Tracheal mucus clearance in high-frequency oscillation: effect of peak flow rate bias


ABSTRACT: We have reported previously that high-frequency oscillation of the chest wall (HFO/CW) enhances the tracheal mucus clearance rate (TMCR) in dogs. This enhancement of TMCR may be due in part to the expiratory bias in peak flow rate (VE/VT>1) that occurs during HFO/CW. We examined this factor in 8 anaesthetized, spontaneously breathing dogs by comparing TMCR during the following manoeuvres: 1) HFO/CW, applied by means of a thoracic cuff; 2) symmetric high-frequency oscillation via the airway opening (HFO/AO), applied by means of a piston pump driven by sinusoidal signal; 3) HFO/AO with an expiratory bias in peak flow, and 4) HFO/AO with an inspiratory bias in peak flow. All manoeuvres were of 5 min duration and were performed at 13 Hz and an oscillatory tidal volume of 1.5 ml/kg. In the latter two manoeuvres, the piston pump was driven by a nonsinusoidal signal such that peak VE/VT was greater than and less than unity, respectively. A high-impedance, cross-current flow of warmed, humidified air was provided at the tracheal tube. The order of manoeuvres 2, 3 and 4 was randomized, while manoeuvre 1 was repeated at the end. TMCR was determined by direct bronchoscopic visualization of charcoal particle transport. Each HFO manoeuvre was bracketed by a control period of spontaneous breathing. We found that TMCR during HFO/CW was 2.4× control (p<0.001), in line with previous results. TMCR was not enhanced over control in any of the three HFO/AO manoeuvres; however, during HFO/AO with an expiratory peak flow bias, TMCR was consistently greater than during symmetric HFO/AO, or during HFO/AO with an inspiratory bias (p<0.01). The increase in TMCR due to expiratory-flow-bias HFO/AO (symmetric HFO/AO) was approximately 30% of the increase in TMCR due to HFO/CW.


We have been examining the effect of high-frequency oscillation applied to the chest wall (HFO/CW) as the method of enhancing the rate of mucus clearance from the lung. In our first study [1], we found that, in anaesthetized dogs, high-frequency chest wall oscillations at 5-17 Hz and of the same magnitude as that used for ventilation led to the enhancement of the tracheal mucus clearance rate. The effect was both sharply frequency dependent and of substantial magnitude, the clearance rate being maximally increased by more than three-fold at 13 Hz. In view of a published report that high-frequency oscillation applied via the airway opening (HFO/AO) reduced the rate of mucus clearance [2], we decided to compare the two forms of HFO directly in the same animals in our second study [3]. We found that HFO/CW at 13 Hz elevated the tracheal mucus clearance rate significantly to 240% of the spontaneous breathing control, in line with our previous observations, while HFO/AO at 13 Hz and the same oscillatory tidal volume did not stimulate tracheal clearance. HFO/AO at 20 Hz (the condition used by McEvoy et al. [2]) also did not stimulate clearance. This second study enabled us to rule out a specific frequency effect; however, it did not allow us to isolate the mechanism since the HFO/CW not only involved vibration of the chest wall but also led to ventilation in which the peak expiratory flow rate was higher than the peak inspiratory flow rate. Hence the stimulation of tracheal mucus clearance could have been due to a cephalad bias in mucus-airflow interaction, or a reflex mechanism involving chest wall vibration, or both. In the present study, we applied, via the airway opening, three forms of HFO/AO at 13 Hz: 1) sinusoidal (symmetric); 2) nonsinusoidal with shortened expiratory duration such that peak expiratory flow rate is greater than peak inspiratory flow rate (bias-out); 3) nonsinusoidal with shortened inspiratory duration (bias-in). We compared the effect of these three forms of HFO/AO on tracheal mucus clearance with the effect of HFO/CW in 8 anaesthetized, spontaneously breathing dogs. We hoped to determine whether the peak airflow bias was
important in enhancing tracheal mucus clearance and, if so, to quantify its importance.

In addition, in a separate experiment with HFO/CW, while maintaining the same oscillatory tidal volume, we modified the pattern of chest wall oscillation such that cephalad bias in peak airflow rate normally associated with HFO/CW was reduced. This enabled us to examine the relationship between airflow bias and mucus clearance in a different fashion.

