UNIVERSITY OF LEEDS

This is a repository copy of *The blood transfer conductance for nitric oxide: infinite vs. finite θNO*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/109866/

Version: Accepted Version

Article:
Coffman, KE, Chase, SC, Taylor, BJ orcid.org/0000-0001-5229-941X et al. (1 more author) (2017) The blood transfer conductance for nitric oxide: infinite vs. finite θNO. Respiratory Physiology & Neurobiology, 241. pp. 45-52. ISSN 1569-9048

https://doi.org/10.1016/j.resp.2016.12.007

© 2016 Published by Elsevier B.V. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International
http://creativecommons.org/licenses/by-nc-nd/4.0/

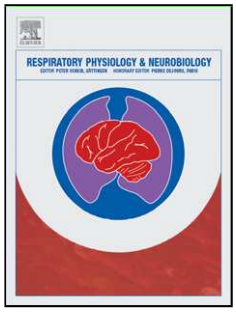
Reuse
Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

White Rose university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

Accepted Manuscript



Title: The blood transfer conductance for nitric oxide: infinite vs. finite θ_{NO}

Author: Kirsten E. Coffman Steven C. Chase Bryan J. Taylor Bruce D. Johnson

PII: S1569-9048(16)30342-1
DOI: http://dx.doi.org/doi:10.1016/j.resp.2016.12.007
Reference: RESPNB 2738

To appear in: *Respiratory Physiology & Neurobiology*

Received date: 9-10-2016
Revised date: 16-12-2016
Accepted date: 19-12-2016

Please cite this article as: Coffman, Kirsten E., Chase, Steven C., Taylor, Bryan J., Johnson, Bruce D., The blood transfer conductance for nitric oxide: infinite vs.finite θNO.Respiratory Physiology and Neurobiology http://dx.doi.org/10.1016/j.resp.2016.12.007

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

The blood transfer conductance for nitric oxide: infinite vs. finite θ_{NO}

Authors
Kirsten E. Coffman[1], Steven C. Chase[1], Bryan J. Taylor[2], Bruce D. Johnson[3]

Author Affiliations
[1]Mayo Graduate School, Mayo Clinic, 200 1st St SW, Rochester, MN, USA
[2]Faculty of Biological Sciences, School of Biomedical Sciences, University of Leeds, UK
[3]Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic, 200 1st St SW, Rochester, MN, USA

Contact Information for Corresponding Author
Bruce D. Johnson, PhD
Professor of Medicine and Physiology
Division of Cardiovascular Diseases
Human Integrative and Environmental Physiology Laboratory
Mayo Clinic
200 1st Street SW
Rochester, MN 55902
Phone: 507-284-4375
E-mail: Johnson.Bruce@mayo.edu

Highlights

- We discuss whether θ_{NO} is finite or infinite
- We calculate Dm_{CO} and V_C under both assumptions at rest and during exercise
- We find that a finite θ_{NO} yields unexpected submaximal exercise results
- We conclude that a finite θ_{NO} should be validated in vivo prior to further use

ACCEPTED MANUSCRIPT

ABSTRACT

Whether the specific blood transfer conductance for nitric oxide (NO) with hemoglobin (θ_{NO}) is finite or infinite is controversial but important in the calculation of alveolar capillary membrane conductance (Dm_{CO}) and pulmonary capillary blood volume (V_C) from values of lung diffusing capacity for carbon monoxide (DLCO) and nitric oxide (DLNO). In this review, we discuss the background associated with θ_{NO}, explore the resulting values of Dm_{CO} and V_C when applying either assumption, and investigate the mathematical underpinnings of Dm_{CO} and V_C calculations. In general, both assumptions yield reasonable rest and exercise Dm_{CO} and V_C values. However, the finite θ_{NO} assumption demonstrates increasing V_C, but not Dm_{CO}, with submaximal exercise. At relatively high, but physiologic, DLNO/DLCO ratios both assumptions can result in asymptotic behavior for V_C values, and under the finite θ_{NO} assumption, Dm_{CO} values. In conclusion, we feel that the assumptions associated with a finite θ_{NO} require further in vivo validation against an established method before widespread research and clinical use.

Keywords

Lung diffusing capacity, alveolar capillary membrane conductance, pulmonary capillary blood volume, exercise, in vivo validation

ACCEPTED MANUSCRIPT

1. INTRODUCTION

There remains significant uncertainty as to the correct specific blood transfer conductance for nitric oxide (NO) with hemoglobin (θ_{NO}) when studying lung diffusing capacity even in light of the ERS task force findings (Zavorsky et al., 2017). Specifically, there is currently a debate as to whether to assume an infinite or finite θ_{NO} when calculating alveolar-capillary membrane conductance (Dm_{CO}) and pulmonary-capillary blood volume (V_C) from measures of lung diffusing capacity for CO (DLCO) and NO (DLNO). While our laboratory has consistently held the original assumption that θ_{NO} is effectively infinite, other groups have begun applying a finite value for θ_{NO} of 4.5 ml_{CO}/min/mmHg/ml_{blood} measured via an in vitro study and supported by animal and human studies not designed to calculate an exact θ_{NO} value (Borland and Cox, 1991; Borland et al., 2010; Guenard et al., 2016; Zavorsky et al., 2014). Based on this limited work, a task force has recently recommended the use of a finite θ_{NO}; however, we believe these recommendations, including those concerning θ_{NO} and also the correct Dm_{NO}/Dm_{CO} ratio, are premature (Zavorsky et al., 2017). In this manuscript, we will begin by summarizing the evolution of, and the scientific reasoning behind, the original assumption that θ_{NO} is effectively infinite. Next, using both published and preliminary data from our laboratory, we will show in detail the effect of assuming an infinite θ_{NO} vs. using a finite value of 4.5 ml_{CO}/min/mmHg/ml_{blood} on calculation of Dm_{CO} and V_C values in vivo in humans. We will do this for lung diffusing capacity data collected using different techniques (i.e. rebreathe and single-breath) and in different populations (i.e. healthy and heart failure, young and old age) at rest and during exercise. Furthermore, we will extend these findings to an investigation of the

ACCEPTED MANUSCRIPT

mathematical limits within which each assumption yields reasonable, physiologic values for Dm_{CO} and V_C; i.e., positive values on the same order of magnitude as values previously reported in the literature. Finally, we will discuss the implications of our findings and offer insight into future research, as well as clinical practice, regarding the use of an infinite versus a finite θ_{NO} in the calculation of Dm_{CO} and Vc in vivo in humans. As we will show, both assumptions yield reasonable values for Dm_{CO} and V_C at rest. However, the assumption that θ_{NO} is finite can yield values which do not increase with submaximal exercise; this is clearly a concern, as Dm_{CO} and V_C would be anticipated to increase during the exercise levels included in these data. Additionally, using a finite θ_{NO} requires more assumptions during calculations (α/Krogh coefficient, θ_{CO} equation, θ_{NO}) than applying an infinite θ_{NO} (α/Krogh coefficient, θ_{CO} equation). As such, while the use of a finite θ_{NO} value may have merit, we urge caution in its application for the calculation of Dm_{CO} and Vc in humans until the method can be properly validated against the multiple O_2 tension method. Overall, we feel that it is more important to interpret Dm_{CO} and V_C as physiologic variables rather than anatomical measurements and to interpret changes over time or between groups rather than to focus on the specific method used.

