Flow limitation and dyspnoea in healthy supine subjects during methacholine challenge

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ABSTRACT: The purpose of this study was to assess whether during standard methacholine (Mch) challenge (concentration up to 128 mg·mL^{-1}) healthy supine subjects a) develop tidal expiratory flow limitation (FL) and hyperinflation, and b) whether the onset of tidal FL is associated with dyspnoea.

Eight healthy subjects were studied. Dyspnoea was assessed using the Borg scale, FL by the negative expiratory pressure (NEP) method and hyperinflation in terms of decrease in inspiratory capacity (IC).

Seven patients became flow limited at Mch doses ranging 4–64 mg·mL^{-1}, with FL encompassing 34–84% of the control tidal volume. In six of them the onset of tidal FL was associated with little or no dyspnoea and a modest degree of hyperinflation (ΔIC <-0.4 L). In one subject, however, onset of FL was associated with a substantial reduction in IC (0.58 L) and moderately severe dyspnoea. In all of these seven subjects FL was transiently reversed after an IC manoeuvre.

In conclusion, the results show that a) most healthy subjects may develop flow limitation and hyperinflation during methacholine challenge in supine position, and b) at onset of flow limitation there is little or no dyspnoea, suggesting that onset of dynamic airway compression *per se* does not elicit significant dyspnoea. Significant dyspnoea probably only occurs with marked dynamic hyperinflation.
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According to PELLEGRINO *et al.* [1], administration of inhaled methacholine (Mch) to seated asthmatic patients readily induces tidal expiratory flow limitation (FL), which represents the starting trigger for the functional residual capacity (FRC) to increase in order to allow breathing at higher flows. PELLEGRINO *et al.* [1] also showed that Mch administration to seated healthy subjects is seldom associated with FL and increased FRC. Since in the supine position the responsiveness to bronchial challenge is enhanced [2], it is possible that Mch challenge in this position may also lead to tidal expiratory FL (*i.e.* inability to further increase flow (V') over the tidal volume (VT) range by increasing transpulmonary pressure) in healthy subjects. The supine position is more susceptible to expiratory FL than the sitting posture mainly because in this position the FRC is low, with a concomitant decrease in expiratory V' reserve over the resting VT range [3, 4]. Dyspnoea is often experienced during bronchial challenge [5]. According to O'DONNELL *et al.* [6] flow-limiting dynamic airway compression may *per se* elicit dyspnoea as a result of afferent activity from the distorted and collapsed airways. According to this hypothesis, the onset of expiratory FL should be associated with dyspnoea.

Conventionally, expiratory FL has been assessed by comparison of tidal with maximal expiratory (V'-volume (V)) curves: FL is present in subjects in whom, at comparable lung V, the tidal V' are similar or higher than those obtained during the forced vital capacity (FVC) manoeuvre [7]. This approach, however, has limitations because as a result of thoracic gas compression during the FVC manoeuvre, the tidal and maximal V'-V curves must be measured with a body plethysmograph [8]. Consequently, such measurements are usually confined to the sitting position. It should be noted, however, that even when a body plethysmograph is used, comparison of tidal with maximal V'-V curves may still lead to erroneous conclusions [8]. Recently, however, an alternate method, the negative expiratory pressure technique (NEP), has been developed, which does not require a body plethysmograph and can be used in the supine posture [3, 4, 8]. Furthermore, this technique does not depend on patient co-operation and co-ordination.

In the present study the NEP technique was used to assess a) whether healthy supine subjects develop tidal expiratory FL during standard bronchial challenge with Mch; and b) if the onset of expiratory FL is associated with dyspnoea in the absence of substantial dynamic hyperinflation. During the bronchial challenge forced expiratory volume in one second (FEV1), and FVC and the inspiratory capacity (IC) were also measured.

