Eur Respir J 2012; 39: 573–581
DOI: 10.1183/09031936.00164410

Respiratory health and endotoxin: associations and modification by *CD14*/-260 genotype

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ABSTRACT: Exposure to endotoxin has been associated with increased respiratory symptoms and decrements in lung function in occupational settings but little is known about the health effects of domestic exposure in adults. Here, we describe the association of respiratory disease, immunoglobulin (Ig)E sensitisation, bronchial reactivity and lung function with mattress endotoxin levels in adults, and determine whether these associations are modified by polymorphisms in *CD14*.

Endotoxin levels in mattress dust from a population-based sample of 972 adults were measured. Associations were examined using generalised linear mixed models, adjusting for individual and household confounders. Effect modification of these associations by *CD14*/-260 (rs2569190) was assessed.

Mattress endotoxin levels varied from 0.1 to 402.6 EU·mg^{-1}. Although there was no overall association of lung function with endotoxin exposure, there was evidence that the association of forced expiratory volume in 1 s and forced vital capacity with endotoxin was modified by *CD14*/-260 genotype (p-value for interaction 0.005 and 0.013, respectively). There was no evidence that symptoms, IgE sensitisation or bronchial reactivity were associated with mattress endotoxin levels.

In this large epidemiological study of adults, there was no evidence that mattress endotoxin level was associated with respiratory symptoms or IgE sensitisation but the association of lung function with endotoxin levels may be modified by *CD14* genotype.

KEYWORDS: Adults, *CD14*/-260 genotype, endotoxin, lung function, respiratory symptoms

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Received:
Oct 20 2010
Accepted after revision:
July 24 2011
First published online:
Sept 01 2011

Endotoxin is a lipopolysaccharide molecule derived from the cell membrane of many Gram-negative bacteria, and is present in indoor [1], occupational [2] and outdoor environments [3]. There is substantial literature suggesting endotoxin exposure is associated with increased respiratory symptoms and decrements in lung function, particularly in environments with very high endotoxin exposure (*e.g.* pig farming and food processing) [2]. In the home environment, endotoxin exposure is much lower, but there have been reports that even at these levels, exposure is associated with an increased prevalence of wheeze in the first year of life [4], increased prevalence of sensitisation in 2-yr-olds [5], increased peak flow variability and asthma exacerbations in children [6], and increased prevalence of asthma [1] and asthma severity [7] in adults. In contrast, other studies suggest that residential exposure may lead to lower levels of allergic disease [8] and lower levels of immunoglobulin (Ig)E sensitisation in children [9] and a lower prevalence of severe IgE sensitisation in adults [10].

There is some evidence that polymorphisms in the *CD14* gene are associated with atopic asthma [11], but more importantly, there is emerging evidence that these polymorphisms modify the effect of endotoxin on atopy and atopic disease. This may, to some extent, explain some of the reported inconsistencies in the association of endotoxin with disease. A recent review [12] identified four studies (three in children and one in families including adults) that supported a protective effect of carriage of the C allele of the *CD14*/-260 genotype for atopy on exposure to endotoxin in the home environment.

We do not know of any reported population-based studies in adults that examined the association of

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

respiratory outcomes with measured domestic endotoxin and possible effect modification by *CD14*. This report uses information collected as part of the European Community Respiratory Health Survey (ECRHS) and HITEA (Health Effects of Indoor Pollutants: Integrating Microbial, Toxicological and Epidemiological Approaches) to explore these associations.

METHODS

Sample

The methodology of the ECRHS II has been described previously [13]. Briefly, 29 centres performed a follow-up investigation of asthma, allergy, and their known or suspected risk factors (ECRHS II) in a random population sample of adults aged 20–44 yrs at the baseline survey (ECRHS I, 1992–1994). In 2000–2002, participants were invited to a testing centre for interview, venesection for assessment of IgE sensitisation and lung function testing (forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and bronchial reactivity to methacholine). Whole blood samples were taken for DNA extraction (except for centres in Italy and Iceland) at Helmholtz-Centrum (Munich, Germany).

