Importance of the time interval between FEV\textsubscript{1} measurements in a methacholine provocation test

P. Malmberg, K. Larsson, B.M. Sundblad, W. Zhiping

**ABSTRACT:** We examined the hypothesis that a forced expiratory volume in one second (FEV\textsubscript{1}) manoeuvre (and the preceding deep inhalation) before inhalation of methacholine might influence FEV\textsubscript{1} measured after methacholine, if the time between measurements was short. Six to nine healthy subjects inhaled a single dose of methacholine, known to cause about 20% decrease in FEV\textsubscript{1}, on different days in different test protocols. If an FEV\textsubscript{1} manoeuvre was performed immediately before methacholine, the first FEV\textsubscript{1} measured 3 min after provocation was higher (77% of basal FEV\textsubscript{1}) than if a pre-methacholine FEV\textsubscript{1} manoeuvre was not performed (64%). This effect of a pre-methacholine FEV\textsubscript{1} manoeuvre was also demonstrated at 2, 4 and 6, but not at 10 min after the start of methacholine inhalation. If an FEV\textsubscript{1} manoeuvre was not performed before methacholine, the second and subsequent FEV\textsubscript{1} measured in constricted airways was higher than the first, and of similar magnitude to the first FEV\textsubscript{1} in tests where a pre-challenge FEV\textsubscript{1} manoeuvre was performed.

In another trial, 10 healthy subjects performed two stepwise methacholine tests, with either 6 or 3 min between dose steps. The percentage decrease in FEV\textsubscript{1} per mg of inhaled methacholine decreased from 2.6 (1.9–5.2) to 1.7 (0.8–2.3) (median, interquartile-range) when the time interval was shortened.

The results suggest that the deep inhalation associated with the FEV\textsubscript{1} manoeuvre decreases the bronchial tone in airways constricted by methacholine for up to 6 min, possibly due to yielding of cross-links in airway smooth muscles. 


The present study was originally motivated by a desire to shorten the time interval between the dose steps from 6 to 3 min in a stepwise methacholine bronchial provocation test. This resulted in the surprising finding (study A, reported below) that, with the short protocol, the subjects appeared less responsive to methacholine measured as change in the forced expiratory volume in one second (FEV\textsubscript{1}).

A possible explanation is that the deep inhalation (DI) associated with measurement of FEV\textsubscript{1}, at one dose step might influence the measurement of FEV\textsubscript{1}, at the following dose step. Such a hypothetical effect could be greater if the time interval between FEV\textsubscript{1} measurements is short.

It is well-known that a DI causes a temporary increase in airflow and conductance in airways constricted by, for example, methacholine [1, 2]. The effect on airways resistance is, however, of short duration (less than 1 min [3, 4]). Measurement of FEV\textsubscript{1} begins with a maximal inhalation, that probably increases forced expiratory flow [5], but this mechanism does not explain why one FEV\textsubscript{1} measurement influences a subsequent measurement of FEV\textsubscript{1}. Yet, we have noted that the second FEV\textsubscript{1} measured after inhalation of methacholine is often higher than the first, even if the time interval between the two FEV\textsubscript{1} measurements is as long as one minute (study B, reported below). We therefore decided to study under what circumstances one FEV\textsubscript{1} manoeuvre influenced subsequent measurements of FEV\textsubscript{1} in airways constricted by methacholine.

Three different study protocols (studies B, C and D below) were designed to measure the size and duration of such an effect in healthy subjects; to investigate whether an FEV\textsubscript{1} manoeuvre had the same effect if it was performed immediately before or after inhalation of methacholine, and to compare effects of FEV\textsubscript{1} manoeuvres on airways resistance and on subsequent FEV\textsubscript{1} measurements.

FEV\textsubscript{1} manoeuvres performed with the sole intention of dilating the airways are abbreviated DI\textsubscript{pre}, rather than DI, since a maximal inhalation followed by a forced expiration may have a different effect to an isolated DI [2, 6].

