

Original article

Rye flour induces a stronger early bronchial response than wheat flour in occupational asthma

Background: Our aims were to compare the doses of wheat and rye flour that induce early bronchial responses in occupationally exposed asthmatic subjects and to assess the effects of the dose of inhaled flour, the duration of exposure and the dose rate.

Methods: Ten patients underwent tests with lactose, wheat flour and rye flour. We compared the decrease in forced expiratory volume in 1 s (FEV_1) observed during the challenge with flour and with lactose. We also calculated the amount of flour that was instantaneously active.

Results: Seven subjects had significantly decreased FEV_1 values following exposure to wheat and rye flour and two subjects only did so for rye flour. The provocative dose (PD, dose required to reduce FEV_1 by 15%) of rye was lower than that of wheat flour (geometric mean; PD₁₅ rye: 95 µg; wheat: 368 µg). The calculated doses of rye and wheat flour were better correlated with the change in FEV_1 than were the cumulative doses.

Conclusion: The bronchial response was greater with rye than with wheat flour. The response was related to the dose of allergen inhaled and to the dose rate.

**L. Bensefa¹, C. Villette¹, F. Tabka¹,
E. Causse-Sounillac¹, J. F. Fabriès²,
D. Choudat¹**

¹Centre Hospitalier Universitaire Cochin, AP-HP-
Université Paris 5, Paris; ²Institut National de
Recherche et de Sécurité (INRS), Vandoeuvre,
France

Key words: asthma; bronchial challenge; flour;
occupation; rye; wheat.

D. Choudat
Hôpital Cochin
Service de Pathologie Professionnelle
27 rue du Faubourg Saint-Jacques
75014 Paris
France

Accepted for publication 8 March 2004

Wheat and rye flour are often involved in baker's asthma. The early asthmatic response to wheat flour depends on levels of specific reactivity and exposure (1, 2). Specific reactivity can be assessed by measuring the cumulated dose of flour that induces a 20 or 15% decrease in forced expiratory volume in 1 s (FEV_1); this dose is known as the provocative dose (PD₂₀ or PD₁₅) (1). However, the early asthmatic response resolves spontaneously within 1 h. The deactivation of the allergen and mediators has to be taken into account if the duration of the specific challenge is extended. Therefore, the bronchial response to wheat flour is related to the inhaled dose of wheat flour, the airborne concentration, the duration of exposure, and the deactivation of inhaled allergens and mediators (2). The level of the specific reactivity and the dose rate of allergen are the main determinants of the bronchial response to wheat flour.

Rye flour is also involved in occupational asthma among bakers. Bakeries use less rye flour than wheat flour, but this amount is increasing because rye flour is becoming increasingly popular in 'special breads'. Cross-reactivity has been demonstrated between wheat and rye allergens (3–8). Baldo et al. reported the case of one baker with a positive challenge test to rye and wheat (6). They concluded that bronchial reactivity was higher to rye than to wheat. However, they did not measure the inhaled

doses. The dose–response relationship for rye flour has not yet been established or compared with that for wheat flour.

Our aims were to compare the doses of wheat and rye flour inducing an early FEV_1 response in occupationally exposed asthmatic subjects and to assess the roles of 1) the inhaled dose of flour, 2) the concentration, 3) the duration of the exposure and 4) the dose rate during specific challenges with wheat and rye flour.

Material and methods

Subjects

Ten patients referred for occupational asthma caused by flour agreed to undergo specific bronchial challenges with wheat and rye. All reported work-related symptoms of asthma and underwent a standard check-up including a questionnaire, spirometry and a methacholine test (9). The patients were challenged with lactose, wheat flour and rye flour on three separate days. Bronchodilator and steroid treatments were stopped 24 h before the challenges.

Specific bronchial challenge

Specific bronchial challenges were performed with an aerosol generator as previously described (1, 2, 10). The equipment was fully computerized to calculate the dose of aerosol inhaled by the patient

through a venturi system and a mouthpiece. The dose of flour or lactose inhaled was calculated from the inspiratory flow rate and the instantaneous aerosol concentration measured by a calibrated photometer. The aerosol concentration inside the inhalation chamber was maintained at 3.0 ± 0.2 or 5.0 ± 0.2 mg/m³ (mean $\pm$ SD).

