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ORIGINAL ARTICLE

Exposure-response relationships for inhalant wheat allergen exposure and asthma

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ABSTRACT

Background A few studies have investigated exposure–response relationships for sensitisation to wheat, work-related symptoms and wheat allergen exposure. IgG4 is suggested to protect against the development of allergic sensitisation. The main aim of this current study was to explore the nature of exposure–response relationships for a range of clinically relevant endpoints among bakery workers, and to investigate the role of IgG4 in these relationships.

Methods A cross-sectional study of 517 supermarket bakery workers in 31 bakeries used a questionnaire, serum-specific IgE and IgG4 to wheat, and methacholine challenge testing. Exposure models were developed previously using job, bakery size, tasks and specific ingredients used. These models were used to predict average personal exposure to wheat allergens.

Results The exposure–response relationships for average exposure followed a linear relationship for sensitisation, but a bell-shaped curve for allergic symptoms and probable occupational asthma, increasing up to 10–15 $\mu g/m^3$ wheat allergen concentration after which they plateau off and decrease at higher exposure concentrations. This relationship was modified by atopic status. IgG4 levels were strongly exposure related: a clear increase in prevalence of higher IgG4 with increase in wheat allergen exposure was observed among those sensitised and non-sensitised to wheat, with IgG4 even more strongly associated with exposure than IgE to wheat.

Conclusions The bell-shaped exposure–response relationship in the current study is consistent with the findings of previous studies. IgG4 showed no protective effect for sensitisation, confirming the findings of previous studies, suggesting that the pattern is probably related to a healthy worker effect.

What the study adds

- Exposure–response relationships between wheat allergen exposure and objective clinical data (bronchial hyper-responsiveness) for asthma endpoints have not been previously described.
- This is the first detailed study exploring the possible role of IgG4 to wheat in explaining the non-linear exposure–response relationship.
- The bell-shaped exposure–response relationship for clinical endpoints incorporating objective measurement of bronchial hyper-responsiveness and sensitisation (probable occupational asthma) observed in this study is consistent with previous studies using other clinical endpoints.
- The study demonstrated that the association between wheat allergen exposure and IgE sensitisation is not modified by IgG4 to wheat nor does IgG4 display a protective effect.

INTRODUCTION

Exposure–response relationships have been previously described for α-amylase and wheat allergen exposures in relation to specific sensitisation in bakery workers.[1 2] Studies investigating the shape of the relationship between flour dust exposure and, more specifically, wheat allergen levels suggest that the dose–response relationship with sensitisation may be non-linear and level off or even decline at higher exposure levels.[3–5] Furthermore, there have been no clear indications of an exposure level below which the risk for sensitisation is zero or negligible. This bell-shaped response has also been observed in studies of laboratory animal workers who have been exposed to rats,[6 7] which have demonstrated an attenuation of specific IgE antibody response at high exposure levels.[8]

Some studies have suggested that the healthy worker effect (HWE) is the likely explanation for the inverted relationship observed at high levels of exposure. Others have postulated that this may be due to the blocking effect or protective role of IgG4 antibodies.[8] A 'modified Th2 response' mechanism has been used to explain the difference in the shape of exposure–response relations in earlier studies of children for house dust mites when compared with those exposed to cat allergens and those receiving immunotherapy.[9 10] The evidence from these studies suggests that IgG4 production is driven by increasing allergen exposure, resulting in a reduction of sensitisation in laboratory animal workers exposed to rats[8] and mice,[11] and children exposed to cats.[9] Others have contested this view and showed that IgG4 antibodies to rat urinary allergen cannot explain the absence of a dose–response relationship and does not protect against the development of respiratory allergy, but is merely considered a marker of exposure.[12–14] This evidence is particularly strong because two of these studies are longitudinal studies, although a confounding role of exposure to laboratory animals before the start of the study cannot be excluded. Aside from allergic sensitisation, there have been a few studies that have considered other endpoints

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such as symptoms, allergic rhinitis or baker's asthma in relation to wheat allergen exposure.[15]

This study is based on the results of a detailed health survey of South African bakery workers.[16] The exposure estimates for wheat allergen used in the current study were based on baseline measurements of a subgroup of supermarket bakery workers previously reported.[17] The main aim of this current study is to explore the nature of exposure–response relationships for a range of clinically relevant endpoints such as allergic sensitisation, work-related allergic upper and lower airway symptoms and bronchial hyper-responsiveness among bakery workers.

METHODS

Health outcome assessment

A cross-sectional study of 517 supermarket bakery workers employed in 31 bakeries used a European Community Respiratory Health Survey questionnaire, skin prick tests for atopy, and specific IgE to flour dust allergens and methacholine challenge testing.[16] While all permanent (n=318) and casual workers (n=168) in the bakery and ex-bakers with asthma redeployed from the bakery section 2 years prior to the study (n=31) were investigated in the initial study, only permanent and casual workers were included in this current analysis since no data on current exposures were available for the ex-baker group.

