0, 20.4 and 13.2 $\mu g/m^3$, respectively) than GINI/LISA North (23.2, 25.2
298 and 17.3 $\mu g/m^3$, respectively). In contrast, median $PM_{2.5}$ absorbance and ozone concentrations were
299 higher in GINI/LISA South (1.6 10^{-5} m^{-1} and 45.0 $\mu g/m^3$, respectively) than GINI/LISA North (1.2 10^{-5}
300 m^{-1} and 38.8 $\mu g/m^3$, respectively). The short-term air pollutant concentrations differed slightly by area
301 with NO_2 and ozone concentrations being higher in GINI/LISA South and PM_{10} mass and $PM_{2.5}$ mass
302 concentrations being higher in GINI/LISA North.
303
304 Spearman correlations between all air pollutant estimates are provided per study area in the
305 Supplementary Information, Table S2. Long- and short-term air pollutant concentrations of NO_2, PM_{10}
306 mass, $PM_{2.5}$ mass and $PM_{2.5}$ absorbance (long-term only) tended to be positively correlated with each
307 other, and negatively correlated with ozone. Correlations were stronger in GINI/LISA North than
308 GINI/LISA South. The long-term and short-term air pollutants were not correlated with each other in
309 either study area. Long-term air pollutant concentrations were correlated across addresses (Spearman
310 correlations > 0.66 between air pollutants estimated to the birth- and 15-year home addresses).
311
312 *3.4 Associations with air pollutants*

313 All pooled and area-specific associations between the long-term air pollution concentrations assessed to
314 the 15-year home addresses and the lung function variables measured before bronchodilation were non-
315 significant, except for a positive association between FVC and $PM_{2.5}$ absorbance in GINI/LISA South
316 (beta = 34.89 ml, 95% confidence interval = [9.37; 60.41]; adjusted associations presented in Table 4).

317 Adjusted associations for the post-bronchodilation lung function variables and for the crude models
318 were similar (not shown). Associations remained null when the long-term air pollutants were assigned
319 to the birth- and ten-year home addresses of participants instead of their current 15-year address
320 (Supplementary Information, Table S3). Using the standard deviation scores (z-scores) for FEV_1, FVC,
321 FEV_1/FVC, FEF_{75}, and FEF_{25-75} as alternative outcomes yielded similar null findings (Supplementary
322 Information, Table S4), as did additional adjustments for secondhand smoke exposure in the home
323 between six and 15 years, and subsequently for use of natural gas for cooking, mold/ dampness in the
324 home or the presence of furry pets in the home, all during the first year of life, daycare attendance
325 during the first two years of life, older siblings and respiratory infections in the week before testing.
326
327 The short-term air pollutants were more consistently associated with the lung function variables. In the
328 complete population, nearly all effect estimates for the associations between short-term $PM_{2.5}$ mass,
329 PM_{10} mass and ozone concentrations and the lung function variables were negative and that between
330 FEV_1/FVC and short-term PM_{10} mass and $PM_{2.5}$ mass concentrations, as well as between FEF_{50} and
331 short-term ozone concentrations reached statistical significance (Supplementary Information, Table
332 S5). These associations were similar after bronchodilation although the association between FEF_{50} and
333 short-term ozone was no longer significant. When stratified by study area, effect estimates appeared
334 somewhat stronger in GINI/LISA North. Estimates for short-term $PM_{2.5}$ absorbance effects are not
335 presented as these data were not available. Results remained similar when short-term air pollution
336 estimates were defined as the average calculated for the day of testing as well as for the three days
337 before testing, instead of the average for the seven days before testing. Exclusion of short-term air
338 pollution exposures from the main models did not change the estimates for the long-term air pollution
339 exposures.
340

3.5 Intermediates, effect modification and sensitivity analyses

Excluding asthma status and birth weight as potential intermediates in the models did not change the null associations observed for the long-term air pollutants. Interaction terms between long-term air pollutants estimated to the 15-year address and asthma status were significant for NO_2 for all lung function variables except FVC and PEF, for PM_{10} mass, $PM_{2.5}$ mass and ozone for FEV_1 and for ozone for FVC (p-values < 0.05). Pooled associations stratified by asthma status revealed a tendency towards negative associations among asthmatics for most of the lung function parameters, especially for NO_2 (Figure 1, for example, -117.03 ml [-250.32, 16.25], -3.51% [-6.02, -1.01] and -297.35 ml/s [-592.63, -2.06] for the associations between NO_2 and FEV_1, FEV_1/FVC and FEF_{25-75} among asthmatics, respectively). Associations with NO_2 among asthmatics were not-attenuated post bronchodilation, were similar when the long-term NO_2 concentrations were estimated to the ten -year home addresses instead of the 15-year addresses, but were attenuated and no longer significant when long-term NO_2 was estimated to the birth addresses. Study area specific associations were not investigated due to the small number of asthmatics per study area (83 and 65 in GINI/LISA South and North, respectively). Possibly because of the negative correlation between NO_2 and ozone, effect estimates between ozone and the lung function variables tended to be positive among asthmatics in the complete population, and were not attenuated after bronchodilation (for example, for FEV_1 (119.3 ml [3.5, 235.1]) and FVC (100.3 ml [-7.1, 207.7] measured before bronchodilation). No associations were observed among non-asthmatics. Long-term air pollutant concentrations were not higher among asthmatics.

