Eur Respir J, 1993, 6, 680–686
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European Respiratory Journal
ISSN 0903 - 1936

Importance of the time interval between FEV_1 measurements in a methacholine provocation test

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Importance of the time interval between FEV_1 measurements in a methacholine provocation test. P. Malmberg, K. Larsson, B.M. Sundblad, W. Zhiping. ©ERS Journals Ltd 1993.

ABSTRACT: We examined the hypothesis that a forced expiratory volume in one second (FEV_1) manoeuvre (and the preceding deep inhalation) before inhalation of methacholine might influence FEV_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_1, on different days in different test protocols. If an FEV_1 manoeuvre was performed immediately before methacholine, the first FEV_1 measured 3 min after provocation was higher (77% of basal FEV_1) than if a pre-methacholine FEV_1 manoeuvre was not performed (64%). This effect of a pre-methacholine FEV_1 manoeuvre was also demonstrated at 2, 4 and 6, but not at 10 min after the start of methacholine inhalation. If an FEV_1 manoeuvre was not performed before methacholine, the second and subsequent FEV_1 measured in constricted airways was higher than the first, and of similar magnitude to the first FEV_1 in tests where a pre-challenge FEV_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_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_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.

Eur Respir J., 1993, 6, 680–686.

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Keywords: Airways resistance
bronchial provocation tests
bronchial responsiveness
forced expiratory volume
muscle relaxation
smooth muscle

Received: August 25 1992
Accepted after revision December 28 1992

This study was supported by grants AMFO:84–1272 and 89–0098 from the National Work Environment Fund.

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_1).

A possible explanation is that the deep inhalation (DI) associated with measurement of FEV_1 at one dose step might influence the measurement of FEV_1 at the following dose step. Such a hypothetical effect could be greater if the time interval between FEV_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_1 begins with a maximal inhalation, that probably increases forced expiratory flow [5], but this mechanism does not explain why one FEV_1 measurement influences a subsequent measurement of FEV_1. Yet, we have noted that the second FEV_1 measured after inhalation of methacholine is often higher than the first, even if the time interval between the two FEV_1 measurements is as long as one minute (study B, reported below). We therefore decided to study under what circumstances one FEV_1 manoeuvre influenced subsequent measurements of FEV_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_1 manoeuvre had the same effect if it was performed immediately before or after inhalation of methacholine, and to compare effects of FEV_1 manoeuvres on airways resistance and on subsequent FEV_1 measurements.

FEV_1 manoeuvres performed with the sole intention of dilating the airways are abbreviated DI_{FE}, 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 FEV_1 ($PD_{20}FEV_1$) 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 $mg \cdot ml^{-1}$ was inhaled, until FEV_1 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 FEV_1 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 FEV_1 was measured after 2 and 2.5 min.

Study B: size and duration of effect of pre-methacholine inhalation DI_{FE} on post-methacholine FEV_1

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 $PD_{20}FEV_1$ 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 FEV_1 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 FEV_1 was measured either 2, 3, or 4 min after the start of the methacholine inhalation. FEV_1 measurements were then repeated at 1 min intervals up to 10 min. Two DI_{FE} 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 DI_{FE} 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 FEV_1. Successive FEV_1 were recorded each minute up to 10 and 15 min, respectively. The design is further illustrated in figure 1.

Table 1. – Characteristics of the subjects participating in the trials

Sex	Age yrs	Smoking habit	FEV_1 % pred	VC % pred	$PD_{20}FEV_1$ mg	PD≥20 mg	Studies
F	36	NS	82	88	9.1	11.8	A, B, C, D
F	40	NS	103	92	2.2	3.0	A, B, C, D
F	46	NS	107	107	1.0	3.0	A, B, C, D
M	38	NS	110	115	6.8	11.8	A, B, D
M	49	NS	108	110	4.7	11.8	A, B, D
M	41	NS	76	71	0.4	0.7	A, B
M	43	S	119	105	>12.0		A
F	37	NS	90	98	1.1		A
F	28	NS	102	93	>12.0		A
F	45	S	97	88	9.6		A
M	39	NS	107	95	1.2	3.0	C, D
F	44	NS	111	103	4.0	5.9	C, D
F	42	NS	107	95	0.8	1.5	C, D
F	47	S	122	118	1.2	3.0	C, D
F	25	NS	92	88	0.7	1.5	C
F	33	NS	92	88	1.1	1.6	C

Study A: shortening of stepwise protocol; B: size and duration of effect of pre-methacholine DI_{FE}; C: effect of pre- *versus* post-methacholine DI_{FE}; D: comparison of effect of DI_{FE} on Raw and FEV_1. FEV_1: forced expiratory volume in one second; VC: vital capacity; $PD_{20}FEV_1$ provocative dose of methacholine producing a 20% decrease in FEV_1 in a stepwise methacholine test (Study A); PD≥20: dose of methacholine given as a single dose in studies B, C, D; DI_{FE}: FEV_1 manoeuvres performed with the sole intention of dilating the airways; Raw: airways resistance; NS: nonsmoking; S: smoking.

