Title

Changes in IgE sensitisation and total IgE over 20 years of follow-up

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ABSTRACT

Background: Cross-sectional studies have reported a lower prevalence of sensitisation in older adults, but few longitudinal studies have examined whether this is an aging or a year-of-birth cohort effect.

Objective: To assess changes in sensitisation and total IgE in a cohort of European adults as they aged over a 20-year period.

Methods: Serum specific IgE to common aeroallergens (house dust mite, cat, grass) and total IgE were measured in 3206 adults, from 25 centres in the European Community Respiratory Health Survey, on three occasions over 20 years. Changes in sensitisation and total IgE were analysed by regression analysis, corrected for potential differences in laboratory equipment, and using inverse sampling-probability weights to account for non-response.

Results: Over the 20-year follow-up, the prevalence of sensitisation to at least one of the three allergens fell from 29.4% to 24.8% (-4.6%, 95%CI: -7.0% to -2.1%). The prevalence of sensitisation to house dust mite (-4.3%, 95%CI: -6.0% to -2.6%) and cat (-2.1%, 95%CI: -3.6% to -0.7%) fell more than sensitisation to grass (-0.6%, 95%CI: -2.5% to 1.3%). Age-specific prevalence of sensitisation to house dust mite and cat did not differ between year-of-birth cohorts, but sensitisation to grass was most prevalent in the most recent ones. Overall, total IgE fell significantly (geometric mean ratio: 0.63, 95%CI 0.58 to 0.68), at all ages, in all year-of-birth cohorts.

Conclusion:

Aging was associated with lower levels of sensitisation, especially to house dust mite and cat, after the age of 20.

Key messages

- Allergen-specific and total IgE decline after the age of 20 as people become older.
- Kinetics of IgE sensitisation decline differ for different allergens and may be faster after 40 years of age.
- The biological mechanism and environmental determinants for IgE sensitisation decline with aging in adulthood need to be explored so that we can improve our understanding of the aetiology of atopy and atopic diseases.

Capsule summary

After following a large multinational population-based cohort over 20 years, we show that the lower prevalence of IgE sensitisation in older adults is explained by aging.

Key words

Allergens; sensitisation; cohort study; epidemiology; immunoglobulin E; longitudinal analysis; aging; immunosenescence

Population-based cross-sectional studies have shown that the prevalence of sensitisation is higher in younger than in older age groups (1-4). Although there have been year-of-birth cohort-related increases in atopy over the last decades, it is hypothesised that these cross-sectional observations may, in addition, reflect decreases in sensitisation with aging-related immunosenescence. Longitudinal studies that have performed skin prick tests or measured serum allergen specific IgE, at baseline and follow-up over periods of up to 14 years, have reported that sensitisation increased with aging, although changes were less evident in middle-aged and older adults (2, 5-7). Two recent longitudinal studies reported no change or a slight decline in sensitisation with aging (4, 8). In one of these studies, changes in sensitisation were based on allergen specific IgE measures (8), while in the other the comparison between time points was based on both specific IgE and skin prick tests (4). Within the European Community Respiratory Health Survey (ECRHS) (9), a multicentre cohort study of over 6000 young and middle aged adults followed for a 10-year period, there was little evidence of substantial change in sensitisation to at least one of cat, grass or house dust mite (as measured by serum specific IgE) over time as the cohort aged. The age-specific prevalence of sensitisation to grass, but not to the other allergens measured, was higher in more recent year-of-birth cohorts. At the time, it was observed that changes in laboratory methods between the baseline and follow-up could influence assessment of change in sensitisation – such biases are even more difficult to quantify when using skin prick tests. Completion of the third phase of the ECRHS has allowed assessment of serum specific IgE on three occasions: at baseline, ten-year and twenty-year follow-up. The aims of this report were to: 1) to assess the changes in IgE sensitisation and in total IgE in this population-based cohort of European adults over a period of 20 years; and 2) to investigate whether these changes were different between year-of-birth cohorts.

METHODS

Study participants

This is a multicentre population-based cohort study. Detailed descriptions of the methods for ECRHS I and ECRHS II have been published elsewhere (10, 11). In ECRHS I, 1500 men and 1500 women age 20 to 44 years were randomly recruited from community-based sampling frames in each centre. After completing a short postal screening questionnaire, a random sample of responders was selected to complete an interviewer-led questionnaire and provided a blood sample (1991-1993). In the majority of centres, an additional sample of people with symptoms highly suggestive of asthma were recruited for study, but these participants are not included in the present analysis.

In ECRHS II (1998-2002), participants who had completed the extended questionnaire in ECRHS I were re-investigated, and again provided a blood sample. In ECRHS III, those who took part in the clinical stages of ECRHS I and II were again contacted, with responders invited to a local testing centre where, once more, blood samples were taken (2008-2013).

Eleven countries are represented in this report: Iceland (Reykjavik), Norway (Bergen), Sweden (Gothenburg, Umeå, and Uppsala), Estonia (Tartu), Belgium (Antwerp South, and Antwerp City), Germany (Hamburg, and Erfurt), UK (Ipswich, and Norwich), France (Bordeaux, Grenoble, Montpelier, and Paris), Spain (Barcelona, Galdakao, Albacete, Oviedo, and Huelva), Italy (Pavia, Turin, and Verona), and Australia (Melbourne).

Ethical approval for the study from local research ethics committees and written consent from participants were obtained.

Measurement of IgE

In all three surveys, blood samples were obtained and processed under similar conditions. After clotting and centrifuging, serum was stored at -20 °C until analysis in a single central

laboratory (Pharmacia Uppsala in 1992, Kings College London in 2002, and AMC Amsterdam in 2013/2014) using the Phadia ImmunoCAP system (now Thermo Fisher Scientific, Uppsala, Sweden).

To assess the effects of potential laboratory bias on prevalence of IgE sensitisation and mean of total IgE estimates, we conducted duplicate assays on 794 samples (tested at ECRHS I, stored, and tested at ECRHS II) and 475 samples (tested at ECRHS II, stored, and tested at ECRHS III) (see Table E1 in the Online Repository). The methods for this correction are described in detail in the Online Repository.

Outcomes

Participants were considered to be sensitised if allergen specific IgE to *Dermatophagoides pteronyssinus* (house dust mite), *Felis silvestris catus* (cat), and *Phleum pratense* (Timothy grass) was present in concentrations >0.35 kU_A/L. A higher threshold (>0.70 kU_A/L) was also considered. 'Atopy' was defined as being sensitised to one of either house dust mite, grass or cat. Total IgE, expressed in kilounits/litre (kU/L), was log-transformed and considered as a continuous outcome for estimation of geometric means and their ratios.

Statistical methods

Statistical analyses were performed using Stata V.13 (StataCorp LP, College Station, TX). Analyses were restricted to the 3206 participants with information on serum specific IgE and total IgE in all three ECRHS surveys (Figure 1). Inverse sampling-probability weights were used to standardise the estimation from this population with data on IgE assays from all three ECRHS surveys to the original target population of participants with data on IgE assays from ECRHS I (see Online Repository for details on the inverse sampling-probability weighted estimation).

The prevalence of sensitisation at each survey was determined using logistic regression with Huber variances considering participants as the clusters. Confidence intervals for prevalences, and their differences (net change) between ECRHS II and I, ECRHS III and II, and ECRHS III and I were estimated using the normalising hyperbolic-arctangent transformation (12). Similarly, using linear regression, we calculated geometric mean (GM) ratios of total IgE between ECRHS II and I, ECRHS III and II, and ECRHS III and I.

Statistical analyses for each outcome were performed in two ways, using uncorrected models and models corrected for potential laboratory bias. Only results of the corrected models are presented in this report. As data came from multiple centres, we tested for between-centre heterogeneity in the uncorrected results using the methods of Cochran (14).

In a final step, analyses were repeated: 1) stratified by gender; 2) restricted to lifetime non-smokers; and c) by year-of-birth cohort. For this latter step, year-of-birth cohorts were defined by date of birth (1964-1973, 1954-1963, 1944-1953). The ages of these participants at 1 January 1992 (the approximate midpoint of ECRHS I data collection) would have been $18 \leq age < 28$, $28 \leq age < 38$ and $38 \leq age \leq 48$ years, respectively (participants from Tartu, Estonia, were recruited aged 20-44 in 1994 and would have been less than 20 years on 1 January 1992, hence 18 years is the lower age limit). Members of each age cohort would have been 10 years older on 1 January 2002 (during the ECRHS II data collection) and 20 years older on 1 January 2012 (during the ECRHS III data collection). This approach allowed comparison of earlier cohorts with later cohorts at approximately the same ages.

RESULTS

A total of 3206 (30.6%) of the 10,478 participants who provided a blood sample in the first survey took part and again provided a sample in both ECRHS II and III. The median age of participants at ECRHS I was 34.9 years (interquartile range: 28.6-40.5), half were males, and forty five percent were lifetime non-smokers. There was variation between centres in the proportion of participants who provided samples at ECRHS I and then went on to provide samples at ECRHS II and ECRHS III (minimum: 13.6% in Pavia; maximum: 58.6% in Reykjavik). Factors associated with response were older age, and being a non-smoker. Response was not associated with sensitisation at baseline, gender, and reporting of wheeze (see Table E2 in the Online Repository), although those who took part in all three surveys did report waking with breathlessness less frequently.

Net change in IgE sensitisation and total IgE

Laboratory-corrected net changes in prevalence of IgE sensitisation to each of the allergens and in geometric mean of total IgE over a period of 20 years are shown in table 1. Between ECRHS I and ECRHS II there was no significant change in the prevalence of IgE sensitisation to any of the allergens using either the low or the high cut-off levels.

Over the 20 years of follow up, i.e. between ECRHS I and ECRHS III, prevalence of IgE sensitisation to house dust mite, cat, and to at least one allergen fell. Using the 0.35 kU_A/L cut-off, the prevalence of sensitisation to grass remained stable, but when the 0.70 kU_A/L cut-off was used there was evidence of a reduction in sensitisation. These changes were similar in men and women (see Table E3 in the Online Repository).

For some estimates there was evidence of heterogeneity between countries, but no clear pattern in this variation was observed by latitude (figure 2), response rate (see Figure E1 in

the Online Repository) or prevalence of sensitisation at baseline (see Figure E2 in the Online Repository).

Overall there was a significant fall in total IgE over the 20 years of follow up (geometric mean ratio: 0.63, 95% CI 0.58 to 0.68). This generalised fall in total IgE occurred in all centres, although the magnitude of the change varied (heterogeneity between centres $P < 0.001$; see Figure E3 in the Online Repository). Patterns were similar in men and women (see Table E3 in the Online Repository).

