Termination of inspiration by phase-dependent respiratory vagal feedback in awake normal humans

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BuSha, Brett F., Martha H. Stella, Harold L. Manning, and J. C. Leiter. Termination of inspiration by phase-dependent respiratory vagal feedback in awake normal humans. J Appl Physiol 93: 903–910, 2002. First published May 10, 2002; 10.1152/japplphysiol.00153.2002.—Imperceptible levels of proportional assist ventilation applied throughout inspiration reduced inspiratory time (TI) in awake humans. More recently, the reduction in TI was associated with flow assist, but flow assist also reaches a maximum value early during inspiration. To test the separate effects of flow assist and timing of assist, we applied a pseudorandom binary sequence of flow-assisted breaths during early, late, or throughout inspiration in eight normal subjects. We hypothesized that imperceptible flow assist would shorten TI most effectively when applied during early inspiration. Tidal volume, integrated respiratory muscle pressure per breath, TI, and TE were recorded. All stimuli (early, late, or flow assist applied throughout inspiration) resulted in a significant increase in inspiratory flow; however, only when the flow assist was applied during early inspiration was there a significant reduction in TI and the integrated respiratory muscle pressure per breath. These results provide further evidence that vagal feedback modulates breathing on a breath-by-breath basis in conscious humans within a physiological range of breath sizes.

vagus; control of respiration; mechanical ventilation; Hering-Breuer reflex

THE HERING-BREUER REFLEX, in which passive overinflation of the lungs or prevention of inspiration or expiration alters respiratory timing (11), has been documented in humans by using airway occlusion during anesthesia (19) or passive overinflation of the lungs during sleep (12). The reflex has been more difficult to elicit during wakefulness in humans. However, airway occlusion and overinflation of the lungs are potent stimuli not typically encountered during eupneic breathing, and these stimuli may evoke behavioral responses if applied during wakefulness, which could mask the effects of the Hering-Breuer reflex. In a previous study in adult human subjects (3), our laboratory investigated the effect of unloading the respiratory system by using imperceptible levels of proportional assist ventilation applied in a pseudorandom binary sequence. We found that a small but significant increase in tidal volume (VT) and inspiratory flow rate (Vt/TI) resulted in a significant reduction in inspiratory time (TI), consistent with the action of the Hering-Breuer reflex. In a subsequent investigation using a similar technique (4), our laboratory examined the separate effects of imperceptible flow and volume-assist ventilation on respiratory timing. Although flow and volume assist resulted in similar increases in VT and Vt/TI, only during flow-assisted breaths was there a significant reduction in TI. Flow-assist ventilation occurs early during inspiration and has a decrementing pressure profile, whereas volume assist has an augmenting pressure profile and reaches a maximum level of assist at the end of the breath. Thus the timing of the respiratory assistance, rather than the type of assist (volume vs. flow assist), may have influenced intrabreath vagal feedback.

In the present study, we investigated the effect of the timing of flow assistance on respiratory activity during wakefulness. We hypothesized that if imperceptible flow assistance were administered during early inspiration, reflex shortening of TI and integrated inspiratory muscle pressure (JPmus) would occur. As in prior studies (3, 4), we applied assistance in a pseudorandom binary sequence of breaths at levels below the threshold of conscious perception. The respiratory response was modeled by using a linear system estimation procedure based on the prediction-error method (15). This technique requires an input (the pseudorandom binary sequence of assisted and unassisted breaths) and an output (the respiratory variables we measured) to develop a mathematical model of the respiratory system. The parameters of the mathematical model are selected to minimize the differences between the actual values of the variables measured and the predicted values of the same variables derived from model calculations. This technique can be used to model the first breath response to an impulse (the flow assist) by using data from every breath of the pseudorandom binary sequence of flow assist and permits, as a result, the quantification of small responses to stimuli that would not be evident by using other methods such as single-
Methods

Subjects. We recruited eight adult volunteers of either sex without prior or current respiratory disease. The consent form stated that the investigators would measure the mechanical characteristics of each subject’s respiratory system, assess each subject's ability to detect when a ventilator was helping the subject breathe, and measure each subject’s response to respiratory assistance from a mechanical ventilator. Neither the consent form nor the investigators revealed the objectives of the study to any of the subjects. None of the subjects had significant knowledge of respiratory psychophysics. The local Institutional Review Board approved the study.

Instrumentation and setup. Each subject was studied while sitting semirecumbent in a dental chair, wearing a nose clip, and breathing though a mouthpiece attached to a prototype ventilator (University of Manitoba, Winnipeg, Canada). Each subject listened to white noise through earphones so that he or she was not distracted or given clues about the activity of the ventilator. The dead space of the breathing circuit was ~0.1 liter. We recorded analog signals from the ventilator proportional to airway pressure and flow. We used software written in LabVIEW (National Instruments, Austin, TX) to calculate the instantaneous flow-assist level and to command the ventilator to give the appropriate assist pressure.

