Optimizing the Calculation of $D_{M,CO}$ and V_C via the Single Breath Single Oxygen Tension $DL_{CO/NO}$ Method

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ABSTRACT

Alveolar-capillary membrane conductance ($D_{M,CO}$) and pulmonary-capillary blood volume (V_C) are calculated via lung diffusing capacity for carbon monoxide (DL_{CO}) and nitric oxide (DL_{NO}) using the single breath, single oxygen tension (single-FiO_2) method. However, two calculation parameters, the reaction rate of carbon monoxide with blood (θ_{CO}) and the $D_{M,NO}/D_{M,CO}$ ratio (α-ratio), are controversial. This study systematically determined optimal θ_{CO} and α-ratio values to be used in the single-FiO_2 method that yielded the most similar $D_{M,CO}$ and V_C values compared to the 'gold-standard' multiple-FiO_2 method. Eleven healthy subjects performed single breath DL_{CO}/DL_{NO} maneuvers at rest and during exercise. $D_{M,CO}$ and V_C were calculated via the single-FiO_2 and multiple-FiO_2 methods by implementing seven θ_{CO} equations and a range of previously reported α-ratios. The RP θ_{CO} equation (Reeves and Park, Respiration physiology 88:1-21, 1992.) and an α-ratio of 4.0-4.4 yielded $D_{M,CO}$ and V_C values that were most similar between methods. The RP θ_{CO} equation and an experimental α-ratio should be used in future studies.

KEYWORDS

Lung diffusing capacity, alveolar-capillary membrane conductance, pulmonary-capillary blood volume, submaximal exercise

1. INTRODUCTION

Lung diffusing capacity (D_L) is the ability of the lungs to transfer gases from the pulmonary alveoli to the pulmonary capillary blood and is typically measured as the rate of uptake of carbon monoxide (CO) during either a breath hold or a rebreathe maneuver (Blakemore et al., 1957; Hsia, 2002; Hughes and Bates, 2003; Macintyre et al., 2005; Sackner et al., 1975). Lung diffusing capacity for CO (DL_{CO}) is commonly assessed in pulmonary function laboratories and provides a useful tool in screening for diseases that affect alveolar-capillary gas transfer such as chronic obstructive pulmonary disease (COPD) and heart failure.

The rate of transfer of CO from the alveoli to the pulmonary capillary blood is dependent on 1) the conductance of the alveolar-capillary membrane ($D_{M,CO}$), and 2) the reaction rate of CO with hemoglobin in blood (θ_{CO}). Thus, DL_{CO} can be modeled as a circuit with two conductances in series, where V_C is the pulmonary capillary blood volume (Roughton and Forster, 1957), as follows:

$$\frac{1}{DL_{CO}} = \frac{1}{D_{M,CO}} + \frac{1}{\theta_{CO} * V_C} \qquad \text{Equation (1)}$$

Due to the fact that CO and O_2 competitively bind to hemoglobin, the value of θ_{CO} varies with the partial pressure of oxygen (PO_2) present in pulmonary capillary blood (Forster et al., 1957a; Forster et al., 1957b; Roughton and Forster, 1957; Roughton et al., 1957), such that DL_{CO} is decreased with increasing PO_2. Because of this relationship, the subcomponents shown in Eq. 1 ($D_{M,CO}$ and V_C) have historically been determined by measuring DL_{CO} at two or more alveolar oxygen tensions (P_AO_2). For this 'multiple-FiO_2' method, $1/DL_{CO}$ is plotted against $1/\theta_{CO}$ and a linear regression is fit to the data, where the y-intercept is equal to $1/D_{M,CO}$ and the slope is equal to $1/V_C$, based on Eq. 1 (Roughton and Forster, 1957). The inverses of these values are then reported as $D_{M,CO}$ (ml/min/mmHg) and V_C (ml), respectively. Further details regarding this calculation are described later (*see 2.5 Methods, "Calculation of $D_{M,CO}$ and V_C via the multiple-FiO_2 method"*).

Although numerous equations have been published describing the mathematical relationship between PO_2 and θ_{CO}, there is currently no consensus regarding the correct or most appropriate equation to use. Many of these previously published equations are of the same form (*see Equation* 2), but the constants used (i.e. *a* and *b*) differ substantially between equations. These differences are due to the fact that studies aimed at determining the correct θ_{CO} equation have been performed under varying test conditions, such as different blood sources and blood pH (Holland, 1967; Reeves and Park, 1992; Roughton and Forster, 1957; Stam et al., 1991; Te Nijenhuis et al., 1996).

$$\frac{1}{\theta_{CO}} = a * P_{cap}O_2 + b \qquad \text{Equation (2)}$$

Another method, termed the 'single-FiO_2 method,' has been established to determine $D_{M,CO}$ and V_C while measuring DL_{CO} at only one alveolar oxygen tension (Borland and Higenbottam, 1989; Guenard et al., 1987). This method utilizes simultaneous assessment of DL_{CO} with lung diffusing capacity of nitric oxide (NO), a gas whose reaction rate with hemoglobin (θ_{NO}) has been cited to be between 250 and 400 times faster than that of CO (Borland and Higenbottam, 1989; Hakim et al., 1996). Because this rate is significantly faster than θ_{CO}, θ_{NO} has been considered infinite, such that the lung diffusing capacity for NO (DL_{NO}) is equal to $D_{M,NO}$. Recently, however, the assumption that θ_{NO} is effectively infinite compared to θ_{CO} has been debated (Borland et al., 2014; Borland et al., 2010; Zavorsky, 2010), with some groups using an experimentally-derived, finite value of 4.5 $(min*mmHg)^{-1}$ for θ_{NO} (Borland et al., 2010; Carlsen and Comroe, 1958). However, the use of a finite θ_{NO} remains somewhat controversial and for several reasons discussed in detail later in this manuscript (*see 4.7 Discussion, "Assumption that θ_{NO} is infinite")* we have chosen to maintain the assumption that θ_{NO} is infinite, as shown in the equations below:

$$\frac{1}{DL_{NO}} = \frac{1}{D_{M,NO}} + \frac{1}{\theta_{NO} * V_C} \qquad \text{Equation (3)}$$

$$\theta_{NO} \approx \infty$$

$$\frac{1}{DL_{NO}} = \frac{1}{D_{M,NO}} + \frac{1}{\infty}$$

$$\frac{1}{DL_{NO}} = \frac{1}{D_{M,NO}}$$

$$DL_{NO} = D_{M,NO}$$

Another important consideration related to the single-FiO_2 method is the assumption regarding the ratio of $D_{M,NO}$ to $D_{M,CO}$. The factor describing this relationship between $D_{M,NO}$ and $D_{M,CO}$, termed the α-ratio, is a physical property that is dependent on both the solubility and molecular weight of NO and CO, as both properties affect the ability of the gas to transfer across the alveolar-capillary membrane. However, it has been shown previously that values of the α-ratio calculated directly in laboratory settings vary substantially from the theoretical value of 1.93 that is calculated using values of gas solubilities in water (Meyer et al., 1990), with experimental values of up to 2.63 being reported (Hughes and Bates, 2003; Magini et al., 2013; Tamhane et al., 2001). For this reason, there is no consensus in the literature regarding the correct α-ratio to be used in calculation of $D_{M,CO}$. Practically, once an α-ratio is chosen, $D_{M,CO}$ can be calculated, and in conjunction with a value of θ_{CO} (Eq. 2), V_C is determined via Eq. 1.

Based on the aforementioned considerations, both θ_{CO} and the α-ratio are critical in the determination of $D_{M,CO}$ and V_C via the single-FiO_2 method. However, various values for constants in the θ_{CO} equation exist in the literature, as does a range of values for the α-ratio. Previously, our laboratory has determined the optimal θ_{CO} equation and α-ratio to be used when calculating $D_{M,CO}$ and V_C from DL_{CO} and DL_{NO} values obtained via a rebreathe technique (Ceridon et al., 2010). Semi-automated units that measure DL_{CO} and DL_{NO} during the single breath hold maneuver are gaining popularity and becoming more widely used (Blakemore et al., 1957; Borland and Higenbottam, 1989; Guarnieri et al., 2015; Guenard et al., 1987; Macintyre et al., 2005; Magini et al., 2013; Pavelescu et al., 2013; Zavorsky et al., 2004). Accordingly, the aim of the present study was to determine the optimal θ_{CO} equation and α-ratio for calculation of $D_{M,CO}$ and V_C when implementing the single breath hold technique.

