Effects of inspiratory flow on diaphragmatic motor output in normal subjects

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Corne, S., K. Webster, and M. Younes. Effects of inspiratory flow on diaphragmatic motor output in normal subjects. J Appl Physiol 89: 481–492, 2000.—Increasing inspiratory flow (V) has been shown to shorten neural inspiratory time (T1n) in normal subjects breathing on a mechanical ventilator, but the effect of V on respiratory motor output before inspiratory termination has not previously been studied in humans. While breathing spontaneously on a mechanical ventilator, eight normal subjects were intermittently exposed to 200-ms-duration positive pressure pulses of different amplitudes at the onset of inspiration. Based on the increase in V above control breaths (ΔV), trials were grouped into small, medium, and large groups (mean ΔV: 0.51, 1.11, and 1.65 l/s, respectively). We measured T1n, transdiaphragmatic pressure (Pdi), and electrical activity (electromyogram) of the diaphragm (EMGdi). Transient increases in V caused shortening of T1n from 1.34 to 1.10 (not significant), 1.55 to 1.11 (P < 0.005), and 1.58 to 1.17 s (P < 0.005) in the small, medium, and large ΔV groups, respectively. EMGdi measured at end T1n of the pulse breaths was 131 (P < 0.05), 142, and 155% (P < 0.05) of the EMGdi of the control breaths at an identical time point in the small, medium, and large trials, respectively. The latency of the excitation was 126 ± 42 (SD) ms, consistent with a reflex effect. Increasing V had two countervailing effects on Pdi: 1) a depressant mechanical effect due primarily to the force-length (11.2 cmH2O/l) relation of the diaphragm, and 2) an increase in diaphragm activation. For the eight subjects, mean peak Pdi did not change significantly, but there was significant intersubject variability, reflecting variability in the strength of the excitation reflex. We conclude that increasing inspiratory V causes a graded facilitation of EMGdi, which serves to counteract the negative effect of the force-length relation on Pdi.

mechanical ventilation; diaphragm force-length relation; reflex control

INSPIRATORY FLOW (V) varies over a very wide range in health and disease. During exercise, for example, V may increase from a resting level of 0.5 l/s to values in excess of 6 l/s. In mechanically ventilated patients, V is an important independent variable that is often adjusted, over a wide range, to accomplish a variety of clinical and physiological objectives. The effect of V on respiratory motor output in humans is not well documented. Such information would be relevant to the understanding of mechanisms of spontaneous hyperpnea in health and disease and of the consequences of changes in ventilator V on respiratory muscle energetics in ventilator-dependent patients.

Based on experiments in anesthetized animals, in which V can be readily manipulated while other relevant variables are controlled, V may influence respiratory motor output in one of three ways.

1) With changes in inspiratory duration [neural inspiratory time (T1n)] consequent to the Hering-Breuer (H-B) volume (V)-related inspiratory inhibitory reflex (6, 16), an increase in V (ΔV) would result in an earlier attainment of the V threshold for inspiratory termination and a shorter T1n. Because inspiratory muscle activity rises in a ramp-like fashion, a deliberate ΔV, with a consequent reduction in T1n, should result, with all else being the same (i.e., no change in rate of rise of inspiratory activity), in lower peak activity and vice versa (6, 31, 33).

2) With changes in inspiratory activity before inspiratory termination, whether the rate of inflation affects inspiratory activity before inspiratory termination is controversial. In several animal studies, the rate of change in inspiratory activity during inspiration was found to be unaffected by V (6, 31, 33). Other studies, however, demonstrated an increase in this rate of rise when V was increased (4, 8, 9, 18, 27). It is very likely that these differences in response to V are related to the depth of anesthesia (9, 27). This suggests that this V-related excitation may be particularly prominent in consciousness.

3) Changes in V, and, consequently, instantaneous V, could affect inspiratory muscle pressure output, independent of muscle activity, via strictly mechanical effects (intrinsic properties of respiratory muscles). Thus at a given activity respiratory muscles generate less pressure in the presence of higher V [via the force-velocity relation (1, 13, 28)] and V [via the force-length relation (10, 14, 28)], and vice versa.

There is no information in humans regarding the possible excitatory effect of V (see 2 above). A small effect of V on T1n (1 above) was found in several human studies (20, 22, 29, 32). However, the range of V exam-
ined was very small [essentially between zero (occlusion) and resting V (∼0.3–0.5 l/s)], and the subjects were anesthetized (29) or asleep (20, 22, 32). Our laboratory has recently demonstrated a marked effect of inspiratory V on T\textsubscript{In} in normal, awake subjects over the V range of 0.8–2.5 l/s (11). Interestingly, T\textsubscript{In} reduction was not related to earlier attainment of a V threshold. In fact, V at inspiratory termination was significantly lower when V was increased. The operation of the intrinsic properties of respiratory muscles has been well demonstrated in humans (1, 13, 14, 28). It is, however, difficult to infer the quantitative impact of these responses during spontaneous breathing in view of the nature of the methods used in these studies (see DISCUSSION).