For analysis, airway pressure measured at the mouth (Pm) and inspiratory airflow were recorded directly from a calibrated analog output supplied by the ventilator, and flow was integrated with a zero-crossing reset to calculate Vt. End-tidal carbon dioxide (ETCO2) was measured at the mouth with a CAPSTAR-100 carbon dioxide analyzer (CWE, Ardmore, PA). During the estimation of respiratory mechanics, airflow at the mouth was occluded with a two-way shutoff valve (9340 series inflatable balloon controlled with an 8230 series automatic controller, Hans Rudolph, Kansas City, MO). Programs written with LabVIEW software controlled the ventilator and balloon valves and displayed and recorded the data.

Experimental protocols. Each experiment consisted of three parts, completed on 2 consecutive days. During the first day, we estimated the passive mechanical characteristics of the respiratory system of each subject [respiratory system resistance (Rrs) and respiratory system elastance (Ers)]. Next, we determined the threshold of detection of respiratory assistance in each subject when the assist was confined to early or late inspiration. Finally, we studied the response of each subject to a pseudorandom binary sequence of flow-assisted ventilation delivered early or late during inspiration. The timing of the assist was accomplished by averaging 20 control breaths before the pseudorandom binary sequence of assisted breaths to determine the average duration of Tt. Assist was applied for a time equal to 50% of the average Tt. For early assist (Eassist), flow was augmented starting at the onset of inspiration. For late assist (Lassist), flow was augmented from the point of the breath equal to 50% of the average Tt until the breath terminated spontaneously. On the second day, the threshold of detection for assist applied throughout inspiration (Tassist) was determined, and flow assist was applied throughout inspiration by using a pseudorandom binary sequence.

Estimation of respiratory mechanics. We used a modification of the interrupter method to estimate Ers and Rrs (7, 22). Each subject was ventilated by using controlled ventilation to achieve passive ventilation. Subjects were invariably hypocapnic during this period. Flow rate, Pm, and Vt were monitored continuously to ensure passive ventilation. The airway was occluded every five to eight breaths for 400–600 ms after delivery of 60–80% of a subject’s Vt. Oclusions were judged satisfactory when no respiratory effort was visible during the breath, when flow and pressure profiles were similar to previous breaths, and when the occlusion plateau was constant. Approximately 20 satisfactory oclusions were recorded from each subject. Ers was calculated from the difference between the plateau pressure and the end-expiratory pressure divided by the Vt of that breath. Rrs was calculated from the difference between Pm measured immediately before the occlusion and the subsequent plateau pressure divided by the flow immediately preceding the occlusion.

Determination of 50% threshold of sensation. We used a forced choice protocol to determine the threshold of perception when flow assist was delivered early, late, or throughout inspiration (13). The levels of flow assistance varied from imperceptible to well above the perceptual threshold. After each assisted breath, the subject was “forced” to decide whether the ventilator “helped” or “did not help” on that breath. The ventilator randomly applied five levels of assist, ranging from 20 to 70%, and three unassisted breaths followed each assisted breath. Each 20-breath sequence of assisted breaths was repeated 11 times in a randomized-block design. The first 20-breath sequence from each protocol was discarded. The analysis. The probability of detection of ventilator assistance (P) was calculated at each level of assistance by using logits [logit = ln[P/(1 − P)]]. We defined the perceptual threshold as the level of assistance that was detected 50% of the time. For each subject, logits were plotted as a function of percent assistance, and linear regression was used to fit the data. The x-axis crossing in this analysis is equal to the 50% threshold of sensation for Eassist, Lassist, or Tassist.

Pseudorandom binary sequence assist protocols. In each breathing trial, 267 sequential breaths were measured. This long sequence of breaths was the sum of three treatment sequences: a control set of 70 unassisted breaths, a test set of 127 breaths that were either assisted or unassisted, as determined by a pseudorandom binary sequence, and a final 70 unassisted control breaths. The control sequences of 70 unassisted breaths recorded before and after the pseudorandom binary sequence improve the estimation of the low-frequency response characteristics of the system that occur during the initiation and conclusion of the pseudorandom binary sequence of breaths. The pseudorandom binary sequence provides a broad-band perturbing input to the respiratory system that has the characteristics of an impulse (an example of a pseudorandom binary sequence and a discussion of the logic of this approach are provided in Fig. 1 of Ref. 2). The order of the Eassist and Lassist trials was randomized, and a 10-min rest period was allowed between trials. On the next day, Tassist trials were conducted.

Determination of first-breath response of respiratory variables. Respiratory variables [VT, Vt/Tt, Tt, expiratory time (Te)], instantaneous respiratory muscle pressure (Pmus) output, Pmus, inspiratory flow integrated per breath (f(flow), and Pm were calculated on-line by using programs written in LabVIEW and MATLAB (Math Works, S. Natick, MA). Continuous measurements of flow, Vt, and Pm and the values of Ers and Rrs determined previously in each subject were used to calculate inspiratory Pmus as follows

\[
P_{\text{mus}} = (E_{\text{rs}} \times V_t) + (R_{\text{rs}} \times \text{flow}) - P_{\text{m}}
\]
Some subjects had augmented breaths (sighs) that would have disproportionately affected the average breath size. Sighs were defined as breaths with a VT greater than the mean plus three times the standard deviation. If a sigh occurred before or after the pseudorandom binary sequence, data for the breath were omitted from analysis. If a sigh occurred during the pseudorandom binary sequence of breaths, individual values for that breath were replaced with mean values calculated from the pseudorandom binary sequence. Breaths were not removed from the pseudorandom binary sequence to avoid altering the randomness of the assisted breaths. Sighs tended to occur more frequently on unassisted breaths simply because there were more unassisted breaths. Within the pseudorandom binary sequence of breaths, the sighs were evenly distributed among assisted and unassisted breaths.