Methods

Subjects

Studies were performed on eight healthy volunteers (7 males) from the laboratory staff. Seven were nonsmokers and one was a former smoker who had stopped smoking 9

yrs prior to this study. None had a recent respiratory infection or a history of chronic respiratory disease. Anthropometric and lung function data in sitting position are summarized in table 1. The FEV1 and FVC were measured with a microspirometer (Micromedical Ltd., Rochester, Kent, UK), and the predicted values are those of KNUDSON et al. [9]. Airway resistance (Raw) was also measured with a body plethysmograph (P.K. Morgan Ltd., Rochester, Kent, UK). The mean±SD value was 1.40 ± 0.35 cmH$_2$O$\cdot$L$^{-1}\cdot$s, and was below the upper normal limit of 2.2 cmH$_2$O$\cdot$L$^{-1}\cdot$s in all individuals. The study was approved by the institutional ethics committee and all subjects gave informed consent.

Negative expiratory pressure method for detection of expiratory flow limitation

The NEP method used to detect tidal expiratory FL has been previously described in detail [4, 10]. A flanged mouthpiece was connected with a Fleisch No. 2 pneumotachograph (Fleisch, Lausanne, Switzerland) and a Venturi device capable of generating a negative pressure during expiration (Aeromech Devices Ltd., Almonte, Ontario, Canada). One end of the device was open to the atmosphere whilst the other was connected to the distal cone of the pneumotachograph. Rigid tubing (id=8 mm) was used to connect a side port on the Venturi device, via an electrically operated solenoid valve, to a tank of compressed air. A pressure (P) regulator was used to obtain a preset level of NEP at the airway opening (~-5 cmH$_2$O). The solenoid valve (Ascots electrical valve model No. 8262G2; Ascolectric Ltd., Toronto, Ontario, Canada) controlled by a computer (Direc Physiologic Recording System; Raytech Instruments, Vancouver, British Columbia, Canada) has an opening time of 29 ms. The solenoid valve was activated when the expiratory V' reached a preset threshold value (30 mL$\cdot$s^{-1} in the present study) and could be kept open for any desired time. With this threshold, the overall time required to trigger the valve and reach the preset level of NEP (tNEP) could be prolonged to any desired time by introducing a computer-controlled delay period between the time when the V' reached the preset threshold and the triggering of the valve. V' was measured with the heated pneumotachograph connected to a differential P transducer (Validyne MP45, $\pm$2 cmH$_2$O; Validyne Corp., Northridge, CA, USA). The pneumotachograph was linear over the experimental range of V'. Artefacts on the flow record caused by common-mode rejection ratio were negligible. V was obtained by numerical integration of the V' signal. P at the airway opening (Pao) was measured through a side port on the mouthpiece using a differential P transducer (Vali-

dyne MP45, $\pm$100 cmH$_2$O; Validyne). The breathing assembly has a dead space of 50 mL, and its P-V' relationship is characterized by the following equation:

$$P = 0.45\ V' + 0.02V'^2\ (\text{R}^2 = 0.996),$$

where P is in cmH$_2$O and V' in L$\cdot$s^{-1}. The P, V, and V' signals were amplified, low-pass filtered at 50 Hz and digitized at 100 Hz by a 16 bit analogue-to-digital converter (Direc Physiology Recording System; Raytech Instruments). The digitized data was stored on the computer hard disk for subsequent analysis. Data analysis was performed using ANADAT software (version 5.1; RHT-InfoDat Inc., Montreal, Canada). During the study the time course of V', V and P were continuously monitored on the screen of the computer, together with the corresponding V'-V loops.

Spirometry

During the experiments IC, FEV1 and FVC were measured in supine position with a microspirometer (Micromedical Ltd.). This device, which meets the American Thoracic Society (ATS) standards, was calibrated every day according to the ATS guidelines [11].

Dyspnoea evaluation

Dyspnoea was defined as "an unpleasant sensation of laboured or difficult breathing". The intensity of dyspnoea was assessed with the modified Borg scale [12] to which the subjects were familiarized prior to the study. Its endpoints were anchored such that zero represented "no breathlessness" and 10 was "the most severe breathlessness that they had ever experienced or could imagine experiencing", respectively.