Several interactions between the air pollutants assessed to the 15-year home address and sex were also observed: with PM_{10} for FEV_1 and FEF_{75}, with $PM_{2.5}$ absorbance for FEV_1, FEF_{25-75}, FEF_{50} and FEF_{75}, and with ozone for FEV_1, FEV_1/FVC, FEF_{25-75}, FEF_{50} and FEF_{75}. The interactions suggested that PM_{10} mass had a negative (adverse) effect in males but a positive effect in females, whereas the opposite

trend was observed for $PM_{2.5}$ absorbance and ozone. No consistent trends in the associations between pollutants and the lung function variables could be observed between sexes in stratified analyses. One potential exception may be that for several lung function variables, effect estimates with $PM_{2.5}$ mass were above one among males but below one among females, although most associations did not reach statistical significance (Figure 1). The adolescents in our study were approximately 15 years of age at the time of lung function testing and nearly all (> 98%) had reached puberty. There was no difference in age or asthma prevalence between sexes.

Differences in associations were not observed between those who had and had not moved since birth. Natural log (ln) transformation of the lung function variables did not change the main results, nor did fitting generalized additive models or excluding potential air pollution outliers (six long-term NO_2 estimates).

4.0 DISCUSSION

In a cross-sectional analysis of 2266 children living in two areas in Germany, we did not find evidence to support an association between long-term TRAP exposure and reduced lung function at 15 years of age in the complete study population. As this null finding was also observed when the long-term air pollutants were assessed to the birth- and ten-year home addresses of participants, we were unable to identify a period of increased susceptibility during lung development in our study. However, we did find evidence of negative associations in children with asthma, with the strongest effects observed for NO_2 estimated to the current (15-year) home address. As these negative associations were observed for lung function measurements taken both before and after bronchodilation, the adverse effects of TRAP exposure on asthmatics may be partly related to structural changes and not solely to increased muscle tone of the airways.

Studies that have examined potential periods of increased susceptibility have reported associations with both early and lifetime exposures (Oftedal et al. 2008), only early but not current exposures (Schultz et al. 2012), and in contrast, only current but not early exposures (Gehring et al. 2013). The overall null findings for the complete population in this study (for both early-life and current exposures) add to the existing inconsistent evidence for a persistent effect of long-term TRAP on lung function development. However, it should be noted that participants in our study were up to ten years older than the participants of these previous studies (Gehring et al. 2013; Oftedal et al. 2008; Schultz et al. 2012). The process of lung development in adolescence and puberty follows a more complex pattern than in younger ages. Lung development follows a linear pattern with age and height in early adolescence, but becomes non-linear after the growth spurt, continuing until the end of puberty in males but being shorter in duration and almost finished following menarche in females (Nevé et al. 2002). This non-

401 linear growth relationship throughout adolescence may make it difficult to disentangle the effect of
402 TRAP exposure on lung development during this age period, although clear effects on lung function
403 growth between 10-18 years have been observed in the Children's Health Study (Gauderman et al.
404 2007). In the current analysis, we examined associations between the lung function variables and the
405 air pollutants assuming a linear relationship in the main models, the results of which were confirmed in
406 sensitivity analyses that examined potential non-linear effects.
407
408 Air pollution exposure might have a reversible effect on lung function leading to retardation in lung
409 development in younger, more vulnerable stages, but less so at older ages or after improvements in air
410 quality. In repeated surveys in East Germany, it was shown that deficits in lung function growth in
411 children were partly compensated after air quality improvements within a few years (Frye et al. 2003)
412 and a recent publication by the Children's Health Study demonstrated associations between
413 improvements in four-year growth in FEV_1 and FVC as levels of ambient air pollutants (NO_2, PM_{10}
414 mass and $PM_{2.5}$ mass) declined in five communities in California (Gauderman et al. 2015). Air quality
415 in our study areas has also improved and the traffic fleet has changed since the inception of the
416 GINIplus and LISAplus birth cohorts. Thus, any potential adverse effects of TRAP on lung function at
417 younger ages may have been compensated by 15 years of age. Indeed, effect estimates for these two
418 cohorts tended to be negative for the associations between $FEV_{0.5}$ at six years of age and long-term
419 exposure to air pollutants in a previously published meta-analysis (Gehring et al. 2013), whereas there
420 appears to be no indication of associations for any lung function variable at 15 years of age in the
421 current study.
422
423 We observed negative associations between long-term NO_2 exposure and reduced lung function among
424 asthmatics, which is in line with some previous studies (Oftedal et al. 2008; Schultz et al. 2012) but in

contrast to others which have found no difference (Gehring et al. 2013). Oxidative stress and inflammation have been hypothesized as the main mechanisms by which TRAP could adversely affect both lung function and asthma. It is thus unclear whether asthma may lie in the causal pathway between TRAP exposure and lung function, or vice versa, or whether asthma status may act as an effect modifier in this relationship. In this study of adolescents, we found no associations between long-term TRAP exposure and lung function among non-asthmatics, although others have observed associations among healthy subjects (Gauderman et al. 2007; Gauderman et al. 2015).

We found some evidence to suggest that sex may modify the association between long-term TRAP exposure and lung function, but it is unclear why this may be. The observed sex-specific associations in this study should be interpreted with caution given the heterogeneity of the effect estimates obtained in the analyses stratified by sex. Previous studies examining potential sex-differences are neither consistent nor conclusive; stronger associations with air pollution indicators have been reported for girls (Frye et al. 2003; Oftedal et al. 2008) and boys (Gauderman et al. 2007; Schultz et al. 2012), whereas others report no difference (Gehring et al. 2013).