1.1 Theory and evidence for an infinite vs. finite θ_{NO}

In our laboratory, we use two methods for the determination of DLCO: 1) a single breath technique, and 2) a rebreathe technique. The single breath technique for measuring DLCO was first described in 1909 (Krogh and Krogh, 1910; Krogh, 1915), but the methodology currently in common use was established in 1954 (Forster et al., 1954). This technique requires the participant to take a deep inspiration from residual volume to total lung capacity of a test gas containing approximately 10% helium, 0.3% CO, 21% oxygen, and balance nitrogen, followed

ACCEPTED MANUSCRIPT

by a short breath hold (typically 4 to 10 seconds), with a subsequent swift expiration. Simply put, the ratio of inspired to expired CO concentrations, after taking into account dead space and alveolar mixing, yields the diffusing capacity of the lungs for CO (DLCO). The rebreathe technique used in our laboratory to measure DLCO requires participants to rebreathe from a bag containing 9% Helium, 0.3% $C^{18}O$, 35% O_2, and balance nitrogen for 8 to 10 tidal breaths (Ceridon et al., 2010; Meyer et al., 1990; Sackner et al., 1975). The assumptions of the rebreathe technique are identical to that of the single breath method. However, the rebreathe technique often yields lower values for Dm_{CO} and V_C; while this inconsistency is not completely understood, it is not the focus of the manuscript and hence, we will separate results for both techniques for clarity.

In 1957, Roughton and Forster extended this technique and established a method for determining Dm_{CO} and V_C by measuring DLCO at multiple O_2 concentrations (Roughton and Forster, 1957). In short, because CO and hemoglobin competitively bind hemoglobin, a higher partial pressure of oxygen (PO_2) in the pulmonary capillaries yields a lower DLCO. Taking advantage of this, it is then possible to separate the contribution of resistances to gas transfer due to both the alveolar capillary membrane (Dm) and the pulmonary capillary blood volume (Vc) by graphing 1/DLCO against $1/\theta_{CO}$ and fitting the resulting points. Specifically, Dm_{CO} will be equal to the inverse of the y-intercept and V_C will be equal to the inverse of the slope of the regression line, according to the following equation developed by Roughton and Forster (Roughton and Forster, 1957):

$$\frac{1}{DLCO} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} * V_C}$$

ACCEPTED MANUSCRIPT

However, this method is less than ideal, as it is not only time-consuming, but also requires the assumption that measurements of DLCO separated by multiple minutes represent an identical physiologic state unaffected by varying oxygen tensions, i.e. the same CO distribution throughout the lung during breath hold and the same cardiac output during the maneuver. Despite these shortcomings, this multiple-O_2 tension method is considered the 'gold standard' for the calculation of Dm_{CO} and V_C.

In the late 1980's, a method was developed in which DLNO is measured simultaneously with DLCO by the addition of 40 ppm NO to the gas mixture and has quickly become the technique of choice as it removes the issues described above associated with the multiple O_2 tension method (Borland and Higenbottam, 1989; Guenard et al., 1987). The theory behind this method is that the rate of reaction of NO with hemoglobin in vitro is extremely rapid, approximately two orders of magnitude greater than θ_{CO} (Borland and Higenbottam, 1989; Hakim et al., 1996).. Furthermore it is assumed that the extremely rapid reaction of NO with hemoglobin extends to the intact red cell in vivo. In this way, the resistance to NO transfer from the alveoli onto hemoglobin can be assumed to be only dependent on alveolar capillary membrane resistance and independent of the volume of pulmonary capillary blood present. This technique simplifies calculation of Dm_{CO} and V_C, as it does not require tests to be performed at several oxygen tensions, therefore removing the necessity of assuming that the physiologic state is the same across multiple trials and also shortening the time required to obtain data (detailed calculations are described in sections 2.1-2 "Assumptions and Calculations").

More recently, however, it has been argued that the assumption that θ_{NO} is infinite is not valid. Indeed, it has been demonstrated by multiple groups that the specific blood transfer conductance

ACCEPTED MANUSCRIPT

for NO with free hemoglobin is anywhere from 100 to over 1000 times faster than that with red blood cells, suggesting that the red blood cell does in fact have a meaningful resistance to the diffusion of NO. These studies have been completed in several animal species and using various methods (Azarov et al., 2011; Borland et al., 2006; Borland et al., 2010; Carlsen and Comroe, 1958; Deonikar and Kavdia, 2010; Liu et al., 1998; Vaughn et al., 2000). Accordingly, it has been argued by some researchers that θ_{NO} cannot, by definition, be infinite as a red cell resistance does in fact exist. Indeed, several in vitro studies find evidence for a finite specific blood transfer conductance for NO (Azarov et al., 2011; Sakai et al., 2008); however, these studies do not provide evidence as to the appropriate value for in vivo experiments. Furthermore, the in vivo value recently recommended for θ_{NO} by the ERS task force is based on only three papers. Each of these papers has limitations; either the studies were performed in vitro or in animals, or the study has a large degree of variability in the value obtained (for further discussion, see section 3.3 "Required assumptions/problems of each method") (Borland et al., 2010; Carlsen and Comroe, 1958; Guenard et al., 2016). Importantly, we recognize that it is likely impossible to directly measure θ_{NO} in vivo in humans, and as such these studies are meaningful first steps in determining the value of θ_{NO} (currently suggested to be 4.5 $ml_{CO}/min/mmHg/ml_{blood}$). However, we feel that this θ_{NO} value needs to be validated and/or optimized with respect to a method that does not utilize NO as a means to gain confidence in its application\.

So, the question becomes: should θ_{NO} be considered infinite or finite in the calculation of Dm_{CO} and V_C? In order to investigate the outcomes of both methods, we have calculated Dm_{CO} and V_C while applying both a finite and infinite θ_{NO} for over 750 DLCO and DLNO measurements from

ACCEPTED MANUSCRIPT

our laboratory. What follows includes an overview of these calculations, details on the dataset used, and the outcomes of this analysis.

2. METHODS

2.1 Assumptions and Calculations - Infinite θ_{NO}

As we and others have demonstrated previously, the calculation of Dm_{CO} and V_C when assuming that θ_{NO} is infinite is critically dependent on two key considerations. First, it is essential that the correct equation for calculating θ_{CO} is chosen. Second, the ratio of Dm_{NO} to Dm_{CO} (termed α) must be established. While there exists a theoretical value for α that is based on the molecular weights and solubilities in water of both NO and CO (termed the Krogh coefficient), numerous studies utilizing an infinite θ_{NO} have experimentally determined that α is actually greater than 1.97 (Magini et al., 2013; Tamhane et al., 2001). Accordingly, we have previously experimentally determined the best θ_{CO} equation and α value to be used, for both the rebreathe and single breath techniques (Ceridon et al., 2010; Coffman et al., 2016), by systematically comparing the resulting Dm_{CO} and V_C values against those obtained via the original multiple O_2 tension method. From both of these studies, our laboratory has concluded that the θ_{CO} equation described by Reeves and Park is ideal, while the optimal α value is dependent on the technique used; ~2.26 for rebreathe and ~4.40 for single breath. This difference can be understood by recognizing that the single breath method, in our laboratory and others, often yields DLNO values somewhat higher than the rebreathe method (Ceridon et al., 2010; Ceridon et al., 2011; Coffman et al., 2016; Snyder et al., 2007; Zavorsky et al., 2014; Zavorsky and Murias, 2006; Zavorsky et al., 2004). Of note, while this inconsistency in DLNO values between the two methods in not completely understood, it is not the focus of this manuscript. Importantly, the

ACCEPTED MANUSCRIPT

chosen α value for each method ensures that calculated Dm_{CO} values are in agreement with those obtained via the original multiple O_2 tension method, which does NOT rely on measures of DLNO. Details of the calculation of Dm_{CO} and V_C, under the assumption that θ_{NO} is effectively infinite, are as follows:

$$\frac{1}{DLNO} = \frac{1}{Dm_{NO}} + \frac{1}{\theta_{NO} * V_C}$$

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

$$\frac{1}{DLNO} = \frac{1}{Dm_{NO}} + \frac{1}{\infty}$$

$$DLNO = Dm_{NO}$$

$$Dm_{CO} = \frac{Dm_{NO}}{\alpha} = \frac{DLNO}{\alpha}$$

$$where\ \alpha = 2.26\ (rebreathe)\ or\ \alpha = 4.40\ (single\ breath)$$

$$\frac{1}{DLCO} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} * V_C}\ so\ that\ V_C = \frac{1}{\theta_{CO}} * \left(\frac{1}{DLCO} - \frac{1}{Dm_{CO}}\right)^{-1}$$

$$where\ \frac{1}{\theta_{CO}} = 0.008 * PO_2 + 0.0156 \qquad \text{(Reeves and Park, 1992)}$$

