Single-Breath, Room-Air Method for Measuring Closing Volume (Phase 4) in the Normal Human Lung*

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The purpose of this study was to evaluate a new method to measure closing volume (CV). This new method does not require oxygen or inert gases to be inhaled to obtain the onset of phase 4. Because there are regional differences in the concentrations of the resident alveolar gases (O₂, CO₂, and N₂), there should be an abrupt change in the concentration of these gases at the terminal portion of a prolonged expired vital capacity (VC) that marks the onset of phase 4. Nine normal healthy subjects, 30 to 65 years of age, inspired room air from residual volume (to mimic the maneuver of the standard single breath N₂ [SN₂] washout test) to total lung capacity. During the expiration (flow constant at 250 ml.s⁻¹) following a 10-s breath hold at total lung capacity, the exhaled gas was analyzed with a mass spectrometer for fractions of O₂, CO₂, and N₂. Although the onset of phase 4 can be shown as the change in concentration of any of the three alveolar resident gases, oxygen was selected because (1) it demonstrates a greater apex to base concentration gradient than that found with CO₂ and N₂, and (2) a clear identification of the onset of phase 4 (minimum value of O₂ fraction). With this method, the mean ± SEM of CV was 16.8 ± 1.52 percent (CV × 100/VC). No significant difference was found among the room air method, SN₂ method, and the helium bolus technique. (Chest 1992; 102:435-43)

CV = closing volume; FCO₂ = fraction of CO₂; FN₂ = fraction of N₂; FO₂ = fraction of O₂; He = helium; IC = inspiratory capacity; RV = residual volume; SN₂ = single breath N₂ washout test; TLC = total lung capacity; V/Q = ventilation-perfusion ratio

D istribution of ventilation in normal human lungs is uneven. The description by Milic-Emili et al.'s of the regional distribution of inspired gas in the lung is one model of this phenomenon. Their model explains the earlier observation by Fowler and associates of an increase in the expired nitrogen concentration in the terminal portion of the expired volume of the vital capacity (VC). Dollfuss et al. labeled this abrupt increase in gas concentration as phase 4. Because it was postulated that this abrupt increase was due to a progressive closure of the airways, Holland and associates coined the term "closing volume" (CV) for the corresponding expired volume beginning at the onset of phase 4 and ending at residual volume (RV). Therefore, the detection of phase 4 is a requirement for the measurement of CV. In order to obtain phase 4, a gas concentration gradient must exist between the dependent and nondependent regions of the lung. In the upright lung, these regions correspond to the basal and apical regions, respectively.

The standard method to measure CV is the single-breath nitrogen washout test (SN₂). However, because of the importance of a reliable measurement of CV, investigators have introduced other methods to better identify the onset of phase 4, such as inhalation of inert gases. The helium⁶,⁷ and argon⁸ bolus techniques are the two most popular tests, but xenon-¹³³⁵ and other inert gases have also been applied. The SN₂ test requires an inspiration of 100 percent O₂ from RV to total lung capacity (TLC).⁹ The inert gas bolus techniques require an inhalation of a bolus of the representative tracer gas at RV, followed by inspiration of room air to TLC. The use of a method for measuring CV that would not require breathing 100 percent O₂ or an inert gas would provide an alternative procedure.

According to the lung model of West,¹⁰ there is a 43 mm Hg difference in the PO₂ between the first and ninth region (apex to base) of the upright lung. The corresponding differences for FCO₂ and PN₂ amount to only 14 and 29 mm Hg, respectively. We reasoned that the phenomena of phase 4 (a sudden change in the slope of the expire gas during the latter part of phase 3) could be demonstrated when the dependent area of the lungs no longer contributes to the expire during a prolonged expiration to RV. This room air CV method was compared with the SN₂ and helium bolus methods.