2. METHODS

2.1 Subjects

Eleven healthy nonsmoking adult males (mean ± SD; age = 25 ± 3 y, height = 180.7 ± 6.6 cm, weight = 74.0 ± 10.5 kg) who had pulmonary function within normal limits participated in the study (mean ± SD % of age and sex predicted; FVC = 98.3 ± 8.5%, FEV_1 = 97.0 ± 7.6%, FEV_1/FVC = 99.0 ± 7.4%). Each participant gave written informed consent after being provided a detailed description of the study requirements. The experimental procedures were approved by the Mayo Clinic Institutional Review Board and were performed in accordance with the ethical standards of the Declaration of Helsinki.

2.2 Experimental procedures

The experimental procedures were conducted during a single laboratory visit (Fig. 1). Resting measures of pulmonary function were assessed according to standard procedures in order to determine the volume of test gas to be delivered to each subject for the assessment of DL_{CO} and DL_{NO}. Next, DL_{CO} and DL_{NO} were assessed in duplicate during a single breath hold technique with the subjects seated upright on a cycle ergometer (Corival, Lode BV, Netherlands) under three different conditions in a random order; 1) FiO_2 of 20%, 2) FiO_2 of 40%, and 3) FiO_2 of 60%. This protocol was completed at rest and during constant-load cycle exercise at 80 W. During exercise, subjects maintained a self-selected but consistent pedal cadence between 60-100 rpm. Steady-state exercise was verified via measures of heart rate and oxygen consumption. Upon collection of DL_{CO} and DL_{NO} values, $D_{M,CO}$ and V_C were calculated according to both the multiple-FiO_2 and single-FiO_2 methods (*see 2.4-7 Methods, "Calculations")*.

2.3 Lung diffusing capacity

DL_{CO} and DL_{NO} were determined by simultaneously measuring the disappearance of CO and NO during a single breath hold technique (Hyp'air Compact, Medisoft, Dinant, Belgium) (de Bisschop et al., 2012; Pavelescu et al., 2013). Before the assessment of DL_{CO} and DL_{NO}, subjects pre-breathed gas of an FiO_2 of 20%, 40%, or 60% for five minutes to ensure adequate alveolar equilibration. This was verified with breath-by-breath measures of $P_{ET}O_2$ (Ultima CPX, MGC Diagnostics, Saint Paul, MN). After five minutes, DL_{CO} and DL_{NO} were measured simultaneously, followed by four minutes of quiet breathing on the test gas (FiO_2 of 20, 40, or 60%) to ensure proper CO washout. DL_{CO} and DL_{NO} were then assessed a second time at the same oxygen tension. This process was repeated for the remaining two oxygen concentrations.

To perform the single breath hold technique, subjects were instructed to breathe normally for 4-5 breaths before exhaling completely to residual volume (RV). Upon reaching RV, the subjects were switched into an inspiratory bag containing 0.28% CO, 40 ppm NO, 14% He, one of three O_2 concentrations, and N_2 balance, and instructed to breathe in rapidly and completely up to total lung capacity. Subjects then held their breath for four seconds before performing a relaxed expiration back to RV. The first 0.9 L of the expired gas was discarded to ensure dead space washout, with the next 0.9 L of the expired gas being collected for analysis.

2.4 Calculation of θ_{CO}

For both the multiple-FiO_2 and single-FiO_2 methods, the reaction rate of CO with hemoglobin in blood (θ_{CO}) is required to calculate $D_{M,CO}$ and V_C. The Roughton and Forster model for calculating θ_{CO} was used as the basis for all calculations, but with seven different sets of constants (i.e. *a* and *b*) found in the literature (Table 1) (Blakemore et al., 1957; Hsia, 2002; Hughes and Bates, 2003; Macintyre et al., 2005; Sackner et al., 1975):

$$\frac{1}{\theta_{CO}} = a * P_{cap}O_2 + b \qquad \text{Equation (2)}$$

where $P_{cap}O_2$ is the mean capillary partial pressure of O_2; $P_{cap}O_2$ in mmHg and θ_{CO} in ml (CO gas)/min/mmHg/ml (blood). First, $P_{cap}O_2$ was calculated for each subject for each of the three O_2

concentrations (20, 40, and 60% O_2), estimated as alveolar PO_2 – VO_2/(DL_{CO} x 1.23); PO_2 in mmHg, VO_2 in ml/min, and DL_{CO} in ml/min/mmHg (Forster, 1987). Next, these $P_{cap}O_2$ values were applied to Eq. 2 and $1/\theta_{CO}$ was calculated using seven sets of values for constants *a* and *b* found in the literature (Table 1). These values for $1/\theta_{CO}$ are used in the following calculations for both the multiple- and single-FiO_2 method.

It should be noted that while we originally included the θ_{CO} equation derived by Forster in 1987 (Forster, 1987), we do not report data for this equation because it yielded highly variable, and sometimes negative, $D_{M,CO}$ and V_C values via the multiple-FiO_2 method, such that any further comparisons to those values calculated via the single-FiO_2 method were meaningless.

2.5 Calculation of $D_{M,CO}$ and V_C via the multiple-FiO_2 method

The multiple-FiO_2 method utilizes DL_{CO} measurements made at multiple oxygen tensions to calculate $D_{M,CO}$ and V_C. Multiple oxygen tensions are used in order to take advantage of the dependency of θ_{CO} on the capillary partial pressure of oxygen, $P_{cap}O_2$ (Eq. 2). Using the multiple-FiO_2 method, $1/DL_{CO}$ is plotted against $1/\theta_{CO}$ and a linear regression is fit to the points, where the area under this line can be considered the total resistance to CO transfer from the alveolar space to pulmonary-capillary blood. According to Eq. 1, the y-intercept of the regression line is equal to $1/D_{M,CO}$, or the inverse of alveolar-capillary membrane conductance, and the slope of the regression line is equal to $1/V_C$, or the inverse of pulmonary capillary blood volume. Thus, $D_{M,CO}$ is calculated as 1/y-intercept and V_C is calculated as 1/slope (Roughton and Forster, 1957). This method of determining $D_{M,CO}$ and V_C is termed the multiple-FiO_2 method and is represented graphically in Fig. 2a.

Applying this knowledge, we first calculated $1/\theta_{CO}$ at the three oxygen tensions studied (20, 40, and 60% O_2) for all seven θ_{CO} equations. Next, we plotted $1/DL_{CO}$ versus $1/\theta_{CO}$, which gives three data points for each subject for each θ_{CO} equation. We then fit a linear regression to these data points for each θ_{CO} equation and calculated $D_{M,CO}$ as 1/y-intercept and V_C as 1/slope. Values of $D_{M,CO}$ and V_C are reported as group averages for each θ_{CO} equation at rest and during exercise.

2.6 Calculations of $D_{M,CO}$ and V_C via the single-FiO2 method

The single-FiO_2 method utilizes DL_{CO} and DL_{NO} measurements made at only one oxygen concentration, presently 20% O_2, to calculate $D_{M,CO}$ and V_C (Borland and Higenbottam, 1989; Guenard et al., 1987). First, $D_{M,CO}$ was calculated as $D_{M,CO}=DL_{NO}/\alpha$-ratio. The α-ratio was used to convert $D_{M,NO}$ ($D_{M,NO} \approx DL_{NO}$) to $D_{M,CO}$ and is based on the solubility (α_B) and molecular weight (MW) of the gases, as follows:

$$\frac{D_{M,NO}}{D_{M,CO}} = \left(\frac{\alpha_{B_{NO}}}{\sqrt{MW_{NO}}} \div \frac{\alpha_{B_{CO}}}{\sqrt{MW_{CO}}}\right) = \left(\frac{\alpha_{B_{NO}}}{\alpha_{B_{CO}}} \times \sqrt{\frac{MW_{CO}}{MW_{NO}}}\right)$$

$$such\ that\ D_{M,CO} = D_{M,NO} \times (\alpha_{ratio})^{-1}$$

$$where\ \ \alpha_{ratio} = \left(\frac{\alpha_{B_{NO}}}{\alpha_{B_{CO}}} \times \sqrt{\frac{MW_{CO}}{MW_{NO}}}\right) = 1.93$$ (Meyer et al., 1990)

However, values for the α-ratio calculated directly in the laboratory setting, referred to as the experimental α-ratio, have been shown to vary substantially with values up to 2.63 reported (Hughes and Bates, 2003; Magini et al., 2013; Tamhane et al., 2001); thus clearly greater than the theoretical value of 1.93 (Borland and Cox, 1991; Hughes and Bates, 2003; Meyer et al., 1990; Tamhane et al., 2001). Thus, we calculated $D_{M,CO}$ for the single-FiO_2 method using a range of α-ratio values from 0.01-5.00 in 0.01 increments (Fig. 3); this range was determined by statistical analysis (*see 3.5 Results, "Optimization of CCC and linear regression"*). Next, $1/\theta_{CO}$ was calculated for each of seven equations (Table 1) according to Eq. 2. However, unlike the multiple-FiO_2 method, only the 20% O_2 trial ($P_{cap}O_2$ at 20%) is used for the *single*-FiO_2 method. Then, the entire range of calculated $D_{M,CO}$ values are used in Eq. 1, in combination with all seven $1/\theta_{CO}$ values at 20% O_2, in order to calculate an array of V_C values via the single-FiO_2 method (Fig. 4).