In the present study, we describe the response of diaphragmatic electrical [electromyogram (EMG)] activity (EMG\textsubscript{di}) and pressure output [transdiaphragmatic pressure (Pdi)] to brief (∼0.2 s) increases in inspiratory V in awake humans. From this information, we extract the magnitude of the intrinsic properties and document the occurrence of substantial V-related inspiratory excitation in awake humans. The response of T\textsubscript{In} to such brief increases in V was also examined in an effort to define the mechanism of reduction in T\textsubscript{In} observed earlier (11) with sustained increases in V.

**METHODS**

Eight normal subjects were studied: four men and four women. Subject age ranged from 27 to 38 yr. No subject had any clinical evidence of respiratory disease. Three subjects were aware of the general nature of the study (to study the effect of inspiratory V) but not the specific protocol or expected results. One subject (S. Corne) was aware of the specific protocol.

A gastroesophageal catheter was placed in all subjects. The catheter was fluid-filled and measured gastric and esophageal pressures from two ports separated by a distance of 20 cm. The catheter also recorded the EMG\textsubscript{di} from two silver electrodes placed between the gastric and esophageal pressure ports. The catheter was inserted through the nose and advanced until negative pressure deflections were observed in both the gastric and esophageal pressure waveforms during inspiration. The catheter was then slowly advanced until positive deflections became visible in the gastric waveform, confirming position in the stomach. Minor adjustments were then made in the catheter position to optimize the EMG\textsubscript{di} waveform and minimize the amplitude of the cardiac artifact in the Pdi tracing. The distance between the proximal pressure port and the EMG electrodes was 8.5 and 13.5 cm (average 11 cm), respectively, for the proximal and distal EMG electrodes. This ensured an appropriate location of the proximal port in the lower one-third of the esophagus when the EMG electrode was at the level of the diaphragm (and hence providing the optimal EMG signal). The Pdi was derived by subtracting esophageal pressure from gastric pressure. The raw EMG signal was filtered by a band-pass filter (30–1,600 Hz).

Subjects were seated and connected to a mechanical ventilator (Winnipeg Ventilator) through a mouthpiece. The ventilator was set to allow spontaneous respiration through the ventilator circuit. Nose clips were applied. Airway pressure (Paw) was measured from a side port near the mouthpiece with a differential pressure transducer. Inspiratory and expiratory V were measured with a heated pneumotachograph (Hans-Rudolph 3700, Kansas City, MO) connected to the mouthpiece, and V was derived from the electronic integration of V. End-tidal carbon dioxide was monitored from a side port near the mouthpiece by using a mass spectrometer (MGA-1100 Medical Gas Analyzer, Perkin-Elmer, Pomona, CA). All waveforms were continuously recorded on two personal computers with the use of a data-acquisition program (WINDAQ, DATAQ Instruments, Akron, OH) for later analysis. EMG\textsubscript{di} and V were sampled at 500 Hz on one computer. All other waveforms, including the identical V signal, were sampled at 125 Hz on a second computer. The V signal, common to both recordings, was used to align the EMG signal to other recorded signals in the second computer.

An external pulse-generating box was connected to the pressure control mechanism of the ventilator. This permitted the delivery of positive Paw pulses of varying amplitudes (0–23 cmH\textsubscript{2}O). The duration of the pulse was 0.2 s.

The ventilator was V\textsubscript{t} triggered, and the V threshold for triggering was set at the lowest level that did not result in autotriggering. This typically resulted in a trigger sensitivity of ∼0.1 l/s. The pulse apparatus sensitivity was set at a level that caused triggering of the pulse to occur at 0.1 l/s as well. Subjects breathed room air, and a period of ∼20 min was allowed for them to adjust to the ventilator.

Subsequently, brief pulses of positive pressure were delivered near the onset of inspiration. Pulses were delivered for one breath, and then ∼30–60 s were allowed to elapse before the next pulse was delivered. The duration between pulses was varied so that the subjects could not anticipate when a pulse was about to be delivered. Pulses of at least three different voltages, small [increase (∆) in Paw, 5–10 cmH\textsubscript{2}O], medium (∆Paw, 10–15 cmH\textsubscript{2}O), and large (∆Paw, 15–20 cmH\textsubscript{2}O), were given to all subjects, with the exception of subject 1, to whom only medium and large pulses were given. Eight to fifteen pulses of a given voltage were applied, and then the voltage was changed. The order of applying the different voltages was randomized. For the purposes of describing the data, we shall refer to a sequence of pulses (8–15 pulses) of the same external voltage in a given subject as a “trial.”

The effect of pulses on peak inspiratory V, inspiratory V at the termination of T\textsubscript{In} (V\textsubscript{E}/T\textsubscript{In}), inspiratory tidal V at the termination of T\textsubscript{In} (V\textsubscript{E}/T\textsubscript{In}), Paw, Pdi, EMG\textsubscript{di}, T\textsubscript{In}, neural expiratory time (T\textsubscript{Ex}), and duration of respiratory cycle (T\textsubscript{tot}) were analyzed. Control breaths, usually the breath immediately preceding the pulse breath, were analyzed as well for comparison.

