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Effects of intrathoracic pressure, inhalation time, and breath hold time on lung diffusing capacity

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Abstract

The single breath hold maneuver for measuring lung diffusing capacity for carbon monoxide (DLCO) and nitric oxide (DLNO) incorporates multiple sources of variability. This study examined how changes in intrathoracic pressure, inhalation time, and breath hold time affect DLCO, DLNO, alveolar-capillary membrane conductance (DmCO) and pulmonary capillary blood volume (Vc) at rest and during submaximal exercise. Thirteen healthy subjects (mean±SD; age=26±3y) performed duplicate tests at rest and during submaximal exercise.

DLCO and Vc were lower with a positive versus negative intrathoracic pressure during the breath hold at rest (DLCO: 22.2±5.5 vs. 22.7±5.5ml/min/mmHg, p=0.028; Vc: 46.5±11.6 vs. 48.2±11.7ml, p=0.018). However, during exercise, DLCO and Vc were higher with positive versus negative pressure (DLCO: 26.7±5.5 vs. 25.7±5.7ml/min/mmHg, p=0.014; Vc: 56.2±12.6 vs. 53.9±13.1ml, p=0.039). The inhalation time did not significantly affect DLCO, DLNO, DmCO or Vc.

Short breath hold times (<4 seconds) may yield high DLNO/DLCO ratios and non-physiologic DmCO values. The single breath hold maneuver is useful for evaluating gas transfer at rest and during exercise, however intrathoracic pressure, inhalation time, and breath hold time should be kept consistent between repeated tests.

Keywords

Alveolar-capillary membrane conductance; pulmonary capillary blood volume; submaximal exercise; single breath hold maneuver

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1. Introduction

Lung diffusing capacity for carbon monoxide (DLCO) measures the disappearance of carbon monoxide (CO) across the alveolar-capillary interface and reflects the ability of the lungs to transfer gases to the blood. DLCO is dependent on alveolar-capillary membrane conductance (DmCO), reaction rate of CO with blood (θ_{CO}) and pulmonary-capillary blood volume (Vc)(Roughton and Forster, 1957; Equation 1).

$$\frac{1}{DLCO} = \frac{1}{DmCO} + \frac{1}{\theta_{CO} * VC}$$

Simultaneous measurement of CO and nitric oxide (NO) disappearance allows for the calculation of DmCO and Vc in a single test (Guenard et al., 1987). However, although the partitioning of DLCO into its components DmCO and Vc yields valuable insight into pulmonary physiology, it has not yet become standard clinical practice due in part to its complexity and lack of standardization.

One method for measuring DLCO, the single breath hold maneuver, involves an inspiration to total lung capacity, a breath hold, and a normal expiration. Stand alone systems are available that can measure DLCO via the single breath hold maneuver, and these systems have become portable enough that they can even be used in field-based studies. One such system, designed by Medisoftware (Hyp'air Compact, Medisoftware, Dinant, Belgium), measures both DLCO and DLNO simultaneously using the single breath hold maneuver. However, results from a previous study in our laboratory support the notion that standardization in the maneuver technique may be necessary for obtaining accurate and reproducible DmCO and Vc values (Coffman et al., 2016b). Specifically, while Vc generally increases with exercise, a previous study in our laboratory showed variable responses in Vc in young healthy males with low intensity activity (Coffman et al., 2016b). These results were not correlated with fitness level, age, or body size. Therefore, we hypothesized that the lack of an observable increase in Vc values from rest to submaximal exercise might be related to variability in the maneuver used to determine DLCO and thus DmCO and Vc.