Restriction of analyses to the 1304 participants who were lifetime non-smokers did not materially alter the results reported above (see Table E4 in the Online Repository).

Association of net change with age and cohort

In ECRHS I, the prevalence of IgE sensitisation to house dust mite, grass, cat, and to at least one allergen was higher in younger adults (i.e. those born more recently) than in older adults (table 2).

Over the 20-year period, the prevalence of sensitisation to house dust mite fell in all age groups to a similar extent, and there was little evidence that the age-specific prevalence of sensitisation to house dust mite was different between those born more recently and those born earlier (figure 3A). Overall the picture was one of a decrease in sensitisation with age, with decreases occurring throughout adult life. This was broadly similar for sensitisation to cat (figure 3C). However, these patterns were different for sensitisation to grass. Although there was evidence of a fall in sensitisation to grass in those who were the oldest at recruitment (i.e. the earlier cohort), falls were not seen in those who were born more recently. As a result, there were marked differences in the age-specific prevalence of sensitisation to grass between cohorts with higher age-specific prevalence in those born after 1964 (figure 3B). The prevalence of IgE sensitisation to at least one of house dust mite, grass and cat

showed a pattern similar to that of sensitisation to house dust mite and cat. The most recent cohort had the highest prevalence at younger ages, but these cohort-related differences were not apparent in later adult life (figure 3D). Similar patterns were observed when using the cut-off of 0.70 kU_A/L (see Table E5 in the Online Repository).

The population GM of total IgE was lower at each follow up, in all cohorts over the 20-year period of follow up, and the more recent cohorts had lower levels of total IgE than those born earlier at the equivalent ages (figure 4, table 2).

DISCUSSION

We have shown that the prevalence of sensitisation to at least one of house dust mite, cat or grass has decreased within a large population-based adult cohort followed over a period of 20 years. There was a decrease in the prevalence of sensitisation to house dust mite, and to cat, and the geometric mean total IgE levels also decreased. Sensitisation to grass did not follow these patterns so clearly, showing instead, an increase in younger ages and aging effects only at older ages.

Strengths of this study are the population-based nature of the sample derived from several parts of Europe and Australia, the prolonged period of follow-up and the standardised handling and testing of samples between centres and over time. Changes in laboratory staff, consumables and methods between surveys could lead to bias in prevalence estimates and to address this we have used information from duplicate assays of hundreds of samples to adjust our estimates. As with all cohorts, there has been attrition during the 20-year period of follow-up and the analyses we present are based on participants who have taken part in all three phases of the study. We are aware that considerable loss to follow up has the potential to induce bias, therefore to account for small differences between these individuals and the initial cohort at baseline and to enhance the external validity of our results, we have corrected our models with inverse sampling-probability weights. This method generates estimates that apply to the population we sampled at baseline. We are unable to say whether the start of the age-related decrease in sensitisation occurs around the age of 20 or earlier because the ECRHS is a cohort of adults only

To date, few other population-based studies have reported on longitudinal changes in sensitisation by measuring serum specific IgE levels (6, 8). These earlier reports, both in Denmark, are on smaller samples and mostly over shorter time periods. Linneberg et al. studied changes over an 8-year period in serum specific IgE to at least one of six allergens in

about 400 adolescents and adults in Copenhagen (6), reporting an increase in prevalence of IgE sensitisation, especially among those born in the 1960s or later. Older adults (above 40 years, n = 695) living in the same city and followed for 20 years showed no change in sensitisation over a 20-year period in prevalence of IgE sensitisation to at least one of 19 allergens (8). Other studies looked at changes in sensitisation by performing skin prick tests and reported increases with aging (2, 4, 5). However, skin prick tests are much more difficult to standardise over different periods as they are prone to fieldworker variation, with changes in skin prick test reagents being difficult to assess (15, 16).

Barbee et al. studied 1100 participants in the US and reported a decrease in levels of total IgE with age in children and young adults, but not in older adults (17). In the ECRHS, total IgE levels fell with aging within each cohort, with more recent cohorts having lower levels of total IgE than earlier ones at the same age. In a previous report, we showed that smoking associated differently with sensitisation to different aeroallergens, and in a dose-response manner with total IgE levels (18). Therefore, we hypothesized that changes in sensitisation over time could be related to declining smoking rates and that lifetime non-smokers would not show changes in sensitisation. Our present findings show that a decline in sensitisation is unlikely to be related to smoking cessation. The fall in total IgE in our study may in part be explained by a decline in helminthic infestation as observed by others in children (19).

We saw no evidence of change in the prevalence of IgE sensitisation to house dust mite, cat, grass, and at least one of these three as the cohort aged over the initial 10 years of follow-up of the ECRHS (9). This observation is confirmed within this second report, but we go on to show that prevalence does decrease over 20 years, and appears greater when people are aged about 40 or older. This finding may be explained by immunosenescence, which seems to be more evident after 50 years of age (20) and corresponds to age-related changes in the number and function of cells from the immune system (21). The production of IgE, which is

386 dependent on an interaction between B cells and T cells (22), may decline as a consequence
387 of the naturally occurring involution of the thymus (23) – the thymic output of T cells per day
388 in a 50-year old is about 33% lower than that of a 25-year old (23). Our findings are
389 supported by animal studies, which suggest that the production of IgE to an allergen
390 challenge is higher in younger than older animals (24, 25). In one of these studies, the
391 transplant of thymocytes into young (8 weeks old) mice resulted in no change in IgE
392 response, whereas that into aged (65 weeks old) mice resulted in an enhanced IgE response
393 similar to that of young mice (25).
394 One might expect all markers of atopy to follow similar age/period/cohort patterns. Our
395 report suggests house dust mites and cat may be different to grass, but we can only speculate
396 as to the reason for this. One explanation for the fall in sensitization to house dust mites and
397 cat could be avoidance by the participants. We cannot assess whether participants avoided
398 house dust mite allergen, but we do know that the prevalence of cat ownership amongst those
399 with IgE at all three time points has not decreased over the 20 years of follow up (16.9% at
400 ECRHS I and 19.5% at ECRHS III). This supports the hypothesis that the decrease in
401 prevalence of sensitisation to cat is more likely due to aging-related immunosenescence.
402 There are differences in the epidemiology of sensitisation to each of the three allergens,
403 particularly with respect to factors associated with the 'hygiene hypothesis'. Larger sibships
404 protect younger siblings from hay fever and from sensitisation to grass more strongly than
405 from asthma and sensitisation to house dust mites (26, 27). Declining family size over the last
406 decades may explain the less marked aging effect for grass than for other allergens. Changes
407 in the level of exposure to pollens may have had a role in our findings (28, 29). There are also
408 reports suggesting that pollens in our more modern society are more allergenic than they have
409 been previously (30, 31), which could be related to the high levels of air pollutants such as

ozone, nitrogen dioxide and carbon dioxide (31-33). The presence of unmeasured factors may also have a role in the different patterns observed in the sensitisation to the three allergens. In summary, over a period of 20 years the prevalence of specific IgE sensitisation to house dust mite and cat, but not grass, significantly fell in the multinational cohort of adults from the ECRHS as a consequence of aging, being more evident among those aged 40 or over.

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505

Table 1. Net change in IgE sensitisation to house dust mite, grass, and cat, and total IgE over 20 years (N = 3206).

	Prevalence (%) ECRHS I	**Net change (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**Net change (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**
House dust mite					
(>0.35 kU_A/L)	16.6	-0.7 (-2.2 to 0.9)	0.051	-4.3 (-6.0 to -2.6)	0.71
(>0.70 kU_A/L)	13.1	-0.7 (-1.9 to 0.4)	0.63	-3.1 (-4.5 to -1.7)	0.21
Grass					
(>0.35 kU_A/L)	17.0	0.5 (-1.0 to 2.0)	0.048	-0.6 (-2.5 to 1.3)	0.009
(>0.70 kU_A/L)	14.2	0.0 (-1.3 to 1.3)	0.48	-2.2 (-3.8 to -0.6)	0.97
Cat					
(>0.35 kU_A/L)	8.8	-0.9 (-2.1 to 0.3)	0.14	-2.1 (-3.6 to -0.7)	0.09
(>0.70 kU_A/L)	6.4	0.0 (-1.0 to 1.1)	0.15	-1.1 (-2.2 to 0.1)	0.04
House dust mite or grass or cat					
(>0.35 kU_A/L)	29.4	0.1 (-2.0 to 2.1)	0.003	-4.6 (-7.0 to -2.1)	0.03
(>0.70 kU_A/L)	24.2	-0.6 (-2.2 to 1.0)	0.11	-4.6 (-6.6 to -2.6)	0.17
	GM ECRHS I	**GM ratio (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**GM ratio (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**
Total IgE (kU/L)	29.8	0.84 (0.78 to 0.90)	< 0.001	0.63 (0.58 to 0.68)	< 0.001

GM, Geometric mean.

Table 2. Net change in IgE sensitisation (>0.35 kU_A/L) to house dust mite, grass, and cat, and total IgE (kU/L) over 20 years, by year-of-birth cohort.

	1964-1973 (N = 736)			1954-1963 (N = 1314)			1944-1953 (N = 1156)		
	Prevalence or GM	Net change (95% CI)		Prevalence or GM	Net change (95% CI)		Prevalence or GM	Net change (95% CI)	
	ECRHS I	ECRHS II vs I	ECRHS III vs I	ECRHS I	ECRHS II vs I	ECRHS III vs I	ECRHS I	ECRHS II vs I	ECRHS III vs I
House dust mite	18.6	-0.6 (-3.0 to 1.8)	-4.1 (-6.7 to -1.5)	17.2	0.2 (-1.9 to 2.4)	-4.5 (-6.9 to -2.1)	13.8	-2.0 (-3.9 to -0.1)	-4.3 (-6.6 to -1.9)
Grass	20.6	3.3 (0.4 to 6.2)	1.5 (-1.8 to 4.9)	15.9	0.5 (-1.4 to 2.3)	-0.1 (-2.5 to 2.3)	15.4	-1.9 (-3.8 to 0.0)	-3.2 (-5.3 to -1.0)
Cat	10.5	0.2 (-2.2 to 2.6)	-0.7 (-3.5 to 2.0)	8.3	-1.4 (-2.9 to 0.1)	-2.0 (-3.6 to -0.3)	8.1	-1.2 (-2.7 to 0.2)	-3.6 (-5.2 to -2.0)
House dust mite or grass or cat	33.5	1.9 (-1.3 to 5.1)	-2.1 (-6.1 to 1.9)	28.7	1.1 (-1.6 to 3.7)	-4.1 (-7.2 to -1.1)	26.5	-3.0 (-5.6 to -0.3)	-7.4 (-10.4 to -4.3)
Total IgE	29.9	0.81 (0.72 to 0.91)	0.61 (0.54 to 0.68)	31.3	0.85 (0.78 to 0.92)	0.61 (0.56 to 0.67)	27.9	0.84 (0.78 to 0.92)	0.68 (0.61 to 0.75)

GM, Geometric mean.