Immunological assessment

Serum-specific IgE levels were available for 513 workers. Quantification of specific IgE antibodies to wheat (f4) was performed using CAP-FEIA (fluorescence enzyme immunoassay) according to the manufacturer's instructions (Thermofisher). An ImmunoCAP result of >0.35 kU/L was regarded as positive. A positive skin prick test (SPT) was regarded as a wheal read 15 min after testing that had a diameter (mean of two perpendicular measures) of ≥3 mm more than the negative control. For the purposes of our study, atopy was considered to be present if the SPT to one or more common aeroallergens was positive. The presence of atopy in workers who did not undergo SPTs (n=10) was defined by a positive Phadiatop test (ImmunoCAP 100 System; Phadia, Uppsala, Sweden).[16]

Specific IgG4 analyses

Serum analyses were performed for the quantitative measurement of wheat-specific IgG4 antibodies using the UniCap assay procedure (ImmunoCap 100 System; Phadia, Uppsala, Sweden), according to the manufacturer's instructions. The fluorescence measured was transformed to concentrations with the use of a calibration curve. The calibrator ranged from 0 to 300 μg/L. Samples that were greater than the highest calibrator (>300 μg_A/L) were further diluted. The fluorescence response value is correlated with the specific IgG4 antibody concentration in the serum specimen. An ImmunoCAP-specific IgG control was included in the analyses for quality control.

Spirometry and challenge testing

Spirometry was performed using the Jaeger Aerosol Provocation System (APS) Pro apparatus according to American Thoracic Society (ATS) guidelines.[18] Methacholine challenge testing was performed on all workers by trained technologists according to an abbreviated protocol using the Medic Aid Pro Nebulizer dosimeter method as previously described.[16] The results of the methacholine challenge test were interpreted as follows: borderline defined as 0.4 mg<PD_{20} methacholine<1.0 mg; mild=0.08 mg>PD_{20} methacholine<0.4 mg; moderate/severe=PD_{20} methacholine<0.08 mg. Borderline values for PD_{20} methacholine were considered negative in the definition of non-specific bronchial hyper-responsiveness (NSBH). In participants in whom PD_{20} methacholine was contraindicated, such as those with acute asthma symptoms or a baseline forced expiratory volume in 1 s (FEV_1) <1.5 L or FEV_1<70% predicted, a bronchodilator (400 μg salbutamol dose) was administered instead. A change in FEV_1 of ≥12% 10 min after administration of bronchodilator was considered suggestive of NSBH. The positive results from methacholine and postbronchodilator tests were used to identify individuals considered to have bronchial hyper-responsiveness.

Operational definitions of outcome variables

Upper and lower airway symptoms were considered to be work-related if they were reported to worsen during the work shift and improve when away from work.

1. *Probable occupational asthma*: Defined as presence of NSBH; *and* sensitisation to wheat allergens.
2. *Work*-related allergic ocular-nasal symptoms: Defined as presence of work-related ocular-nasal symptoms and sensitisation to wheat allergens.
3. *Work*-related allergic chest symptoms: Defined as presence of work-related chest symptoms and sensitisation to wheat allergens.

Sensitisation to wheat was defined as a positive SPT or elevated serum IgE to wheat.

EXPOSURE ASSESSMENT

Exposure metrics were derived from personal inhalable samples collected during the baseline survey.[17] During exposure assessment, information on job title, supermarket store size, tasks and products used was also collected to yield a total of 211 samples from 18 bakeries. Environmental samples were analysed for wheat allergens using the rabbit IgG inhibition EIA.[19]

Statistical analysis

Exposure models

All statistical analyses were performed using STATA V.8 and SAS V.9.1 (SAS 2002). Since the exposure data followed a skewed distribution, they were log transformed prior to statistical analysis. Mixed effects models were used to evaluate the association between exposure either to inhalable dust, wheat, rye or fungal α-amylase allergens and fixed effect covariates such as job title, supermarket store size, tasks and products, taking into account worker and supermarket store as random effects, as previously described.[17] The information on work characteristics was obtained from the questionnaire interview and combined with the exposure models to predict individuals' average current exposures to occupational allergens for each worker in the past year. Ex-bakers were not included in the analysis of exposure–response relationships as it was not possible to assign a predicted exposure level to this group, as there were no objective exposure measures since they were not currently working in the bakery. The exposure estimates were therefore reported on only 466 observations. The standardised questionnaire included, which had been designed for the investigation of asthma (respiratory symptoms, family history of allergy), was modified to include detailed questions relating to current and previous employment, and degrees of exposure to flour dust and tobacco smoke were also included.

The following three exposure assessment approaches were considered according to methods employed in previous studies[4]: (1) measured exposure for each individual worker, (2) estimated exposure based on the predictive model (which accounts for bakery size, job title and tasks performed) and (3) a combination of the previous two approaches, referred to as the variance-weighted estimator of exposure, based on measured and estimated exposure.