The patient was asked to breathe normally (tidal volume). The inhaled dose was increased by increasing the number and duration of exposure periods. The duration of the inhalation period was increased in a step-wise manner. A maximum of nine periods was used (15 s, 30 s, 1 min, 2 min, 5 min, 15 min, 20 min, 30 min and 30 min). Therefore, the cumulative duration of exposure was 15 s to 104 min, according to specific bronchial reactivity. Each period was separated from the next by an interval of 10 min. During each inhalation period, the dose inhaled was continuously determined by integration of the instantaneous concentration and inspiratory flow rate. The cumulative inhaled dose from the beginning of the challenge was determined at the end of each period (Fig. 1B).

FEV_1 was measured by flow-volume curves with an automatic electronic spirometer 1 and 10 min after each inhalation period. The time-response curve was immediately plotted by computer (Fig. 1A). The lower of the lung function values 1 or 10 min after each inhalation period and the cumulative dose since the beginning of the challenge were automatically plotted to establish dose-response curves (Fig. 2). If the FEV_1 changed by less than 20% the patient was subjected to another inhalation period. The test was stopped when the FEV_1 decreased by 20% or after 104 min of exposure.

On the first day, a control test with lactose was performed. If lung function was stable, the flour challenges were performed on two other days. The Parisian hospitals central pharmacy (Assistance Publique, Hopitaux de Paris, France) provided the lactose powder. The wheat and rye flours were unmixed commercially available products.

Expression and interpretation of the specific challenge results

Changes in lung function were evaluated according to the criterion of the American Thoracic Society for spirometry: if several maximal expiratory manoeuvres were performed, the FEV_1 was the highest value recorded (11). Therefore, we used the highest FEV_1 value recorded separately on each day (lactose or flour) and calculated the change in FEV_1 at the end of each period of exposure (1).

The percentage of variation of the FEV_1 during the tests with lactose or flour, the confidence interval limit of the percentage of variation of the FEV_1 during the test with lactose and the PD of flour causing a 20 or 15% decrease in FEV_1 (wheat: PD_{20w} and PD_{15w}; rye: PD_{20r} and PD_{15r}, respectively) were automatically calculated during the challenges and used to define the level of bronchial reactivity:

- If FEV_1 decreased by more than 15%, the result of the specific bronchial challenge was expressed as the PD causing a 20 or 15% decrease. The PD was determined from the logarithm of the cumulative dose from the beginning of the challenge as for the methacholine test (9). Patients were considered to be highly reactive to wheat or rye flour if the FEV_1 decreased more than 20%.
- Changes in FEV_1 during the lactose test and the flour tests were also compared using confidence intervals (1, 12). Patients were considered to show intermediate reactivity to wheat or rye flour when the FEV_1 decreased by less than 20% but significantly more than with the lactose challenge.