Figures legends

Figure 1. Participant flow in the European Community Respiratory Health Survey (only centres that took part in all three surveys are included).

Figure 2. Net change in prevalence of IgE sensitisation (cut-off: 0.35 kU_A/L) to house dust mite [(I^2 (heterogeneity) = 0.0%, *P* = 0.71)], grass (I^2 = 44.9%, *P* = 0.009), cat (I^2 = 29.0%, *P* = 0.09), and at least one of these allergens (I^2 = 38.6%, *P* = 0.03). Centres are sorted by latitude (from North to South).

Figure 3. Prevalence of IgE sensitisation to (A) house dust mite, (B) grass, (C) cat, and (D) at least one of these three allergens, over 20 years of follow up, by year-of-birth cohort.

Figure 4. Changes in total IgE (kU/L) over 20 years of follow up, by year-of-birth cohort.

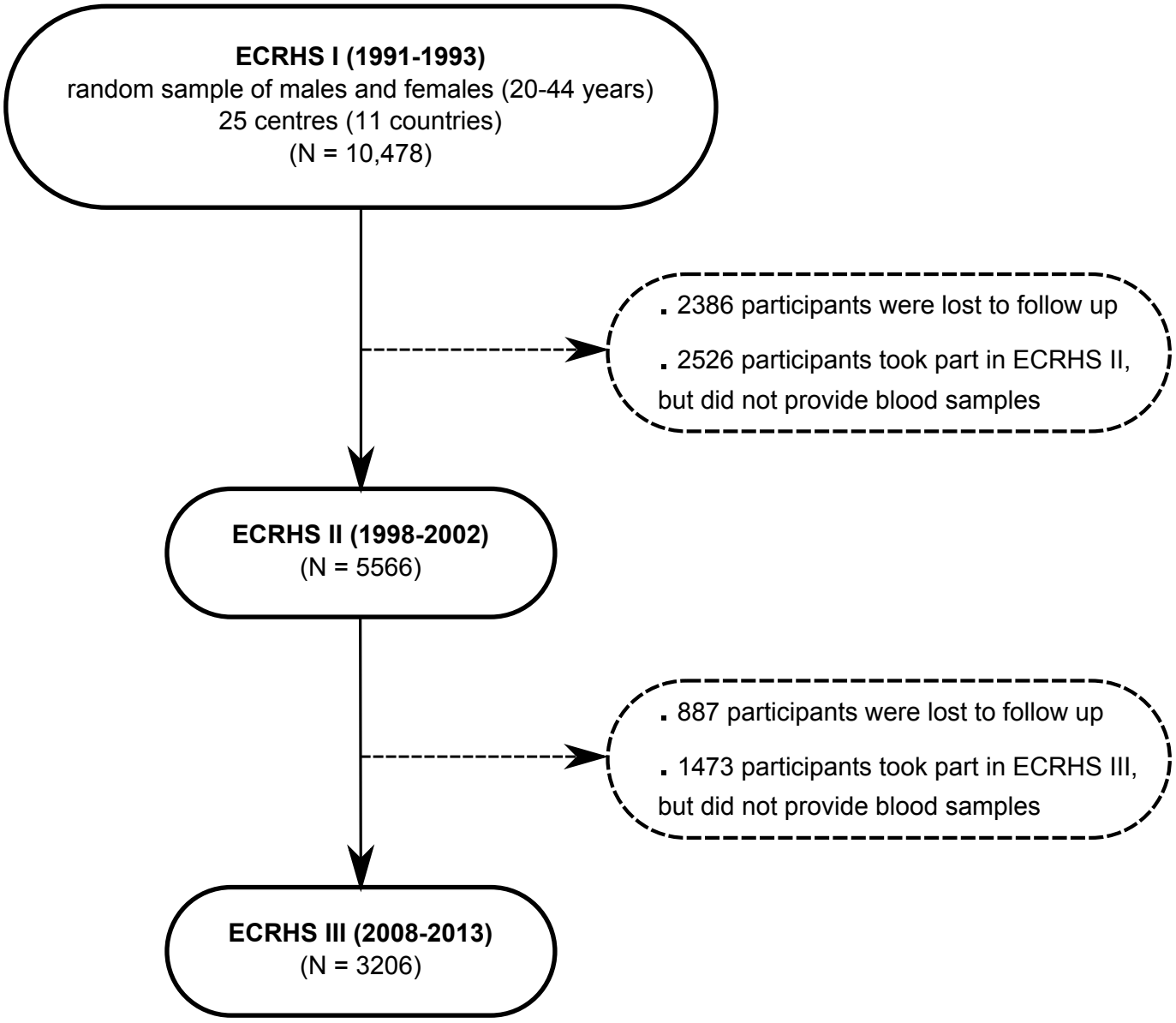
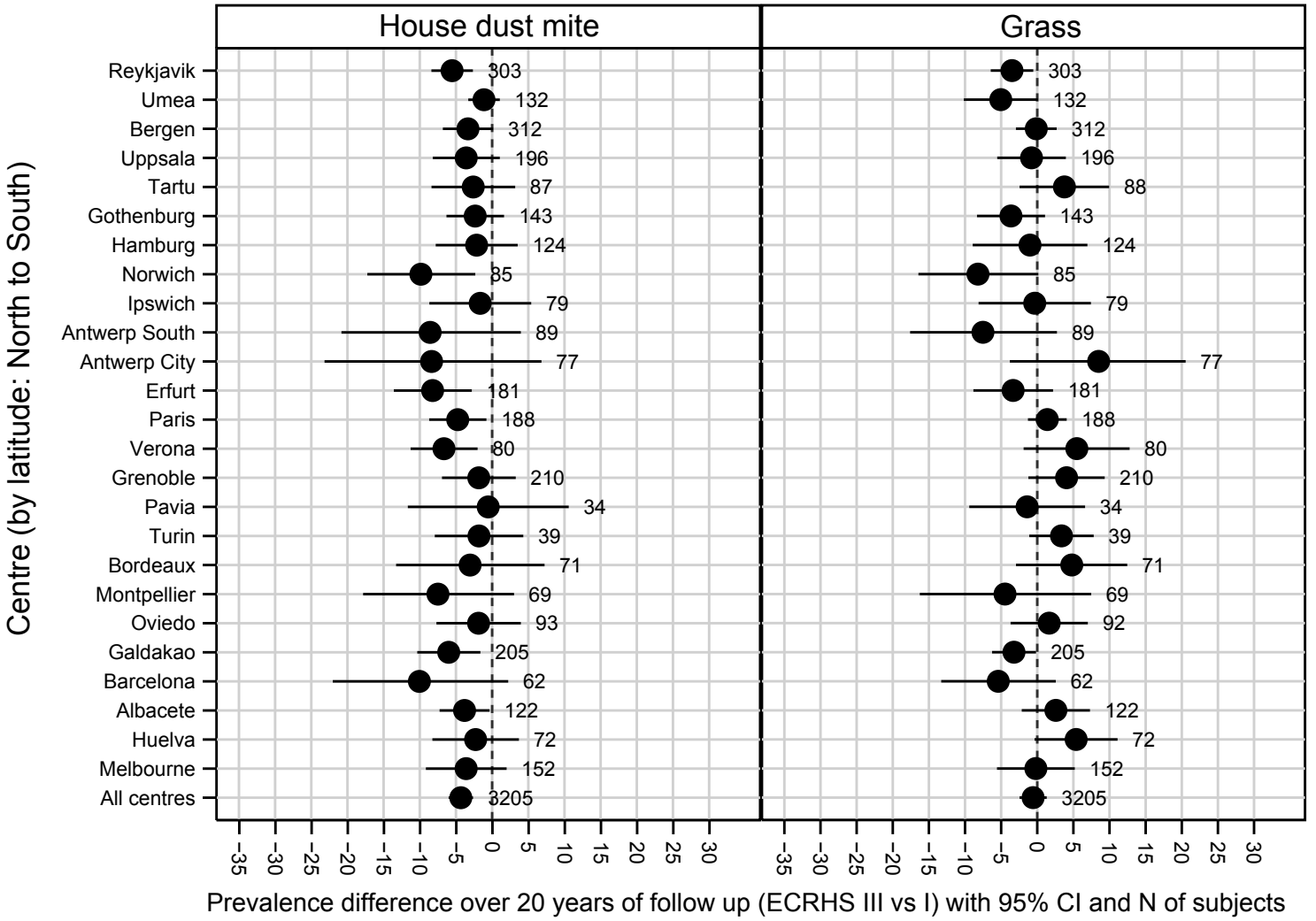
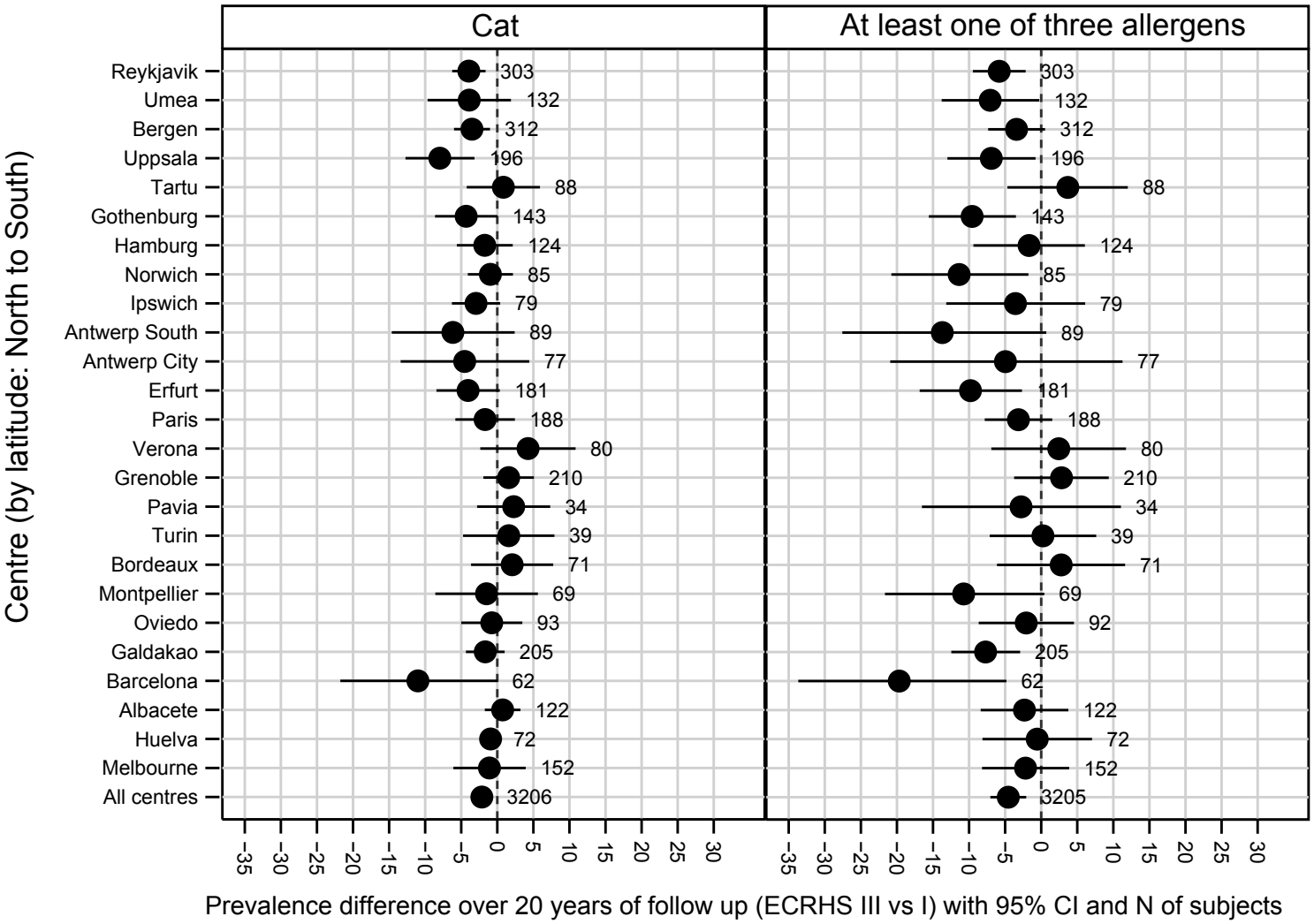
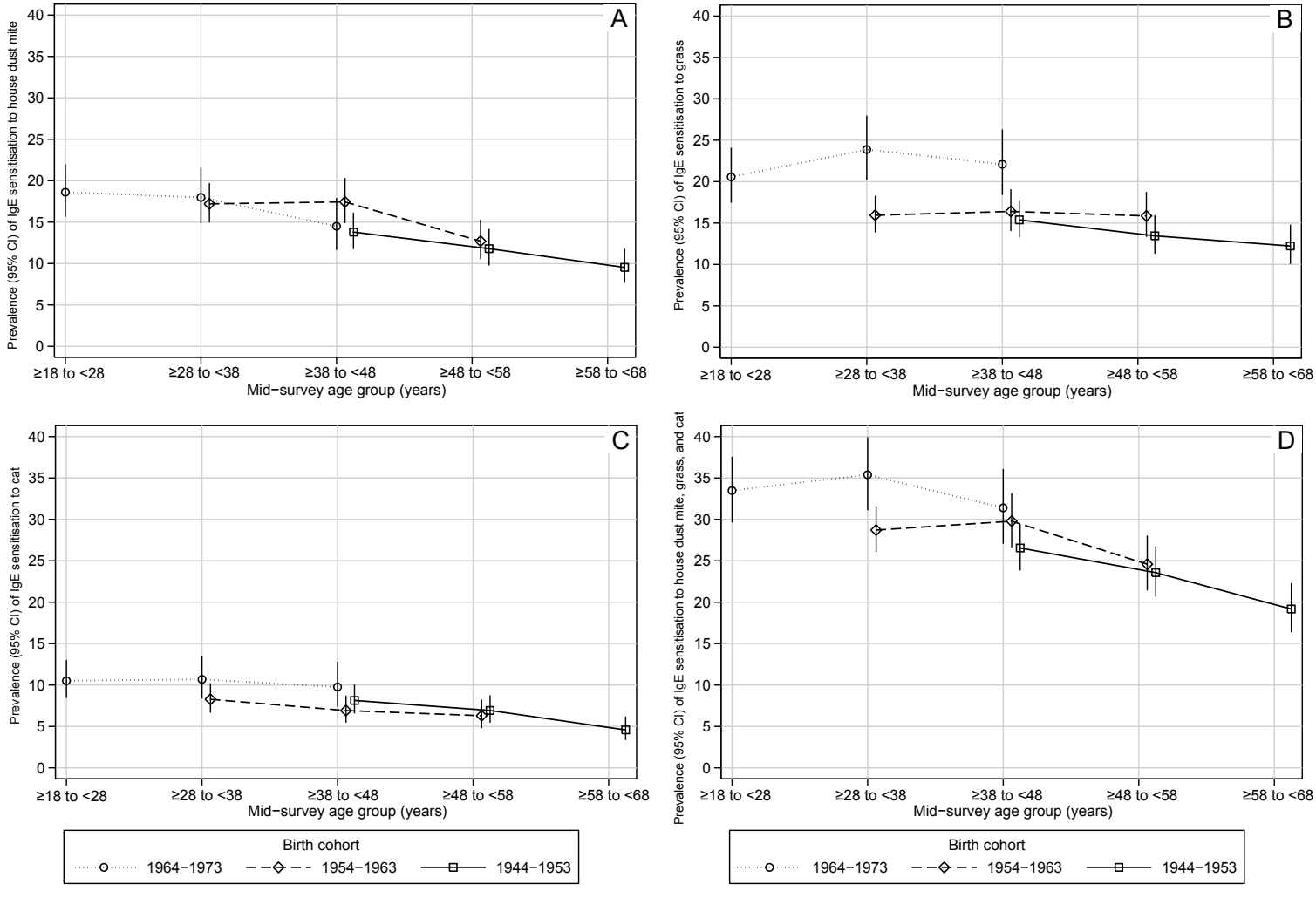
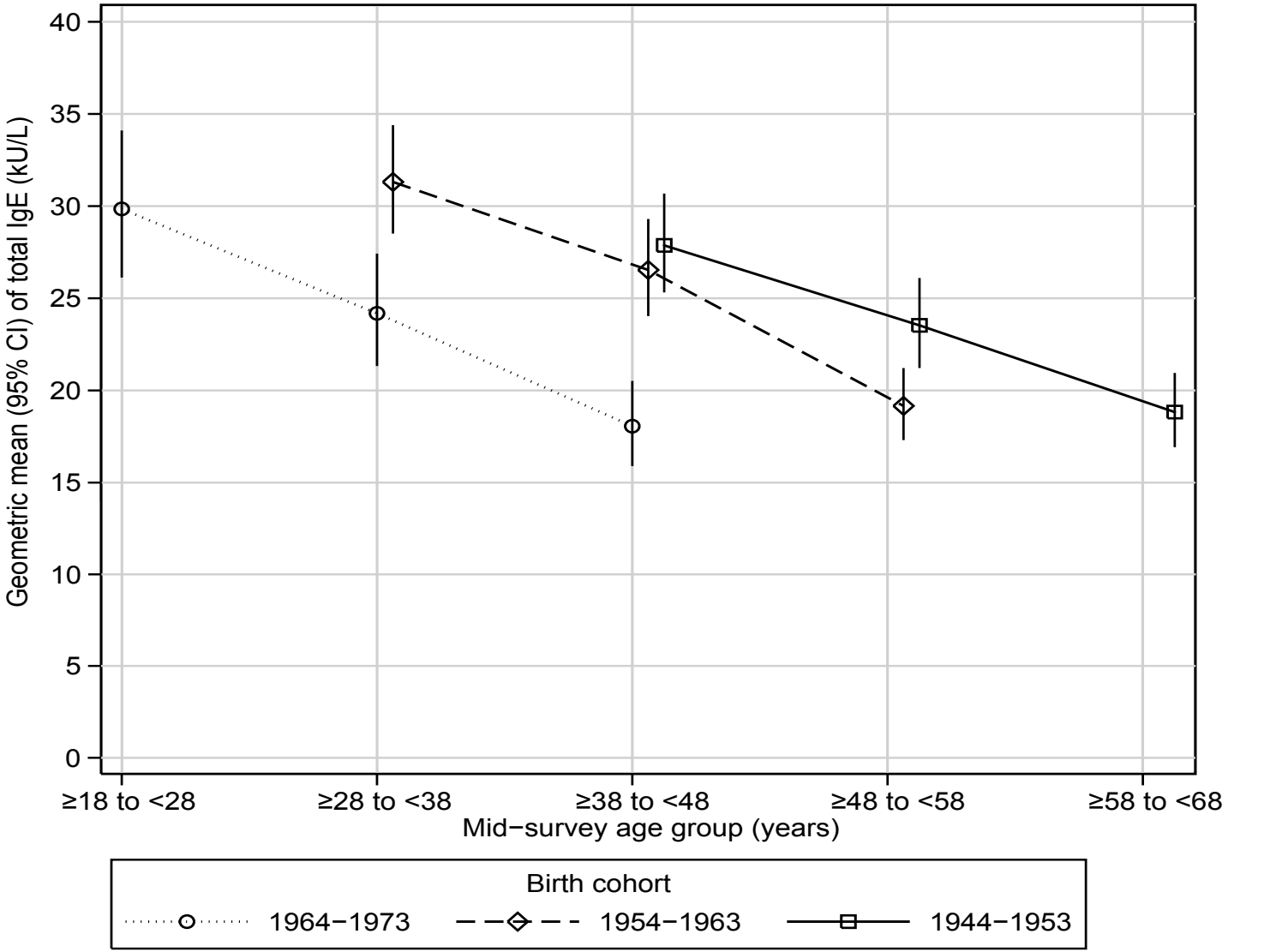


Figure 1. Participant flow in the European Community Respiratory Health Survey (only centres that took part in all three surveys are included).