**Materials and methods**

Random source dogs of either sex weighing from 15 to 25 kg were employed in this study. They were premedicated with xylazine (2mg·kg⁻¹ i.m.) and anaesthetized with sodium pentobarbital (10–15 mg·kg⁻¹ i.v.). Supplemental doses of sodium pentobarbital were given as required to suppress ocular reflexes. No paralysing agent was administered. The dogs were intubated as shallowly as possible with a shortened endotracheal tube and placed supine. A heating pad was used to minimize the fall in core temperature.

![Diagram](image)

Fig. 1. – Diagram of combined set-up for HFO in anesthetized, supine dog. The HFO/CW is generated by wrapping a modified double blood-pressure cuff around the lower thorax, partially inflating the cuff with a constant air inflow and variable outflow leak, and oscillating the pressure in the cuff by means of a heavy-duty reciprocating pump. The HFO/AO is generated by a second, variable duty-cycle piston pump leading to the endotracheal tube. A transverse flow of warmed, humidified air for ventilation is provided by passing wall air through a heated nebulizer. Mean Pm is maintained near atmospheric by means of a needle valve. The bronchoscope port is closed during the periods of ventilation.

The experimental set-up used is illustrated in fig. 1. The high-frequency mouth oscillations (HFO/AO) were generated by a servo-controlled linear magnetic motor (Info-Mag Model 15) with an attached piston. The oscillations could be made nonsinusoidal, i.e. with a variable duty-cycle, by means of a wave-form generator (Wave Tek). A high impedance, transverse flow of about 8 l·min⁻¹ was used to provide fresh humidified air for ventilation. This was driven from a wall air source via a heated nebulizer (Ohio Medical Products) and was regulated with a needle valve to maintain mean mouth pressure (Pm) near atmospheric. The nebulizer was set to provide air at approximately 30°C with a total water content of 0.04–0.05 g·l⁻¹, i.e. near 100% RH for air of 37°C. Pm was measured using a Validyne DP45 pressure transducer and short tubing connections. Oesophageal pressure was also monitored, using a standard 5 cm balloon and a second Validyne pressure transducer. Flow was determined with a Fleisch No. 2 pneumotachograph; volume was obtained by integrating the flow signal electronically.

High-frequency chest wall oscillation (HFO/CW) was provided by the same apparatus used previously [1], namely a thoracic cuff (Pneumoband, Bird Corp.) which was inflated with a constant air flow and oscillated with a heavy duty piston pump, driven by a 3 HP DC motor. As in our previous study [3], this combined set-up enabled us to circumvent a number of problems common to all HFO experiments: the frequency response of the equipment (since we could compare the two modes of ventilation at the same frequency), and the boundary effects associated with air flowing from one type of tubing to another (since the same connections between the endotracheal tube and pneumotachograph were used for both HFO/CW and HFO/AO). Other common features which might otherwise have been problematic included the airway humidification system and the bronchoscopic technique of determining the clearance rate.

As in our previous studies [1, 3] the tracheal mucus clearance rate (TMCR) was determined by depositing a drop (10 μl) of a suspension of powdered charcoal in saline onto the lower tracheal wall. The position of the leading edge of the charcoal spot was located by advancing the bronchoscope until the spot just passed out of view. The bronchoscope was withdrawn and then reintroduced after a period of time, usually five min. TMCR was computed as the distance travelled divided by the time elapsed. Displacements were recorded to the nearest 0.5 cm, the limit of precision for determining the position of the leading edge.

We studied the effect of HFO/CW at 13 Hz on TMCR and compared it with the effects of three HFO/AO manoeuvres: 1) HFO/AO 13 Hz, symmetrical, i.e. where the pump was driven with a sinusoidal signal; 2) HFO/AO 13 Hz, bias-out, where the expiratory duration was shorter than the inspiratory duration leading to a peak expiratory flow rate greater than peak inspiratory flow rate (Vₑ/Vᵢ>1); 3) HFO/AO 13 Hz, bias-in, where the expiratory duration was longer than the inspiratory duration (Vₑ/Vᵢ<1). For each of these three HFO/AO manoeuvres, the stroke amplitude was set to give the same oscillatory tidal volume (Vₖo) as that achieved by the HFO/CW manoeuvre in the same dog. The HFO manoeuvres were all of 5 minutes duration.

The order of application of manoeuvres was: HFO/CW, the three HFO/AO manoeuvres in randomized order, HFO/CW again. In each case, the dog was allowed to breathe spontaneously throughout the application of the HFO/AO. TMCR was measured during 5 min of spontaneous breathing before and after each HFO/AO manoeuvre. Between HFO/AO manoeuvres, the cuff of the endotracheal tube was deflated to avoid excessive...
build-up of secretions. A total of 8 dogs were studied using this protocol.