Although the current study is not optimally designed to examine the effects of recent short-term air pollution exposure on acute changes in lung function (for example, compared to a panel design), nearly all effect estimates for the associations between the lung function variables and short-term $PM_{2.5}$ mass, PM_{10} mass and ozone concentrations were negative in the complete population. Short-term air pollution effects on acute changes in lung function are fairly well established, but the relative importance of short-term versus long-term effects is unclear and warrants further investigation. Stronger effects with short-term variations in air pollution could suggest short-term reversibility, whereas associations with long-term concentrations may cause structural changes, potentially leading to a lower lung function

449 plateau phase in young adults and an increased rate of lung function decline during adulthood. It is
450 important to note that in this study, the estimated long-term air pollutants (with the possible exception
451 of ozone) were markers of TRAP exposure whereas the short-term air pollutants were derived from
452 data from a single monitoring station in each study area and better represent changes in ambient
453 concentrations over time.
454
455 An important strength of this study is the availability of a full range of objectively measured and
456 visually inspected lung function variables indicative of airway inflammation or obstruction (FEV_1 and
457 FEV_1/FVC), lung volume and size (FVC), as well as more rarely investigated measures of small
458 airways and airway narrowing (FEF_{25}, FEF_{50} and FEF_{75}) and mean flow rate (FEF_{25-75}). Altered small-
459 airway function is considered an early pre-clinical marker of disease, and may be especially relevant
460 for TRAP as fine and ultrafine particles may be able to penetrate into the peripheral airways and ozone,
461 as an oxidative gas, can penetrate deep into the respiratory system. In our study, associations were
462 observed among asthmatics for some measures indicative of small airways and airway narrowing, as
463 well as of airway inflammation and obstruction. This study is rather unique in its ability to report on
464 associations with lung function variables taken both before and after bronchodilation, thus allowing an
465 examination of whether air pollution effects may be due to structural (irreversible) changes and not
466 solely to increased muscle tone of the airways (potentially reversible changes). The long-term effects
467 observed among asthmatics and the short-term effects observed in the complete population remained
468 after bronchodilation.
469
470 The standardized objective assessment of individual-level TRAP estimates to the home addresses of
471 participants at several ages, allowing potential periods of increased lung susceptibility to be examined,
472 is a further strength of this study. The long-term air pollution estimates for NO_2, $PM_{2.5}$ mass and $PM_{2.5}$

absorbance were derived from LUR models built using measurement data from 2008-2009. These models were used to predict air pollution concentrations to the home addresses at the time of birth (1995-1999) up to the age of 15 years (2010-2014) of participants under the implicit assumption that the spatial variability in the air pollutants would not have changed over this time period. Consequently, this likely means that our assessment of exposures at the birth address are more imprecise, although this approach has been used in several recent large birth cohort studies and LUR models for NO_2 have been shown to be temporally stable over 7-12 years by studies in Europe and Canada (Cesaroni et al. 2012; Eeftens et al. 2011; Wang et al. 2013). Nevertheless, as it is challenging to accurately capture within-city variability in air pollutants, model uncertainties, such as the choice of geocoding technique or resolution of land use data, may still affect exposure-health relationships (Jacquemin et al. 2013). The ozone estimates were derived from a database that represents background air pollution rather than TRAP, and it had a lower resolution (1 x 1km) than for the other pollutants. This, combined with the fact that the spatial distribution of ozone is more even than for the other pollutants, with the exception of areas very close to traffic, and that ozone concentrations are likely higher in rural areas compared to areas with greater traffic densities, likely made detecting true associations with this pollutant more difficult.

Unfortunately, information on air pollution exposures that occurred away from the home (at schools, during commutes, indoors) could not be assessed in this study, and these are likely to increase in importance as participants become older and spend more time away from the home. We were nonetheless able to adjust for self-reported tobacco smoking and secondhand smoke exposure in the home during early-life. The availability of ambient air pollutant concentrations on the day of and preceding the lung function measurements also allowed short-term effects of air pollution exposures to be considered. However, as these monitoring stations were located in different surroundings in each

study area (a suburban area nine kilometers from the centre of Munich city in GINI/LISA South and a suburban area two kilometers northeast from the centre of Wesel city), between-study comparisons of the associations with the short-term air pollutants should be done with caution.

Given this study's large sample size and the adequate ranges of modeled air pollutant levels in the complete data, which were similar to that used in previous studies (i.e. Gehring et al. 2013), the confidence intervals around the effect estimates were relatively narrow, with the potential exception of the analyses restricted to asthmatics, for which the sample size was more limited. The prospective collection of information reduced the potential for recall bias, although residual confounding can never be completely excluded. Although the air pollution exposures in this study were calculated for the participants without knowledge of potential confounders and outcomes, uncertainty in the exposures estimates is still possible and likely random, which would drive the results towards the null. Participation bias is always a concern in cohort studies with long follow-up periods. As children included in this study differed from the initial birth cohorts in several characteristics, the generalizability of our results, particularly to those of lower socioeconomic status, is limited. Furthermore, we are unable to examine a potential modifying effect by socioeconomic status as there are few children of true low socioeconomic status in our study. All children included in this study were Caucasian, further limiting the generalizability of the results. Potential gene-air pollution interactions were also not examined. Nevertheless, effect modification by asthma status and sex could be considered and the residential history data (at birth, ten and 15 years) allowed associations to be examined among those who had and had not moved between birth and 15 years of age.