For each pulse trial, we derived averaged waveforms for pulse and control breaths for each of the relevant parameters, V, V\textsubscript{E}, Pdi, Paw, and EMG\textsubscript{di}. This was done by converting each individual waveform into a series of numerical values. These values were then averaged for the 8–15 breaths analyzed and subsequently reconverted back to waveforms and graphed against time.

Quantitative analysis of the EMG\textsubscript{di} waveform necessitated removal of the electrocardiogram (ECG) artifact. In all subjects but one, this required removal of only the QRS artifact. The QRS artifact, typically 100 ms in duration, was first removed from the signal. The EMG signal was then rectified. Subsequently, the deleted 100-ms segment corresponding to the ECG artifact was replaced with a straight line that began at an amplitude equal to the average of values obtained for 40 ms before the QRS artifact and ended in a value equal to the average of values obtained 40 ms after the QRS artifact. In one subject (subject 2), the T-wave
artifact was of sufficient magnitude that it required removal as well. The T-wave artifact was also ~100 ms in duration and was removed with a process identical to that described for the QRS artifact. The EMG signal was then averaged (100-ms moving average). An example of the raw EMG and the processed EMG with artifact removed is shown in Fig. 1. For the purposes of averaging EMGdi values among trials, we used normalized data, whereby the average peak control EMGdi in each trial was assigned a value of 100 (%).

For the purposes of analysis, the onset of TIn, in an individual breath was defined as the initial negative deflection in the Paw tracings and a rapid change in the direction of V from expiratory (or zero) to inspiratory. Where possible, the Pdi and EMGdi tracings were also inspected to help define precisely where inspiration began simply from inspection of the EMGdi waveform. The very gradual increase in EMGdi, typically observed during this isoactivity interval and creating a linear regression equation by calculating the difference in Pdi, V, and V˙ acting via the force-length and force-velocity relationships of the diaphragm (see RESULTS and DISCUSSION). We attempted to quantitate this relationship by calculating the difference in Pdi, V, and V˙ between pulse and control breaths at multiple data points during this isoactivity interval and creating a linear regression equation

$$\Delta \text{Pdi}/\Delta V = A + B \Delta V/\Delta V$$

where A is equal to the force-length relationship (in cmH2O/l) and B is equal to the force-velocity relationship (in cmH2O · l−1 · s) of the diaphragm.

RESULTS

As expected, the application of positive pressure pulses resulted in increased inspiratory V during the duration of the pulse. As outlined in METHODS, trials were sorted into three groups based on the peak ∆V achieved: small (mean ∆V, 0.51 l/s), medium (mean ∆V, 1.11 l/s), and large (mean ∆V, 1.65 l/s). Figure 2 shows responses of EMGdi, Pdi, V, and V˙ to a pulse application in a representative subject. These responses can be described in terms of three major effects.

Effects of Increased Inspiratory V on Respiratory Timing

TIn decreased in breaths when pulses were applied (Table 1), and the decrease was larger in the medium and large ∆V trials. TIn decreased from 1.34 to 1.10 s (P = 0.08), from 1.55 to 1.11 s (P = 0.003), and from 1.58 to 1.17 s (P = 0.0004) in the small, medium, and large ∆V groups, respectively. TEn decreased along with TIn, with the result statistically significant in the medium and large ∆V groups (P < 0.05). As a result of decreases in both TIn and TEn, Ttot decreased as well (Table 1).

There was no significant difference in V@TIn between pulse and control breaths in the small and medium ∆V trials. In the large ∆V trials, V@TIn was significantly larger in the pulse breaths (Table 1). For the group of 23 trials, V@TIn was larger in the control breaths in 11 trials and larger in the pulse breaths in 12 trials. In the large ∆V group, there was no significant correlation between the extent of increase in V@TIn and the extent of decrease in TIn (r = −0.32).

Excitatory Effects of V on Respiratory Motor Output

In seven of eight subjects (the exception being subject 6), there was clear evidence of an increased rate of rise in
EMGdipls over that of EMGdicon shortly after the onset of ΔV (see Figs. 1–3). Although the increase in EMGdi began during the period of increased V, EMGdipls remained greater than in the EMGdicon in these seven subjects, even after V had decreased to below the level found in the control breaths (Fig. 3).

We measured the amplitude of the EMGdipls signal at the cessation of Tn and compared it with the EMGdicon at an identical time point in inspiration, with this time point being referred to as EMGdiisotime. EMGdipls was almost invariably greater than EMGdicon in the eight subjects (See EMGdiisotime Table 1). EMGdipls was 131 (P = 0.02), 142 (P = 0.055), and 155% (P = 0.007) of EMGdicon in the small, medium, and large ΔV trials, respectively. We also compared the mean values for EMGdipls and EMGdicon at 0.1-s intervals from the onset of the ΔV. Results for the large ΔV trials are shown in Fig. 4. The increase in EMGdipls was statistically significant at 0.3 and 0.4 s.