We have identified three potential aspects of the single breath hold maneuver that may alter DLCO, and particularly calculated DmCO and Vc values. First, subjects might hold their breath with different intrathoracic pressures—some performing a Valsalva-type maneuver and some continuing to try to breathe inward while the valve at the mouth is occluded (Müller type maneuver). Previous studies have shown that a Valsalva breath hold causes a decrease in Vc during breath holds longer than 5 seconds (Smith and Rankin, 1969). However, this has not been tested at shorter breath holds such as the 4–6 s breath hold recommended for this test. Second, the subject's inhalation is assumed to be instantaneous in the calculation of DLCO and DLNO. To accomplish this, subjects are instructed to inspire as rapidly as possible (MacIntyre et al., 2005). This likely causes a large negative intrathoracic pressure excursion, which could markedly alter central hemodynamics and blood flow through the pulmonary vasculature, thus altering Vc and/or ventilation distribution. Third,

there is significant variability in how quickly a subject inspires to total lung capacity. Since the set breath hold time on an automated system incorporates part of the inhalation time, this likely influences the amount of time the DLCO and DLNO test gas has to reach and mix in the functioning alveoli, thus influencing how long the gas is exposed to perfused capillaries.

Therefore, the aim of this study was to separately investigate how intrathoracic pressure during the breath hold, inhalation time, and breath hold time affect DLCO, DLNO, DmCO and Vc values at rest and during submaximal exercise. We hypothesized that lung diffusing capacity values would be increased with positive intrathoracic pressure and a fast inhalation, but unaffected by changes in breath hold time.

2. Methods

2.1 Subjects

Thirteen healthy, non-smoking, males and females between 18–35 years of age were recruited for this study (mean $\pm$ SD; age = 26 ± 3 y, height = 178 ± 8 cm, weight = 71.6 ± 9.2 kg, BMI = 22.5 ± 1.5 kg/m²; Table 1). All participants had normal pulmonary function values (mean $\pm$ SD % of age- and sex-predicted; FVC = 96 ± 9 %, FEV₁ = 95 ± 8 %, FEV₁/FVC = 99 ± 9 %). All subjects provided written informed consent prior to study start. The experimental protocol was approved by the Mayo Clinic Institutional Review Board.

2.2 Experimental Procedures

Subjects completed the study during a single visit to Mayo Clinic, Rochester. First, resting measures of forced vital capacity were obtained to determine the volume of gas to be inhaled in the single breath DLCO/DLNO test. Next, single breath DLCO/DLNO maneuvers were performed in duplicate at rest and during submaximal cycling exercise utilizing five technique variations (see *section 2.3*). The five conditions were randomized and counterbalanced. All resting and exercise measures were performed on an upright cycle ergometer (Corival, Lode BV, Netherlands). Submaximal exercise was defined as the workload at which a subject maintained 70% of their age-predicted maximum heart rate (136 ± 2 bpm resulting 90 ± 28 watts).

2.3 Experimental variations of the DLCO/DLNO maneuver

The single breath DLCO/DLNO maneuver is measured with the subject sitting in the upright position. Typically, the subject is coached to perform a complete exhalation to residual volume, a fast inhalation of the test gas to total lung capacity, a 4 second breath hold and finally a normal exhalation. However, the purpose of this study was to determine how changing the technique of the maneuver affects the values obtained for DLCO and calculated DmCO and Vc values. To accomplish this, three aspects of the maneuver were altered. First, subjects were trained to achieve either a positive or negative intrathoracic pressure during the breath hold via visual feedback from a real time mouth pressure tracing (see *section 2.6* and Figure 1). Second, subjects were instructed either to breathe in as fast as possible or to breathe in half as fast during the inhalation segment of the maneuver. Third, the breath hold time was changed from 4 seconds to either 2 or 6 seconds. Apnea time was measured from the closure of the automated system valve which initiates breath holding

until the moment the valve re-opened to terminate the breath hold. The “Control” for all 3 conditions being tested was the typical technique; a maneuver that included a negative intrathoracic pressure, fast inhalation and a 4 second breath hold. Each of the three aspects (intrathoracic pressure, rate of inhalation, breath hold time) was tested individually and compared to the control condition (Table 2).