For each respiratory variable, we used a general system-identification technique known as the prediction-error minimization algorithm. Models of different order and with a binary sequence to avoid altering the randomness of the data for the breath were omitted from analysis. If a sigh occurred during the pseudorandom binary sequence, the optimal order of the model and the coefficients with the low difference between the model estimates and real response data). The coefficients producing the model with the lowest AIC value were used to define the impulse-response characteristics of the first through fifth breaths (15). All system identification procedures were analyzed on a computer using MATLAB and the MATLAB System Identification Toolbox (Math Works, S. Natick, MA).

Statistics. Data were expressed as means ± SD. A two-tailed one-sample t-test was used to determine whether the change in a respiratory variable (assisted breaths compared with control breaths) was significantly different from zero. Changes in respiratory variables as a result of flow assist applied at different times during inspiration and the change in ETCO2 among the control periods before and after the pseudorandom binary sequence were assessed with a one-way repeated-measures ANOVA. When the ANOVA indicated that significant differences existed among treatment conditions, paired comparisons were made by using t-tests with P values adjusted by the Bonferroni method. A P value of <0.05 was considered significant.

RESULTS

All eight subjects completed all of the protocols. Subject characteristics, Ers, Rs, 50% threshold of sensation for Eassist, Lassist, and Tassist are shown in Table 1. The threshold of detection of flow assist was significantly different among all treatments (Eassist, Lassist, and Tassist; P < 0.05 for each of the three comparisons). Subjects were least able to detect Lassist and best able to detect Tassist.

Response to respiratory system unloading with flow assist. The changes in inspiratory flow and VT from a single subject are shown in Fig. 1. During Eassist, flow was augmented only during the first half of inspiration, and, during Lassist, flow was augmented only during the second half of inspiration; however, during Tassist, flow was augmented throughout inspiration. In this subject, flow assist resulted in significant increases in peak inspiratory flow (relative to unassisted breaths) during all three assist protocols (Eassist, Lassist, and Tassist). Although Lassist and Tassist resulted in a small increase in VT, Eassist did not change VT significantly. The average changes in the inspiratory flow and VT waveforms for all subjects are shown in Fig. 2, and the average first-breath responses from all subjects are shown in Tables 2 and 3. There was a significant increase in Pm during all three types of assisted

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Ers, cmH2O/l</th>
<th>Rs, cmH2O·l−1·s−1</th>
<th>E50%</th>
<th>L50%</th>
<th>T50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>170</td>
<td>59</td>
<td>8.8</td>
<td>3.4</td>
<td>44.3</td>
<td>49.1</td>
<td>33.0</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>173</td>
<td>68</td>
<td>9.9</td>
<td>2.5</td>
<td>45.4</td>
<td>58.0</td>
<td>34.4</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>168</td>
<td>70</td>
<td>13.6</td>
<td>3.4</td>
<td>50.4</td>
<td>57.9</td>
<td>25.3</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>180</td>
<td>80</td>
<td>8.2</td>
<td>3.2</td>
<td>57.1</td>
<td>59.1</td>
<td>37.8</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>173</td>
<td>68</td>
<td>7.1</td>
<td>2.7</td>
<td>47.4</td>
<td>52.0</td>
<td>31.3</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>188</td>
<td>86</td>
<td>7.1</td>
<td>4.0</td>
<td>38.2</td>
<td>43.7</td>
<td>22.7</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>173</td>
<td>70</td>
<td>9.4</td>
<td>2.6</td>
<td>40.5</td>
<td>53.7</td>
<td>28.6</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>178</td>
<td>66</td>
<td>7.1</td>
<td>2.0</td>
<td>58.0</td>
<td>85.7</td>
<td>57.7</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>30 ± 5</td>
<td>175 ± 6</td>
<td>71 ± 8</td>
<td>8.9 ± 2.2</td>
<td>3.0 ± 0.6</td>
<td>47.7 ± 7.2a</td>
<td>57.4 ± 12.6a</td>
<td>33.9 ± 10.8a</td>
</tr>
</tbody>
</table>

Ers, estimated static respiratory elastance; Rs, estimated static respiratory resistance; E50%, threshold of sensation with flow assist applied during early inspiration; L50%, threshold of sensation with flow assist applied during late inspiration; T50%, threshold of sensation with flow assist applied throughout inspiration. *Significantly different from all other patterns of assist, P ≤ 0.05.
breaths ($P \leq 0.031$). Although peak inspiratory flow increased significantly regardless of the timing of the flow assist, there was no significant change in Vt during Eassist ($P = 0.895$). On the other hand, Lassist and Tassist significantly increased Vt ($P = 0.035$ and 0.001, respectively), but the size of the increase in Vt between Lassist and Tassist was not significantly different. A repeated-measures ANOVA and a multiple-comparison analysis indicated that the increase in Vt during Lassist and Tassist was significantly greater than during Eassist ($P < 0.05$). The Vt/Ti of the first-breath Ti response did not overlap zero. For control values, thick solid lines indicate average responses, and thin dashed lines indicate the 95% confidence intervals. For test values, the thick dashed lines indicate average responses, and the thin solid lines indicate the 95% confidence intervals. The 95% confidence intervals for Ti were omitted for clarity.