5.0 CONCLUSIONS

This study did not identify associations between early-life or current long-term TRAP exposure and lung function variables at the age of 15 years in the complete study population. Among asthmatics, we found associations with current long-term air pollution exposures, especially NO_2, and lung function. We also observed associations with short-term air pollution concentrations in the complete study population, providing further support for the acute effects of air pollution on lung function.

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528 **7.0 REFERENCES**
529

Beelen R, Hoek G, Pebesma E, Vienneau D, de Hoogh K, Briggs DJ. 2009. Mapping of background air pollution at a fine spatial scale across the European Union. Sci. Total Environ. 407:1852–1867; doi:10.1016/j.scitotenv.2008.11.048.

Beelen R, Hoek G, Vienneau D, Eeftens M, Dimakopoulou K, Pedeli X, et al. 2013. Development of NO2 and NOx land use regression models for estimating air pollution exposure in 36 study areas in Europe – The ESCAPE project. Atmos. Environ. 72:10–23; doi:10.1016/j.atmosenv.2013.02.037.

Carlsen KCL, Håland G, Devulapalli CS, Munthe-Kaas M, Pettersen M, Granum B, et al. 2006. Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study*. Allergy 61:454–460; doi:10.1111/j.1398-9995.2005.00938.x.

Cesaroni G, Porta D, Badaloni C, Stafoggia M, Eeftens M, Meliefste K, et al. 2012. Nitrogen dioxide levels estimated from land use regression models several years apart and association with mortality in a large cohort study. Environ. Health 11:48–57; doi:10.1186/1476-069X-11-48.

Cyrys J, Eeftens M, Heinrich J, Ampe C, Armengaud A, Beelen R, et al. 2012. Variation of NO2 and NOx concentrations between and within 36 European study areas: results from the ESCAPE study. Atmos. Environ. 62:374–390; doi:10.1016/j.atmosenv.2012.07.080.

Eeftens M, Beelen R, de Hoogh K, Bellander T, Cesaroni G, Cirach M, et al. 2012a. Development of land use regression models for PM2.5, PM2.5 absorbance, PM10 and PM coarse in 20 European study areas; results of the ESCAPE project. Environ. Sci. Technol. 46:11195–11205; doi:10.1021/es301948k.

Eeftens M, Beelen R, Fischer P, Brunekreef B, Meliefste K, Hoek G. 2011. Stability of measured and modelled spatial contrasts in NO2 over time. Occup. Environ. Med. 68:765–770; doi:10.1136/oem.2010.061135.

Eeftens M, Tsai M-Y, Ampe C, Anwander B, Beelen R, Bellander T, et al. 2012b. Spatial variation of PM2.5, PM10, PM2.5 absorbance and PM coarse concentrations between and within 20 European study areas and the relationship with NO2 – results of the ESCAPE project. Atmos. Environ. 62:303–317; doi:10.1016/j.atmosenv.2012.08.038.

Frye C, Hoelscher B, Cyrys J, Wjst M, Wichmann H-E, Heinrich J. 2003. Association of lung function with declining ambient air pollution. Environ. Health Perspect. 111:383–387; doi:10.1289/ehp.5355.

Gauderman WJ, Vora H, McConnell R, Berhane K, Gilliland F, Thomas D, et al. 2007. Effect of exposure to traffic on lung development from 10 to 18 years of age: a cohort study. The Lancet 369:571–577; doi:10.1016/S0140-6736(07)60037-3.

Gauderman WJ, Urman R, Avol E, Berhane K, McConnell R, Rappaport E, et al. 2015. Association of improved air quality with lung development in children. N. Engl. J. Med. 372(10): 905-913; doi: 10.1056/NEJMoa1414123.

Gehring U, Gruzieva O, Agius RM, Beelen R, Custovic A, Cyrys J, et al. 2013. Air pollution exposure and lung function in children: the ESCAPE project. Environ. Health Perspect. 121:1357–1364; doi:10.1289/ehp.1306770.

Götschi T, Heinrich J, Sunyer J, Künzli N. 2008. Long-term effects of ambient air pollution on lung function: a review. Epidemiology 19:690–701; doi:10.1097/EDE.0b013e318181650f.

Guarnieri M, Balmes JR. 2014. Outdoor air pollution and asthma. The Lancet 383:1581–1592; doi:10.1016/S0140-6736(14)60617-6.

Håland G, Carlsen KCL, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M, et al. 2006. Reduced lung function at birth and the risk of asthma at 10 years of age. N. Engl. J. Med. 355:1682–1689; doi:10.1056/NEJMoa052885.

Heinrich J, Bolte G, Hölscher B, Douwes J, Lehmann I, Fahlbusch B, et al. 2002. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. Eur. Respir. J. 20:617–623; doi:10.1183/09031936.02.02322001.

Jacquemin B, Lepeule J, Boudier A, Arnould C, Benmerad M, Chappaz C, et al. 2013. Impact of geocoding methods on associations between long-term exposure to urban air pollution and lung function. Environ. Health Perspect. 121:1054–1060; doi:10.1289/ehp.1206016.

Künzli N, Tager IB. 2000. Long-term health effects of particulate and other ambient air pollution: research can progress faster if we want it to. Environ. Health Perspect. 108: 915–918.

