

Skin test and RAST responses to wheat and common allergens and respiratory disease in bakers

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Summary

Interrelationships between skin and humoral tests for immediate hypersensitivity to wheat and indicators of respiratory disease were examined in 176 male bakers. Skin tests were assessed by measuring the diameter of the weal resulting from prick inoculation of allergen extract and circulating allergen-specific IgE by radioallergo-sorbent test (RAST). Fifteen per cent of subjects showed positive skin-prick test responses to wheat extracts. These subjects demonstrated an increased prevalence of respiratory symptoms and of measurable bronchial responsiveness to methacholine. Thirty per cent of subjects had positive skin test responses to common allergens but negative responses to whole wheat. Compared to subjects with no positive skin test responses they had an increased prevalence of bronchial responsiveness to methacholine but a similar prevalence of respiratory symptoms.

There was a significant association between skin test responses to whole wheat and skin test responses to common allergens suggesting that bakers with pre-existing sensitivity to common allergens are at increased risk of developing wheat flour sensitization.

There was no significant difference between skin-prick test and RAST responses to wheat, water-soluble wheat protein and common allergens. Both tests showed similar relationships with indices of respiratory disease. The associations between skin test and RAST responses to wheat extracts and indices of respiratory disease was stronger for the water-soluble wheat proteins than for other wheat grain extracts.

These results suggest that immediate hypersensitivity to wheat flour is important in the development of non-specific bronchial hyperreactivity in bakers and that the water-soluble fractions of wheat flour are the most important allergenic components. It agrees with *in vitro* results showing that water-soluble wheat proteins bind IgE in sensitive bakers more closely than other grain extracts.

Introduction

Immediate skin reactivity to wheat was demonstrated in bakers with asthma as early as 1909 (Bonnievie, 1958). Herxheimer (1973) reported that the prevalence of positive skin tests to wheat increased from 9% in bakers' recruits to over 30% by the fifth year of baking. Other authors (Kallos & Kallos-Deffner, 1971; Theil & Ulmer, 1980) have demonstrated similar frequencies of skin reactivity to wheat in regular bakers. In addition Thiel and Ulmer (1980) showed that 91% of bakers with respiratory symptoms related to exposure to flour had positive skin test responses to wheat and rye whereas less than 5% of non-exposed control subjects had positive skin reactions to these materials. The level of exposure to flour, the relative potencies of different wheat-flour antigens and a personal or family history of allergic disease all appear to increase the risk of developing wheat flour sensitization and asthma (Theil & Ulmer, 1980; Jarvinen *et al.*, 1979).

Apart from skin testing, methods that have been used to identify cereal-specific IgE to wheat flour include radioallergosorbent tests (RAST) (Baldo Krilis & Wrigley, 1980; Block *et al.*, 1983) and basophil leucocyte histamine release (Blands *et al.*, 1976). Attempts to identify the major allergens in wheat flour through solvent extraction and RAST inhibition have shown that the water-soluble proteins, particularly albumin, are the antigens to which flour-specific IgE from subjects with bakers' asthma binds most strongly (Baldo, Sutton & Wrigley, 1982).

The aims of this study were to examine the associations between evidence of sensitization to baking-related allergens and indices of respiratory disease including bronchial reactivity, and the relationships between skin reactivity to common allergens and the prevalence of sensitization to the various extracts of wheat flour measured by RAST and skin tests.

Subjects

One hundred and seventy-six male subjects from eighteen metropolitan bakeries were studied (Prichard, Ryan & Musk, 1984). This population represented 90% of all bakers in Perth, Western Australia. Bakery employees engaged only to slice, wrap or deliver bread were not included. Sixteen eligible subjects declined to participate in the study. Two subjects were excluded due to incomplete data and the only female baker was excluded from analysis. The mean age of the bakers was 35.2 years (range 15–64 years).

Methods

All bakers were tested at their place of work between 0400 hr and 0800 hr, towards the end of their working shift. Studies were performed in the middle of the working week during spring and summer (October to December).

Respiratory symptoms

Subjects completed a physician-administered questionnaire based on the 1976 British Medical Research Council questionnaire on respiratory symptoms and smoking history (Medical Research Council, 1960). 'Chronic bronchitis' was defined as a cough productive of sputum on most days for at least 3 months of each year for two or more consecutive years (American Thoracic Society, 1962). Attacks of wheeze and dyspnoea were recorded if subjects responded positively to the question: 'Have you ever had attacks of shortness of breath with wheezing?' Work-related asthma was defined as the presence of attacks of wheeze and shortness of breath in subjects who believed that

work affected their chests. This group included all those subjects with physician-diagnosed asthma which had commenced since they had become bakers.