In 22 centres in 10 countries, the homes of a sample of participants were visited to assess home exposures and to obtain a mattress dust sample. The objective was to assess 200 homes per centre, with home visits occurring as soon as possible after clinical assessment. Priority was given to participants who had not moved home between 1992–1994 and 2000–2002, and who, in 1992–1994, provided a blood sample for serum specific IgE testing.

Our study included 7,122 randomly selected individuals living in centres that took part in the indoor assessment (Italy: two centres; Belgium: two centres; Germany: two centres; Spain: five centres; France: two centres; UK: two centres; Sweden: three centres; Iceland: one centre; Switzerland: one centre; and Estonia: one centre). Of these, 3,043 had their mattress sampled, and 2,889 had sufficient dust for analysis for house dust mite and cat allergens. Only 2,124 had dust left after these tests and of these, 974 were randomly selected for endotoxin measures. Two participants were excluded (one for missing data and another because they were later identified as not randomly selected).

Dust sampling

Homes visits occurred from July 2000 to November 2002 in random order covering all seasons (median time between clinic visit and home visit 158 days, interquartile range 46–263 days). Fieldworkers were trained using a short video. The participant's bed was stripped of bedding, but mattress covers or protectors that had been in place for $\geqslant 3$ months were left. A template of 80×125 cm was placed on the area where the participant usually slept. An ALK dust collection filter (ALK-Abello, Hørsholm, Denmark) was attached to an Electrolux Mondo vacuum cleaner (1,300 W; Electrolux, Stockholm, Sweden) and the area within the template (1 m^2) was vacuumed for 2 min. Within 3 days, samples were frozen for 24 h (to kill mites) and then stored at room temperature until transported with silica gel desiccant to a central laboratory. Samples were sieved to obtain fine dust for extraction and frozen at -20°C. The total dust weight was not recorded.

Endotoxin measurement

A random subsample of dust samples were thawed in 2008, split into approximately equal and homogeneous fractions of about 50–70 mg, and transferred to pre-weighed 10-mL polystyrene vials with screw caps (Sterilin, Newport, UK). Samples were sent within days to Utrecht University (Utrecht, the Netherlands) and extracted in the same vials using a three-step procedure [14]. Endotoxin analysis was performed using the *Limulus* amoebocyte lysate assay (Lonza, Verviers, Belgium) with *Escherichia coli* endotoxin as the standard [15]. Results were expressed as endotoxin units (EU) per mL extract, and converted to an endotoxin concentration in $EU \cdot mg^{-1}$ sieved dust by multiplication by the extraction volume (2.5 mL) and division by the vial dust weight (in mg).

Genotyping

In 2006, stored DNA was tested for single-nucleotide polymorphisms (SNPs) within the *CD14*, *TLR2* and *TLR4* genes using the SNPlex™ platform (Applied Biosystems, Foster City, CA, USA) according to manufacturer instructions, and analysed on an Applied Biosystems 3730/3730xl DNA Analyser. Allele calling was performed by clustering analysis using Genemapper® version 4.0 (Applied Biosystems). The genotype call rate was >98%. Genotyping quality was controlled by inclusion of internal positive and negative controls provided by the manufacturer in the reaction plates, and incorporation of six duplicate samples of two HapMap reference trios in the genotyping process. Both genotype concordance and correct Mendelian inheritance were verified.

Asthma score

A continuous measure of asthma symptoms ranging from 0 to 5 was used [16]. It is the sum of positive responses to questions regarding the following symptoms in the previous 12 months: wheeze with breathlessness, chest tightness, attack of shortness of breath (SOB) at rest, SOB after exercise and being woken by SOB.

Lung function

Each participant was given up to nine attempts to provide two technically satisfactory forced expiratory manoeuvres. The highest recorded FEV_1 and FVC were used to derive age-, sex- and height-standardised residuals based on the prediction equations of QUANJER *et al.* [17] for FEV_1, FVC and FVC/ FEV_1.

Other respiratory outcomes

IgE sensitisation was defined as presence of allergen-specific IgE >0.35 $kU \cdot L^{-1}$ (Pharmacia, Uppsala, Sweden) in 2000–2002. Atopy was defined as any positive response to any of the three allergens tested (grass pollen, house dust mite and/or cat).

