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Title: Determinants of change in airway reactivity over 11 years in a population study (SAPALDIA)

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Abstract

Study question:
We investigated determinants of change in bronchial reactivity in SAPALDIA, a population-based cohort with wide age range (29-72 years at follow-up).
Materials and Methods:
The role of sex, age, atopic status, smoking and BMI on percent change in bronchial reactivity slope from baseline value was analysed in 3005 participants with methacholine tests in 1991 and 2002 and complete covariate data. Slope was defined as percentage decline in FEV1 from its maximal value per µmol methacholine.
Results:
Bronchial hyper-reactivity prevalence fell from 14.3% to 12.5% during follow-up. Baseline age was non-linearly associated to change in reactivity slope: participants below age 50 years experienced a decline, those above an increase during follow-up. Atopy was not associated with change, but accentuated the age pattern ($p_{interaction}$=0.038). Smoking significantly increased slope by 21.2%, as did weight gain (2.7% increase per BMI unit). Compared to persistent smokers, quitters before baseline or during follow-up experienced a significant decrease in slope (-27.7% and -23.9%, respectively). Differing, but not statistically different age-relationships and effect sizes for smoking and BMI between sexes were found.
Conclusions:
Mean bronchial reactivity increases after age 50 years, possibly due to airway remodelling or ventilation perfusion disturbances related to cumulating lifetime exposures.

Abbreviations

BHR: bronchial hyper-reactivity, BMI: body mass index, COPD: Chronic obstructive pulmonary disease, ECRHS: European Community Respiratory Health Survey, ETS: environmental tobacco smoke, FEV1: forced expiratory flow in 1 second, FVC: forced vital capacity, FEF_{25-75}: forced expiratory flows between 25% and 75% of the FVC, L: litres, UK: United Kingdom,

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Adult, bronchial hyperreactivity, cohort studies, epidemiologic determinants, methacholine chlorine, population,

Introduction

Although elevated bronchial reactivity plays a major role in asthma[1], the determinants of its change over time are not extensively researched.

In cross-sectional studies, bronchial reactivity was inversely associated with airway size[2, 3] and positively with atopy[4, 5]. Smoking has not consistently been shown to increase reactivity[5-8] and a gender difference beyond airway size is debated[3, 8, 9].

Longitudinal change in bronchial reactivity in the general population has only been investigated by a few cohort studies. In the Normative Aging Study, bronchial reactivity at follow-up was positively correlated with baseline blood basophile counts[10]. New-onset bronchial hyper-reactivity (BHR) was associated with both, low and high baseline body mass index (BMI), and linearly with change in BMI[11]. In the European Community Respiratory Health Survey (ECRHS), baseline smokers had higher bronchial reactivity at follow-up[12] and an interaction between allergic sensitization and gender was found: persistent sensitisation was associated with a reactivity decrease in women but not men[12]. Baseline allergic rhinitis was associated with incident BHR and increased bronchial reactivity at follow-up[13]. Finally, a small study on volunteers from UK general practices showed seasonal patterns with higher reactivity during summer and winter months[14].

These findings suggest that change in bronchial reactivity might differ between sexes and is influenced by both, allergic conditions and inflammatory processes. Still, knowledge on longitudinal determinants is limited: bronchial reactivity is highly variable in repeated assessments[15], and the cited studies had either short follow-up[14, 11] or restricted age range[13] or sex[11]. There is thus a knowledge gap on longitudinal determinants in older populations including men and women. As major pulmonary diseases such as COPD and asthma increase either in prevalence[16] or severity[17] with age and exhibit important gender-differences[18, 19], investigating the time course of bronchial reactivity in aged populations is important.

The Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) with its population-based sample aged 19-72 years at follow-up, detailed health questionnaire data, allergy testing, standardized spirometry and methacholine testing, provides an opportunity to fill this gap.

We thus aimed to assess the longitudinal impact of sex, age, atopic status, smoking and BMI on change in bronchial reactivity over the whole age range of the SAPALDIA population.

Methods and materials

Study design and population

The SAPALDIA study methodology has been published previously[20]. Briefly, 6966 randomly selected, 18-60 year old adults from eight areas of Switzerland underwent health interview, spirometry and bronchial reactivity testing with methacholine in 1991. At follow-up in 2002, 3358 underwent the same assessments (figure 1). 3005 participants with complete covariate data and no asthma medication were available for multivariable analysis.
All participants gave written consent, and the study was approved by the Swiss Academy of Medical Sciences and local ethics committees.

