Influences of the breathing route on upper airway dynamics properties in normal awake subjects with constant mouth opening

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ABSTRACT
MB (mouth breathing) promotes the occurrence of sleep-disordered breathing even in non-apnoeic subjects. Considering that MO (mouth opening) contributes to an increase in UA (upper airway) collapsibility independently of MB, the aim of the present study was to assess the influence of breathing route on UA dynamics in the presence of MO. Bilateral anterior magnetic phrenic nerve stimulation was performed 2 s after expiratory onset in 12 healthy male subjects during wakefulness (age, 50 ± 5 years; body mass index, 27.8 ± 2.4 kg/m²) during MB through a mouthpiece and during exclusive NB (nasal breathing) with the same mouthpiece in place. Twitch-induced $\dot{V}_I$ (instantaneous flow), $P_{ph}$ and $P_{es}$ (pharyngeal and oesophageal pressures respectively) were recorded and the corresponding resistances were measured. A polynomial regression model, $\dot{V}_I = k_1P_d + k_2P_d^2$, was used to characterize flow–pressure relationship and to determine the $P_d$ value at which UA collapses. There was no difference in UA dynamic properties between NB and MB when UA collapse occurred above the pharyngeal catheter. For twitches where UA collapse occurred lower in the UA, pharyngeal resistance decreased from NB to MB (2.0 ± 0.3 and 1.5 ± 0.2 cmH₂O · l⁻¹ · s respectively; $P = 0.02$; values are means ± S.D.), whereas closing pressure increased (−25.7 ± 10.1 and −18.0 ± 3.0 cmH₂O respectively; $P = 0.04$). We conclude that (i) in the presence of MO the dynamic properties of the proximal UA free of phasic activity do not differ between NB and MB, and (ii) MB decreases the upstream resistance and increases collapsibility of the distal UA.

INTRODUCTION
The UA (upper airway) contributes largely to determine breathing characteristics by modulating respiratory airflow, regulating the duration of inspiration and expiration and influencing expiratory lung volumes [1,2]. It accounts for approx. 50% of the resistance of the whole respiratory system [3]. The pharynx is the most compliant part of the UA, because it is not supported by any rigid cartilaginous or bony structures. In this condition, besides the intrinsic tension of the pharyngeal airway walls, the force developed by contraction of the UA dilator muscles plays

Key words: airway resistance, breathing route, instantaneous flow, mouth opening, obstructive sleep apnoea, phrenic nerve stimulation, upper airway collapsibility.

Abbreviations: BAMPS, bilateral anterior magnetic phrenic nerve stimulation; BMI, body mass index; EMG, electromyogram; GG, genioglossus; MB, mouth breathing; MO, mouth opening; NB, nasal breathing; OSA, obstructive sleep apnoea; $P_d$, driving pressure; $P_{es}$, oesophageal pressure; $P_{es, max}$, peak $P_{es}$ twitch; $P_{ph}$, pharyngeal pressure; $P_{ph, max}$, peak $P_{ph}$ twitch; UA, upper airway; $\dot{V}_I$, instantaneous flow; $\dot{V}_{max}$, maximal $\dot{V}_I$; $\dot{V}_{max, lim}$, $\dot{V}_{max}$ of flow-limited twitches; $P_{es, lim}$, $P_{es}$ at $\dot{V}_{max, lim}$; $P_{ph, lim}$, $P_{ph}$ at $\dot{V}_{max, lim}$; $V_{lim}$, minimal $\dot{V}_I$; $k_1$, counterpart of the resistance measured at $\dot{V}_{max}$; $k_2$, resistance measured at $\dot{V}_{lim}$.

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a crucial role to counterbalance the collapsing forces represented by the pharyngeal transmural negative pressure gradient and tissue weight [4]. This accounts for the fact that UA dilator muscles play a determinant role in maintaining UA patency (UA resistance) and stability (UA collapsibility).

Breathing route influences the level of UA dilator muscle neuromuscular activity. In animals, bypassing the nasal route during tidal breathing produces a decrease in EMG (electromyogram) activity of the GG (genioglossus) [5,6]. In humans, EMG activities of alae nasi decrease during voluntary MB (mouth breathing) compared with NB (nasal breathing) [7]. Such effects of MB may be ascribed to the role of flow-sensitive receptors in the nasal mucosa and of the reflex-mediated increase in UA neuromuscular activity in response to negative airway pressure [7,8] on UA dilator muscles activity. During wakefulness, the similarity of UA resistance during NB and MB [9,10] can be accounted for by the related changes in UA muscle activity. During sleep, the GG EMG response to UA negative pressure is markedly reduced [11], and MB is associated with an increase in UA resistance after sleep onset when compared with NB [12]. This detrimental effect of MB is supported further by the higher incidence of OSA (obstructive sleep apnoea) in normal subjects who intend to breathe by the mouth [11]. On the other hand, the respective effects of MB and MO (mouth opening) are not easy to differentiate, because MB implies MO, and obstructive breathing events are frequently associated with the transition from NB to MB [11,13,14]. Such detrimental effects of MO may be due to an increase in UA collapsibility independent of the breathing route [15] but, to date, no studies have investigated the influence of MB on UA stability independently of MO.