**Subjects and study design**

All subjects were healthy nonsmokers, used no medication, and denied present or past symptoms of asthma. A random sample of office workers, who met the inclusion criteria
above, participated in the study of effects of shortening of the methacholine test. In the three subsequent studies, the subjects were chosen among those who in a pretrial methacholine test, had a cumulated dose of methacholine (provocative dose) causing a 20% decrease in FEV₁ (PD₂₀FEV₁) of <10 mg (about 70% of those investigated), (table 1). If the subjects had a cold, the tests were postponed for at least 6 weeks. Coffee or tea was not allowed before the tests on the day of the test. All participants gave their informed consent. The study had the approval of the local Ethics Committee.

All studies involved multiple provocations with methacholine, which for a given subject were performed on different days at the same time of the day. The order of the tests was strictly randomized.

**Study A: shortening of the methacholine protocol**

Ten healthy subjects performed a methacholine provocation test on two occasions. Physiological saline followed by methacholine in doubling concentrations from 0.5 to 32 mg·ml⁻¹ was inhaled, until FEV₁ had decreased 20% or more compared to the value obtained after saline. In one of the tests, the interval between the dose steps was 6 min, and FEV₁ was measured 4 and 5 min after the start of methacholine inhalation. In the other provocation test, the interval between the dose steps was 3 min and FEV₁ was measured after 2 and 2.5 min.

**Table 1. — Characteristics of the subjects participating in the trials**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Smoking habit</th>
<th>FEV₁ % pred</th>
<th>VC % pred</th>
<th>PD₂₀FEV₁ (mg)</th>
<th>PD≥20 (mg)</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>36</td>
<td>NS</td>
<td>82</td>
<td>88</td>
<td>9.1</td>
<td>11.8</td>
<td>A, B, C, D</td>
</tr>
<tr>
<td>F</td>
<td>40</td>
<td>NS</td>
<td>103</td>
<td>92</td>
<td>2.2</td>
<td>3.0</td>
<td>A, B, C, D</td>
</tr>
<tr>
<td>F</td>
<td>46</td>
<td>NS</td>
<td>107</td>
<td>107</td>
<td>1.0</td>
<td>3.0</td>
<td>A, B, C, D</td>
</tr>
<tr>
<td>M</td>
<td>38</td>
<td>NS</td>
<td>110</td>
<td>115</td>
<td>6.8</td>
<td>11.8</td>
<td>A, B, D</td>
</tr>
<tr>
<td>M</td>
<td>49</td>
<td>NS</td>
<td>108</td>
<td>110</td>
<td>4.7</td>
<td>11.8</td>
<td>A, B, D</td>
</tr>
<tr>
<td>M</td>
<td>41</td>
<td>NS</td>
<td>76</td>
<td>71</td>
<td>0.4</td>
<td>0.7</td>
<td>A, B</td>
</tr>
<tr>
<td>M</td>
<td>43</td>
<td>S</td>
<td>119</td>
<td>105</td>
<td>&gt;12.0</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>37</td>
<td>NS</td>
<td>90</td>
<td>98</td>
<td>1.1</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>NS</td>
<td>102</td>
<td>93</td>
<td>&gt;12.0</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>45</td>
<td>NS</td>
<td>97</td>
<td>88</td>
<td>9.6</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>39</td>
<td>NS</td>
<td>107</td>
<td>95</td>
<td>1.2</td>
<td>3.0</td>
<td>C, D</td>
</tr>
<tr>
<td>F</td>
<td>44</td>
<td>NS</td>
<td>111</td>
<td>103</td>
<td>4.0</td>
<td>5.9</td>
<td>C, D</td>
</tr>
<tr>
<td>F</td>
<td>42</td>
<td>NS</td>
<td>107</td>
<td>95</td>
<td>0.8</td>
<td>1.5</td>
<td>C, D</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>S</td>
<td>122</td>
<td>118</td>
<td>1.2</td>
<td>3.0</td>
<td>C, D</td>
</tr>
<tr>
<td>F</td>
<td>25</td>
<td>NS</td>
<td>92</td>
<td>88</td>
<td>0.7</td>
<td>1.5</td>
<td>C</td>
</tr>
<tr>
<td>F</td>
<td>33</td>
<td>NS</td>
<td>92</td>
<td>88</td>
<td>1.1</td>
<td>1.6</td>
<td>C</td>
</tr>
</tbody>
</table>