2.7 Calculation of the experimental α-ratio

An experimental α-ratio was calculated from the data for each θ_{CO} equation by combining 1) $D_{M,CO}$ calculated solely via the multiple-FiO_2 method and 2) the measured DL_{NO} value (at 20%) as follows: α-ratio=measured $DL_{NO}/D_{M,CO}$ (multiple-FiO_2 method). This calculation effectively uses both the single- and multiple-FiO_2 methods to determine an α-ratio, as opposed to using an estimated α-ratio to then calculate $D_{M,CO}$ (and V_C) without use of the multiple-FiO_2 method. Generally when the single-FiO_2 method is used in experimental studies, data is not collected in order to allow for calculation of $D_{M,CO}$ via the multiple-FiO_2 method. Therefore, it is often not feasible to directly calculate the experimental α-ratio in studies in which only the single-FiO_2 method will be used. However, because this study incorporated measurements allowing for calculation of $D_{M,CO}$ and V_C via both the multiple- and single-FiO_2 methods, the experimental α-ratio could be calculated.

2.8 Optimization of CCC and linear regression

2.8.1 Concordance Correlation Coefficient

Lin's concordance correlation coefficient (CCC) was used to compare calculated values of $D_{M,CO}$ and V_C by the single-FiO_2 to those calculated via the 'gold-standard' multiple-FiO_2 method across all subjects. The CCC is calculated as follows:

$$CCC = \frac{2 * s_{x_1 x_2}}{s^2_{x_1} + s^2_{x_2} + (\overline{X_1} - \overline{X_2})^2} \quad \text{Equation (4)}$$

In Eq. 4, sx_ix_j is the covariance between groups i and j, sx_i is the variance of group i, and X_i is the mean of group i, where the two groups are the multiple- and single-FiO_2 methods. The CCC works as a scoring system and outputs a value between 0 and 1, with 1 being perfect agreement between the two groups (Lin, 1989). Presently, the CCC was calculated in order to determine 1) which of seven θ_{CO} equations and 2) which α-ratio (used to calculate $D_{M,CO}$ values in the single-FiO_2 method) resulted in best agreement between both methods, where the $D_{M,CO}$ and V_C values calculated via the multiple-FiO_2 method were considered accurate.

First, the CCC was calculated for each of seven θ_{CO} equations across a range of α-ratios found in the literature (α-ratio = 1.90 - 2.70) (Hughes and Bates, 2003; Magini et al., 2013; Meyer et al., 1990; Tamhane et al., 2001) using both $D_{M,CO}$ and V_C values. Next, the CCC value was plotted as a function of the α-ratio for each θ_{CO} equation, yielding seven plots which used $D_{M,CO}$ values and seven plots which used V_C values, at rest and during exercise. After analyzing these plots, it was determined that a wider range of α-ratios would yield more complete information regarding the α-ratio which resulted in the highest CCC value. Therefore, the range of α-ratios used to calculated $D_{M,CO}$ (and therefore V_C) was widened and these CCC plots were recreated (α-ratio = 0.01 – 5.00, 0.01 increments). This range allowed for clear maxima in the CCC vs. α-ratio plot to be seen (see Fig. 5). The α-ratio at which the CCC was at a maximum was termed the 'CCC-optimized α-ratio' for that θ_{CO} equation, and the CCC-optimized α-ratio was determined for each

θ_{CO} equation using either $D_{M,CO}$ or V_C values at rest and during exercise. The CCC-optimized α-ratio implies that $D_{M,CO}$ or V_C values calculated via the single-FiO_2 method using that α-ratio and that θ_{CO} equation yielded the most similar results to the 'gold-standard' multiple-FiO_2 method values.

Additionally, the CCC-optimized α-ratios were compared to the group mean experimental α-ratios, calculated as DL_{NO} divided by the multiple-FiO_2 method $D_{M,CO}$. The experimental α-ratio can be used to give insight into how close the CCC-optimized α-ratio approaches the experimental value, and therefore how well the CCC-optimized α-ratio approximates the true values of $D_{M,CO}$ and V_C based on the 'gold-standard' multiple-FiO_2 method. All in all, the experimental α-ratio takes both methods into account but is mathematically different than the CCC optimization that has been performed here. In comparing the α-ratios determined experimentally and through CCC optimization of $D_{M,CO}$ and V_C values calculated from both the single- and multiple-FiO_2 methods, the validity of the CCC optimization protocol can be addressed.

2.8.2 Linear Regression

Linear regression was also performed on $D_{M,CO}$ and V_C values calculated via both the single- and multiple-FiO_2 method. The statistics obtained via linear regression allowed for analysis of specific linear fit characteristics that might have affected the CCC calculated previously. First, sets of individual subject values for $D_{M,CO}$ or V_C were plotted as single-FiO_2 method values vs. multiple-FiO_2 method values; these plots were created for all seven θ_{CO} equations for all α-ratios at rest and during exercise. Next, a linear regression was fit to the data and the following linear regression statistics were collected for each plot: slope, y-intercept (y-int), sum of squared errors (SSE), and coefficient of determination (R^2). Then, a plot was created for each of these statistics vs. the range of α-ratios for each θ_{CO} equation. Subsequently, the value of each statistic at the α-ratio which was determined to be optimal according to the CCC statistic was collected for each θ_{CO} equation.

From this set of statistics, the α-ratio that was determined to be optimal via the CCC can be further analyzed by comparing these four statistics (slope, y-int, SSE, R^2). Additionally, the best set of linear regression statistics for a given θ_{CO} equation can be compared against those for other θ_{CO} equations in order to yield the 'best' θ_{CO} equation. The best θ_{CO} equation is considered that which, in combination with its optimal α-ratio (determined via the CCC statistic), gives the slope closest to 1, y-int closest to 0, lowest SSE, and highest R^2. This combination of θ_{CO} equation and α-ratio will be optimal as these parameters signify strongest agreement between the single-FiO_2 method and the gold-standard multiple-FiO_2 method values for $D_{M,CO}$ and V_C.

3. RESULTS

3.1 Subjects

Subject characteristics and DL_{CO} and DL_{NO} measurements at rest and during exercise are shown in Table 2 and Table 3, respectively. There was a significant increase in group mean DL_{CO} and DL_{NO} from rest to exercise at all oxygen concentrations. As expected, an increase in inspired O_2 concentration was associated with a decrease in DL_{CO}. By contrast, measured DL_{NO} was independent of inspired oxygen concentration in all cases but one (rest 20% vs. rest 60%). Heart rate increased significantly from rest to exercise (Table 3).

3.2 Calculation of $D_{M,CO}$ and V_C via the multiple-FiO_2 method

An example plot for the calculation of $D_{M,CO}$ and V_C via the multiple-FiO_2 method is shown in Fig. 2a, while representative group data for the RP θ_{CO} equation is given in Fig. 2b. $D_{M,CO}$ values were significantly increased with exercise for all θ_{CO} equations, while V_C values were not significantly increased for any equation (Table 4). All equations yielded similar values to other studies for $D_{M,CO}$ and V_C (Ceridon et al., 2010; de Bisschop et al., 2012; Snyder et al., 2008; Taylor et al., 2014).

3.3 Calculation of $D_{M,CO}$ and V_C via the single-FiO_2 method

Values of $D_{M,CO}$ and V_C calculated via the single-FiO_2 method for each θ_{CO} equation are represented graphically in Fig. 3 and Fig. 4, respectively. $D_{M,CO}$ decreased with an increasing α-ratio whereas V_C increased with an increasing α-ratio. Both $D_{M,CO}$ and V_C increased with exercise for each θ_{CO} equation across all α-ratios.

3.4 Calculation of the experimental α-ratio

An experimental α-ratio was calculated for each θ_{CO} equation by combining 1) $D_{M,CO}$ calculated solely via the multiple-FiO_2 method and 2) the measured DL_{NO} value (at 20%) as follows: α-ratio=measured $DL_{NO}/D_{M,CO}$ (multiple-FiO_2 method). These experimental α-ratios are shown for each θ_{CO} equation in Table 5 both at rest and during exercise.

3.5 Optimization of CCC and linear regression

3.5.1 Concordance Correlation Coefficient

Lin's concordance correlation coefficient (CCC) was calculated for each of seven θ_{CO} equations across all α-ratios using both $D_{M,CO}$ and V_C values calculated via both methods. The resulting relationship of the CCC statistic to the α-ratio for each θ_{CO} equation is shown in Fig. 5. The α-ratio at which the CCC was at a maximum was termed the 'CCC-optimized α-ratio' for that θ_{CO} equation; these CCC-optimized α-ratios are given in Table 5. Additionally, the corresponding experimental α-ratios for each θ_{CO} equation are depicted as tick marks at the maxima of the CCC vs. α-ratio plot in Fig. 5.