Procedure and data analysis

Mch chloride provocation studies were carried out using the standardized protocol of COCKCROFT et al. [13] while the subjects were lying supine on a comfortable couch. Before the Mch provocation test, each subject had a 5 min trial in order to become accustomed to the NEP apparatus and procedure. After this, each subject was studied in the prechallenge state breathing air and next inhaling an aerosol of phosphate buffered saline (PBS) solution. This was followed by inhalation of increasing concentrations of Mch chloride aerosol. For aerosol inhalation the jet Wright nebulizer was used (Aerosol Medical Ltd., Colchester, Essex, UK), whose output was 0.13 mL$\cdot$min^{-1} at a V' of 5 L$\cdot$min^{-1}. Each aerosol dose was administered during 2 min of tidal breathing. After each dose of aerosol the subjects, wearing a noseclip, were asked to breathe room air through the NEP equipment assembly for ~3 min. During this period they were instructed to avoid deep inspirations or sighing. During the last minute of this period, when breathing had become regular, the ventilatory variables were recorded and the subjects were asked to rate dyspnoea by pointing to the Borg scale. This was followed by three NEP tests, which were made at intervals of ~5 regular breaths. The NEP was applied 0.2 s after the onset of expiration and was maintained throughout the ensuing expiration. After the third NEP test, the subjects were asked to perform an IC manoeuvre with

Table 1. – Anthropometric characteristics and lung function of eight healthy subjects

	Age yrs	Height cm	Weight kg	FEV1 % pred	FVC % pred	FEV1/FVC % pred
Mean	37	175	74	91	94	97
SD	7	6	7	13	13	8

FEV1: forced expiratory volume in one second; FVC: forced vital capacity.

$V' < 2$ L·s^{-1}. Since during the pharmacological challenge the total lung capacity does not change [14, 15], the increase in FRC (ΔFRC) induced by Mch was assessed in terms of changes in IC [10, 16]. Next, they were connected to the microspirometer and performed an FVC manoeuvre after a rapid maximal inspiration without an end-inspiratory pause in order to obtain FEV1 and FVC under standardized conditions [17]. The Mch challenge was stopped either when expiratory FL was detected with the NEP test or the inhaled Mch concentration reached the maximal allowed value of 128 mg·mL^{-1}. If FL was present, a NEP test was also performed ~30 s after the FVC manoeuvre in order to assess whether FL could be reversed by the maximal inspiration prior to FVC. During the NEP tests and FVC measurements the neck was held in a fixed neutral position to avoid changes associated with neck extension [18]. At the end of the Mch trial, an aerosol of 400 µg of albuterol was administered to reverse bronchoconstriction.

During the application of NEP air may leak around the lips into the expired line and contribute to the expiratory V'. Such leaks, however, are easily detected because they result in a sustained decrease of the end-expiratory lung V during the tidal breaths following NEP application. Great care was taken to avoid such leaks by proper positioning of the mouthpiece and by asking the subjects to hold their lips tight.

Analysis of the data obtained with NEP consisted of comparing the expiratory V'-V curves of control tidal expirations with those obtained during subsequent expirations in which NEP was applied [3, 4, 10]. Subjects in whom V' was greater with NEP over the entire range of the control tidal expiration were considered as not flow limited (NFL) (fig. 1). By contrast, subjects in whom the application of NEP did not elicit an increase in V', except for a transient increase (spike) coincident with NEP application, were considered FL (fig. 2). This spike reflects reduction in V of the airways and heralds FL [3, 4, 10]. The FL portion was expressed as percentage of the control expired VT (FL, %VT). In the example in figure 2, FL amounted to 51% VT. It should be noted that during the early part of tidal expiration, which is characterized by increasing V' (fig. 1 and 2), there cannot be FL [3, 4]. Accordingly, in spontaneously breathing subjects FL is necessarily always <100% VT.

In line with previous studies [3, 10], the application of NEP was not associated with unpleasant sensations or cough. With NEP the expiratory V' either increased (reflecting absence of FL) or did not change during part of the expiration (reflecting presence of FL). There was no instance in which the application of NEP resulted in a sustained decrease of expiratory V' below control as a consequence of upper airway collapse or narrowing.