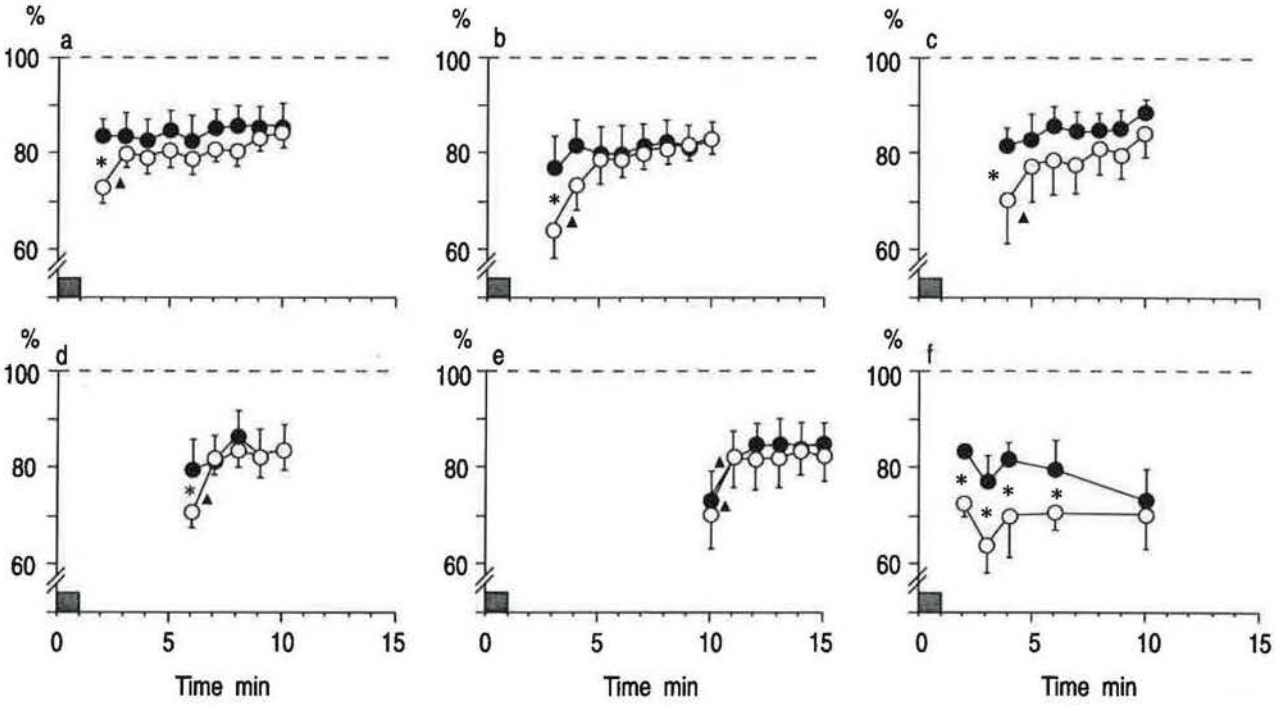

Fig. 1. – Effects of pre-methacholine DI_{FE} on FEV_1 measured at different times after the start of 1 min of methacholine inhalation (Study B). Panels a–e show the first and subsequent FEV_1 in trials where the first post-methacholine FEV_1 was measured 2, 3, 4, 6 or 10 min, respectively, after the start of methacholine inhalation (shaded box). Panel f summarizes the first post-methacholine FEV_1 mean values. The symbols show mean (±SEM) of FEV_1 in percentage of basal values. Filled symbols indicate tests where two DI_{FE} manoeuvres were performed immediately before methacholine inhalation. *: $p<0.05$ for differences with and without DI_{FE}: ▲: $p<0.05$ for differences between successive FEV_1. FEV_1: forced expiratory volume in one second; DI_{FE}: FEV_1 manoeuvres performed with the sole intention of dilating the airways.

In studies B, C and D, basal FEV_1 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 DI_{FE} on subsequent FEV_1 measurements*

Nine subjects inhaled the PD≥20 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, DI_{FE} manoeuvres were not performed and FEV_1 was measured 3 and 4 min after the start of the methacholine administration. The second test was identical, except that two DI_{FE} were performed 10 and 15 s before methacholine. In the third test, two DI_{FE} were performed 10 and 15 s after methacholine, and FEV_1 was measured at 3.5 and 4.5 min after the start of methacholine administration.