MATERIAL AND METHODS

Nine normal, healthy male volunteers, 30 to 65 years of age, whose general physical characteristics and vital capacity (VC) values are given in Table 1, voluntarily consented to participate in this study. The protocol for this research was reviewed and approved by
Table 1—Physical Characteristics of the Experimental Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Vital Capacity, L</th>
</tr>
</thead>
<tbody>
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<td>1</td>
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<td>178</td>
<td>75</td>
<td>5.01</td>
</tr>
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<td>2</td>
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<tr>
<td>4</td>
<td>44</td>
<td>170</td>
<td>82</td>
<td>4.45</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>170</td>
<td>76</td>
<td>4.46</td>
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<tr>
<td>6</td>
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<td>175</td>
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<td>5.01</td>
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<tr>
<td>7</td>
<td>55</td>
<td>188</td>
<td>75</td>
<td>5.76</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>183</td>
<td>86</td>
<td>5.37</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>176</td>
<td>66</td>
<td>4.43</td>
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<tr>
<td>Mean*</td>
<td>43</td>
<td>176</td>
<td>77</td>
<td>4.65</td>
</tr>
<tr>
<td>SEM</td>
<td>± 3.8</td>
<td>± 1.9</td>
<td>± 2.4</td>
<td>± 0.24</td>
</tr>
</tbody>
</table>

*Mean of the nine subjects.

the Institutional Review Board of the Medical College of Ohio, and the research was conducted in accordance with the Declaration of Helsinki.

Subjects were asked to perform a series of slow VC maneuvers (see below). Expired gases were analyzed with a mass spectrometer (Perkin Elmer, Medical Gas Analyzer 1100, Pomona, Calif). The mass spectrometer outputs were corrected for time delay of 0.2 s between expired gas flows and the gas concentration outputs. Expired gas was sampled at the lips through a needle located in the mouthpiece 1 cm from the subject's incisors. Both inspiratory and expiratory gas flows were measured with a pneumotachograph (Fleisch, Rockford, Ill) that was inserted between a four-way valve and the mouthpiece. Gas volume was obtained by integration. A 9-L respirometer (Collins, Braintree, Mass) was also connected to the four-way valve and was used as a reservoir for the foreign gases, 100 percent helium (bolus technique), or 100 percent O2 (SBN1 test). The voltage output from the mass spectrometer and the pneumotachograph were sampled at 50 Hz by an analog-to-digital converter. The gas concentrations (O2, CO2, N21, and helium) and the flow signals were sampled and processed on line by an eight-channel acquisition system (MacLab, New Haven, Conn) and a computer (Apple Macintosh IIx, Cupertino, Calif).

Room-Air Method for Measuring CV

Subjects initially performed four or five normal tidal breaths with room air. Subsequently they expired to RV and in the following breath inspired room air to TLC. The breath was held for 10 s and then exhaled to RV at approximately 250 ml·s⁻¹. The expired flow rate was kept constant with the aid of a visual flowmeter. Fractional concentrations of exhaled CO2, O2, and N2, and the expired gas flow were recorded. The lowest value of fraction of O2 (F(O2)) was taken as the landmark delimiting the end of phase 3 and beginning of phase 4. The onset of phase 4 was then used to measure the closing volume.

SBN1 and the Helium Bolus Tests for Measuring CV

After four or five normal breaths of room air, the same subjects studied above expired to RV. At the end of this expiration, the subject was connected to the spirometer via a mouthpiece and the four-way valve. Each subject then either inspired 100 percent O2 to TLC or a bolus of 100 percent helium 250 ml of pure helium at the beginning of the breath, i.e., from RV followed by inhalation of room air to TLC. In both experiments, the exhalation proceeded at a rate of approximately 250 ml·s⁻¹ from TLC to RV. For the SBN2 method, CV was measured following the recommendations of the National Heart, Lung, and Blood Institute guidelines. In brief, a best fit line was drawn through phase 3 by visual inspection, then the first convincing departure from this line was taken as the indicator of the onset of phase 4. A similar technique was applied to the helium bolus method.