Statistical analysis

Comparison between different experimental conditions was made using the Student's paired t-test for the lung function measurements, and the Wilcoxon matched paired signed ranks test for the Borg score. Regression analysis between the Borg scores and the changes in IC relative to PBS was performed using the Spearman rank-order method. Values are expressed as mean±SD, and p<0.05 was taken as statistically significant.
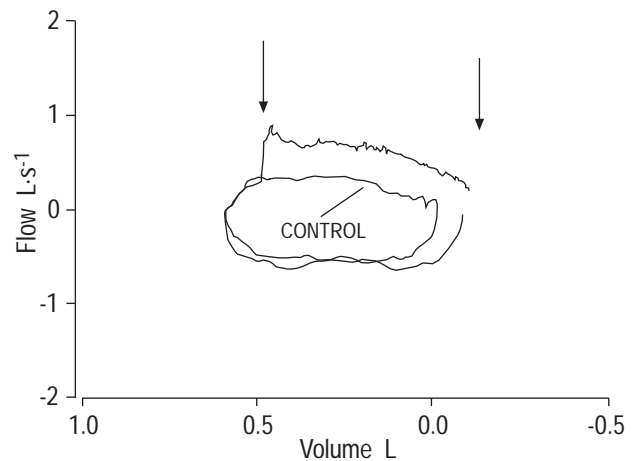

Fig. 1. – Flow-volume loops of subject 3 during resting breathing in supine position after inhalation of the phosphate buffered saline solution (PBS). A control breath is shown together with the subsequent expiration during which negative expiratory pressure (NEP) was applied at the point indicated by the first arrow. The second arrow indicates termination of NEP. Expiratory flow increased throughout NEP application, indicating absence of flow limitation. Zero volume corresponds to functional residual capacity in supine position with PBS.

Results

Both before and after inhalation of PBS there was no tidal expiratory FL in any of the eight subjects studied, as shown by the NEP test in figure 1. Seven individuals showed FL at Mch threshold concentrations ranging 4–64 mg·mL^{-1} (table 2), as depicted in figure 2. The FL encompassed 62±18% of control VT (range 35–84%). Only subject 2 did not exhibit FL up to the maximal Mch concentration used in the present study (128 mg·mL^{-1}). In all subjects, at the Mch threshold concentration, FL was

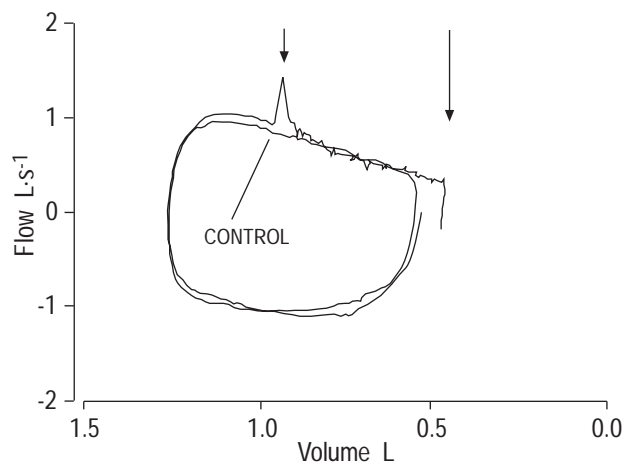Fig. 2. – Flow-volume loops after subject 3 had reached the threshold methacholine (Mch) concentration (=64 mg·mL^{-1}) at which flow limitation appeared. In this case negative expiratory pressure (NEP) did not increase flow, except for a brief initial transient (spike), which is mainly due to sudden reduction in volume of the upper airways and heralds flow limitation. A control breath is shown together with the subsequent expiration during which NEP was applied at the point indicated by the first arrow. The second arrow indicates termination of NEP. Zero volume corresponds to functional residual capacity (FRC) in supine position with phosphate buffered saline (PBS). After Mch the FRC increased by 575 mL, reflecting dynamic hyperinflation.

Table 2. – Threshold concentrations of methacholine (Mch) eliciting tidal expiratory flow limitation (FL) and corresponding changes in lung function and Borg dyspnoea score in eight supine subjects

Subject No.	Mch threshold for FL mg·mL^{-1}	ΔFEV1 % PBS	ΔFVC % PBS	ΔIC mL	Borg dyspnoea Score
1	16	-4.9	-2.8	-363	0
2	>128*	-9.4	-7.5	-36	0
3	64	-8.7	7.5	-575	4.5
4	16	-4.4	2.0	-387	0
5	4	0.0	2.1	-84	0
6	32	-5.4	1.6	-330	2
7	16	-8.3	0.7	-323	1
8	16	-4.9	-2.6	-243	1
Mean$^{\#}$		-5.2	1.2	-329	1.2
SD$^{\#}$		2.9	3.5	149	1.6
p$^{\#}$	6	<0.001	NS	<0.001	<0.005

$^{\#}$: excluding subject 2; *: only in subject 2 was flow limitation not achieved at the highest Mch concentration used (128 mg·mL^{-1}). ΔFEV1: decrease in forced expiratory volume in one second; % PBS: per cent change relative to values obtained after inhalation of phosphate buffered saline solution ΔFVC: decrease in forced vital capacity; ΔIC: decrease in inspiratory capacity.