Assessment procedures

Health questionnaire

Study participants underwent a health interview on respiratory symptoms, pre-existing pulmonary diseases, smoking behaviour, environmental tobacco smoke (ETS) exposure at home or work, medication use and socio-economic factors.
Never smokers had smoked less than 20 packs of cigarettes or 360g of tobacco during their lifetime[21]. Ex-smokers had quit smoking at least 30 days before the interview, and current smokers reported active smoking[21]. We defined six categories of longitudinal smoking behaviour: *persistent never smokers*, *ex-smokers* and *smokers* for those with unchanged exposure, *quitters* for baseline smokers becoming ex-smokers at follow-up, *up-takers* for baseline never smokers becoming current or ex-smokers at follow-up, and *intermittent smokers* for the rest.
Asthma was defined as an affirmative answer to both questions 'Have you ever had asthma?' and 'Was this confirmed by a doctor?'. Asthma medication was defined by current intake of inhalers, aerosols or tablets for asthma. Chronic cough was defined as cough during the day or in the morning for at least 3 months a year in the last 2 years. Wheezing was present if occurring unrelated to a cold in the 12 months prior to examination.

Spirometry

Following the ECRHS protocol[22], participants underwent three to eight forced expiratory lung function manoeuvres to achieve a minimum of two measurements complying with American Thoracic Society criteria[23].

Bronchial reactivity testing

After a starting inhalation of physiological saline solution, methacholine was administered by MEFAR® aerosol dosimeters using progressive 4-fold solutions of 0.39, 1.56, 6.25 and 25mg per millilitre. Starting from functional residual capacity, participants inhaled to the total lung capacity and held their breath for 4 seconds. Two forced expiratory manoeuvres were performed one and two minutes after inhalation, and the larger forced expiratory volume in 1 second (FEV1) measurement was recorded. If FEV1 fell more than 10% from post-saline level, intermediate, 2-fold concentrations were applied. No testing was done if FEV1 after saline inhalation fell below 90% of the maximum spirometry value. The test was stopped if FEV1 fell by 20% or more from post-saline measurement, or if a cumulative methacholine dose of 2mg was reached.
Bronchial reactivity was defined as dose-response slope, similarly to the method employed by O'Connor and co-workers[24]: percentage decline in FEV1 (relative to the maximal test value) divided by the cumulative methacholine dose in μmol. BHR was defined as FEV1-decline of 20% or more from post-saline measurement up to 2mg of methacholine.

Test exclusion criteria were myocardial infarction within the last 3 months, severe cardiac failure, beta-blocker medication (including eye-drops), epilepsy, pregnancy, lactation, FEV1/FVC ratio below 80% and FEV1 below 70% of the predicted value.

Allergic sensitization

In both examinations, atopic sensitization was assessed by Phadiatop® tests (Phadia Uppsala Sweden), radio-immune assays detecting serum IgE antibodies to common inhalatory antigens such as pollen, household dust mite, and animal epithelia[25]. Positive tests had activity levels >=0.35 kU/L (for any allergen) and defined atopy.

Statistical analysis

After adding a small constant (0.01) to each, bronchial reactivity slopes were naturally logarithmized to achieve a more symmetrical distribution[3].The difference between follow-up minus baseline logarithm was used as outcome measure and corresponds to the logarithmized follow-up over baseline slope ratio, as shown below:

$$\text{Change in bronchial reactivity slope} = \ln(\text{slope}_{\text{follow-up}} + 0.01) - \ln(\text{slope}_{\text{baseline}} + 0.01)$$
$$= \ln\left[(\text{slope}_{\text{follow-up}} + 0.01) \ / \ \ln(\text{slope}_{\text{baseline}} + 0.01) \right]$$

Exponentiation of regression estimates thus yields geometric means and confidence limits of slope ratios. For clearer interpretation, exponentiated coefficients were expressed as percent changes from baseline slope (e.g. an exponentiated coefficient of 1.03 as 3% increase).
To study the effect of selection processes at different stages, characteristics of participants with follow-up methacholine testing, complete covariate data and no asthma medication were compared to those assessed only at baseline, either solely by spirometry (n=2084) or including methacholine test (n=3127), using X^2-, Wilcoxon rank sum and Student's t-tests.
BHR prevalence and median reactivity slopes at both examinations were described.
Determinants of the logarithmized follow-up over baseline slope ratio were investigated by multivariable mixed linear models including sex, age, allergic sensitization, BMI, change in BMI, current and ex-smoking, exposure to passive smoke, adjusting for concurrent colds at either examination, sinus and cosinus terms modelling seasonal cyclicity, and random effects for study areas. Continuous covariates such as age, BMI and pack-years were also modelled using natural cubic splines specifying 5 knots positioned as recommended by Harrell[26].
Packyears smoked in smokers, ex-smokers or both were entered instead of smoking variables. Changes in smoking behaviour were assessed by replacing baseline smoking variables with categories *persistent never smokers* (n=1349), *ex-smokers* (n=606), *up-takers* (n=99) *quitters* (n=250) and *intermittent smokers* (n=100) in a model relating to *persistent smokers* (n=601) as reference. Effects of airway calibre and lung size were not of primary interest, but models were controlled for baseline FEV1, FVC and FEF_{25-75} (the latter pre-adjusted for sex and FEV1). . Participants reporting asthma medication at either examination were expected to influence the observed associations significantly and were thus excluded (n=56).
Robust standard errors were computed to account for heteroscedasticity in residuals. Two-sided significance levels were chosen at α=0.05 for main effects and α=0.1 for interactions.

