

Bronchial allergen challenge: dose versus concentration

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Summary

This study was designed to investigate if two equivalent doses of allergen administered by different dosing regimes—two breaths and 10 breaths of each concentration—would result in the same magnitude of the early and late asthmatic response. Fifteen patients with extrinsic allergic asthma were challenged twice with either two or 10 breaths of twofold increasing allergen concentrations. The challenge was continued until a 20% decrease in FEV₁ had been achieved. A non-cumulative PC₂₀FEV₁ allergen was derived, and the cumulative dose of allergen given was similarly derived. In order to assess the reproducibility of the challenge, seven patients were challenged twice with two-breath regime. The mean value of allergen PC₂₀ obtained by the two-breath regime was 4.1 fold (95% CI: 2.3–7.1 fold) greater than those obtained by the 10-breath regime ($P < 0.05$), whereas the difference was 1.4 fold (95% CI: –3.3–0.5 fold) for the cumulative dose ($P > 0.05$). A statistically significant larger magnitude of the early asthmatic response, as determined by the maximum per cent fall in FEV₁, and late asthmatic response determined by the maximum per cent fall in peak expiratory flow domiciliary recorded during the following 24 hr after challenge, was observed in favour of the 10-breath regime compared to the two-breath regime (mean difference 6%, 95% CI: 0.6–11%). The reproducibility of the provocation test was acceptable (± 1.8 two-fold concentration difference). These results confirm the 'equivalent dose hypothesis', and demonstrates that dosage rather than concentration appears to determine the early and late asthmatic response after bronchial allergen challenge.

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Introduction

Since 1873 bronchial allergen provocation has been used to evaluate the relevance of a specific allergen in patients suspected to suffer from extrinsic asthma. During the recent years it has been used, primarily in research, particularly into the pathogenesis of allergic asthma, and the protective effect of various medications [1].

The data on allergen bronchoprovocation tests, has been either expressed as cumulative dose of allergen administered [2,3], or as the provocative concentration PC, calculated by linear interpolation on the log concentration-response curve [4]. A case report has indicated a dose-dependency of the allergen-induced asthmatic early

and late responses [5], and results have indicated that it is possible to induce a late asthmatic response in a subject who previously demonstrated only an early response by increasing the dose of allergen inhaled [6,7]. Furthermore, it has been reported that natural exposure to house dust can induce immediate bronchoconstriction in asthmatic patients allergic to house dust mite, in a dose-dependent manner [8].

The following study was conducted to investigate whether the effect of inhaled antigen is due solely to the cumulative dose inhaled, or whether the concentration of antigen may contribute to the response [4] by investigating if two equivalent doses of allergen administered by different dosing regimes—two breaths and 10 breaths of each concentration—would result in the same magnitude of early asthmatic response (EAR), as well as to record the late asthmatic response (LAR) induced by the two regimes.

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Patients and methods

Study population

Bronchial allergen challenge (BAC) was performed in 15 patients with mild atopic asthma, three female and 12 male patients (mean age 24 years [range 16–39 years], mean height 177 cm [range 156–180 cm], mean FEV₁ % predicted 83% [range 69–100%]) after informed consent had been obtained. All patients had chest X-rays without infiltrates and showed more than 15% variation in FEV₁ spontaneously or after inhaled bronchodilator [9]. All demonstrated higher peak expiratory flow rates in the afternoon, rather than in the morning. The asthma was controlled with inhaled beta₂-agonists alone, and all demonstrated satisfactory inhaler technique. All patients completed a standard questionnaire of allergic symptoms [10], and a skin prick test with a standard panel was performed on the volar side of the forearm using a Dome Hollister-Stier needle, following Nordic Guidelines [11]. The allergen extract was used in concentrations of 10 Histamine Equivalent Prick (HEP) (ALK A/S, Hørsholm, Denmark) [12]. All patients showed a more than four SE (Standard Units) by RAST analysis [13] for the allergen used for bronchial provocations. The investigation was approved by the local Ethics Committee.

Lung function measurements

Forced expiratory volume in the first second (FEV₁) was measured on a calibrated dry wedge spirometer (Vitalograph Ltd, Buckingham, U.K.) and at least two technically correct forced maximal expiratory manoeuvres with a variation less than 5% in FEV₁ were obtained, the highest value was used for further calculations. Results were reported at ambient temperature, atmospheric pressure and saturated with water vapour (ATPS). Peak expiratory flow (PF) was determined by means of a Mini Wright peak flow meter, and the patient was instructed to perform a forced maximal expiratory effort through the mouth after maximal inspiration. The best of three readings with a variation of less than 10% was reported.