Fig. 1. Average flow (A) and tidal volume (VT; B) 95% confidence interval for 70 control breaths preceding the pseudorandom binary sequence (solid lines) and the 64 flow-assisted breaths (dashed lines) from subject 7. Late flow assist and flow assist throughout inspiration resulted in small increases in Vt. However, inspiratory time (Ti) decreased only during early assist when the 95% confidence interval of the first-breath Ti response did not overlap zero. For control values, thick solid lines indicate average responses, and thin dashed lines indicate the 95% confidence intervals. For test values, the thick dashed lines indicate average responses, and the thin solid lines indicate the 95% confidence intervals. The 95% confidence intervals for TI were omitted for clarity.

Fig. 2. Average flow (A) and VT (B) preceding the pseudorandom binary sequence (solid lines) and the 64 flow-assisted breaths (dashed lines) for all eight subjects. Flow was significantly increased by all assist conditions. Late flow assist and assist throughout Ti significantly increased VT. Early flow assist modified the timing of VT acquisition but did not change the final VT achieved.

A

Early Assist

Late Assist

Full Assist

B

Time (sec)

Time (sec)

Time (sec)

Flow (L/sec)

VT (L)

0.0

0.5

1.0

1.5

0.0

0.5

1.0

1.5

0.0

0.5

1.0

1.5

0.0

0.5

1.0

1.5
significant decrease in Tt during Eassist (P = 0.011) but no significant change during Lassist or Tassist. A repeated-measures ANOVA and a multiple-comparison analysis indicated that Eassist resulted in a significantly greater reduction in Tt than did Lassist and Tassist (P < 0.05). There were no significant changes in Tt during any type of flow assist.

Average changes in ETCO2 with Eassist, Lassist, and Tassist are shown in Fig. 3. Although ANOVA indicated that significant differences existed between conditions in the Eassist protocol (P = 0.048), specific comparisons between treatment conditions failed to detect any differences in ETCO2 values among any of the three phases of the Eassist protocol. There were no significant differences among ETCO2 levels in any of the treatment conditions during Lassist or Tassist.

**Fit of the linear model.** To determine whether the modeled responses were fully described with the linear model, a cross-correlation function was calculated between the model residuals and the input data for each of the six variables analyzed on each of the 3 experimental days for each of the eight subjects. Results from the cross-correlation analysis of these 144 impulse-response analyses indicated that 15 (10.4%) contained an attribute of the response that was not described fully by the linear approximation. There was no clear pattern to the distribution of trials in which nonlinear elements contributed to the response. Hence, these data do not change the findings of this study but do indicate a small, nonlinear component in the modeled responses in some of the variables for some of the subjects.

**DISCUSSION**

We reinvestigated the Hering-Breuer reflex in awake human subjects by using a pseudorandom binary sequence of imperceptible flow-assist ventilation applied early, late, or throughout inspiration. The respiratory responses to the pseudorandom binary sequence of flow assist (VT, VT/Tt, TE, fPmus, and Pm) were modeled with linear difference equations to construct the impulse responses of the respiratory system. The application of Eassist, Lassist, or Tassist resulted in a significant increase in VT during Tt. Although the largest increase in VT occurred when flow assist was applied late or throughout inspiration, Tt and/or fPmus were reduced only when flow-assist ventilation was applied during early inspiration. These data suggest that vagal feedback can modulate breathing on a breath-by-breath basis.