Several sensitivity analyses were conducted: To assess to which extent effects of study covariates were mediated by lung function and its change, we applied different adjustments to the models: including change in FEV1, FVC and FEF_{25-75}, calculated as follow-up minus baseline value, to the baseline variables, replacing lung function variables by percent predicted values for FEV1 and FVC[27] and their corresponding change, and leaving out all lung function variables. We checked whether covariate coefficients and p-values were unaltered by the adjustments.

To study the impact of selection processes, we reran multivariable analyses while giving more weight to under-represented study participants. Weights consisted of the inverse probability of having methacholine testing at both examinations, as calculated by regressing participation on the same baseline covariates as in the multivariable analyses plus doctor diagnosed asthma and baseline BHR. Finally, regression analyses were rerun after excluding doctors-diagnosed asthma at either examination.

All statistical analyses were performed using STATA version 9.2 (StataCorp, College Station, Texas, USA) and SAS Software, Version 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Characteristics of the study populations

Briefly, 49.1% of our study participants with complete data on covariates and no asthma medication (n=3005) were female, 28.3% current smokers, 29.4% atopic, 12.9% hyper-reactive and 3.7% asthmatic (see online table O1). Subjects of this sample were significantly less smoking, asthmatic, hyper-reactive, had better lung function values and less wheezing and chronic cough than participants with only baseline spirometry or methacholine test. Main reasons for missing baseline methacholine tests were medical exclusions (n=918) and technical/performance problems (n=763), while at follow-up, missing was largely due to complete non-participation (n=1019) or questionnaire assessment only (n=1017). 481 follow-up participants met exclusion criteria for methacholine testing (figure 1). Expectedly, they fared worse regarding smoking, asthma prevalence, hyper-reactivity, lung function and respiratory symptoms than our multivariable analysis sample (data not shown).

Prevalence of BHR and bronchial reactivity slope at either examination

3358 participants with methacholine testing at both examinations but not necessarily complete data on covariates were analysed descriptively (online table O2). BHR prevalence fell significantly by -1.8% from 14.3 to 12.5% ($p_{McNemar}$=0.0054). Only 46.8% of the 479 BHR cases at baseline persisted to follow-up. 6.8% of 2879 previously normo-reactive participants became hyper-reactive (n=195).
The median bronchial reactivity slope fell from 1.00 to 0.93 percent FEV1 decline per µmol methacholine.

Determinants of change in bronchial reactivity slope

Analyses were based on 3005 participants with follow-up methacholine testing, complete covariate data and no asthma medication

Main effects of determinants

No significant association between sex and change in reactivity slope presented after controlling for baseline lung function (table 1). When modelled with spline-functions, age was non-linearly associated with the change in reactivity slope: subjects aged <50 years at baseline experienced a decline over the subsequent 11 years of follow-up (figure 2, part a). The decline was largest around 30 years, diminished continuously afterwards, and at 50 years, a change in direction occurred, with an observable increase thereafter. In participants aged 30 years and older, the reactivity slope increased significantly by 1.5% (95%-CI: 0.7-2.3) per year. BMI increase, but not its baseline value, was associated with an increase in reactivity slope by 2.7% (95%-CI: 0.3-5.2) per BMI-unit. Current baseline smokers experienced a 21.2% (95%-CI: 7.5-36.7) increase in reactivity slope compared to never-smokers, corresponding to a 3.6% (95%-CI: 1.6-5.6) increase per 5 pack-years. There were no associations between exposure to ETS or atopy at baseline and change in reactivity slope.

Interactions between determinants

Sex

Plotting covariate-adjusted age estimates using spline functions suggested different time courses of change in reactivity slope between men and women (figure 2, parts b and c). Smoking at baseline appeared to increase slope more in women than men (25.0% versus 16.7% respectively) while or BMI increase , the opposite was observed (5.4% increase in men versus 0.9% in women) (table 2). However, none of these gender-differences were statistically significant.

Atopy
Atopic sensitisation showed a significant interaction with age (table 3): The non-linear age relationship detected in the whole study sample was more pronounced in atopic, but weaker in non-atopic subjects. When modelling age with polynomial functions, a significant interaction between atopy and the quadratic age term was observed (p=0.038). This model was significantly better than assuming no interaction (p=0.027).
Smoking at baseline was associated with a large and significant increase of 39.1% (95%-CI 12.2-72.6) only in atopic persons. The interaction with atopy turned however only significant when analysing packyears ($p_{interaction}$=0.042).

S***moking***
The non-linear relationship between age and change in reactivity slope was most pronounced in non-smokers (figure 3), and appeared differently in ever-smokers ($p_{interaction}$=0.073). In current and ex-smokers, the turning-point to increasing slope occurred earlier, around age 40 years, and no linear increase in slope was observed thereafter. Effect estimates for the other covariates remained unaffected by smoking status.
Compared to persistent smokers, participants quitting smoking before the first examination or during follow-up experienced a 27.7% (95%-CI 15.9 to 37.9) and 23.9% (95%-CI 6.8 to 37.8) decline in bronchial reactivity slope respectively (table 4), which was comparable to persistent neversmokers (-24.6% decline, 95%-CI: 14.5 to 33.5).
BMI
No significant interactions were found for BMI at baseline or its change.

