ORIGINAL ARTICLE

High exposure to endotoxin in farming is associated with less new-onset pollen sensitisation

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ABSTRACT

Objectives Little is known about risk factors for new onset and loss of atopic sensitisation in adulthood. The aim is to examine the longitudinal effect of quantitatively assessed endotoxin exposures on changes in specific allergen sensitisation in young adults.

Methods The cohort consisted of 1113 young Danish farmers and rural controls, with a mean age of 19 years at baseline. Sensitisation to birch pollen, grass pollen, cat dander and house dust mite was measured by specific IgE levels in serum samples from baseline and at 15 years' follow-up. Changes in sensitisation were analysed in relation to cumulative endotoxin exposure during follow-up, considering early life farm exposure.

Results Endotoxin exposure during follow-up was significantly associated with less new onset of specifically grass and birch pollen sensitisation. For the highest versus lowest quartile of cumulative endotoxin exposure, the OR for new-onset IgE sensitisation was 0.35 (0.13–0.91) for birch and 0.14 (0.05–0.50) for grass. On the other hand, loss of pollen sensitisation showed a positive, although mostly non-significant, association with increased levels of endotoxin exposure. Endotoxin exposure was not associated with significant changes in cat dander and house dust mite sensitisation.

Conclusions High exposure to endotoxin during young adulthood appears to protect against new onset of pollen sensitisation, independent of childhood farm exposure.

What this paper adds

- The beneficial effect of farming exposure on allergy and asthma has been widely studied and confirmed in several studies of children and their exposures.
- Narrowing down the specific beneficial constituents of farming exposure has however proven quite challenging. This study adds valuable information on timing of exposure, as adult farming exposure is found to have a beneficial effect independent of childhood exposure. Furthermore, we are able to relate specific endotoxin levels to change in sensitisation to specific allergens.
- Hopefully these results will provide further understanding of the complexity of allergic sensitisation and the development of allergy for the clinicians, which will help them improve their medical advice to patients within and outside the farming trade.

INTRODUCTION

Increased occurrence of allergic disease has been evident during recent decades, the reasons for which continue to be heavily debated. It has been claimed that lifestyle and environmental changes, which began around 1960, have caused subsequent generations to experience more allergies.[1] Prevalence of symptomatic allergy and allergic sensitisation has been found to diminish with age,[1–4] but this decrease in prevalence among older populations might be due to a cohort effect in these particular studies,[1–5] and therefore prevalence in future ageing generations could allegedly increase and allergy could continue to be a problem also for the ageing populations.[6] However, studies on individual changes in sensitisation among adults are sparse, and thus knowledge of the determinants of new onset or loss of sensitisation in adulthood is limited.

Apart from established risk factors such as a genetic predisposition for atopy, it is widely accepted that farm-related exposures such as endotoxin in early childhood confer a lifelong protective effect against atopic disease.[7] In addition, studies of farmers and other agricultural workers have suggested that current farm work and its associated microbial exposures such as endotoxin also protect against sensitisation in adulthood,[8 9] although healthy worker selection could not be completely excluded. A recent longitudinal study conducted in Sweden found that risk factors for new onset of sensitisation were young age and a family history of allergy, whereas the presence of furry pets during childhood appeared to be protective.[4]

Similar cross-sectional results have been found in the SUS cohort,[10] which comprises farming students and controls first studied in the early 1990s. At baseline a decreased prevalence of atopic sensitisation was seen among farming students and among those with a farm childhood.[11] We recently reported that in the follow-up of the same population, the overall risk of new-onset sensitisation to common allergens was negatively associated with farm work and farm animal contact, also after taking childhood exposure into consideration.[12]

In the present study, we go beyond investigating farming exposures using categorical variables. Instead we investigate the impact of quantified occupational endotoxin exposure levels, during adulthood, on change in sensitisation over time, to each of the four common allergens (grass pollen, birch pollen, cat dander and house dust mite (HDM)).

MATERIALS AND METHODS

Study population at follow-up

In 2007 a follow-up of the SUS cohort (n=2371),[10] first studied in 1992–1994, was initiated using the Danish Civil Registration System. The participants initially included in the cohort were all farming students at their first year at farming schools across Denmark and as unexposed controls; a group of young men just drafted for the army without farming exposure were included. At follow-up 26 participants had passed away, 51 participants had emigrated and 32 participants could not be found, and thus 2262 participants were available for follow-up. Of those a further 1092 were lost, as some participants chose not to continue in the follow-up study, while others did not respond to letters or phone calls, or failed to appear for scheduled appointments. A total of 1166 participants were re-examined at follow-up, and blood samples were obtained from 1113 participants for IgE serology. A flow chart of the cohort constitution can be seen in figure 1.