We defined the onset of excitation as the point at which the rate of rise of the EMGdipls clearly deviated from that of EMGdicon (arrow in EMGdi tracing, Fig. 2). This point could be identified in 21 of 23 trials. The latency of the EMGdi response to a pulse was measured as the interval between the increase in inspiratory V above control and the point at which excitation of EMGdi occurred. The mean latency for the 21 trials was 126 ± 42 (SD) ms. The latencies for the small, medium, and large trials were not statistically significantly different.

**Inhibitory Effects of V Due to Force-Velocity and Force-Length Relationship**

The averaged individual responses of Pdi to the increased V during the highest V transients are shown in Fig. 5. In six of eight subjects, the Pdi tracing typically demonstrated a negative concave deflection at the onset of the V pulse that, in five of these subjects, persisted for the duration of the higher inspiratory V (see Figs. 2, 5, and 8). Inspection of the EMGdi tracings for this same segment of inspiration failed to reveal any inhibition of the EMGdipls tracing (Fig. 3).

In fact, to the extent that any changes in the EMGdipls tracing relative to EMGdicon were visible during this segment of early inspiration, they involved an

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**Table 1. Trial values**

<table>
<thead>
<tr>
<th>Pulse Trial</th>
<th>Small (n = 7)</th>
<th>Medium (n = 8)</th>
<th>Large (n = 8)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Pulse</td>
<td>Control</td>
</tr>
<tr>
<td>Tn, s</td>
<td>1.34 ± 0.08</td>
<td>1.1 ± 0.14</td>
<td>1.55 ± 0.25</td>
</tr>
<tr>
<td>TEx, s</td>
<td>4.44 ± 0.82</td>
<td>4.08 ± 0.69</td>
<td>3.61 ± 0.5</td>
</tr>
<tr>
<td>Tot, s</td>
<td>5.77 ± 0.84</td>
<td>5.18 ± 0.78</td>
<td>5.16 ± 0.56</td>
</tr>
<tr>
<td>Peak Pdi, cmH2O</td>
<td>12.4 ± 1.5</td>
<td>11.4 ± 2.4</td>
<td>11.9 ± 1.5</td>
</tr>
<tr>
<td>Peak V, l/s</td>
<td>0.65 ± 0.06</td>
<td>1.16 ± 0.10†</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>V@Tn, l/s</td>
<td>0.46 ± 0.08</td>
<td>0.44 ± 0.09</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td>V@TEx, liters</td>
<td>0.54 ± 0.05</td>
<td>0.50 ± 0.10</td>
<td>0.72 ± 0.13</td>
</tr>
<tr>
<td>EMGdi, liters</td>
<td>80 ± 6</td>
<td>105 ± 11*</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>Peak EMGdi</td>
<td>100‡</td>
<td>105 ± 11</td>
<td>100‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of trials. Trial groups are based on increase in peak flow (ΔV): mean ΔV of 0.51, 1.11, and 1.65 l/s for small, medium, and large trials, respectively. Tn, neural inspiratory time; TEx, neural expiratory time; Tot, duration of respiratory cycle; Pdi, transdiaphragmatic pressure; V@Tn, V at the end of Tn; V@TEx, volume at the end of TEx; EMGdi, diaphragmatic electromyogram; EMGdiisotime, EMGdi measured in both pulse and control breaths at the end of Tn in the pulse breath. Significantly different vs. control: *P < 0.05, †P < 0.005. §Normalized value (normalized refers to adjustment of EMGdi values in each trial based on assigning a value of 100 to the mean peak EMGdi control value in each trial).
excitation of the motor output of the diaphragm ~40–200 ms after the onset of the pulse, as described above. This concave deflection, observed in the pulse breath Pdi (Pdi_{pls}) relative to the control breath Pdi (Pdi_{con}), therefore, appears to represent a change due to the force-velocity and force-length relationship of the diaphragm. The magnitude of this depression effect was quantified as described earlier in METHODS. Some trials were not suitable for analysis. The two major reasons for excluding trials were 1) very early onset of EMGdi excitation, such that the interval in which EMGdi_{pls} and EMGdi_{con} were visually comparable was too short to permit sufficient data points for analysis, and 2) excessively large cardiac artifact in the Pdi tracings, such that the signal-to-noise ratio was unacceptably low. Twelve trials (each trial consisting of the averaging of 8–15 pulse and control breaths) in six subjects were deemed suitable for analysis. The mean values for the force-length and force-velocity-relationship were 11.2 \pm 2.5 and 0.2 \pm 0.6 (SD) cmH_2O \cdot 1^{-1} \cdot s, respectively. An example of the results of analysis in one trial is shown in Fig. 6. The intercept (11.2 in this case) represents the change in Pdi per unit change in V, whereas the slope (0.8 in this case) reflects the change in Pdi per unit change in V. Excellent correlation coefficients were obtained in all cases, with a mean r of 0.95 \pm 0.05 (SD).