Sensitivity analyses

Effect estimates and strengths of associations for age, smoking, change in BMI and atopy were unaffected by the method of adjustment for change in lung function (online table O3). However, when using percent predicted values and corresponding change for FEV1 and FVC, female sex was significantly associated with a decrease in reactivity slope by 13.8% (95%-CI: 4.2 to 22.4). As the only lung function variable significantly associated with change in reactivity slope, decline in FEV1 percent predicted increased slope by 1.2% (95%-CI 0.3 to 2.1) for each percent decline.
Repeating regression analyses with weights for each observation inverse to the probability of participation yielded the same results.
Exclusion of participants with doctor diagnosed asthma resulted in similar smoking effect estimates for men and women, but no significant alterations of other estimates (data not shown).

Discussion

In our general population sample of 18-60 year old adults, we found a 1.8% decrease in BHR prevalence and 7% decrease in median reactivity slope over the course of 11 years. We observed a significant negative association of female sex with change in reactivity slope only after controlling for lung function using percent predicted values. These reflect the sex-specific deviation in lung function from an age- and height dependent expected value, and preclude the assumption of equal effects of a given absolute lung volume or change in both sexes. But they do not take account of smaller absolute airway sizes in women, which might importantly influence the concentration of the stimulus at the airway walls. In accordance with other studies[12], we have found no sex effect once baseline lung function values were controlled for. The observed sex-specific age-relationships of reactivity suggest different age-courses of bronchial reactivity between sexes, maybe due to a higher level of reactivity as a consequence of smaller airway size in women. Additionally, the apparent stronger smoking effect suggests higher susceptibility to environmental exposures in women. ..

Our findings relating to the non-linear association of change in bronchial reactivity slope with age are new and not described by previous longitudinal studies. These mostly focused on other determinants[11-13] and differed regarding age-distribution. An age-associated increase in bronchial reactivity has been described in cross-sectional studies[28]. At the time of our own cross-sectional analysis[3], , participants were 11 years younger and the switch occurring in older age was not detectable. Based on the limited literature on bronchial reactivity in older populations, we can only speculate about possible mechanisms Airway remodelling or ventilation/perfusion disturbances, which increase bronchial reactivity [29, 30],might be induced by cumulative, lifetime exposures to different noxious substances Further, loss of tissue elasticity in the ageing lung might cause a tendency for airway closure and air trapping with increased residual volume. It is likely that these mechanisms are not adequately captured in our models, even those including change in lung function.

In accordance with other studies[10, 12] , we did not observe a direct effect of atopy on change in reactivity slope. But atopy accentuated the relationship with age and enhanced smoking effects. We found a strong, positive relationship between smoking and change in reactivity slope, a finding inconsistently described[10, 12], possibly due to shorter follow-up times. Profiting from the large sample size of our study, we could also show that quitting smoking has a profound beneficial effect on change in reactivity. Power was however limited to assess effects of up-taking of smoking.

Finally, we observed that change in BMI but not its baseline value was positively associated with change in reactivity slope. These effects could be mediated by increased levels of subclinical inflammation from adipose tissue. Our study benefitted from a relatively large sample size, wide age distribution for both sexes, standardized spirometry and methacholine testing, and detailed health interviews. However, it also had limitations: The substantial loss-to-follow-up over 11 years caused by non- or partial participation, technical problems, health-related exclusions or refusal, and associated with smoking, lower lung function values and higher bronchial reactivity slope, anticipated bias. Our sensitivity analyses giving more weight to underrepresented groups within the study sample yielded the same results, though. Effect estimates for age, smoking, BMI increase and atopy also remained stable after exclusion of known asthmatics or adjustment for lung function in different ways. Loss to follow-up is thus unlikely to invalidate our findings, but due to the stringent selection processes taking place their generalizability is limited to relatively healthy participants with no or only mild to moderate asthmatic disease or mild lung function impairment. Our results thus mostly represent the natural course of bronchial reactivity in a general population sample of healthy adults.

In conclusion, in its natural course, bronchial reactivity slope tends to increase in middle to old aged persons after showing a favourable attenuation during young adulthood – a pattern more pronounced in atopics, but less evident in smokers. Airway remodelling, ventilation/ perfusion disturbances and airway closure associated with the ageing lung possibly underlie the increase in older age. Women might present a different age-course in airway reactivity due to their smaller lung and airway size or greater susceptibility to environmental exposures.