Pulmonary function

Forced expiratory volume in 1 sec (FEV₁) and forced vital capacity were recorded on a dry-bellows spirometer (Vitalograph, Buckingham, U.K.). The mean of the best two measurements of FEV₁ from three technically satisfactory attempts after one or more practice attempts was taken for analysis and corrected to BTPS (Knudson *et al.*, 1976). Standardized FEV₁ values were calculated for each subject using the age and height regression of the whole group and correcting the measured volume to the mean age and height of all subjects.

Bronchial reactivity was assessed using increasing doses of methacholine aerosol administered every 90 sec from hand-operated, calibrated Devilbiss No. 40 nebulizers. The starting dose was 0.04 μ mol in subjects with an FEV₁ less than 60% of the predicted value or a history of asthma and 0.15 μ mol for other subjects (Yan, Salome & Woolcock, 1983). The challenge was terminated when the FEV₁ fell by more than 20% from the initial (post saline) value or a total cumulative dose of 30 μ mol of methacholine had been administered. Reactivity was expressed as the cumulative dose producing a 20% fall in FEV₁ (PD20).

Skin-prick tests

Skin-prick tests were performed with fifteen common and thirteen baking-related allergens. Extracts of whole wheat, rye, barley and oats, bakers' yeast, grass pollens, house dust, and animal danders were obtained from Hollister-Stier (Spokane, Washington, U.S.A.). House dust mite (*Dermatophagoides pteronyssinus*) extract was obtained from the Australian Commonwealth Serum Laboratories (CSL) and wheat flour extracts, including water-soluble wheat flour protein, glutenin, gliadin and gluten digest, from the Bread Research Institute of the Australian Commonwealth Scientific and Industrial Research Organization (CSIRO). Histamine in a solution of 1 mg/ml (John Bull Laboratories, Victoria, Australia) was used as a positive control and the Hollister-Stier diluent as a negative control. The wheat flour extracts were supplied as a powder and made up to concentrations of 1 mg/ml and 0.1 mg/ml in 50% glycerine. For all skin tests a weal of 3 mm or more and greater than the negative control was measured as positive.

Radioallergosorbent tests (RAST)

Serum samples were obtained for RAST testing to whole wheat flour (Pharmacea Wheat Disc), water-soluble wheat flour protein (CSIRO Wheat Research Unit), perennial rye grass (Hollister-Stier) and *D. pteronyssinus* (CSL). The water-soluble wheat flour protein, perennial rye grass and *D. pteronyssinus* extracts used in the RAST testing were the same agents used for the skin test. RAST tests were performed by the CSIRO Wheat Research Unit (Baldo & Wrigley, 1978).

Statistical methods

Continuous variables were compared using an unpaired *t*-test. Comparisons of categorical variables employed the chi-square test except if the expected values were less than five when Fisher's exact test was used (Armitage, 1971). Cross-product ratios were used to indicate the degree of association between skin test or RAST response and

indices of respiratory disease; a ratio of one indicating complete lack of association (Bishop, Feinberg & Holland, 1975). The logarithm of these ratios were compared using an unpaired *t*-test.

Results

The twenty-six bakers with positive skin responses to whole wheat were of similar age and smoking habit to those bakers who did not react to whole wheat but had a greater prevalence of chronic bronchitis, attacks of wheeze and dyspnoea and work-related asthma (Table 1). They also had a greater prevalence of increased bronchial reactivity to methacholine.

Within the groups determined by their skin test responses to whole wheat there was no demonstrable relationship between common allergens and respiratory symptoms (Table 2). However, within the group of bakers with negative skin tests to whole wheat, subjects who reacted to common antigens more often had a PD₂₀ < 30 μ mol than those with no positive skin tests. There was no difference in the prevalence of higher degrees of bronchial hyperreactivity (PD₂₀ < 1 μ mol) between subjects who reacted to common allergens only on skin testing and those who were negative to all skin tests.