Bronchial challenge

Bronchial hyperresponsiveness (BHR) to methacholine was measured using a dosing schedule that delivered methacholine to a maximum dose of 1 mg. Methacholine was delivered *via* a Mefar dosimeter (Mefar, Bovezzo, Italy), FEV_1 was recorded 2 min after each inhalation and the test stopped when either a 20% fall in FEV_1 had been achieved or the final dose had been given [18–20]. The term ''slope'' is used for transformed logarithmic slope as used in the ECRHS [18–20] with a low slope indicative of high BHR, with values ranging from 1 to 20 [21].

Statistical analysis

The association of asthma score with the level of exposure to mattress endotoxin (log-transformed to account for skewness) controlling for personal factors (age, sex and smoking status in pack-yrs) and for household variables (keeping a cat/dog, age of mattress, presence of mould in the bedroom, presence of dampness in the bedroom, household density and age of home) was assessed using a random-intercept, negative binomial model set to take account of clustering of individuals within countries.

The association of IgE sensitisation to any of grass pollen, house dust mite or cat allergen was assessed using generalised linear mixed models controlling for the same personal and household factors. Similar analyses were conducted to examine associations with: geometric mean (GM) total IgE; BHR; age-, sex- and height-standardised residuals of FEV_1; age-, sex- and height-standardised residuals of FVC; and age-, sex- and height-standardised residuals of FEV_1/FVC.

Effect modification of the association of respiratory outcomes with endotoxin level by *CD14*/-260 genotype was tested by the inclusion of an interaction term assuming an additive genetic model.

Linearity of observed associations was tested with the use of generalised additive mixed models (GAMMs).

Statistical analyses were conducted using STATA 10 (Stata Corporation, College Station, TX, USA) with GAMMs also performed in R (www.r-project.org).

In all centres, permission to conduct this study was obtained from appropriate local ethics committees.

RESULTS

Participants

This analysis included 972 adults living in 21 centres in 10 countries. Compared with participants in the ECRHS II, but who did not have endotoxin measured (n=6,150; see Methods section), our sample had a similar proportion of males (50.6% *versus* 50.5%; p=0.400) and was older (mean age 44.4 *versus* 42.5 yrs; p<0.001). There was a similar proportion of smokers (29.1% *versus* 29.6%; p=0.440) and of those reporting wheeze in the last year (21.7% *versus* 19.9%; p=0.107). Asthma score was similar (Chi-squared test p=0.297). Genotyping was not performed in Italy or Iceland; in the remaining centres, 63.7% of participants agreed to genotyping.

Endotoxin levels varied from 0.1 to 402.6 EU·mg^{-1} (GM 2.4 EU·mg^{-1}, 95% CI 2.2–2.6 EU·mg^{-1}; 5th percentile 0.3 EU·mg^{-1}, 95th percentile 17.8 EU·mg^{-1}).

Table 1 presents demographic data of those included in this analysis and descriptive statistics for the outcomes considered.

Asthma score

Overall, there was no association of asthma score with mattress endotoxin level (table 2), even when asthma was considered separately in atopic and nonatopic participants (all p>0.1; data not shown). There was no evidence of a non-linear association (using a smoothing spline in GAMM, p=0.207). There was no evidence that the association was present in some countries and not in others (test of between country heterogeneity, p>0.1), although the low number of subjects in some countries limited the extent to which this could be explored. Results were similar whether asthma score was analysed in its recommended form (a continuous variable) and as a binary variable (positive answer to any of the five questions compared to negative answers to all) (table 2).

The association of asthma score with endotoxin level was not modified by *CD14* genotype (interaction p>0.05) using an additive genetic model. However, there were differences in the association between the genotypes, such that the adjusted ratio of mean asthma score per 10-fold change in endotoxin level was 1.86 (95% CI 1.19–2.91) for the CT allele (table 2).