Miller M, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. 2005. Standardisation of spirometry. Eur. Respir. J. 26:319–338; doi:10.1183/09031936.05.00034805.

Nève V, Girard F, Flahault A, Boulè M. 2002. Lung and thorax development during adolescence: relationship with pubertal status. Eur. Respir. J. 20;1292-1298; 10.1183/09031936.02.00208102.

Oftedal B, Brunekreef B, Nystad W, Madsen C, Walker S-E, Nafstad P. 2008. Residential outdoor air pollution and lung function in schoolchildren: Epidemiology 19:129–137; doi:10.1097/EDE.0b013e31815c0827.

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. 2005. Interpretative strategies for lung function tests. Eur. Respir. J. 26:948–968; doi:10.1183/09031936.05.00035205.

Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. 2012. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur. Respir. J. 40:1324–1343; doi:10.1183/09031936.00080312.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, www.R-project.org/.

Schultz ES, Gruzieva O, Bellander T, Bottai M, Hallberg J, Kull I, et al. 2012. Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study. Am. J. Respir. Crit. Care Med. 186:1286–1291; doi:10.1164/rccm.201206-1045OC.

Sin DD, Wu L, Man SFP. 2005. The relationship between reduced lung function and cardiovascular mortality: A population-based study and a systematic review of the literature. Chest 127:1952–1959; doi:10.1378/chest.127.6.1952.

Tager I, Demerjian K, Frampton M, et al. 2010. Traffic-related air pollution: a critical review of the literature on emissions, exposure, and health effects. No. 17. Health Effects Institute.

Von Berg A, Krämer U, Link E, Bollrath C, Heinrich J, Brockow I, et al. 2010. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course – the GINIplus study up to the age of 6 years. Clin. Exp. Allergy 40:627–636; doi:10.1111/j.1365-2222.2009.03444.x.

Wang R, Henderson SB, Sbihi H, Allen RW, Brauer M. 2013. Temporal stability of land use regression models for traffic-related air pollution. Atmos. Environ. 64:312–319; doi:10.1016/j.atmosenv.2012.09.056.

Ward DJ, Ayres JG. 2004. Particulate air pollution and panel studies in children: a systematic review. Occup Environ Med. 61:e13; doi:10.1136/oem.2003.007088.

Zutavern A, Brockow I, Schaaf B, Bolte G, von Berg A, Diez U, et al. 2006. Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. Pediatrics. 117;401-411. doi:10.1542/peds.2004-2521.

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CONFLICTS OF INTEREST

All co-authors have no conflicts of interest.

532 **Table 1:** Characteristics of the complete and study area specific populations

	Complete (N = 2266)	GINI/LISA South (N = 1274)	GINI/LISA North (N = 992)
	% (n/N)	% (n/N)	% (n/N)
Boys	48.8 (1106/2266)	49.3 (628/1274)	48.2 (478/992)
Birth weight[a] (g)	3460 (456.5)	3400 (426.7)	3530 (484.9)
Breast feeding (6 months)	57.8 (1256/2174)	65.2 (802/1230)	48.1 (454 / 944)
Older siblings	48.9 (1107/2262)	46.0 (586/1274)	52.7 (521/988)
Parental atopy	62.3 (1406/2254)	69.8 (885/1268)	52.8 (521/986)
Smoking			
Mother during pregnancy	12.8 (285/2235)	11.5 (144/1254)	14.4 (141/981)
In home (birth to 4 years)	38.0 (820/2157)	28.9 (353/1220)	49.8 (467/937)
In home (6 to 15 years)	31.3 (652/2081)	22.7 (269/1184)	42.7 (383/897)
Parental education			
< 10 years	6.3 (143/2261)	3.9 (49/1271)	9.5 (94/990)
= 10 years	27.0 (611/2261)	17.5 (223/1271)	39.2 (388/990)
> 10 years	66.7 (1507/2261)	78.6 (999/1271)	51.3 (508/990)
Study			
GINIplus observation	38.7 (876/2266)	30.3 (386/1274)	49.4 (490/992)
GINIplus intervention	38.4 (870/2266)	37.4 (476/1274)	39.7 (394/992)
LISAplus	22.9 (520/2266)	32.3 (412/1274)	10.9 (108/992)
Use of gas for cooking in 1st year of life	7.2 (163/2251)	9.4 (119/1270)	4.5 (44/981)
Mold/dampness in home in 1st year of life	28.3 (491/1732)	34.5 (296/859)	22.3 (195/873)
Furry pets in 1st year of life	15.4 (335/2174)	14.0 (171/1224)	17.3 (164/950)
Daycare care in first 2 years of life	9.2 (204/2210)	14.1 (176/1244)	2.9 (28/966)
Moved between birth and 15 years	57.8 (1306/2262)	64.8 (825/1274)	48.7 (481.988)
At 15-year physical examination			
Age (years)[a]	15.2 (0.3)	15.2 (0.3)	15.2 (0.3)
Weight (kg)[a]	60 (11.8)	59 (10.6)	62 (12.9)
Height (cm)[a]	171 (8.3)	170 (8.2)	172 (8.2)
Smoker at age 15 years	7.1 (152/2131)	8.9 (105/1186)	5.0 (47/945)
Positive bronchodilation[b]	3.8 (82/2146)	3.5 (43/1229)	4.3 (39/917)
Asthma	6.6 (148/2242)	6.6 (83/1260)	6.6 (65/982)
Asthma and/or positive bronchodilation[b]	10.3 (219/2127)	9.9 (120/1217)	10.9 (99/910)
Respiratory infections in past week	19.2 (436/2266)	20.0 (255/1274)	18.2 (181/992)