Because mucus-airflow interaction may depend on mucus layer thickness, as it does in vitro [4, 5] we estimated the thickness of the mucus layer [6]. Prior to the first TMCR measurement in each dog, a cytology brush (Mill-Rose 151) was inserted through the endotracheal tube and placed in contact with the lower tracheal mucosa for 10 min. During this time mucus was collected on the brush by mucociliary flow. The brush was then withdrawn and the mucus removed by scraping it off with a scalpel blade. The weight of mucus collected divided by the contact time was used as an index of tracheal mucus flux. Then, from the collection rate and the initial reading of TMCR, an estimate of the depth of mucus was computed:

Mucus depth = Mucus collection rate ($\text{mm}^2\text{min}^{-1} \equiv \text{mg}\text{min}^{-1}$) 
$\times$ Linear velocity ($\text{mm}\text{min}^{-1}$) $\times$ brush width (mm) 

In three additional dogs we attempted to alter the pattern of filling and emptying of the thoracic cuff by inserting a parallel pathway in the tubing connecting it to the chest wall pump. One side of the pathway consisted of wide-bore tubing with a one-way flap valve; the other side contained a variable constriction, (fig. 2). We matched the V\(_{\text{to}}\) achieved by unaltered HFO/CW by adjusting the constant flow leak of the thoracic cuff, and monitored the change in V\(_{\text{Ve}}/V_{\text{t}}\) at the mouth. We tested two altered HFO/CW patterns: 1) manoeuver A - slow filling, rapid emptying of the thoracic cuff - designed to reduce V\(_{\text{Ve}}/V_{\text{t}}\) and 2) manoeuver B - rapid filling, slow emptying - designed to enhance the natural bias in V\(_{\text{Ve}}/V_{\text{t}}\). We compared the TMCR during these two manoeuvers with that achieved during unaltered HFO/CW, randomizing the order of application. We were only successful in modifying V\(_{\text{Ve}}/V_{\text{t}}\) at 8 Hz and not at 13 Hz, due to the failure of the flap valve to function at the higher frequency. (The enhancement of TMCR by HFO/CW at 8 Hz is considerably less than that achieved at 13 Hz [1]). However, the data are reported here because they support the findings of the main part of the study.

Finally, we studied the effect of atropine pretreatment on the stimulation of tracheal mucus clearance by HFO/CW, comparing it with the effect prior to atropine administration in the same dogs. Atropine was administered at a dose of 0.6 mg 15 to 30 min prior to HFO/CW. Five trials were made at 13 Hz and four at a frequency of 8 Hz.

Comparisons between data for statistical purposes were made using the paired t-test. A value of $p<0.05$ was considered significant.

**Results**

We found, in confirmation of our two previous studies, that HFO/CW at 13 Hz led to a substantial elevation of TMCR. This is shown in fig. 3, in which TMCR during 5 min of HFO/CW at 13 Hz is compared with TMCR during bracketing control periods of spontaneous breathing. For each dog, two points are shown, since the HFO/CW manoeuver was performed at the beginning and end of the experiment. In every case, TMCR during
TRACHEAL MUCUS IN HIGH FREQUENCY OSCILLATION

Fig. 3. - TMCR during a 5 min period of HFO/CW at 13 Hz versus mean TMCR during 5 min periods of spontaneous breathing before and after HFO/CW. Two points are shown for each of 8 dogs, with an arrow indicating the second point, which was taken 2-3 hours after the first. The solid line is the line of best fit, and the shaded line is the line of identity.

HFO/CW was higher than in control (p<0.001), the mean elevation being 2.35 times (±0.28 SE) (table 1). From the two sets of control measurements, it can be seen that the dogs tended to show a fall in baseline clearance rate over the course of the experiment (2-3 hours), by an average of 30% of original control. This did not affect our conclusion regarding the effectiveness of HFO/CW, as all points lie above the identity line. It is interesting to note that the increase in TMCR due to HFO/CW appears to be partly additive and partly multiplicative (i.e. with a positive intercept and a slope greater than unity).