House dust mite
Grass
Reykjavik 303
Umea 132
Bergen 312
Uppsala 196
Tartu 87
Gothenburg 143
Hamburg 124
Norwich 85
Ipswich 79
Antwerp South 89
Antwerp City 77
Erfurt 181
Paris 188
Verona 80
Grenoble 210
Pavia 34
Turin 39
Bordeaux 71
Montpellier 69
Oviedo 93
Galdakao 205
Barcelona 62
Albacete 122
Huelva 72
Melbourne 152
All centres 3205
Reykjavik 303
Umea 132
Bergen 312
Uppsala 196
Tartu 88
Gothenburg 143
Hamburg 124
Norwich 85
Ipswich 79
Antwerp South 89
Antwerp City 77
Erfurt 181
Paris 188
Verona 80
Grenoble 210
Pavia 34
Turin 39
Bordeaux 71
Montpellier 69
Oviedo 92
Galdakao 205
Barcelona 62
Albacete 122
Huelva 72
Melbourne 152
All centres 3205
-35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30
Centre (by latitude: North to South)
Prevalence difference over 20 years of follow up (ECRHS III vs I) with 95% CI and N of subjects


Cat
At least one of three allergens
Reykjavik 303
Umea 132
Bergen 312
Uppsala 196
Tartu 88
Gothenburg 143
Hamburg 124
Norwich 85
Ipswich 79
Antwerp South 89
Antwerp City 77
Erfurt 181
Paris 188
Verona 80
Grenoble 210
Pavia 34
Turin 39
Bordeaux 71
Montpellier 69
Oviedo 93
Galdakao 205
Barcelona 62
Albacete 122
Huelva 72
Melbourne 152
All centres 3206
Reykjavik 303
Umea 132
Bergen 312
Uppsala 196
Tartu 88
Gothenburg 143
Hamburg 124
Norwich 85
Ipswich 79
Antwerp South 89
Antwerp City 77
Erfurt 181
Paris 188
Verona 80
Grenoble 210
Pavia 34
Turin 39
Bordeaux 71
Montpellier 69
Oviedo 92
Galdakao 205
Barcelona 62
Albacete 122
Huelva 72
Melbourne 152
All centres 3205
-35 -30 -25 -20 -15 -10 -5 0 5 10 15 20 25 30
Centre (by latitude: North to South)
Prevalence difference over 20 years of follow up (ECRHS III vs I) with 95% CI and N of subjects


A
Prevalence (95% CI) of IgE sensitisation to house dust mite
0
5
10
15
20
25
30
35
40
≥18 to <28
≥28 to <38
≥38 to <48
≥48 to <58
≥58 to <68
Mid−survey age group (years)
B
Prevalence (95% CI) of IgE sensitisation to grass
C
Prevalence (95% CI) of IgE sensitisation to cat
D
Prevalence (95% CI) of IgE sensitisation to house dust mite, grass, and cat
Birth cohort
1964−1973
1954−1963
1944−1953


Geometric mean (95% CI) of total IgE (kU/L)
40
35
30
25
20
15
10
5
0
≥18 to <28
≥28 to <38
≥38 to <48
≥48 to <58
≥58 to <68
Mid−survey age group (years)
Birth cohort
1964−1973
1954−1963
1944−1953

Online methods

Statistical analyses were performed using Stata V.13 (StataCorp LP, College Station, TX).

Laboratory bias (duplicate measurements)

To assess the effects of potential laboratory bias on prevalence of IgE sensitisation and mean of total IgE estimates, we conducted duplicate assays on 794 samples (tested at ECRHS I, stored, and tested at ECRHS II) and 475 samples (tested at ECRHS II, stored, and tested at ECRHS III). Confidence intervals for Cohen's kappa statistics for each comparison between two measurements of the same sample were computed using the kap command in Stata, together with delta-method standard errors, using the normalising and variance-stabilising transformation ln(1-kappa) (see Table E1 in the Online Repository).

Elimination of laboratory bias

To correct our estimates for laboratory bias, we included in the models:

- the three main-assessment assays for each participant (GMs or odds for each combination of centre and ECRHS survey);
- four extra parameters (GM ratios or odds ratios) regarding the paired method-comparison assays:
    - two indicating an assay's membership in the two method-comparison studies;
    - two indicating that an assay was carried out using the method of ECRHS II or III, respectively, instead of the method of ECRHS I.

Analysis of outcomes

To determine the difference in prevalence of sensitisation and geometric mean ratios of total IgE between surveys we used the 'margins' and 'nlcom' commands and the 'regpar' add-on package (E1) as required.

Inverse sampling-probability weighted estimation

Inverse sampling-probability weights were used to standardise the estimation from the population with data on IgE assays in all three ECRHS surveys to a target population of participants with data on IgE assays from ECRHS I, which was randomly sampled from the general adult population in different European and Australian centres.

The inverse sampling-probability weights were calculated using a logistic regression model (E2) with a separate set of parameters for each centre with any IgE data responders, predicting response to all three surveys from baseline characteristics, adapted from the response-regression model of Jarvis et al. (E3). The parameters for each centre were a baseline odds, an exponential per-decade odds ratio for age at 01 January 1992, an odds ratio for female gender (compared to a baseline of male gender), odds ratios for self-reported smoking status at ECRHS I ('ex' and 'current' compared to a baseline of 'never'), an odds ratio for wheeze at ECRHS I, an odds ratio for waking with shortness of breath at ECRHS I, and an odds ratio for IgE sensitisation to house dust mite, cat, or grass at ECRHS I. When we meta-analysed the parameters using randomly-variable-effects meta-analysis (E4), we found that participants who have taken part in all three phases of the study were slightly older, less likely to be smokers and less likely to have reported shortness of breath than participants who did not have serum IgE in all three surveys (see Table E2 in the Online Repository).

The use of inverse sampling-probability weights to standardise the estimates to the target population in ECRHS I seemed to work, as indicated by a Somers' D of response-propensity score (E5) with respect to response of 0.008 when inverse sampling-probability weighted versus one of 0.239 when unweighted.

References

E1. Newson RB. Attributable and unattributable risks and fractions and other scenario comparisons. The Stata Journal 2013;13:672–98.

E2. Robins JM, Rotnitzky A, Zhao LP. Estimation of regression coefficients when some regressors are not always observed. Journal of the American Statistical Association. 1994;89:846-66.

E3. Jarvis D, Luczynska C, Chinn S, Potts J, Sunyer J, Janson C, et al. Change in prevalence of IgE sensitization and mean total IgE with age and cohort. J Allergy Clin Immunol. 2005;116:675-82.

E4. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7:177-88.

E5. Newson R. Confidence intervals for rank statistics: Somers' D and extensions. Stata Journal. 2006;6:309.

Online Figures legends

Figure E1. Net change in prevalence of IgE sensitisation (cut-off: 0.35 kU_A/L) to house dust mite, grass, cat, and at least one of these allergens. Centres are sorted by descending response rate.

Figure E2. Net change in prevalence of IgE sensitisation (cut-off: 0.35 kU_A/L) to house dust mite, grass, cat, and at least one of these allergens. Centres are sorted by ascending prevalence of sensitisation at baseline.

Figure E3. Net change in geometric mean ratio of total IgE (kU/L). Centres sorted by latitude (north to south, left) and by descending response rate (right).

Table E1. Results from comparability study in which replicate samples from 1992 were tested in 2002, and from 2002 were tested in 2013/14.

	IgE in 1992		**IgE in 2002**		**% difference 2002 vs 1992 (95% CI)**	**Cohen kappa 2002 vs 1992**	**IgE in 2002**		**IgE in 2013/14**		**% between 2013/14 vs 2002 (95% CI)**	**Cohen kappa 2013/14 vs 2002**
	N (of 794)	**%**	**N (of 794)**	**%**	**N = 794**		**N (of 475)**	**%**	**N (of 475)**	**%**	**N = 475**	
House dust mite (0.35 kU_A/L)	241	30.4	247	31.1	0.8 (-1.3 to 2.8)	0.80	129	27.2	133	28.0	0.8 (-0.6 to 2.3)	0.94
(0.70 kU_A/L)	193	24.3	195	24.6	0.3 (-1.1 to 1.6)	0.89	106	22.3	104	21.9	-0.4 (-1.4 to 0.6)	0.96
Grass (0.35 kU_A/L)	229	28.8	224	28.2	-0.6 (-2.3 to 1.1)	0.86	119	25.1	115	24.2	-0.8 (-2.1 to 0.5)	0.94
(0.70 kU_A/L)	187	23.6	196	24.7	1.1 (-0.3 to 2.6)	0.88	99	20.8	98	20.6	-0.2 (-1.6 to 1.2)	0.93
Cat (0.35 kU_A/L)	116	14.6	133	16.8	2.1 (0.7 to 3.6)	0.83	60	12.6	63	13.3	0.6 (-0.7 to 2.0)	0.90
(0.70 kU_A/L)	94	11.8	102	12.8	1.0 (-0.3 to 2.3)	0.85	51	10.7	54	11.4	0.6 (-0.5 to 1.7)	0.92
Sensitisation to at least one allergen (0.35 kU_A/L)	336	42.3	338	42.6	0.3 (-1.8 to 2.3)	0.82	182	38.3	186	39.2	0.8 (-0.9 to 2.6)	0.92
(0.70 kU_A/L)	278	35.0	293	36.9	1.9 (0.4 to 3.4)	0.89	159	33.5	162	34.1	0.6 (-0.7 to 2.0)	0.95
	GM in 1992 N = 794		**GM in 2002 N = 794**		**GM ratio 2002 vs 1992 (95% CI)**		**GM in 2002 N = 475**		**GM in 2013/14 N = 475**		**GM ratio 2013/14 vs 2002 (95% CI)**	
Total IgE (kU/L)	36.1		52.75		1.46 (1.38-1.55)		42.7		43.2		1.01 (0.98-1.05)	

GM, Geometric mean.

Table E2. Baseline characteristics of subjects with IgE measurements in all three surveys of ECRHS versus subjects with IgE measurements in baseline survey only from same centres.

	With IgE measurements in baseline survey only (n = 7272)	**With IgE measurements in all three surveys (n = 3206)**	**Adjusted* odds for responding (95% CI)**	***P* for heterogeneity#**
Age at baseline (per 10 years)	-	-	1.40 (1.29-1.52)	0.036
Female (%)	49.9	50.0	1.00 (0.19-1.11)	0.17
Smoking status at baseline (%)				
Lifetime non-smoker	41.6	45.1	1.00	
Ex-smoker	21.1	22.6	0.88 (0.78-1.01)	0.29
Current smoker	37.3	32.3	0.65 (0.58-0.73)	0.38
Symptoms in the last 12 months				
Wheeze	22.2	19.8	0.97 (0.84-1.11)	0.12
Woken with shortness of breath	6.4	4.8	0.76 (0.61-0.94)	0.40
Sensitised to at least one allergen** (%)	29.5	27.9	1.05 (0.91-1.22)	0.0017

*From meta-analysis by centre, adjusting for all other factors in table.