2.2 Assumptions and Calculations - Finite θ_{NO}

When calculating Dm_{CO} and V_C using a finite θ_{NO}, three assumptions must be established. First, the correct θ_{CO} equation must be determined; Zavorksy et al. have chosen to use the equation reported by Guenard in 2016 (Forster, 1987; Guenard et al., 2016). Second, the α ratio must be determined; most groups that assume a finite θ_{NO} have chosen to use an approximation of the Krogh coefficient, equal to 2. This Krogh coefficient, and the α ratio used under the infinite θ_{NO} assumption, are similar, as they both convert Dm_{NO} to Dm_{CO}. The relationship of α and the

ACCEPTED MANUSCRIPT

Krogh coefficient and why α differs between the methods is discussed later (see Fig. 4, as well as section 3.3 "Required assumptions/problems of each method"). Third, the value for θ_{NO} must be chosen; θ_{NO} is currently set to a value of 4.5 $ml_{CO}/min/mmHg/ml_{blood}$ (see section 3.3 "Required assumptions/problems of each method*"* for more details) (Carlsen and Comroe 1958). Details of the calculation of Dm_{CO} and V_C, under the assumption that θ_{NO} is finite, are as follows:

$$\frac{1}{DLCO} = \frac{1}{Dm_{CO}} + \frac{1}{\theta_{CO} * V_C}$$

$$\frac{1}{DLNO} = \frac{1}{Dm_{NO}} + \frac{1}{\theta_{NO} * V_C}$$

$$Krogh\ coefficient = 2\ such\ that\ Dm_{CO} = \frac{Dm_{NO}}{2}$$

$$rearranging;\ Dm_{NO} = \frac{\theta_{NO} - 2 * \theta_{CO}}{\frac{\theta_{NO}}{DLNO} - \frac{\theta_{CO}}{DLCO}}\ where\ \theta_{NO} = 4.5$$

$$Dm_{CO} = \frac{Dm_{NO}}{2}$$

$$V_C = \frac{1}{\theta_{CO}} * \left(\frac{1}{DLCO} - \frac{1}{Dm_{CO}}\right)^{-1}$$

$$where\ \frac{1}{\theta_{CO}} = 0.0062 * PO_2 + 1.16 \quad \text{(Guenard et al., 2016)}$$

3. RESULTS

3.1 Effect of infinite vs. finite θ_{NO} assumption on actual data

In order to determine the effect of a finite vs. infinite θ_{NO} on calculated values of Dm_{CO} and V_C, the data discussed next includes over 750 observations (an observation is a single, simultaneous measurement of DLCO and DLNO on a study participant) from our laboratory using both the

ACCEPTED MANUSCRIPT

rebreathe and single breath methods, from which we have calculated Dm_{CO} and V_C using both the infinite and finite θ_{NO} assumptions described above (see sections 2.1-2 "Assumptions and Calculations"). Table 1 details the source of these data points; this includes data from healthy and heart failure participants, younger and older individuals, and rest as well as submaximal or incremental exercise trials. Therefore, these data span multiple variations in potential values of DLCO and DLNO, as well as Dm_{CO} and V_C (Table 2).

Figure 1 and Figure 2, as well as Table 2, show the median and distribution for Dm_{CO} and V_C values calculated assuming an infinite θ_{NO} and a finite θ_{NO} using a rebreathe (Table 2, Figure 1) or single breath (Table 2, Figure 2) technique. Using the rebreathe method (Figure 1), both the infinite and finite θ_{NO} assumptions yield Dm_{CO} and V_C values that qualitatively appear to be grouped in a physiological range and incorporate very few outliers. However, the Dm_{CO} values are statistically higher using the finite vs. infinite assumption. Quantitatively, the means, standard deviations, and number of outliers (defined as ± 2.7 standard deviations from the mean), are as follows: for Dm_{CO}: Infinite Dm_{CO}, 41.2 ± 17.7 ml/min/mmHg, 4 outliers; Finite Dm_{CO}, 63.8 ± 31.1 ml/min/mmHg, 12 outliers (Infinite vs. Finite, $p < 0.001$); for V_C: Infinite V_C, 85.8 ± 46.3 ml, 14 outliers; Finite V_C, 89.8 ± 43.3 ml, 6 outliers (Infinite vs. Finite, $p = 0.111$). Using the single breath method (Figure 2), the findings are similar; Dm_{CO} and V_C under both an infinite and finite θ_{NO} assumption are grouped in a physiologic range with few outliers. However, the mean Dm_{CO} values are statistically higher, and the V_C values are statistically lower, using the finite vs. infinite assumption. The means, standard deviations, and number of outliers for the single breath method are as follows: for Dm_{CO}: Infinite Dm_{CO}, 49.5 ± 8.4 ml/min/mmHg, 3

ACCEPTED MANUSCRIPT

outliers; Finite Dm_{CO}, 281.7 ± 81.5 ml/min/mmHg, 3 outliers ($p < 0.001$); for V_C: Infinite V_C, 223.2 ± 141.2 ml, 5 outliers; Finite V_C, 82.5 ± 18.7 ml, 2 outliers ($p < 0.001$).

Such a difference in results between the finite and infinite assumption may complicate the ability to implement measures of Dm_{CO} and V_C clinically. This is in contrast to the research environment, where we feel that the absolute values are of less importance, whereas the ability to observe changes between research groups or after an intervention is of the utmost importance. However, in order to implement Dm_{CO} and V_C clinically, a single method to be implemented across the entire practice would simply need to be chosen. We discuss this idea further later (see section 4.3 "Conclusions").

3.2 Exercise responses

In healthy humans, exercise is associated with an increase in cardiac output and pulmonary perfusion pressure that causes both recruitment of under-perfused pulmonary capillaries and distension of already perfused pulmonary blood vessels, as evidenced by an increase in DLCO, Dm_{CO}, and V_C (La Gerche et al., 2010; Tamhane et al., 2001; Taylor et al., 2014). Therefore, it is crucial that measures of Dm_{CO} and Vc increase accordingly in response to exercise, regardless of whether θ_{NO} is assumed to be infinite or finite.

We have simultaneously assessed DLCO and DLNO using the single-breath technique at rest and during submaximal exercise in two separate studies in our laboratory (Coffman et al., 2016). In the first study, DLCO and DLNO were measured in duplicate at rest and during cycle exercise at 80W in 11 healthy subjects (Table 1, #5). In the second study, DLCO and DLNO were again

ACCEPTED MANUSCRIPT

measured in duplicate at rest and during cycle exercise at an intensity designed to elicit a doubling of resting cardiac output and at least ~70% of age predicted heart-rate maximum. This study was performed in 8 healthy subjects (Table 1, #6). For the present analyses, the data from both studies were pooled, yielding a mean submaximal workload of 80 W, range 40 – 130 W.

In order to determine if Dm_{CO} and V_C significantly increased with submaximal exercise, we performed a student's paired t-test on the data calculated under both infinite and finite θ_{NO} assumptions. Table 3 shows that, when assuming an infinite θ_{NO}, Dm_{CO} and V_C increased significantly with submaximal exercise, as would be expected (Dm_{CO} at rest 45.6 ± 6.5 vs. exercise 53.7 ± 8.2 ml/min/mmHg, $P < 0.001$; V_C at rest 162.6 ± 68.6 vs. exercise 288.9 ± 168.9 ml, $P < 0.001$). On the other hand, only V_C significantly increased under the finite θ_{NO} assumption, whereas Dm_{CO} was statistically unchanged (Dm_{CO} at rest 279.1 ± 95.4 vs. exercise 284.5 ± 64.2 ml/min/mmHg, $P = 0.498$; V_C at rest 72.7 ± 12.9 vs exercise 93.1 ± 18.3 ml, $P < 0.001$). This is clearly concerning, as Dm_{CO} is expected to rise in concert with Vc with increasing exercise intensity. Thus, the lack of response suggests a likely flaw with the determination of these values when assuming a finite θ_{NO} (Lewis et al., 1958; Tamhane et al., 2001).