The effect of inspiratory \( \dot{V} \) on peak Pdi was minimal when it was looked at for the groups as a whole (Table 1). Mean peak Pdi decreased from 12.4 to 11.4, from 11.9 to 10.2, and from 12.1 to 11.8 cmH_2O in the small, medium, and large \( \Delta V \) trials, respectively. None of these differ-

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**Fig. 3.** Control (solid lines) and pulse EMGdi (dotted lines) plotted against time in subjects 1–8. Each waveform represents averaging of 8–15 breaths. Vertical lines (1st and 2nd) mark the onset and termination, respectively, of the increased flow in the pulse breaths. Note excitation in the pulse breaths.

**Fig. 4.** Average (means \( \pm \) SE) EMGdi pulse (dotted line) and EMGdi control (solid line) of all large flow increase trials. Time refers to the onset of flow increase in the pulse breath relative to control breath. *P < 0.05.
Fig. 5. Control Pdi (solid lines) and pulse Pdi (dotted lines) plotted against time in subjects 1–8. Each waveform represents averaging of 8–15 breaths. Vertical lines (1st and 2nd) mark the onset and termination, respectively, of the increased flow in the pulse breaths. Note initial negative deflection in the pulse Pdi waveform, reflecting the force-velocity and force-length relation of the diaphragm, as well as a subsequent increase in rate of rise in the Pdi, particularly in subjects 1, 2, 7, and 8, reflecting diaphragmatic excitation.

Fig. 6. Mechanical relationship between diaphragmatic pressure output and flow (force-velocity) and volume (force-length) in 1 subject. Results shown here represent averaging of 15 breaths. Note the importance of the force-length relation (intercept = 11.2 cmH2O/l) relative to the force-velocity relation (slope = 0.8 cmH2O·l·s−1). ΔP, ΔV, and ΔV represent differences in pressure, flow, and volume, respectively, between control and pulse breaths at different points following the increase in V.
ences was statistically significant. However, there was a great deal of intersubject variability in the response of peak Pdi to V. In those with a large decrease in TIn and a weak excitatory response to V, peak Pdi decreased at higher V (subjects 5 and 6, Fig. 5). Conversely, in those with only a small decrease in TIn and a stronger excitatory response to V (subjects 1, 2, 7, and 8), peak Pdi increased at the higher V. We also analyzed the difference in Pdi_plos and Pdi_con at 100-ms intervals from the onset of ΔV. The results for the large ΔV group are shown in Fig. 7. There were statistically significant decreases in Pdi_plos at 0.1 and 0.2 s, reflecting the force-length and force-velocity effect. There was also a subsequent increase in Pdi_plos over Pdi_con from 0.4 to 0.7 s, reflecting the excitatory effects of V. This failed to reach statistical significance, because of the large variability in responses (SE bars shown).

Figure 8 demonstrates the change in Paw, V, V, Pdi, and EMGdi between pulse and control breaths in the small, medium, and large ΔV groups. The time period analyzed begins with the first data point at which pulse V

![Figure 7](image)

**Fig. 7.** Average (means ± SE) Pdi pulse (dotted line) vs. average Pdi control (solid line) of all large flow increase trials. Time refers to the time from onset of flow increase in pulse breath relative to control breath. *P < 0.05.

![Figure 8](image)

**Fig. 8.** Average (means ± SE) differences (Δ) between control and pulse breaths in various variables in the small (dotted lines), medium (dashed lines), and large (solid lines) flow increase groups. Note that the response in each variable is graded. EMGdi values were normalized in reference to the mean peak control EMGdi in each trial, which was assigned a value of 100. *Significant difference between responses in the three groups by ANOVA (P < 0.05). Changes in Paw, flow, and volume were significant (by design) at all time points (asterisks not shown).
increased over that of control and extends to the end of T_in for the individual trial with the shortest T_in in that group. This limited the period of analysis to \(-0.2\) s for the small ΔV group and to \(-0.4\) s for the medium and large ΔV groups. By definition, there was a graded increase in ΔV and ΔV from the small to the large ΔV groups associated with a graded increase in ΔPaw. There was also a graded increase in the negative ΔPdi deflection, reflecting the force-velocity and force-length relation of the diaphragm. Conversely, there was a graded increase in ΔEMGdi from the small to the large ΔV group. The EMGdi was significantly different at 0.2 s among the three groups using ANOVA (P < 0.05).

**DISCUSSION**

We have examined the effect of changes in inspiratory V on respiratory motor output in normal subjects. These effects can be grouped into three categories: 1) effects on respiratory timing, 2) effects on excitation of diaphragmatic motor output, and 3) the force-length and force-velocity relationship of the diaphragm.

**Respiratory Timing**

In a recent study in awake, normal subjects, our laboratory found that deliberate increases in inspiratory V, maintained until inspiratory termination, result in shortening of T_in (11). The gain of the response was substantial, and earlier termination of inspiratory activity was not related to earlier attainment of a V threshold, as per the classical H-B reflex. The changes in T_ex were inconsistent. The present study compliments these earlier findings by documenting the effects on respiratory timing of transient increases in V where the stimulus (i.e., increased V) terminates before the end of neural inspiration. We found that such transient increases in V continue to reduce T_in, even though V at the time of inspiratory termination was not significantly different between pulse and control breaths (Table 1). These responses imply the presence of delayed effects in the aftermath of transient inspiratory interventions.