**House dust mite, cat, grass.

#From random effects meta-analysis.

Table E3. Net change in IgE sensitisation to house dust mite, grass, and cat, and total IgE over 20 years, by gender.

	Males (n = 1604)					Females (n = 1602)				
	Prevalence (%) ECRHS I	**Net change (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**Net change (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**	**Prevalence (%) ECRHS I**	**Net change (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**Net change (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**
House dust mite										
(>0.35 kU_A/L)	19.7	-0.5 (-2.7 to 1.6)	0.20	-5.0 (-7.2 to -2.8)	0.59	13.5	-0.8 (-2.5 to 0.9)	0.038	-3.7 (-5.7 to -1.7)	0.34
(>0.70 kU_A/L)	15.1	-0.3 (-2.0 to 1.4)	0.95	-2.9 (-4.9 to -0.9)	0.26	11.0	-1.1 (-2.3 to 0.1)	0.096	-3.3 (-5.0 to -1.6)	0.057
Grass										
(>0.35 kU_A/L)	18.5	0.4 (-1.6 to 2.4)	0.18	-0.9 (-3.2 to 1.3)	0.11	15.6	0.6 (-1.2 to 2.4)	0.94	-0.2 (-2.5 to 2.1)	0.74
(>0.70 kU_A/L)	15.8	-0.3 (-2.0 to 1.5)	0.16	-3.1 (-5.1 to -1.0)	0.82	12.7	0.3 (-1.2 to 1.8)	0.91	-1.3 (-3.3 to 0.6)	0.95
Cat										
(>0.35 kU_A/L)	8.7	-0.3 (-1.9 to 1.3)	0.21	-2.1 (-3.8 to -0.4)	0.40	8.9	-1.5 (-2.9 to -0.1)	0.54	-2.2 (-3.9 to -0.5)	0.074
(>0.70 kU_A/L)	6.4	0.2 (-1.2 to 1.6)	0.22	-1.2 (-2.7 to 0.3)	0.27	6.4	-0.1 (-1.4 to 1.1)	0.071	-1.0 (-2.3 to 0.4)	0.013
House dust mite or grass or cat										
(>0.35 kU_A/L)	32.5	0.8 (-1.8 to 3.5)	0.74	-5.6 (-8.6 to -2.5)	0.39	26.2	-0.7 (-3.0 to 1.6)	0.46	-3.6 (-6.4 to -0.7)	0.089
(>0.70 kU_A/L)	26.5	0.3 (-2.0 to 2.5)	0.81	-4.6 (-7.2 to -2.0)	0.25	21.9	-1.5 (-3.2 to 0.3)	0.40	-4.5 (-6.8 to -2.2)	0.056
	GM ECRHS I	**GM ratio (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**GM ratio (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**	**GM ECRHS I**	**GM ratio (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**GM ratio (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**
Total IgE (kU/L)	34.3	0.82 (0.75 to 0.88)	< 0.001	0.65 (0.59 to 0.71)	< 0.001	26.0	0.86 (0.79 to 0.93)	0.004	0.61 (0.56 to 0.67)	< 0.001

GM, Geometric mean.

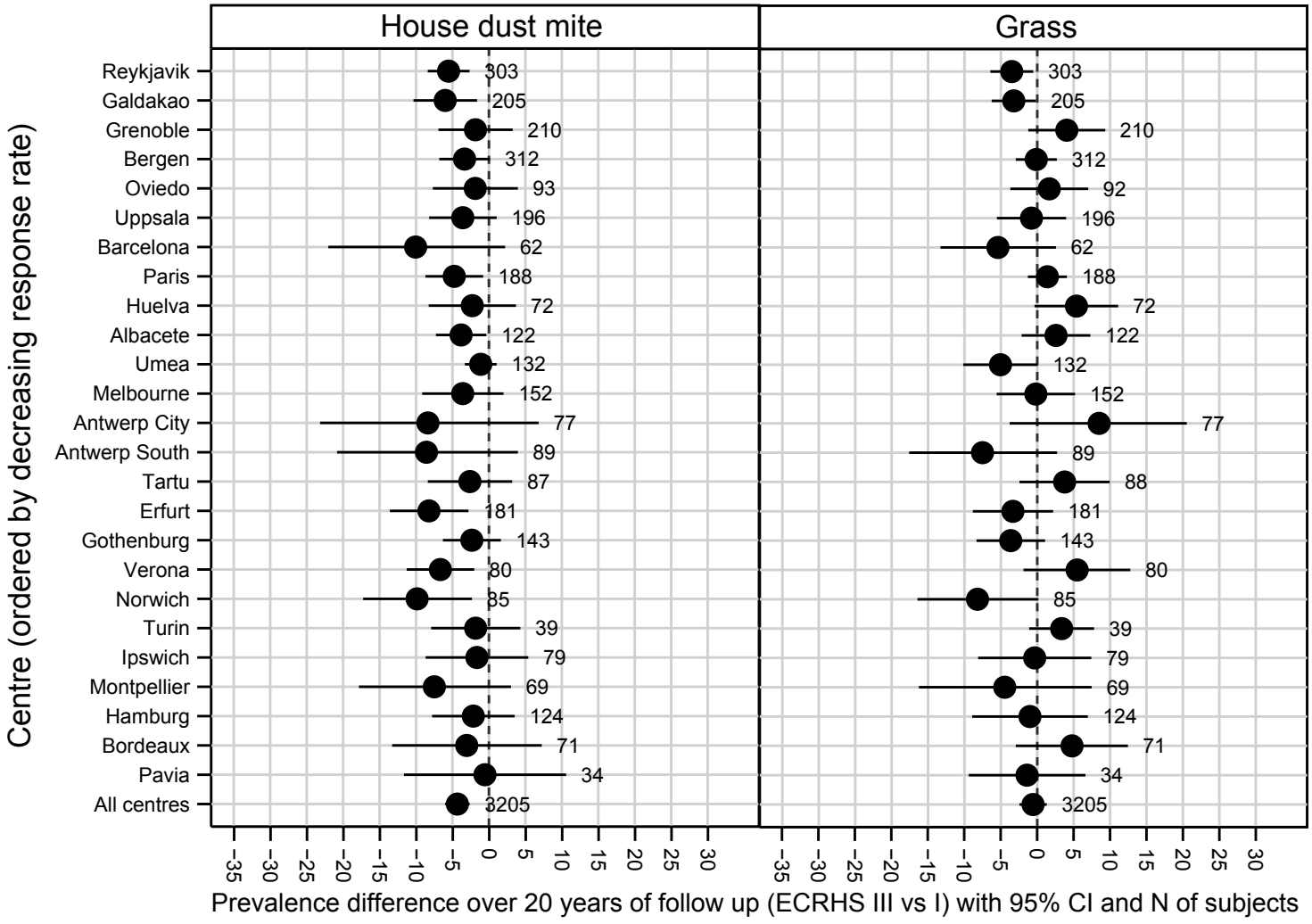
Table E4. Net change in IgE sensitisation to house dust mite, grass, and cat, and total IgE over 20 years: Persistent lifetime non-smokers only (N = 1304).

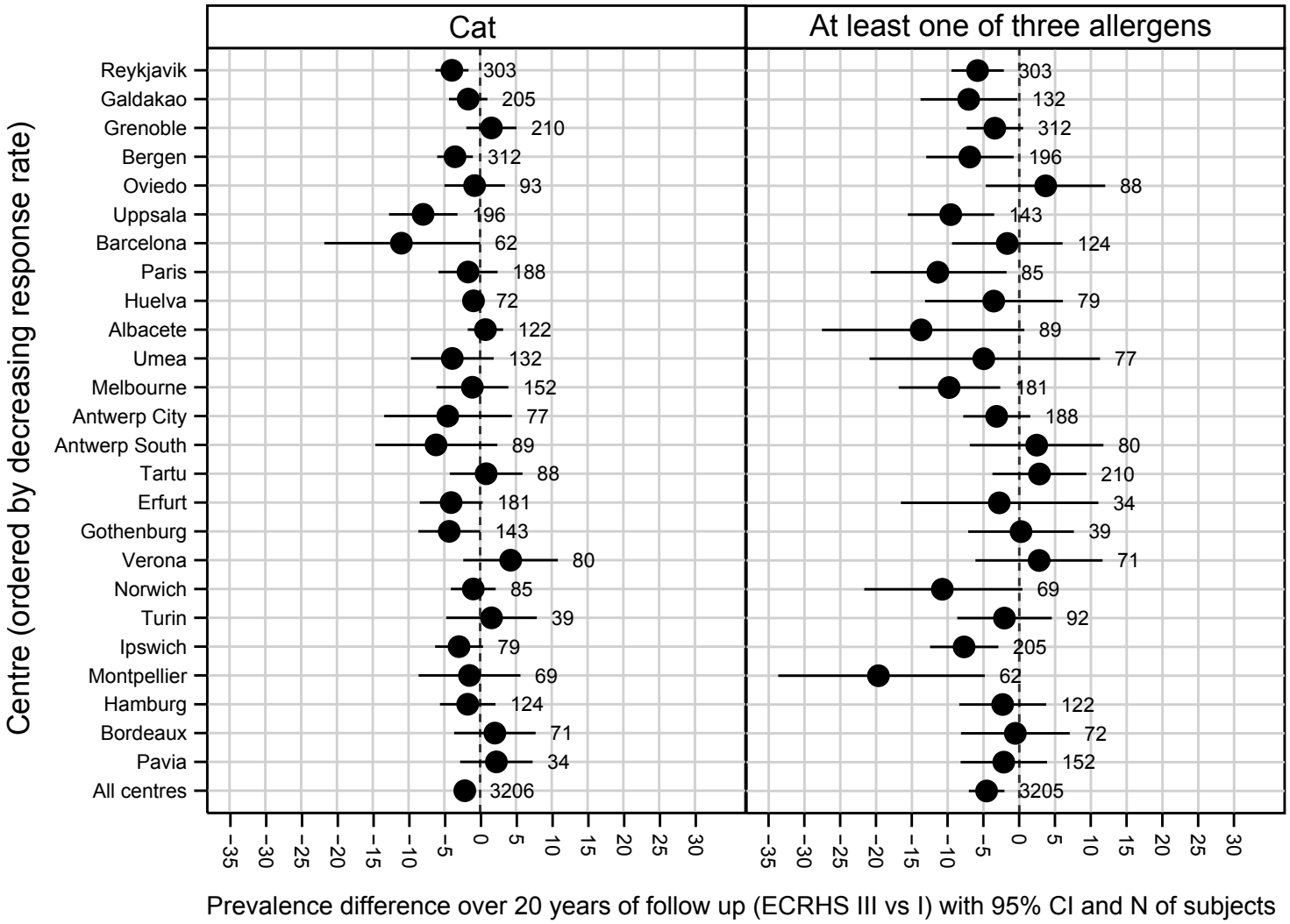
	Prevalence (%) ECRHS I	**Net change (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**Net change (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**
House dust mite					
(>0.35 kU_A/L)	15.8	0.0 (-1.9 to 2.0)	0.005	-3.4 (-5.5 to -1.4)	0.08
(>0.70 kU_A/L)	12.4	-0.9 (-2.2 to 0.5)	0.79	-2.0 (-3.8 to -0.2)	0.41
Grass					
(>0.35 kU_A/L)	20.5	1.1 (-1.0 to 3.3)	0.75	-0.4 (-3.0 to 2.2)	0.26
(>0.70 kU_A/L)	17.9	0.2 (-1.6 to 2.1)	0.65	-2.5 (-4.9 to -0.1)	0.98
Cat					
(>0.35 kU_A/L)	10.5	-0.6 (-2.3 to 1.1)	0.78	-2.0 (-4.1 to 0.0)	0.42
(>0.70 kU_A/L)	8.0	0.4 (-1.2 to 2.0)	0.71	-0.8 (-2.5 to 1.0)	0.42
House dust mite or grass or cat					
(>0.35 kU_A/L)	31.4	1.9 (-0.8 to 4.5)	0.002	-2.9 (-6.0 to 0.2)	0.03
(>0.70 kU_A/L)	26.7	0.1 (-1.9 to 2.2)	0.21	-3.3 (-5.9 to -0.6)	0.21
	GM ECRHS I	**GM ratio (95% CI) ECRHS II vs I**	***P* for heterogeneity between centres**	**GM ratio (95% CI) ECRHS III vs I**	***P* for heterogeneity between centres**
Total IgE (kU/L)	27.8	0.82 (0.75 to 0.89)	< 0.001	0.62 (0.56 to 0.68)	< 0.001