We have also simultaneously assessed DLCO and DLNO using the rebreathe technique at rest and during incremental exercise in two separate studies in our laboratory. In the first study, DLCO and DLNO were measured at rest and during cycle exercise at 0, 10, 15, 30, 50, and 70% of Wpeak (determined during a maximal exercise test at a prior study visit). This study was performed in 7 healthy subjects (Table 1, #3). In the second study, DLCO and DLNO were measured in duplicate at rest and during cycle exercise at 25, 50, 75, and 90% of Wpeak

ACCEPTED MANUSCRIPT

(determined during a maximal exercise test at a prior study visit). This study was performed in 31 healthy subjects (Table 1, #4). For the present analysis, the data from both studies were pooled, and Dm_{CO} or V_C was then plotted as a function of workload. Because these data incorporate repeat measures, a linear mixed effects model was used to separate the individual and group effects on either Dm_{CO} or V_C with exercise. The group effect was then plotted for both variables under the infinite and finite assumptions (Figure 3). Whether an infinite or a finite θ_{NO} is assumed, Dm_{CO} and V_C significantly increased with increasing workload (all $P < 0.001$). All in all, this suggests that assuming both an infinite or finite θ_{NO} yields statistically significant increases in Dm_{CO} and V_C with incremental exercise when using the rebreathe method, as would be anticipated.

3.3 Required assumptions/problems of each method

Both the finite and infinite methods for calculating V_C and Dm_{CO} from DLCO and DLNO rely on several values that likely impossible to measure directly in vivo in humans. The infinite method has two assumptions that must be optimized, the θ_{CO} coefficients and α, while the finite method has the additional term, θ_{NO}. Because these values cannot be directly measured, our laboratory has taken the approach of systematically verifying these terms using the gold-standard multiple O_2 tension method, which does not rely on measures of DLNO. By contrast, this rigorous optimization does not appear to have been done for the finite method.

Both methods are very sensitive to the values chosen for the α ratio/Krogh coefficient, θ_{NO}, and θ_{CO} coefficients. While the determination of the optimal θ_{CO} coefficients is essential to the use of either method, the following section will focus on the α ratio/Krogh coefficient. When θ_{NO} and

ACCEPTED MANUSCRIPT

θ_{CO} are required in the theoretical discussion going forward, we will use the values that have either been determined as optimal by our group for the infinite assumption, or the values currently used in the literature for the finite assumption.

The Krogh coefficient used with the finite assumption and the α ratio used with the infinite assumption are very similar, as they both convert in Dm_{NO} to Dm_{CO}. Here, we compare the values used by each assumption and explore their relationship. The Krogh diffusion constant for CO versus NO is 1.97 in water. However, studies in our laboratory and others have suggested that a value greater than 1.97 should be used when converting Dm_{NO} to Dm_{CO} (Magini et al., 2013; Tamhane et al., 2001). For this reason, we are wary of the conclusion by the recent task force that a value of 1.97 should be utilized (Zavorsky et al., 2017). Below, we have calculated an 'effective α' for the finite method (see below) which can be used to directly convert DLNO to Dm_{CO} as the α ratio does for the infinite method. The value of this 'effective α' is shown in Figure 4 over a range DLNO/DLCO ratios.

$$\alpha_{eff} = 2 * \left(\frac{DL_{NO}}{\theta_{NO} - 2\theta_{CO}} * \left(\frac{\theta_{NO}}{DL_{NO}} - \frac{\theta_{CO}}{DL_{CO}} \right) \right)$$

This effective α is less than 1.97 for most DLNO/DLCO ratios using the finite method. The lower 'effective α' of the finite method represents the resistance to the transfer of NO through the red blood cell that is not taken into account by the infinite method. Some have argued that it is inappropriate to vary α as it is a chemical property based on the solubility of NO and CO. However, others have shown that α should be greater than 1.97 and have suggested that confounders such as uptake by the airway epithelium, conversion of NO to N_2O, or differences

ACCEPTED MANUSCRIPT

in solubility of NO and CO in biological tissues may have a larger effect on DLNO than red blood cell resistance (Tamhane et al., 2001). Thus, we feel that it is appropriate for the α ratio to vary away from the Krogh coefficient when performing a systematic optimization.

4. DISCUSSION

The determination of θ_{NO} has been chosen based on three studies. First was an in vitro study from 1958 determining the second order rate constant of NO with red blood cells(Carlsen and Comroe, 1958). Later, the value of 4.5 ml_{CO}/min/mmHg/ml_{blood} was determined from this earlier study (Borland and Cox, 1991). A major methodological concern with this method is that the concentration of NO used was very high relative to that used for DLNO measurements, possibly altering the reaction kinetics and underestimating the rate constant observed in vivo (see CD Borland, this issue, *Hypothesis: Why* θ_{NO} could be finite in vitro but infinite in vivo). Second, a 2010 study in dogs where oxyglobin was exchanged with red blood cells found an increase in DLNO with progressively greater exchange (Borland et al., 2010). These results suggest that θ_{NO} is finite, but the study was not designed to calculate the actual value. Finally, a 2016 study in humans measured DLCO and DLNO while participants breathed 15% and 21% oxygen and attempted to estimate θ_{NO}; however, this study was not designed to precisely calculate θ_{NO} and therefore only suggests the continued use of the 4.5 ml_{CO}/min/mmHg/ml_{blood} value (Guenard et al., 2016). Based on only these three studies, a recent task force has recommended use of a finite θ_{NO} for calculation of Dm_{CO} and V_C (Zavorsky et al., 2017). Furthermore, to the best of our knowledge, there have been no studies optimizing θ_{NO} to the multiple O_2 tension method, a method which does not utilize NO, or any other method. Therefore, we suggest that further

ACCEPTED MANUSCRIPT

validation is necessary to determine the correct values for θ_{NO}, as well as θ_{CO} coefficients, before the finite method enters standard practice.

4.1 Theoretical breaking points for each assumption

In our analysis, both the infinite and finite θ_{NO} assumptions yielded extreme outliers in a total of four instances (approximately 0.5% of all observations). Though we removed these outliers from further analysis, this finding led us to ask under which circumstances each assumption would fail to yield reasonable physiologic values for Dm_{CO} and V_C. Figures 5-6 demonstrates the values that would be obtained for Dm_{CO} (Figure 5) and V_C (Figure 6) over a range of DLNO/DLCO values for both methods assuming PO_2 of 80 mmHg, 100 mmHg, and 120 mmHg, DLCO of 20 $ml*min^{-1}*mmHg^{-1}$ and a DLCO/DLNO ratio of up to 10.

For Dm_{CO}, assuming an infinite θ_{NO} yields stable Dm_{CO} values for any DLNO/DLCO ratio. However, assuming a finite θ_{NO} causes an asymptote to occur, in this example around DLNO/DLCO equals 7, upon which Dm_{CO} values increase rapidly to a non-physiologic range. This asymptote occurs when DLNO/DLCO is equal to θ_{NO}/θ_{CO} (note that θ_{CO} is dependent on PO_2). The asymptote location is very sensitive to the choice of θ_{CO} equation in the finite method and care must be taken to ensure that the asymptote does not occur in the physiological range of DLNO/DLCO ratio. For V_C, both the infinite and finite θ_{NO} assumptions are stable unless DLNO/DLCO approaches α, where an asymptote occurs yielding rapidly increasing V_C values which climb to a non-physiologic range. It is important to note that these asymptotes will shift depending on the specific values entered into the calculations.