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Figure legends

Figure 1 Selection of study participants

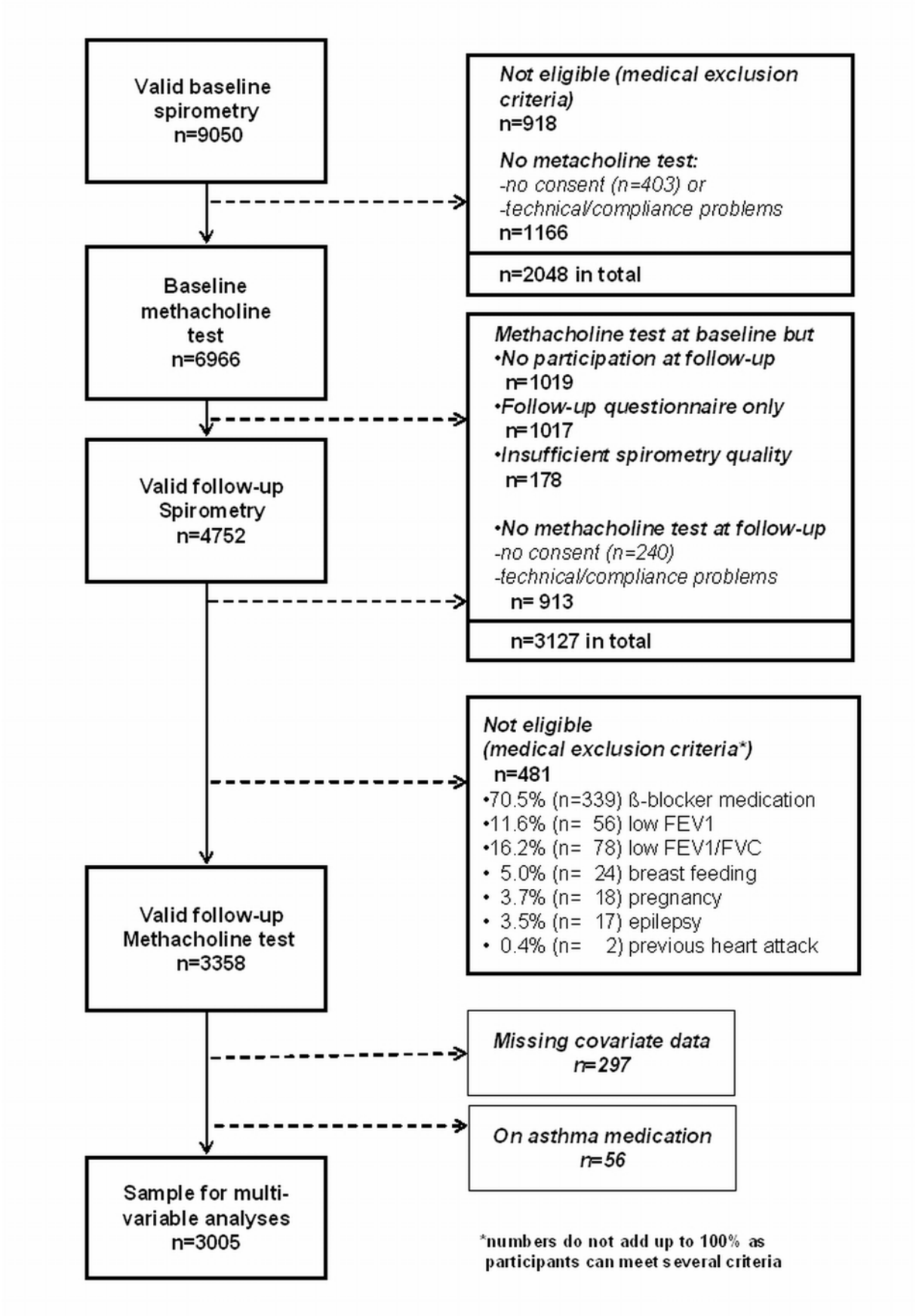
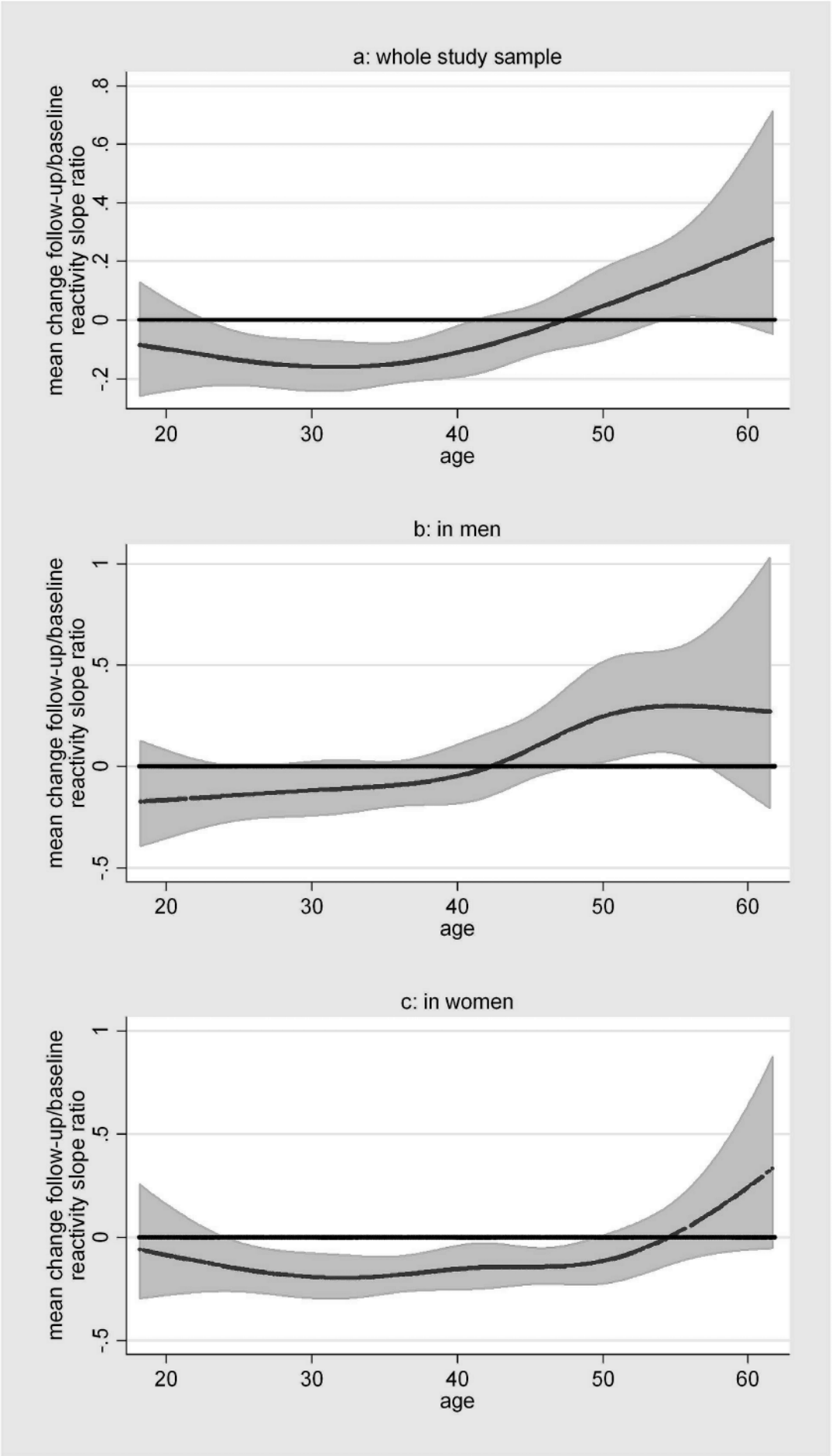
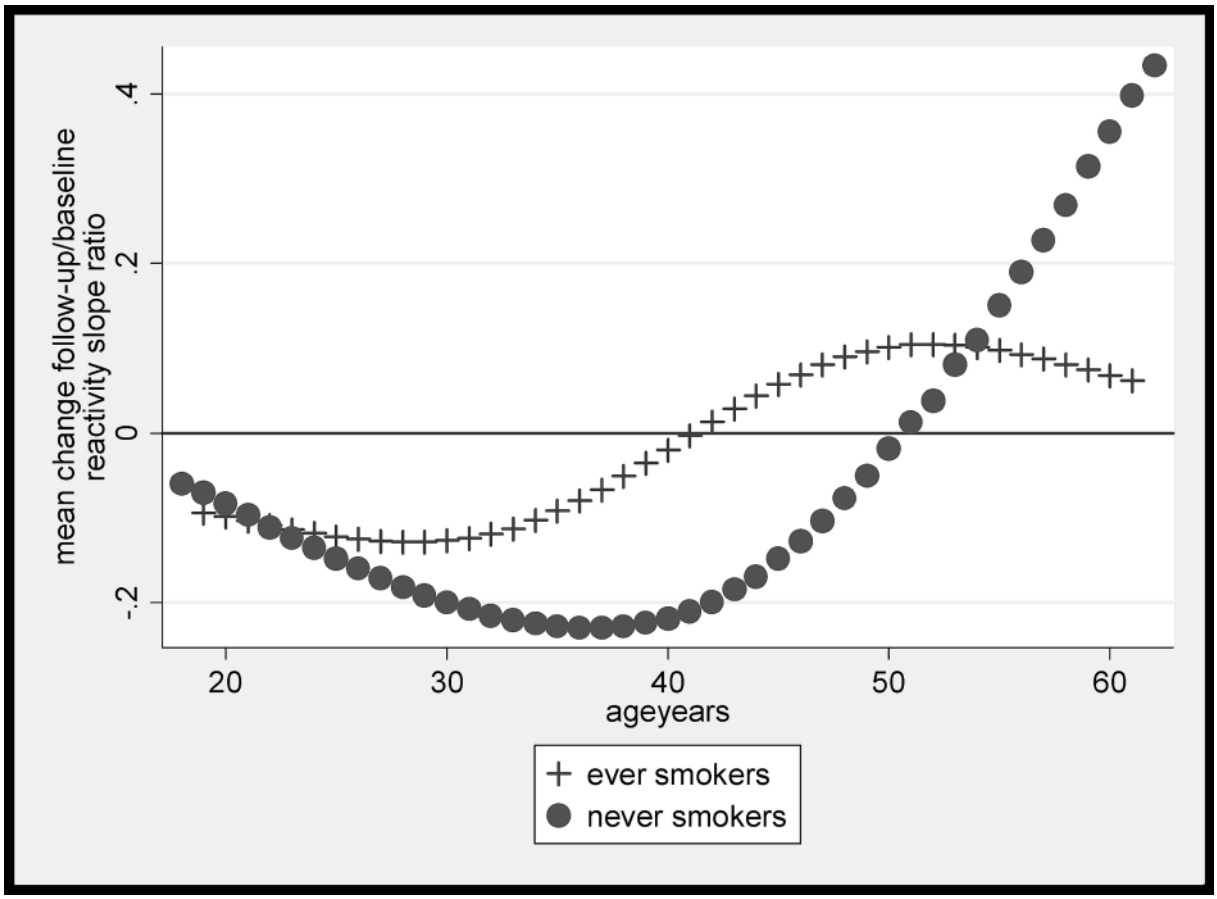


