

Fixed ventilation during tidal volume breathing bronchial challenge may improve repeatability

F. MADSEN, L. FRØLUND, N. H. NIELSEN, U. G. SVENDSEN & B. WEEKE

Allergy Unit, Medical Department TTA, State University Hospital, Copenhagen, Denmark

Tidal breathing during bronchial challenge shows both between- and within-subject variation. A challenge protocol permitting controlled ventilation was designed in order to improve repeatability of the method. Twenty-five patients were challenged twice with an interval of 2 h. The 2 min ventilation was kept constant at each concentration administered. During the 2 min inhalation of aerosol all expired air (V_E) was led through a dry gas meter and respiratory frequency was counted concomitantly. The patients were instructed to achieve the same 2 min ventilation at each dosage. All patients accomplished the two challenges without problems. A PC_{20} was determined by interpolation on the log dose-response curve. Repeatability was improved compared to what has been found in previous studies on adults, although statistically non-significant when compared to one study. The 95% confidence interval for PC_{20} based on a single determination was rather narrow, being the observed value ± 0.58 two-fold concentration differences.

Key words: bronchial challenge; bronchial provocation; repeatability.

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CLINICAL ASPECTS

Bronchial challenge has shown to be an effective diagnostic test in the detection of bronchial hyperreactivity and bronchial asthma. The bronchial challenge has also proved effective in confirmation of an allergic etiology in bronchial asthma. This study tests a new method for tidal volume challenge. Ventilation was controlled during tidal volume breathing and repeatability of the method was higher than previously reported.

Non-specific (nBC) and specific bronchial challenge (sBC) have become highly repeatable tests by careful standardization of the procedures (6, 9, 10, 11, 12, 15, 18, 19, 20). The influence of prechallenge conditions (7, 8), bronchoconstrictors (4), nebulizers (15, 20), aerosol delivery systems (18, 20), and challenge protocols on the outcome of a bronchial challenge, has recently been described (3, 5, 16).

One of the major determinants for the results of a BC is the dose of bronchoconstrictor delivered to the mouth (5). In the tidal volume breathing method for BC (tBC) the dose of bronchoconstrictor delivered to the mouth depends on the concentration of bronchoconstrictor, the output of the

nebulizer, the ventilation (V_E), and the inspiratory time (T_i) (20).

Recently it has become clear that the 2 min tidal volume ventilation during BC not only demonstrates variation between subjects but also shows large variation within subjects (13).

The aim of the present study was to test whether fixed ventilation tidal volume breathing BC (fBC) would improve the repeatability of BC.

MATERIAL AND METHODS

After informed consent was obtained, 25 outpatients were included in the study. Anthropometric data, medication and clinical diagnosis are given in Table 1.

Design

The patients were challenged twice with an interval of 120 min. First-second forced expiratory volume (FEV₁) was not allowed to deviate more than 15 % between the first and second challenge.

Prechallenge conditions

BC was performed only if the patients had abstained from antihistaminics for 72 h before challenge, sustained release methylxanthines for 48 h, plain methylxanthines for 24 h, ipratropin and β_2 -adrenergic agonists for 12 h, and inhaled β_2 -adrenergic agonists and sodium cromoglycate for 8 h and smoking for 4 h. Local and systemic steroids were continued unchanged. Chest roentgenograms were normal, and none of the patients had had respiratory tract infections or received vaccination within 2 months prior to the study. The pre-challenge conditions were controlled by a standard questionnaire.

Aerosol generation

The same Wright nebulizer (15, 22) was used for aerosol generation throughout the study. The nebulizer was filled with 2 ml solution and driven by compressed air at a pressure of 1.10 bar which was continuously monitored on an

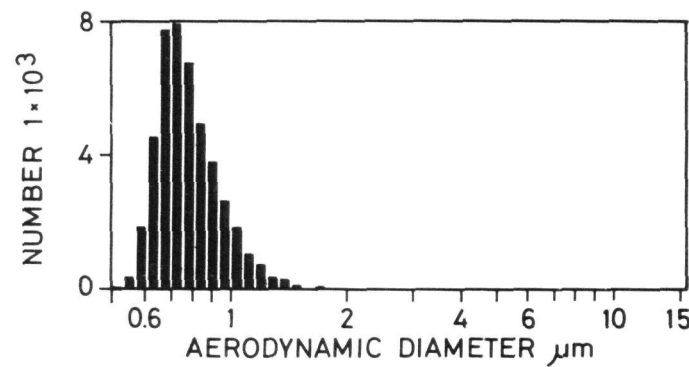


Fig. 1. The Wright nebulizer. Number of particles after dilution 1:10⁴ versus count median aerodynamic diameter.