ACCEPTED MANUSCRIPT

All in all, Figures 5-6 demonstrates the mathematical constraints that are incorporated into the determination of Dm_{CO} and V_C from values of DLCO and DLNO, regardless of the assumptions made as to the correct value of θ_{NO}. While Dm_{CO} and V_C are themselves physiologic variables, the calculations that have been established to determine these variables incorporate an unavoidable complication. The DLNO/DLCO values at which each assumption yields an asymptote is potentially concerning in two cases. First, Dm_{CO} values calculated using a finite θ_{NO} under normal conditions are unlikely to encroach upon the asymptote, as a physiologic DLNO/DLCO value is generally around 4-5 (Hughes and van der Lee, 2013). However, in instances such as altitude, where PO_2 is lower and the asymptote is therefore shifted to lower DLNO/DLCO ratios, the DLNO/DLCO ratio may fall within the range where Dm_{CO} rapidly increases to large, non-physiologic values. Second, V_C values calculated using the infinite θ_{NO} assumption and the single breath method, where the optimal α value has been optimized at ~ 4.4, also causes the asymptote to occur at a physiologic range for DLNO/DLCO. However, as can be seen in Table 2, the single breath method tends to yield higher DLNO/DLCO ratios than that of the rebreathe method (presently, mean 5.39 ± 0.43 for single breath vs. 3.58 ± 0.59 for rebreathe), such that approaching the asymptote is not usually an issue. The observation that DLNO/DLCO is slightly higher using the single breath method is found in our laboratory and others (Ceridon et al., 2010; Ceridon et al., 2011; Coffman et al., 2016; Zavorsky and Lands, 2005; Zavorsky and Murias, 2006).

4.2 Key Points

To be clear, we agree that θ_{NO} is, in a strict biochemical sense, not infinite, as no biological process can occur instantaneously. Indeed, as we have highlighted above, there is experimental

ACCEPTED MANUSCRIPT

data which demonstrates a resistance to combination of NO with hemoglobin that resides in the red cell (Azarov et al., 2011; Sakai et al., 2008). In this sense, we concede that a finite θ_{NO} may be a more accurate representation of gas transfer from the environment to hemoglobin in blood. However, in the case of calculating Dm_{CO} and V_C, the value of θ_{NO} has not been optimized relative to the gold standard multiple O_2 method or in any other way. Therefore, the assumption of an infinite θ_{NO} relative to θ_{CO} is still appropriate. While our laboratory recognizes that considering θ_{NO} infinite may not be ideal, we argue two main points that cause our group to be wary of the use of a finite θ_{NO}, in its current form, at this point.

First, when we have applied the finite θ_{NO} value to our calculations of Dm_{CO} and V_C using the single breath technique, we obtain values which do not increase as expected during exercise. This is concerning, as much of the research performed in our laboratory relies on accurate measures of the change in Dm_{CO} and V_C during submaximal and maximal exercise bouts. Second, as is the case in any scientific field, new methodology must be validated against a gold-standard. While some groups may argue that there is no gold standard in the case of Dm_{CO} and V_C, we feel it is important that the finite θ_{NO} calculations be validated against a method that does not utilize NO. We have performed such optimizations on our calculations, which assume an infinite θ_{NO}, using both the single breath and rebreathe techniques (Ceridon et al., 2010; Coffman et al., 2016). We feel it is important that those groups that are invested in the use of a finite θ_{NO} apply a similar method, where the θ_{NO} value would be systematically varied with respect to the other two required assumptions (the θ_{CO} equation and Krogh coefficient) of the finite θ_{NO} calculations. This methodology would produce optimized values for all three calculation parameter assumptions, thus adding confidence to the use of the finite method.

ACCEPTED MANUSCRIPT

All in all, while the finite θ_{NO} calculations have merit and may move into standard practice in the future, we feel that two main issues remain with the use of a finite θ_{NO} in practice, including the lack of validation of the currently established θ_{NO} value as well as the non-physiological response to exercise.

Additionally, we cannot stress enough that while alveolar-capillary membrane conductance and pulmonary-capillary blood volume are anatomical phenomena, the calculations that have been established to determine these variables from measured values of DLNO and DLCO are entirely dependent on the mathematical relationships underlying the given assumptions. In this sense, perhaps it is better to consider Dm_{CO} and V_C functional variables that can be used to observe changes over time, whether that be during exercise studies or clinically in disease, instead of true representations of anatomy.

4.3 Conclusions

The nuances of the calculations required for both assumptions can be discussed endlessly, but our ability to utilize Dm_{CO} and V_C to contribute meaningfully to the field is the overriding goal. We have shown that the values themselves are very dependent on the mathematical relationships, and therefore are likely not entirely in agreement with the physiology regardless of the assumptions. However, by using a consistent method for calculation of Dm_{CO} and V_C, it is entirely possible to observe changes within study participants or differences between study groups. If Dm_{CO} and V_C are to eventually enter clinical practice, an assumption regarding θ_{NO} must be determined, in addition to a θ_{CO} equation and α ratio, and standardized across the

ACCEPTED MANUSCRIPT

practice. Once the optimal calculation parameters have been chosen, it will then be possible to systematically investigate appropriate cutoffs for the determination of disease states. At the current time, with a number of conflicting assumptions and methods, the results are simply too variable to determine how a particular result should be interpreted. In the research arena, the choice of calculation parameters is of little concern as long as the resulting data remain within the physiologic range (i.e. positive) and have appropriate response to stimuli, such as pulmonary-capillary blood volume increasing with exercise. While we respect the work of the recent ERS task force, we believe their findings are premature as no study has been designed to calculate an exact θ_{NO} in vivo in humans, and we are also uncomfortable with their conclusions regarding the correct Dm_{NO}/Dm_{CO} (α-) ratio. Additionally, the ERS task force has not presented any new evidence for use of a finite θ_{NO} beyond the evidence discussed above (Zavorsky et al., 2017). Therefore at this time, we suggest the continued use of the assumption that θ_{NO} is infinite until further optimization of the finite θ_{NO} method can be performed.

5. ACKNOWLEDGEMENTS

KEC and SCC are supported by Mayo Graduate School. KEC is supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health grant F31HL131076. BJT is supported by a Fulbright Commission UK Distinguished Scholar Award and American Heart Association Grant AHA12-POST12070084. This study was funded by NIH grant HL71478.

REFERENCES

Azarov, I., Liu, C., Reynolds, H., Tsekouras, Z., Lee, J.S., Gladwin, M.T., Kim-Shapiro, D.B., 2011. Mechanisms of slower nitric oxide uptake by red blood cells and other hemoglobin-containing vesicles. The Journal of biological chemistry 286, 33567-33579.

ACCEPTED MANUSCRIPT

Borland, C., Dunningham, H., Bottrill, F., Vuylsteke, A., 2006. Can a membrane oxygenator be a model for lung NO and CO transfer? Journal of applied physiology 100, 1527-1538.

Borland, C.D., Cox, Y., 1991. Effect of varying alveolar oxygen partial pressure on diffusing capacity for nitric oxide and carbon monoxide, membrane diffusing capacity and lung capillary blood volume. Clinical science 81, 759-765.

Borland, C.D., Dunningham, H., Bottrill, F., Vuylsteke, A., Yilmaz, C., Dane, D.M., Hsia, C.C., 2010. Significant blood resistance to nitric oxide transfer in the lung. Journal of applied physiology 108, 1052-1060.

Borland, C.D., Higenbottam, T.W., 1989. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. The European respiratory journal 2, 56-63.

Carlsen, E., Comroe, J.H., Jr., 1958. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. The Journal of general physiology 42, 83-107.

Ceridon, M.L., Beck, K.C., Olson, T.P., Bilezikian, J.A., Johnson, B.D., 2010. Calculating alveolar capillary conductance and pulmonary capillary blood volume: comparing the multiple- and single-inspired oxygen tension methods. Journal of applied physiology 109, 643-653.

Ceridon, M.L., Morris, N.R., Hulsebus, M.L., Olson, T.P., Lalande, S., Johnson, B.D., 2011. Influence of bronchial blood flow and conductance on pulmonary function in stable systolic heart failure. Respiratory physiology & neurobiology 177, 256-264.

Coffman, K.E., Taylor, B.J., Carlson, A.R., Wentz, R.J., Johnson, B.D., 2016. Optimizing the calculation of DM,CO and VC via the single breath single oxygen tension DLCO/NO method. Respiratory physiology & neurobiology 221, 19-29.