533

[a] Mean (standard deviation)
[b] Defined according to ATS/ERS standards (Pellegrino et al. 2005)

534 **Table 2:** Characteristics of spirometric lung function variables measured before and after
535 bronchodilation in the complete and study area specific populations [mean (standard deviation)].
536

	Complete	**GINI/LISA South**	**GINI/LISA North**
Before bronchodilation	(N = 2266)	(N = 1274)	(N = 992)
FEV_1 (ml)	3503 (621)	3525 (628)	3475 (611)
FVC (ml)	4063 (762)	4054 (760)	4075 (765)
FEV_1 / FVC (%)	86.6 (6.4)	87.4 (6.3)	85.7 (6.4)
PEF (ml/s)	7103 (1279)	7165 (1268)	7022 (1290)
FEF_{25} (ml/s)	6213 (1171)	6301 (1168)	6101 (1165)
FEF_{50} (ml/s)	4438 (1047)	4522 (1045)	4330 (1039)
FEF_{75} (ml/s)	2202 (713)	2272 (737)	2111 (670)
FEF_{25-75} (ml/s)	3899 (924)	3978 (936)	3799 (898)
After bronchodilation	(N = 2146)	(N = 1229)	(N = 917)
FEV_1 (ml)	3600 (635)	3618 (645)	3576 (622)
FVC (ml)	4053 (761)	4041 (760)	4070 (762)
FEV_1 / FVC (%)	89.2 (5.6)	89.9 (5.4)	88.3 (5.7)
PEF (ml/s)	7268 (1268)	7346 (1262)	7163 (1268)
FEF_{25} (ml/s)	6454 (1168)	6561 (1161)	6310 (1161)
FEF_{50} (ml/s)	4854 (1033)	4929 (1032)	4757 (1026)
FEF_{75} (ml/s)	2539 (786)	2614 (808)	2440 (744)
FEF_{25-75} (ml/s)	4304 (927)	4385 (937)	4195 (903)

537

538 **Table 3:** Distribution of long-term and short-term air pollutants in the complete and study area specific
539 populations.
540

	Long-term air pollutants			Short-term air pollutants		
	Min, Max	**Median**	**IQR**	**Min, Max**	**Median**	**IQR**
NO_2 (µg/m^3)						
Complete	11.46, 59.76	21.86	5.92	6.70, 62.94	18.47	10.58
GINI/LISA South	11.46, 58.04	19.04	6.78	8.06, 62.94	18.69	8.86
GINI/LISA North	19.66, 59.76	23.15	3.21	6.70, 53.67	17.67	13.86
PM_{10} mass (µg/m^3)						
Complete	14.80, 32.70	22.32	4.74	3.68, 67.33	16.67	11.48
GINI/LISA South	14.80, 32.04	20.42	2.97	3.68, 62.89	15.25	9.75
GINI/LISA North	23.88, 32.70	25.19	1.53	6.70, 67.33	19.00	12.52
$PM_{2.5}$ mass (µg/m^3)						
Complete	10.66, 21.38	14.28	4.08	2.70, 67.33	12.00	9.48
GINI/LISA South	10.66, 18.06	13.21	1.04	2.70, 56.58	11.18	6.78
GINI/LISA North	15.76, 21.38	17.25	0.86	4.70, 67.33	14.00	11.77
$PM_{2.5}$ absorbance (10^{-5} m^{-1})						
Complete	0.97, 3.07	1.48	0.40	na	na	na
GINI/LISA South	1.34, 2.98	1.57	0.14	na	na	na
GINI/LISA North	0.97, 3.07	1.16	0.22	na	na	na
Ozone (µg/m^3)						
Complete	31.82, 59.26	44.08	5.80	2.00, 171.00	69.94	42.00
GINI/LISA South	38.82, 59.26	44.95	4.83	2.00, 154.06	72.12	43.25
GINI/LISA North	31.82, 47.16	38.81	6.96	2.70, 171.00	68.00	36.00

541
542 na = not available.

Table 4: Pooled and study area specific estimated change in lung function variables before bronchodilation per corresponding interquartile range increase in long-term air pollution concentration assessed to the home address at 15 years of age*