The SUS study was approved by the ethics committee of Aarhus County (AA-19912197) and the Danish Data Protection Agency. The SUS follow-up study was also approved by the ethics committee of Aarhus County (AA-20070074) and the Danish Data Protection Agency. Informed written consent was obtained from all participants.

IgE serology

All the participants were examined at baseline and at 15-year follow-up using the same methodology.[10] The serum was stored at −80°C until analyses. IgE analyses were carried out at the ALK Abelló laboratory in Copenhagen in 2010, applying the ADVIA Centaur method for allergen-specific serum IgE.[13] The specific allergens investigated were cat dander (*Felis domesticus*), birch pollen (*Betula verrucosa*), grass pollen (*Phleum pratense*) and HDM (*Dermatophagoides pteronyssinus*).

Baseline and follow-up serum aliquots from each participant were tested in parallel within the same test run and in duplicate. The specific IgE (sIgE) level was measured for each allergen. Sensitisation was defined as a mean sIgE concentration ≥0.35 kU/L. The duplicate results were highly correlated (r>0.95 for log-transformed values above the cut-off point).

Questionnaires

At baseline, a modified British Medical Research Council questionnaire on respiratory symptoms[14] with additional questions on allergy, asthma, familial history of allergy, smoking and occupational history was used. At follow-up the questionnaire was extended to include further questions on respiratory impairment from the European Community Respiratory Health Survey.[15] Atopic predisposition was defined as a participant's report of at least one parent with asthma and/or rhinitis. Every period of farm employment was registered with duration of work, stable or fieldwork and the type of livestock production involved.

Exposure assessment

Inhalable dust and endotoxin exposure levels were available from 507 personal measurements collected within the follow-up cohort as described elsewhere.[16] Briefly, monitoring was seasonal (summer/winter) involving 327 pig, poultry, mink and cattle farmers employed in 86 farms selected mostly (n=83) from the remaining active population of the cohort. Dust was collected at a flow of 3.5 L/min using Conical Inhalable sampling heads mounted with glass fibre filters. Samples were stored at 20°C until extraction, which was performed in pyrogen-free water with 0.05% (v/v) Tween-20. The amount of dust collected in the filter was estimated gravimetrically, whereas its endotoxin content was estimated with a kinetic chromogenic limulus amebocyte lysate test (Kinetic-QCL 50–650U Kit, Lonza, Walkersville, Maryland, USA) as previously described.[17] Measured inhalable dust levels had an overall geometric mean of 2.5 mg/m^3 (range <lower limit of detection (LOD) to 47.8) and for endotoxin 988 EU/m^3 (range <LOD to 374 000), with pig farmers being exposed on average threefold higher than cattle farmers (geometric mean (GMs) of 3.4 vs 1.0 mg/m^3 and 1490 vs 358 EU/mg^3 for dust and endotoxin, respectively). Mean personal time-weighted (8 hours) average exposure levels for pig and cattle work were estimated from the included measurements on the corresponding type of farmers, whereas for fieldwork the results from 21 task-based measurements performed within the same measurement series were used. Information on the participants' working history, including the number of years of employment and the working hours spent on each type of work for every job held since the beginning of his farming career, was available from the follow-up questionnaire. The cumulative exposure during each employment was calculated as the product of the time-weighted average (TWA) concentration and corresponding work duration in hours per week for stable and fieldwork separately.[18] Assuming a 40 hours workweek, the number of work years multiplied by the relevant TWAs was calculated and summed up for each employment period. The total cumulative exposure was computed throughout each participant's follow-up period as the sum of exposure during all employment periods. For endotoxin and dust the median cumulative exposure was 6.4 (0–70.9) mg/m^3 × years and 1803 (0–31,677) EU/m^3 × years, respectively.

Analyses

New onset and loss of sensitisation to the four specific allergens were considered the outcomes of interest. '*New onset of specific allergen sensitisation*' was defined as participants changing from no sensitisation to the specific allergen at baseline to being sensitised at follow-up. '*New onset sensitisation*' was defined as participants changing from no sensitisation to any of the four allergens at baseline to sensitisation to one or more of the four allergens on follow-up. Analogously '*Loss of specific allergen sensitisation*' was defined as participants changing from being sensitised to a specific allergen at baseline to not being sensitised to the same allergen at follow-up. In addition, '*Loss of sensitisation*' was defined as participants changing from sensitisation to one or several of the allergens at baseline to no sensitisation to any of the four allergens at follow-up.