At rest, the RP θ_{CO} equation gave the best agreement between $D_{M,CO}$ values calculated via the single- and multiple-FiO_2 method (CCC = 0.92) at an α-ratio of 4.44 (Table 5, Fig. 5a); there is no clear superiority of any θ_{CO} equation for similarity in the V_C values (Table 5, Fig. 5c). During exercise, the RP θ_{CO} equation gave the best agreement between $D_{M,CO}$ values calculated via the two methods (CCC=0.92, α-ratio=3.99, Table 5, Fig. 5b) but there is no clear superiority of any θ_{CO} equation for similarity in V_C values (Table 5, Fig. 5d). Additionally, the disparity between the CCC-optimized and experimental α-ratios is small, ranging from 0.01-0.16 across all θ_{CO} equations. For this reason, we are confident in the validity of the CCC optimization protocol presented here.

3.5.2 Linear Regression

Sets of individual $D_{M,CO}$ and V_C values were plotted against one another as those calculated via the single-FiO_2 method vs. multiple-FiO_2 method for each combination of θ_{CO} equation and α-ratio. A linear regression was fit to each set of data and the following variables were collected for each plot: slope, y-intercept (y-int), sum of squared errors (SSE), and coefficient of determination (R^2). The value of each of these variables at the CCC-optimized α-ratio for each θ_{CO} equation is given in Table 6.

The RP θ_{CO} equation provided the most optimal linear regression variables (i.e. slope closest to 1, y-intercept closest to 0, smallest SSE, largest R^2) at its CCC-optimized α-ratio when $D_{M,CO}$ is used as the independent variable; the θ_{CO} equation which gives the best linear regression variables when using V_C as the independent variable is inconsistent (Table 6). This suggests that, overall, the RP θ_{CO} equation, when its CCC-optimized α-ratio is applied, gives best agreement between $D_{M,CO}$ and V_C values calculated via the single- and multiple-FiO_2 methods at rest as well as during exercise.

4. DISCUSSION

This study aimed to determine both the optimal θ_{CO} equation and α-ratio to be used in the single-FiO_2 DL_{CO}/DL_{NO} method by comparing $D_{M,CO}$ and V_C values calculated via this method to those calculated via the 'gold-standard' multiple-FiO_2 method using a single breath hold technique.

4.1 Major findings

The RP θ_{CO} equation gave the best overall agreement between the single- and multiple-FiO_2 methods when using $D_{M,CO}$ as the independent variable, with a CCC of 0.92 at an α-ratio of 4.44 at rest and a CCC of 0.92 at an α-ratio of 3.99 during exercise. However, when V_C is used as the independent variable, there is no θ_{CO} equation that is clearly optimal; those with the highest CCC statistic have an α-ratio between 2.74 and 3.70 at rest and between 3.10 and 3.30 during exercise. Overall, the RP θ_{CO} equation shows best agreement when optimizing via linear regression statistics (slope, y-int, SSE, R^2); however, the θ_{CO} equation that shows the best agreement when V_C is used as the independent variable is inconsistent. Nevertheless, those equations which are considered optimal via the linear regression statistics show minimal differences between their CCC-optimized α-ratios and their experimental α-ratios. Taking all of this into account, we recommend using the RP θ_{CO} equation when calculating $D_{M,CO}$ and V_C via the single-FiO_2 single breath DL_{CO}/DL_{NO} method.

Regarding the α-ratio, our data consistently show that the CCC-optimized α-ratio is decreased during exercise, perhaps suggesting that the α-ratio should be chosen based on activity level. Additionally, the experimental α-ratio closely matches the CCC-optimized α-ratio in all cases for the RP θ_{CO} equation. Therefore, we recommend that each laboratory determine an experimental α-ratio using their equipment (α-ratio=DL_{NO}/multiple-FiO_2 $D_{M,CO}$, *see 2.7 Methods, "Calculation of the experimental α-ratio"*) and apply this to their calculation of $D_{M,CO}$ and V_C in order to ensure that accurate measures of $D_{M,CO}$ and V_C are reported.

4.2 $D_{M,CO}$ and V_C values: multiple-FiO_2 method

The $D_{M,CO}$ values calculated via the multiple-FiO_2 method in the present study (Table 4) are similar to those previously reported (Ceridon et al., 2010; de Bisschop et al., 2012; Snyder et al., 2008; Taylor et al., 2014). However, the V_C values found presently are similar to some (de Bisschop et al., 2012) but somewhat greater than other (Ceridon et al., 2010; Snyder et al., 2008; Taylor et al., 2014) previously reported data. For example, when using a rebreathe technique to assess DL_{CO} and DL_{NO}, our laboratory has previously reported group mean resting V_C values ~60 ml lower than found in the present study (Snyder et al., 2008; Taylor et al., 2014). By contrast, when implementing the same single breath hold technique as used in this study, deBisschop et al. found V_C values much more similar to those reported here (100 ml vs. 104-156 ml) (de Bisschop et al., 2012). Accordingly, it appears that the divergence between V_C values reported previously and the V_C values found in the present study is at least in part due to the technique used to assess DL_{CO} and DL_{NO}. It is well known that large changes in intrathoracic pressure during the respiratory cycle transiently influence cardiac filling and cardiac output (Charlier et al., 1974; Guz et al., 1987). The single breath hold technique used in the present study requires a relatively fast transition from residual volume to total lung capacity, thus generating a large negative pressure swing and transiently increasing venous return. An increase in venous return may increase right ventricular outflow (Charlier et al., 1974), thus potentially increasing V_C to values above that which are reported when the rebreathe technique is utilized.

While it might be expected that both $D_{M,CO}$ and V_C would show significant increases with exercise, this was only true for $D_{M,CO}$. The exact reason for this somewhat unexpected finding is unknown, but it may again be related to the single breath hold maneuver used to assess DL_{CO} and DL_{NO}. First, it is possible that subjects naturally performed a small Valsalva maneuver during the breath hold at total lung capacity, with this effect likely greater during exercise when the desire to exhale is stronger than it is at rest. If subjects were in fact naturally performing a Valsalva maneuver, the increase in intrathoracic pressure could have limited right ventricular outflow and subsequently decreased V_C during the 4 s breath hold (Guz et al., 1987; Parisi et al., 1976; Stark-Leyva et al., 2004), such that any increase in V_C with exercise was blunted. Second, as mentioned previously, the single breath hold technique requires a relatively fast transition from residual volume to total lung capacity, thus generating a large negative pressure swing and transiently increasing venous return with a subsequent increase in V_C (Charlier et al., 1974). Although speculative, it is possible that any small, exercise-induced increase in V_C may have been masked by a transient increase in V_C due to the nature of the single breath hold maneuver. Third, 80 W on an upright cycle ergometer is relatively low level exercise for young healthy males, as evidenced by the modest increase in heart rate with exercise (78 ± 11.9 bpm at rest vs. 117.5 ± 27.9 bpm during exercise). Despite no statistically significant change, group mean V_C does in fact show an upward trend with exercise in the present study (Table 4). This finding is in line with previous studies which have shown that low level exercise yields minimal increases in V_C (Ceridon et al., 2010). When changes in $D_{M,CO}$ and V_C with exercise are taken into account simultaneously, DL_{CO} increases significantly with exercise, as does DL_{NO} (Table 3).

4.3 Experimental and CCC-optimized α-ratio

The experimental α-ratio is calculated by combining 1) $D_{M,CO}$ calculated solely via the multiple-FiO_2 method and 2) the measured DL_{NO} value as follows: α-ratio=measured $DL_{NO}/D_{M,CO}$ (multiple-FiO_2 method). While the absolute difference between the experimental and CCC-optimized α-ratios are small (0.01-0.16), we found CCC-optimized α-ratios (α-ratio=3.99-4.44) that are substantially higher than other α-ratios used and calculated in previous literature, which range from ~1.90 to ~2.70 (Hughes and Bates, 2003; Magini et al., 2013; Meyer et al., 1990; Tamhane et al., 2001). The greater α-ratios observed in the present study are due to the fact that we also observed substantially greater DL_{NO} values compared to those previously reported (Ceridon et al., 2010; Magini et al., 2013; Wheatley et al., 2011), with the α-ratio effectively proportional to $DL_{NO}/D_{M,CO}$.