ACCEPTED MANUSCRIPT

Deonikar, P., Kavdia, M., 2010. An integrated computational and experimental model of nitric oxide-red blood cell interactions. Annals of biomedical engineering 38, 357-370.

Forster, R.E., (1987). Diffusion of Gases Across the Alveolar Membrane, Comprehensive Physiology. John Wiley & Sons, Inc.

Forster, R.E., Fowler, W.S., Bates, D.V., Van Lingen, B., 1954. The absorption of carbon monoxide by the lungs during breath-holding. The Journal of clinical investigation 33, 1135-1145.

Guenard, H., Varene, N., Vaida, P., 1987. Determination of lung capillary blood volume and membrane diffusing capacity in man by the measurements of NO and CO transfer. Respiration physiology 70, 113-120.

Guenard, H.J., Martinot, J.B., Martin, S., Maury, B., Lalande, S., Kays, C., 2016. In vivo estimates of NO and CO conductance for haemoglobin and for lung transfer in humans. Respiratory physiology & neurobiology 228, 1-8.

Hakim, T.S., Sugimori, K., Camporesi, E.M., Anderson, G., 1996. Half-life of nitric oxide in aqueous solutions with and without haemoglobin. Physiological measurement 17, 267-277.

Hughes, J.M., Bates, D.V., 2003. Historical review: the carbon monoxide diffusing capacity (DLCO) and its membrane (DM) and red cell (Theta.Vc) components. Respiratory physiology & neurobiology 138, 115-142.

Hughes, J.M., van der Lee, I., 2013. The TL,NO/TL,CO ratio in pulmonary function test interpretation. The European respiratory journal 41, 453-461.

Krogh, A., Krogh, M., 1910. On the Rate of Diffusion of Carbonic Oxide into the Lungs of Man1. Skandinavisches Archiv Für Physiologie 23, 236-247.

ACCEPTED MANUSCRIPT

Krogh, M., 1915. The diffusion of gases through the lungs of man. The Journal of physiology 49, 271-300.

La Gerche, A., MacIsaac, A.I., Burns, A.T., Mooney, D.J., Inder, W.J., Voigt, J.U., Heidbuchel, H., Prior, D.L., 2010. Pulmonary transit of agitated contrast is associated with enhanced pulmonary vascular reserve and right ventricular function during exercise. Journal of applied physiology 109, 1307-1317.

Lewis, B.M., Lin, T.H., Noe, F.E., Komisaruk, R., 1958. The measurement of pulmonary capillary blood volume and pulmonary membrane diffusing capacity in normal subjects; the effects of exercise and position. The Journal of clinical investigation 37, 1061-1070.

Liu, X., Miller, M.J., Joshi, M.S., Sadowska-Krowicka, H., Clark, D.A., Lancaster, J.R., Jr., 1998. Diffusion-limited reaction of free nitric oxide with erythrocytes. The Journal of biological chemistry 273, 18709-18713.

Magini, A., Apostolo, A., Salvioni, E., Italiano, G., Veglia, F., Agostoni, P., 2013. Alveolar-capillary membrane diffusion measurement by nitric oxide inhalation in heart failure. European journal of preventive cardiology.

Meyer, M., Schuster, K.D., Schulz, H., Mohr, M., Piiper, J., 1990. Pulmonary diffusing capacities for nitric oxide and carbon monoxide determined by rebreathing in dogs. Journal of applied physiology 68, 2344-2357.

Reeves, R.B., Park, H.K., 1992. CO uptake kinetics of red cells and CO diffusing capacity. Respiration physiology 88, 1-21.

Roughton, F.J., Forster, R.E., 1957. Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true

ACCEPTED MANUSCRIPT

diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. Journal of applied physiology 11, 290-302.

Sackner, M.A., Greeneltch, D., Heiman, M.S., Epstein, S., Atkins, N., 1975. Diffusing capacity, membrane diffusing capacity, capillary blood volume, pulmonary tissue volume, and cardiac output measured by a rebreathing technique. The American review of respiratory disease 111, 157-165.

Sakai, H., Sato, A., Masuda, K., Takeoka, S., Tsuchida, E., 2008. Encapsulation of concentrated hemoglobin solution in phospholipid vesicles retards the reaction with NO, but not CO, by intracellular diffusion barrier. J Biol Chem 283, 1508-1517.

Snyder, E.M., Beck, K.C., Turner, S.T., Hoffman, E.A., Joyner, M.J., Johnson, B.D., 2007. Genetic variation of the beta2-adrenergic receptor is associated with differences in lung fluid accumulation in humans. Journal of applied physiology 102, 2172-2178.

Tamhane, R.M., Johnson, R.L., Jr., Hsia, C.C., 2001. Pulmonary membrane diffusing capacity and capillary blood volume measured during exercise from nitric oxide uptake. Chest 120, 1850-1856.

Taylor, B.J., Carlson, A.R., Miller, A.D., Johnson, B.D., 2014. Exercise-induced interstitial pulmonary edema at sea-level in young and old healthy humans. Respiratory physiology & neurobiology 191, 17-25.

Vaughn, M.W., Huang, K.T., Kuo, L., Liao, J.C., 2000. Erythrocytes possess an intrinsic barrier to nitric oxide consumption. The Journal of biological chemistry 275, 2342-2348.

Zavorsky, G.S., Hsia, C.C.W., Hughes, J.M.B., Borland, C.D.R., Guenard, H.J., Van der Lee, I., Steenbruggen, I., Naeije, R.C., J., Dinh-Xuan, A.T., 2017. Standardization and application of the

ACCEPTED MANUSCRIPT

single-breath determination of nitric oxide uptake in the lung. European Respiratory Journal [In press] doi: 10.1183/13993003.00926-2016.

Zavorsky, G.S., Lands, L.C., 2005. Lung diffusion capacity for nitric oxide and carbon monoxide is impaired similarly following short-term graded exercise. Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society 12, 31-38.

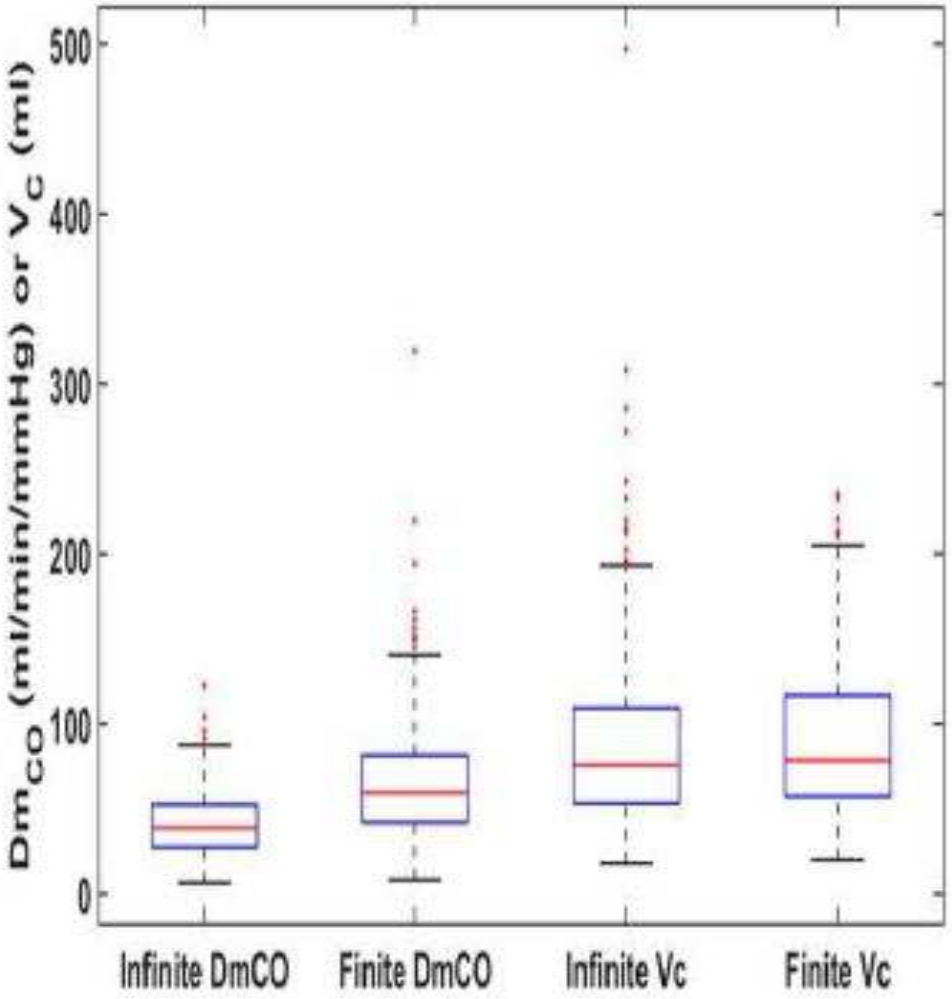
Zavorsky, G.S., Milne, E.N., Lavorini, F., Rienzi, J.P., Cutrufello, P.T., Kumar, S.S., Pistolesi, M., 2014. Small changes in lung function in runners with marathon-induced interstitial lung edema. Physiological reports 2.