Non-specific bronchial responsiveness

Less than one month before the allergen challenge, the non-specific bronchial responsiveness was assessed by performing a histamine challenge carried out as a non-cumulative dose-response study [14].

Aerosol generation

The aerosols were generated from the aerosol generation unit, in the Bronchoscreen (Erik Jaeger G.m.b.H., Würz-

burg, F.R.G.). The nebulizer (Sandoz 1500 Jet Nebulizer, Sandoz Products, Rockby, Warwickshire, U.K.) was filled with 2 ml of solution and driven by compressed air at a pressure of 1.5 bar delivered from serially connected 2 and 5 litre tanks. The pressure was controlled continuously, and the output of the nebulizer was determined before and after the study by weighing the complete nebulizer on a Mettler balance before and after delivering 10 doses. A solenoid valve, controlled by a timer, delivered 1 dose of aerosol (5.00 µl, standard deviation 0.16 µl, *n*=10) during 500 ms. The dosimeter was triggered 180 ml after the beginning of inspiration. The aerodynamic diameters, of 99% of the dry particles, varied between 0.5 µm and 2.0 µm (mass median aerodynamic diameter = 0.92 µm, geometric standard deviation = 1.30) [15].

Allergen extracts

Allergen extracts standardized according to the Nordic Guidelines [11] were used in stock solutions of 100 000 biological units per millilitre (BU/ml) (Pharmalgen®, ALK A/S, Hørsholm, Denmark). One batch for each allergen of the lyophilized final product was used throughout the study. Extracts were reconstituted and diluted immediately before use in Albumin Diluent® (0.03% human albumin, 0.9% saline, 0.4% phenol) obtained from the manufacturer (ALK), by twofold dilutions in concentrations of 100 000 BU/ml to 98 BU/ml.

The allergens used for challenges were: cat (*n*=6); timothy (*Phleum pratense*) (*n*=4); house dust mite (*Dermatophagoides pteronyssinus*) (*n*=3); dog (*n*=1); and mould (*Alternaria alternata*) (*n*=1).

Pre-challenge procedures

The patients were enrolled in the study when it was assured that, within the last 4 weeks, no exposure to the allergen in question had taken place (except house dust mite). No patients had suffered from respiratory tract infections or received immunotherapy within 4 weeks, nor been smoking or drinking caffeine containing beverages 6 hr before the challenge. Nebulized beta₂-agonist were withheld 6 hr prior to the challenge (*n*=15) [16, 17].

Prior to the first challenge, a skin prick test was performed using a twofold diluted extract of the allergen in question, from 100 000 BU/ml to 98 BU/ml. The allergen concentration giving a skin weal response of 2 mm in diameter was chosen to be the starting concentration for inhalation at this first and subsequent challenges [2].

The challenge tests were performed with an interval of 2 weeks, and took place at the same time of the day, 8 a.m., or 1 p.m. The challenge was called off if FEV₁ was less than 60% of the predicted value, and at each rechallenge variation in start FEV₁ had to be within $\pm 10\%$.

The patients were allocated by random numbers to be challenged with either the two-breath or 10-breath regime at the first challenge and the reverse at the second.

Challenge protocol

After receiving either two breaths or 10 breaths of diluent, twofold increasing concentrations of allergen extract were delivered to the patient with either two breaths or 10 breaths of each concentration. The interval between the inhalation cycles was 10 min. The patient was instructed to breath at normal rate, as the dosimeter operates at tidal volume breathing, and after inhalation of 180 ml, the dosimeter triggers to deliver one dose of aerosol.

In order to assess the reproducibility of the method used, the patients who were randomized to the two-breath regime at the second challenge ($n = 7$), were rechallenged 2 weeks later under the same conditions as previously described.

Measurements of response

After termination of each inhalation period, FEV₁ was measured with 0, 5, and 10 min intervals, the lowest FEV₁ plotted on a log dose-response curve. Allergen inhalation was discontinued at the first concentration that produced at least 20% decrease in FEV₁. Measurements of FEV₁ were then performed every 5 min, until the lung function had returned to at least 85% of pre-challenge value. A PC₂₀ FEV₁ allergen was derived by linear interpolation between the two last points on the log dose-response curve [4,18], and the total cumulative dose of allergen given similarly derived [2]. The challenge was considered negative, if a decrease in FEV₁ less than 20% was observed within 30 min after inhalation of 100 000 BU/ml.