Closing volume was measured as the expired volume beginning at the onset of phase 4 and ending at RV. A minimum of three tracings were obtained for each subject and for each method. In all three methods, 10 min were allowed between trials to wash out the

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21652/ on 06/27/2017)
inhalable gases. All tests were performed in random order. Three photocopies were made of each expired curve and distributed to three different readers.

This study employed a within-subject design in order to examine the equivalence of three methods for determining CV. Multivariate analysis of variance (using Proc GLM of the SAS statistic package) was used to test for differences in means related to observer and test type. Significance level for statistical tests was set at 0.05.

RESULTS

The expired gas concentrations corresponding to each of the three methods—room air, SBN₂ and helium—in a representative subject, have been compiled and are shown in Figure 1. As expected, the gas concentrations in phase 1 were identical to those in the inspired gases. A sudden change in the fractional concentrations marked the beginning of phase 2. In this phase, all three gas fractions (F₀₂, FN₂, and FHe) produced sigmoidal-shaped curves. While in the SBN₂ washout test and helium bolus technique there is an increase in the fractional gas concentrations (FN₂ and FHe, respectively), the opposite was observed with the Fo₂ (room air method), a decrease in the fractional concentration. All gases show the alveolar gas plateau, phase 3. The slope of this phase for FN₂ and for FHe increased slightly, but the slope for Fo₂ decreased significantly. Phase 4, marked by a change in the slope of phase 3, was observed with all the three gases. In many cases only a visual inspection, without the drawing of the best fit line, was required by the observer to determine the phase 3-4 intercept. However, because the slopes of phases 3 and 4 were positive, in both the N₂ and helium methods, the drawing of the best fit line became essential especially when the slopes of the phases 3 and 4 are only separated by a few units. Because the O₂ concentration fell during phase 3 and then rose, changing the sign of the slope created a minimum gas fraction value, which allowed the onset of phase 4 to be determined more easily and without the above problem.

The CV mean ± SD of each subject read by three different observers for all three methods are presented in Table 2. No significant differences were found among methods, observers, or interaction between observer and method (Table 3).

DISCUSSION

As early as the turn of the century, investigators have suggested that the inhalation of atmospheric air could not be considered feasible to elucidate questions concerning the mixing of inspired gases with the resident alveolar gases. Therefore, bolus of inert gases, such as hydrogen, helium, and argon, have been used to study the distribution of inspired gases. Contrary to accepted assumptions, our study, using room air, demonstrates that resident alveolar gases can be used to study the distribution of inspired gases. Due to the apex to base difference in the concentrations of the resident alveolar gases, we reasoned that the phenomena of phase 4 could be demonstrated. Flores demonstrated that during room-air breathing, the onset of phase 4 can be identified during a prolonged expiration to RV with all resident gases. The experiments reported in the present study show no significant difference in the onset of phase 4 by the inhalation of room air, oxygen, or helium.

The rise in O₂ and the fall in CO₂ at the end of a prolonged expiration, now known as phase 4, were observed more than 50 years ago. More recently, these paradoxic findings have been documented by others, although the latter authors did not use these observations to directly quantitate CV. In reviewing those concepts in pulmonary physiology dealing with the onset of phase 4, a rational approach was designed to employ alveolar gases (O₂, CO₂ and N₂) as tracers to measure CV. The following lung model helps to clarify the rationale employed.

Model Used for the Room-Air Method

The present model is constructed by a combination of three models found in the literature. This model takes into account the regional O₂ and CO₂ gas exchange in the lung at steady state conditions. Alveolar N₂ concentration is set by the other two gases, thus: FN₂ = 1 - (FAO₂ + FCOCO₂). The alveolar gas concentration values for O₂ and CO₂ at steady-state conditions are determined from a balance between