**Table 2. Summary of first-breath responses expressed as the difference between control and assisted breaths**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Eassist</th>
<th>Lassist</th>
<th>Tassist</th>
<th>Eassist</th>
<th>Lassist</th>
<th>Tassist</th>
<th>Eassist</th>
<th>Lassist</th>
<th>Tassist</th>
<th>Eassist</th>
<th>Lassist</th>
<th>Tassist</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.297</td>
<td>0.250</td>
<td>0.261</td>
<td>0.023</td>
<td>0.026</td>
<td>0.027</td>
<td>0.056</td>
<td>0.030</td>
<td>0.042</td>
<td>0.004</td>
<td>0.048</td>
<td>0.057</td>
</tr>
<tr>
<td>2</td>
<td>0.309</td>
<td>0.474</td>
<td>0.426</td>
<td>-0.072</td>
<td>0.005</td>
<td>0.062</td>
<td>-0.004</td>
<td>0.048</td>
<td>0.057</td>
<td>0.004</td>
<td>0.013</td>
<td>0.034</td>
</tr>
<tr>
<td>3</td>
<td>0.179</td>
<td>0.422</td>
<td>0.162</td>
<td>0.027</td>
<td>0.035</td>
<td>0.031</td>
<td>0.004</td>
<td>0.013</td>
<td>0.034</td>
<td>0.038</td>
<td>0.039</td>
<td>0.045</td>
</tr>
<tr>
<td>4</td>
<td>0.518</td>
<td>0.897</td>
<td>0.311</td>
<td>-0.035</td>
<td>0.145</td>
<td>0.033</td>
<td>0.063</td>
<td>0.098</td>
<td>0.047</td>
<td>0.038</td>
<td>0.039</td>
<td>0.045</td>
</tr>
<tr>
<td>5</td>
<td>0.202</td>
<td>0.275</td>
<td>0.173</td>
<td>0.011</td>
<td>0.059</td>
<td>0.026</td>
<td>0.086</td>
<td>0.255</td>
<td>0.112</td>
<td>0.038</td>
<td>0.039</td>
<td>0.045</td>
</tr>
<tr>
<td>6</td>
<td>0.491</td>
<td>2.378</td>
<td>0.640</td>
<td>0.022</td>
<td>0.200</td>
<td>0.065</td>
<td>0.086</td>
<td>0.255</td>
<td>0.112</td>
<td>0.038</td>
<td>0.039</td>
<td>0.045</td>
</tr>
<tr>
<td>7</td>
<td>0.264</td>
<td>0.430</td>
<td>0.238</td>
<td>0.008</td>
<td>0.056</td>
<td>0.060</td>
<td>0.047</td>
<td>0.066</td>
<td>0.058</td>
<td>0.043</td>
<td>0.058</td>
<td>0.052</td>
</tr>
<tr>
<td>8</td>
<td>0.276</td>
<td>0.312</td>
<td>0.376</td>
<td>0.003</td>
<td>0.035</td>
<td>0.080</td>
<td>0.054</td>
<td>0.058</td>
<td>0.052</td>
<td>0.054</td>
<td>0.058</td>
<td>0.052</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.317</td>
<td>0.680</td>
<td>0.323</td>
<td>0.002</td>
<td>0.053</td>
<td>0.048</td>
<td>0.043</td>
<td>0.076</td>
<td>0.056</td>
<td>0.054</td>
<td>0.058</td>
<td>0.052</td>
</tr>
<tr>
<td>P value</td>
<td>0.001</td>
<td>0.031</td>
<td>0.001</td>
<td>0.895</td>
<td>0.035</td>
<td>0.001</td>
<td>0.005</td>
<td>0.027</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For all variables, the values reported are the difference between the assisted and unassisted response. VT, tidal volume; VT/Tt, inspiratory air and/or VT, inspiratory air and/or VT/Tt, inspiratory time; TE, expiratory time. *Significantly different from zero, P = 0.05. ††Significantly different [i.e., † is different from †, and ‡ is different from ‡] (P = 0.05).

**Table 3. Summary of first-breath responses expressed as the difference between control and assisted breaths**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>fPmus, cmH2O s</th>
<th>Tt, s</th>
<th>TE, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.085</td>
<td>-0.015</td>
<td>-0.014</td>
</tr>
<tr>
<td>2</td>
<td>-0.653</td>
<td>0.084</td>
<td>-0.059</td>
</tr>
<tr>
<td>3</td>
<td>-0.124</td>
<td>0.224</td>
<td>0.007</td>
</tr>
<tr>
<td>4</td>
<td>-1.101</td>
<td>-0.168</td>
<td>-0.193</td>
</tr>
<tr>
<td>5</td>
<td>-0.150</td>
<td>0.004</td>
<td>-0.078</td>
</tr>
<tr>
<td>6</td>
<td>-0.501</td>
<td>-0.293</td>
<td>-0.049</td>
</tr>
<tr>
<td>7</td>
<td>-0.340</td>
<td>0.149</td>
<td>-0.050</td>
</tr>
<tr>
<td>8</td>
<td>-0.091</td>
<td>0.309</td>
<td>-0.092</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-0.383</td>
<td>0.200</td>
<td>-0.070</td>
</tr>
<tr>
<td>P value</td>
<td>0.020</td>
<td>0.618</td>
<td>0.011</td>
</tr>
</tbody>
</table>

For all variables, the values reported are the difference between the assisted and unassisted response. fPmus, calculated inspiratory muscle pressure integrated per breath; Tt, inspiratory time; TE, expiratory time. *Significantly different from zero, P = 0.05. ††Significantly different [i.e., † is different from †, and ‡ is different from ‡] (P = 0.05).
It is our hypothesis that the reduction was mediated by flow assist. The results of the present study indicate that a pseudorandom binary sequence of imperceptible flow \( T_{assist} \) reduced \( \Delta P_{mus} \), whereas a similar application of volume assist did not change respiratory timing (4). In the present study, we demonstrated that flow assist is a more potent inhibitory stimulus in early inspiration compared with late inspiration. The application of flow assist throughout inspiration did not duplicate our previous findings (3) in that \( T_i \) was not significantly reduced by the \( T_{assist} \) protocol in the present study.