Study A: shortening of stepwise protocol; B: size and duration of effect of pre-methacholine DIFE; C: effect of pre– versus post-methacholine DIFE; D: comparison of effect of DIFE on Raw and FEV₁; FEV₁: forced expiratory volume in one second; VC: vital capacity; PD₂₀FEV₁: provocative dose of methacholine producing a 20% decrease in FEV₁ in a stepwise methacholine test (Study A); PD≥20: dose of methacholine given as a single dose in studies B, C, D; DIFE: FEV₁ manoeuvres performed with the sole intention of dilating the airways; Raw: airways resistance; NS: nonsmoking; S: smoking.

**Study B: size and duration of effect of pre-methacholine inhalation DIFE on post-methacholine FEV₁**

Six subjects inhaled methacholine in six repeated tests on different days. The methacholine dose was identical in all tests in a given subject, and corresponded roughly to the PD₂₀FEV₁ of the subject (table 1). Thus, the subjects inhaled nebulized methacholine solution with twice the concentration of the methacholine solution causing a ≥20% decrease in FEV₁ in a stepwise (6 min interval) methacholine test (this dose is abbreviated PD≥20 in the following text). The methacholine was administered during 1 min, using a device controlling inspiratory flow and volume [7].

In three of the tests the first post-methacholine FEV₁ was measured either 2, 3, or 4 min after the start of the methacholine inhalation. FEV₁ measurements were then repeated at 1 min intervals up to 10 min. Two DIFE manoeuvres were performed 60 and 30 s before the start of the methacholine inhalation. In three additional tests, the protocols were the same as in the first three tests, except that the pre-methacholine DIFE manoeuvres were omitted.

Since it was not anticipated that the effect would remain 4 min after methacholine, an analogous randomized study was subsequently performed in the same subjects, with 6 or 10 min intervals between the start of the methacholine inhalation and the first FEV₁. Successive FEV₁ were recorded each minute up to 10 and 15 min, respectively. The design is further illustrated in figure 1.
In studies B, C and D, basal FEV₁ was measured 20 min before the methacholine provocation, as the highest value from three blows. After this measurement, and for the remaining time, the subjects were asked not to make any deep tidal breaths unless specifically instructed.

Study C: effect of pre- versus post-methacholine inhalation D₁₀₀ on subsequent FEV₁ measurements

Nine subjects inhaled the PD₂₀ of methacholine over 30 s (twice the concentration and half the time compared to the above-mentioned trials) in three tests on different days. In one test, D₁₀₀ manoeuvres were not performed and FEV₁ was measured 3 and 4 min after the start of the methacholine administration. The second test was identical, except that two D₁₀₀ were performed 10 and 15 s before methacholine. In the third test, two D₁₀₀ were performed 10 and 15 s after methacholine, and FEV₁ was measured at 3.5 and 4.5 min after the start of methacholine administration.

Study D: comparison of effect of pre-methacholine inhalation D₁₀₀ on post-methacholine airway resistance (Raw) and FEV₁

Nine subjects inhaled their PD₂₀ of methacholine in two tests. Airways resistance and thoracic gas volume (TGV) were measured before and 3 min after the start of the provocation. FEV₁ was measured 4 min after provocation. On one of the two occasions, two D₁₀₀ manoeuvres were performed, 30 and 60 s before provocation.

Methods

Aerosol generation and inhalation

A jet nebulizer (Astra Meditec, Gothenburg, Sweden), driven by dry compressed air (390 kPa) producing 0.1 l aerosols·s⁻¹ was used in all studies. The output of the nebulizer was 0.38 (so 0.013) ml·min⁻¹ and was measured at the beginning and end of all test days. The mass median aerodynamic diameter of dried nebulisate was 1.7 μm (geometric mean) according to measurements with a Aerodynamic Particle Sizer (APS-3300, TSI inc. Saint Paul, Minnesota, USA). The nebulizer was connected to a drying device [7], where inspiratory flow was controlled at 0.4 l·s⁻¹. Inspiratory time was 2 s, and a total of 15 inspirations were performed, unless otherwise stated. The inhaled dose was computed from the output of the nebulizer, inhalation time, and the concentration of the nebulisate.
Measurement of response