2.4 Calculation of DLCO and DLNO

DLCO and DLNO values were calculated from the disappearance of inhaled CO and NO during the breath hold of the single breath maneuver. First, the subject breathed in from a bag containing the test gas, a mixture of 0.3% CO, 40 ppm NO, 14% He, 21% O₂ and balance N₂. After the breath hold, the subject exhaled into a second bag. The inhaled and exhaled concentrations of CO, NO, and He were measured using a small portable system (Hyp’air Compact system with Exp’air software, version 1.30.02, Medisoft, Dinant, Belgium). The Medisoft system samples a volume of 0.9L (after 0.9L washout) with a sample flow of 400 ml/min over a period of 30–35 seconds. Anatomical dead space was estimated as 2.2 multiplied by the subject’s weight in kilograms (MacIntyre et al., 2005). Total dead space (DS) included anatomical dead space, instrument dead space (100 ml) and the dead space of the filter and mouthpiece (60 ml).

In general, the test gas has three possible fates after it is inhaled. It either crosses the alveolar-capillary membrane, mixes with the dead space of the lung, or it is exhaled in the subsequent breath. Helium is an inert tracer gas that does not cross the alveolar-capillary membrane appreciably, thus by measuring the dilution of helium, the amount of dead space can be determined. Alveolar volume (V_A), then, is the inspired volume (V_I) minus the volume of air that mixes with DS, determined by the ratio of inspired to expired helium (mean ± SD; V_A = 4.8 ± 0.9L; Equation 2). The diffusing capacity (DL) of the lung for a gas, X, is calculated by Equation 3 (denoted DL_X). In this equation, t is the time between the first third of the inhalation and the first half of the exhalation, and b is a correction factor that converts V_A from BTPS to STPD (Jones and Meade, 1961). Inhaled and exhaled concentrations of the gases were imported into MATLAB to perform the calculations in Equations 2–8 (version R2016a, Mathworks, Inc, Natick, MA).

$$V_A = V_I - DS * \frac{He_{insp}}{He_{exp}} \quad 2$$

$$DL_X = \frac{V_A \cdot b}{t} \cdot \log_{10} \left[\frac{X_{insp}}{X_{exp}} \cdot \frac{He_{exp}}{He_{insp}} \right] \quad 3$$

2.5 Calculation of Vc and DmCO

DL is dependent on the gas conductance of the alveolar-capillary membrane (Dm) and the reaction rate (θ) of the gas with hemoglobin. Therefore, DL can be modeled as two resistors

in series (Roughton and Forster, 1957; Equation 4). The value of θ_{CO} is dependent on P_{AO_2} and can be calculated by Equation 5 ($a=0.0062$, $b=1.16$; Guénard et al. 2016). Next, V_c can be solved for in Equation 4 and the resulting equation for NO is set equal to the same equation for CO since V_c must be the same regardless of whether CO or NO was used as the test gas (Equation 6). $DmCO$ is related to $DmNO$ by the α ratio (or Krogh coefficient) of 1.97 (Wilhelm et al., 1977; Equation 7), and thus $DmNO/\alpha$ can be substituted for $DmCO$ to get Equation 8. $DmNO$ can be solved for using a finite θ_{NO} of 4.5 ml/min/mmHg/ml_{blood} (Borland et al., 2010; Borland and Cox, 1991; Carlsen and Comroe, 1958; Guénard et al., 2016). Finally, V_c is calculated using Equation 6

$$\frac{1}{DL_x} = \frac{1}{Dm_x} + \frac{1}{\theta_x \cdot V_c} \quad 4$$

$$\frac{1}{\theta_{co}} = a \cdot P_{AO_2} + b \quad 5$$

$$V_c = \frac{DL_x \cdot Dm_x}{\theta_x \cdot (Dm_x - DL_x)} \quad 6$$

$$DmCO = \frac{DmNO}{\alpha} \quad 7$$

$$DmNO = \frac{\frac{\theta_{NO} - \alpha\theta_{co}}{\theta_{NO}}}{\frac{DLNO}{DLNO} - \frac{DLCO}{DLCO}} \quad 8$$