We are unsure of exactly why DL_{NO} assessed in our laboratory using the single breath hold technique yields greater values compared to DL_{NO} values reported previously. In two studies that found lower DL_{NO} values compared to the present study, DL_{NO} was assessed using a rebreathe method as opposed to the single breath hold technique used in the present study (Ceridon et al., 2010; Magini et al., 2013). Similar to the present study, Magini et al. assessed DL_{NO} in healthy subjects using a 2-4 s breath hold technique and reported group mean DL_{NO} to be ~110 ml/min/mmHg lower than that found presently (~89 vs. 201 ml/min/mmHg) (Magini et al., 2013). However, in the Magini et al. study, DL_{NO} measurements were made using a different experimental apparatus that utilized slightly different gas concentrations within the test gas mixture (9% vs. 14% He). In addition, two recent studies in which the same experimental apparatus and single breath hold technique were used also found somewhat lower DL_{NO} values compared to those reported presently (de Bisschop et al., 2012; Pavelescu et al., 2013); once again, however, the gas concentrations used in the test gas mixture differed between studies (Pavelescu et al.: 1600 vs. 2800 ppm CO, 8% vs. 14% He, 19% vs. 21% O_2). Nevertheless, the NO concentration used by Pavelescu et al. was the same as that used presently (40 ppm NO); therefore, it is unlikely that other differences in the test gas mixture would be responsible for the higher values of DL_{NO} reported presently.

Because the single breath hold maneuver is performed at total lung capacity, it is possible that the test gas is able to fill the pulmonary alveoli sooner and/or more completely, thus allowing for greater NO transfer into the pulmonary capillary blood via better ventilation/perfusion matching. More specifically, a maximal breath to total lung capacity will likely open more airways and fill alveoli more completely than would a tidal inspiration. While speculative, it is possible that the

NO in the test gas may be able to reach the pulmonary capillary blood more effectively, thus allowing for a greater disappearance of NO than is observed using other methods such as the rebreathe technique. All in all, although we have observed DL_{NO} values that are greater than might be expected based on previous studies, we are confident that the α-ratios determined here allow for calculation of $D_{M,CO}$ and V_C values that are comparable to those obtained via the gold-standard multiple-FiO_2 method. Therefore, the present study provides crucial data for the proper calculation of lung diffusion parameters using the single breath hold technique, a method which is increasing in popularity.

4.4 RP θ_{CO} equation

We determined that $D_{M,CO}$ and V_C values calculated via the RP θ_{CO} equation showed best agreement between the single-FiO_2 method and the 'gold-standard' multiple-FiO_2 method. The RP θ_{CO} equation was derived in 1991, when Reeves and Park (Reeves and Park, 1992) studied the rate at which CO displaces oxygen from hemoglobin. The presence of a diffusion limitation to CO in prior experiments was hypothesized to be due to a red cell membrane resistance (Roughton et al., 1957), but that idea was eventually rejected (Forster, 1987). Consequently, Reeves and Park hypothesized that this diffusion limitation actually arose from unstirred blood layers surrounding the red cells, and this notion was later supported by a mathematical model by Chakraborty et al. (Chakraborty et al., 2004). Therefore, the authors designed a novel method which would minimize this limitation. Blood was drawn from human subjects and a small amount was spread between two membranes creating a thin film of blood. A background oxygen pressure was held around the films, and a step change in CO pressure was then applied while spectrophotometry was used to measure the rate of CO uptake. From these measurements a $1/\theta_{CO}$

vs. PO_2 plot was created and fit to a linear regression, where the equation of the line is the RP θ_{CO} equation (Reeves and Park, 1992).

Taking all of this into account, there are two reasons why the RP θ_{CO} equation might give the best results here. First, the RP θ_{CO} equation removes the complication of unstirred red cell layers, making the measured uptake rates more likely to be due strictly to binding competition of CO and O_2 on hemoglobin. Second, unlike several of the other equations studied here (Table 1), the RP θ_{CO} equation uses blood directly from human subjects. This removes the uncertainty of studying uptake kinetics with non-human blood sources and the potential need to make assumptions regarding blood pH, which is the case for other θ_{CO} equations reported in the literature.

4.5 Optimization of re-breathe DL_{CO}/DL_{NO} method

In the present study, we found that the RP θ_{CO} equation yielded the best agreement between values of $D_{M,CO}$ or V_C calculated by the multiple- and single-FiO_2 methods. These calculations were performed using values of DL_{CO} and DL_{NO} determined using the single breath hold technique. Previously, our laboratory conducted a similar study that aimed to determine the optimal θ_{CO} equation and α-ratio to be used in the rebreathe method for determination of DL_{CO} and DL_{NO} (Ceridon et al., 2010). The DL_{CO}/DL_{NO} rebreathe method implements a different technique for measurement of DL_{CO} and DL_{NO} but uses the same model for its calculation of $D_{M,CO}$ and V_C (Sackner et al., 1975). Specifically, the rebreathe method requires subjects to rebreathe a gas mixture containing 9% He, 0.6% C_2H_2, 23% $C^{18}O$, 45 ppm NO, O_2 and balance N_2 for 8-10 breaths. The rate of change in expired CO levels after each breath is used to determine the rate of CO uptake and thus DL_{CO} (an identical method is used for determination of DL_{NO}).

In the previous study, DL_{CO}/DL_{NO} rebreathe maneuvers were performed at three oxygen tensions (18, 35, and 55% O_2) during rest and submaximal exercise. The same calculations presented here were performed for determination of $D_{M,CO}$ and V_C via both the single- and multiple-FiO_2 method. It was concluded that the RP θ_{CO} equation should be used as it gave the best agreement between values of $D_{M,CO}$ and V_C calculated via both methods (Ceridon et al., 2010). Values of $D_{M,CO}$ and V_C at rest and during exercise were also similar between both studies. While this study was performed on different subjects using different equipment and an entirely different technique for measurement of DL_{CO} and DL_{NO}, the conclusion regarding the best θ_{CO} equation to use was the same. This gives us further confidence that the RP θ_{CO} equation should be implemented in future studies.

4.6 Statistical analysis

Regarding the statistical analysis used in this study, it should be noted that testing for equivalence, or determining if two methods give the same value, is rather challenging. While there are several statistical tests not used here which were developed to test for equivalence, most require application of a threshold value *a priori* that is used to determine whether the difference in values between two methods falls within an 'acceptable' range. Because there is no clear method to determine the acceptable error in calculated values of $D_{M,CO}$ and V_C, we chose here to focus on the CCC statistic. The CCC statistic works like a scoring system, where the agreement between the multiple- and single-FiO_2 methods is given as a value between 0 and 1, with 1 signifying perfect agreement and 0.8-1 signifying excellent agreement (Lin, 1989). In this way, we cannot say with certainty that any θ_{CO} equation and α-ratio combination gives $D_{M,CO}$ and V_C values by both methods that are statistically the same; however, we can determine which α-ratio is strongest for each θ_{CO} equation and which θ_{CO} equation is strongest overall.

4.7 Assumption that θ_{NO} is infinite

The single-FiO_2 method calculates $D_{M,CO}$ as DL_{NO}/α-ratio under the assumption that the reaction rate of NO with hemoglobin (θ_{NO}) can be considered infinite. θ_{NO} has been cited to be between 250 and 400 times that of θ_{CO} (Borland and Higenbottam, 1989; Hakim et al., 1996), such that any resistance to NO uptake provided by hemoglobin in blood can be considered insignificant. If θ_{NO} is considered infinite, then DL_{NO} is only dependent on the resistance of the alveolar-capillary membrane and not the pulmonary-capillary blood volume.

This assumption was originally introduced by Guenard et al. (Guenard et al., 1987) and has since been challenged by others (Borland et al., 2014; Borland et al., 2010; Zavorsky, 2010). The primary evidence for the existence of a finite θ_{NO} comes from numerous studies that have found that the reaction rate of NO with free hemoglobin is up to 1250 times faster than with intact red blood cells (Carlsen and Comroe, 1958; Deonikar and Kavdia, 2010); thus, the reaction rate of NO with intact red blood cells must not be infinite. A finite value for θ_{NO} of 4.5 $(min*mmHg)^{-1}$ was first reported by Carlsen and Comroe (Carlsen and Comroe, 1958) using *in vitro* methods. Later, Borland et al. found that DL_{NO} increased *in vivo* when blood was replaced with a cell-free bovine hemoglobin glutamer-200 solution in anesthetized dogs. In this study, successive exchange transfusions were performed and an estimated value of the *in vivo* θ_{NO} was found to be

5.1 $(min*mmHg)^{-1}$, thus approaching the *in vitro* value of 4.5 $(min*mmHg)^{-1}$ (Borland et al., 2010).