<table>
<thead>
<tr>
<th>Subjects</th>
<th>He</th>
<th>SBN₂</th>
<th>Room Air</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.8 ± 0.65</td>
<td>12.8 ± 0.80</td>
<td>16.5 ± 0.70</td>
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<tr>
<td>2</td>
<td>13.9 ± 0.11</td>
<td>17.2 ± 0.74</td>
<td>16.4 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>29.7 ± 4.00</td>
<td>29.2 ± 2.08</td>
<td>18.2 ± 0.92</td>
</tr>
<tr>
<td>4</td>
<td>20.6 ± 0.77</td>
<td>18.5 ± 3.02</td>
<td>19.0 ± 1.71</td>
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<tr>
<td>5</td>
<td>19.2 ± 0.58</td>
<td>19.2 ± 1.54</td>
<td>13.2 ± 1.02</td>
</tr>
<tr>
<td>6</td>
<td>13.6 ± 0.02</td>
<td>17.8 ± 2.08</td>
<td>13.2 ± 0.00</td>
</tr>
<tr>
<td>7</td>
<td>25.7 ± 2.11</td>
<td>25.0 ± 0.53</td>
<td>27.2 ± 1.44</td>
</tr>
<tr>
<td>8</td>
<td>15.4 ± 0.59</td>
<td>15.8 ± 0.65</td>
<td>14.4 ± 0.00</td>
</tr>
<tr>
<td>9</td>
<td>12.0 ± 1.60</td>
<td>13.3 ± 1.58</td>
<td>12.4 ± 0.00</td>
</tr>
<tr>
<td>Mean † ± SEM</td>
<td>18.3 ± 2.02</td>
<td>18.7 ± 1.78</td>
<td>16.8 ± 1.52</td>
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</tbody>
</table>

*Values represent the mean ± SD of three observers.
†Mean of the nine subjects.

<table>
<thead>
<tr>
<th>Source</th>
<th>Value</th>
<th>df*</th>
<th>F</th>
<th>p</th>
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<tr>
<td>Test observer</td>
<td>1.133879</td>
<td>4.5</td>
<td>1.4173</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*df = degrees of freedom.
alveolar ventilation and pulmonary perfusion (VA/Q). The alveolar $\text{Po}_2$ and $\text{PCO}_2$ at the apex and base of the lung, obtained from the West lung model, are shown as gas fractions in Table 4 (resting conditions). These values were altered first, by the gas inspired, and second, by $\text{O}_2$ and $\text{CO}_2$ gas exchange. Room air inspiration was simulated from RV and FRC, and gas exchange 70 s was allowed for the RV maneuver only. Thus, it is demonstrated that the magnitude of these changes will depend on the preinspiratory volume, ie, RV or FRC, and the time of gas exchange. These calculations are described in the appendix.

**Room-Air Method vs Other Methods**

The maneuver of exhaling first to RV and then inhaling room air to TLC was carried out to pattern or mimic the same maneuvering that is performed with the SBN$_2$ washout test and helium bolus technique. Therefore, comparable maneuvers were analyzed.

Because expired alveolar N$_2$ concentration changes very little during normal expiration, a volume of $\text{O}_2$ corresponding to a VC is inhaled to dilute the resident alveolar N$_2$ and magnify the apex to base concentration differences. Using the model described above, the calculated apex to base N$_2$ concentration gradient after a VC inspiration of oxygen (SBN$_2$ test) has been calculated (Table 5). Unfortunately, with the SBN$_2$ washout test not all subjects show a clear onset to phase 4, probably due to variations in the magnitude of the apex to base N$_2$ gradient. Consequently, investigators have developed other methods to identify the onset of phase 4. Thus, the inert gas technique was derived. The SBN$_2$ washout test and the helium bolus technique are now the two most practiced methods. Herein, we are demonstrating an alternative method that does not require the inhalation of 100 percent $\text{O}_2$ or an inert gas. We evaluated this new method for measuring CV by comparing it with the standard SBN$_2$ washout test and the helium bolus technique. The multivariate analysis of variance showed no significant difference among the three methods (Table 3). Due to large variations in the size of lung volumes, the amount of inhaled tracer gas from residual volume will produce a small or large concentration difference between the apex and the base of the lungs. From the work of Laviolette and Cormier and our modeling of their experiments (unpublished), the magnitude of the slope for phase 4 is directly dependent on the amount of trace gas inhaled. A lesser rise of phase 4 (slope) in N$_2$ when the SBN$_2$ test is used as compared with a steeper and better resolution of the intersection point between phases 3 and 4 using the bolus technique has been predicted by estimating the changes in the relative concentrations of regional alveolar gases to the predicted alveolar concentrations of phase 3. This difference is evident in Figure 1. However, the estimates of CVs are not different among methods (Table 2). This is also true in the model of Kaneko et al (see their Fig 5). Other investigators also failed to show a