2.6 Mouth Pressure

Mouth pressure during the breath hold was used as a surrogate for intrathoracic pressure. A pressure sensor was connected to a port in the pneumotach and the data was captured in LabChart 8 (ADInstruments, Colorado Springs, CO). Before beginning the study, subjects were trained to reach a mouth pressure of +40 cmH₂O via visual feedback from LabChart in the form of a region bounded by dotted lines on the pressure trace. During the *Positive* condition, subjects were instructed to adjust their mouth pressure to between the dotted lines (40–50 cmH₂O) as quickly as possible and maintain it for the duration of the breath hold (Figure 1). For all other conditions, mouth pressure measurements were used to ensure that subjects were maintaining a negative intrathoracic pressure, defined as a mouth pressure less than 5 cm H₂O during the breath hold period. A valve in the heated pneumotach of the

Medisoft closes at the start of the breath hold preventing air flow. Since a difference in pressure is dependent on flow and resistance, the mouth pressure measured will be closer to zero than the actual intrathoracic pressure. While there are limitations of using mouth pressure as a surrogate for intrathoracic pressure, the purpose of this study was to generate two distinct intrathoracic pressure conditions and look at within-subject DLCO differences; thus, the relative change in pressure was more important than the absolute values (see Discussion, *4.5 Technical Considerations*).

2.7 Statistical methods

Differences in mouth pressure, inhalation time, and apnea time between the 5 different tests were compared using a one-way ANOVA. Changes in DLCO, DLNO, DLNO/DLCO, DmCO, and Vc within a technique variation were evaluated using a paired t-test, comparing all conditions to *Control* (see *section 2.3* and Table 2). A paired t-test was also used to test for changes between rest and exercise for all conditions. Differences in DLCO, DLNO, DLNO/DLCO, DmCO, and Vc across the three different breath hold times were analyzed using linear regression. All statistical tests were performed using Matlab (version R2016a, Mathworks, Inc, Natick, MA) or GraphPad Prism (Prism 7, GraphPad Software, Inc., La Jolla, CA, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Intrathoracic pressure during breath hold

At rest, subjects successfully maintained either a positive or negative intrathoracic pressure, as measured by mouth pressure (MP), during the breath hold of the single breath hold maneuver (Table 3; Figure 1). At rest, DLCO and Vc were lower when subjects held a positive vs. negative pressure (DLCO: 22.2 ± 5.5 vs. 22.7 ± 5.5 ml/min/mmHg, $p = 0.028$; Vc: 46.5 ± 11.6 vs. 48.2 ± 11.7 , $p = 0.018$; Table 4). There was no difference in DLNO or DmCO values between positive and negative intrathoracic pressure at rest.

During submaximal exercise, subjects successfully maintained a positive or negative intrathoracic pressure during the breath hold (Table 3). Unlike at rest, DLCO values were higher with positive vs. negative intrathoracic pressure (26.7 ± 5.5 vs. 25.7 ± 5.7 , $p = 0.014$; Table 4) due to an increase in Vc (56.2 ± 12.6 vs 53.9 ± 13.1 , $p = 0.039$; Table 4). DLNO was also higher during exercise with positive vs. negative intrathoracic pressure (146.4 ± 33.4 vs. 141.7 ± 30.9 , $p = 0.030$; Table 4). There was no difference in DmCO between positive and negative intrathoracic pressure during exercise.

3.2 Inhalation time

Subjects were instructed to take twice as long to inhale to total lung capacity, thus doubling their inhalation time (IT) (Table 3). Theoretically, this might be sufficient to reduce any negative intrathoracic pressure excursion experienced during the fast inhalation and potentially alter measures of lung diffusing capacity. However, both at rest and during submaximal exercise, there were no differences in DLCO, DLNO, DmCO or Vc with a slow versus fast inhalation rate (Table 5). This might suggest that the static lung conditions at the

beginning and end of the inhalation are more important than the rate of inhalation in determining DLCO and its components Dm and Vc.