Zavorsky, G.S., Murias, J.M., 2006. A small amount of inhaled nitric oxide does not increase lung diffusing capacity. The European respiratory journal 27, 1251-1257.

Zavorsky, G.S., Quiron, K.B., Massarelli, P.S., Lands, L.C., 2004. The relationship between single-breath diffusion capacity of the lung for nitric oxide and carbon monoxide during various exercise intensities. Chest 125, 1019-1027.

ACCEPTED MANUSCRIPT

Figure 1: Dm_{CO} and V_C values calculated from rebreathe data using both the finite and infinite θ_{NO} assumptions. Both assumptions yield reasonable values for Dm_{CO} and V_C – i.e., positive and within range of previously reported values. Outliers (red +) are defined as ± 2.7 standard deviations from the mean. Finite Dm_{CO} values are significantly greater that infinite Dm_{CO} values ($p < 0.001$); V_C values are not statistically different. Dm_{CO}, alveolar capillary membrane conductance; V_C, pulmonary capillary blood volume.



ACCEPTED MANUSCRIPT

Figure 2: Dm_{CO} and V_C values calculated from single breath data using both the finite and infinite θ_{NO} assumptions. Both assumptions yield reasonable values for Dm_{CO} and V_C – i.e., positive and within range of previously reported values. Outliers (red +) are defined as ± 2.7 standard deviations from the mean. Finite Dm_{CO} values are significantly greater, and finite V_C values are significantly lower, that the infinite assumption values (both $p < 0.001$). Dm_{CO}, alveolar capillary membrane conductance; V_C, pulmonary capillary blood volume.

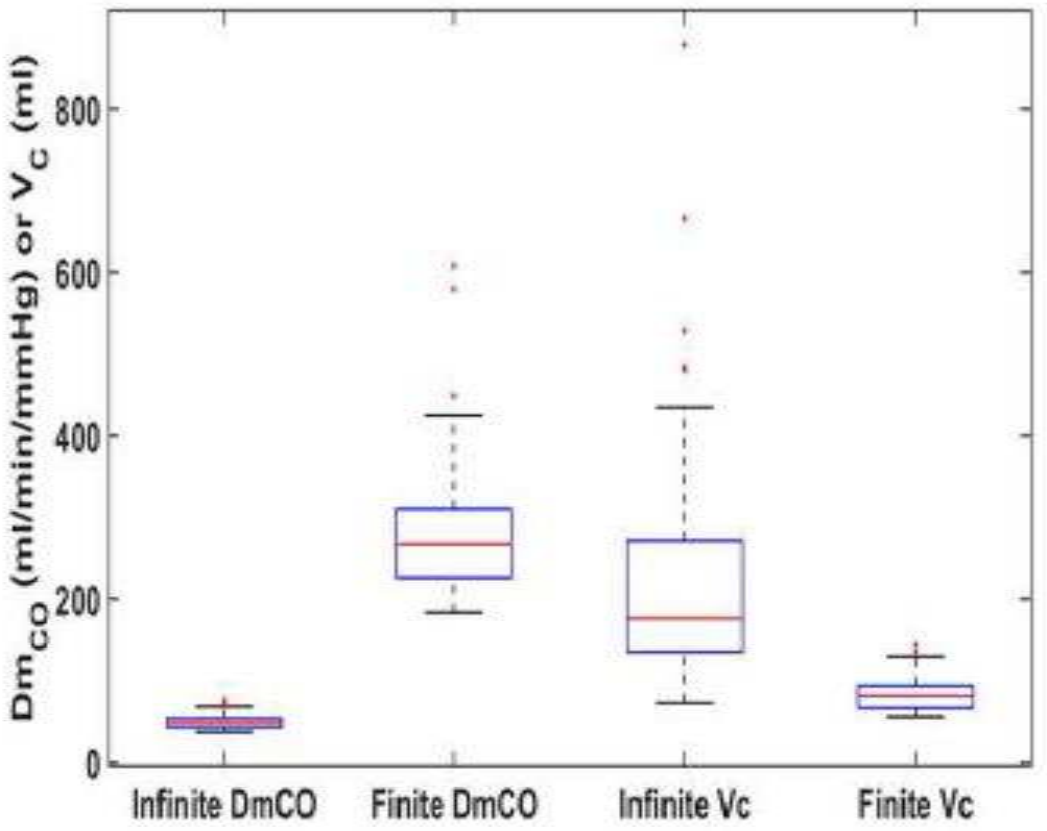


Figure 3: Response of Dm_{CO} and V_C to incremental exercise calculated from rebreathe data. A linear mixed effects model was implemented to separate the individual and group effects on either Dm_{CO} or V_C throughout incremental cycling exercise. The group effects for both the infinite and finite θ_{NO} assumptions are plotted as a function of workload. For both assumptions,

ACCEPTED MANUSCRIPT

Dm_{CO} and V_C increased significantly throughout exercise (all $p < 0.001$). Dm_{CO}, alveolar capillary membrane conductance; V_C, pulmonary capillary blood volume.