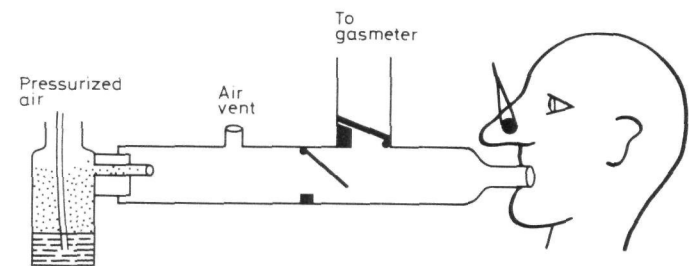


Fig. 2. Nebulizer and valve system for challenge with fixed ventilation.

anaeroid manometer calibrated against a mercury column. Under these conditions the flow was 13.0 l/min and the output 152 μg /min, SD 7 μg/min. The output was determined by calibration of the actual set up and reported as the mean of 10 determinations. Under these condi-

Table 1
Anthropometry, atopy and medication

	Sex	Age (years)	Height (cm)	RAST class	SPT (pos./10)	IgE (U/l)	Eosinophil leukocytes × 10 ⁹ l ⁻¹	Clinical diagnosis	Medication (n)
Mean	13 M 12 F	36	173	1	1.4	640	291	16 asthmatics 6 rhinitis	beta ₂ -agonists (12) local steroid (8)
Range		11-74	155-192	0-4	0-7	4-5940	19-1481	3 non-asthmatics	cromoglycate (1) systemic steroid (1) no medication (8)

RAST: serum radioallergosorbent test, values above 1 were considered positive
SPT: skin prick test (*Alternaria*, *Cladosporium*, cat, dog horse, house-dust, *Derm. pteronyssinus*, *Phleum pratense*, *Artemisia vulgaris* and birch)
IgE: serum immunoglobulin E
beta₂-agonist: nebulized salbutamol or terbutalin and theophylline

tions the count aerodynamic diameter of 95 % of the dry particles varied between 0.5 and 1.8 μm (Fig. 1).

Aerosol composition was analysed during nebulization of saline (15). The aerosol was analysed during 100 s sampling. The aerosol was delivered directly into a Kr 85 neutralizer (TSI model 3054) coupled to a diffusion dryer. The dry particles were led to a mixing chamber and in two steps diluted 1:10⁴ (TSI diluter 3302) and hereafter analysed in an aerodynamic particle sizer (TSI Aps 3300) calibrated with latex particles (15).

The aerosol was led through a unidirectional valve (Astra Meditec) (Fig. 2). Because the inspiratory flow during inhalation of aerosol was higher than the flow through the nebulizer, accessory air was led to the system through an air vent (Fig. 2).

Ventilation

Exhalations were performed through a dry gas meter (Vedras type IIE, Copenhagen, Denmark) calibrated against a wet Tissot spirometer before and after the study. Repeatability of 2 min V_E -measurements during spontaneous tidal breathing (without aerosol inhalation) was determined in 10 patients after habituation to the procedure. Repeatability was expressed as the residual standard deviation (RSD) for replicated measurements; $\text{RSD} = 2.07 \text{ l per } 2 \text{ min}$.

Measurement of response

FEV_1 and forced vital capacity (FVC) were measured with a low resistance heated Lilly pneumotachograph (Eric Jaeger, GmbH, Würzburg, W. Germany) which had a linearity of 2 % at a flow below 12 l/sec, and volume was obtained by electronical integration of the signal over time. Calibration was performed before each challenge, and volumes are reported at body temperature, atmospheric pressure, and saturated with water vapour (BTPS). At least two technically correct forced expiratory manoeuvres with a variation of less than 5 % in FEV_1 were obtained and the highest value was used for further calculation.

Challenge protocol

Patients were accustomed to the procedures and their baseline pulmonary function was determined. Through a mouthpiece and with the nose clipped, 2 min tidal volume breathing inhalation of nebulized isotonic saline was then performed and V_E and respiratory frequency (f) were measured. FEV_1 was determined 90 s after termination of aerosol inhalation. The procedure was repeated 5 min after termination of aerosol inhalation, and mean FEV_1 (saline) and mean 2 min ventilation (saline) were used for further calculation of the percentage fall in FEV_1 after inhalation of histamine chloride (HC).

