

# Methacholine bronchial challenge using a dosimeter with controlled tidal breathing

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**ABSTRACT** A new inhalation synchronised dosimeter triggered by low inspiratory flow rates has been assessed. The methacholine challenge test using dosimeter nebulisation with controlled tidal breathing was compared with continuous nebulisation using De Vilbiss No 40 nebulisers with deep inhalations in 11 asthmatic subjects. Within subject  $PD_{20} FEV_1$  values were lower with the dosimeter method than with the continuous nebulisation method (geometric means 158 and 588  $\mu g$ ). The repeatability of the dosimeter method with controlled tidal breathing was studied in 11 asthmatic subjects, and the 95% range for a single measurement was  $\pm 0.72$  doubling doses of methacholine. The dosimeter method has greater efficacy because aerosol is delivered during the first part of an inhalation, minimising loss of aerosol outside the respiratory tract. The dosimeter technique combined with controlled tidal breathing appears to be a useful method for carrying out standardised non-specific bronchoprovocation tests.

## Introduction

Measurement of airway responsiveness to inhaled non-specific agonists such as histamine and methacholine are used increasingly for research, for clinical assessment, and for epidemiological surveys of obstructive lung disease. The way in which the aerosol for the bronchial challenge test is produced and inhaled may influence the results. The physical characteristics of the aerosol generation system, the breathing pattern used, and the anatomical and physiological characteristics of the airways are important factors determining the dose and distribution of aerosol to the lung.<sup>1,2</sup>

For aerosol delivery continuous nebulisation with deep inhalation or with tidal breathing is used most commonly, but many centres use dosimeter techniques as these may be more accurate.<sup>1-3</sup> Although the values obtained from the various combinations of inhalation and aerosol delivery techniques may be highly reproducible, their use creates difficulties when comparison of the results from different laboratories is required.<sup>4,5</sup>

When Ryan *et al*<sup>6</sup> compared dosimeter delivery plus deep inhalation with continuous nebulisation plus tidal breathing for histamine challenge testing, they found no major differences in the measurement of bronchial reactivity, although aerosol deposition was shown to be more central with the dosimeter and deep inhalation method. Using the same methods for methacholine challenge, Bennett and Davies,<sup>7</sup> found dosimeter delivery with deep inhalation to be less reproducible than continuous nebulisation with tidal breathing.

In the present study we assessed a new inhalation synchronised dosimeter, Spira Elektro 2, which, because it is triggered by a very low inspiratory flow rate, can be used with tidal breathing.<sup>8</sup> Dosimeter nebulisation with controlled tidal breathing was compared with continuous aerosol delivery with deep inhalations for methacholine bronchial challenge in asthmatic subjects. The reproducibility of the dosimeter method was also studied.

## Methods

### SUBJECTS

Twenty two non-smoking patients with chronic, stable asthma were recruited from the outpatient clinic of the pulmonary department at Tampere University Central Hospital. There were 14 women and 8 men.

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with a mean age of 40.5 (range 16–63) years. All subjects gave informed consent according to the Helsinki Declaration, and the study was approved by the Ethics Committee of Tampere University Central Hospital.

All had a documented variation in forced expired volume in one second (FEV<sub>1</sub>) of more than 20%, either spontaneously or after medication, and fulfilled the criteria of bronchial asthma as defined by the American Thoracic Society.<sup>9</sup> None had had an upper respiratory tract infection or an exacerbation of their asthma in the preceding six weeks. At the time of the study patients were receiving beta agonists or inhaled corticosteroids only as medication and the dose regimen did not change during the study. The pre-challenge FEV<sub>1</sub> was over 70% of the predicted value in two patients, and over 75% of predicted in the remainder. Data on the individual patients are available from the authors on request. Beta agonists were withheld for at least eight hours before each challenge.

#### BRONCHIAL CHALLENGE TECHNIQUES

Two methods were used for methacholine challenge: a dosimeter technique with controlled tidal breathing<sup>8</sup> and continuous nebulisation with deep inhalations.<sup>10,11</sup> Eleven subjects were tested with both the methods in random order. The other 11 subjects were tested twice with the dosimeter technique to assess the reproducibility of the method. The provocation tests were performed at the same time of day, two weeks apart. The baseline FEV<sub>1</sub> was required to be within 10% of the value on the first day.