By primarily stimulating the diaphragm without previous UA dilator muscle activation, phrenic nerve stimulation delivered at the end of expiration provides a practical way to evaluate the dynamic properties of the non-phasically active UA during wakefulness [16]. Using this technique, we have demonstrated previously that UA dynamics may be altered by MO during NB [17]. We reasoned that phrenic nerve stimulation could be used to evaluate the influence of breathing route on UA dynamics independently of MO. To reach this goal, the present study was designed to compare the UA dynamic response with phrenic nerve stimulation during NB and MB with constant MO.

**METHODS**

**Subjects**

Twelve healthy volunteers (all males) were recruited in the present study. They had no symptoms suggestive of respiratory or neurological disease, and were not taking any medication. A conventional in-lab polysomnographic study (Sandman 4.1; Nellcor Puritan Bennett) was completed in all subjects and confirmed the absence of sleep-related respiratory disorders. The Internal Review Board of our institution approved this protocol, and informed consent was obtained from every subject.

**Protocol**

Silver cup electrodes were placed on the mid-clavicular line in the seven to eight right and left intercostal spaces for surface recording of right and left costal diaphragmatic EMG activities. After administration of a local anaesthesia (1 ml of 2% xylocaine) to the nostrils, an oesophageal balloon was inserted through one nostril and located in the lower third of the oesophagus, as assessed by the occlusion technique [18]. A pressure-tipped catheter (CT/S X1058; Gaeltec) was passed through the other nostril and positioned at 16 cm from the nares to record hypopharyngeal pressure. The two catheters were secured on the nose. Although the two pressure measurement techniques are different, they only lead to a few milliseconds delay in the balloon catheter response compared with the Gaeltec technique. This did not result in any measurable lag in the flow–pressure response when the tracings obtained with these two pressure recordings were superimposed, as found previously using an air-infused catheter to measure UA pressure [19–22]. A plastic nasal stent was placed in the anterior nares to prevent nasal collapse. A mouthpiece (inner diameter, 25 mm) was placed into the mouth and connected to the oral port of a tight-fitting oro-nasal CPAP mask (Spectrum reusable full face mask; Respironics). This mask has two separate nasal and oral ports, thus allowing a separate assessment of MB and NB with MO (Figure 1). The mask was then placed over the nose and the mouth. The airtightness of each part of the mask was assessed by occluding its opening during maximal inspiratory efforts. Two catheters were inserted separately into the oral and nasal ports of the mask to measure oral and nasal pressures respectively. $P_{es}$ and $P_{ph}$ (oesophageal and pharyngeal pressures...
respectively) were referenced to mask pressure according to the breathing route. Instantaneous flow was obtained from a pneumotachograph (model 112467-3850A; Hans Rudolph) connected to the opened breathing route. Pressures and flow were recorded digitally at a 2000 Hz sample rate (Digidata 1320; Axon Instrument). Subjects were seated in a comfortable armchair inclined to 60°. The head was supported by a firm pre-moulded pillow to ensure that the head and neck were maintained in the same position during the experiment.

**Study design**

BAMPS (bilateral anterior magnetic phrenic nerve stimulation) was performed with two Magstim 200 stimulators connected to two 45-mm figure-of-eight-shaped coils, according to a technique described previously [16, 23]. Each stimulating coil was positioned anterolaterally over the anatomical landmark of the phrenic nerve in the neck by a high-precision multipositional support consisting of two articulated arms (MAN 143; Manfrotto Trading). Twitch stimuli were applied 2 s after the onset of expiration. The stimulators were triggered by a timer driven by the changes in flow direction. Measurements were made in two separate conditions applied in random order: exclusive NB and MB. During NB, the oral port was sealed with an airtight plastic plug, and vice versa for MB. The mouth remained open constantly because of the mouthpiece, whatever the breathing route. For each condition, one series of five stimuli was applied at 100% of the maximum output intensity.