Thereafter patients inhaled HC in doubling concentrations beginning with 0.03 mg/ml. They were instructed to breathe (for 2 min) as much as was necessary to reach the mean 2 min V_E obtained during inhalation of saline. The patients were under constant observation by a trained technician and encouraged to obtain V_E as close as possible to the V_E -saline. If V_E -saline was reached within 2 min, the inhalation was terminated and the remaining period of time recorded.

HC was inhaled in doubling concentrations until FEV_1 had dropped below 20 % of the mean postsaline value (FEV_1 -saline) or 16 mg/ml was reached. For patients with a baseline FEV_1 above 80 % of the predicted (2) after abstaining from antiasthmatic drugs, the following modifications were used: patients without need of medication started by inhaling HC 2 mg/ml. A start concentration of 0.25 mg/ml was used if the patient used bronchodilators only and 0.125 mg/ml if they used steroids.

Data analyses

Linear interpolation was performed between the last two points on the log dose-response curve. The HC-concentration inducing a 20 % fall in FEV_1 of the postsaline value was calculated and reported as the PC_{20} -value.

Statistical analyses

Results are reported as the mean with the range in parentheses. Repeatability was defined and

calculated according to Altman & Bland (1). The positive difference between the first (x_1) and the second (x_2) observation was plotted against the mean $|x_1 - x_2|$ vs $((x_1 + x_2) \times 0.5)$ to determine whether the variation was related to the size of the measurement. The correlation coefficient between the positive differences and the mean was then tested against the hypothesis of $R = 0$. If the within-subject repeatability was found independent of the size of the measurement, the 95 % confidence interval for the mean difference was calculated. Logarithmic transformation of results was carried out to obtain independence between within-subject variation and the size of the measurement. Relative differences in prechallenge FEV₁ were calculated according to Oldham (17) taking differences in initial value into consideration.

Variation of replicated measurements was determined by analyses of variance (21). Variances were compared by the F-test (21). *P*-values below 0.05 were considered statistically significant (two-tailed test).

RESULTS

Prechallenge pulmonary function as well as 2 min ventilation, fR and PC₂₀ are given in Table 2. After 10 min habituation to the procedures, all patients were able to perform fixed volume tidal breathing (Table 2). Large between-subject differences in V_E during challenge were observed. The within-subject variation in V_E was practically eliminated, since all patients after training were able to reproduce V_E within 200 ml. Tidal volume (V_T) varied between 0.34 l and 1.35 l. There was no statistical difference

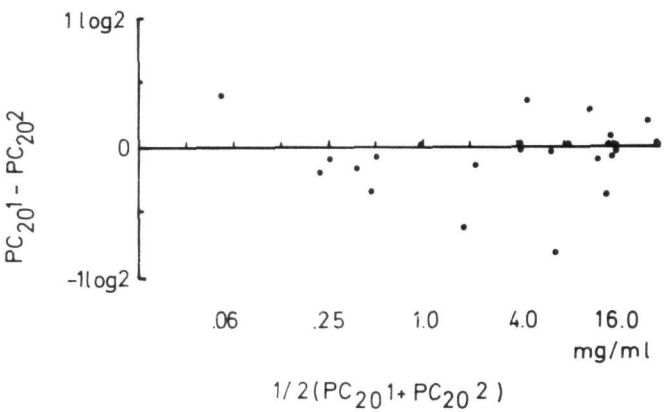


Fig. 3. Repeatability of PC₂₀. Differences versus mean: log 2 = one two-fold concentration difference.

between f and V_T, respectively, between the two challenges. The PC₂₀-values were equally distributed over the testing range, the majority of PC-values being between 0.25 and 32 mg/ml. The repeatability did not depend on the size of the measurement as the positive differences between replicates did not correlate with the mean of the replicates ($r = 0.22$, $P > 0.10$). Fig. 3 shows the repeatability of the new method. It is seen that none of the differences between replicates was larger than 0.9 two-fold concentration differences. After logarithmic transformation the mean difference between replicates was -0.05 mg/ml, SD 0.189. Therefore, the 95 % confidence interval for PC₂₀ based on a single determination (95 % CI) was the observed value ± 0.58 two-fold concentration differences.