Cumulative endotoxin exposure was the primary exposure variable of interest divided into exposure quartiles. Analyses were also carried out with three other exposure variables: farm work, contact with animals during follow-up and cumulative organic dust exposure quartiles. '*Current farmer*' was defined as working as a farmer at the point of follow-up, '*ex-farmer*' as having worked as a farmer during the follow-up period but not being a farmer at the follow-up assessment and '*never farmer*' as never having worked on a farm during the follow-up period. The univariate analyses were performed with χ^2 test for categorical variables and the Student's t-test or Kruskal-Wallis test for

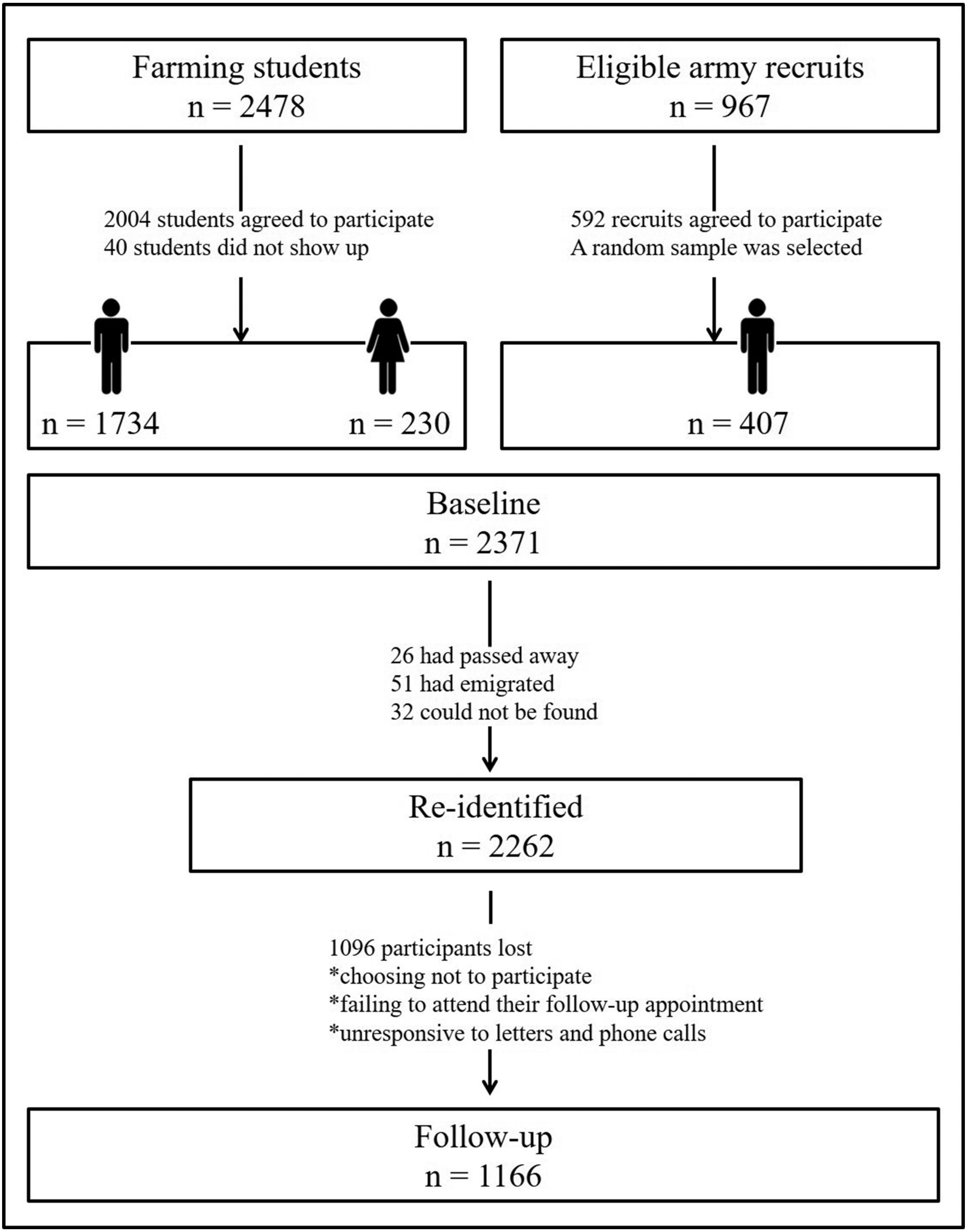

Figure 1 The SUS (Healthy Stable) study population recruitment and dropout flow chart overview.

the continuous variables. Crude risk estimates were calculated as cumulative incidence proportions (CIP). Logistic regression analyses were performed both unadjusted and adjusted for farm childhood (born and raised on a farm), pets during childhood (exposure to cats, dogs or other furry pets in the childhood home) and the participants' baseline smoking status (whether they had ever smoked). The model was not adjusted for age because the age of the participants was almost identical. Models both with and without stratification by atopic predisposition were performed. Unadjusted risk ratios (RR) with 95% CIs were also calculated (online supplementary material 1).