Study D: comparison of effect of pre-methacholine inhalation DI_{FE} on post-methacholine airway resistance (Raw) and FEV_1

Nine subjects inhaled their PD≥20 of methacholine in two tests. Airways resistance and thoracic gas volume (TGV) were measured before and 3 min after the start of the provocation. FEV_1 was measured 4 min after provocation. On one of the two occasions, two DI_{FE} 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 (SD 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 an 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 FEV_1, 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 $Raw^{-1} \cdot TGV^{-1}$.

Analysis

The average change in FEV_1 per mg of inhaled methacholine (cumulated dose) in the stepwise methacholine provocation test ("slope") was calculated by linear regression with the percentage change in FEV_1 as independent variable, and the cumulated dose (linear scale) of nebulized methacholine as dependent variable [8]. The first FEV_1 measured at each dose step was used in these calculations.

Statistics

Wilcoxon's signed rank test was used (Statview II®) on FEV_1 values measured after methacholine provocation, expressed in percentage of basal values (measured 20 min before methacholine). The first FEV_1 value measured after methacholine from trials with or without a pre-methacholine DI_{FE} was compared, pairing results from each individual. Similarly the difference between the first and second FEV_1 measured after methacholine from trials with or without a pre-methacholine DI_{FE} 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 (SD) 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 FEV_1 measurements was 5 min (6 min per dose step), FEV_1 decreased by 2.6 (1.9–5.2)% per mg of inhaled methacholine. When the time interval was halved, FEV_1 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 FEV_1 at the highest dose (measured at 1 min intervals) were 78 (8.6)% and 81 (8.9)% (nonsignificant difference) of basal FEV_1, respectively. With the short protocol the first and second FEV_1 (30 s interval) at corresponding methacholine concentrations were 87 (6.5)% and 90 (5.2)% ($p<0.05$) of basal FEV_1, respectively. The difference between the first and second FEV_1 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 DI_{FE} on post-methacholine FEV_1

The results are summarized in figure 1. Three minutes after the start of inhalation of the PD≥20 dose of methacholine, the first FEV_1 was 64 (15)% of basal values if deep inhalations had been avoided ("no-DI_{FE} test"). In trials where two DI_{FE} were performed immediately before the methacholine inhalation ("pre-methacholine DI_{FE} test") the first FEV_1 was 77 (16)% of basal values. The first FEV_1 after methacholine was significantly ($p<0.05$) lower in no-DI_{FE} tests compared to pre-DI_{FE} tests, when measured at either 2, 3, 4 or 6 min, but not 10 min after the start of methacholine inhalation.

In no-DI_{FE} tests, the first FEV_1 was significantly lower than the second (and subsequent) FEV_1 ($p<0.05$) at all times after methacholine inhalation (fig. 1a–e). Thus, 3 min after the start of methacholine inhalation the first FEV_1 was by average 2.45 *l*. The second FEV_1 measured one minute later was 2.82 *l*, an increase of 0.37 (0.20) *l* ($p<0.05$).

In pre-methacholine DI_{FE} tests, there was no significant difference between the first and subsequent post-methacholine FEV_1 measured at 2–6 min after methacholine inhalation (fig. 1a–d). Thus, 3 min after the start of methacholine inhalation, the first FEV_1 was by average 2.94 *l*, the second FEV_1 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 FEV_1, the difference between the first and the second FEV_1 was significant; first FEV_1 2.75 *l*, second 3.06 *l*, difference 0.31 (0.26) *l*, $p<0.05$ (fig. 1e).

The increase in FEV_1 between the first and second measurement after methacholine was significantly greater in no-DI_{FE} tests than in pre-methacholine DI_{FE} tests, when the first FEV_1 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 DI_{FE} on subsequent FEV_1 measurements*

The results are summarized in figure 2. When DI_{FE} was performed immediately after the methacholine inhalation the attenuation of the methacholine induced decrease in FEV_1 was less marked than if DI_{FE} was performed immediately before the inhalation of methacholine. The first

post-methacholine FEV_1 was 84 (9)% of basal values in the pre-methacholine DI_{FE} test, 78 (10)% in the immediately post-methacholine DI_{FE} test, and 71 (13)% in the no-DI_{FE} test, respectively. The difference between pre- and post-methacholine DI_{FE} tests was of borderline significance (p=0.051). In the post-methacholine DI_{FE} test, the increase between the first and second post-methacholine FEV_1 was 0.11 (0.14) *l*, and of borderline significance (p=0.06).