**Table 4 — Theoretical Alveolar Gas Fractions at the Apex and Base of the Lung at Rest and after Respiratory Maneuvers* **

<table>
<thead>
<tr>
<th>Region</th>
<th>Rest</th>
<th>From RV†</th>
<th>From FRC‡</th>
<th>Breath Hold, 70 s</th>
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<tbody>
<tr>
<td></td>
<td>$\text{O}_2$</td>
<td>$\text{CO}_2$</td>
<td>$\text{O}_2$</td>
<td>$\text{CO}_2$</td>
</tr>
<tr>
<td>Apex</td>
<td>0.185</td>
<td>0.039</td>
<td>0.201</td>
<td>0.013</td>
</tr>
<tr>
<td>Base</td>
<td>0.125</td>
<td>0.059</td>
<td>0.202</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Assumptions: TLC = 7.5 L; FRC = 3.2 L; RV = 1.4 L; $V_d = 0.208$ L.
†Simulated VC inspiration of 6.1 L of room air.
‡Simulated IC inspiration of 4.3 L of room air.

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**Table 5 — Estimated Apex to Base Gas Concentration Difference for the SBN$_2$, Test, Helium (He) Bolus Technique, and Room-Air Method* **

<table>
<thead>
<tr>
<th>Test</th>
<th>Gas</th>
<th>Apex, %</th>
<th>Base, %</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBN$_2$</td>
<td>N$_2$</td>
<td>25.5</td>
<td>6.6</td>
<td>18.9</td>
</tr>
<tr>
<td>He†</td>
<td>He</td>
<td>2.9</td>
<td>2.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Air‡</td>
<td>$\text{O}_2$</td>
<td>18.9</td>
<td>12.5</td>
<td>6.4</td>
</tr>
</tbody>
</table>

*Assumptions for lung volumes and capacities are the same as Table 4.
†Helium inhaled from RV = 200 mL.
‡Gas exchange of 70 s is considered (see Table 4).
difference in CV determinations between the helium bolus technique and the SBN washout test.\textsuperscript{6,7} The variability observed among subjects is in agreement with observations by others.\textsuperscript{22}

In conclusion, the onset of phase 4 can be easily identified by using the new room-air method while exhibiting no significant differences between the two most common methods (SBN\textsubscript{2} and helium bolus). Furthermore, the room-air method appears to be the simplest of all three modalities. First, no foreign gas (100 percent O\textsubscript{2} or inert gases) needs to be inhaled. Second, the beginning of phase 4 is self-determined when resident O\textsubscript{2} is analyzed; thus, the need to draw a best fit line through phase 3 of the gas curve is eliminated. This study was conducted in healthy subjects; the assessment of our method in subjects with pulmonary-impaired disease still needs to be addressed.

ACKNOWLEDGMENTS: The authors gratefully acknowledge Professor Dr. Johannes Piiper for reading this manuscript and for his invaluable comments, and Dr. Michael Weaver for his assistance with the statistical analyses. This study was supported in part by a Biomedical Research Grant from the Medical College of Ohio.