TABLE 1 Description of sample

	Total with information n	Proportion
Age yrs	972	44.4 (38.0–53.6)
Females	481/972	49.4
Asthma score		
0	673/972	69.1
1	172/972	17.7
2	75/972	7.7
3	23/972	2.3
4	18/972	1.8
5	12/972	1.2
Smokers		
Never	408/972	41.9
Ever	280/972	28.8
Current	283/972	29.1
Hay fever/nasal allergies	251/972	25.8
IgE to HDM	140/972	14.4
IgE to grass pollen	117/972	12.0
IgE to cat	66/972	6.7
IgE to HDM, grass pollen or cat	235/972	24.1
Geometric mean IgE kU·L^{-1}	874	39.8
Proportion with FEV_1<LLN#	54/909	6.9
Proportion with FVC<LLN#	46/909	5.1
Cat or dog in house	316/972	32.5
Mould in bedroom	63/972	6.4
Damp in bedroom	43/972	4.4
House built before 1970	467/972	48.0
Mattress <1 yr old	53/972	5.5
Household density	961	0.75 (0.3–1.3)
***CD14*/-260 rs2569190¶**		
CC	126/561	22.4
CT	282/561	50.2
TT	153/561	27.3

Data are presented as median (interquartile range) or %, unless otherwise stated. Ig: immunoglobulin; HDM: house dust mite; FEV_1: forced expiratory volume in 1 s; LLN: lower limit of normal; FVC: forced vital capacity. #: LLN from [22]; ¶: in addition, two tagging single-nucleotide polymorphisms (tagSNPs) in *TLR2* (rs1816702 and rs1898830) were found in 562 and 560 individuals, respectively, and five tagSNPs in *TLR4* (rs1554973, rs1927914, rs2737191, rs10759930 and rs11536889) in 567, 564, 567, 566 and 516 individuals, respectively.

TABLE 2 Unadjusted and adjusted ratios of asthma score per 10-fold increase of endotoxin levels with 95% confidence intervals for the entire sample and stratified by *CD14* genotype

	Participants			*CD14/-260* genotype			p-value	
	All	Full confounder information	Full confounders and genotype	CC	CT	TT	Heterogeneity of effect	Trend of interaction[#]
Participants n	972	872	552	120	283	149		
Asthma score								
Continuous[¶]								
Unadjusted	1.16 (0.93–1.46)	1.14 (0.90–1.44)	1.15 (0.85–1.54)	0.55 (0.27–1.10)	1.82 (1.20–2.74)	1.02 (0.59–1.77)	0.011	0.291
Adjusted[+]		1.12 (0.88–1.43)	1.12 (0.82–1.52)	0.63 (0.31–1.27)	1.86 (1.19–2.91)	1.11 (0.61–2.01)	0.032	0.374
Binary[§]								
Unadjusted	1.18 (0.89–1.57)	1.14 (0.84–1.54)	1.11 (0.76–1.63)	0.44 (0.18–1.06)	2.01 (1.13–3.56)	1.00 (0.5–2.00)	0.015	0.201
Adjusted[+]		1.14 (0.83–1.57)	1.10 (0.73–1.65)	0.49 (0.18–1.31)	2.12 (1.15–3.91)	0.96 (0.43–2.13)	0.033	0.267

[#]: assuming additive model; [¶]: continuous measure ranging from 0 to 5; [+]: confounders were age, sex, smoking status in pack-yrs and household variables (keeping a cat or dog, age of mattress, presence of mould in bedroom, presence of damp in bedroom, household density, and age of home); [§]: binary measure comparing positive answers to any of the five questions with negative answers to all five.

IgE sensitisation and BHR

There was no significant association of IgE sensitisation to house dust mite, grass or cat allergen with endotoxin level or any association of total IgE and BHR slope with endotoxin level (table 3).There was no evidence that these associations were modified by *CD14/-260* genotype (data not shown).

Lung function

Overall, there was no evidence of an association of either FEV1, FVC or FEV1/FVC with endotoxin levels (table 4), even if analyses were stratified by atopy (data not shown).

There was, however, strong evidence that the association of FEV1 and FVC with endotoxin levels was modified by *CD14* genotype (test for heterogeneity of effect p=0.008 and p=0.029, respectively). For a 10-fold increase in mattress endotoxin level, those with the CC genotype showed an improvement in FEV1 residuals of 0.62 standard deviations (95% CI 0.21–1.04) greater than the predicted value. In those carrying the TT genotype, a similar increase in exposure was associated with a nonsignificant decrement in lung function (-0.22; 95% CI -0.59–0.13). The CT genotype showed an intermediate association (trend across the three genotypes p=0.005).