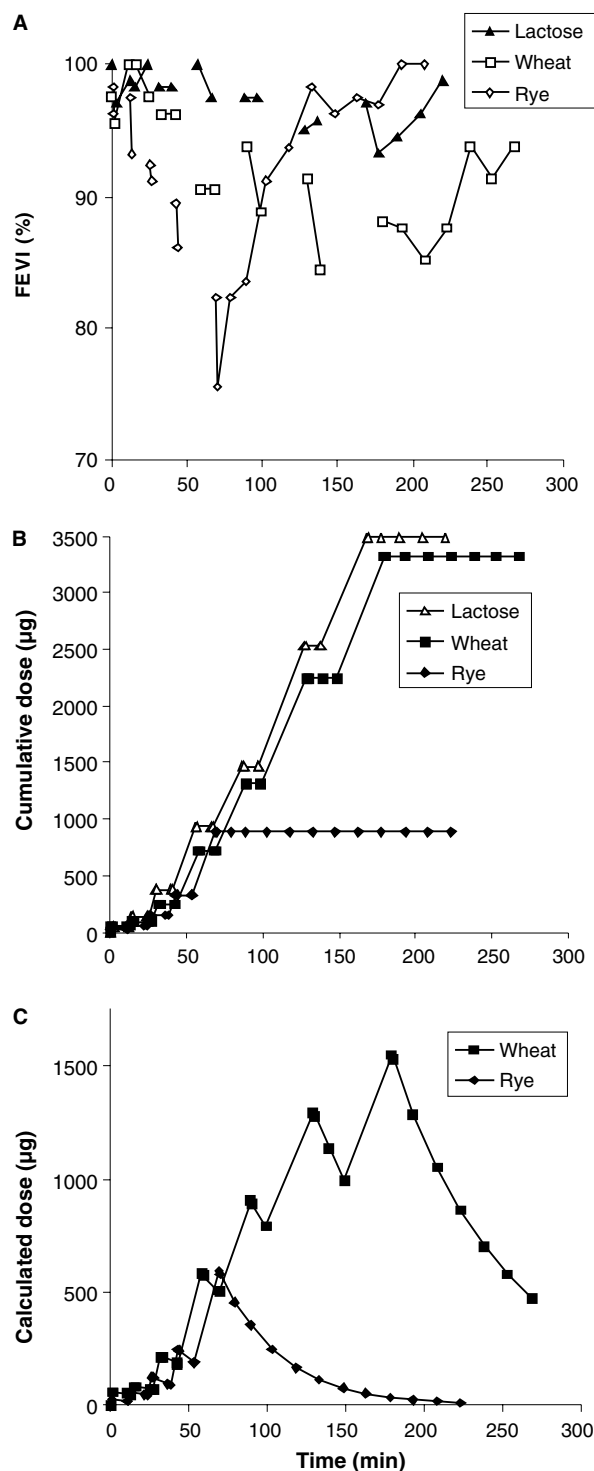


Figure 1. (A) Time-response curves showing changes in FEV_1 over time during the test with lactose ($\blacktriangle$), wheat flour ($\square$) and rye flour ($\diamond$) for one subject (no. 6). (B) Time-dose curves showing the variation of the cumulative dose of lactose ($\triangle$), wheat flour ($\blacksquare$) and rye flour ($\blacklozenge$). (C) Time-dose curves showing the variation of the calculated dose of wheat flour ($\blacksquare$) and rye flour ($\blacklozenge$) taking into account deactivation.