The robustness of the analyses results was further examined for farmers only excluding the controls from the analyses. General additive modelling (smoothing) with cumulative endotoxin exposure (log-transformed) as a continuous variable was used to retest the shape of the exposure–response relationship as previously described. In these models a logit-link function was used to compute the smoothed curves with the degree of smoothing determined using the Akaike's information criterion.[8 19]

All statistical analyses were performed using Stata, except for the smoothing analyses, which were performed with the GAM procedure in SAS V.9.3. The p value used to define significance was 0.05 unless otherwise stated.

RESULTS

Baseline characteristics of the cohort by gender are shown in table 1. The male farming students were younger than both the female farming students and the controls, they were slightly taller than the controls, and they had less hay fever and asthma. Participants lost to follow-up generally smoked more but otherwise did not differ considerably from the participants who stayed in the cohort.

Overall atopic sensitisation at baseline and follow-up

The majority of the participants (n=841) were not sensitised to any of the four common allergens at baseline or at follow-up. A limited group (n=181) was sensitised at both baseline and follow-up, and a relatively small group was sensitised only at the point of follow-up (n=62) or at baseline only (n=30).

Farming exposures and change in allergen-specific sensitisation

Table 2 shows unadjusted CIP and unadjusted ORs of new onset overall and allergen-specific sensitisation by cumulative endotoxin exposure in quartiles during the follow-up period. The results are given for never farmers, ex-farmers and current farmers and for the four endotoxin exposure quartiles. The unadjusted OR for new-onset sensitisation among current versus never farmers and for the higher endotoxin exposure quartiles (vs the lowest) was in general below 1.00 and significantly so for pollen sensitisation (OR range 0.13–0.55). Similar associations were seen using early life farm exposure and quantitatively measured dust as exposure variables (data not shown). Unadjusted RR analyses were carried out for new-onset sIgE and skin prick test (SPT) sensitisation, and a similar apparently protective effect of farm work and the associated endotoxin exposures was seen (see online supplementary tables S1 and S2). RRs were performed for loss of sIgE and SPT sensitisation (see online supplementary tables S3 and S4). Both childhood and adult farm exposures appear to be associated with a loss of sensitisation to the specific allergens.

Figure 2 shows the results of adjusted logistic regression analyses of the associations between cumulative endotoxin exposure during follow-up and new onset and loss of sensitisation measured by sIgE. Increased endotoxin exposure was associated with a significantly decreased risk for new onset of sIgE sensitisation to birch pollen (fourth quartile exposure OR 0.35 (0.13–0.91)), grass pollen (fourth quartile exposure OR 0.14 (0.05–0.5)) and non-significantly cat allergens (fourth quartile exposure OR 0.21 (0.02–2.05)). In the adjusted analyses of loss among participants with sensitisation at baseline, an association appeared for increased loss of sIgE sensitisation to birch and grass pollen with increased endotoxin exposure levels, with a p value ranging from 0.07 to 0.9. No such associations were noted for changes in HDM sensitisation; most ORs were not far from 1, except for the third quartile of endotoxin exposure, which showed a non-significant trend to decreased loss of sensitisation.

Both new onset and loss analyses were also carried out for SPT sensitisation (see online supplementary figure S1) and similar associations were seen.

All analyses of changes in sIgE were also carried out with a cut-off of 0.70 kU/L; this however barely changed the results (online supplementary table S5).

Sensitivity analysis of the data with non-parametric methods (smoothing), after excluding rural controls, confirmed the negative effect of endotoxin exposure on new-onset IgE sensitisation against any common allergen (figure 3).

DISCUSSION

This is the first longitudinal study to follow changes in allergic sensitisation for more than a decade in young adults. The most remarkable finding was the inverse association between quantitatively assessed work-related high exposure to endotoxin and new onset of pollen sensitisation. Increased endotoxin exposure levels also showed a negative relation with new-onset cat sensitisation, with similarly low ORs, although not reaching statistical significance. In contrast, loss of HDM sensitisation did not appear to be related with the level of endotoxin exposure and neither to job history of farm work or farm animal exposure. The observed effects were independent of childhood farm exposure.

Almost half of the original cohort was lost to follow-up. However, the dropout analysis, showed relatively small differences with regard to general demographics and baseline sensitisation, also between sexes. To scrutinise the potential gender imbalance, the main analyses were also carried out for male participants only, and the same associations were seen (online supplementary table S6, endotoxin exposure and OR for new-onset sensitisation for women only). The male farmers who were lost to follow-up did have slightly more asthma than the male farmers who stayed. The participants who were lost to follow-up though generally smoked more; however, currently there is no scientific consensus regarding the association between smoking and atopy, and in this study smoking was not associated with atopy. Therefore, it appears that the effect of selection bias would be relatively small.