Spirometry was performed with a wedge spirometer (Vitalograph®, Buckingham, UK). The highest of three reproducible measurements of FEV₁, and the highest of three slow vital capacity (VC) manoeuvres were chosen as basal values. Raw was measured in study D during tidal volume, breathing at a rate of 0.5 Hz from the inspiratory limb of the curve, using a volume constant body plethysmograph (E. Jaeger, Würzburg, Germany). In addition, TGV was measured by closing the shutter at the end of an expiration, while the subject was breathing for the resistance measurements. Specific conductance of the airways (sGaw) was computed as Raw×TGV⁻¹.

Analysis

The average change in FEV₁ per mg of inhaled methacholine (cumulated dose) in the stepwise methacholine provocation test ("slope") was calculated by linear regression with the percentage change in FEV₁ as independent variable, and the cumulated dose (linear scale) of nebulized methacholine as dependent variable [8]. The first FEV₁ measured at each dose step was used in these calculations.

Statistics

Wilcoxon's signed rank test was used (Statview II®) on FEV₁ values measured after methacholine provocation, expressed in percentage of basal values (measured 20 min before methacholine). The first FEV₁ value measured after methacholine from trials with or without a pre-methacholine DIFE was compared, pairing results from each individual. Similarly the difference between the first and second FEV₁ measured after methacholine from trials with or without a pre-methacholine DIFE was compared, pairing results from each individual. The difference in slope obtained from the long and short protocols in study A was also analysed, by pairing results from each individual. A p-value <0.05 was considered significant. The results are given as mean (so) or median and interquartile (IQ) range. In the figures, means and standard error of the mean (SEM) bars of the values expressed in percentage of basal values (20 min before methacholine inhalation) are shown.

Results

Study A

Shortening of the methacholine protocol reduced the response to methacholine. When the interval between FEV₁ measurements was 5 min (6 min per dose step), FEV₁ decreased by 2.6 (1.9–5.2)% per mg of inhaled methacholine. When the time interval was halved, FEV₁ decreased by 1.7 (0.8–2.3)% per mg of methacholine. The difference between the long and the short protocol was significant (p<0.01). In the long protocol, the first and second FEV₁ at the highest dose (measured at 1 min intervals) were 78 (8.6)% and 81 (8.9)% (nonsignificant difference) of basal FEV₁, respectively. With the short protocol the first and second FEV₁ (30 s interval) at corresponding methacholine concentrations were 87 (6.5)% and 90 (5.2)% (p<0.05) of basal FEV₁, respectively. The difference between the first and second FEV₁ in the long protocol was not significantly different from that of the short protocol.

Study B: size and duration of effect of pre-methacholine inhalation DIFE on post-methacholine FEV₁

The results are summarized in figure 1. Three minutes after the start of inhalation of the PD20 dose of methacholine, the first FEV₁ was 64 (15)% of basal values if deep inhalations had been avoided ("no-DIFE test"). In trials where two DIFE were performed immediately before the methacholine inhalation ("pre-methacholine DIFE test") the first FEV₁ was 77 (16)% of basal values. The first FEV₁ after methacholine was significantly (p<0.05) lower in no-DIFE tests compared to pre-DIFE tests, when measured at either 2, 3, 4 or 6 min, but not 10 min after the start of methacholine inhalation.

In no-DIFE tests, the first FEV₁ was significantly lower than the second (and subsequent) FEV₁ (p<0.05) at all times after methacholine inhalation (fig. 1a–e). Thus, 3 min after the start of methacholine inhalation the first FEV₁ was by average 2.45 l. The second FEV₁ measured one minute later was 2.82 l, an increase of 0.37 (0.20) l (p<0.05).