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Modelling exposure–response relationships

Non-parametric regression modelling (smoothing) was performed by using generalised additive models (proc gam) to explore and visualise the association between exposure and health for atopics as well as non-atopics separately. Generalised cross validation was used to select the degrees of freedom for the smoothing component. In a number of cases, the curves in generalised additive models showed large fluctuations that were not biologically plausible. In those cases the degrees of freedom were limited to a maximum of two. Estimates from the generalised additive models were exported to SigmaPlot (V.9.0) to create smoothed spline plots for the different exposure–response relationships explored. Confidence limits for most plots were relatively narrow and constant between 0 and 20–25 wheat concentration level, making the splines about the shape of the relation between the health endpoint and wheat exposure more informative in this range. Above 20–25 wheat concentration level the CIs are generally wider, making this part of the plot less informative.

RESULTS

Health outcomes

A total of 466 workers were included in the analysis, on average 32 years old; 52% were female. The prevalence of atopy, defined as a positive SPT to one or more common aeroallergens, was 42%. A total of 115 (25%) were sensitised to wheat, with 74% (85/115) having high levels of IgG4 (table 1). A weak but significant correlation was observed for wheat IgE and wheat IgG4 (Spearman r=0.40, p<0.001). The prevalence of work-related ocular-nasal symptoms (30%) was higher than work-related chest symptoms (14%), while wheat-related allergic symptoms were present in approximately half of these workers. These symptoms were more often reported by atopics compared with non-atopics for work-related ocular-nasal symptoms (OR 2.1; p<0.001) and work-related chest symptoms (OR 1.6; p=0.076). IgG4 levels were not significantly different for atopics and non-atopics. Self-reported work-related symptoms were associated with wheat-specific IgE but not with IgG4 levels. The mean (geometric) IgG4 levels were significantly higher among sensitised (2235 μg/L), than among non-sensitised workers (411 μg/L; p<0.001). Furthermore, IgG4 levels were also higher in those with work-related chest symptoms (947 vs 591 μg/L; p<0.001) and work-related ocular-nasal symptoms (873 vs 548 μg/L; p<0.001).

Table 1 Demographic and health outcome characteristics of supermarket bakery workers

Demographic and health outcomes	Overall (n=466)	Non-atopics (n=270)	Atopics (n=196)
Age (years)	32±9	32±9	30±9
Gender (%F:M)	52:48	51:49	53:47
Atopy	196 (42%)	0 (0)	196 (100%)
Specific IgE (n=462)			
Wheat IgE*	115 (25%)	35 (13%)	80 (41%)
Wheat IgE (kU/L)	0.45 (4.14)	0.32 (2.85)	0.72 (5.52)
IgG4 (μg/L)	630.91 (6.09)	596.77 (6.35)	680.27 (5.76)
IgE/IgG4 ratio	0.85 (1.23)	0.81 (1.17)	0.92 (1.29)
High IgG4†	211 (47%)	117 (45%)	94 (49%)
High IgE/IgG4‡	227 (50%)	110 (42%)	117 (61%)
Work-related symptoms (n=466)			
Work-related ocular-nasal symptoms	140 (30%)	63 (23%)	77 (39%)
Work-related chest symptoms	63 (14%)	30 (11%)	33 (17%)
Work-related allergic symptoms (n=462)			
Work-related ocular-nasal symptoms	62 (13%)	19 (7%)	43 (22%)
Work-related chest symptoms	25 (5%)	8 (3%)	17 (8%)
Probable occupational asthma (n=411)	37 (9%)	8 (3%)	29 (17%)

Work-related symptoms: upper and lower airway symptoms were considered to be work-related if they were reported to worsen during the work shift and improve when away from work. *Allergic symptoms*: work-related symptoms *and* sensitisation (specific IgE to wheat). *Probable occupational asthma*: non-specific bronchial hyper-responsiveness *and* sensitisation (specific IgE) to wheat.
Data are presented as per cent or geometric mean and geometric SD.
*Serum-specific IgE>0.35 kU/L.
†*High IgG4*=calculated using the GM+1SD as cut-off value for high versus low.
‡*High IgE/IgG4*=calculated using the median of the ratio as cut-off value.

Exposure

The geometric mean predicted exposure for inhalable dust particulate was approximately 0.7 mg/m^3 (range 0.12–5.42 mg/m^3) and 5 μg/m^3 (range 0.32–44.18 μg/m^3) for wheat allergens. The median duration of employment was 4 years (IQR of 3–8 years).

Exposure–response relationships

Tables 2–3 and figures 1–2 present the results of exposure–response modelling. An increase in average current wheat exposure level was associated with a significant continuous monotonic

Table 2 Association between wheat allergen exposure and clinical endpoints in supermarket bakery workers, stratified by atopic status in generalised additive models

	Average current exposure			
	Non-atopic		Atopic	
Clinical endpoint	Estimate	p Value	Estimate	p Value
Wheat sensitisation				
Regression model (parametric part)				
Intercept	−2.258	<0.001	−0.693	0.001
Linear component	0.038	0.052	0.045	0.023
Smoothing model (non-parametric part)				
Spline component	df=1	0.291	df=1	0.047
Work-related allergic ocular-nasal symptoms				
Regression model (parametric part)				
Intercept	−2.938	<0.001	−1.367	<0.001
Linear component	0.038	0.112	0.014	0.524
Smoothing model (non-parametric part)				
Spline component	df=1	0.433	df=1	0.215
Work-related allergic chest symptoms				
Regression model (parametric part)				
Intercept	−3.627	<0.001	−2.261	<0.001
Linear component	0.018	0.655	−0.009	0.845
Smoothing model (non-parametric part)				
Spline component	df=1	0.135	df=1	0.157
Probable occupational asthma				
Regression model (parametric part)				
Intercept	−3.380	<0.001	−1.436	<0.001
Linear component	0.007	0.896	−0.017	0.654
Smoothing model (non-parametric part)				
Spline component	df=1	0.227	df=1	0.045