3.3 Breath hold time

While the single breath system allows breath hold time to be changed, the actual breath hold time (“apnea time”) is dependent on variability in the subject’s performance (specifically, their inhalation time, which factors into the breath hold time). Thus, given the variability in apnea time during the 2, 4, and 6 second trials (see Table 3), the results have been analyzed using scatter plots and a linear regression (Figures 2–3). The linear regression analysis was performed on the individual data points, however, for simplicity the data is presented as mean and standard deviation in the figures (Figures 2–3). Of note, two non-physiologic values have been removed from the analysis ($DmCO = -281$ and 3400 ml/min/mmHg; $DLNO/DLCO = 7.3$ and 8.1). At rest, changing the breath hold time did not affect DLCO, DLNO, or Vc significantly (Figures 2A-B & 3A). However, the ratio of DLNO/DLCO decreased significantly with increasing apnea time ($p = 0.007$, Figure 2C). Further, DmCO decreased by 30 ml/min/mmHg for each extra second of the breath hold during rest ($p = 0.019$, Figure 3B).

Similar to rest, there was no significant change in DLCO, DLNO or Vc with increasing apnea time during exercise (Figures 1A-C). DLNO/DLCO and DmCO decreased with increasing apnea times during exercise (DLNO/DLCO, $p = 0.006$, Figure 2C; DmCO, $p = 0.050$, Figure 3B).

Overall, DLCO and Vc were significantly greater at exercise versus rest across all apnea times (DLCO, $p = 0.010$, Figure 2A; Vc, $p = 0.017$, Figure 3A). The DLNO/DLCO ratio was decreased at rest versus exercise across all apnea times ($p = 0.011$, Figure 2C). There was no significant difference in DmCO between rest and exercise at any apnea time (using the finite θ_{NO} assumption) (Coffman et al., 2016a; Zavorsky et al., 2017).

4. Discussion

4.1 Main findings

The purpose of this study was to determine how subject variability in intrathoracic pressure, inhalation time, and breath hold time affect measurements of DLCO, DLNO, DmCO, and Vc during the single breath DLCO/DLNO maneuver. The main findings were: 1) DLCO and DLNO increased from rest to submaximal exercise despite changes in intrathoracic pressure, inhalation time or breath hold time; however, DmCO did not increase from rest to submaximal exercise, 2) short breath hold times (<4 seconds) may yield high DLNO/DLCO ratios and therefore non-physiologic DmCO values and 3) Vc was lower during positive versus negative intrathoracic pressure at rest, but higher during positive versus negative intrathoracic pressure during exercise. Our findings highlight the importance of keeping intrathoracic pressure, inhalation time, and breath hold time consistent between repeated tests, days, and across subjects and patient populations.

4.2 Physiologic effects of exercise on DLCO, DmCO and Vc

The primary effects of exercise on the cardiopulmonary system are well-established. Exercise increases cardiac output from the right ventricle into the pulmonary circulation which accommodates the increased cardiac output by distension of under-perfused capillaries or recruitment of additional capillaries (Hsia et al., 1995; Johnson et al., 1960; Rahul M Tamhane et al., 2001). Recruitment and distension increase pulmonary capillary blood volume (Vc) and the surface area available for gas exchange (a major factor in DmCO), thereby increasing DLCO and DLNO. Indeed, DLCO, DmCO, and Vc have been shown to increase linearly with exercise (Johnson et al., 1960; Rahul M. Tamhane et al., 2001). In line with these well-established effects of exercise on lung diffusing capacity, our results show an increase in DLCO during exercise, caused by an increased Vc.

This study diverges from previous studies in that DmCO did not increase with submaximal exercise (Coffman et al., 2016b). However, it is important to note that the calculation of DmCO and Vc is not consistent between this study and previous studies. Specifically, the present study calculated DmCO using the finite θ_{NO} assumption, which has recently been adopted into the current ERS guidelines and was therefore implemented here (Zavorsky et al., 2017). However, when using the finite θ_{NO} assumption, DmCO is sensitive to changes in both DLNO and DLCO, and particularly to changes in the DLNO/DLCO ratio. The implication of the finite θ_{NO} assumption on calculation of DmCO is discussed below in more depth.