Six subjects had positive skin test responses to water-soluble wheat flour protein. All of these subjects reacted to whole wheat on skin testing. There was a closer association between a positive skin test response to water-soluble wheat protein and bronchial hyper-reactivity to methacholine than between a positive skin test response to whole wheat and bronchial hyperreactivity. Four-sixths of the subjects with positive skin test responses to water-soluble wheat protein had a PD₂₀ < 1 μ mol, while only three of the 170 subjects with negative skin test responses to water-soluble wheat flour protein had a PD₂₀ < 1 μ mol ($P = 0.00026$). Seven subjects showed a positive skin test

Table 1. Characteristics of bakers and skin-prick test responses to whole wheat

	Skin test response to whole wheat	
	Positive	Negative
Number	26	150
Smokers (%)	42	50
Ex-smokers (%)	27	19
Never smoked (%)	31	31
Attacks of wheeze and dyspnoea (%)	46	15‡
Work-related asthma (%)	35	7‡
Chronic bronchitis (%)	42	21*
Bronchial reactivity to methacholine		
PD ₂₀ < 30 μ mol (%)	65	37†
PD ₂₀ < 1 μ mol (%)	19	2‡

Statistical significance of difference from bakers with positive skin-prick test responses to whole wheat. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Table 2. Skin-prick test responses to whole wheat and common allergens, symptoms and bronchial reactivity

	Skin test response			
	Wheat positive		Wheat negative	
	Common-allergen		Common-allergen	
	Positive	Negative	Positive	Negative
Number	18	8	52	98
Attacks of wheeze (%) and dyspnoea	50	38	23	11
Work-related asthma (%)	33	38	8	6
Chronic bronchitis (%)	44	38	23	23
Bronchial reactivity to methacholine				
PD ₂₀ < 30 micromols (%)	72	50	58	26*
PD ₂₀ < 1 micromol (%)	22	13	2	2

*Statistical significance of difference from subjects with positive skin-prick test responses to one or more common allergens (but not to whole wheat). $P < 0.001$.

response to gliadin, gluten digest or glutenin. Responses to these substances showed no relationship to bronchial hyper-reactivity. Only three subjects (1.7%) reacted to bakers' yeast on skin tests. All of these also reacted to one or more common allergens.

There was a significant association between skin test responses to whole wheat and common allergens. Sixty-nine per cent of subjects who reacted to whole wheat also had positive skin test responses to one or more common allergens while only 35% of subjects with negative skin test responses to whole wheat reacted to common allergens ($P < 0.001$).

The associations between indices of respiratory disease and RAST responses to whole wheat, water-soluble wheat protein, perennial rye grass and house dust mite were not significantly different from that association already demonstrated for skin-prick test responses (Table 3).

Sensitization to wheat flour was related to the duration of occupational exposure. Subjects with positive skin-prick test responses to whole wheat had a longer duration of baking (mean 20.6 years, s.d. 12.8 years) than those with negative responses to wheat (mean 15.4 years, s.d. 12.3 years) ($P < 0.05$). No significant association was found between baking duration and bronchial hyper-reactivity in the whole study population. However, in the group of bakers with positive skin-prick test responses to whole wheat but not to common allergens those who had increased bronchial reactivity to methacholine (PD₂₀ < 30 μ mol) had worked longer in bakeries (24.4 years, s.d. 10.7 years) than those with normal reactivity (14.2 years, s.d. 12.0 years) ($P < 0.05$). In the group of bakers with negative skin-prick test responses to whole wheat, subjects with positive skin test responses to common allergens and childhood asthma had a shorter duration of baking (6.6 years, s.d. 5.6 years) than other atopic bakers (16.6 years, s.d. 12.6 years) ($P < 0.05$).

Table 3. Associations between indices of respiratory disease* and RAST and skin-prick tests

Allergen	Disease index	Skin test	RAST
Whole wheat	Attacks of wheeze	4.73§	3.15§
	Work-related asthma	6.69§	7.62§
	Chronic bronchitis	2.70†	2.34
	PD20 < 30 μ mol	3.17‡	5.74§
	PD20 < 1 μ mol	11.67§	6.65‡
Water-soluble wheat protein	Attacks of wheeze	8.97‡	8.00§
	Work-related asthma	19.25§	11.46§
	Chronic bronchitis	6.72†	4.21‡
	PD20 < 30 μ mol	4.1	1.57
	PD20 < 1 μ mol	38.0 §	12.3
Common allergens (wheat negative)	Attacks of wheeze	2.37	2.14
	Work-related asthma	1.28	2.93
	chronic bronchitis	0.98	0.41
	PD20 < 30 μ mol	3.78§	3.58‡
	PD20 < 1 μ mol	0.94	7.71

*Cross-product ratios.