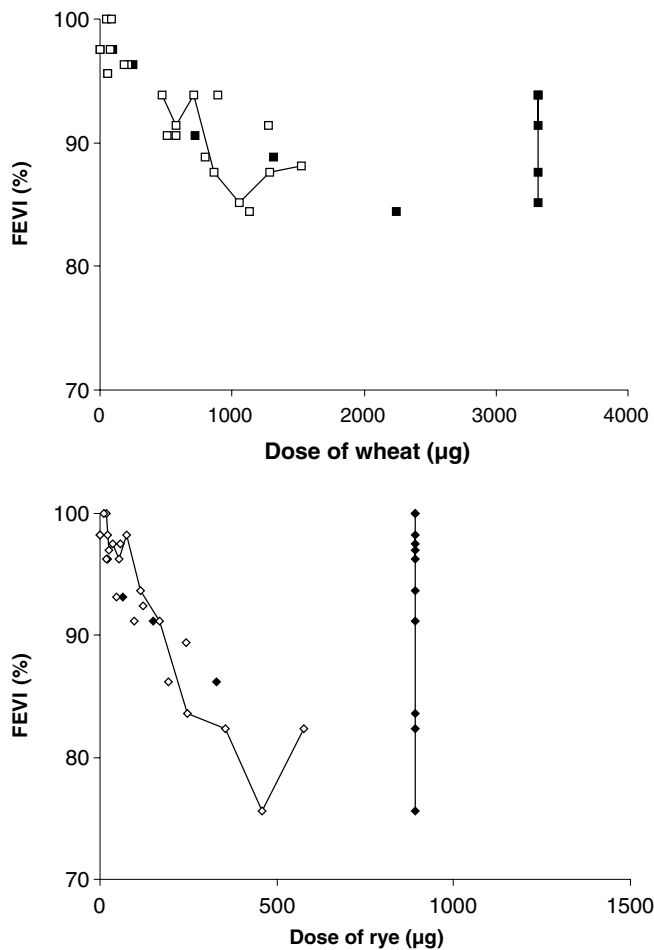


Figure 2. Dose-response curves for subject (no. 6). (A) For the cumulative doses of wheat, the dose-response curve became parallel to the abscissa with increasing dose from 2000 to 3000 μg (■), and the cumulative dose remained constant while the FEV_1 normalized after the end of exposures (—■—). The optimal value of k (0.013 min^{-1}) was determined as that giving the best correlation between the calculated dose of wheat and the FEV_1 during the periods of exposure (□) and recovery (—□—). (B) For the cumulative doses of rye, no plateau was observed because the periods of exposure (◆) were short, but the cumulative dose remained constant while the FEV_1 normalized after the end of exposures (—◆—). The optimal value of k (0.026 min^{-1}) was determined as that giving the best correlation between the calculated dose and the FEV_1 during the periods of exposure (◇) and recovery (—◇—).

Expression of the dose

Two expressions of dose were used to establish dose-response relationships:

1. The cumulative dose from the beginning of the test. The dose-response was analysed as if all the inhaled flour was always bio-available to induce bronchoconstriction (Fig. 2). However, after the exposure, the cumulative dose remained constant while the FEV_1 increased during a period of recovery.

2. The dose of flour that was instantaneously biologically active. This was calculated from the quantity of flour inhaled and by taking into account deactivation of the allergens contained in the flour and catabolism of mediators (2). This model is based on exponential deactivation and links between concentration, inhaled dose and time (2). For each subject, several dose-time curves were constructed for different values of the deactivation coefficient k . These curves were located between the abscissa and the cumulative dose: for the lower values of k , the inhaled allergen was slowly deactivated and the curves were close to the cumulative dose; for the higher values of k , the inhaled allergen was quickly deactivated and the values of the calculated doses were low, close to the abscissa. The dose-response curves were then analysed according to the observed changes in FEV_1 taking into account both the data of period of exposure and of recovery (Fig. 2). The correlations between the calculated doses and the variations of the FEV_1 during the exposure and recovery periods were studied for each subject and each specific challenge. The optimal value of k was determined from the best correlation between the calculated dose and the observed variations of the FEV_1 (annexe of Ref. 2). If the variations of the FEV_1 were not significant, they could not be correlated to the dose and the coefficient of deactivation could not be determined.

Statistical analysis

Descriptive statistics were used to summarize the patients' demographic characteristics. Data that were not normally distributed (e.g. PDs, optimal deactivation coefficient) were log-transformed. Means and standard deviations of the transformed data were calculated and the results are expressed as geometric means. Student's t -test was used to compare the results of wheat and rye challenges. A P -value of <0.05 was considered as significant.

Results

The characteristics of the 10 subjects are described in Table 1.

The inhalation of lactose did not significantly affect the FEV_1 (Table 2). The cumulative dose inhaled was $2467 \pm 670 \mu\text{g}$ (mean $\pm$ SD). The slight changes in FEV_1 observed were consistent with the typical reproducibility of FEV_1 determinations and did not depend on the inhaled dose of lactose.

The total amount of wheat and rye flour inhaled by each subject depended on the subject's specific hyperreactivity (Table 2). All the subjects with change in FEV_1 higher than 15% had distribution of FEV_1 significantly different for the challenge with flour from the distribution for the test with lactose. The decrease in FEV_1 was significantly greater with wheat ($n = 7$) and with rye ($n = 9$) than with lactose. The maximum decrease in airflow was observed about 10 min after the last inhalation period. Reversal of airway obstruction was spontaneous within 1 h of the end of exposure. The seven patients with a FEV_1 decrease higher than 15% during the wheat challenge also had a FEV_1 decrease higher than 15% during the rye challenge (subjects 1–7). Their PD_{15w} was higher than their PD_{15r} (Fig. 3). In two cases, the FEV_1 decreased slightly but not significantly during the

Table 1. Characteristics of the 10 subjects according to specific bronchial reactivity to wheat flour