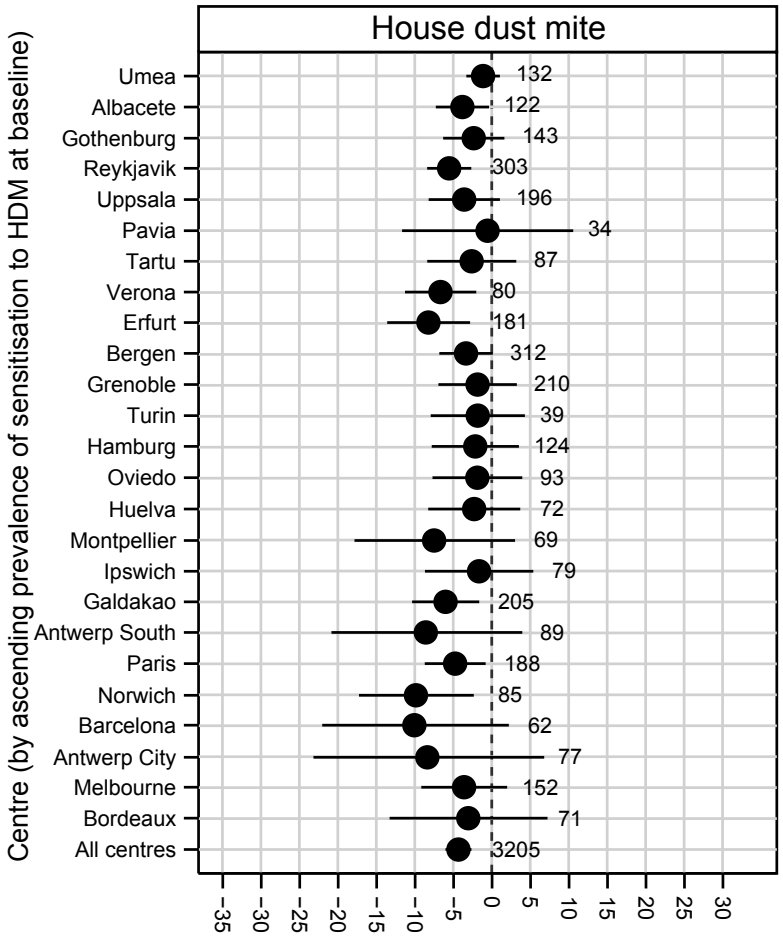
GM, Geometric mean.

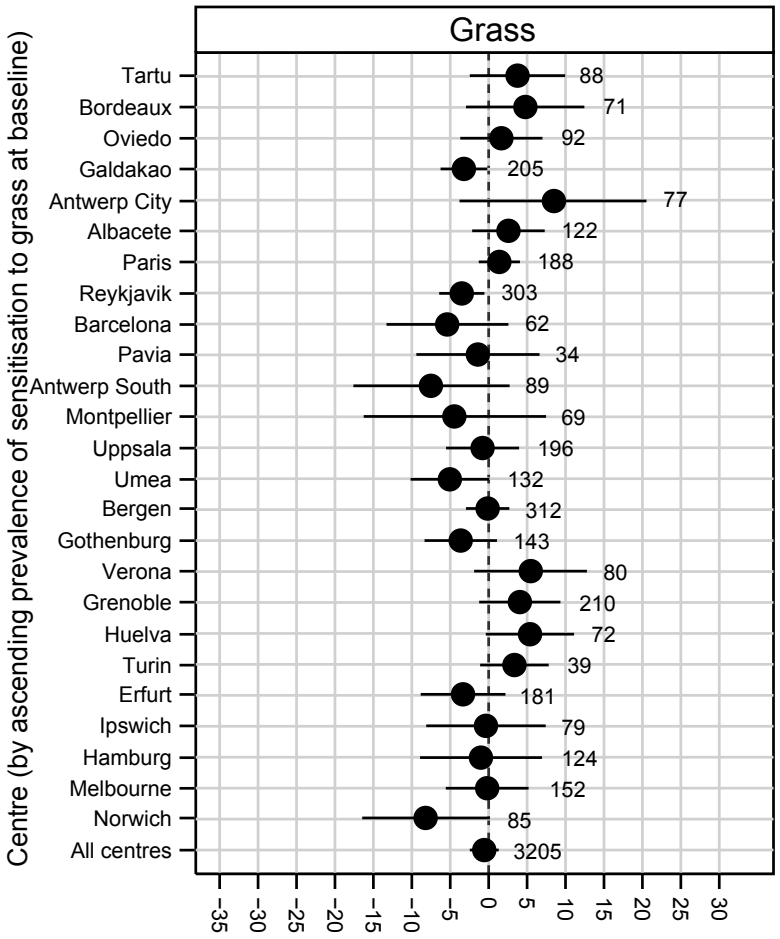
Table E5. Net change in IgE sensitisation (>0.70 kU_A/L) to house dust mite, grass, and cat over 20 years, by birth cohort.

	1964-1973 (N = 736) Net change (95% CI)			1954-1963 (N = 1314) Net change (95% CI)			1944-1953 (N = 1156) Net change (95% CI)		
	Prevalence or GM	Net change (95% CI)		Prevalence or GM	Net change (95% CI)		Prevalence or GM	Net change (95% CI)	
	ECRHS I	ECRHS II vs I	ECRHS III vs I	ECRHS I	ECRHS II vs I	ECRHS III vs I	ECRHS I	ECRHS II vs I	ECRHS III vs I
House dust mite	15.0	0.3 (-1.9 to 2.4)	-1.5 (-4.2 to 1.2)	14.1	-0.9 (-2.6 to 0.8)	-4.4 (-6.4 to -2.4)	9.9	-1.3 (-2.7 to 0.0)	-2.7 (-4.5 to -0.9)
Grass	18.2	1.7 (-0.8 to 4.2)	-0.7 (-3.7 to 2.4)	13.8	0.1 (-1.6 to 1.7)	-2.2 (-4.3 to -0.2)	11.4	-1.6 (-3.2 to 0.0)	-3.5 (-5.3 to -1.7)
Cat	7.7	1.0 (-1.2 to 3.1)	-0.1 (-2.3 to 2.1)	5.8	-0.3 (-1.5 to 0.9)	-0.8 (-2.2 to 0.7)	5.9	-0.3 (-1.6 to 1.0)	-2.3 (-3.6 to -1.0)
House dust mite or grass or cat	29.5	1.2 (-1.7 to 4.1)	-2.3 (-6.0 to 1.4)	24.1	-0.6 (-2.7 to 1.6)	-5.4 (-7.9 to -2.9)	19.6	-2.2 (-4.2 to -0.3)	-5.4 (-7.8 to -3.1)