APPENDIX

The apex and base regions of the seven regional lung model\textsuperscript{18} are herein used to quantitate the alveolar O\textsubscript{2} and CO\textsubscript{2} changes that took place upon inspiration (from RV or FRC) and the changes that occurred with gas exchange. Briefly, this model\textsuperscript{18} consists of seven regions with similar volumes at TLC (each has \(\frac{1}{7}\) of TLC). Only the apex and base regions are considered for these calculations. At RV they have 32.7 percent and 7.6 percent of regional TLC for the apex and base, respectively. Each region inflates/deflates in a similar manner, \textit{ie}, the Milic-Emili et al\textsuperscript{1} model. However, a polynomial of third order is used for the apex (\(V_a = 2X - 1.4X^2 + 0.4X^3\)) and base (\(V_b = 0.23X + X^2 - 0.23X^3\)), where X is the lung volume as a fraction of TLC. \(V_a\) and \(V_b\) are regional volumes as fractions of regional TLC. The alveolar O\textsubscript{2} and CO\textsubscript{2} values at rest for the apex and base regions are taken from the lung model of West.\textsuperscript{19} These values are expressed as fractions in Table 4.

Firstly, with known regional alveolar gas fractions and the RV, as well as the FRC of the subject, the amount of O\textsubscript{2} and CO\textsubscript{2} can be calculated. Adding the amount of O\textsubscript{2} inspired (no CO\textsubscript{2} is inhaled with room air), the alveolar O\textsubscript{2} and CO\textsubscript{2} fraction at RV or FRC level can be calculated. The resulting calculations for alveolar O\textsubscript{2} and CO\textsubscript{2} fractions following the inspiration of room air (VC = 6.1 L, at sea level) after the complete expiration to RV are shown in Table 4. No gas exchange is considered during this inspiratory maneuver. Furthermore, the assumption is made that the inspired gas mixes completely with the alveolar resident gas (the volume of the regional dead space is not included: 5.8 ml for the apex and 60 ml for the base). As can be seen, there is a substantial gain of alveolar oxygen and a significant dilution of alveolar CO\textsubscript{2}. The dilution for CO\textsubscript{2} at the base is more marked. If we simulate a CO\textsubscript{2} expirogram with these alveolar gases, an end-tidal CO\textsubscript{2} of 6.6 mm Hg, or 0.0092 is obtained. This is at variance with experimental findings (end-tidal CO\textsubscript{2} of 30 mm Hg or 0.0421 at 0 s breath holding) whereby the effect of breath holding on end-tidal CO\textsubscript{2} was studied.\textsuperscript{23} The difference between the model and the experiments suggests that physiologically a significant amount of O\textsubscript{2} and CO\textsubscript{2} was exchanged at the alveolar-capillary level during the inspiratory maneuver, due to an enlarged mixed venous to alveolar CO\textsubscript{2} gradient.

Secondly, in order to calculate the effect of gas exchange with the model, the ratio of CO\textsubscript{2} to O\textsubscript{2} of the differences between the alveolar gases at FRC and those after inspiration allow us to calculate the gas exchange ratio in each region. A total pulmonary blood flow of 5.784 L is assumed, with a pulmonary perfusion at the apex of 0.236 L/min and at the base of 1.397 L/min. It is further assumed that neither total blood flow nor the distribution of the pulmonary perfusion is altered by the VC maneuver. Taking these conditions into consideration and the standard O\textsubscript{2} and CO\textsubscript{2} dissociation curves, the O\textsubscript{2} and the CO\textsubscript{2} fluxes can be calculated. Thus, assuming a steady-state gas exchange, the time required for the alveolar gases to return to the resting values can be calculated. The results (Table 4) show that this process takes 70 s.

Thirdly, if a volume of room air is inhaled from FRC to TLC, instead of from RV, the effect of gas dilution is also observed (Table 4, inspiration from FRC), but to a lesser degree. Therefore, if normal tidal breathing is maintained, the expected changes will be even less, perhaps closer to the resting values, which explains why phase 4 is seen (Fig 2) without a VC maneuver.

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