Figure 1 shows the smoothed association using GAMM, confirming linearity in each of the three genotypes (using a smoothing spline, p>0.05).

Table 5 presents some results stratified by atopy. Table 5 should be interpreted cautiously as the effect estimates in atopics and nonatopics by each genotype are based on small numbers (as shown by the wide confidence intervals). In both atopics and nonatopics, the relationship of lung function with endotoxin was different in the three *CD14* genotypes for FEV1 (p=0.028 and p=0.001, respectively) (table 5). These different effects by genotype were seen for FVC in nonatopics (p=0.008) and for FEV1/FVC ratio (p=0.018) in atopics. However, statistical testing showed that any apparent differences between atopics and nonatopics could have arisen by chance, and that the effect of *CD14* genotype on lung function responses to endotoxin was the same in atopics and nonatopics.

There was no evidence of between-country heterogeneity in this gene–environment interaction, although the number of individuals with complete data in each country was relatively small. In figure 2, we show the change in FEV1 standardised residuals per 10-fold increase in mattress endotoxin levels by northern (Sweden, Estonia and Iceland), middle (UK, Belgium, France, Germany and Switzerland) and southern (Spain and Italy) Europe. Variation in the association of FEV1 with endotoxin by *CD14* genotype was most clearly seen in the northern European centres, but in each of the three regions, data were consistent with the overall findings.

Other genotypes

TLR genes have also been proposed to modify the response to the endotoxin-rich farming environment [23]. As participants had also been genotyped for SNPs within *TLR2* (rs1816702 and rs1898830) and *TLR4* (rs10759930, rs1927914, rs1554973, rs2737191 and rs11536889) associations were tested in a similar manner to *CD14*. In this *post hoc* analysis, there was no evidence that any of the SNPs modified the association of

TABLE 3 Unadjusted and adjusted associations with 95% confidence intervals of serum specific immunoglobulin (Ig)E, total IgE and bronchial hyperresponsiveness (BHR) per 10-fold increase in mattress endotoxin level

Health outcome	Unadjusted	Adjusted#
OR		
IgE¶ to HDM	0.96 (0.65–1.43) (n=972)	0.98 (0.65–1.49) (n=790)
IgE¶ to grass	1.01 (0.67–1.53) (n=876)	0.99 (0.63–1.58) (n=790)
IgE¶ to cat	0.81 (0.47–1.39) (n=876)	0.63 (0.34–1.15) (n=790)
IgE¶ to HDM, grass or cat	0.93 (0.68–1.27) (n=876)	0.91 (0.64–1.28) (n=790)
Regression coefficient		
Log total IgE	-0.0007 (-0.08–0.08) (n=874)	0.008 (-0.07–0.09) (n=788)
BHR slope	-0.16 (-0.49–0.15) (n=716)	-0.02 (-0.37–0.32) (n=642)

HDM: house dust mite. #: confounders were age, sex, smoking status in pack-yrs and household variables (keeping a cat or dog, age of the mattress, presence of mould in bedroom, presence of damp in bedroom, household density and age of home); ¶: cut-off for IgE >0.35 kU·L^{-1}.

health outcomes with exposure to endotoxin (all tests for interaction, p>0.06).

DISCUSSION

In this multinational study of middle-aged adults, we found no overall association of lung function with mattress endotoxin levels, although we did find evidence that this association may be modified by *CD14* genotype. There was no evidence of an association of respiratory symptoms or sensitisation to environmental allergen with mattress endotoxin levels.