The cohort consists of all farming students entering into farming schools and a group of young men drafted for the army in Denmark over a 2-year period in the early 1990s. Due to the limited number of women pursuing farm education or military service at that time, the cohort comprises primarily of young Danish men with a mean age of 19 years at baseline. The farming production methods used in Denmark were and still are comparable to those in other countries such as The Netherlands and Iowa, USA. We have applied standard methods for blood sampling and sIgE analyses, and a major strength was the parallel

Table 1 Demographic and health characteristics of the original cohort (n=2371)

	Participants (n=1166)			Lost to follow-up (n=1205)		
	Female students (n=109)	Male students (n=885)	Controls (n=172)	Female students (n=121)	Male students (n=849)	Controls (n=235)
Demographics						
Age, years*	19.2 (17.3–40.9)	18.6 (16.8–38.8)§	19.0 (18.5–23.1)	19.1 (16.9–46.9)	18.5 (16.8–48.9)§	18.9 (17.8–23.2)
Height, cm†	169.3 (±7.13)	182.1 (±6.99)¶	180.8 (±6.88)	169.9 (±6.83)	181.6 (±6.75)¶	180.4 (±7.02)
Weight, kg*	75 (52–173)**	90 (55–186)	87 (58–181)	NR	NR	NR
Smokers, N (%)‡	28 (25.7%)	245 (27.7%)††	49 (28.5%)	45 (37.2%)	309 (36.4%)††	87 (37.0%)
Relevant exposures						
Farm childhood, N (%)‡	36 (33.0%)	514 (58.1%)	29 (16.9%)	NR	NR	NR
Pets	93 (85.3%)	763 (86.2%)	153 (88.9%)‡‡	105 (86.8%)	725 (85.4%)	182 (77.5%)[5]
Atopy						
Sensitisation prevalence, N (%)	14 (12.2%)	122 (13.8%)	43 (25.0%)	13 (10.9%)	131 (15.6%)	59 (25.1%)
Maternal predisposition, N (%)‡	11 (10.1%)	81 (9.2%)	15 (8.7%)	17 (14.0%)	81 (9.5%)	21 (8.9%)
Paternal predisposition, N (%) ‡	12 (11.0%)	77 (8.7%)	25 (14.5%)	8 (6.6%)	55 (6.5%)	22 (9.4%)
Respiratory status, N (%)‡						
Asthma (ever)	9 (8.3%)	39 (4.4%)[4]	11 (6.4)	9 (7.4%)	60 (7.1%)††	15 (6.4%)
BHR	7 (6.4%)	80 (9.0%)	11 (6.4%)	13 (10.7%)	86 (10.1%)	21 (8.9%)
Hay fever (ever)	16 (14.7%)	74 (8.4)[1]	25 (14.5%)	18 (14.9%)	83 (9.8%)§	35 (14.9%)
Lung function, mean (±SD)						
FEV_1†	3.56 (±0.54)	4.68 (±0.59)	4.78 (±0.64)	3.58 (±0.49)	4.54 (±0.60)	4.66 (±0.62)
FVC†	4.09 (±0.66)	5.48 (±0.70)	5.58 (±0.75)	4.09 (±0.59)	5.39 (±0.72)	5.47 (±0.75)

*Median (min – max).

†Mean (±SD).

‡May vary due to missing values. NR, no record, as weight was only measured at follow-up. Differences within the participants and the 'lost to follow-up'.

§p≤0.05, male farmers versus controls and female farmers by Kruskal-Wallis test.

¶p≤0.05, male farmers versus controls by Student's t-test.

**p≤0.05, female farmers versus male farmers and controls by Kruskal-Wallis test. Differences between participants versus lost to follow-up.

††p≤0.05 male farmers versus male farmers lost to follow-up by X^2 test.

‡‡p≤0.05 controls versus controls lost to follow-up by X^2 test.

BHR, bronchial hyperresponsiveness; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Table 2 New onset of specific IgE sensitisation depending on exposure as unadjusted CIP and OR