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Table 3 Association between wheat allergen exposure, IgG4 and clinical endpoints among supermarket bakery workers in generalised additive models

Clinical endpoint	Estimate	p Value
High IgG4		
Regression model (parametric part)		
Intercept	−0.550	<0.001
Linear component	0.052	<0.001
Smoothing model (non-parametric part)		
Spline component	df=1	0.001
Wheat sensitised group		
Regression model (parametric part)		
Intercept	0.463	0.164
Linear component	0.066	0.045
Smoothing model (non-parametric part)		
Spline component	df=1	0.261
Non-wheat sensitised group		
Regression model (parametric part)		
Intercept	−0.850	<0.001
Linear component	0.044	0.003
Smoothing model (non-parametric part)		
Spline component	df=1	0.009
Probable occupational asthma		
Regression model (parametric part)		
Intercept	−2.126	<0.001
Linear component	−0.021	0.499
Smoothing model (non-parametric part)		
Spline component	df=1	0.029
High IgG4 group		
Regression model (parametric part)		
Intercept	−1.267	<0.001
Linear component	−0.062	0.135
Smoothing model (non-parametric part)		
Spline component	df=1	0.188
Low IgG4 group		
Regression model (parametric part)		
Intercept	−3.240	<0.001
Linear component	0.012	0.815
Smoothing model (non-parametric part)		
Spline component	df=1	0.567
Probable occupational asthma		
Regression model (parametric part)		
Intercept	−2.126	<0.001
Linear component	−0.021	0.499
Smoothing model (non-parametric part)		
Spline component	df=1	0.030
High IgE/IgG4 ratio		
Regression model (parametric part)		
Intercept	−0.940	0.003
Linear component	−0.076	0.055
Smoothing model (non-parametric part)		
Spline component	df=1	0.187
Low IgE/IgG4 ratio		
Regression model (parametric part)		
Intercept	−5.837	0.716
Linear component	0.119	0.860
Smoothing model (non-parametric part)		
Spline component	df=1	0.380

High IgG4=calculated using the GM+1SD as cut-off value for high versus low.
High IgE/IgG4=calculated using the median of the ratio as cut-off value.

increase in the prevalence of wheat sensitisation (see online supplementary figure S1) in a linear pattern. This relationship was more pronounced in atopics (p=0.047) compared with non-atopics (p=0.291).

Wheat allergen exposure was significantly associated with an increase in probable occupational asthma when using smoothing spline models (table 2). Figure 1A illustrates that in atopic workers increased exposure to wheat allergens was associated with a higher prevalence of work-related allergic chest symptoms, work-related allergic ocular-nasal symptoms and probable occupational asthma (NSBH and sensitisation to wheat) in a dose-dependent manner. However, the prevalence of these symptoms only showed an increase up to 10–15 μg/m^3 of wheat allergen concentration, and levelled off and decreased at higher exposure concentrations. The increased prevalence of upper respiratory symptoms appeared at a lower exposure than lower respiratory symptoms. The graph also shows a lower prevalence of occupational asthma (and also allergic chest symptoms) in those who are highly exposed (above 10 μg/m^3); however, the splines presented have a large CI at higher exposure levels. The relationship between wheat allergen exposure, sensitisation and symptoms in non-atopic workers, however, showed insignificant associations (figure 1B). A further investigation of the exposure–response relationship for probable occupational asthma (NSBH and sensitisation to wheat) showed a relatively flat relationship when compared with the pattern observed in atopic workers.

To explain this bell-shaped relationship, the impact of IgG4 on the observed exposure–response relationship was investigated further. Table 3 demonstrates that an increase in wheat allergen concentration was significantly associated with IgG4 levels in wheat sensitised and non-sensitised workers, both for linear regression analysis and splines. The results clearly illustrated that IgG4 is strongly exposure related: a clear increase in prevalence of higher IgG4 with increase in wheat allergen exposure is seen (see online supplementary file 2). A similar exposure–relationship was observed among individuals sensitised and non-sensitised to wheat, with IgG4 more strongly exposure related than IgE to wheat.

Evaluating the relationship among those with probable occupational asthma showed that individuals with high IgG4 titres have a higher baseline prevalence of probable occupational asthma than those with low IgG4 (figure 2A). However, at higher exposure levels the prevalence of occupational asthma decreases in those with high Ig4 as well as in those with low IgG4. This seems to indicate that IgG4 is merely a marker of exposure. A similar trend was observed for those with a high IgE/IgG4 ratio (figure 2B). We conducted further analysis using the 75th and 90th centile, respectively, as cut-offs for high versus low. This sensitivity analysis demonstrated that the overall conclusions were not affected by changes in the cut-off points for IgG4 or IgE/IgG4 ratio.