Figure 2: Mean change in bronchial reactivity slope* over 11 years of follow-up according to age at baseline in the whole study sample and stratified by sex


a: whole study sample
mean change follow-up/baseline reactivity slope ratio
-.2
0
.2
.4
.6
.8
20
30
40
50
60
age
b: in men
mean change follow-up/baseline reactivity slope ratio
-.5
0
.5
1
20
30
40
50
60
age
c: in women
mean change follow-up/baseline reactivity slope ratio
-.5
0
.5
1
20
30
40
50
60
age

* Bronchial reactivity slope is defined as % decline in FEV1 / µmol methacholine
Shaded areas represent 95% confidence intervals

Figure 3 Mean change in bronchial reactivity slope* over 11 years of follow-up according to baseline smoking status



* Bronchial reactivity slope is defined as % decline in FEV1 / µmol methacholine

Tables

Table 1 Determinants of change in bronchial reactivity slope in participants with complete covariate data and no asthma medication (n=3005)

Covariate		%-change in b. r. slope[1]	95%-CI	P-value
Female sex	***(yes/no)***	-3.9	-21.1 *to* 16.9	0.689
Age centered at 40 years	**(*per year)***	0.9	0.4 *to* 1.5	**0.001**
Age cent. at 40 years squared	***(per year squ)***	0.0	0.0 *to* 0.1	**0.024**
Age centered at 40 years, only linear term	**(per year)**	1.5	0.7 *to* 2.3	**<0.001**
Exposure to ETS neversmoker	***(yes/no)***	-1.0	-16.0 *to* 16.7	0.908
Exposure to ETS ever smoker	***(yes/no)***	10.7	-10.1 *to* 36.3	0.340
Positive Phadiatop Test at baseline	***(yes/no)***	-6.3	-15.8 *to* 4.2	0.230
BMI (centered at 25kg/m2)	***(per unit)***	-0.1	-1.6 *to* 1.4	0.847
Change in BMI between surveys	***(per unit)***	2.7	0.3 *to* 5.2	**0.030**
Being a smoker at baseline	***(yes/no)***	21.2	7.5 *to* 36.7	**0.002**
Being an ex-smoker at baseline	***(yes/no)***	-5.1	-18.2 *to* 10.1	0.487
Pack-years in ever smokers[2]	**(per 5 years)**	2.1	0.0 *to* 4.2	**0.048**
Pack-years in smokers[2]	**(per 5 years)**	3.6	1.6 *to* 5.6	**0.000**
Pack-years in ex-smokers[2]	**(per 5 years)**	-2.4	-6.5 *to* 1.9	0.267

[1] %-change in b.r. slope: percent change in bronchial reactivity slope from the baseline value. Bronchial reactivity slope is defined as percent change in FEV1 per µg methacholine. Estimates are expressed in percent change from baseline slope and adjusted for all other covariates in the table plus concurrent colds at both examinatins, seasonal terms and study area.

[2] Pack-years in ever smokers and in smokers/ex-smokers were entered into the model instead of the smoking variables.