**Data and statistical analysis**

All EMG, flow and pressure tracings were recorded on a microcomputer (AxoScope software 9.0; Axon Instruments). The twitch-induced breaths were considered flow-limited when \( V_1 \) (instantaneous flow) plateaued or decreased, despite a persistent increase in \( P_d \) (driving pressure). For evaluating the dynamic characteristics of UA, the following variables were measured: (i) \( V_{\text{max,lim}} \) (maximal \( V_1 \)) of flow-limited twitches; (ii) \( P_{\text{ph,lim}} \) and \( P_{\text{es,lim}} \) (\( P_{\text{ph}} \) and \( P_{\text{es}} \) at \( V_{\text{max,lim}} \) respectively); (iii) \( V_{\text{lim}} \) (minimal \( V_1 \), reached while increasing \( P_d \) during flow-limited twitches); (iv) \( P_{\text{ph,max}} \) and \( P_{\text{es,max}} \) (peak \( P_{\text{ph}} \) and \( P_{\text{es}} \) twitches respectively); and (v) the corresponding pharyngeal and total airway resistance values. In addition, the \( P_d–V_1 \) relationship of flow value ranging from 0 to \( V_{\text{lim}} \) was characterized using a polynomial regression model, \( V_1 = k_1 P_d + k_2 P_d^2 \), as described previously [24], where \( k_1 \) (counterpart of resistance measured at \( V_{\text{max}} \)) and \( k_2 \) (resistance measured at \( V_{\text{lim}} \)) relate to airway conductance. Depending on the site of UA collapse, \( P_{\text{ph}} \) or \( P_{\text{es}} \) were considered as \( P_d \) (see below). The \( P_d \) at which UA collapses represents the closing pressure, and it can be determined by solving this relationship for \( V_1 = 0 \) and corresponds to the \( k_1/k_2 \) ratio. Polynomial model fitting and determinations of \( k_1 \) and \( k_2 \) values were performed semi-automatically using custom-made software (Matlab; The Mathworks) or using a statistical software (JMP IN version 5.1; SAS Institute). The results provided by these two methods were found to be highly consistent (\( r^2 \) ranges were from 0.79–0.99).

For each subject and each condition, the percentage of flow-limited twitches and the parameters used to characterize the flow–pressure phrenic nerve stimulation responses were determined for each collapsing site. All data are reported as means ± S.D. Differences in UA dynamic parameters between MB and NB were analysed with a paired Student’s \( t \) test. The anthropometric characteristics of the subjects with exclusive proximal or distal flow limitation in either breathing route were compared with an unpaired Student’s \( t \) test. The results were considered statistically significant when the \( P \) value was < 0.05.

**RESULTS**

The mean age of the subjects was 50 ± 5 years, the height was 174 ± 8 cm, BMI (body mass index) was 27.8 ± 2.4 kg/m², and neck circumference was 40.1 ± 2.2 cm.

There was no flow limitation during spontaneous breathing. \( P_{\text{es}} \) values just preceding twitch-induced flow limitation did not differ between NB (2.5 ± 2.1 cmH₂O) and MB (1.8 ± 1.4 cmH₂O; \( P = 0.43 \)). Pharyngeal resistance measured at peak flow during tidal breathing was 3.4 ± 2.0 cmH₂O·l⁻¹·s during NB and 2.5 ± 2.1 cmH₂O·l⁻¹·s during MB (\( P = 0.18 \)). BAMPS induced a typical pattern of flow limitation in all subjects during both NB and MB (Figure 2A). The percentage of flow-limited twitches was 94.9 and 98.3% of all stimuli in NB and MB respectively (\( P = 0.3 \)). During these twitches, the \( V_1–P_{\text{es}} \) and \( V_1–P_{\text{ph}} \) relationships were analysed separately. Occlusion was identified to occur above the pharyngeal catheter (proximal UA level) when the two pressures tracings both diverged from the flow tracing (Figure 2A). In this condition, a fall in flow occurred while the two pressures continued to decrease, as illustrated by the flow–pressure relationships (Figure 3A). When \( P_{\text{ph}} \) and flow plateaued similarly and both dissociated from the \( P_{\text{es}} \) tracing, which continued to decrease, UA closure was identified to occur in the distal UA which was below the pharyngeal catheter (Figure 2B). In such a condition, flow paralleled the \( P_{\text{ph}} \) tracing, whereas \( P_{\text{es}} \) diverged from the flow signal (Figure 3B). Pharyngeal resistance then represented upstream resistance.