Table 3. – Breathing pattern of seven subjects at methacholine (Mch) threshold for flow limitation (FL) and after inhalation of phosphate buffered saline solution (PBS)

	VT L	tI s	tE s	f min^{-1}	V'E L·min^{-1}
Mch threshold for FL	0.54 ±0.09	1.51 ±0.68	2.22 ±1.06	18.8 ±6.8	10.5 ±4.7
PBS	0.62 ±0.12*	1.68 ±0.45	2.48 ±0.71	15.3 ±3.6^{+}	9.6 ±2.9

Values are expressed as mean±SD. *: p<0.025; $^{+}$: p<0.05. VT: tidal volume; tI and tE: inspiratory and expiratory times respectively; f: respiratory frequency; V'E: minute ventilation

consistently found in all three repeated NEP tests. After these tests, the subjects performed an IC manoeuvre followed by resting breathing for about 30 s at which time the NEP test was repeated. In all instances, FL was no longer present 30 s after the IC manoeuvre. At the Mch threshold levels for FL, relative to PBS values, there was no change in FVC and a small, though statistically significant, decrease in FEV1 (table 2). In all subjects, however, the decrease in FEV1, (ΔFEV1) was <10%. Tidal FL was associated with a small (p<0.001) decrease in IC, which amounted to 329±149 mL. Since total lung capacity is independent of bronchomotor tone [14, 15], the decrease in IC implies that there was a concomitant increase of the end-expiratory lung volume (EELV), reflecting dynamic hyperinflation. There was no significant correlation between the changes in IC and FEV1 at the Mch threshold concentration for FL.

At PBS all subjects exhibited a Borg score of zero. In the seven subjects who demonstrated FL the Borg dyspnoea score was 1.2±1.6, a significant change (p<0.005) relative to PBS (table 2). In three of the FL subjects, however, the Borg score remained zero while in the other four the degree of dyspnoea ranged from very slight (score 1) to somewhat severe to severe (score 4.5) The Borg dyspnoea score was not significantly correlated with the concomitant decrease in IC. At the Mch concentrations immediately preceding the Mch threshold for FL, there were no significant changes in IC, FVC and Borg dyspnoea score relative to PBS. Only FEV1 was slightly (3±3%) though significantly decreased (p<0.02).

In the seven subjects who exhibited FL during Mch challenge there was a significant decrease in VT (p<0.025) and a significant increase in breathing frequency (p<0.005) while the other ventilatory variables did not change (table 3). At the Mch concentrations immediately preceeding the Mch threshold for FL, none of the ventilatory variables differed significantly relative to PBS.

Discussion

The main findings of the present study are that a) in supine position most of our the healthy subjects developed tidal expiratory FL at Mch levels at which the reduction in FEV1, relative to PBS, was small (<10%); b) tidal FL could be reversed by a deep inspiration; and c) onset of FL was associated with little or no dyspnoea.

Administration of Mch to healthy subjects results in increased Raw, and reduction of FEV1 and maximal expiratory flows [19, 20]. The responsiveness to Mch has been found to be enhanced in the supine position [2]. Since in supine position there is a reduction in FRC and hence in expiratory V' reserve over the VT range [21], this position should be susceptible to development of FL during Mch challenge. Indeed, the current study found that in supine position seven subjects developed FL, at Mch concentrations ranging 4–64 mg·mL^{-1}. Only one subject remained NFL at the highest concentration allowed in this study (128 mg·mL^{-1}). At FL threshold, the reduction of FEV1 was, on average, only 5% relative to PBS. This supports the notion that there is a dissociation between the response to Mch measured in terms of changes of Raw or FEV1 [22]. This discrepancy is probably due to the fact that in healthy subjects with pharmacologically-induced bronchoconstriction a deep inspiration transiently reduces Raw, and consequently the FEV1 returns toward the pre-challenge values [19, 22]. The bronchodilation following a deep inhalation is due to a reduction in bronchial smooth muscle tone [23], which lasts for 45–60 s, as measured by specific airway conductance [20, 24, 25]. Consistent with these studies, this study found that 30 s after a maximal inspiration all seven subjects who were FL before it, became NFL.