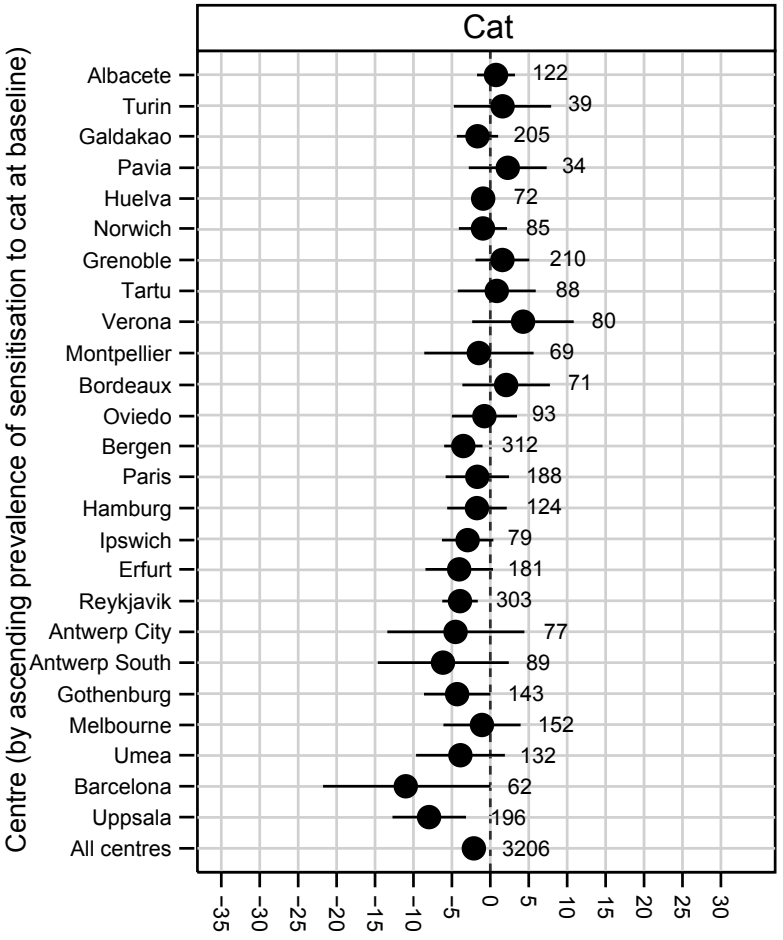

House dust mite
Grass
Reykjavik 303 303
Galdakao 205 205
Grenoble 210 210
Bergen 312 312
Oviedo 93 92
Uppsala 196 196
Barcelona 62 62
Paris 188 188
Huelva 72 72
Albacete 122 122
Umea 132 132
Melbourne 152 152
Antwerp City 77 77
Antwerp South 89 89
Tartu 87 88
Erfurt 181 181
Gothenburg 143 143
Verona 80 80
Norwich 85 85
Turin 39 39
Ipswich 79 79
Montpellier 69 69
Hamburg 124 124
Bordeaux 71 71
Pavia 34 34
All centres 3205 3205
−35 −30 −25 −20 −15 −10 −5 0 5 10 15 20 25 30
Centre (ordered by decreasing response rate)
Prevalence difference over 20 years of follow up (ECRHS III vs I) with 95% CI and N of subjects

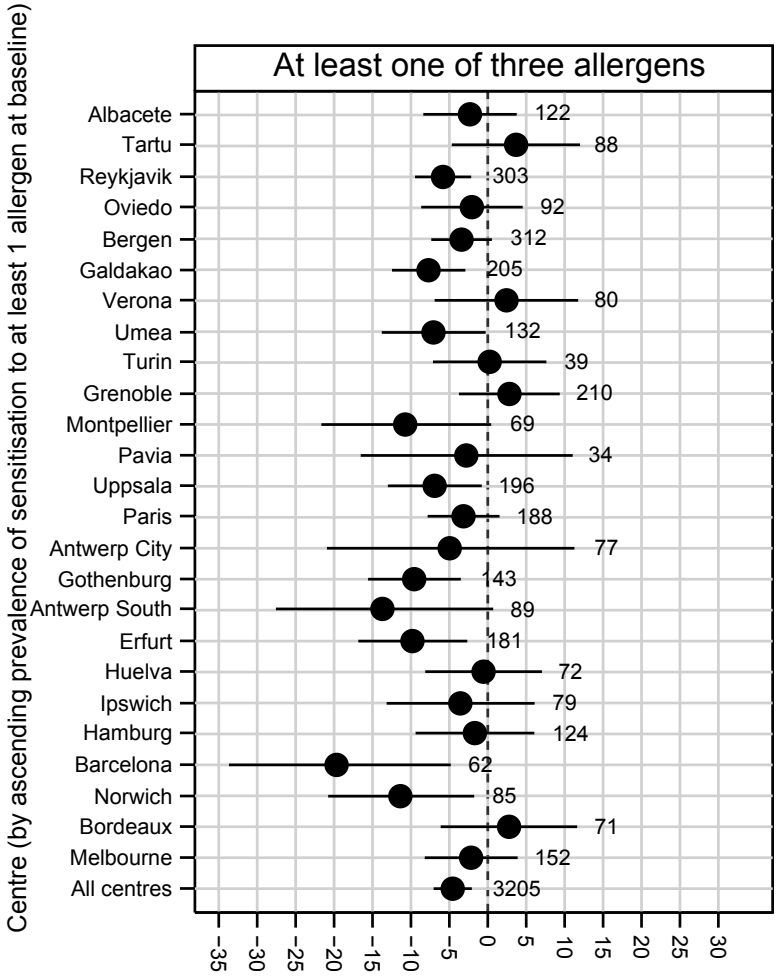

Cat
At least one of three allergens
Reykjavik 303 303
Galdakao 205 132
Grenoble 210 312
Bergen 312 196
Oviedo 93 88
Uppsala 196 143
Barcelona 62 124
Paris 188 85
Huelva 72 79
Albacete 122 89
Umea 132 77
Melbourne 152 181
Antwerp City 77 188
Antwerp South 89 80
Tartu 88 210
Erfurt 181 34
Gothenburg 143 39
Verona 80 71
Norwich 85 69
Turin 39 92
Ipswich 79 205
Montpellier 69 62
Hamburg 124 122
Bordeaux 71 72
Pavia 34 152
All centres 3206 3205
−35 −30 −25 −20 −15 −10 −5 0 5 10 15 20 25 30
Centre (ordered by decreasing response rate)
Prevalence difference over 20 years of follow up (ECRHS III vs I) with 95% CI and N of subjects



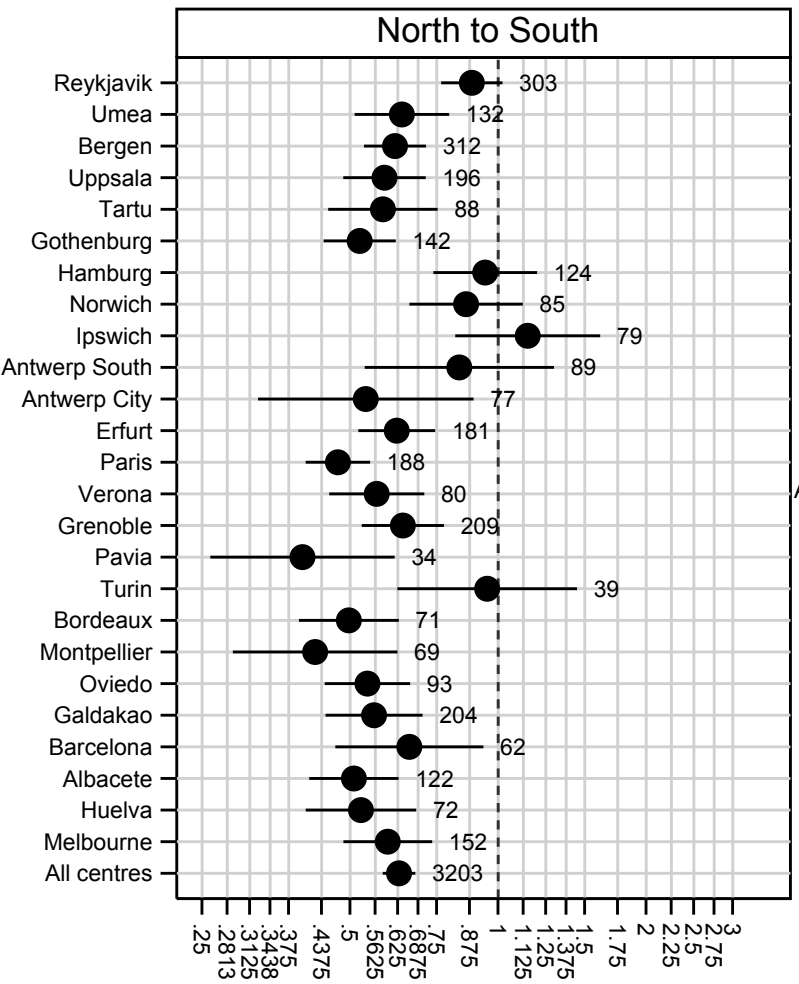


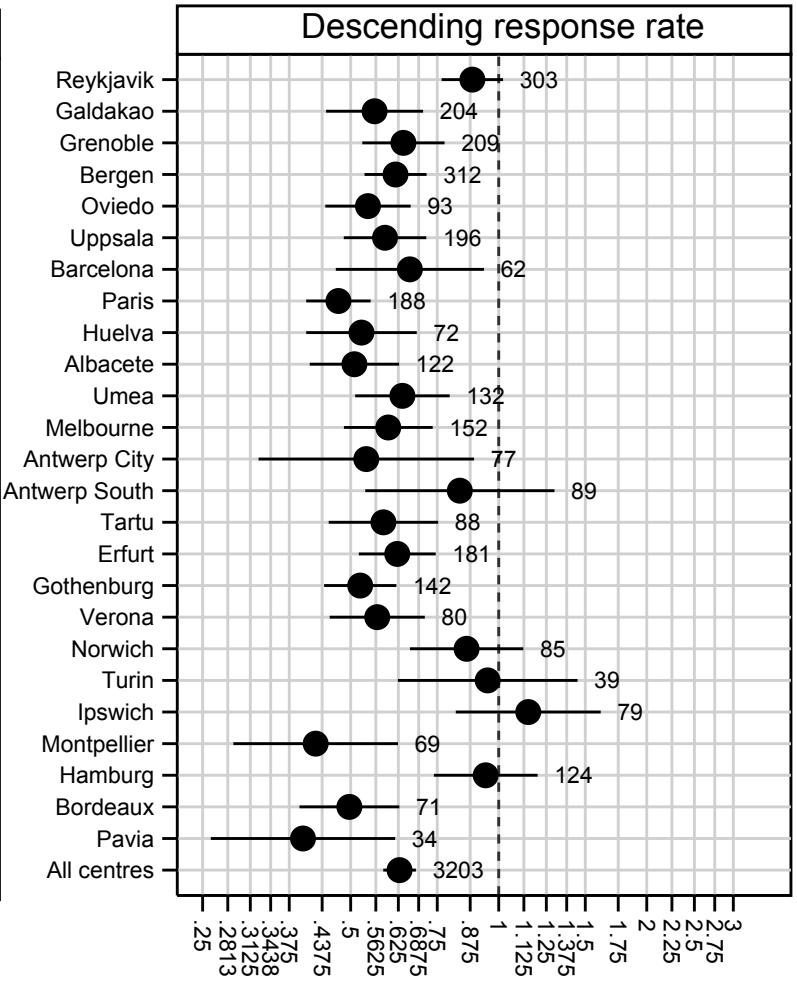
Prevalence difference over 20 years of follow up (ECRHS III vs I) with 95% CI and N of subjects





Prevalence difference over 20 years of follow up (ECRHS III vs I) with 95% CI and N of subjects





Geometric mean ratio of total IgE over 20 years of follow up (ECRHS III vs I), with 95% CI and N of subjects