Statistical significance of association between the presence of allergen-specific IgE detected either by RAST or skin-prick test and indicators of respiratory disease. † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$.

Discussion

The results of this study are in accord with earlier studies demonstrating a relationship between skin reactivity to wheat flour and symptoms of airway disease in bakers (Theil & Ulmer, 1980; Block *et al.*, 1983). Positive skin-prick test and RAST responses to whole wheat were related to a history of attacks of wheeze and dyspnoea, work-related asthma and chronic bronchitis. Furthermore, wheat flour sensitization was significantly associated with non-allergic bronchial hyper-reactivity, whether the level of reactivity was taken as PD20 < 30 μ mol (the limit of the test) or PD20 < 1 μ mol. Non-allergic bronchial hyper-reactivity has been shown to be associated with the presence of current symptoms of attacks of wheeze and dyspnoea in asthmatics (Yan *et al.*, 1983) and randomly selected subjects in population surveys (Woolcock *et al.*, 1983). The present study adds to this information in relating non-allergic bronchial reactivity to the presence of wheat-specific IgE.

The specific fraction of whole wheat which was most closely related to the development of non-allergic bronchial hyper-reactivity was the water-soluble wheat protein. This supports the findings of Baldo and Wrigley (1978) that water-soluble wheat proteins (especially albumins) have the greatest affinity for wheat flour specific IgE in the sera of bakers with asthma. However, Walsh, Wrigley & Baldo (1984) have drawn attention to the deficiencies of conventional skin-prick testing and RAST in effectively testing allergy to water-insoluble protein, particularly the gluten of wheat flour. They have devised a modified RAST procedure that accomodates both soluble and insoluble allergens. This method will be applied in the present survey to evaluate the relevance of RAST analysis further.

There was a significant association between positive skin-prick test responses to common allergens and increased non-allergic bronchial responsiveness in bakers with negative skin-prick test responses to whole wheat. Thus although the development of immediate cutaneous hyper-sensitivity to whole wheat and its water-soluble fraction is quantitatively more important, hyper-sensitivity to common allergens may also contribute to the development of non-allergic bronchial hyper-reactivity in some bakers.

The significant association between skin-prick test responses to common allergens and whole wheat in this study may be due in part to partial antigenic identity between grass pollens and wheat grain (Blands *et al.*, 1976; Baldo *et al.*, 1982). It is more likely that it indicates a greater degree of wheat flour sensitization in bakers who have pre-existing sensitivity to common allergens since subjects with positive skin-prick test responses to common allergens and negative responses to whole wheat had shorter duration of baking than subjects with positive skin-prick test responses to whole wheat. This is consistent with the findings of Jarvinen *et al.* (1979) that a family or personal history of 'atopic disease' increases the risk of developing respiratory symptoms related to baking.

Significant positive relationships existed between baking duration and skin-prick test response to whole wheat, and between baking duration and non-allergic bronchial reactivity, in the group of bakers who reacted to whole wheat but not to common allergens on skin testing. These findings support the concept that the degree of exposure (in this case, duration) to wheat flour is related to the development of indices of respiratory disease and that wheat flour sensitization is an important pathogenic factor (Theil & Ulmer 1980). The failure to demonstrate the latter relationship in all bakers may have been due to the confounding influence of the relationship between skin-prick test responses to common allergens and non-allergic bronchial hyper-reactivity. Additionally atopic subjects entering the trade who have pre-existing non-allergic bronchial hyper-reactivity may develop respiratory symptoms and leave the industry prematurely. This is supported by the influence of a history of childhood asthma on baking duration in subjects who reacted to common allergens (but not to whole wheat) on skin testing.

In this study there was no significant difference between skin-prick tests and RAST in detecting an association between responses to baking-related or common allergens and indices of respiratory disease. Not only does this agreement strengthen the validity of the associations demonstrated but it suggests that either test could be used in future field studies of bakers.

While other studies have emphasized the prevalence of sensitization and of allergic respiratory disease in bakers, this study extends previous information in that it provides further evidence for a causal relationship between sensitization to a specific occupational agent and bronchial hyper-reactivity. In addition, hyper-sensitivity to common allergens probably contributes to the development of bronchial hyper-responsiveness in some bakers.

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