	Any common allergen*					Grass pollen					Birch pollen					Cat dander					House dust mite (DerP1)				
	N	n	CIP	OR	95% CI	N	n	CIP	OR	95% CI	N	n	CIP	OR	95% CI	N	n	CIP	OR	95% CI	N	n	CIP	OR	95% CI
Farmer status	885	58				994	30				1038	37				1068	9				969	32			
Current farmer	370	22	0.06	0.61	(0.28 to 1.29)	405	4	0.01	0.13†	(0.04 to 0.42)	420	11	0.03	0.33†	(0.14 to 0.78)	426	1	0.002	0.12	(0.01 to 1.16)	395	13	0.03	0.88	(0.31 to 2.53)
Ex-farmer	398	25	0.06	0.65	(0.31 to 1.36)	449	16	0.04	0.48	(0.21 to 1.08)	472	15	0.03	0.40†	(0.18 to 0.90)	486	5	0.01	0.53	(0.13 to 0.24)	439	14	0.03	0.86	(0.30 to 2.42)
Never farmer	117	11	0.09	1		140	10	0.07	1		146	11	0.08	1		156	3	0.02	1		135	5	0.04	1	
Endotoxin (EU/m^3 × years)	881	58				989	30				1033	37				1063	9				965	32			
Fourth quartile	233	13	0.06	0.60	(0.29 to 1.26)	254	4	0.02	0.19†	(0.06 to 0.58)	261	7	0.03	0.38†	(0.15 to 0.92)	261	1	0.004	0.25	(0.03 to 2.26)	244	7	0.03	0.96	(0.33 to 2.77)
Third quartile	228	10	0.04	0.47	(0.21 to 1.04)	254	3	0.01	0.14†	(0.04 to 0.50)	263	3	0.01	0.16†	(0.05 to 0.54)	269	1	0.004	0.24	(0.03 to 2.19)	246	7	0.03	0.95	(0.33 to 2.75)
Second quartile	219	17	0.08	0.86	(0.43 to 1.71)	245	5	0.02	0.25†	(0.09 to 0.69)	260	10	0.04	0.55	(0.24 to 1.22)	268	3	0.01	0.74	(0.16 to 3.33)	241	11	0.05	1.55	(0.59 to 4.07)
First quartile	201	18		1		36	18	0.08	1		249	17	0.07	1		265	4	0.02	1		234	7	0.03	1	

*Any common allergen: grass, birch, cat and/or house dust mite.
†p<0.05.
CIP, cumulative incidence proportion; N, population; n, cases.

testing of baseline and follow-up serum samples in the same test runs to achieve maximal precision. The constitution of the cohort, the longitudinal study design and the techniques applied are comparable with other studies in the field, and we therefore expect the results to be valid also for other population in countries that apply the same farming practices as in Denmark.

The vast majority of participants in the investigated cohort were born in the 1970s and were therefore mostly in their mid-30s at the time of follow-up. Overall, a small increase in sIgE sensitisation prevalence (from 19% to 22%) was observed at follow-up, which according to Linneberg *et al*[1] would be an expected sIgE sensitisation increase in this age group during the same time period. The CIP for new onset of sIgE sensitisation to any common allergen in our study was 0.07 (table 2), which corresponds to an underlying annual incidence rate of CIP/15×100%=0.47% per year.

Our results indicate that farming and the related endotoxin exposure can have a protective effect on sensitisation even in young adults, and that much of this effect may be mediated by this farm work-associated airborne exposure to endotoxin and other organic dust agents. This supports previous results from cross-sectional studies that adult farming exposure can have a protective effect on atopy.[19 20] At this point, it cannot be predicted whether the prevalence of sensitisation will continue to increase with advancing age, although data from other cohorts indicate that the highest prevalence would occur in early adulthood.[21–24]

New-onset allergen sensitisation in childhood can be attributed to both the host constitution and environmental exposures.[25] Endotoxin being a part of the cell wall of Gram-negative bacteria is a common environmental exposure both outdoor and indoor. Endotoxin is therefore also present in our home environment. Population-based studies have reported endotoxin levels in European and American homes to range from 0.005 to 17.74 EU/m^3 measured by active stationary sampling.[26–28] In the present study the measured occupational endotoxin exposure in the pig farms reached a mean of 988 EU/m^3, which must be considered substantially higher than the reported domestic levels. As there are few published longitudinal studies on changes in sensitisation in large adult populations, specific determinants such as endotoxin for persistent or new-onset sensitisation in adulthood have not yet been carefully studied. This study demonstrated that continuous occupational farm-related endotoxin exposure is associated with both a decreased risk of new onset and a tendency of increased loss of pollen sIgE sensitisation, and similar results were seen for SPT. We also demonstrated the beneficial farming effect using the categorical variables of 'current farm work' and 'contact with swine and/or cattle' during follow-up.[16] A negative association between endotoxin exposure and new-onset sensitisation was also confirmed with non-parametric analyses. The latter analysis was restricted to exposed individuals, which further support that the demonstrated association is not simply a result of a difference between farmers and non-farmers.

The protective effect of endotoxin exposure on new atopic sensitisation was strongest for grass and birch pollen. In general the inverse relations between farming and farm life exposures and atopy are in most studies more evident for pollen than for mite sensitisation.[8 9 29] However, changes in sensitisation to cat allergens appeared to be similarly dependent on farm-related exposures. New-onset cat IgE positivity showed for current farming and at high endotoxin exposure ORs <0.3–0.5, but these were non-significant (table 2, figure 2). In contrast, new HDM sensitisation was apparently not influenced by farm work exposures (figure 2, table 2), and loss of HDM sensitisation seemed even less likely at higher farm-related exposures. These