Comparison with previous studies. We have previously shown that passive overinflation of the lungs with proportional-assist ventilation resulted in a small but significant reduction in \( T_i \) (3); however, the relative contributions from flow or volume assist could not be separated. We subsequently found that a pseudorandom binary sequence of imperceptible flow \( T_{assist} \) reduced \( \Delta P_{mus} \), whereas a similar application of volume assist did not change respiratory timing (4). In the present study, we demonstrated that flow assist is a more potent inhibitory stimulus in early inspiration compared with late inspiration. The application of flow assist throughout inspiration did not duplicate our previous findings (3) in that \( T_i \) was not significantly reduced by the \( T_{assist} \) protocol in the present study. In our original study, we applied combined volume and flow assist throughout \( T_i \), and the total \( P_m \) was almost twice that present during the flow-only \( T_{assist} \) protocol in the present study. Despite the markedly reduced level of total assist, \( T_i \) actually diminished in seven of eight subjects, although the magnitude of the change was small and not statistically significant. Thus we feel that the failure to demonstrate a reduction in \( T_i \) during \( T_{assist} \) with flow assist alone probably reflects the lower level of assist both early (since \( E_{assist} \) did decrease \( T_i \)) and over the entire breath (since combined assist of greater magnitude shortened \( T_i \) in our previous study).

Much larger steady-state increases in inspiratory flow in mechanically ventilated subjects also shorten \( T_i \) (6, 16, 20). The results of the present study indicate that more subtle changes in inspiratory flow may also modulate the timing of inspiratory efforts on a breath-by-breath basis if the assist is given early during inspiration.

We were surprised that \( L_{assist} \) did not shorten \( T_i \) given that the stimulus (flow assist) was greatest in this condition. As noted above, the high threshold of detection of \( L_{assist} \) may indicate that the respiratory system does not receive or does not attend to respiratory afferent information as effectively late in \( T_i \). The volume threshold of inspiratory termination in anesthetized animals decreases steeply as inspiration progresses (5). Hence, large changes in volume are necessary to terminate inspiration until very late in \( T_i \). Furthermore, there is not much \( T_i \) left to modify when the stimulus is delivered late in inspiration. It may be

### Percepción de asistencia de flujo.

La percepción de la asistencia de flujo fue la más baja durante \( T_{assist} \), y la mayor contribución de asistencia subliminar se aplicó durante \( L_{assist} \). Sin embargo, no hubo diferencias significativas entre los promedios \( P_m \) durante cada protocolo de asistencia, aunque mayores presiones fueron a menudo aplicadas durante \( L_{assist} \). Estos datos sugieren que la percepción de \( P_m \) varía durante la inspiración. El estímulo asociado con la asistencia de flujo se refleja en \( P_m \), y parece que los sujetos eran más sensibles a \( P_m \) durante la inspiración temprana que durante la inspiración tardía. To the extent that perception of assist is based on the same stimuli that mediate respiratory reflexes, this finding suggests that there is some gating of sensory information in which the respiratory system attends to or is more sensitive to afferent information early during the inspiratory cycle. It is also possible that sensory receptors may be more sensitive in the lower pressure range, but we know of no studies of pulmonary receptors demonstrating differential sensitivity in the range of pressures we applied.

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**Fig. 3.** Average end-tidal CO₂ (±SD) during pseudorandom binary-sequence protocols (PRBS). Although a repeated-measures ANOVA indicated that significant differences existed among end-tidal CO₂ levels during early assist (\( P = 0.048 \)), there were no significant differences identified by using a multiple-comparison analysis in which each condition was compared with all others. There were no significant differences between end-tidal CO₂ levels during late or total inspiratory assist.

**Interpretación de flujo.** La percepción de la asistencia de flujo fue la más baja durante \( T_{assist} \), y la mayor contribución de asistencia subliminar se aplicó durante \( L_{assist} \). Sin embargo, no hubo diferencias significativas entre los promedios \( P_m \) durante cada protocolo de asistencia, aunque mayores presiones fueron a menudo aplicadas durante \( L_{assist} \). Estos datos sugieren que la percepción de \( P_m \) varía durante la inspiración. El estímulo asociado con la asistencia de flujo se refleja en \( P_m \), y parece que los sujetos eran más sensibles a \( P_m \) durante la inspiración temprana que durante la inspiración tardía. To the extent that perception of assist is based on the same stimuli that mediate respiratory reflexes, this finding suggests that there is some gating of sensory information in which the respiratory system attends to or is more sensitive to afferent information early during the inspiratory cycle. It is also possible that sensory receptors may be more sensitive in the lower pressure range, but we know of no studies of pulmonary receptors demonstrating differential sensitivity in the range of pressures we applied.