In a simple logistic regression model for work-related chest and ocular-nasal symptoms, atopy, specific IgE and a high IgE/IgG4 ratio were significantly associated with increased risk of symptoms. High IgG4 levels were also independently associated with a positive relationship with sensitisation (OR 5.03; p<0.001), ocular-nasal symptoms (OR 1.61; p=0.013), and not chest symptoms (OR 1.29; p=0.273).

DISCUSSION

The current study is one of few studies investigating the exposure–response relationship for occupational wheat allergen

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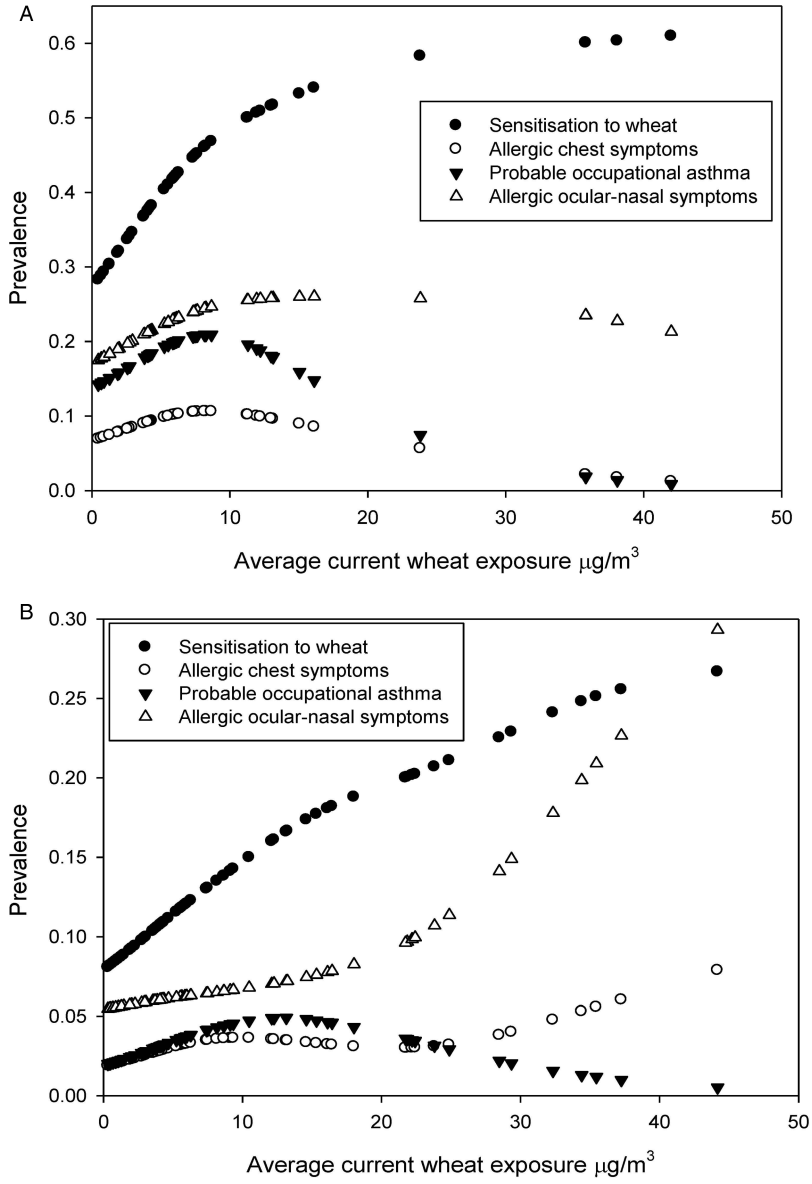

Figure 1 (A) Relationship between various clinical endpoints and wheat allergen concentration among atopic supermarket bakery workers. (B) Relationship between various clinical endpoints and wheat allergen concentration among non-atopic supermarket bakery workers.

exposure in relation to sensitisation, symptoms and probable occupational asthma in bakers. While exposure–response relationships have been previously described for the former two outcomes, these studies were limited in that a critically relevant endpoint of baker's asthma was not modelled. The study is also the first detailed exploration of the role of IgG4 in explaining the non-linear exposure–response relationships observed in bakery workers.

The prevalence of probable occupational asthma (10%) in this study is at the upper end of the prevalence range (5–13%) of studies in industrial bakeries in which a similar definition of occupational asthma (bronchial hyper-responsiveness and sensitivity to flour) was used.[20 21] However, a higher rate of sensitisation to wheat (26%) was found in the current study, when compared with earlier studies of industrial and supermarket bakeries (11–12%)[22 23] as well as in other studies of exposure–response relationships for wheat allergen exposures.[5]