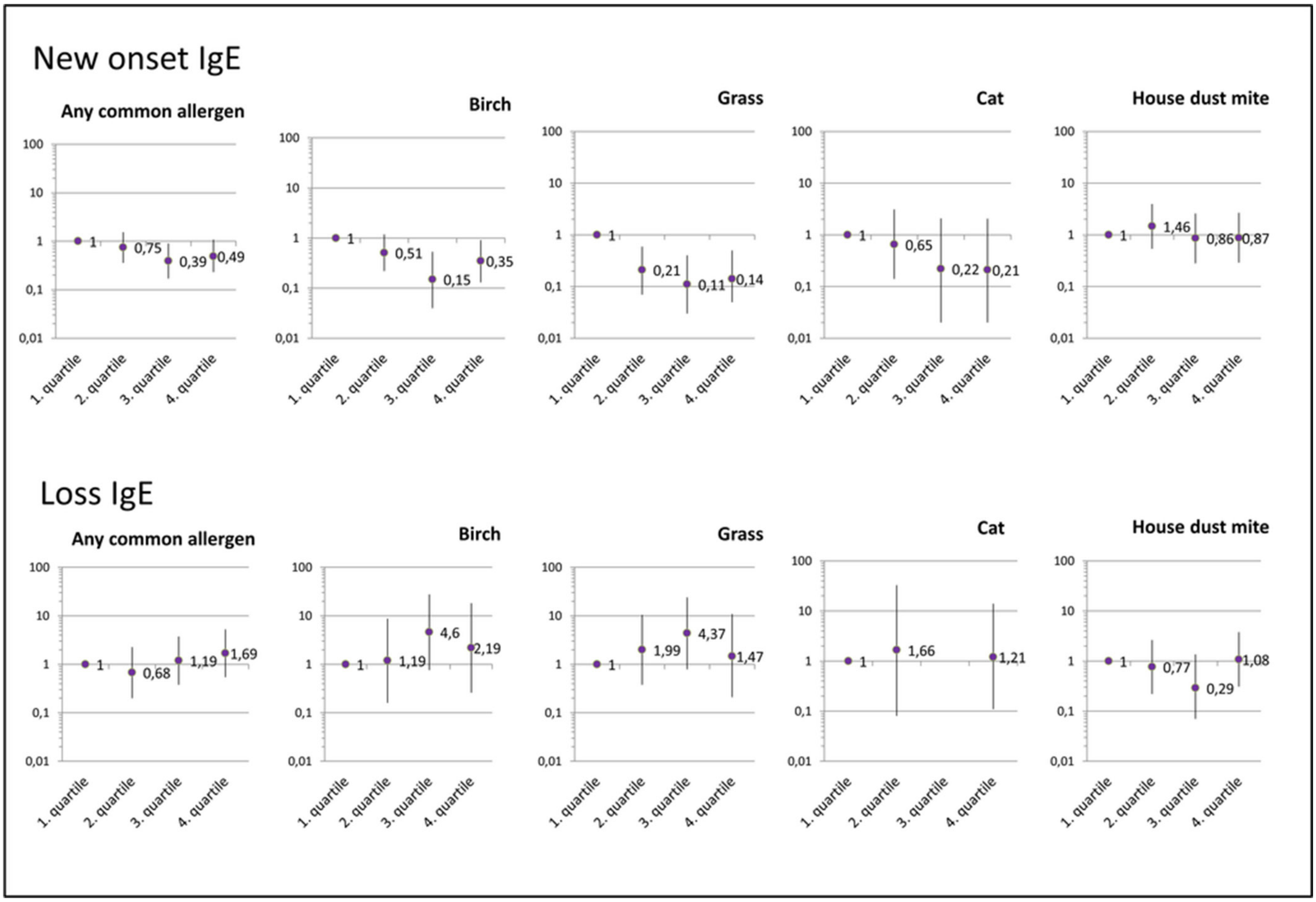

Figure 2 The effect of endotoxin exposure on new onset and loss of specific allergen sensitisation measured by specific IgE, presented as adjusted OR with 95% CIs. Any common allergen refers to sensitisation to birch, grass, cat and/or house dust mite. The analysis is adjusted for, farm childhood, exposure to pets during childhood and smoking.

findings are in fact in line with those of various other studies in both children and adults, as summarised in a recent systematic review and meta-analysis by Campbell *et al*[30] of studies with IgE serological data from farm children, farmers and respective control groups.