**Comparación con estudios previos.** Hemos previamente mostrado que la pasiva overinflación de los pulmones con asistencia de flujo-proportional resultó en un pequeño pero significativo reducción en \( T_i \) (3); sin embargo, las contribuciones relativas de flujo o asistencia de volumen no fueron separadas. Posteriormente, encontramos que una secuencia pseudorandom de impulso imperceptible de flujo \( T_{assist} \) redujo \( \Delta P_{mus} \), mientras que un aplicado similar de asistencia de volumen no cambió el tiempo respiratorio (4). En el estudio actual, demostramos que la asistencia de flujo es un estímulo inhibidor más potente en la inspiración temprana que con la inspiración tardía. El aplicado de asistencia de flujo durante todo el \( T_i \), resultó en un \( P_m \) total casi dos veces que presentó durante el protocolo de asistencia de flujo en el estudio original. Aunque el reducido nivel de asistencia total, \( T_i \) se disminuyeron en siete de los siete sujetos, aunque la magnitud del cambio fue pequeña y no significativa estadísticamente. Así, sentimos que la incapacidad para demostrar una reducción en \( T_i \) durante el \( T_{assist} \) con asistencia de flujo solo probablemente refleja el menor nivel de asistencia tanto temprano como durante toda la inspiración (ya que combinado asistencia de mayor magnitud redujo \( T_i \) en nuestro estudio anterior).

Muchas mayores aumentos estacionarios en flujo inspiratorio en sujetos ventilados mecánicamente también redujeron \( T_i \) (6, 16, 20). Los resultados de este estudio indican que los cambios más sutiles en el flujo inspiratorio pueden también modulación el tiempo de esfuerzos inspiratorios en un grado de inspiración-breath basis si la asistencia es dada temprano durante la inspiración.

Nos sorprendió que \( L_{assist} \) no disminuyeron \( T_i \) dado que el estímulo (asistencia de flujo) fue más grande en este condición. Como se mencionó anteriormente, el alto umbral de detección de \( L_{assist} \) puede indicar que el sistema respiratorio no recibe o no presta atención a la información aferente respiratoria de manera efectiva tardía en \( T_i \). El umbral de volumen de terminación inspiratoria en animales anestesiados disminuye agudamente a medida que la inspiración progresa (5). Por lo tanto, grandes cambios en volumen son necesarios para terminar la inspiración hasta muy tarde en \( T_i \). Además, no quedaba mucho \( T_i \) para modificar cuando el estímulo es entregado tarde en la inspiración. Podría ser
that the variability of the respiratory pattern in our awake subjects prevented detection of a $L_{\text{assist}}$ effect. However, these explanations are not entirely satisfying since we have detected quite subtle changes in $T_I$ in previous studies.

**Reflex modulation of $T_I$ and $P_{\text{mus}}$.** The reduction in $T_I$ is probably reflex in origin, and the afferent information is probably carried by the vagus nerve. Previous studies of steady-state increases in inspiratory flow demonstrated a decrease in $T_I$ (6) and an increase in respiratory rate during mechanical ventilation that was not affected by breathing route (oral or nasal) or upper airway anesthesia. This suggests that at least some of the receptors mediating the flow-related responses are located in the lungs or chest wall (9). There are chest wall reflexes that modify $T_I$ (21), but $T_I$ is shortened by distorting the chest wall and increasing the load on the chest wall. In the present study and in previous studies of steady-state changes in inspiratory flow, the effect of increased flow was to unload the system, which one would not expect to shorten $T_I$ on the basis of the chest wall reflex described above. Thus we conclude that the reflex shortening of $T_I$ and reduction in $P_{\text{mus}}$ are mediated by vagal mechanisms.

Among vagally mediated reflexes, there are two prime candidates: a phrenic augmenting reflex associated with increased inspiratory flow (18) and the Hering-Breuer reflex. Pack et al. (18) described phrenic augmentation and shortening of $T_I$ in anesthetized dogs when the $V\tau/T_I$ was increased. The response was abolished by vagotomy. Augmentation of phrenic activity was most apparent at flow rates two to three times the basal $V\tau/T_I$. We believe that this phrenic augmentation is not likely to contribute to the responses we observed. First, the low flow rates we studied are only slightly above the baseline flow chosen by each subject. Second, the temporal profile of $P_{\text{mus}}$, which reflects in part the drive to the diaphragm, was reduced (not augmented) at a time when inspiration ceased. Results similar to those we report were obtained when steady-state unloading was studied and inspiratory flow was increased; $T_I$ decreased, but the profile of calculated $P_{\text{mus}}$ and measured transdiaphragmatic pressure were not augmented (10). On the other hand, augmenting inspiratory flow by approximately twofold during assist-control mechanical ventilation in normal subjects decreased duration of respiratory cycle, and the rate of inspiratory pressure generation necessary to trigger the ventilator, which reflects the pattern of activation of respiratory muscles, increased as the $V\tau/T_I$ increased (8). This response is consistent with the phrenic augmentation seen in anesthetized dogs. However, the effect of increased inspiratory flow on duration of respiratory cycle was present during sleep, but rate of inspiratory pressure generation was not changed by increasing inspiratory flow during sleep. We conclude that the most consistent effect of increased inspiratory flow is reflex shortening of $T_I$, and phrenic augmentation is not a necessary component of this response. Therefore, the phrenic augmentation and reflex shortening of $T_I$ described by Pack et al. (18) probably do not contribute to the reflex modulation of $T_I$ and $P_{\text{mus}}$ that we observed.