Post-challenge procedures

In order to get an impression, after each challenge, of the late asthmatic response (LAR) (defined as a more than 15% decrease compared to pre-challenge peak flow), the patients were instructed to record PF every hour when awake during the following 24 hr in a diary card. The patients were instructed to use bronchodilator medication (beta₂-agonists) only, if both symptoms of breathlessness and wheezing occurred, and a decrease in PF observed. Any use had to be indicated in the diary.

Statistics

Since results varied over several orders of magnitude, the natural logarithm of the results were used for calculations. Student's *t*-test was used for paired observations. Reproducibility was defined and calculated according to Altmann and Bland [19,20]. The 95% confidence interval (CI) for the overall mean difference between replicated observations was calculated. The single determination, 95% range (tolerance limits), for the difference between replicated estimations, was calculated [21]. Linear relationship was examined by least square linear regression. Calculations were performed by Minitab [22]. Results outside the testing range (not reacting to challenge) were excluded from final analysis. Probability values of $P < 0.05$ were considered statistically significant.

Results

Table 1 shows the PC₂₀ histamine, FEV₁ % predicted, individual maximum % fall in FEV₁ after challenge (EAR), and the maximum % fall in PF registered during the following 24 hr after challenge (LAR) for each patient. A statistically significant larger magnitude of the EAR, as determined by the maximum % fall in FEV₁, was observed after the 10-breath regime compared to the two-breath regime (mean difference 8%, 95% CI: 1–15%). A PC₂₀ allergen, and consequently the cumulative dose inhaled was determined for 12 patients at both challenges (Table 1). Three patients reached the PC₂₀ value at the 10-breath but not at the two-breath regime.

Figure 1 shows the PC₂₀ values compared for the two-, and 10-breath regimes. PC₂₀ FEV₁ (two-breath) was found to be mean 4.1 fold (95% CI: 2.3–7.1 fold) concentration difference higher than PC₂₀ FEV₁ (10-breath) ($P < 0.05$). Figure 2 shows the corresponding cumulative doses inhaled at the two regimes, respectively. No statistically significant difference was seen, being 1.4 fold (95% CI: –3.3–0.5 fold), ($P > 0.05$). A linear relationship between PC₂₀ FEV₁ (10-breath) and PC₂₀ FEV₁ (two-breath) was found ($r^2 = 0.6$, $P < 0.05$). For the corresponding calculated cumulative results, the *P* value almost reached significance ($P = 0.06$).

The late asthmatic reaction (LAR) was observed 6 hr (4–8 hr) after termination of the bronchial allergen challenge. Ten patients developed a 26% (range 15–61%) decrease in PF after the 10-breath regime, six of these patients showed no significant decrease, 6% (range 1–12%), after the two-breath regime (mean difference 6%, 95% CI: 0.6–11%). This was consistent with the smaller use of beta₂-agonists registered during the post-challenge period after the two-breath regime. The remaining four patients developed a LAR after both regimes. One patient

Table 1. Patient characteristics, and effects of the 10-, and two-breath regimes on the magnitude of the EAR and LAR

Patient	Allergen	Baseline FEV ₁ % predicted	Max %-fall in FEV ₁ EAR 10-breath	Max %-fall in FEV ₁ EAR two-breath	Max %-fall in PF LAR 10-breath	Max %-fall in PF LAR two-breath	PC _{histamine}
1	Cat	75	24	20	22	9	0.14
2	Cat	69	22	20	16	7	0.20
3	Cat	106	66	26	14	9	0.75
4	Cat	100	30	28	10	1	1.25
5	Cat	90	27	20	3	11	0.25
6	Cat	80	25	22	15	16	0.03
7	Grass	77	31	23	25	15	2.00
8	Grass	81	24	—	22	1	16.0
9	Grass	90	29	—	23	1	16.0
10	Grass	80	34	—	20	20	0.24
11	Mite	77	42	23	61	55	0.14
12	Mite	80	26	21	26	12	1.73
13	Mite	78	23	21	12	16	0.62
14	Dog	96	25	21	6	9	0.70
15	Mould	87	24	21	25	7	1.45
Mean			30.1	22.2	20.0	12.6	
±			±	±	±	±	
s.e.m.			2.9	0.7	3.5	3.4	