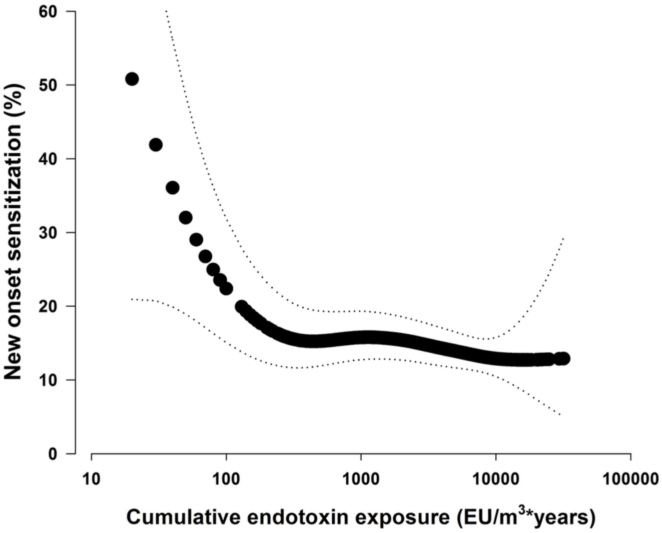

Figure 3 Effect of cumulative endotoxin exposure on new-onset sensitisation against any of the four common allergens as defined by the IgE level among exposed participants. Dotted lines represent 95% confident limits. Results are adjusted for pets, smoking and farm childhood.

In several cross-sectional studies, a farm childhood and specifically exposure to farm animals and endotoxin in childhood have been shown to decrease the prevalence of allergic disease[8 31–34] and may even facilitate a lifelong protective effect.[35] Cross-sectional studies have also found adult occupational exposures to have a protective effect on allergy, independent of childhood exposures.[19 20] With its longitudinal perspective, this study supports that childhood farming exposure is protective, but furthermore that adult farming and endotoxin exposures are associated with an independent protective effect.

Continued farming exposure and increased exposures to endotoxin in adulthood seemed to promote loss of pollen sensitisation, although the effects were not significant, likely due to the low absolute numbers in these analyses. Loss of sensitisation has been described in only a few studies.[6 36] In 2002 a small longitudinal study found that children of farming parents had a significantly enhanced chance of losing their SPT positivity over time.[37] It has been suggested that such reversal of allergic sensitisation might occur as a result of childhood exposure to endotoxins and other pathogen-associated molecular patterns (PAMPs) acting as environmental immunoregulatory factors.[7] Endotoxin is thought to activate innate immunity by increasing the expression of Toll-like receptors[38] and is also associated with higher regulatory T-cell numbers.[39] Generally exposure to PAMPs upregulates Interleukin-10 (IL-10), which can lead to a downregulation of the immune response.[40] This is corroborated by an extended downregulation of tumour necrosis factor alpha (TNF-α) for weeks, which can be seen in naïve subjects after only 3 hours of exposure to the environment inside a swine confinement,[41] although the precise mechanism still remains elusive. Our findings may be regarded as indication that such mechanisms are operative in childhood and in young adulthood, and it emphasises that continued farming exposure might be beneficial for the immune system.

Our results using quantitatively assessed work-related exposure support the hypothesis that ongoing exposure to PAMPs such as endotoxin modifies immune responses to environmental allergens towards a non-atopic phenotype, also in adulthood, as suggested previously.[8,19] Ege *et al* argued that, most likely, it is the diversity and wider range of microbial strains present in the farming environment that contribute to the beneficial effects of farming exposure, rather than a single agent such as endotoxin.[42] Thus, although we have found endotoxin exposure to be associated with the current sensitisation status, we recognise that the beneficial effect of farming exposure is likely to be a combination of diversity and quantity of microbial strains.

CONCLUSIONS

Our study indicates that adult exposure to endotoxin attenuates the pollen sensitisation rate. The high levels of endotoxin or other microbial exposures encountered on a farm has a protective effect against new-onset sensitisation to common allergens, especially grass and birch pollen, independent of childhood farm exposure.

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Contributors GE has carried out the analyses and interpretation of the data included in this manuscript. Furthermore, she has drafted the entire manuscript as the first author, and has been responsible for including and amending the manuscript according to the coauthors' suggestions. VS took part in the acquisition and interpretation of data. She also took part in drafting and revising it critically for important intellectual content and final approval of the version to be published. GD contributed to analysis and interpretation of data. He also took part in drafting and revising it critically for important intellectual content and final approval of the version to be published. IB took part in the acquisition and interpretation of the data. He also took part in revising it critically for important intellectual content and final approval of the version to be published. ACSB took part in the acquisition and interpretation of the data. She also took part in revising it critically for important intellectual content and final approval of the version to be published. CH contributed to the conception and design of the study and acquisition of data. She also took part in revising it critically for important intellectual content and final approval of the version to be published. PMG contributed to the acquisition of data. She also took part in revising it critically for important intellectual content and final approval of the version to be published. ØO contributed to the conception and design of the study, and also the acquisition and interpretation of data. He also took part in revising it critically for important intellectual content and final approval of the version to be published. TS contributed to conception and design of the study. He also contributed to the acquisition of data, analysis and interpretation of data. He additionally took part in drafting the article and revising it critically for important intellectual content. Furthermore, he also took part in the final approval of the version to be published.

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