4.3 Importance of breath hold time to the proper measurement of DLNO and DmCO

In the present study, DmCO did not increase from rest to submaximal exercise. There are two potential reasons for this discrepancy with previous studies (Rahul M. Tamhane et al., 2001; Taylor et al., 2014). First, as we have shown previously, using the finite θ_{NO} assumption causes calculated values of DmCO to fall or rise rapidly when DLNO/DLCO is outside of a certain range (Coffman et al., 2016a). Specifically, this asymptote occurs when the DLNO/DLCO ratio approaches θ_{NO}/θ_{CO} , which given the most recent ERS guidelines occurs around 8.2 (depending on P_{AO_2}). The rapid increase or decrease in calculated DmCO values as the θ_{NO}/θ_{CO} ratio approaches this asymptote likely explains the high coefficient of variation in DmCO values (on average CV ~ 11.1%) in comparison to calculated Vc values (on average CV ~ 4.3%) (see Table 6).

Second, non-physiologic DmCO values were observed only in the 2 second breath hold condition. When the breath hold is shorter, the ratio of DLNO to DLCO is greater, encroaching upon the asymptote in DmCO that occurs when using the finite θ_{NO} assumption. High DmCO values in Figure 3 cause the linear regression to have a large negative slope. Furthermore, the recommended breath hold time for the single breath hold maneuver is 4–6 seconds. However, the effective breath hold time, the apnea time, using an automated system will be less than the set time due to partial incorporation of the inhalation time (see *Technical Considerations*). Thus, our data suggests that the breath hold time using an automated system should be closer to 6 seconds when using the finite θ_{NO} assumption to avoid high DLNO/DLCO ratios and non-physiologic values of DmCO.

4.4 Positive intrathoracic pressure alters pulmonary capillary blood volume

In this study, V_c was lower during positive versus negative intrathoracic pressure at rest, but higher during positive versus negative intrathoracic pressure during exercise. The reason for the reversal of which intrathoracic pressure condition yielded a greater V_c value between rest and exercise is unclear. At rest and during exercise, performing a Valsalva maneuver (positive intrathoracic pressure) has been shown to reduce pulmonary capillary blood flow and V_c , whereas a negative intrathoracic pressure increases V_c (Kawakami et al., 1970; Kowallick et al., 2014; Smith and Rankin, 1969). Theoretically, it is possible that venous return is impeded less during exercise than at rest during a positive intrathoracic pressure condition. Additionally, there may be an initial effect of positive intrathoracic pressure which normalizes after a few heartbeats; this is important because the 4s breath hold encompasses a greater number of heartbeats during exercise compared to rest. The V_c response to positive intrathoracic pressure at rest is in agreement with previous studies, however the opposite response during exercise requires further study.

4.5 Technical considerations

Automated systems such as the Medisoft utilize the Jones-Meade method to automatically apply the breath hold time, which includes 70% of the inspiratory time. Therefore, subjects who take longer to breathe in may have a shorter apnea time, potentially resulting in higher DLNO/DLCO ratios. There is a large degree of variability in subjects' inhalation time between tests (CV ~ 11% presently) which, using the Jones-Meade breath hold method, will cause variability in the apnea time between tests. Therefore, the variability in subject performance could affect the reproducibility of DLCO values. All in all, it is important to recognize that if a breath hold time of 4s is desired, as is suggested by the ERS standards (Zavorsky et al., 2017), the actual apnea time might be short enough to cause high DLNO/DLCO ratios.

In this study, we used mouth pressure as a surrogate for intrathoracic pressure. An esophageal balloon is the gold standard for measuring intrathoracic pressure. However, because the valve at the mouth was occluded during the breath hold in the present study, thoracic pressure was expected to trend with mouth pressure. Furthermore, we were more interested in the relative change in intrathoracic pressure rather than the absolute values.