It has been suggested that the dynamic compression of airways downstream from the flow-limiting segment, which underlies FL, may elicit a reflex mechanism that influences the breathing pattern by terminating expiration prematurely, thus increasing the FRC [1]. In this way, onset of tidal FL during Mch challenge should be associated with an increase in EELV sufficient to confine FL to near end-expiration. In the current supine healthy subjects, however, at the onset of tidal FL there was no significant change in expiratory time (table 3) while the increase in FRC, measured in terms of ΔIC, was in most instances very small (table 2). Furthermore, tidal FL was not confined to near expiration but encompassed a relatively large portion of the control VT (35–84%).

While in supine control subjects tidal FL appears to be the main cause of dynamic hyperinflation during Mch challenge, this is not the case for seated patients with asthma in whom dynamic hyperinflation may occur in the absence of FL [26].

Although in the seven patients tidal FL encompassed 35–84% V_T, their degree of dynamic hyperinflation, as reflected by the reduction in IC (table 2), was small (<0.4 L, except in subject 3). This implies that at the onset of tidal FL during Mch challenge the subjects were still able to sustain their resting ventilatory requirements without the need to increase the FRC, in spite of the prevailing reduction in maximal flows. Consequently there was also little increase in inspiratory efforts due to intrinsic positive end-expiratory pressure (PEEP) and impairment in inspiratory muscle function [8, 26].

At the Mch threshold for FL there was a small but significant increase in dyspnoea score relative to PBS (table 2). According to O'DONNELL et al. [6] the sensation of dyspnoea may occur as a result of afferent activity from the mechanoreceptors of the intrathoracic airways which collapse when reaching FL. In the current population, however, there were three subjects (subjects 1, 4 and 5) in whom the dyspnoea score was zero in spite of the presence of FL (table 2). Furthermore, in three FL subjects (subjects 6–8) the Borg dyspnoea score was only 1–2 (very slight dyspnoea). Thus, at the onset of FL, the afferent activity from the receptors of the collapsed and distorted intrathoracic airway does not appear to elicit dyspnoea. Under these conditions, however, the transpulmonary pressure probably barely exceeds the critical value (P_{crit}) required to elicit dynamic airway compression [7]. However, it is possible that under conditions in which P_{crit} is more markedly exceeded, the increased afferent activity from the more extensively collapsed and distorted intrathoracic airways may actually contribute to dyspnoea. It should be stressed that in most of the current subjects the degree of dynamic hyperinflation at the FL threshold for Mch was relatively small, and hence little or no dyspnoea would be expected because of increased inspiratory effort due to intrinsic PEEP [27, 28]. It should be stressed that in this study the Mch challenge was terminated at the onset of FL. If further doses of Mch had been administered, the severity of both dynamic hyperinflation and dyspnoea should become more pronounced [5]. In this connection it is noteworthy that subject 3, who exhibited the largest reduction in IC (575 mL) at the FL threshold, exhibited the highest degree of dyspnoea (4.5 Borg score).

The present study has shown that, with methacholine challenge in supine position, all but one of the eight healthy subjects exhibited tidal expiratory flow limitation, which occurred in the absence of substantial changes in forced expiratory volume in one second and forced vital capacity, reflecting the bronchodilation which occurs during the maximal inspiration prior to the forced vital capacity manoeuvre. In fact, flow limitation was in all instances transiently abolished by the maximal inspiration. The onset of tidal flow limitation elicited little or no dyspnoea in most subjects, suggesting that onset of dynamic airway compression per se does not elicit significant dyspnoea. Unlike patients with asthma, the increase in functional residual capacity in healthy subjects during methacholine challenge appears to be related to tidal flow limitation.

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