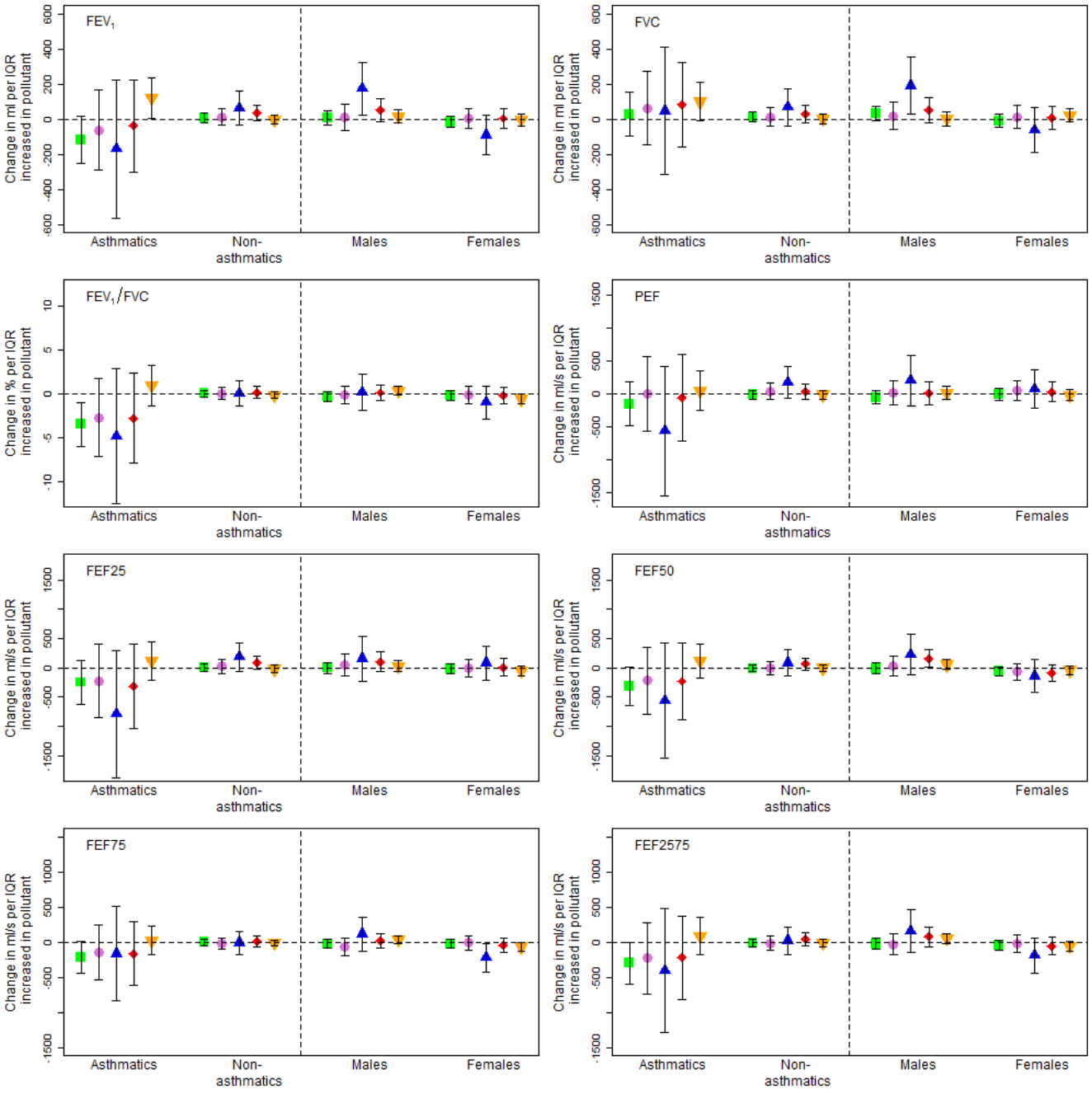
	Pooled	GINI/LISA South	GINI/LISA North
	Beta [95% CI]	**Beta [95% CI]**	**Beta [95% CI]**
NO_2			
FEV_1 (ml)	-3.14 [-29.22; 22.94]	-15.07 [-48.54; 18.40]	15.53 [-15.57; 46.63]
FVC (ml)	11.51 [-17.07; 40.09]	0.77 [-36.00; 37.54]	23.25 [-10.85; 57.35]
FEV_1 / FVC (%)	-0.30 [-0.68; 0.08]	-0.35 [-0.86; 0.16]	-0.11 [-0.55; 0.33]
PEF (ml/s)	-40.16 [-105.64; 25.32]	-76.46 [-159.26; 6.35]	38.26 [-42.17; 118.70]
FEF_{25} (ml/s)	-15.17 [-81.26; 50.91]	-42.41 [-126.93; 42.11]	45.62 [-34.65; 125.88]
FEF_{50} (ml/s)	-36.95 [-98.15; 24.25]	-59.48 [-138.50; 19.54]	19.27 [-53.86; 92.40]
FEF_{75} (ml/s)	-22.27 [-65.65; 21.11]	-31.56 [-90.69; 27.58]	-8.31 [-55.76; 39.13]
FEF_{25-75} (ml/s)	-34.61 [-88.87; 19.64]	-53.92 [-125.25; 17.40]	8.18 [-55.07; 71.44]
PM_{10} mass			
FEV_1 (ml)	4.66 [-42.69; 52.01]	3.23 [-29.65; 36.11]	-0.82 [-35.35; 33.72]
FVC (ml)	15.39 [-36.47; 67.26]	14.94 [-21.15; 51.03]	-8.75 [-46.69; 29.19]
FEV_1 / FVC (%)	-0.21 [-0.90; 0.49]	-0.21 [-0.71; 0.29]	0.10 [-0.39; 0.59]
PEF (ml/s)	21.65 [-97.48; 140.79]	-15.05 [-96.53; 66.44]	64.08 [-25.45; 153.61]
FEF_{25} (ml/s)	9.90 [-110.25; 130.05]	-20.34 [-103.39; 62.70]	60.30 [-28.97; 149.56]
FEF_{50} (ml/s)	-26.74 [-137.57; 84.09]	-26.56 [-104.27; 51.14]	19.46 [-61.13; 100.04]
FEF_{75} (ml/s)	-42.49 [-121.41; 36.43]	-30.37 [-88.47; 27.73]	-10.75 [-63.90; 42.40]
FEF_{25-75} (ml/s)	-33.59 [-131.99; 64.82]	-26.71 [-96.82; 43.40]	2.71 [-67.31; 72.74]
$PM_{2.5}$ mass			
FEV_1 (ml)	40.05 [-54.40; 134.50]	6.59 [-22.91; 36.10]	12.19 [-22.34; 46.73]
FVC (ml)	63.09 [-40.35; 166.53]	17.62 [-14.75; 49.99]	10.48 [-27.46; 48.42]
FEV_1 / FVC (%)	-0.48 [-1.86; 0.91]	-0.22 [-0.67; 0.23]	0.04 [-0.46; 0.53]
PEF (ml/s)	128.38 [-109.24; 366.0]	-5.56 [-78.68; 67.56]	74.00 [-15.51; 163.50]
FEF_{25} (ml/s)	114.88 [-124.76; 354.5]	-9.99 [-84.52; 64.54]	78.18 [-11.03; 167.39]
FEF_{50} (ml/s)	37.93 [-183.20; 259.06]	-7.51 [-77.25; 62.23]	30.46 [-50.16; 111.08]
FEF_{75} (ml/s)	-48.46 [-205.98; 109.05]	-20.02 [-72.16; 32.13]	-1.49 [-54.70; 51.73]
FEF_{25-75} (ml/s)	-19.87 [-216.24; 176.50]	-20.05 [-82.96; 42.87]	13.48 [-56.59; 83.55]
$PM_{2.5}$ absorbance			
FEV_1 (ml)	31.13 [-13.72; 75.97]	22.13 [-1.16; 45.42]	-2.51 [-34.71; 29.69]
FVC (ml)	33.81 [-15.31; 82.94]	**34.89 [9.37; 60.41]**	-15.48 [-50.83; 19.87]
FEV_1 / FVC (%)	-0.04 [-0.70; 0.62]	-0.21 [-0.56; 0.15]	0.20 [-0.26; 0.66]
PEF (ml/s)	12.59 [-100.30; 125.49]	-5.50 [-63.31; 52.31]	9.47 [-74.10; 93.05]