In the current study, a strong exposure–response relationship was demonstrated between wheat allergen exposure and the prevalence of wheat sensitisation, work-related allergic symptoms and probable occupational asthma. However, the exposure–response relationship was not linear, but had a bell-shaped curve. These findings are consistent with previous studies, showing a similar bell-shaped exposure–response relationship. In one of these studies, the prevalence of sensitisation and symptoms increased with increasing wheat allergen concentrations up to 10 μg/m^3,[3] as has been shown in the current study, whereas another study demonstrated increased sensitisation up to 25 μg/m^3 wheat allergen concentration,[4] which was followed by declining risks at higher exposures. Bell-shaped curves for sensitisation are not consistently observed. For instance, Houba *et al*[2] had no bell shape, while Peretz *et al*[4] reported that the relationship for sensitisation to wheat may be non-linear but differs between industries. Recently, Jacobs *et al*[5] also observed a bell-shaped exposure–response relationship. The differences in the observed exposure–response relationships could be ascribed to contextual differences between studies (exposure distribution, HWE, pre-employment selection, etc). A detailed comparison shows that populations indeed differ in exposure distributions (higher in the flour millers and bakery product workers) and atopy (differences between bakery workers and flour workers) indicative of differences observed in the HWE.

The current study demonstrates an increase in sensitisation risk with increasing exposure, showing a linear relationship. However, a non-linear bell-shaped exposure relationship is observed for symptoms and probable occupational asthma

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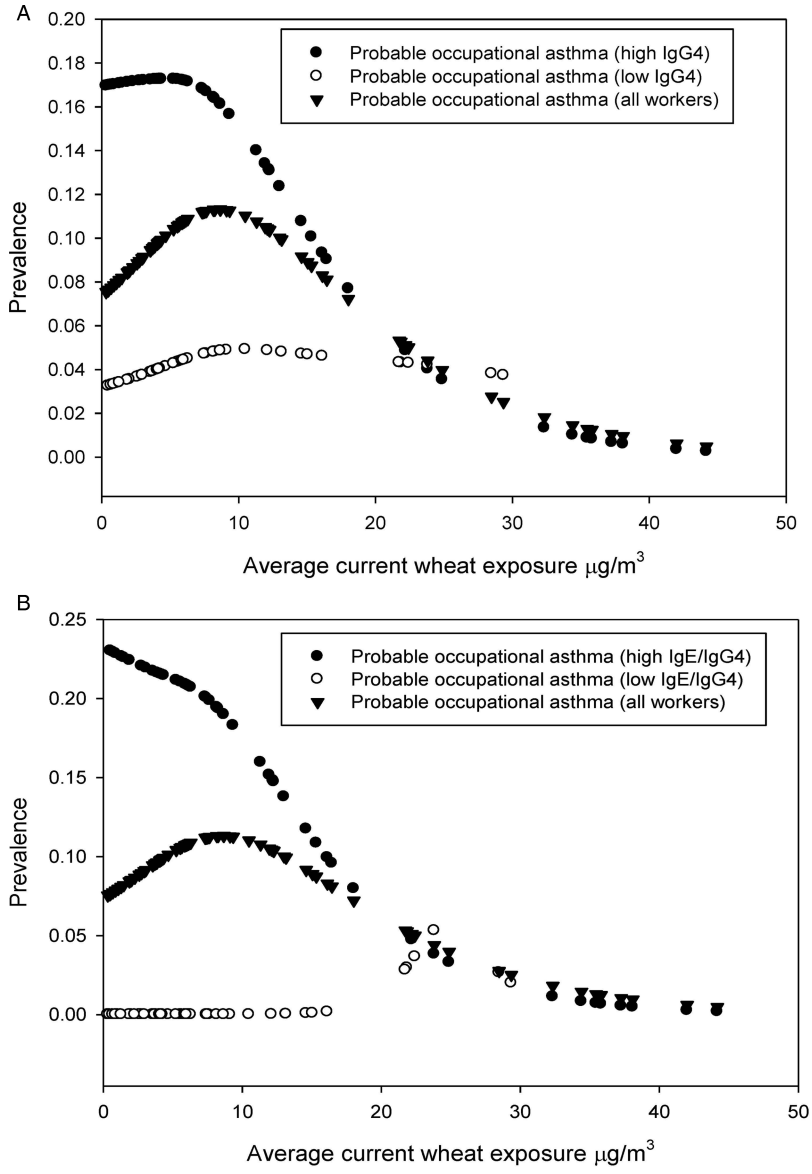

Figure 2 (A) Relationship between probable occupational asthma and wheat allergen concentration among supermarket bakery workers, stratified by level of IgG4 (B) Relationship between probable occupational asthma and wheat allergen concentration among supermarket bakery workers, stratified by IgE/IgG4 ratio.

(when NSBH is taken into account). A re-analysis of the earlier studies by Heederik and Houba[3] also reported a bell-shaped relationship when symptoms were taken into account. A possible explanation for these observations could be due to the fact that those with symptoms or NSBH cannot continue working at higher exposure levels, and therefore move to lower exposed areas. However, longitudinal studies are required to allow for the direct observation of selective forces in this population. Furthermore, pooling of these various data sets in a pooled analysis may also contribute to a better understanding of these relationships.