In pre-methacholine DIFE tests, there was no significant difference between the first and subsequent post-methacholine FEV₁ measured at 2–6 min after methacholine inhalation (fig. 1a–d). Thus, 3 min after the start of methacholine inhalation, the first FEV₁ was by average 2.94 l, the second FEV₁ was 3.1 l, and the difference was 0.16 (0.26) l (nonsignificant, p>0.1). However, in the test where 10 min had elapsed until the first measurement of FEV₁, the difference between the first and the second FEV₁ was significant; first FEV₁ 2.75 l, second 3.06 l, difference 0.31 (0.26) l, p<0.05 (fig. 1c).

The increase in FEV₁ between the first and second measurement after methacholine was significantly greater in no-DIFE tests than in pre-methacholine DIFE tests, when the first FEV₁ was measured 2 or 6 min after the start of methacholine inhalation (p<0.05). However, at 3 and 4 min the difference did not reach statistical significance (p=0.12).

Study C: effect of pre- versus post-methacholine inhalation DIFE on subsequent FEV₁ measurements

The results are summarized in figure 2. When DIFE was performed immediately after the methacholine inhalation the attenuation of the methacholine induced decrease in FEV₁ was less marked than if DIFE was performed immediately before the inhalation of methacholine. The first
post-methacholine FEV₁ was 84 (9)% of basal values in the pre-methacholine DIₑₑₑₐₑₐₑ test, 78 (10)% in the immediately post-methacholine DIₑₑₑₐₑₐₑ test, and 71 (13)% in the no-DIₑₑₑₑₑₑₑ test, respectively. The difference between pre- and post-methacholine DIₑₑₑₑₑₑₑ tests was of borderline significance (p=0.051). In the post-methacholine DIₑₑₑₑₑₑₑ test, the increase between the first and second post-methacholine FEV₁, was 0.11 (0.14) l, and of borderline significance (p=0.06).

The findings of study B were confirmed. Thus, the first FEV₁ was significantly lower in the no-DIₑₑₑₑₑₑₑ tests (2.47 (0.69) l), than in the pre-methacholine DIₑₑₑₑₑₑₑ test (2.88 (0.55) l), difference 0.43 (0.46) l (p<0.05). The first FEV₁ was lower than the second in the no-DIₑₑₑₑₑₑₑ test (difference 0.33 (0.22) l, p<0.01), but there was no significant difference between the first and the second FEV₁ in the pre-methacholine DIₑₑₑₑₑₑₑ test (0.09 (0.12) l, p=0.1)

**Study D: comparison of effect of pre-methacholine inhalation DIₑₑₑₑₑₑₑ on post-methacholine Raw and FEV₁**

Pre-methacholine inhalation DIₑₑₑₑₑₑₑ attenuated the decrease in FEV₁ caused by methacholine, but did not significantly influence Raw or sGaw. Four minutes after methacholine, FEV₁ was 71 (18)% of basal values if pre-methacholine DIₑₑₑₑₑₑₑ was not performed, and 84 (9)% if pre-methacholine DIₑₑₑₑₑₑₑ was performed. Thus, the average methacholine induced FEV₁ change with DIₑₑₑₑₑₑₑ was 54% of the FEV₁ change without DIₑₑₑₑₑₑₑ (p<0.01). Raw increased from 0.13 (0.06) to 0.56 (0.36) kPa·s⁻¹·l⁻¹ after inhalation of methacholine if pre-methacholine DIₑₑₑₑₑₑₑ was not performed, and from 0.15 (0.11) to 0.52 (0.28) kPa·s⁻¹·l⁻¹ if DIₑₑₑₑₑₑₑ was performed. Thus, the average change in airways resistance with pre-methacholine DIₑₑₑₑₑₑₑ was 85% of the resistance change without DIₑₑₑₑₑₑₑ (nonsignificant). There was no significant increase in TGV at end-expiration in either group following methacholine, and the specific airways conductance was nearly the same after methacholine, whether a pre-methacholine DIₑₑₑₑₑₑₑ had been performed or not (0.65 (0.37) and 0.65 (0.47) l·s⁻¹·kPa⁻¹, respectively).