Presently, we have chosen to perform our calculations using the original method introduced by Guenard et al. (Guenard et al., 1987) for three reasons. First, when we do assume a finite θ_{NO} of 4.5 $(min*mmHg)^{-1}$ we obtain unreliable values for $D_{M,CO}$. Specifically, application of a finite θ_{NO} of 4.5 $(min*mmHg)^{-1}$ results in excessively large or even negative $D_{M,CO}$ values which actually decrease with exercise in some cases (Table 7). For example, using the RP θ_{CO} equation, $D_{M,CO}$ falls significantly from -86 ml/min/mmHg at rest to -144 ml/min/mmHg during exercise. Alternatively, using the RF2.5 θ_{CO} equation, $D_{M,CO}$ falls significantly from 1203 ml/min/mmHg at rest to 716 ml/min/mmHg during exercise. Therefore, the fact that we do not obtain realistic data in humans is strong evidence that we should still treat the finite value for θ_{NO} with caution. Second, while the use of a finite θ_{NO} is perhaps gaining popularity, this change in practice remains preliminary and, more importantly, has not become common practice in physiological studies. That is, studies aimed at simply using $D_{M,CO}$ and V_C as markers of the effect(s) of a given intervention (as opposed to those studies which are performed with the sole purpose of deriving improved calculations) have not adopted use of a finite θ_{NO}. Thus, the present study offers crucial data which helps to ensure that $D_{M,CO}$ and V_C values which are calculated assuming an infinite θ_{NO} are trustworthy and consistent. Third, the calculations which incorporate a finite θ_{NO} still include two assumptions: (1) the ratio of $D_{M,NO}$ to $D_{M,CO}$ (α-ratio) and (2) the proper θ_{CO} equation to use, both of which are optimized in the current study using the calculations which assume an infinite θ_{NO}. The finite θ_{NO} calculations use a value of 2 for the α-ratio, based on the molecular weights and water solubilities of CO and NO (Meyer et al., 1990), but it has been shown multiple times that this value does not hold up experimentally in vivo (Hughes and Bates, 2003; Magini et al., 2013; Tamhane et al., 2001). Additionally, as discussed previously, it is not clear what θ_{CO} equation should be used to obtain trustworthy results for $D_{M,CO}$ and V_C. Therefore, until a value for the ratio of $D_{M,NO}$ to $D_{M,CO}$ is determined using an *in vivo* system and the literature agrees upon the proper θ_{CO} equation to use, the finite θ_{NO} calculations are not without uncertainty and variability. We fully recognize that there is increasing evidence that θ_{NO} may in fact be finite, and that as more studies are performed the calculation of $D_{M,CO}$ and V_C will likely adopt this change. That being said, the purpose of the current study was to optimize the θ_{CO} equation and α-ratio for use in calculation of $D_{M,CO}$ and V_C assuming an infinite θ_{NO}, as these calculations are still being used abundantly in important physiological studies.

4.8 Potential Clinical Significance

Generally, $D_{M,CO}$ and V_C are not calculated clinically, and only values of DL_{CO} are used to assess lung function in patients. Nevertheless, values of $D_{M,CO}$ and V_C could be advantageous in discerning disease prognosis in clinical populations. For example, Guazzi *et al.* calculated $D_{M,CO}$ and V_C in 106 stable chronic heart failure patients and concluded that $D_{M,CO}$ is a strong predictor of worse prognosis, with patients that have a $D_{M,CO} < 24.7$ ml/min/mmHg at high risk for adverse outcomes (Guazzi et al., 2002). It is likely that $D_{M,CO}$ and/or V_C carry similar significance in other diseases of the cardiopulmonary system. Therefore, it is essential that reliable values of $D_{M,CO}$ and V_C can be calculated for clinical use. While these calculations are not yet commonplace clinically, it is crucial to tease out all of the possible underlying questions before the use of $D_{M,CO}$ and V_C values potentially become standard practice. Importantly, the single breath maneuver used here is already the technique of choice in pulmonary function laboratories.

4.9 Conclusions

The RP θ_{CO} equation gives the best agreement between $D_{M,CO}$ and V_C values calculated via the single-FiO_2 method as compared to the multiple-FiO_2 method. The α-ratio corresponding to the best agreement between the single- and multiple-FiO_2 methods is variable, but consistently agrees with the experimental α-ratio, calculated as α-ratio=DL_{NO}/single-FiO_2 method $D_{M,CO}$. For this reason, each laboratory should calculate an α-ratio to be used in their laboratory with their equipment and setup.

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Figure 1: Timeline of the laboratory visit. First, a forced vital capacity (FVC) was performed. Next, subjects breathed either 20, 40, or 60% O_2. Subjects breathed this oxygen tension for about 5 minutes to ensure adequate alveolar equilibration. Next, subjects performed a DL_{CO}/DL_{NO} single-breath maneuver, followed by about 4 minutes of quiet breathing to ensure CO washout. Then, subjects performed a second DL_{CO}/DL_{NO} single-breath maneuver. This process was repeated at the remaining two oxygen concentrations (randomized and counterbalanced). Subjects were then prepped for exercise and began cycling at 80W at a pedal cadence of their choice between 60-100 rpm. The entire process was then repeated during steady-state exercise.

Figure 2: Multiple-FiO$_2$ method – RP θ_{CO} equation. The relationship between $1/DL_{CO}$ and $1/\theta_{CO}$ during rest and exercise plotted for the Reeves and Park θ_{CO} equation (Reeves and Park, 1992) for subject 1 only (A) and group mean data (B). A linear regression is fit to the data to determine the y-intercept and slope, where $D_{M,CO}$=1/y-intercept and V_C=1/slope by Eq. 1. The three data points represent values for 20, 40, and 60% oxygen, where θ_{CO} is determined by $1/\theta_{CO}=a*PO_2+b$ (Eq. 2). Horizontal and vertical errors bars signify standard deviation from the mean. $D_{M,CO}$ and V_C values for all equations are given in Table 4.

Figure 3: Single-FiO$_2$ method – $D_{M,CO}$. Group mean $D_{M,CO}$ calculated via the single-FiO$_2$ method as a function of the α-ratio during rest and exercise, where $D_{M,CO}=DL_{NO}/\alpha$-ratio. This relationship applies to all θ_{CO} equations. Vertical error bars signify standard deviation from the mean.

Figure 4: Single-FiO$_2$ method – V$_C$. Group mean V_C calculated via the single-FiO$_2$ method as a function of the α-ratio during rest (A) and exercise (B) for each of seven θ_{CO} equations. V_C is calculated via Eq. 1, where DL_{CO} is measured, $D_{M,CO}$ is calculated as $D_{M,CO}$=DL_{NO}/α-ratio (Fig. 2), and θ_{CO} is determined by $1/\theta_{CO}$=a*PO_2+b. Error bars have been left off for clarity.

Figure 5: Optimization of D$_{M,CO}$ and V$_C$ values. CCC (concordance correlation coefficient) (Lin, 1989Lin, 1989) between $D_{M,CO}$ (A, B) or V_C (C, D) values calculated via both the multiple- and single-FiO$_2$ method as a function of the α-ratio at rest (A, C) and during exercise (B, D) for each of seven θ_{CO} equations. The experimental α-ratio for each equation, calculated as DL_{NO}/multiple-FiO$_2$ method $D_{M,CO}$, is depicted as a tick mark with horizontal error bars signifying its standard deviation from the mean.

Table 1. θ_{CO} equations and their references

Equation Abbreviation	a	b	Reference	Assumptions
RF1.5	0.0058	1	(Roughton and Forster, 1957)	pH=7.8; λ=1.5
RF2.5	0.0058	0.73	(Roughton and Forster, 1957)	pH=7.8; λ=2.5
RFinf	0.0058	0.33	(Roughton and Forster, 1957)	pH=7.8; λ=∞
Hol	0.0065	1.08	(Holland, 1967)	
Stam	0.0054	0.33	(Stam et al., 1991)	pH=7.4; λ=∞
RP	0.008	0.0156	(Reeves and Park, 1992)	
Fors	0.0084	0.63	(Te Nijenhuis et al., 1996)	pH=7.4

θ_{CO}, reaction rate of CO with hemoglobin; a and b, constants in the equation $1/\theta_{CO}=a*PO_2+b$ (Eq. 2); pH, blood pH; λ, ratio of permeability of the red cell membrane to the permeability of the red cell interior.

Table 2. Subject characteristics

Characteristics			
No. of Subjects		11	
Age, y	25.3	±	2.5
Height, cm	180.7	±	6.6
Weight, kg	74.0	±	10.5
BMI, kg/m^2	22.6	±	2.5
BSA, m^2	1.9	±	0.2
Vital capacity, L	5.7	±	0.8

Values are reported as mean ± SD. BMI, body mass index; BSA, body surface area.