Patient no.	Age (years)	Smoking	Treatment	Job	Duration of exposure (years)	IgE (specific wheat) (kIU/l)	IgE (specific rye) (kIU/l)	PD ₂₀ (methacholine) (μg)
1	31	Smoker	B + C	Pastry maker	15	30.1	27.0	456
2	32	Nonsmoker	B	Baker	16	35.3	31.4	300
3	27	Smoker	B	Baker	7	15.3	9.18	184
4	27	Nonsmoker	B + C	Baker	10	29.0	14.8	84
5	31	Nonsmoker	B + C	Baker	16	7.8	8.8	Reversibility
6	27	Smoker	H + B	Pastry maker	8	8.2	5.2	1260
7	28	Smoker	H	Pastry maker	12	72.0	36.3	>3100
8	32	Ex-smoker	B + C	Baker	18	3.9	3.9	155
9	44	Ex-smoker	B + C	Baker	35	0.4	0.6	Reversibility
10	29	Nonsmoker	B + C	Baker	12	2.6	1.5	Reversibility
Mean	31				15	20	13	791
SD	5				8	22	12	1094
Geometric mean	31				13	10	8	386

B, inhaled bronchodilator; C, inhaled corticosteroid; H, antihistamine; IgE, specific immunoglobulin E to flour; reversibility, significant increase of FEV_1 after inhaled bronchodilator; PD₂₀, provocative dose of methacholine causing a 20% fall in FEV_1 .

Table 2. Characteristics of the challenge with lactose, wheat and rye among the 10 subjects

Patients number	Lactose challenge	Wheat flour challenge				Rye flour challenge			
	Cumulative dose (μg)	Cumulative dose (μg)	PD _{15W} (μg)	PD _{20W} (μg)	Deactivation coefficient (min ⁻¹)	Cumulative dose (μg)	PD _{15r} (μg)	PD _{20r} (μg)	Deactivation coefficient (min ⁻¹)
1	3116	140	50	69	0.024	39	4	6	0.007
2	2059	258	83	118	0.038	19	4	6	0.023
3	2965	947	356	471	0.011	299	134	152	0.018
4	2204	1388	398	604	0.012	697	378	525	0.010
5	2539	958	776	851	0.032	435	294	395	0.023
6	3494	3318	633	NC	0.013	891	423	671	0.026
7	2776	4593	3175	NC	0.028	138	98	122	0.008
8	2440	4233	NC	NC	NC	1529	688	855	0.015
9	1185	4367	NC	NC	NC	4045	NC	NC	0.023
10	1888	3149	NC	NC	NC	3082	NC	NC	NC
Mean	2467	2335	782	423	0.023	1117	252	341	0.017
SD	670	1774	1088	330	0.011	1386	238	320	0.007
Geometric mean	2372	1447	368	288	0.020	416	95	129	0.015

NC, not calculable.

wheat challenge and significantly during the rye challenge (subjects 8 and 9). The variations in FEV_1 during wheat and rye challenges were not significant for one subject (subject 10). The results for patients 1–9 suggest that they had a higher specific bronchial reactivity for rye flour than for wheat flour.

The deactivation coefficient was calculated when the FEV_1 decreased significantly during the specific challenge (seven wheat challenges and nine rye challenges). The calculated doses of rye and wheat flour were better correlated with the change in FEV_1 than were the cumulative doses. The deactivation coefficient was similar for rye flour and for wheat flour (rye: $0.017 \pm 0.007 \text{ min}^{-1}$; wheat: $0.023 \pm 0.011 \text{ min}^{-1}$; $P = \text{NS}$).

Discussion

We investigated the relationship between the dose of wheat and rye flour inhaled and the early bronchial response in 10 subjects occupationally exposed to flour. A wide range of specific reactivity was observed for rye and wheat flour, consistent with previous results (1, 2). As for house dust, isocyanate and wheat flour, the early asthmatic response was related both to the individual's specific reactivity and to the inhaled doses (1, 13–16). We performed challenge with flour in subjects occupationally exposed to allergen, to confirm the diagnosis of occupational asthma. As negative tests were observed, a control group of healthy unexposed subjects to study the bronchial responsiveness with rye and wheat flour was not necessary.