FEF_{25} (ml/s)	59.91 [-53.90; 173.73]	11.16 [-47.76; 70.09]	36.12 [-47.16; 119.40]
FEF_{50} (ml/s)	50.36 [-54.65; 155.36]	15.33 [-39.81; 70.46]	24.17 [-50.98; 99.32]
FEF_{75} (ml/s)	-8.78 [-83.61; 66.04]	-9.05 [-50.29; 32.19]	-5.26 [-54.86; 44.34]
FEF_{25-75} (ml/s)	19.63 [-73.63; 112.90]	4.21 [-45.55; 53.96]	6.94 [-58.38; 72.26]
Ozone			
FEV_1 (ml)	7.18 [-18.88; 33.25]	10.01 [-16.29; 36.31]	-9.76 [-64.78; 45.25]
FVC (ml)	10.86 [-17.65; 39.37]	17.76 [-11.11; 46.64]	-18.14 [-78.34; 42.06]
FEV_1 / FVC (%)	-0.10 [-0.48; 0.28]	-0.16 [-0.56; 0.24]	0.07 [-0.71; 0.84]
PEF (ml/s)	-6.80 [-72.13; 58.54]	13.10 [-51.97; 78.16]	-79.73 [-221.08; 61.63]
FEF_{25} (ml/s)	-2.66 [-68.55; 63.23]	5.25 [-61.17; 71.67]	-41.71 [-182.79; 99.37]
FEF_{50} (ml/s)	11.10 [-49.73; 71.92]	7.61 [-54.51; 69.74]	0.75 [-126.98; 128.47]
FEF_{75} (ml/s)	-9.18 [-52.53; 34.16]	-13.77 [-60.25; 32.71]	4.57 [-79.65; 88.79]
FEF_{25-75} (ml/s)	4.38 [-49.66; 58.42]	0.31 [-55.78; 56.40]	3.72 [-107.22; 114.66]

*Models are adjusted for age, height and weight at the time of lung function testing, sex, birth weight, breast feeding up to six months, parental atopy, maternal smoking during pregnancy, secondhand smoke exposure in the home during early life (up to four years), personal smoking at the age of 15 years, history of asthma or a bronchodilator response during the lung function testing, parental education, cohort, city (pooled models only) and short-term air pollution exposure.

Bold: p-value <0.05

554 **FIGURE LEGEND**
555



557
558 **Figure 1:** Estimated change in lung function variables before bronchodilation per corresponding
559 interquartile range increase in long-term air pollution concentrations assessed to the 15-year home
560 address (NO_2 = green squares, PM_{10} mass = purple circles, $PM_{2.5}$ mass = blue triangles, red diamonds =
561 $PM_{2.5}$ absorbance, orange inverse triangles = ozone) in the complete population stratified by asthma
562 status and sex. Models are adjusted for age, height and weight at the time of lung function testing, birth
563 weight, breast feeding up to six months, parental atopy, maternal smoking during pregnancy,
564 secondhand smoke exposure in the home during early life, personal smoking at age 15 years, parental

education, cohort, city and short-term air pollution exposure. Models stratified by asthma are also adjusted for sex, and those stratified by sex are also adjusted for asthma or a bronchodilator response during the lung function testing.