The percentage of flow-limited twitches occurring in the proximal UA was 79.6 ± 35.2% in NB and 71.7 ± 44.7% in MB (\( P = 0.45 \)). When considering the characteristics of flow and pressure responses and the corresponding resistances of these twitch-induced flow-limited events, no difference was observed between the two breathing routes in any of the parameters studied (Table 1). The percentage of flow-limited twitches associated with
Figure 2 Representative flow and pressure responses of BAMPS-induced flow-limited twitches

(A) Flow-limited twitch occurring in the proximal UA as identified both by \( P_{ph} \) (Pph) and \( P_{es} \) (Pes) recordings. (B) Twitch-induced flow-limited response identified in the distal UA as identified by the \( P_{es} \) recording only. The arrow represents the application of the twitch. Pnose, nasal pressure.

distal UA closure did not differ between NB and MB (20.4 ± 35.2 and 28.3 ± 44.7 % respectively; \( P = 0.45 \)). UA dynamic properties differed between the two breathing routes, with a lower upstream resistance at \( V_{\text{Imax,lim}} \), \( R_{ph} \), lim and a higher fall in flow at maximal \( P_{es} \) during MB (Table 2). It was noticeable that \( V_{\text{Imax,lim}} \) did not differ significantly between these two conditions. When compared with NB, the resistance of the respiratory system below the pharyngeal catheter increased during MB at both \( V_{\text{Imax,lim}} \) (11.6 ± 0.8 and 13.5 ± 1.7 cmH2O·l\(^{-1}\)·s\(^{-1}\) respectively; \( P = 0.05 \)) and \( V_{\text{Imin}} \) (30.4 ± 1.5 and 47.6 ± 5.9 cmH2O·l\(^{-1}\)·s\(^{-1}\) respectively; \( P = 0.0002 \)).

The \( V_{l} = k_{1} P_{d} + k_{2} P_{d}^{2} \) model adequately characterized the flow–pressure relationship, with the \( r^{2} \) values ranging from 0.68–0.99. For twitches associated with proximal UA closure, \( P_{ph} \) was entered as the \( P_{d} \) and the \( k_{1}/k_{2} \) ratio corresponded to the \( P_{ph} \) at which UA closed. In these situations, no difference was found in \( k_{1} \) and \( k_{2} \) (Table 1) and the \( k_{1}/k_{2} \) ratio between NB and MB (Figure 4).

For flow-limited twitches associated with distal UA collapse, \( P_{es} \) determined the downstream pressure in the polynomial model. In those cases, \( k_{2} \) was significantly less during MB (Table 2). The \( k_{1}/k_{2} \) ratio was significantly less during MB compared with NB (Figure 4). During NB, the difference in \( k_{1}/k_{2} \) between the proximal and distal sections of the UA was significant (\( P = 0.01 \), with

Table 1 Characteristics of flow-limited twitches identified by \( P_{ph} \) during NB and MB

<table>
<thead>
<tr>
<th>Breathing route</th>
<th>NB</th>
<th>MB</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{Imax,lim}} ) (ml/s)</td>
<td>861 ± 511</td>
<td>698 ± 445</td>
<td>0.09</td>
</tr>
<tr>
<td>( V_{\text{Imin}} ) (ml/s)</td>
<td>460 ± 340</td>
<td>396 ± 411</td>
<td>0.43</td>
</tr>
<tr>
<td>( P_{ph,lim} ) (cmH2O)</td>
<td>-10.2 ± 4.3</td>
<td>-8.0 ± 4.8</td>
<td>0.45</td>
</tr>
<tr>
<td>( k_{1} ) (ml·s(^{-1})·cmH2O(^{-1}))</td>
<td>281 ± 117</td>
<td>255 ± 199</td>
<td>0.77</td>
</tr>
<tr>
<td>( k_{2} ) (ml·s(^{-2})·cmH2O(^{-2}))</td>
<td>-27 ± 25</td>
<td>-47 ± 81</td>
<td>0.002</td>
</tr>
</tbody>
</table>

In the first case, \( P_{ph} \) (Pph) and \( P_{es} \) (Pes) both diverge from the flow tracing, whereas this is only observed for \( P_{es} \) in the second case.
the proximal UA demonstrating a higher collapsibility (less negative $k_1/k_2$ ratio) than the distal one. This difference in collapsibility between the two sections was no longer observed when considering MB.

The anthropometric characteristics of the four subjects in whom flow limitation exclusively occurred in the proximal UA in both conditions were compared with the three whose flow limitation exclusively occurred in the distal UA in either breathing route. No difference was observed in age, height or BMI, but neck circumference was less in the latter group ($40.7 \pm 1.5$ and $37.0 \pm 3.3$ cm respectively; $P = 0.04$).