Again in confirmation of our previous study [3], we found that HFO/AO at 13 Hz (symmetric flow) did not elevate TMCR above spontaneous breathing control. In fact it produced a slight but non-significant depression of clearance rate (0.77 x control±0.14). Neither of the two new AO manoeuvres produced any significant shift from spontaneous control (table 1). However, by paired analysis of the TMCR results, we found that the HFO/AO with expiratory flow bias elevated the clearance rate when compared with HFO/AO symmetric (p<0.01). This result is shown in fig. 4. For comparison, fig. 4 also shows the mean elevation in TMCR due to the HFO/CW manoeuvres in the same dogs. The HFO/AO manoeuvre with inspiratory flow bias did not result in any significant alteration in TMCR from symmetric HFO/AO.

The ventilatory parameters, Vro and peak Vr/Vt for the four HFO manoeuvres are also listed in table 1. The Vro values achieved were all similar except for HFO/AO, bias-in manoeuvre, where Vro was limited in two cases to only 10 ml because of the high mouth pressures developed. Although it was our desire to match both the Vro and Vr/Vt values for the HFO/AO, bias-out manoeuvre with those achieved with HFO/CW, this was not accomplished because flow amplitude and bias were not independently variable when there was a significant respiratory load.

The computed tracheal mucus depth, determined from equation 1, varied from 1 to 16 μm in 7 dogs (mean depth=7 μm; sd=5 μm). In the eighth dog, the depth could not be computed because control TMCR was near zero. The mucus collection rates varied from 0.04 to 0.26 mg·ml⁻¹, consistent with previous observations on anesthetized dogs [7]. There was no correlation between computed depth and the stimulation of TMCR by HFO/CW.

Table 1. - TMCR and ventilatory parameters for various HFO Manoeuvres

<table>
<thead>
<tr>
<th>Manoeuvre</th>
<th>TMCR</th>
<th>Vro</th>
<th>(Vr+Vt)/2</th>
<th>Vr/Vt</th>
<th>Pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFO/CW</td>
<td>235</td>
<td>24.7</td>
<td>±0.97</td>
<td>1.34</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>±28</td>
<td>±2.4</td>
<td>±0.07</td>
<td>±0.041</td>
<td></td>
</tr>
<tr>
<td>HFO/AO</td>
<td>77</td>
<td>27.2</td>
<td>±0.99</td>
<td>0.91</td>
<td>2.8</td>
</tr>
<tr>
<td>symmetric</td>
<td>±14</td>
<td>±2.0</td>
<td>±0.09</td>
<td>±0.034</td>
<td></td>
</tr>
<tr>
<td>HFO/AO</td>
<td>99</td>
<td>24.3</td>
<td>±0.97</td>
<td>1.65</td>
<td>3.7</td>
</tr>
<tr>
<td>bias-out</td>
<td>±11</td>
<td>±1.8</td>
<td>±0.07</td>
<td>±0.14</td>
<td></td>
</tr>
<tr>
<td>HFO/AO</td>
<td>81</td>
<td>19.1</td>
<td>±0.87</td>
<td>0.39</td>
<td>6.7</td>
</tr>
<tr>
<td>bias-in</td>
<td>±17</td>
<td>±3.1</td>
<td>±0.19</td>
<td>±0.031</td>
<td></td>
</tr>
</tbody>
</table>
We were unsuccessful in our attempts to further enhance the expiratory flow bias by maneuver B (rapid filling, slow emptying of the cuff), nor did this maneuver result in increased TMCR.

Finally, the results of the experiments in which the dogs were administered atropine prior to HFO/CW are illustrated in table 2. These results indicate that atropine reduced the baseline clearance rate, on average, and that it reduced the stimulation of clearance by HFO/CW by an even greater percentage, particularly at 13 Hz, where the effect prior to atropine treatment was greatest.

We were unsuccessful in our attempts to further enhance the expiratory flow bias by maneuver B (rapid filling, slow emptying of the cuff), nor did this maneuver result in increased TMCR.

Finally, the results of the experiments in which the dogs were administered atropine prior to HFO/CW are illustrated in table 2. These results indicate that atropine reduced the baseline clearance rate, on average, and that it reduced the stimulation of clearance by HFO/CW by an even greater percentage, particularly at 13 Hz, where the effect prior to atropine treatment was greatest.