Table 2 Determinants of change in bronchial reactivity slope in men and women separately

		Males (n=1530)			Females (n=1475)		
Covariate		**%-change in b.r. slope[1]**	**95%-CI**	**P-value**	**%-change in b.r. slope[1]**	**95%-CI**	**P-value**
Age centered at 40 years	***(per year)***	1.4	(0.5 *to* 2.3)	**0.003**	0.6	(-0.1 *to* 1.3)	**0.094**
Age cent. at 40 years squared	***(per year sq.)***	0.0	(0.0 *to* 0.1)	0.473	0.1	(0.0 *to* 0.1)	**0.022**
Exposure to ETS neversmoker	***(yes/no)***	-5.2	(-27.0*to*23.0)	0.686	2.3	(-16.5*to*25.3)	0.825
Exposure to ETS ever smoker	***(yes/no)***	11.5	(-17.7*to*51.0)	0.483	8.5	(-16.7*to*41.4)	0.545
Positive Phadiatop Test at baseline	***(yes/no)***	-9.9	(-23.3*to* 5.9)	0.207	-3.2	(-15.4*to*10.6)	0.629
BMI (centered at 25kg/m2)	***(per unit)***	-1.3	(-3.9 *to* 1.3)	0.316	0.9	(-0.9 *to* 2.8)	0.325
Change in BMI between surveys	***(per unit)***	5.4	(1.1 *to* 9.9)	**0.012**	0.9	(-1.9 *to* 3.8)	0.545
Being a smoker at baseline	***(yes/no)***	16.7	(-2.9 *to*40.2)	0.100	25.0	(6.6 *to*46.6)	**0.006**
Being an ex-smoker at baseline	***(yes/no)***	-8.6	(-28.1*to*16.2)	0.461	-4.0	(-19.2*to*14.0)	0.641

[1] %-change in b.r. slope: percent change in bronchial reactivity slope from the baseline value. Bronchial reactivity slope is defined as percent change in FEV1 per µg methacholine.. Estimates are expressed in percent change from baseline slope and adjusted for all other covariates in the table plus concurrent colds at both examinatins, seasonal terms and study area.

Table 3 Determinants of change in bronchial reactivity slope according to Phadiatop test result at baseline

		Phadiatop negative (n=2122)			Phadiatop positive (n=883)		
Covariate		**%-change in b.r. slope[1]**	**95%-CI**	**P-value**	**%-change in b.r. slope[1]**	**95%-CI**	**P-value**
Female sex	***(yes/no)***	-5.6	(-25.5 *to* 19.7)	0.634	0.2	(-29.3 *to* 42.1)	0.991
Age centered at 40 years	**(*per year)***	0.8	(0.1 *to* 1.5)	**0.023**	1.5	(0.3 *to* 2.8)	**0.012**
Age cent. at 40 years squared	***(per year squ)***	0.0*	(-0.0 *to* 0.1)	0.450	0.1*	(0.0 *to* 0.2)	**0.009**
Exposure to ETS neversmoker	***(yes/no)***	-1.5	(-18.7 *to* 19.5)	0.881	2.7	(-24.5 *to* 39.7)	0.866
Exposure to ETS ever smoker	***(yes/no)***	11.9	(-12.4 *to* 42.8)	0.368	4.7	(-30.7 *to* 58.0)	0.828
BMI (centered at 25kg/m2)	***(per unit)***	-0.2	(-1.9 *to* 1.6)	0.853	-0.5	(-3.3 *to* 2.4)	0.731
Difference in BMI between surveys	***(per one unit change)***	3.5	(0.6 *to* 6.5)	**0.020**	0.9	(-3.5 *to* 5.4)	0.702
Being a smoker at baseline	***(yes/no)***	13.9	(-1.6 *to* 31.8)	**0.081**	39.1	(12.2 *to* 72.6)	**0.003**
Being an ex-smoker at baseline	***(yes/no)***	-6.0	(-21.1 *to* 12.0)	0.486	-3.3	(-27.0 *to* 28.1)	0.815
Pack-years in ever smokers	**(per 5 years)**	0.8**	(-1.6 *to* 3.3)	0.502	6.5**	(3.0 *to* 10.2)	**0.000**

[1] %-change in b.r. slope: percent change in bronchial reactivity slope from the baseline value. Bronchial reactivity slope is defined as percent change in FEV1 per µg methacholine. Estimates are expressed in percent change from baseline slope and adjusted for all other covariates in the table plus concurrent colds at both examinatins, seasonal terms and study area.

* significant interaction with p=0.038

** significant interaction with p=0.044

Table 4 Effects of change in smoking behaviour during 11-years of follow-up on change in bronchial reactivity slope

Category of change in smoking behaviour		**%-change in b.r. slope[1]**	95%-confidence limits	P-value
Persistent smokers	(n= 601)	ref.		
Persistent ex-smokers	(n= 606)	-27.7	-37.9 to -15.9	<0.001
Quitters	(n= 250)	-23.9	-37.8 to -6.8	0.008
Up-takers	(n= 99)	-17.3	-37.6 to 9.4	0.202
Intermittent smokers	(n= 100)	-12.8	-30.0 to 8.8	0.156
Persistent never smokers	(n=1349)	-24.6	-33.5 to -14.5	<0.001

ref.: reference category

[1] %-change in b.r. slope: percent change in bronchial reactivity slope from the baseline value. Bronchial reactivity slope is defined as percent change in FEV1 per µg methacholine. Estimates are from a mixed linear regression model with random effects for area and adjusting for sex, age, age squared, bmi, change in bmi, atopy, FEV1, FEF25-75, FVC, occurrence of colds and seasonal terms