There are a limited number of studies in adults in which the association of allergic and respiratory disease with directly measured domestic endotoxin has been examined. The largest of these reported an association of current wheeze and asthma medication use with endotoxin levels in bedding in the USA [1]. The methods used to assess endotoxin levels were similar to ours but the range of measured endotoxin levels was higher than we observed in our study (GM endotoxin level in bedding 18.7 EU·mg^{-1}, 5th percentile 2.0 EU·mg^{-1} and 95th percentile 142 EU·mg^{-1}). Their analysis suggested that associations of symptoms with endotoxin level were present in adults, but not in children. In another study in Germany, the homes of 350 adults taking part in the first phase of the ECRHS I had living room dust samples taken for endotoxin assay in 1995–1996 (GM 4.4 EU·mg^{-1}, range 0.2–1,661 EU·mg^{-1}) [24]. There was some evidence that higher levels of endotoxin were associated with a lower prevalence of IgE sensitisation, which was most evident when IgE sensitisation was defined by a higher cut-off than we have used (>3.5 rather than 0.35 kU·L^{-1}) and was particularly strong for grass pollen. Even when we looked in our data for a relationship with endotoxin with this higher level of sensitisation to grass pollen, we saw no effect (adjusted OR 0.90, 95% CI 0.48–1.69). A family based study in Barbados, which included adults, showed no overall association of living room endotoxin levels with asthma, asthma severity or total IgE [25].

The aforementioned studies sampled endotoxin from a variety of indoor sites. Living room floor dust levels tend to be higher than mattress levels but the two have been shown to be weakly or moderately correlated in several studies conducted in Europe and the USA [4, 14, 26–28]. THORNE *et al.* [1], in the USA, measured endotoxin in the living room (floor and sofa) and in the bedroom (floor and bedding). Symptoms suggestive of asthma were associated with endotoxin in all locations, but this was most strongly and significantly seen for the bedroom measurements. This might reflect that exposure in the bedroom is longer (~8 h·day^{-1}) and, for mattress levels, is more intense (as the endotoxin is closer to the breathing zone). We have only measured mattress dust levels, but further studies could include other indoor sites, particularly as the relative contribution of each source of endotoxin may differ between locations [29].

None of the studies that have examined health effects of domestic exposure in adults have examined lung function. We did not see an overall association of lung function with mattress endotoxin, even though there is a substantial body of literature suggesting that low lung function is associated with workplace endotoxin exposure [2] and that there is a dose-related inflammatory response in the lung to inhaled endotoxin [30]. It is highly likely that exposures in workplace settings are higher than those in the domestic environment, but direct comparison of our results with these other studies is hindered by differences in sampling methods.

We did see strong evidence that the association of endotoxin with lung function may be modified by *CD14*/-260 genotype. This gene–environment interaction did not exactly follow the pattern suggested by previous studies, which have examined related respiratory outcomes. In the nonoccupational environment, increasing endotoxin exposure has been associated with better health outcomes in those carrying the C allele of *CD14*/-159 (more recently referred to as *CD14*/-260) when compared with those with the T allele (*e.g.* less IgE sensitisation in UK children [12], lower total IgE in rural and farm children in Germany [31], less asthma in families in Barbados [25], and lower total IgE in pregnant mothers [32] and their offspring [33] in the USA). This has been interpreted as evidence that the presence of the C allele confers suppression of atopic T-helper cell type-2 responses in the presence of endotoxin. We anticipated that carriage of the C allele would protect against endotoxin-related decrements in lung function, a protection that would not be seen in those with the T allele. However, we observed an improvement in lung function with endotoxin exposure in those carrying the C allele, with little or no change

TABLE 4 Unadjusted and adjusted change in mean standardised residual[#] per 10-fold increase in endotoxin level for forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC and endotoxin levels for the entire sample and stratified by *CD14* genotype

	Participants			*CD14*/-260 genotype			p-value	
	All	Full confounder information	Full confounders and genotype	CC	CT	TT	Heterogeneity of effect	Trend of interaction[¶]
Participants n	902	814	527	110	271	146		
FEV_1								
Unadjusted	-0.01 (-0.16–0.15)	0.01 (-0.15–0.18)	0.07 (-0.11–0.26)	0.51 (0.11–0.92)	0.04 (-0.21–0.30)	-0.24 (-0.61–0.11)	0.020	0.003
Adjusted[+]		0.04 (-0.11–0.19)	0.08 (-0.11–0.27)	0.62[§] (0.21–1.04)	0.01 (-0.25–0.27)	-0.22 (-0.59–0.13)	0.008	0.005
FVC								
Unadjusted	-0.09 (-0.24–0.06)	-0.06 (-0.22–0.09)	-0.02 (-0.22–0.16)	0.31 (-0.14–0.77)	-0.03 (-0.30–0.23)	-0.33 (-0.68–0.02)	0.008	0.059
Adjusted[+]		-0.05 (-0.22–0.10)	0.00002 (-0.20–0.20)	0.49 (0.03–0.96)	-0.04 (-0.32–0.23)	-0.29 (-0.65–0.06)	0.029	0.013
FEV_1/FVC								
Unadjusted	0.15 (0.02–0.29)	0.15 (0.01–0.29)	0.14 (-0.03–0.31)	0.24 (-0.16–0.65)	0.09 (-0.14–0.33)	0.20 (-0.11–0.52)	0.775	0.976
Adjusted[+]		0.13 (-0.01–0.27)	0.13 (-0.005–0.28)	0.18 (-0.24–0.60)	0.05 (-0.18–0.29)	0.27 (-0.03–0.59)	0.538	0.809