#### *Dosimeter technique with controlled tidal breathing*

We used an automatic, inhalation synchronised dosimeter jet nebuliser, Spira Elektro 2 (Respiratory Care Center, Hameenlinna, Finland; fig 1). The aerosol delivery time can be adjusted from 0.2 to 2.9 seconds and the start of aerosolisation, which is determined by a threshold volume of inspiration, from

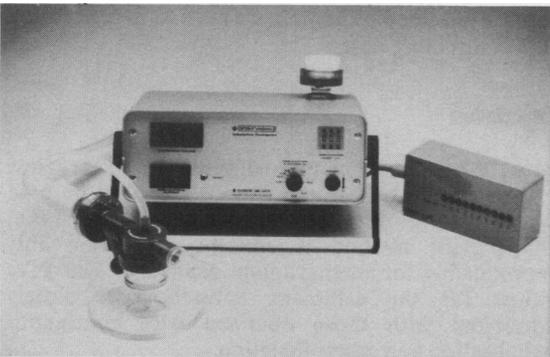


Fig 1 *Spira Elektro 2* dosimeter.

0 to 1000 ml. The volume of each inhalation and the number of nebulisations are displayed digitally; inhalation flow rate is indicated on a detachable flow indicator. An adjustable flow restrictor on the inspiratory side of the one way breathing valve controls inspiratory flow rate. The breath actuated, variable timing circuit regulates air through a solenoid valve to a standard Spira nebuliser with a flow rate of 7.5 l/min to give aerosol particles with a mass median aerodynamic diameter (MMAD) of 1.6 (geometric SD 1.4)  $\mu\text{m}$ .<sup>8</sup>

The dosimeter was adjusted to nebulise for 0.5 second from the beginning of each inhalation. With a 0.5 second nebulisation period the mean (SD) output was 7.1 (0.5)  $\mu\text{l}$ /breath, determined by five weighings on a gravimetric balance. Each patient practised the dosimeter nebulisation before the study. The mouthpiece of the nebuliser was held firmly between the teeth and a noseclip applied. Patients controlled their tidal breathing with the flow indicator and digital readout so that inspiratory flow rate reached but did not exceed 0.5 l/s, and the intraindividual variation in tidal volume was within  $\pm 10\%$ .

After nebulisation of 36  $\mu\text{g}$  of saline, methacholine was delivered in 10 successive, increasing doses ranging from 18  $\mu\text{g}$  to a cumulative dose of 2300  $\mu\text{g}$  (table).

With both methods the inhaled methacholine dose means the amount of agonist delivered to the mouth during inhalation.

#### *Continuous aerosol delivery technique with deep slow mode of inhalation*

The output of several DeVilbiss No 40 glass nebulisers (De Vilbiss Co, Pennsylvania) was determined by five weighings on a gravimetric balance so that we could select two nebulisers with a mean output of 0.18 (SD 0.01) ml/min. Thus the volume delivered to the mouth during each two second inhalation was 6  $\mu\text{l}$ . The DeVilbiss nebulisers were driven by air at a flow rate of 6 l/min, to give a particle size of MMAD 3.5 (GSD 3.0)  $\mu\text{m}$ .<sup>2</sup> The mouthpiece was held between the teeth, and a chronometer used to control the time of inhalation. The subjects exhaled to slightly below functional residual capacity and then inhaled slowly for two seconds, towards total lung capacity, followed by a normal exhalation. The inspiratory flow rate was about 0.5–0.8 l/s.

Inhalation of 30  $\mu\text{g}$  of saline aerosol was followed by successive inhalations of methacholine in concentrations from 0.25 to 25 mg/ml, to give six cumulative doses ranging from 7.5 to 4000  $\mu\text{g}$  (table).

#### MEASUREMENT OF BRONCHIAL REACTIVITY AND EXPRESSION OF DATA

FEV<sub>1</sub> was measured by rolling seal spirometer (Ohio

## Details of methacholine doses delivered by the two methods

Dose number	1	2	3	4	5	6	7	8	9	10
<b>DOSIMETER METHOD</b>										
Methacholine concentration (mg/ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
No of inhalations	1	1	2	2	4	1	1	2	4	4
Cumulative dose ( $\mu\text{g}$ )	18	36	71	110	180	360	530	890	1600	2300
<b>CONTINUOUS NEBULISATION METHOD</b>										
Methacholine concentration (mg/ml)	0.25	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
No of inhalations	5	5	10	5	10	10	10	10	10	10
Cumulative dose ( $\mu\text{g}$ )	7.5	83	230	980	2500	4000	4000	4000	4000	4000

800, Airco, Ohio) before the challenge and three minutes after inhalation of normal saline and each dose of methacholine with the subject seated. The best of three attempts was taken. Bronchial challenge was terminated when FEV<sub>1</sub> fell by at least 20% from the post-saline value. The fall in FEV<sub>1</sub> was plotted against methacholine dose on a log scale, and the provocative dose causing a 20% fall in FEV<sub>1</sub> (PD<sub>20</sub>FEV<sub>1</sub>) was calculated.