With respect to T_in shortening, the delayed effect may be due to the higher V accrued during the period of increased V (e.g., Figs. 2 and 8). Because such excess V is retained past the period of the transient, it could cause earlier termination of T_in via the traditional V-related H-B reflex. Although this may have contributed to some extent in some cases, it is very unlikely that this mechanism provides the entire explanation. According to the H-B reflex, the V threshold for inspiratory termination declines progressively with inspiratory time (6, 16). It would thus require a higher V to terminate inspiration sooner. In the small and medium ΔV groups, V@T_in was not higher (Table 1). Although V@T_in was higher with the large pulses, the difference was too small to account for the T_in shortening. Thus, with the large pulses, T_in was reduced by 25% (1.17 vs. 1.58 s, Table 1), whereas V@T_in increased by \(-25\%\) (0.92 vs. 0.74 liter, Table 1). Even in animals in which this reflex is very strong (e.g., pentobarbital-anesthetized cats (6, 16)), V@T_in increases much more for equivalent reductions in T_in. Furthermore, as mentioned, there was no correlation between the extent of increase in V@T_in and the extent of decrease in T_in. These observations suggest that the delayed effects were primarily related to neural processing.

Information from animal studies regarding delayed central effects of inspiratory-terminating inputs is highly contradictory. In pentobarbital-anesthetized cats, removal of V (36) or inspiratory inhibitory vagal stimulus (34) before the end of inspiration results in paradoxical delayed effects: T_in would have been lengthened relative to its duration had the stimulus not been introduced at all. By contrast, in chloralose-anesthetized dogs, Cross et al. (8) found that T_in continued to be shortened when lung inflation was withdrawn before inspiratory termination. These observations clearly indicate that inspiratory inputs can produce central effects that outlast the stimulus but that the directions of these effects can be diametrically opposite (concordant with, or paradoxical to, the primary effect of the stimulus) in different species and/or under different experimental conditions (type or depth of anesthesia). The present findings indicate that, in awake, normal humans, the delayed effects are concordant with the primary effect of the stimulus; there is memory for the inspiratory-terminating influence of increased V. Whether the same response will hold under other conditions, for example, during sleep or anesthesia, or in the presence of disease remains to be determined.

Earlier studies in anesthetized animals demonstrated a linkage between T_in and T_ex (6, 16, 35). When T_in is shortened by an inspiratory-terminating input, the following T_ex is also shortened. The reduction in T_ex observed in the present study can thus be explained on the basis of this central linkage. In our previous study, T_ex was not consistently shortened when V increased and T_in decreased (11). In this latter study, however, inflation usually continued past the end of T_in, representing further inflation into expiration. Because inflation during expiration lengthens T_ex via the expiratory-prolonging component of the H-B reflex (23–25), extension of inflation into expiration would obscure the reduction in T_ex that might otherwise have occurred as a result of T_in shortening [for a more detailed discussion, please see Fernandez et al. (11)]. By limiting the inflation to the inspiratory phase in the present study, the T_in-T_ex linkage was demonstrated.

**Excitation of Diaphragmatic Activity**

In addition to the effects on respiratory timing, our results also demonstrate that higher inspiratory V caused neural excitation of the diaphragm, as evidenced by an increased rate of rise in the EMGdi waveform. An increase in the rate of rise of EMGdi_pls relative to EMGdi_con was usually evident 50–200 ms after the onset of the inspiratory ΔV. This increase in EMGdi appeared proportionate to the ΔV achieved
over that of control breaths (Fig. 8, lowest panel). Furthermore, the mean \( \text{EMG}_{\text{dipl}} \) at the end of \( T_{\text{In}} \) was significantly greater than the mean \( \text{EMG}_{\text{di con}} \) measured at an identical time point in inspiration. This excitation effect was present in all subjects but showed large intersubject variability in the degree of excitation.

To our knowledge, the present study is the first to deliberately look for and demonstrate \( V \)-related inspiratory excitation in humans. Accordingly, it is necessary to address some technical issues.