Data are presented with 95% confidence intervals. [#]: a male of age 50 yrs and height of 180 cm is predicted to have a FEV_1 of 3.8 L using the equation of QUANJER *et al.* [17]. If his observed FEV_1 is 4 L, then the difference between observed and predicted FEV_1 is 0.2 L. Following standardisation (again using the standard deviation provided by QUANJER *et al.* [17]), his standardised residual is 0.38. This means the difference between his measured and predicted FEV_1 is 0.38 standard deviations greater than the mean of all of these values. [+]: assuming additive model. [¶]: confounders were smoking status in pack-yrs and household variables (keeping a cat or dog, age of the mattress, presence of mould in bedroom, presence of damp in bedroom, household density and age of home). [§]: a coefficient of 0.62 in this table means that for a 10-fold increase in endotoxin levels (it is 10-fold because endotoxin levels have been logarithmically transformed using base 10) there is a change of 0.62 standard deviations in the difference between measured and predicted FEV_1.

FIGURE 1. Generalised additive mixed model (GAMM) plot, after the fitting of a GAMM adjusted for smoking status and household variables, of the relationship between log-transformed dust endotoxin levels and forced expiratory volume in 1 s (FEV_1) standardised residuals by *CD14* genotype. TT denotes individuals homozygous for the cytosine (C) to thymine (T) transition at position -260 of *CD14*, CT denotes individuals heterozygous for the transition and CC denotes individuals homozygous for cytosine at both alleles.

seen in those with the T allele. Our data support there being differential responses to endotoxin between the *CD14* genotypes and that the C allele is beneficial in the presence of high levels of endotoxin; although we find it difficult to understand why better lung function with increasing exposure is seen in this group, we make the following observations. First, in nonatopic subjects with the TT genotype, endotoxin was significantly associated with reduced lung function. The association was of borderline significance in nonatopics carrying the CC genotype. Secondly, the genotype effects predicted by the GAMM plot (fig. 1) among subjects heavily exposed to endotoxin were largely consistent with results from occupationally exposed

FIGURE 2. Estimated effect of a 10-fold increase in mattress endotoxin level in lung function (forced expiratory volume in 1 s (FEV_1) standardised residuals) across the three genotypes of *CD14*/-260 (rs2569190) for three European regions. Endotoxin levels by region were as follows. South: median 3.1 (5th to 95th percentile 0.5–17.2); middle: median 2.7 (5th to 95th percentile 0.5–19.7); north: median 1.5 (5th to 95th percentile 0.3–18.2). Whiskers represent 95% confidence intervals.

TABLE 5 Adjusted change in mean standardised residual[#] per 10-fold increase in endotoxin level for forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC and endotoxin levels for atopics and nonatopics and stratified by *CD14* genotype