After logarithmic transformation of PD<sub>20</sub> values the Spearman rank correlation test and the paired *t* test were used to compare measurements obtained with the two challenge methods. A probability value of 0.05 was considered to be significant. The repeatability of the dosimeter method was evaluated by the method of Altman and Bland<sup>12</sup> by relating the difference between the first and the second measurement with their mean value in log<sub>10</sub> units to ensure that within subject variation was independent of the size of the measurement. From the standard deviation of the differences between measurements the 95% range for a single measurement was calculated from the formula  $t_{0.05}(\text{SD})/\sqrt{2}$ .

## Results

Although PD<sub>20</sub>FEV<sub>1</sub> measurements with the two methods were related (fig 2), intraindividual PD<sub>20</sub>FEV<sub>1</sub>

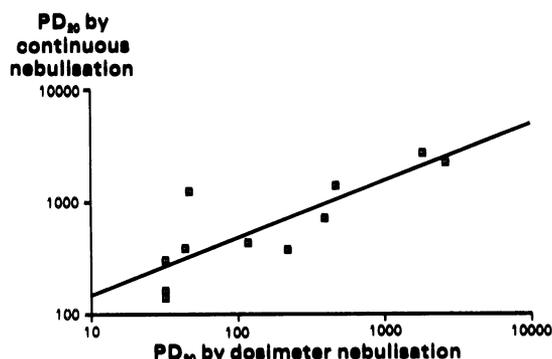


Fig 2 Comparison of PD<sub>20</sub>FEV<sub>1</sub> ( $\mu\text{g}$ ) values from methacholine challenge performed by dosimeter nebulisation with tidal breathing and by continuous nebulisation with deep inhalations in 11 asthmatic patients.

## Difference in log PD<sub>20</sub> by dosimeter method

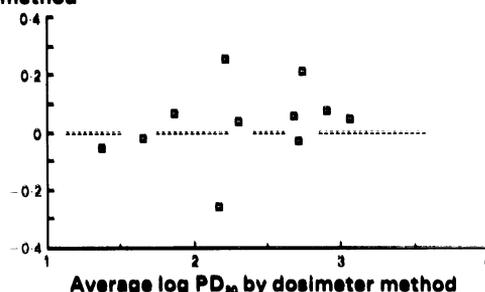


Fig 3 Difference against mean for repeated measures of PD<sub>20</sub>FEV<sub>1</sub> during the dosimeter nebulisation with tidal breathing for methacholine challenge in 11 asthmatic patients (after logarithmic transformation).

values were significantly less with the dosimeter method (geometric mean 158, range 32–2260  $\mu\text{g}$ ) than with the continuous nebulisation technique (588, range 138–2720  $\mu\text{g}$ ,  $p < 0.005$ ).

There was no relation between within subject variation and the size of the PD<sub>20</sub>FEV<sub>1</sub> measurement (fig 3). The mean difference between replicates was 0.03 (SD 0.14) log<sub>10</sub> units. The 95% confidence interval of PD<sub>20</sub>FEV<sub>1</sub> based on a single measurement was the observed value  $\pm 0.72$  doubling doses. When the provocative doses were corrected for vital capacity (VC) and expressed as PD<sub>20</sub>FEV<sub>1</sub>/VC, the 95% range for a single determination was the observed value  $\pm 0.64$  doubling doses.

## Discussion

Most dosimeters are not triggered by a very low inspiratory flow rate, and cannot therefore be used with controlled tidal breathing. Our data suggest that the present method is efficient and reasonably reproducible for methacholine challenge. The PD<sub>20</sub> values for the asthmatic subjects were closely correlated with those obtained with continuous nebulisation and deep inhalation.

There are few comparisons of the response to bronchial challenges using different methods and

comparison is sometimes difficult because the airway responses have been measured in terms of the concentration of bronchoconstrictor given rather than the dose inhaled.<sup>4</sup> Beaupré and Malo compared the airway response of 20 asthmatic subjects to histamine inhaled either by tidal breathing for two minutes from a Wright's nebuliser or by taking five deep slow breaths from a DeVilbiss 646 nebuliser actuated by a dosimeter for 0.6 seconds during inspiration.<sup>13</sup> They found a reasonably close relation between the concentrations of histamine producing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>FEV<sub>1</sub>) with the two methods, as did Ryan *et al*<sup>6</sup> in a similar study. In this study, however, the nebuliser output was found to be 294  $\mu$ l with continuous nebulisation and only 45  $\mu$ l with the dosimeter.