**DISCUSSION**

Our present results have shown that, with the same degree of MO, MB decreases the stability of the distal UA when compared with NB without interfering with the dynamic characteristics of the more proximal UA-collapsing sites. This is the first study to demonstrate a possible deleterious effect of MB on UA stability, independent of MO, and identifies further that MB may influence the level of UA collapse.

Independently of UA changes related to the breathing route itself, one could ask about the possible contribution of differences in lung volumes on our present findings. The link between lung volume and the size of the UA has been reported for both animals and humans [25–27]. As the lung volume increases, caudal displacement of the trachea exerts a dilating force on the UA [25,26] by changing UA length and longitudinal tension [27,28]. The deleterious effect of passive reduction in lung volume on UA collapsibility is seen independently of the increase in GG activation [29]. In the present study, no measurement of lung volume was obtained to assess if differences in end-expiratory lung volume occurred between NB and MB. However, $P_{es}$ measured just before the application of the twitches did not differ between the two breathing routes. Therefore we believe that this predominantly excludes systematic changes in end-expiratory lung volume that could have interfered with the interpretation of our results.

Although we are not able to identify precisely the location of the collapse by using flow–$P_{ph}$ and flow–$P_{es}$ analysis, the absence of differences in anthropometric characteristics between subjects whose flow-limitation events occurred in the proximal and distal parts of UA suggests that $P_{ph}$ measurements corresponded to a similar UA level in the population studied. One could question to what extent methodological considerations would contribute to the differences in UA dynamic properties that we observed between the two breathing routes. It is of primary importance to concede that it is obviously impossible to document what is the influence of the change in breathing route on UA collapsibility at a given UA location, because twitches were applied at a fixed timing during expiration. We did not consider applying repetitive twitches while changing the breathing route, because we have found that UA dynamic features are influenced by twitch expiratory timing [22]. Therefore comparisons have to be made on the characteristics of the distinct twitches applied in different experimental conditions. However, flow–pressure analyses were performed on a twitch-by-twitch basis, and the changes we observed reflect differences in UA mechanical properties of all the twitches occurring at a given UA level.

In order to characterize the UA dynamic properties, flow and the corresponding $P_{es}$ were measured at two specific flow values corresponding to the beginning and end...
of flow limitation. We were not able to find any difference in $V_{\text{Imax,lim}}$ and in the corresponding $P_d$ between NB and MB. On the other hand, $V_{\text{Imax}}$ and the corresponding $P_d$ values differed between NB and MB for flow-limited twitches occurring at the distal UA. Therefore the characterization of the flow–pressure relationship by the polynomial regression model, $V_1 = k_1 P_d + k_2 P_d^2$, which takes into account the changes in $V_1$ from 0 up to $V_{\text{Imax}}$, is a more relevant way to describe the UA dynamic properties during phrenic nerve stimulation [30]. According to this model, the more negative the $k_1/k_2$ ratio is, the higher the UA stability. Although phrenic nerve stimulation mimics the termination of $\dot{V}$ during phrenic nerve stimulation [30]. According to this model, this can be accounted for by the relative role of $P_{\text{crit}}$ (critical pressure) and upstream resistance in the determination of $V_{\text{Imax,lim}}$ during flow-limited events [37]. In the present case, MB was associated with an increase in $k_1/k_2$ ratio as well as a decrease in pharyngeal resistance. As mentioned above, in such situations this resistance represents that upstream of the site of collapse. Therefore the combined increase in lower airway collapsibility, along with the fall in pharyngeal resistance, can account for the absence of a difference in $V_{\text{Imax,lim}}$ between NB and MB. The decrease in pharyngeal resistance when changing from NB to MB may be related to the by-pass of nasal passages and velopharyngeal structures in this last condition. This is not in disagreement with similar values of pharyngeal resistance during NB and MB [8], because UA resistance values obtained during tidal breathing depend on UA muscle activity which is influenced by breathing route. Therefore it is important to mention again that the present investigations were conducted in non-phasically active UA. In such a condition, our measurements are not conditioned by any compensatory changes in UA muscle activity in response to modifications in UA patency and subsequent changes in $P_{\text{ph}}$.

Conclusions

When compared with NB, MB decreases the stability of the lower airway independent of MO. Taking into account that phrenic nerve stimulation applied at the end of expiration is used to investigate UA properties free of phasic inspiratory activity, changes in dimension/shape in the distal UA may account for the deleterious influence of MB on UA stability. This effect of MO and moving from NB to MB may add to the effects of sleep on UA muscle tonic activity to worsen UA stability and favour the occurrence of UA obstruction during sleep.

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References