	CD14/-260 genotype			p-value		
	CC	CT	TT	Heterogeneity of effect	Trend of interaction[¶]	Test of difference in effect of *CD14* on lung function response to endotoxin[+]
FEV_1, adjusted[§]						
Nonatopics	0.46 (-0.03–0.96) (n=84)	0.08 (-0.21–0.38) (n=191)	-0.39 (-0.78– -0.01) (n=104)	0.028	0.004	0.091
Atopics	1.11 (0.67–1.55) (n=26)	-0.13 (-0.71–0.43) (n=79)	0.16 (-0.67–1.00) (n=41)	0.001	0.273	
FVC, adjusted[§]						
Nonatopics	0.57 (0.01–1.13) (n=84)	0.004 (-0.31–0.32) (n=190)	-0.45 (-0.82– -0.08) (n=104)	0.008	0.009	0.260
Atopics	0.42 (-0.35–1.20) (n=26)	-0.02 (-0.59–0.55) (n=80)	-0.04 (-0.82–0.73) (n=41)	0.615	0.900	
FEV_1/FVC, adjusted[§]						
Nonatopics	-0.12 (-0.58–0.33) (n=84)	0.06 (-0.20–0.33) (n=190)	0.28 (-0.07–0.65) (n=104)	0.362	0.194	0.107
Atopics	1.44 (0.60–2.27) (n=26)	0.02 (-0.50–0.55) (n=80)	0.31(-0.25–0.88) (n=41)	0.018	0.221	

Data are presented with 95% confidence intervals. [#]: a male of age 50 yrs and height 180 cm is predicted to have a FEV_1 of 3.8 L using the equation of QUANJER *et al.* [17]. If his observed FEV_1 is 4 L, then the difference between observed and predicted FEV_1 is 0.2 L. Following standardisation (again using the standard deviation provided by QUANJER *et al.* [17]), his standardised residual is 0.38. This means the difference between his measured and predicted FEV_1 is 0.38 standard deviations greater than the mean of all of these values. [¶]: additive model. [+]: comparing atopics and nonatopics, p-value of hypothesis that a linear effect of a three-way interaction term (endotoxin × *CD14*/-260C to T × atopy) is equal to 0. [§]: confounders were age, sex, smoking status in pack-yrs and household variables (keeping a cat or dog, age of the mattress, presence of mould in bedroom, presence of damp in bedroom, household density and age of home).

cohorts [34]. Thirdly, this gene–environment interaction is worthy of further consideration in light of the strong evidence against the null hypothesis (p=0.003). Finally, our analysis clearly indicates that associations of lung function with endotoxin in heterozygotes are intermediate of those for the two homozygote states and that this pattern of association is consistent across different parts of Europe.

In those with the CC genotype, we also saw that endotoxin exposure was nonsignificantly (p>0.05) protective for asthma score, a finding consistent with that for lung function. There was also some evidence that the association of asthma score with endotoxin was different in the CT genotype compared with the other two homozygous states. This might be interpreted as indicative of heterosis (the biological phenomenon in which an effect is most pronounced in the heterozygote), and a small study has suggested heterosis could exist with respect to *CD14* genotype and interleukin-6 levels [35]. Replication in other large studies of adults is required, although, at present, few studies have collected necessary data on exposure, symptoms as defined and genotype.

The role of lipopolysaccharide and the bioactive moiety endotoxin have recently been extensively reviewed by SIMPSON and MARTINEZ [12], and they argue that discrepancies in the literature on the relation of endotoxin exposure to atopy may have arisen due to differential effects in those carrying different genotypes for *CD14*. Although we found no evidence of this for atopy or symptoms, we did observe this interaction for lung function. This did not appear to be mediated by downregulation of atopic responses, as we saw no evidence that endotoxin exposure was associated with less atopy. Our definition of atopy relied on sensitisation to the three major aeroallergens in Europe, which identify most individuals with IgE sensitisation to aeroallergens [36].

In conclusion, this multicentre European study has provided a valuable resource to examine the health effects of residential exposure to endotoxin in almost 1,000 adults with detailed information on respiratory disease and *CD14*/-260 genotype. We found little evidence that exposure to mattress endotoxin was associated with reported respiratory symptoms. There was evidence, however, that the association of lung function with mattress endotoxin level may be modified by the *CD14*/-260 genotype.

SUPPORT STATEMENT

The coordination of the ECRHS II was supported by the European Commission, as part of their Quality of Life programme, who also funded the indoor environmental assessment (grant code QLK4-CT-1999-01237). This work was supported by the European Commission as part of HITEA (Health Effects of Indoor Pollutants: Integrating Microbial, Toxicological and Epidemiological Approaches) (grant agreement number 211488 under the Seventh Framework Programme, Topic ENV.2007.1.2.1.1: ''Indoor air pollution in Europe: an emerging environmental health issue'').

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

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