Table 3. Lung diffusing capacity and heart rate at each oxygen tension during rest and exercise

	Rest	Exercise
DL_{CO} (ml/min/mmHg)		
20	37.4 ± 5.8	46.9 ± 7.6*
40	28.8 ± 4.6	34.2 ± 5.5*
60	24.2 ± 4.6	28.0 ± 4.7*
DL_{NO} (ml/min/mmHg)		
20	200.8 ± 24.5	237.8 ± 36.7*
40	202.7 ± 28.0	240.2 ± 26.0*
60	209.2 ± 30.4	245.8 ± 25.3*
Heart Rate (bpm)		
20	78.0 ± 11.9	117.5 ± 27.9*
40	74.0 ± 11.5	121.2 ± 24.5*
60	75.4 ± 12.2	117.3 ± 26.0*

Values are reported as mean ± SD. DL_{CO}, lung diffusing capacity for carbon monoxide (CO); DL_{NO}, lung diffusing capacity for nitric oxide (NO). *Denotes value is significantly increased from rest to exercise ($P < 0.05$).

Table 4. $D_{M,CO}$ and V_C values calculated via the multiple-FiO_2 method

	Rest		Exercise	
	$D_{M,CO}$	**V_C**	**$D_{M,CO}$**	**V_C**
RF1.5	79.1 ± 18.1	112.5 ± 27.6	131.6 ± 35.5*	115.3 ± 23.6
RF2.5	65.1 ± 11.2	112.5 ± 27.6	97.9 ± 20.3*	115.3 ± 23.6
RFinf	52.1 ± 7.5	112.5 ± 27.6	71.8 ± 12.2*	115.3 ± 23.6
Hol	76.8 ± 16.8	126.1 ± 30.9	125.6 ± 32.4*	129.2 ± 26.5
Stam	52.7 ± 7.6	104.7 ± 25.7	72.9 ± 12.5*	107.3 ± 22.0
RP	45.1 ± 6.3	155.2 ± 38.1	59.4 ± 9.4*	159.0 ± 32.6
Fors	54.9 ± 8.1	162.9 ± 40.0	77.1 ± 13.6*	166.9 ± 34.2

Values are reported as mean ± SD. $D_{M,CO}$ (ml/min/mmHg), alveolar-capillary membrane conductance; V_C (ml), pulmonary-capillary blood volume. *Denotes value is significantly increased from rest to exercise ($P < 0.05$).

Table 5. Optimized α-ratios during rest and exercise via CCC statistic

	Rest			Exercise		
	Experimental α-ratio	**$D_{M,CO}$** *α-ratio (CCC)*	**V_C** *α-ratio (CCC)*	**Experimental α-ratio**	**$D_{M,CO}$** *α-ratio (CCC)*	**V_C** *α-ratio (CCC)*
RF1.5	2.64 ± 0.49	2.49 (0.27)	**2.74 (0.73)**	1.92 ± 0.47	1.76 (0.40)	2.00 (0.70)
RF2.5	3.14 ± 0.41	3.06 (0.54)	**3.20 (0.73)**	2.49 ± 0.39	2.41 (0.64)	2.54 (0.71)
RFinf	3.88 ± 0.30	3.84 (0.82)	3.87 (0.72)	3.34 ± 0.29	3.30 (0.85)	3.33 (0.71)
Hol	2.71 ± 0.48	2.57 (0.31)	**2.81 (0.73)**	2.00 ± 0.46	1.85 (0.44)	2.08 (0.70)
Stam	3.84 ± 0.31	3.79 (0.81)	3.83 (0.72)	3.29 ± 0.29	3.25 (0.85)	**3.28 (0.72)**
RP	4.47 ± 0.23	**4.44 (0.92)**	4.36 (0.63)	4.01 ± 0.24	**3.99 (0.92)**	3.93 (0.62)
Fors	3.69 ± 0.33	3.64 (0.77)	**3.70 (0.73)**	3.12 ± 0.31	3.07 (0.81)	**3.13 (0.72)**

Experimental α-ratio values are reported as mean ± SD. α-ratio is calculated as $DL_{NO}/D_{M,CO}$ (multiple-FiO_2 method). $D_{M,CO}$ (ml/min/mmHg), alveolar-capillary membrane conductance; V_C (ml), pulmonary-capillary blood volume; CCC, concordance correlation coefficient (Lin, 1989). Bolded values indicate the highest CCC for a given independent variable ($D_{M,CO}$ or V_C).

Table 6. Linear regression statistics at CCC-optimized α-ratios during rest and exercise

	$D_{M,CO}$				V_C			
	Rest							
	slope	**y-int**	**SSE**	**R^2**	**slope**	**y-int**	**SSE**	**R^2**
RF1.5	0.17	66.9	29.4	0.11	0.59	47.0	**48.4**	**0.56**
RF2.5	0.40	39.7	20.6	0.34	0.65	40.4	53.9	0.55
RFinf	0.68	16.8	11.0	0.70	0.80	21.4	69.5	0.52
Hol	0.20	62.7	28.0	0.14	0.60	52.0	55.2	**0.56**
Stam	0.67	17.7	11.5	0.69	0.78	21.5	63.4	0.53
RP	**0.78**	**10.1**	**6.2**	**0.87**	**1.06**	**-20.7**	133.9	0.50
Fors	0.62	20.9	13.1	0.62	0.75	40.6	93.1	0.53
	Exercise							
	slope	**y-int**	**SSE**	**R^2**	**slope**	**y-int**	**SSE**	**R^2**
RF1.5	0.26	100.4	58.3	0.22	0.58	49.2	**45.3**	0.50
RF2.5	0.49	51.1	35.3	0.46	0.62	44.3	46.9	**0.52**
RFinf	0.75	18.3	17.8	0.74	0.71	32.1	55.0	0.51
Hol	0.29	91.6	54.3	0.25	0.59	54.5	51.0	0.51
Stam	0.74	19.4	18.6	0.73	0.71	30.9	50.2	0.51
RP	**0.86**	**8.3**	**11.1**	**0.86**	**0.84**	**18.7**	106.5	0.42
Fors	0.69	24.0	21.3	0.68	0.69	52.5	75.2	**0.52**

$D_{M,CO}$ (ml/min/mmHg), alveolar-capillary membrane conductance; V_C (ml), pulmonary-capillary blood volume; y-int, y-intercept; SSE, sum of squared errors; R^2, coefficient of determination. Bolded values indicate the best statistic for a given independent variable ($D_{M,CO}$ or V_C), where 'best' implies the slope closest to 1, y-int closest to 0, smallest SSE, or highest R^2.

Table 7. $D_{M,CO}$ and V_C values assuming finite θ_{NO}=4.5 (min*mmHg)$^{-1}$

	Rest		Exercise	
	$D_{M,CO}$	**V_C**	**$D_{M,CO}$**	**V_C**
RF1.5	311.8 ± 32.0	66.5 ± 11.1	310.1 ± 66.0	87.3 ± 13.5*
RF2.5	1202.6 ± 524.7	50.4 ± 8.3	715.8 ± 337.2*	66.4 ± 10.1*
RFinf	-149.9 ± 37.6	26.5 ± 4.3	-269.8 ± 97.7*	35.3 ± 5.2*
Hol	249.5 ± 24.0	75.3 ± 12.5	260.2 ± 50.1	98.9 ± 15.3*
Stam	-120.9 ± 27.9	24.2 ± 3.9	-201.2 ± 58.6*	32.3 ± 4.7*
RP	-86.0 ± 18.8	20.4 ± 3.3	-143.5 ± 48.8*	27.7 ± 4.4*
Fors	422.5 ± 60.5	59.4 ± 9.7	390.4 ± 109.5	78.4 ± 11.7*

Values are reported as mean ± SD. $D_{M,CO}$ (ml/min/mmHg), alveolar-capillary membrane conductance; V_C (ml), pulmonary-capillary blood volume. *Denotes value is significantly different vs. rest ($P < 0.05$).

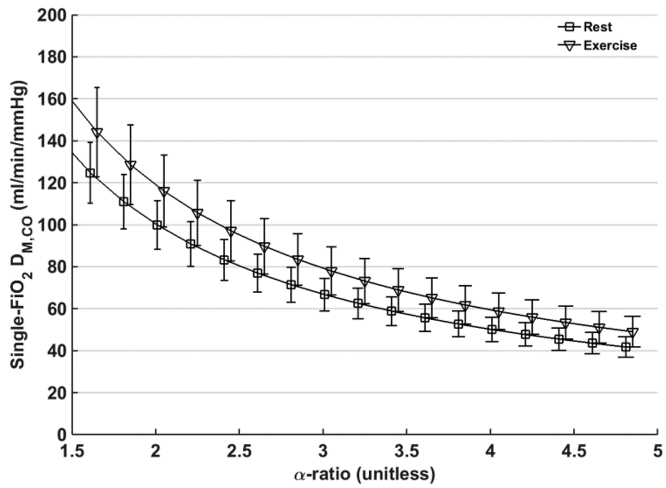
Rest
Exercise
Single-FiO2 DM,CO (ml/min/mmHg)
200
180
160
140
120
100
80
60
40
20
0
1.5
2
2.5
3
3.5
4
4.5
5
α-ratio (unitless)

Figure 1.