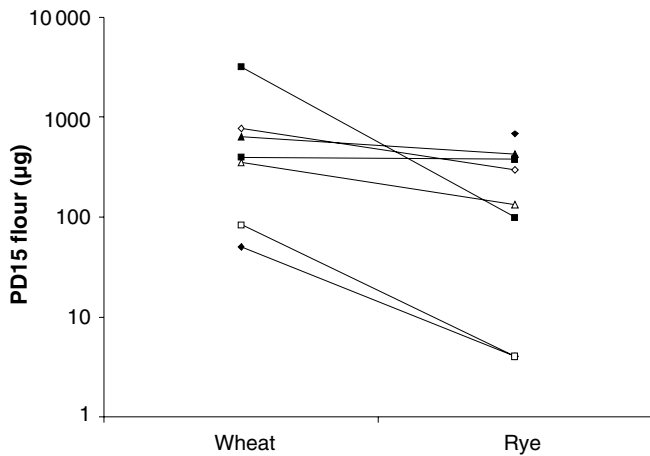


Figure 3. The provocative dose of flour necessary to induce a 15% decrease in FEV_1 (PD_{15}) was greater for wheat than for rye for eight subjects.

We measured the PD_{15} for each patient to allow us to compare the wheat and rye flour challenges. We used PD_{15} rather than PD_{20} because the number of subjects with 15% decrease is higher than the number of subjects with 20% decrease. Moreover, all the subjects with change in FEV_1 higher than 15% had distribution of FEV_1 significantly different during the challenge with flour from the distribution during the test with lactose. In each reactive subject and for the same decrease in FEV_1 , the PD of rye was always lower than that of wheat. Therefore, reactivity was higher for rye than for wheat. This finding may be due to a quantitative and/or qualitative difference:

- Similar allergens may be present in wheat and rye at different concentrations.
- Rye flour may contain specific allergens.

Wheat and rye flour contain similar concentrations of proteins (11.5–13 g/100 g of wheat flour and 12.5–14 g/100 g of rye flour) and cross-reactivity has been demonstrated between wheat and rye proteins. However, rye contains different proteins to wheat.

Baldo et al. showed that one baker had more severe attacks of breathlessness following inhalation of rye flour than wheat flour (6). The bronchial challenge induced a higher decrease in FEV_1 for rye than for wheat. These authors concluded that this baker was more reactive to rye but they did not measure the inhaled doses.

Moreover, we have taken into account deactivation of the inhaled allergen during the specific challenge tests and determined the coefficients of deactivation for each asthmatic subject. The range of the coefficient of deacti-

vation was small and no significant difference was observed between subjects with high and moderate specific reactivity.

The main factors for bronchoconstriction were 1) the level of specific bronchial reactivity of the subject and 2) the dose rate of allergens to take into account the inhaled dose, concentration, duration of exposure and deactivation of allergens and mediators. Our study on rye confirms the findings of Tiffeneau and our previous results on wheat (2, 13). Changes in FEV_1 may be largely a consequence of the dose remaining in the lung, taking into account the deactivation of the flour allergens and mediators. Few published cases suggested deactivation for isocyanates, *Dermatophagoides pteronyssinus*, cat allergen, etc. (2). But, in that cases, deactivation was not studied because the specific challenges were usually not extended, because only the decrease of the lung function was analysed. This deactivation may be neglected for challenges involving a quick increase of the inhaled doses and lasting less than 1 h (2). In contrast, the deactivation may decrease the early response if the test is extended more than 1 h. We have constructed a model to take into account this deactivation and to calculate the dose of flour that remains biologically active. This mathematical model may be used for other allergens such as rye flour, allowing comparisons between allergens in dust form and also aqueous allergens.

In summary, the bronchial response to wheat and rye flour depends on the individual's specific reactivity. This specific reactivity varies greatly from one subject to another. The 10 subjects studied were more reactive to rye than to wheat. Moreover, the bronchial response was related to the dose of allergen inhaled and to deactivation. This deactivation of the allergen and mediators has to be taken into account if the duration of the specific challenge is extended. The mathematical model with exponential decrease established for wheat may be extended to rye flour. This model explains the respective roles of the concentration and duration of exposure. Therefore, the decrease of the lung function is related to the inhaled dose and the dose rate. Quantification of the dose-response may also have consequences for the prevention of asthma, whatever the type of allergen and the type of exposure, occupational or environmental.