The Hering-Breuer reflex is usually described in terms of feedback effects of lung volume on inspiratory or expiratory duration, as if the reflex operated only as an “on” or “off” switch at certain volume thresholds. However, vagal feedback consistent with the Hering-Breuer reflex inhibits respiratory nerve activity within each breath (1, 14). The inhibition of respiratory nerve activity within a breath is greater in nerves innervating the upper airway (the hypoglossal and laryngeal nerves) than in the phrenic nerve, but even in the phrenic nerve vagal feedback may reduce phrenic activity by as much as 10% (14). Vagal feedback also reduces peak end-inspiratory activity of the phrenic nerve by terminating inspiration earlier. The shortening of $T_I$ and reduction in $P_{\text{mus}}$ during $E_{\text{assist}}$ are consistent with these effects of the Hering-Breuer reflex but inconsistent with previous work, suggesting that lung volume feedback terminated inspiration prematurely by earlier attainment of a greater volume threshold (5). There was no increase in $V_T$ at the end of inspiration in the $E_{\text{assist}}$ protocol and, therefore, no possibility of earlier attainment of a greater end-inspiratory volume. Fernandez et al. (6) have pointed out that it may be more appropriate to view the Hering-Breuer reflex as a response to flow-sensitive receptors since previous experiments that augmented volume to shorten $T_I$ necessarily increased inspiratory flow (5). Our findings, which used single-breath unloading to increase inspiratory flow early in inspiration, are consistent with the results of Fernandez et al. using step changes in inspiratory flow, and we agree with their interpretation: Flow-related inhibition of $T_I$ and $P_{\text{mus}}$ is probably a manifestation of the Hering-Breuer reflex.

$E_{\text{assist}}$ shortened $T_I$ and reduced $P_{\text{mus}}$ more effectively than $L_{\text{assist}}$ did. This may reflect greater sensitivity to flow-related information early in the inspiratory cycle, and the greater threshold of detection of assist late during inspiration points toward this conclusion. However, there simply is not much $T_I$ left to modify during $L_{\text{assist}}$, and we cannot conclude for certain that the different effects of $E_{\text{assist}}$ and $L_{\text{assist}}$ derive from a temporal variation in the sensitivity to flow within $T_I$. Moreover, it would be peculiar to develop a negative-feedback system with decreasing sensitivity to the stimulus as the threshold was approached. We think it is more likely that the $E_{\text{assist}}$ acts as a conditioning subthreshold stimulus that actually reduces the threshold for termination of $T_I$. This phenomenon was investigated by Younes and Polachek (23), who found that subthreshold electrical stimulation of the vagus nerve early in $T_I$ reduced the level of vagal stimulation necessary to terminate inspiration later in $T_I$. Thus $E_{\text{assist}}$ may provide similar subthreshold, vagally mediated feedback that “sensitizes” the system to volume- or flow-related feedback later during inspiration. If this is correct, the lower threshold of detection of flow assist early in $T_I$ may indicate that a conditioning stimulus early in $T_I$ also enhances the sensory

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**References:**


information or cortical processing of sensory information used by subjects to detect the flow assist. Younes and Polachek (23) also studied the effect of vagal stimulation within a breath on subsequent breaths. They described late “paradoxical” responses in subsequent breaths. The responses were paradoxical in that shortening Ti by electrical stimulation of the vagus nerve prolonged subsequent inspiratory durations, although the paradoxical effect waned over 6–8 s after the stimulus. We observed no effect of flow assist on Ti or Te of subsequent breaths in this study, although we did see a trend toward paradoxical responses in Ti in a previous study (3). Detecting the effects of vagal stimulation on subsequent breaths requires a stable respiratory drive (the cats used by Younes and Polachek were anesthetized, ventilated, and chemoreceptor and baroreceptor denervated to reduce nonvagal inputs to respiratory drive). In intact humans, the moment-to-moment variation in respiratory drive is complex, and this may reduce our ability to identify the delayed effects of vagal feedback consistently, although they may be apparent in some subjects (3).

In summary, the Hering-Breuer reflex has been demonstrated in anesthetized or sleeping humans by using either airway occlusions or large step changes in inspired volume. These methods cannot be applied to humans during wakefulness without the activation of cortical and/or humoral responses. Therefore, we chose a technique that would allow the application of a stimulus with a magnitude small enough not to elicit cortical or humoral responses, but strong enough to evoke a reflex response. The application of an imperceptible pseudorandom binary sequence of mechanical flow-assist ventilation resulted in a significant increase in inspiratory flow, regardless of the timing of the application; however, only during Eassit was there a small, but significant, reduction in Ti and Pmus. These findings are consistent with previous studies of vagal control of inspiratory duration and respiratory motor output in anesthetized or decerebrate animals. Thus the Hering-Breuer reflex appears to be active in conscious humans, and vagal feedback modulates breathing on a breath-by-breath basis.

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