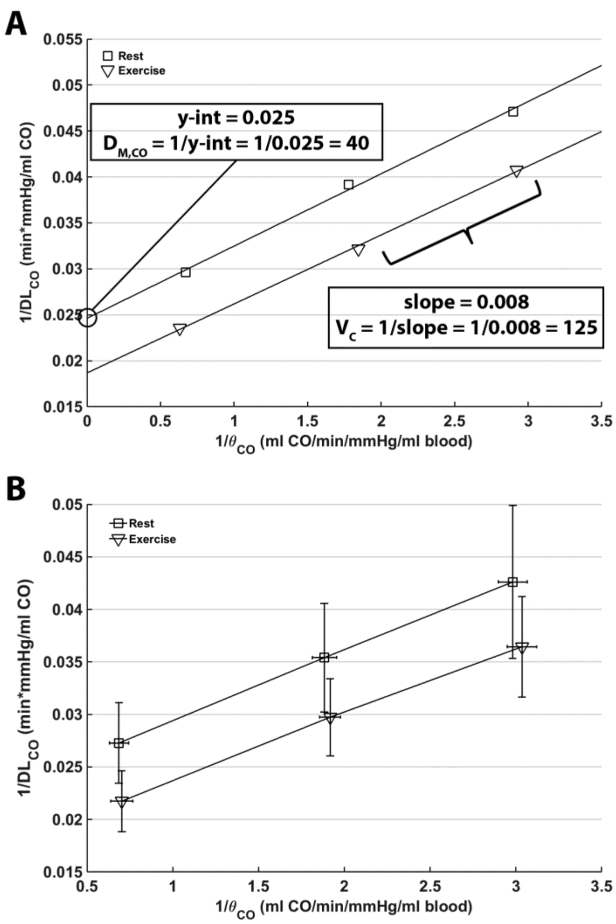
A
Rest
Exercise
y-int = 0.025
D$_{M,CO}$ = 1/y-int = 1/0.025 = 40
slope = 0.008
V$_c$ = 1/slope = 1/0.008 = 125
1/DL$_{CO}$ (min*mmHg/ml CO)
1/θ$_{CO}$ (ml CO/min/mmHg/ml blood)
B
Rest
Exercise
1/DL$_{CO}$ (min*mmHg/ml CO)
1/θ$_{CO}$ (ml CO/min/mmHg/ml blood)

Figure 2.

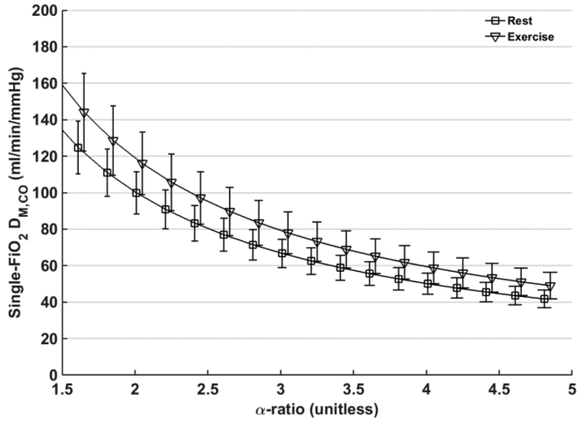
Rest
Exercise
Single-FiO2 DM,CO (ml/min/mmHg)
α-ratio (unitless)
200
180
160
140
120
100
80
60
40
20
0
1.5
2
2.5
3
3.5
4
4.5
5

Figure 3.

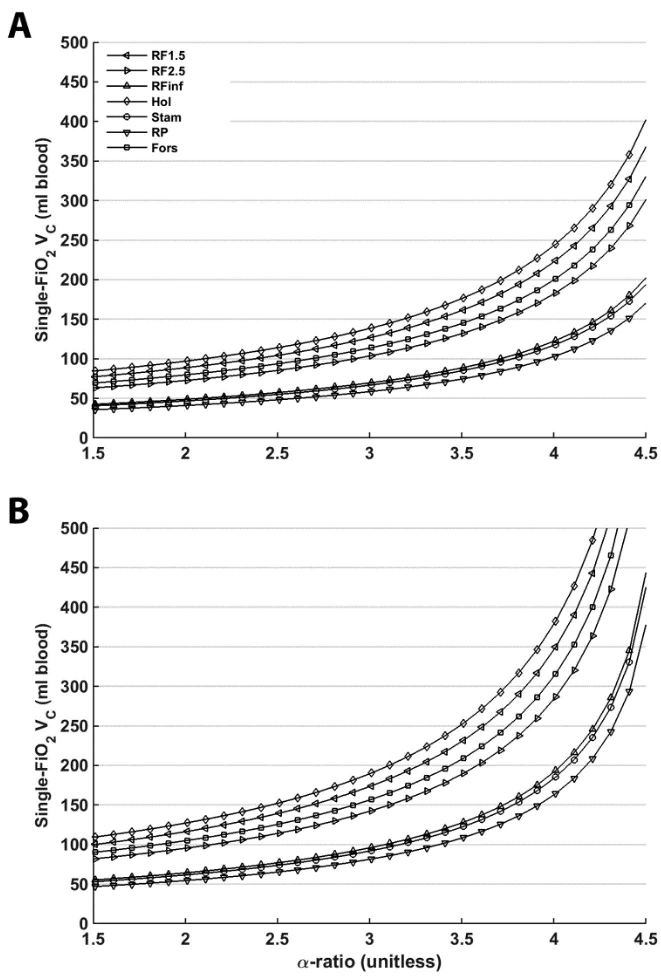
A
RF1.5
RF2.5
RFinf
Hol
Stam
RP
Fors
Single-FiO2 VC (ml blood)
B
α-ratio (unitless)

Figure 4.

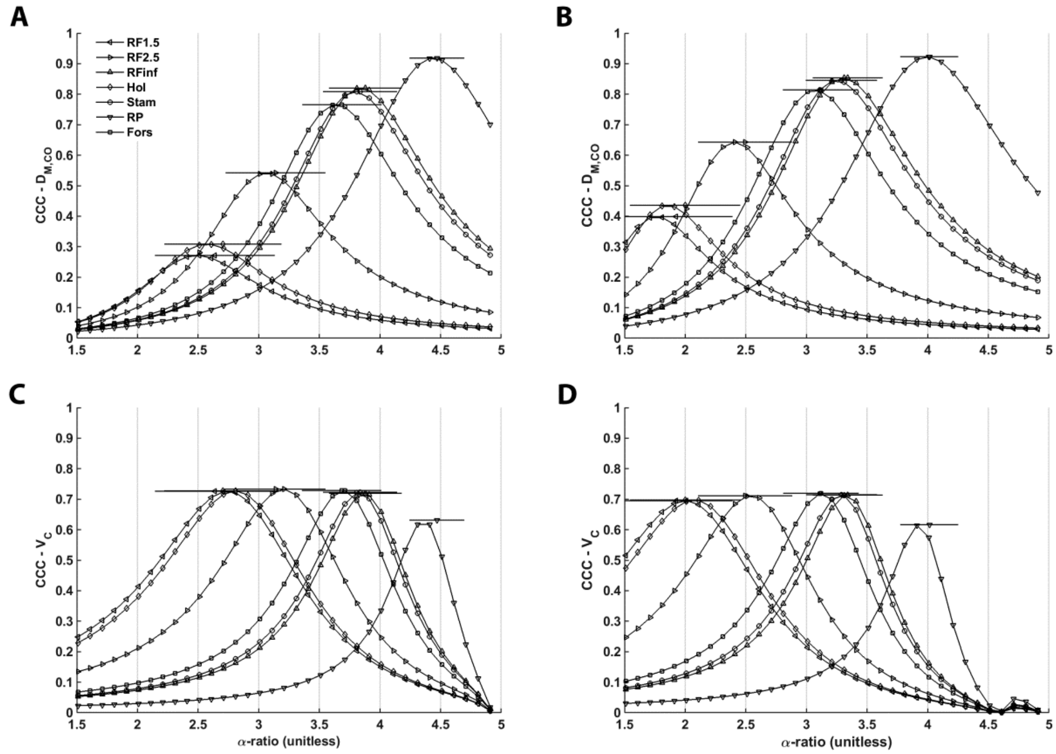
A
B
C
D
RF1.5
RF2.5
RFinf
Hol
Stam
RP
Fors
CCC - $D_{M,CO}$
CCC - V_C
α-ratio (unitless)

Figure 5.