Acknowledgments

The authors would like to thank Marie-France Combalot and Richard Wrobel for technical assistance and all the workers included in this study. This study was supported by grants from *Caisse Nationale d'Assurance Maladie des Travailleurs Salariés* and *Caisse Régionale d'Assurance Maladie d'Ile-de-France*.

References

1. CHOUDAT D, FABRIÈS JF, MARTIN JC, VILLETTE C, TABKA F, DESSANGES JF et al. Quantification of the dose of inhaled flour: relation with non-specific bronchial and immunological reactivities. *Eur Respir J* 1999;**14**:328–334.
2. CHOUDAT D, FABRIÈS JF, MARTIN JC, VILLETTE C. Bronchial challenge with flour: early response is dependent on the dose of activated allergen inhaled. *Eur Respir J* 2002;**20**:409–416.
3. SANDIFORD CP, TEE RD, NEWMAN-TAYLOR AJ. Identification of crossreacting wheat, rye, barley and soya flour allergens using sera from individuals with wheat-induced asthma. *Clin Exp Allergy* 1995;**25**:340–349.
4. BLANDS J, DIAMAND B, KALLOS-DEFFNER L, LOWENSTEIN H. Flour allergy in bakers. I. Identification of allergenic fraction in flour and comparison of diagnostic methods. *Int Arch Allergy Appl Immunol* 1976;**52**:392–406.
5. GARCIA-CASADO G, ARMENTIA A, SANCHEZ-MONGE R, SANCHEZ LM, LO OTIN C, CALCEDO G. A major baker's asthma allergen from rye flour is considered more active than its barley counterpart. *FEBS Lett* 1995;**364**:36–40.
6. BALDO BA, KRILIS S, WRIGLEY CW. Hypersensitivity to inhaled flour allergens. Comparison between cereals. *Allergy* 1980;**35**:45–56.
7. SAVOLAINEN H. Flour protein antigens in occupational flour hypersensitivity. *Occup Med* 1997;**47**:341–343.
8. PALOSUO K, ALENUS H, VARJONEN E, KALKKINEN N, REUNALA T. Rye gamma-70 and gamma-35 secalins and barley gamma-hordein cross-react with omega-5 gliadin, a major allergen wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2001;**31**:466–473.
9. STERK PJ, FABBRI LM, QUANJER PH, COCKCROFT DW, O'BYRNE PM, ANDERSON SD et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur Respir J* 1993;**6**(Suppl. 16):53–83.
10. FABRIÈS JF, CHOUDAT D, WROBEL R, CLOUTIER Y, DESSANGES JF, D'ALL AVA J et al. Computerized equipment for the delivery of inhaled doses of solid particles in specific bronchial challenge. *J Aer Med* 2000;**13**:1–10.
11. American Thoracic Society Statement. Standardization of spirometry. 1994 update. *Am J Respir Crit Care Med* 1995;**152**:1107–1136.
12. CHOUDAT D, MARTIN JC, FABRIÈS JF, VILLETTE C, DESSANGES JF. Test de provocation bronchique spécifique avec aérosols solides. Quantification des résultats. *Rev Mal Resp* 2001;**18**:157–162.
13. TIFFENEAU R. Hypersensibilité cholinergo-histaminique pulmonaire de l'asthmatique. Relation avec l'hypersensibilité allergénique pulmonaire. *Acta Allergologica* 1958;**1**(Suppl. V):187–221.
14. van der VEEN MJ, LOPUHAA CE, AALBERSE RC, JANSEN HM, van der ZEE JS. Bronchial allergen challenge with isolated major allergens of *Dermatophagoides pteronyssinus*: the role of patient characteristics in the early asthmatic response. *J Allergy Clin Immunol* 1998;**102**:24–31.
15. VANDENPLAS O, CARTIER A, GHEZZO H, CLOUTIER Y, MALO JL. Response to isocyanates: effect of concentration, duration of exposure, and dose. *Am Rev Respir Dis* 1993;**147**:1287–1290.
16. NGUYEN B, WEYTIJENS K, CLOUTIER Y, GHEZZO H, MALO JL. Determinants of the bronchial response to high molecular weight occupational agents in a dry aerosol form. *Eur Respir J* 1998;**12**:885–888.