Repeatability of ftBC was found improved when compared with previously published studies by Madsen et al. (14) (95 % CI = ± 1.72

Table 2
Pulmonary function, ventilation during challenge, and PC₂₀

	FEV ₁ (1)	FVC (1)	FEV ₁ % predicted %	V _E -2 min (1)	f (1) (min ⁻¹)	f (2) (min ⁻¹)	V _T (1) (l)	V _T (2) (l)	PC ₂₀ (1) (mg/ml)	PC ₂₀ (2) (mg/ml)
Mean	3.04	3.79	83	20.3	14	13	0.78	0.78	8.50	8.60
Range	1.3-5.00	2.20-6.60	43-118	12.5-26.4	8-27	7-23	0.34-1.15	0.39-1.35	0.06-30.00	0.06-30.00

V_E-2 min: ventilation during 2 min inhalation of aerosol
f (1): respiratory frequency during first challenge
f (2): respiratory frequency during second challenge
V_T: tidal volume inhalation of aerosol

two-fold concentration difference, $n = 25$, F-test: $P < 0.01$), Dehaut et al. (9) (95 % CI = ± 1.59 two-fold concentration differences, $n = 18$, F-test: $P < 0.05$), Ryan et al. (20) (95 % CI = ± 1.09 two-fold concentration differences, $n = 10$, F-test: $P < 0.05$) and Ruffin et al. (19) (95 % CI = ± 0.94 two-fold concentration differences, $n = 12$, F-test: $P < 0.05$). However, an improved repeatability was not found when ftBC was compared to the study by Juniper et al. (12) (95 % CI = ± 0.83 two-fold concentrations differences, $n = 10$, F-test: $P > 0.05$).

DISCUSSION

Compared to other studies (9, 14, 19, 20) repeatability of PC_{20} was statistically significantly improved by controlled ventilation during challenge. However, the improvement was statistically non-significant when compared to a previous study by Juniper presenting the highest repeatability (12). This could be due to the patient selection or an error of the second order, since only few patients were examined. During tBC there is no regulation or determination of f , V_E or T_i . The tBC not only implies large between-subject variation, but also large within-subject variations are seen (13). Hitherto, careful regulation of the tidal volume breathing, however, has been considered unimportant (12).

Repeatability of a BC depends basically on the patient selection, and spontaneous variation in PC_{20} in patients will set a lower limit for the repeatability that can be obtained. Subsequently, patient selection is important in studies dealing with repeatability and reproducibility of BC.

The patients in this study were comparable to patients in our own previous study on BC repeatability (14) as well as patients in other repeatability studies (9, 12, 19, 20) with regard to diagnosis, atopy, medication and especially stability of their asthma.

In two studies with high repeatability (12, 20), the patients had a higher $FEV_1\%$ of predicted than in this study and in the study by Dehaut et al. (9). The patients in our study were not familiar with BC.

In this study care was taken to standardize prechallenge conditions together with challenge protocol and equipment. The only difference from our previous study (14) was the use of a Wright nebulizer (15, 22) which was used to make results comparable to previously reported results (9, 11, 12, 19, 20).

The response to a challenge is a function of the dose delivered to the mouth and therefore determined by the bronchoconstrictor concentration, nebulizer output and the inspiratory time, and subsequently the breathing pattern. In this study both the nebulizer output was well characterized in terms of solution delivered per unit time (152 $\mu\text{g}/\text{min}$) and per unit airflow (152 μg per 13 l). The dilution of aerosol from accessory air through air vent (Fig. 2) depends on the relation between nebulizer flow and inspiratory flow, but the dose available to the mouth is determined by the T_i and the output (dose = output $\times T_i$). Thus T_i may be more important than V_E for the dose delivered to the mouth and it may show less within-subject variation than does V_E . Therefore T_i during BC may be an interesting factor for further investigation. We counted spontaneous f which showed relative stability (Table 2). In a repeatability study by Dehaut (9) f was controlled by a preset metronome ($14 \times \text{min}^{-1}$) but repeatability of BC was not improved when compared with other studies (11, 12, 14, 19, 20).

The dosimeter method is operated with a carefully controlled dose delivered to the mouth and therefore a higher repeatability could be expected, but this has not been documented (19, 20).

In the tidal volume breathing challenge the total dose at each concentration is distributed over 20–40 breaths, reducing the relative importance of each breath to 1/20–1/40. In the standard dosimeter and reservoir method (18, 19, 20) the dose is administered in five breaths, and compared with the tidal volume breathing method the relative importance of each breath will then be four to eight times higher. This may contribute to the explanation of the comparable repeatability of the methods.

Comparison of repeatability of a standard-

ized BC performed on patients who may differ in several ways should be carefully considered.

At present it seems safe to conclude that control of ventilation during tBC may lead to improved repeatability. For routine challenge careful regulation of tidal breathing may not be essential. However, this may not hold when bronchodilators or other drugs influencing ventilation are administered before challenge.

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Address:

Flemming Madsen, M.D.

Ferringvej 12

DK-2720 Vanløse

Denmark

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