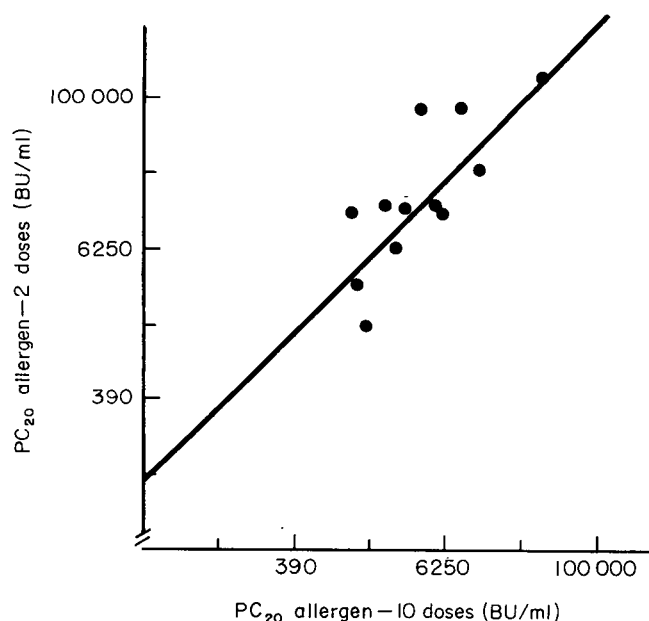


Fig. 1. The results of two bronchial allergen challenges. Non-cumulative PC₂₀ values obtained by inhalation of the same cumulative dose of allergen in different concentrations. PC₂₀ FEV₁ allergen was calculated by linear interpolation between the two last points on the dose-response curve, representing the lowest FEV₁ on the log dose-response curve resulting in a positive challenge. A 4.1 fold shift in the dose-response curve is seen between PC₂₀ values obtained at 10- and two-breath regimes ($n=12$) ($\ln y = 1.2 + 1.0 \ln x$) ($P < 0.05$).

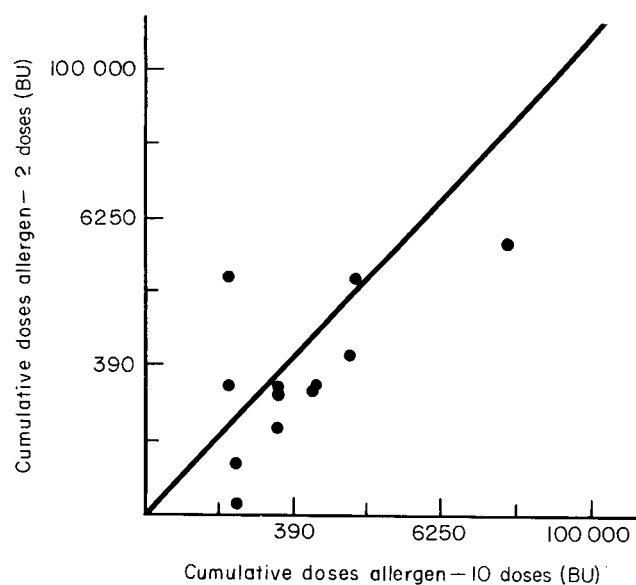


Fig. 2. The result of two bronchial allergen challenges. The same cumulative dose of allergen was administered in different concentrations. The results are reported as the cumulative dose resulting in a positive challenge. The regression line did not differ from the line of identity ($n=12$) ($\ln y = 0.9 + 0.8 \ln x$) ($P > 0.05$).

Table 2. Reproducibility of PC₂₀ FEV₁, and cumulative dose, two-breath regime

Two-breath regime	95% CI overall mean*	95% range single determination†
PC ₂₀ FEV ₁	±0.68	±1.80
Cumulative dose	±0.67	±1.76

*95% confidence interval for overall mean over subjects expressed as a single twofold concentration difference for PC and cumulative dose.

†95% range (tolerance limits) for subjects based on a single determination expressed as a single twofold concentration difference for PC and cumulative dose.

showed a LAR after the two-breath regime only, and four did not develop any LAR.

The non-specific bronchial responsiveness to histamine, expressed as PC₂₀ histamine, is given in Table 1. PC₂₀ histamine was 0.66 mg/ml (range 0.03–2.00 mg/ml) for the group achieving a PC₂₀ allergen at the two regimes, whereas for the remaining three patients, two patients had a PC₂₀ histamine of 16 mg/ml, one patient a PC₂₀ histamine of 0.24 mg/ml, the latter was the patient who developed a LAR, which the other two patients did not.