**Discussion**

The above results demonstrate that the rate of mucus clearance in vivo can be changed by altering the duty-cycle or inspiratory/expiratory ratio of high-frequency oscillation. This is shown most clearly by the two experiments involving direct comparisons of HFO with altered duty-cycle, in the one case where HFO was applied by mouth (fig. 4) and the other where HFO was applied to the chest wall (fig. 2). The magnitude of alteration of TMCR due to altering the airflow pattern was not large compared with that achieved by HFO/CW with regard to spontaneous breathing. For the data shown in fig. 4, the elevation in TMCR due to the cephalad airflow bias was only about 30% of the total elevation associated with HFO/CW, despite the fact that the airflow bias applied by mouth was even greater than that produced by chest wall oscillation. The lack of a significant reduction in TMCR with caudal airflow bias may have been due to excessive dispersion of the charcoal marker spot, since only the progress of the leading edge was used to determine TMCR. Fragmentation of the marker was particularly evident during this maneuver, and retrograde movement was observed on occasion, an occurrence not noted during the other maneuvers. These findings suggest that although airflow interaction is an integral component of the stimulation of mucus clearance by chest wall oscillation, its mechanism also involves other important factor(s).

One possibility is that HFO/CW could lead to the release of acetylcholine by vagal stimulation via a reflex pathway, perhaps involving chest wall proprioceptors. This in turn could stimulate ciliary beating and hence

![Graph](image-url)
TRACHEAL MUCUS IN HIGH FREQUENCY OSCILLATION

We found some evidence to support this in the experiments in which atropine was delivered prior to HFC/AO, as shown in table 2. The results indicated that atropine reduces the stimulation of clearance due to HFC/AO, particularly at 13 Hz where it was strongest. Unfortunately, this finding does not unequivocally establish a vagal efferent pathway for the HFC/AO stimulation of clearance, because atropine probably also reduced the volume of mucus in the airways, and would thus have inhibited any mechanism that depended on mucus depth. Another possibility is that the high airway pressures associated with HFC/AO (see table 1) lead to inhibition of tracheal mucus clearance and counteract the enhancement due to airflow bias that would otherwise be seen. Evidence for stimulation of clearance beyond that achievable by airflow interaction can also be found by examining the relationship between enhancement in TMCR and baseline TMCR (fig. 3). It can be seen that the relationship is partly additive and partly multiplicative, with a finite positive intercept. The positive intercept demonstrates that there is at least some enhancement of clearance, even where baseline mucociliary clearance is negligible. If enhancement were due to biased airflow interaction alone, one might expect a strictly additive effect, which would be seen as a line parallel to the line of identity. This does not seem to be the case.

Additional evidence for a flow-dependent and flow-independent mechanism can be found in an analysis of TMCR enhancement at different frequencies, using the data from the original HFC/AO study [1]. Fig. 5 shows that enhancement was dependent on spontaneous TMCR at 11 and 13 Hz, where enhancement was greatest, and independent of it at other frequencies. By plotting the intercept and slope of the lines (fig. 6), we can see that the enhancement that is independent of baseline (i.e., intercept) is also independent of frequency, while the baseline-dependent enhancement (i.e., slope) accounts for most of the sharp frequency dependence seen.

In a separate study, we examined the effect of nonsymmetrical oscillatory airflow on the rate of transport of mucus stimulants lining a rectangular horizontal trough [9]. We found that mucus transport at constant frequency and oscillatory tidal volume could be augmented by increasing the bias of airflow interaction through alterations in the duty-cycle of the pump driving the air column. Kim et al. [10], in a different model system, have also demonstrated that altering airflow bias can alter the rate of mucus flow.

It should be stressed that the conditions of the present study and those that preceded it apply strictly to healthy anaesthetized dogs, in which the volume or depth of tracheal mucus is small. (The computed mucus depth in 7 dogs was 7 ± 5 \( \mu \)m, which is well within the usual concept of normalcy. This value may in fact be an overestimate of the real depth because of the mechanostimulation that occurs during the mucus collection [11]). It is likely that the relative importance of the airflow mechanism would increase with increasing volume of mucus in the airway, and in fact, one should anticipate this, based on the in vitro experiments that have been
presented. Various in vivo studies have demonstrated that enhancement of mucus clearance with HFO applied via the airway opening [12] or by chest wall vibration [13, 14] is indeed possible. The present work suggests that in the absence of mucus hypersecretion or excessive airway narrowing, the mechanism for enhancement is principally through reflex stimulation of clearance and not through mucus-airflow interaction.

References
des trois manoeuvres HFO/AO; toutefois, au cours du HFO/AO avec un biais au cours du débit expiratoire de pointe, TMCR s'avère nettement plus élevée qu'au cours de HFO/AO symétrique, ou au cours de HFO/AO avec un biais inspiratoire (p<0.01). L'augmentation de TMCR due au biais de débit expiratoire par rapport au HFO/AO symétrique, est de l'ordre de 30% de l'augmentation de la TMCR due à HFO/CW. 