**Discussion**

In this study, we found that a shortened time interval between dose steps in a methacholine bronchial provocation test reduced the decrease in FEV₁ caused by methacholine. This raised the question of whether or not FEV₁ measured at one dose step could influence the FEV₁ measured at the subsequent dose step. Studies B and C clearly showed that this is the case; however, the results of these studies did not establish whether the special case of shortening the methacholine test and, thus, the interval between FEV₁ measurements from 5 to 2.5 min can be fully explained by this mechanism.

An alternative hypothesis to explain the effect of shortened time intervals between dose steps is that in the short protocol there may have been insufficient time for full effect of nebulized methacholine when the first post-methacholine FEV₁ was measured. In vitro the smooth muscles of canine bronchi and trachea require 2 min to develop 90% of full isometric force, after stimulation with methacholine [9]. The "peak action" of inhaled methacholine on specific lung conductance varies between 1 and 4 min (mean 2.0 min) after the end of a 2 min long period of methacholine inhalation [10]. The peak action time was, thus, 3 min after mid-exposure time, which is comparable to the peak action time of 2.5 min after mid-exposure time found in the present study (fig. 1f). In the short methacholine provocation protocol the interval was 1.5 min between the mid-point of nebulization and the FEV₁ measurement, which suggests that the apparent lower sensitivity with the shortened protocol could be due, in part, to insufficient time for full effect of nebulized methacholine.

Shortening of the methacholine protocol (study A) thus caused an unexpected finding. This prompted further studies (studies B–D) to test the hypothesis that a pre-methacholine FEV₁ might influence the post-methacholine FEV₁, and more so if the time interval between the FEV₁ measurements was short. Although this hypothesis appears to be correct, this mechanism may only have partly contributed to the finding of study A. However, while testing this hypothesis, data emerged that illustrate an important mechanism affecting bronchomotor tone, which to our knowledge has not been systematically documented before in humans.

The effects of a single DI have been extensively studied by measuring changes in airway conductance, or the relationship between partial and maximal forced expiratory flow, in healthy and asthmatic subjects with and without pharmacological interventions [1, 2, 11–17]. The effect of a DI on airways resistance has a time constant of only 9–11 s and can normally not be seen after one minute [3, 4]. It has been explained as a consequence of differences between airway and lung tissue hysteresis [18].

---

**Fig. 2.** Effects of two DIₑₑₑₑₑₑₑ manoeuvres performed either immediately before (○), or immediately after (●) methacholine inhalation, on FEV₁ (in % basal values) measured 3–5 min after methacholine. Tests where no DIₑₑₑₑₑₑₑ were performed are denoted by O. *: p<0.05 for differences with and without DIₑₑₑₑₑₑₑ; †: p<0.05 for differences between successive FEV₁. See figure 1f for further details and abbreviations.
Thus, the increase in forced expiratory flow caused by the DI preceding expiration in a FEV₁ manoeuvre has been extensively studied, but the circumstance that the second DI preceding the second measurement of FEV₁ causes a further increase in forced expiratory flow has received less attention. The long duration (6 but not 10 min) of the effect and the greater effect of a DI performed before, rather than immediately after, inhalation of methacholine, has not been described previously according to our knowledge.

A "trivial" explanation to some of the findings in the present study is that a deep inhalation prior to inhalation of methacholine could influence the deposition of the bronchoconstrictor in the airways. However, we have no evidence of this, and such a mechanism cannot explain why the first FEV₁ influences the second FEV₁ measured after methacholine in no-DI trials, or why the third and subsequent FEV₁s are of similar magnitude, whether a pre-methacholine DI₁ had been performed or not. The most convincing evidence against different deposition of methacholine is the finding that 10 min after the inhalation of methacholine (when the effect of methacholine is still maximal [10]), the level of the first FEV₁ was almost identical in tests with or without pre-methacholine DI₁, and of the same magnitude as the first FEV₁ measured 4 or 6 min after no-DI tests (fig. 1f). Furthermore, the second FEV₁ increased by a similar amount, regardless of any pre-methacholine DI₁. We therefore conclude that the first FEV₁, measured 10 min after methacholine was no longer influenced by the pre-methacholine DI₁, and that the effects of a pre-methacholine DI₁ cannot be explained by altered deposition of methacholine in the airways.