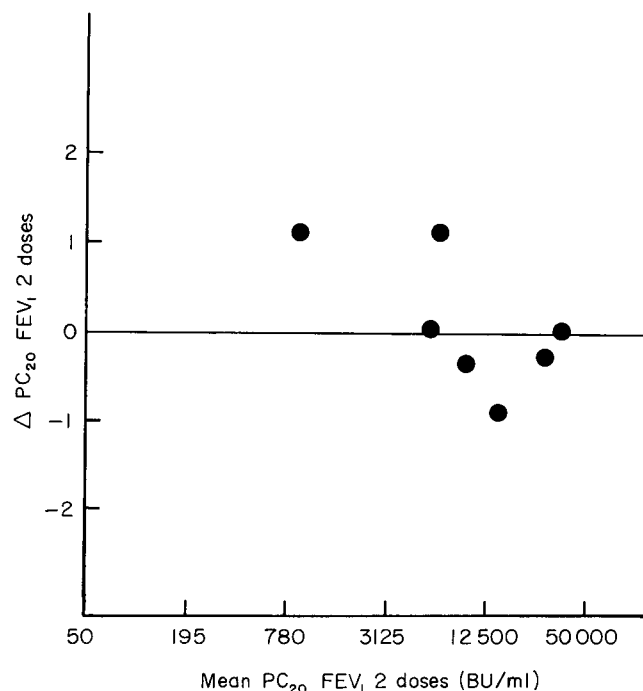
Reproducibility of the two-breath regime (Table 2, Figs 3 and 4), was ±1.8 twofold concentration difference for both PC₂₀, and cumulative dose.

No systemic reactions were observed or other complications reported.

Discussion

In this study we have confirmed the dose-dependency of the early and the late asthmatic response after bronchial allergen challenge, in accordance with other studies [5,6,7]. Furthermore, we have confirmed that result on allergen bronchoprovocation ought to be expressed as cumulative dose of allergen administered [2,3], and that a bronchoprovocation with allergen is highly reproducible when performed under standardized conditions [2,18].

The patients included all had extrinsic asthma in a stable phase controlled by beta₂-agonists alone, and the provocation took place outside the symptom season. All patients, except two, demonstrated hyperreactivity to histamine (PC₂₀ histamine < 2.0 mg/ml). Since the EAR is dependent on non-specific bronchial hyperresponsiveness [23], an assessment of histamine bronchial responsiveness within 1–2 days before each challenge could have been performed to see if there was any significant difference of this airway response between the two study days. How-

**Fig. 3.** Reproducibility of the bronchial allergen challenge expressed as the difference between the PC₂₀ FEV₁ allergen obtained at the first and the second provocation, interval in twofold concentration differences versus mean PC₂₀ FEV₁ ($n=7$) (±1.8 twofold concentration difference).

ever, FEV₁ had to be within ±10% at the two study days, and one could then assume a steady PC₂₀ histamine [24]. It would have improved the design if the dosing regimes were double-blind and placebo-controlled. This could have been achieved by adding some extra breaths.

A linear relationship between PC₂₀ FEV₁ (10-breath) and PC₂₀ FEV₁ (two-breath) was found. For the corresponding calculated cumulative results, the *P* value almost reached significance ($P=0.06$). This may be a result of the relatively limited number of observations. If the 'equivalent' dose hypothesis holds, one would expect one fivefold difference in the intercept (Fig. 1) with a slope of the regression line = 1. This was found for the non-cumulative observations (PC-values, Fig. 1), whereas for the cumulative data (Fig. 2) no such difference was found for the intercept, and the slope was not different from 1 ($P>0.05$). In other words: for the cumulative data, the regression line corresponds to the line of identity, whereas for the non-cumulative data the line is shifted 4.1 fold.