The findings of this study demonstrated that an increase in wheat allergen concentration was significantly associated with IgG4 production in wheat sensitised as well as non-sensitised workers (in regression and smoothing models), with IgG4 demonstrating even stronger exposure correlations than IgE to wheat. Furthermore, individuals with high IgG4 titres had a higher baseline prevalence of probable occupational asthma, and a similar trend was also observed for those with a high IgE/IgG4 ratio. However, at levels >10–20 μg/m^3, there are no significant differences between the models, given the wide CIs. These data suggest that increased IgG4 antibodies to wheat do not explain the bell-shaped exposure–response relationship between wheat allergen exposure and wheat allergy among bakers. This suggests that there is insufficient evidence of IgG4 having a protective effect for becoming sensitised or developing allergic symptoms or occupational asthma in this study population. The data further show that at higher exposure levels the prevalence of occupational asthma decreases in those with high IgG4 and low IgG4. This observation is certainly at odds with the hypothesis that IgG4 has a protective effect on the development of symptoms, and suggests that IgG4 may be irrelevant when highly exposed, or lastly because of reduced power at higher levels of exposure. The relationship appears to be driven more by the IgE component of the equation.

These findings are consistent with previous occupational studies, which also demonstrated that IgG4 does not protect against the development of sensitisation or allergic symptoms. In the first longitudinal study investigating the role of IgG4 in laboratory animal workers, titres of IgG4 showed a strong and positive dose–response relationship with exposure to rat urinary allergens and were the highest in participants who were symptomatic and sensitised to rat allergens, although previous exposure cannot be excluded. The subsequent longitudinal study also

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found no protective effect of IgG4 against the development of IgE to rat allergens. This study further demonstrated that IgG4 responses develop prior to IgE responses and responses remained stable over time.[13] Studies among bakery workers by Tiikkainen and Klockars[24] suggested that the levels of IgG and IgG subclasses to wheat flour in bakers reflected exposure, but were not related to any specific clinical situation. Hur *et al*[25] also demonstrated that the levels of wheat-specific IgG1 and IgG4 antibodies were directly correlated with the levels of exposure to wheat and the concentration of wheat dust in the workplace.

Studies that have suggested a protective role for IgG4 have, however, also been reported. Jeal *et al*[8] argued for the protective role on the basis that this could be explained by the fact that exposure to laboratory animal allergens did not only occur through inhalation but also from intradermal routes through bites and scratches. The same mechanism is also thought to explain the response in studies on domestic cat exposures[9] and beekeepers,[26] but not among bakers, as it has been suggested that they are only exposed via the inhalation route. However, it is possible that intradermal contact with wheat can also occur, as bakers are known to have dermal symptoms and skin diseases related to allergen exposure. Furthermore, bakers also experience dermal trauma due to minor cuts and repeated hand washing. A recent study has demonstrated a possible exposure–response relationship between exposure to wheat allergens and work-related skin symptoms.[27]

Conversely, other studies have suggested that high IgG4 antibodies may, indeed, be a risk factor for sensitisation,[11 12] as has been demonstrated in the current study, in that high IgG4 was, in fact, associated with an increased risk for wheat sensitisation in this group of bakers. A longitudinal study among laboratory animal workers also demonstrated that high levels of IgG4 predicted newly occurring sensitisation and development of respiratory symptoms in atopic workers during follow-up.[12] This is consistent with a Korean study among bakery workers also demonstrating that the presence of specific IgG4 antibodies was associated with the occurrence of work-related symptoms.[25]

The association between allergen-specific IgG4 and beneficial responses observed in relation to specific immunotherapy is well documented. A variety of regulatory cell types, including interleukin (IL-10)-secreting T and B cells, play an important role in suppressing allergic responses to inhaled, ingested and injected allergens. Protective antibodies, including IgG4, Fc sialylated IgG and IgA have been shown to modulate this allergic response and promote tolerance induction.[28] The effects of IL-10 and related cytokines are said to be important, since IL-10 interferes with the class switch, which affects both IgE and IgG4 production,[29] and IL-10 increases IgG4 production and has been suggested to play a role in the maintenance of tolerance.[10 28 30–32] Furthermore, another study that investigated baker's asthma in two different populations (Spanish and French[33]) concluded that the presence of higher levels of IgG4, IL-10 and the diversity of sources of sensitisation in French bakery worker patients may have helped them in reducing disease expression. However, since IL-10 levels were not measured in the current study, its role in explaining the patterns observed could not be explored further.

Some studies of gene-environment interactions have also suggested that exposure to occupational allergens such as endotoxin can significantly influence the exposure–response relationship.[34–36] However, an experimental study in mice has demonstrated that endotoxin does not play a role in the inflammatory response to flour dust.[37] Finally, the possibility of non-allergic mechanisms as has been suggested by Skjold *et al*[38] may play a role.