Technical considerations. The \( \text{EMG}_{\text{di}} \) signal is subject to being artifactually increased and will appear larger if it is measured at a larger \( V \) (12). Therefore, one may question whether the increase in \( \text{EMG}_{\text{di}} \) we observed was due to the fact that \( V \) rose more quickly in the pulsed breath. We do not feel that the increase in \( \text{EMG}_{\text{di}} \) was an artifact for a number of reasons. First, there was a finite delay before the excitation was evident. Second, the increase in \( V \) observed in the pulsed breaths, which was typically only a few hundred milliliters (e.g., Fig. 8), would be insufficient to account for the large increases observed in \( \text{EMG}_{\text{di}} \), based on the relation between \( V \) and \( \text{EMG}_{\text{di}} \) reported by Gandevia and McKenzie (12). Third, the \( P_{\text{di}} \) response was biphasic, initially showing a decreased rate of rise, followed by an increased rate of rise (Fig. 8). The secondary increase in the rate of rise of \( P_{\text{di}} \) began at a time when \( V \) was continuing to rise at a faster rate (Fig. 8) and despite the negative effect this would have on \( P_{\text{di}} \) secondary to the diaphragm's intrinsic properties. An increase in the rate of rise of \( P_{\text{di}} \) under such conditions can only result from the increased rate of rise in \( \text{EMG}_{\text{di}} \). In four subjects, absolute \( P_{\text{di pl}} \) ultimately exceeded \( P_{\text{di con}} \), despite \( V \) being higher (Fig. 5). This can only occur if \( \text{EMG}_{\text{di}} \) increased enough to more than offset the depressant effect of the larger \( V \) via the force-length relation. Finally, some studies in animals (4, 8, 9, 18, 27) have documented a similar excitation while recording was done directly from the phrenic nerve, a measurement that is not subject to this artifact.

Given that the subjects were alert, it may be argued that the excitatory response was a voluntary, as opposed to reflex, response. This is quite unlikely for several reasons. First, the latency for the response was generally <150 ms and was often <100 ms, whereas a minimum latency of 200 ms is required to mount a voluntary respiratory response to an intervention of this sort (17, 38). Second, pulses were applied in random order to avoid anticipatory responses. Third, behavioral responses of this kind are generally inconsistent within and among subjects (3). This was not the case here. Fourth, a similar response was demonstrated in anesthetized animals in which behavioral responses are expected to be absent (4, 8, 9, 19, 27).

Several previous studies described the occurrence of augmented breaths (sighs) when lung inflation is artificially increased during inspiration (5, 23, 30). The excitation observed in the present study was not sighing for the following reasons. In spontaneous or induced augmented breaths, inspiratory activity is similar to “control” breaths over the period corresponding to “control” \( T_{\text{In}} \) (5). Augmentation occurs at the point at which inspiration would normally terminate and results in a breath in which neural inspiration is invariably longer and peak inspiratory activity is invariably much higher than control breaths. In the present experiments, excitation occurred very early in inspiration, soon after the onset of the pulse, \( T_{\text{In}} \) of the stimulated breath was shorter (Table 1), and the increase in peak diaphragm activity was small (Table 1) relative to the increase observed with sighs (>200%). Augmented breaths are also followed by a refractory period (20- to 45-s duration) during which it is difficult to elicit another sigh (5). No refractory period was observed in the present study, as illustrated in Fig. 9. Finally,
sighs appear to be all-or-none responses. The response described here was graded (Fig. 8).

**Mechanism of reflex, \( V \)-related inspiratory excitation.** Animal studies on the effect of inflation rate on inspiratory activity before inspiratory termination produced very conflicting results. In early work, it was found that the pattern or intensity of inspiratory activity was not affected by \( V \) until shortly before termination (6, 31, 33, 36). This led to the concept that \( V \) (and \( V \)) affects inspiratory activity in an all-or-none fashion (6, 31). In several later experiments, however, a vagally mediated, \( V \)-related increase in inspiratory activity was demonstrated before termination of the inspiratory phase (4, 8, 9, 27). The earlier lack of such effect was likely related to the depth of anesthesia because, in at least two studies (9, 27), the excitatory response was eliminated by additional anesthetic doses. It is very likely that the response observed here is the same as the one described in the above-cited animal studies and is, therefore, vagal in origin. \( V \)-sensitive upper airway receptors are not likely to be responsible; McBride and Whitelaw (24) found that increased \( V \) through the upper airway (without lung inflation) in awake humans inhibits inspiratory motor output. Accordingly, any excitation we observed represents the net effect of excitation produced by lower receptors, less any inhibition produced by upper airway receptors. A possible contribution from muscle receptors cannot be entirely discounted, although it is unlikely to be significant. Newsom Davis and Sears (26) monitored external intercostal activity during strong, sustained voluntary contractions against a closed airway (Mueller maneuver). On sudden release of occlusion, there was a short-latency (22–25 ms) inhibition of activity, which they attributed to unloading of the inhibitory tendon organs. The excitation we observed could, therefore, theoretically be due to reduction in the tension of diaphragmatic tendon organs produced by unloading during the pulse. Pdi at the time of pulse application was, however, of the order of 1–4 cmH\(_2\)O (Fig. 5), a mere 1–3% of maximum Pdi. It is highly improbable that tendon organ inhibition at these very low tensions is such that its elimination (by increased \( V \) and unloading) caused a >40% increase in activity (EMGdiisotime, Table 1).