An FEV₁ manoeuvre has little or no effect on subsequent measurements of FEV₁ in normal airways. Relaxation of airways with beta-agonists increases airways conductance, but does not necessarily result in increased FEV₁ [19, 20]. Several mechanisms have been suggested [19], including the possibility that the deep inhalation preceding the first forced expiration causes maximal dilatation, and that a second FEV₁, thus, cannot be further increased [20].

The effect of a DI may be larger in peripheral airways than in central airways [14, 21]. The structures in membranous bronchi are subjected to traction by surrounding tissues, and a maximal inspiration may cause a larger distending force in the peripheral airways than in larger bronchi. The total airway resistance is only partially determined by the resistance in the lower airways [22]. A partial relaxation of constricted peripheral airways smooth muscles due to a DI may, thus, cause a greater increase in forced expiratory flow than in total airways resistance.

The mechanical properties of airways that are constricted by methacholine are probably strongly influenced by the properties of airway smooth muscles [23]. Moderate increases in distending pressures cause a minor increase of the airway circumference in isolated airway segments [23]. There is probably elongation of elastic elements. The cross-links in airways smooth muscles are strained but change little in size. During a simulated maximal inhalation, there is a sudden and large increase in the circumference, and the airway preparation becomes grossly hysteric [23]. Presumably, the cross-links in the smooth muscles yield, resulting in a "breaking up" of muscle filaments, causing a pronounced and long-lasting loss of tension. Similar findings have been made with isolated bronchial smooth muscles [9, 24, 25]. The return of tension after "break-up" is slow (90% in 7-8 min) [9], which is similar to the duration of the effect of a pre-methacholine DIₜₐ observed in the present study. Thus, it appears that in constricted airways the airway smooth muscles do not change in length during normal tidal breathing. A maximal inspiration may, however, at least in the periphery, "break-up" smooth muscles, causing a pronounced loss in their tension.

A constricted muscle that has been partially "broken up" may be more susceptible to further "break-up". A second deep breath within a few minutes may cause a further reduction in tension. When contracted muscle strips are slowly cycled, the first cycle (stretch and relaxation) occurs at relatively high tension. The subsequent cycles occur at progressively lower tensions, and from cycle 5-6 and the loops of tension-length are superimposable [9]. Similar findings were obtained in the isolated airways segment [23]. Airway smooth muscles that are constricted by methacholine may, thus, be more resistant to "breaking-up", which might explain why a pre-methacholine challenge DIₜₐ may appear to be somewhat more effective in increasing a post-challenge FEV₁ value than a DIₜₐ performed immediately after methacholine challenge. However, if the time interval between deep inhalations is increased to 10 min, the tension in smooth muscles may have been fully restored, and the first (pre-challenge) DIₜₐ no longer influences the 10 min post-challenge FEV₁.

Tidal breathing is normally interrupted by deep inhalations, which are increased in frequency during airways constriction [5]. In most studies on effects of DI, the airway smooth muscles may have been partly relaxed due to deep inhalations performed within 6 min of measurements of effects. This may also have been the case in a study showing constancy of repeated partial flow measurements [26]. In most stepwise methacholine tests, the interval between FEV₁ measurements is less than 6-10 min. Many patients will have difficulty avoiding coughs and sighs for long time intervals. The aim of a methacholine challenge test is usually to discriminate between asthmatic and normal bronchi. It is possible that most traditional protocols, where the attenuating effect of successive FEV₁ manoeuvres is near maximal, can better discriminate between asthmatic and normal airways than a protocol with long intervals between FEV₁ measurements, but this has not, to our knowledge, been investigated.

The present study, thus, emphasises the importance of exact timing between successive FEV₁ measurements in bronchial provocation tests. There are several possible contributory mechanisms for the effects of serial DI manoeuvres in contracted airways, such as changes in lung volumes, elastic fibres, surfactant, or release of mediators. An attractive hypothesis is that repeated DI may cause...
progressive yielding of cross-bridges in the smooth muscles of airways and peripheral airspaces, as has been suggested from in vitro studies.

Acknowledgement: The authors gratefully acknowledge the expert assistance from laboratory technician C. Müller-Suur.

References