Our study, instead, suggests that the HWE, rather than tolerance at higher exposure levels, is a more plausible explanation for the bell-shaped exposure–response relationship observed, but longitudinal observations are needed to confirm this explanation. Some evidence suggesting a HWE in the current study can be drawn from the observation that workers with probable occupational asthma were more likely to be supervisors/managers (OR=4.0; p=0.017), rather than bakers (OR=1.6; p=0.312) despite the fact that[16] our environmental exposure studies[17] have shown that bakers indeed had higher exposures to inhalable dust than supervisors/managers. Furthermore, bakers with probable occupational asthma were also more likely to be transferred from their high exposure jobs to less exposed jobs (supervisors/managers) rather than those who worked in the least exposed jobs (counterhands). The baseline health study also demonstrated that at least 6% of workers had to be transferred from their jobs due to them experiencing work-related chest symptoms.[16] Finally, to overcome the HWE in the statistical analysis, the shape of the curves remained unchanged when the analysis was restricted to individuals with employment duration of less than 3 years (see online supplementary file 3).

The HWE may particularly affect epidemiological studies on work-related asthma in that while it is commonly appreciated that work in high-risk exposure settings causes asthma, the asthma may have substantial impact on work.[39 40] To our knowledge, this has not been studied in bakeries but, recently, Dumas *et al*[41] have used marginal structural models (MSMs) to control for this HWE to assess the magnitude of bias due to the HWE. The study found that when using MSMs, associations increased for exposure to known asthmagens/sensitisers, and reached statistical significance for the relationship between exposure to low levels of chemicals/allergens and asthma attacks, while these associations could not be demonstrated using standard conventional analytical techniques.

In conclusion, the bell-shaped exposure–response relationship in the current study is in agreement with the findings of previous studies. A strong positive exposure–response relationship between IgG4 and wheat allergen exposures, as well as for the IgE/IgG4 ratio, was found in sensitised and non-sensitised workers. These findings indicate that IgG4 has no protective effect on the development of sensitisation, and that this pattern is more readily explained by the HWE commonly observed in cross-sectional studies.

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Patient consent Obtained.

Ethics approval University of Cape Town, Research Ethics Committee.

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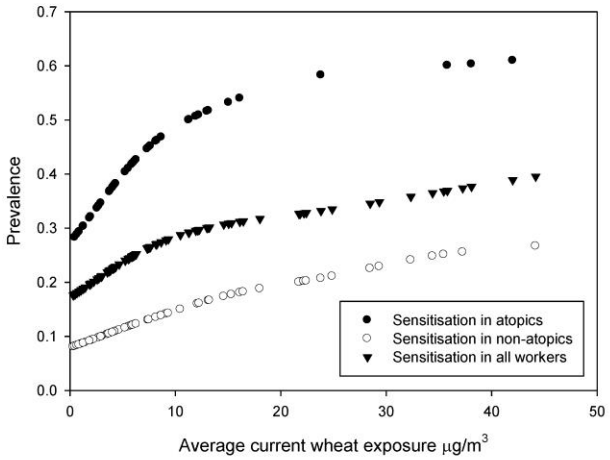
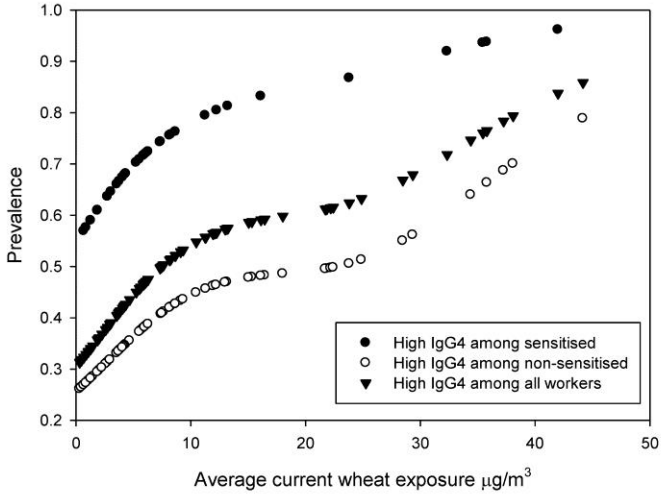
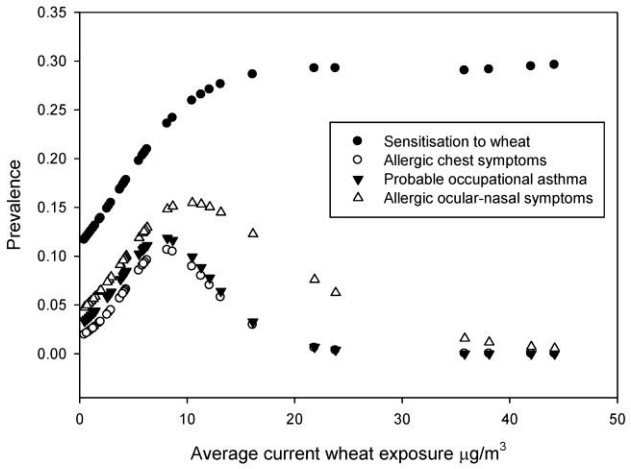


Supplementary Figure 1. Relationship between wheat sensitisation and wheat allergen concentration among supermarket bakery workers, stratified by atopic status



Supplementary Figure 2. Relationship between IgG4 and wheat allergen concentration among supermarket bakery workers stratified by sensitisation



Relationship between various clinical endpoints and wheat allergen concentration among supermarket bakery workers employed for less than 3 years