A previous study [4], compared the dosimeter method, as described in this study, and the tidal volume breathing method. The study showed high comparability of the EAR despite the difference in the cumulative allergen dose delivered from the nebulizer. This discrepancy might be accounted for by the fact that the actual dose of

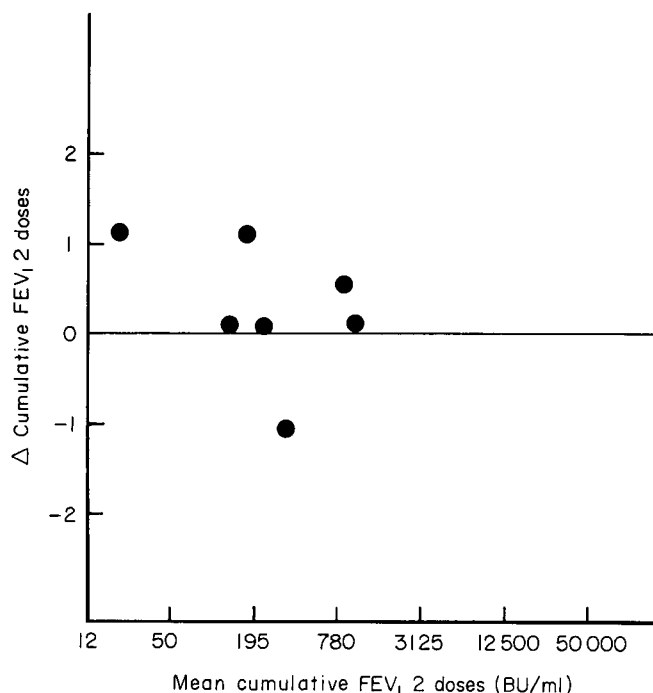


Fig. 4. Reproducibility of the bronchial allergen challenge (two-breath regime) expressed as the difference between the cumulative dose inhaled at the first and second provocation, interval in twofold concentration differences versus mean cumulative dose inhaled ($n=7$) (± 1.8 twofold concentration difference).

allergen reaching the receptor sites during the tidal volume breathing with continuous nebulization, is much smaller than estimated, and a greater part is exhaled than compared to the dosimeter procedure. A recent study has shown that the output of the continuously operated jet-nebulizer may be overestimated when determined by weighing, because of evaporation [25]. This seems to be consistent with the findings concerning the non-specific provocations [17,26,27]. For both methods, however, the site of deposition of inhaled aerosol in the human lung depends on physical characteristics of the aerosol. Such a difference has not been established [15] between the two nebulizers used in the study and, furthermore the mode of inhalation, and the degree of airway obstruction may also influence the bronchial allergen responsiveness [28,29]. The degree of airway obstruction was not different, neither was the mode of inhalation, as tidal volume breathing was used at both provocations.

The LAR of the patients were measured by the use of domiciliary peak flow recording, and although not ideal compared to other studies where FEV₁ has been used [6,7], a significant difference was found. We defined an unequivocal LAR as a maximum fall in PF 3–6 hr after allergen challenge of 15% from baseline (pre-challenge value). As PF recordings prior to inclusion in the study

were always higher in the afternoon than those in the morning, and the patients included were not to use beta₂-agonists unless a fall in PF, as well as breathlessness, was developed after challenge, it is therefore unlikely that a significant spontaneous drop in PF from baseline would have occurred at the time points defined for the LAR. Lung function documented in the laboratory or a control day included for LAR assessment, would have improved the data, bearing in mind the relatively small number of patients. The significant increase in the magnitude of LAR, just reaching significance after the 10-breath regime compared to the two-breath regime, despite the same total cumulative dose given, could reflect the larger variation in the dose inhaled during the two-breath regime, and thereby the dose-dependency. A difference between methods is the relatively longer span of time it takes to reach the same cumulated dose using the two-breath regime than with the 10-breath regime. This leads to a smaller amount of allergen eliminated per time unit. The three patients who did not develop an EAR at the two-breath regime but at the 10 breath regime inhaled a smaller amount of cumulative allergen during the two-breath regime, as the challenge was terminated and considered negative when the dose of 100 000 BU was given (Table 1), for two of these patients a LAR was demonstrated after the 10-breath regime confirming the dose-dependency.

Reproducibility of inhalation challenge results is an important feature of standardization of such tests [16,17]. The reproducibility for the two-breath regime was found acceptable and comparable with results previously reported [4,18]. We examined the two-breath regime as there may be a larger percentage of error (variability), whatever the reason, during the shorter inhalation regime [27].

In conclusion, we have demonstrated the bronchial allergen dose-dependency of the early and late asthmatic response and the acceptable reproducibility of the provocation test used. The findings indicate that the data on allergen bronchoprovocation should be expressed as cumulative dose of allergen administered. The results indicate that standardization of inhalation challenge tests is an important goal as little uniformity in important aspects of method still make comparison of results between centres for practical purposes extremely tenuous.

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