These two reports suggest that a continuous nebulisation technique with tidal breathing requires a four to six fold increase in bronchoconstrictor dose to achieve an airway response similar to that with the dosimeter technique with deep inhalations.<sup>4</sup> This estimate of bronchoconstrictor dose from continuous aerosol delivery, however, was for the whole nebulisation period of two minutes. As 66–75% of nebulisation occurs during expiration,<sup>14</sup> the inhaled dose will be considerably smaller.

In the present study we measured the airway response to the dose of methacholine delivered to the mouth during inhalation. In the case of continuous nebulisation the inhaled dose was calculated from the output of the nebuliser and the recorded inhalation period; with the dosimeter method the dose was determined from the nebuliser output and time of nebulisation. The dosimeter method required a significantly smaller dose of methacholine to produce the same degree of bronchial obstruction as continuous nebulisation. The difference may be due to several factors.

With continuous nebulisation aerosol is delivered throughout inhalation and the aerosol nebulised towards the end of inspiration will be deposited in the main airways or, because of the "last in first out" principle, will be exhaled.<sup>15</sup> The loss of aerosol from large airways, up to 20% of the total amount nebulised, is independent of the mode of inhalation.<sup>16</sup> In contrast, the dosimeter technique, by delivering aerosol only during the first part of inhalation, reduces the exhaled loss of aerosol to 1–2% of the nebulised solution.<sup>8</sup> More effective use of inspiratory time with the dosimeter method may explain in part why less bronchoconstrictor drug is required.

A substantial advantage of our dosimeter technique is the breath by breath control of inspiratory flow rate and volume. Within subject variation in tidal breathing of up to 48% was found by Madsen *et al* during 187 bronchial challenges.<sup>17</sup> When ventilation

was controlled the reproducibility of bronchial challenge improved.<sup>18</sup> With our dosimeter method the intraindividual variation in tidal volume was within 10%, and the inspiratory flow rate did not exceed 0.5 l/s. With continuous nebulisation the maximum inhaled flow rate was about 0.5–0.8 l/s. This may favour more central aerosol deposition and a smaller fall in FEV<sub>1</sub>, as aerosol penetration to smaller airways is inversely related to inspiratory flow rates.<sup>6,19</sup>

Our data might also be explained by the larger droplet size produced by the DeVilbiss 40 nebuliser than by the Spira Elektro 2 (3.5 versus 1.6  $\mu$ m MMAD). This would favour more central aerosol deposition and a smaller airway response, although Ryan *et al*<sup>2</sup> reported that with particle sizes of 1.3–3.6  $\mu$ m the effect on methacholine response is minor.

The reproducibility of non-specific bronchial provocation tests has been documented in subjects with stable asthma with continuous aerosol delivery methods and with the dosimeter techniques with deep inhalations.<sup>20–23</sup> The present data suggest that repeatability with the dosimeter technique with controlled tidal breathing is acceptable, though we did not compare it directly with the repeatability of other methods in the same patients.

A possible pitfall of current dosimeter methods is that, although the aerosol doses are fixed, the difference in airway surface areas between patients is neglected. When aerosol is delivered by continuous nebulisation during the whole of inspiration, the amount of bronchoconstrictor inhaled is "automatically" related to the subjects' lung volumes. With the dosimeter this correction is abolished and there may be a need for a new unit—for example dose/vital capacity (VC), to cover both the dose and the area of challenged airways. Our present data did not, however, indicate any substantial improvement in repeatability when the results were expressed in  $\mu$ g/VC.

With the dosimeter method we gave 10 cumulative doses of methacholine and the test took up to 45 minutes to complete. In clinical practice the test might be shortened by combining some of the doses if the patient has no history of increased bronchial responsiveness and has shown no response to the previous dose. An additional advantage of the dosimeter method is the minimal pollution of the working environment with the challenge aerosol during the provocation test, thereby avoiding non-specific conjunctival, nasal, and lower airways irritation in the technicians. The efficacy of the method also favours its use for the delivery of new experimental compounds, which are often expensive and hence dose limited.

We conclude that accurate aerosol delivery by dosimeter combined with controlled tidal breathing is

an efficient and reproducible method for non-specific bronchoprovocation. Further work is needed to determine whether the response to dosimeter nebulisation should be corrected to airway size.

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