Conclusions

In conclusion, we found that intrathoracic pressure has an effect on V_c measurements, and we hypothesize that this may be due to differences in blood flow and distribution within the lungs during exercise. Properly setting the automated breath hold time is necessary to yield reasonable DLNO/DLCO ratios such that calculated $DmCO$ values are in the physiological range. Alterations in breath hold time appear to have the greatest impact on measures of lung diffusing capacity, particularly $DmCO$. All in all, the single breath hold maneuver can be utilized to measure DLCO, $DmCO$, and V_c , but care needs to be taken regarding the methodological aspects.

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Highlights

- We measure DLCO and DLNO using the single breath hold technique.
- We evaluate the effects of changing intrathoracic pressure, inhalation time, and breath hold time on DLCO, DLNO, DmCO and Vc.
- DLCO and Vc increase with submaximal exercise regardless of changes in intrathoracic pressure, inhalation time, and breath hold time.
- DmCO does not increase from rest to submaximal exercise using the finite θ_{NO} assumption.

Figure 1.

A representative trace of mouth pressure over time during a Positive (black) and Negative (grey) breath hold period of a single breath DLCO/DLNO maneuver. Subjects were asked to generate a mouth pressure between 40–50 cmH₂O and guidelines provided visual feedback during the test. The arrows indicate the times at which the automated system valve opened and closed.

Figure 2.

DLCO, DLNO, DLNO/DLCO ratio as a function of breath hold time at rest and during submaximal exercise. A) DLCO, lung diffusing capacity for carbon monoxide; B) DLNO, lung diffusing capacity for nitric oxide; C) DLNO/DLCO ratio.

Figure 3.

V_c, and DmCO as a function of breath hold time at rest and during submaximal exercise. A) V_c, pulmonary capillary blood volume; B) DmCO, membrane conductance. R = rest; E = exercise

Table 1.

Subject characteristics.

Characteristics					
Number of subjects (males)	13 (8)				
Age, years	26	±	3		
Height, cm	178	±	8		
Weight, kg	71.6	±	9.2		
BMI, kg/m ²	22.5	±	1.5		
FEV ₁ , l (% predicted)	4.3	±	0.6	(94.6	± 7.8)
FVC, l (% predicted)	5.4	±	0.9	(96.1	± 9.2)
FEV ₁ /FVC ratio, % (% predicted)	82	±	8	(98.9	± 9.1)

Values are reported as mean ± SD. BMI, body mass index; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity. FEV₁, FVC, and FEV₁/FVC ratio are presented in absolute values and as a percent of age- and sex-predicted values.

Table 2.

The grey box indicates the aspect of the maneuver being tested. *Positive*, *Slow*, *2 second* and *6 second* were all compared to the *Control* test. The five tests were randomized and counterbalanced.

Test Name	Intrathoracic Pressure	Rate of Inhalation	Breath Hold Time (s)
<i>Control</i>	Negative	Fast	4
<i>Positive</i>	Positive	Fast	4
<i>Slow</i>	Negative	Slow	4
<i>2 second</i>	Negative	Fast	2
<i>6 second</i>	Negative	Fast	6

Table 3.

Testing parameters at rest and exercise.

	Negative		Positive		Slow	2 second		6 second	
<i>Rest</i>									
MP (cmH ₂ O)	-4.6	± 7.8	36.2	± 10.7*	-3.1 ± 6.6	-2.5 ± 6.0	-2.4 ± 10.9		
IT (s)	1.8	± 0.4	1.7 ± 0.4	3.6 ± 0.6*	1.6 ± 0.3	1.7 ± 0.4			
BHT (s)	3.9	± 0.3	3.8 ± 0.4	3.8 ± 0.4	2.1 ± 0.4*	6.0 ± 0.4*			
<i>Exercise</i>									
MP (cmH ₂ O)	-0.5	± 10.4	43.9	± 10.6*	-5.5 ± 9.6	-3.8 ± 8.1	-5.4 ± 11.2		
IT (s)	1.5	± 0.2	1.6 ± 0.3	2.8 ± 0.7*	1.6 ± 0.3	1.6 ± 0.4			
BHT (s)	3.9	± 0.3	3.7 ± 0.5	4.1 ± 0.6	2.2 ± 0.4*	5.9 ± 0.4*			

MP, mouth pressure; IT, inhalation time; BHT, breath hold time. Altering the desired aspect of the single breath hold maneuver was successful in all conditions.