The findings of study B were confirmed. Thus, the first FEV_1 was significantly lower in the no-DI_{FE} tests (2.47 (0.69) *l*), than in the pre-methacholine DI_{FE} test (2.88 (0.55) *l*), difference 0.43 (0.46) *l*, ($p<0.05$). The first FEV_1 was lower than the second in the no-DI_{FE} test (difference 0.33 (0.22) *l*, $p<0.01$), but there was no significant difference between the first and the second FEV_1 in the pre-methacholine DI_{FE} test (0.09 (0.12) *l*, p=0.1)

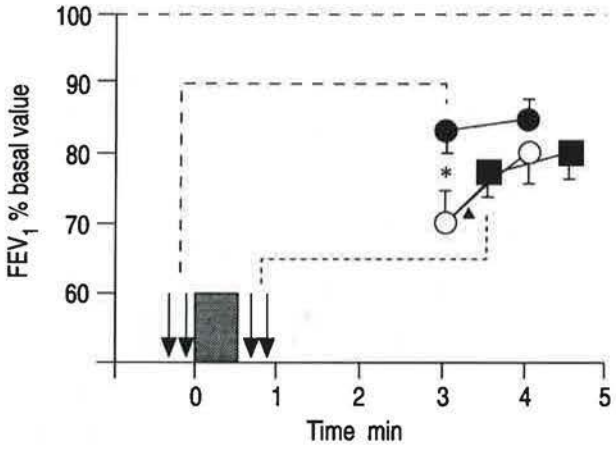

Fig. 2. – Effects of two DI_{FE} manoeuvres performed either immediately before (●), or immediately after (■) methacholine inhalation, on FEV_1 (in % basal values) measured 3–5 min after methacholine. Tests where no DI_{FE} were performed are denoted by ○. *: $p<0.05$ for differences with and without DI_{FE}: ▲: $p<0.05$ for differences between successive FEV_1. See figure 1 for further details and abbreviations.

Study D: comparison of effect of pre-methacholine inhalation DI_{FE} on post-methacholine Raw and FEV_1

Pre-methacholine inhalation DI_{FE} attenuated the decrease in FEV_1 caused by methacholine, but did not significantly influence Raw or sGaw. Four minutes after methacholine, FEV_1 was 71 (18)% of basal values if pre-methacholine DI_{FE} was not performed, and 84 (9)% if pre-methacholine DI_{FE} was performed. Thus, the average methacholine induced FEV_1 change with DI_{FE} was 54% of the FEV_1 change without DI_{FE} ($p<0.01$). Raw increased from 0.13 (0.06) to 0.56 (0.36) $kPa \cdot l^{-1} \cdot s$ after inhalation of methacholine if pre-methacholine DI_{FE} was not performed, and from 0.15 (0.11) to 0.52 (0.28) $kPa \cdot s \cdot l^{-1}$ if DI_{FE} was performed. Thus, the average change in airways resistance with pre-methacholine DI_{FE} was 85% of the resistance change without DI_{FE} (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 DI_{FE} had been performed or not (0.65 (0.37) and 0.65 (0.47) $l \cdot s^{-1} \cdot kPa^{-1}$, 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 FEV_1 caused by methacholine. This raised the question of whether or not FEV_1 measured at one dose step could influence the FEV_1 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 FEV_1 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 FEV_1 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 FEV_1 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 FEV_1 might influence the post-methacholine FEV_1 and more so if the time interval between the FEV_1 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].

Thus, the increase in forced expiratory flow caused by the DI preceding expiration in a FEV_1 manoeuvre has been extensively studied, but the circumstance that the second DI preceding the second measurement of FEV_1 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 FEV_1 influences the second FEV_1 measured after methacholine in no-DI_{FE} trials, or why the third and subsequent FEV_1 are of similar magnitude, whether a pre-methacholine DI_{FE} 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 FEV_1 was almost identical in tests with or without pre-methacholine DI_{FE}, and of the same magnitude as the first FEV_1 measured 4 or 6 min after no-DI_{FE} tests (fig. 1f). Furthermore, the second FEV_1 increased by a similar amount, regardless of any pre-methacholine DI_{FE}. We therefore conclude that the first FEV_1 measured 10 min after methacholine was no longer influenced by the pre-methacholine DI_{FE}, and that the effects of a pre-methacholine DI_{FE} cannot be explained by altered deposition of methacholine in the airways.

An FEV_1 maneouvre has little or no effect on subsequent measurements of FEV_1 in normal airways. Relaxation of airways with beta-agonists increases airways conductance, but does not necessarily result in increased FEV_1 [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 FEV_1, 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 bronchioli 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 hysteretic [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 DI_{FE} 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 DI_{FE} may appear to be somewhat more effective in increasing a post-challenge FEV_1 value than a DI_{FE} 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) DI_{FE} no longer influences the 10 min post-challenge FEV_1.

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 FEV_1 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 FEV_1 manoeuvres is near maximal, can better discriminate between asthmatic and normal airways than a protocol with long intervals between FEV_1 measurements, but this has not, to our knowledge, been investigated.

The present study, thus, emphasises the importance of exact timing between successive FEV_1 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.

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