**Physiological significance.** The \( V \)-related excitatory response may be a mechanism that serves to counteract the negative consequences of the obligatory intrinsic properties of respiratory muscles. Without such an excitatory mechanism, the ventilatory response to a given respiratory stimulus would be attenuated, because as \( V \) increases, the efficiency of the respiratory muscles as pressure generators decreases (secondary to the force-length and force-velocity relation). Regardless of whether this response was developed for this specific purpose, its net effect is in the direction of compensating for reduced respiratory muscle efficiency with increased ventilatory demand. Our results permit an analysis of the balance between the two opposite \( V \)-related responses. To the extent that respiratory drive is not altered during the brief period of the pulse, a perfect compensatory response would result in Pdi remaining constant as \( V \) is artificially increased; the reduction in Pdi produced by the obligate mechanical properties of muscles would be exactly offset by an increase in muscle activation. Figure 8 shows that, on average, the two mechanisms canceled each other out at \(~0.35\) s after the onset of \( \Delta V \) (\( \Delta Pdi \) returning to zero, Fig. 8). Before this time, compensation was inadequate, resulting in a decrease in Pdi. This shortfall is, to a large extent, related to the fact that the operation of the intrinsic properties of muscles is instantaneous, whereas neural delays preclude an instantaneous excitatory response. Given the abrupt \( V \) transition in this study, such an initial shortfall is unavoidable. It may be expected, however, that, with less abrupt changes in \( V \), the discrepancy would be less pronounced.

An important observation in the present study is that the extent to which the excitatory response, once developed, manages to offset the intrinsic properties varies considerably among subjects. In two subjects, Pdi continued to be below control throughout the common period of observation, indicating inadequate compensation (subjects 3 and 5, Fig. 5). In two subjects, Pdi remained close to Pdi\(_{\text{con}}\) until the end of the breath (subjects 4 and 6), whereas in the remaining four subjects there was overcompensation, with Pdi exceeding control for the balance of inspiration (subjects 1, 2, 7, and 8; Fig. 5). It is tempting to speculate that such interindividual differences in response gain contribute to differences among subjects in ventilatory response to physiological stimuli. Furthermore, to the extent that the excitatory response was found to be vulnerable to anesthesia in animals, it is possible that its gain is less during sleep and obtundation in humans, and this may contribute to depressed ventilatory responses in these states.

**Intrinsic Properties of the Diaphragm**

At a given level of activation, all skeletal muscles, whether respiratory or not, generate less force when they shorten at a greater velocity, according to the force-velocity relation [for review see Younes and Riddel (37)]. These characteristics are structural, within the muscles themselves, and occur instantly and in the absence of any feedback from other sources. Within the respiratory system, these properties have the effect of reducing the pressure-generating ability of respiratory muscles at higher \( V \) and higher \( V \) values. To determine the magnitude of these effects, it is necessary to measure respiratory muscle pressure output at the same activity while \( V \) and \( V \) are altered. A variety of techniques have been used to obtain this information in humans. These include measurement of pressure output at different \( V \) values and \( V \) during maximum voluntary efforts (1, 2, 19), during electrical stimulation of the phrenic nerves in the neck (28), or during voluntary activation of the dia-
phragm to specified EMGdi levels using visual feedback of the EMGdi signal (13, 14). These studies have produced widely different quantitative estimates, indicating that the results are greatly influenced by technique (see Ref. 37 for detailed discussion of these results). In view of the dependence of results on technique and the fact that the technique used to obtain isoelectric activity was, in each case, far removed from the situation obtained during spontaneous breathing, none of the estimates obtained from these studies can be reliably applied to spontaneous breathing in humans.

In the present study, we took advantage of the fact that EMGdi was not affected for a period of time (latency of the excitatory response), whereas V and V changed, to calculate the mechanical effects of V and V on Pdi. We believe that this approach has several advantages over previously used techniques and that the results should, therefore, be closer to the situation during spontaneous breathing. First, activation of the diaphragm was spontaneous. Therefore, there is no reason to believe that the distribution of activity within the diaphragm was anything but normal. Second, the distribution of activity between diaphragm and other muscles, which affects thoracoabdominal configuration and hence pressure output (14), was normal (i.e., not constrained by protocol). Third, although we did not monitor thoracoabdominal configuration, expansion during the imposed pulses must have closely followed the relaxed thoracoabdominal configuration. Although some deviation from passive configuration may occur during large, spontaneous increases in ventilation (15), chest expansion usually follows the resting configuration at resting and moderately increased levels of ventilation (13). Thus the changes in Pdi when the chest expands at different rates along its relaxed configuration should provide a closer approximation of what happens during spontaneous increases in ventilation.

Our results indicate that the effect of V per se on pressure output (the force-velocity relation) is negligible (0.2 ± 0.6 cmH2O · 1−1 · s) over the V range studied (0.65−2.4 l/s, Table 1). This is in agreement with findings in spontaneously breathing dogs in which EMGdi and diaphragm velocity of shortening were recorded by using implanted sensors (25). The independent effect of V via the force-length relation and configuration factors was, however, large. Its magnitude (11.2 ± 2.5 cmH2O/l) is comparable to the passive elastance in normal subjects (2). When uncompensated for, via the excitatory response, for example, V would have the same effect on ventilatory responses as would the doubling of the passive elastance. Given these findings, it is of interest to note that the time course of the excitatory response follows much more closely the time course of V (Fig. 8). This suggests that the excitatory response is well suited to offset the unavoidable effects of intrinsic muscle properties.

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