* p<0.05; test condition compared to all other groups

Table 4.

Differences in measures of lung diffusing capacity when subjects held a positive versus negative intrathoracic pressure during the breath hold period of the single breath hold maneuver.

	Positive				Negative			
	Rest		Exercise		Rest		Exercise	
DLCO (ml/min/mmHg)	22.2	± 5.5	26.7	± 5.5 [*]	22.7	± 5.5 [†]	25.7	± 5.7 ^{*†}
DLNO (ml/min/mmHg)	129.1	± 34.3	146.4	± 33.4 [*]	132.4	± 34.2	141.7	± 30.9 ^{*†}
Vc (ml)	46.5	± 11.6	56.2	± 12.6 [*]	48.2	± 11.7 [†]	53.9	± 13.1 ^{*†}
DmCO (ml/min/mmHg)	183.7	± 71.6	194.7	± 86.9	183.2	± 67.5	188.6	± 74.7
DLNO/DLCO	5.8	± 0.5	5.5	± 0.5 [*]	5.8	± 0.4	5.5	± 0.5

DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; Vc, pulmonary capillary blood volume; DmCO, membrane conductance. Values are reported as mean ± SD.

^{*} p<0.05; rest vs. exercise within either positive or negative condition

[†] p<0.05; positive vs. negative within either rest or exercise condition

Table 5.

Differences in measures of lung diffusing capacity with a fast versus a slow inhalation during the single breath hold maneuver.

	Slow inhalation						Fast inhalation					
	Rest			Exercise			Rest			Exercise		
DLCO (ml/min/mmHg)	22.7	±	6.7	27.0	±	9.3 [*]	22.7	±	5.5	25.7	±	5.7 [*]
DLNO (ml/min/mmHg)	127.0	±	36.0	137.3	±	38.9	132.4	±	34.2	141.7	±	30.9 [*]
Vc (ml)	50.6	±	13.2	60.4	±	26.0 [*]	48.2	±	11.7	53.9	±	13.1 [*]
DmCO (ml/min/mmHg)	164.1	±	69.3	152.6	±	42.3 [*]	183.2	±	67.5	188.6	±	74.7
DLNO/DLCO	5.6	±	0.5	5.2	±	0.5 [*]	5.8	±	0.4	5.5	±	0.5

DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; Vc, pulmonary capillary blood volume; DmCO, membrane conductance. Values are reported as mean ± SD.

^{*} p<0.05; rest vs. exercise within either slow or fast condition

[†] p<0.05; slow vs. fast within either rest or exercise condition

Table 6.

Coefficient of variation (CV) analysis for measures of lung diffusing capacity

REST	<i>Positive</i>	<i>Negative</i>	<i>Slow</i>	<i>2 second</i>	<i>6 second</i>
DLCO (%)	2.8	2.7	2.4	2.0	2.8
DLNO (%)	3.5	4.1	5.4	4.1	3.2
Vc (%)	4.6	3.5	2.5	3.5	3.8
DmCO (%)	13.4	8.9	14.1	20.8	7.6
DLNO/DLCO (%)	2.8	2.4	3.2	3.8	1.7
EXERCISE	<i>Positive</i>	<i>Negative</i>	<i>Slow</i>	<i>2 second</i>	<i>6 second</i>
DLCO (%)	2.0	2.8	5.7	4.1	3.1
DLNO (%)	2.9	2.9	6.9	5.2	4.2
Vc (%)	2.7	4.0	7.7	5.5	4.7
DmCO (%)	10.3	4.9	8.3	11.2	11.2
DLNO/DLCO (%)	3.0	1.2	2.0	3.6	4.0

DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; Vc, pulmonary capillary blood volume; DmCO, membrane conductance. The high CV of DmCO relative to other variables is discussed in *section 4.3*.