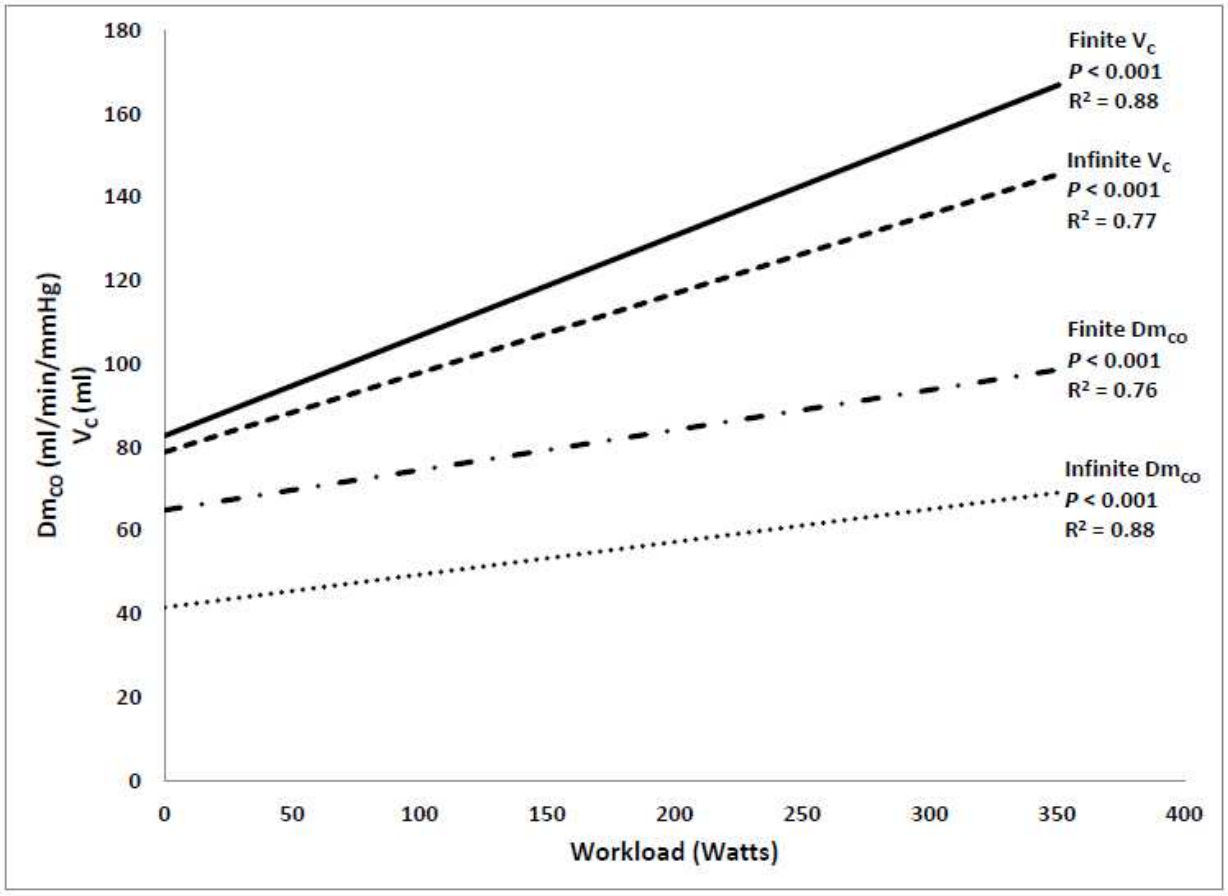


Figure 4: Effective α ratio for the conversion of DLNO to Dm_{CO} for the finite θ_{NO} assumption. The effective α ratio, which converts DLNO directly to Dm_{CO} for the finite θ_{NO} assumption, was calculated for a range for DLNO/DLCO ratios. The resulting effective α ratio is lower than the Krogh Coefficient (1.97) because the finite θ_{NO} assumption calculations only factor in the theoretical red blood cell resistance to the transfer of NO. The resulting effective α ratio is also lower than the α ratio used under the infinite θ_{NO} assumption (2.26) for DLNO/DLCO ratios below ~6.5.

ACCEPTED MANUSCRIPT

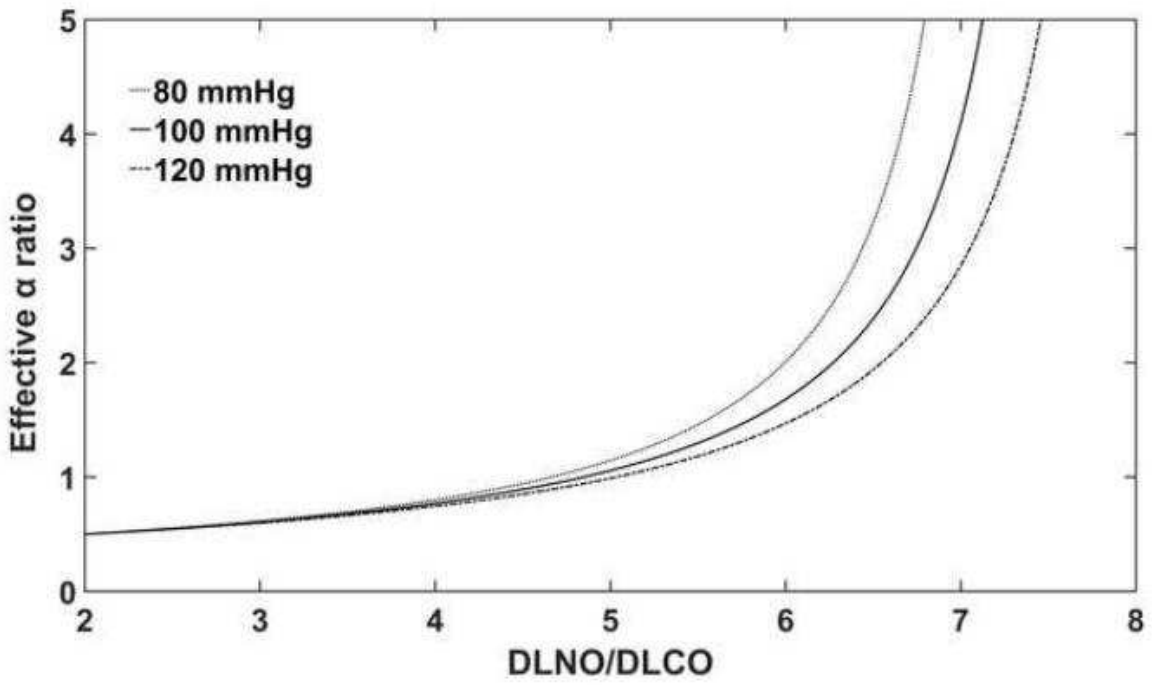


Figure 5: Theoretical Dm_{CO} values over a range of DLNO/DLCO ratios. Dm_{CO} was calculated under both the infinite and finite θ_{NO} assumptions using DLCO = 20 ml/min/mmHg. The finite calculation of Dm_{CO} is also dependent on PO_2; values of 80, 100, and 120 mmHg are show here. While the infinite calculation of Dm_{CO} is stable over a large range of DLNO/DLCO ratios, the finite calculation of Dm_{CO} rapidly increases as the DLNO/DLCO ratio increases. DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; Dm_{CO}, alveolar capillary membrane conductance.

ACCEPTED MANUSCRIPT

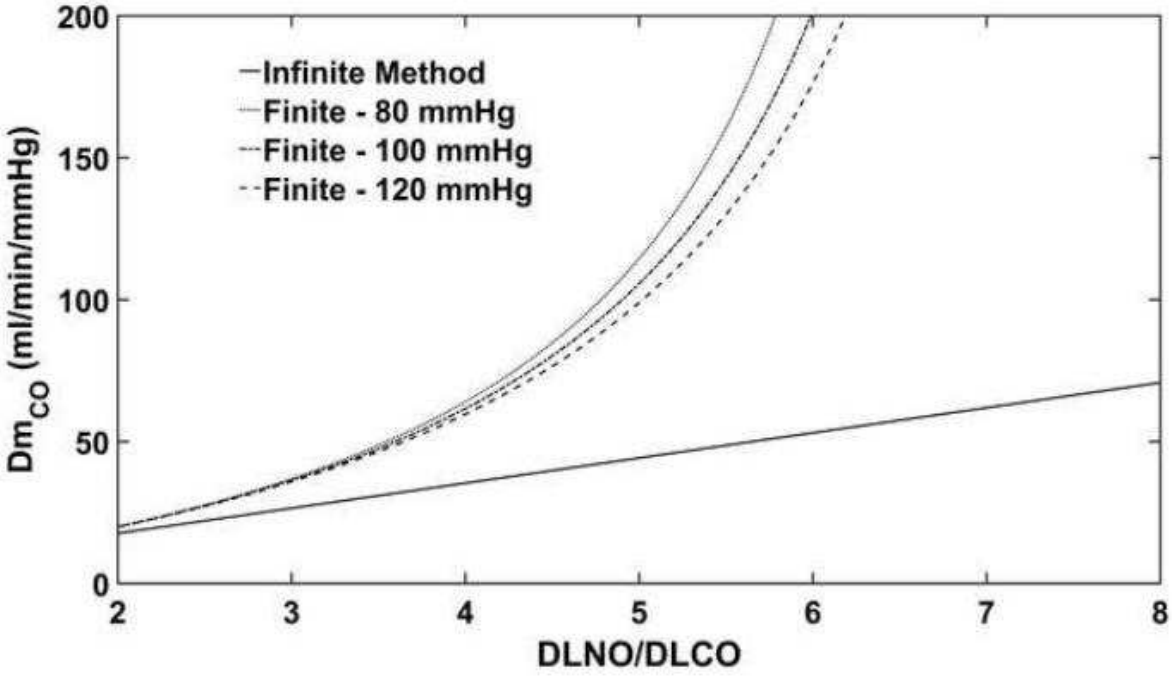
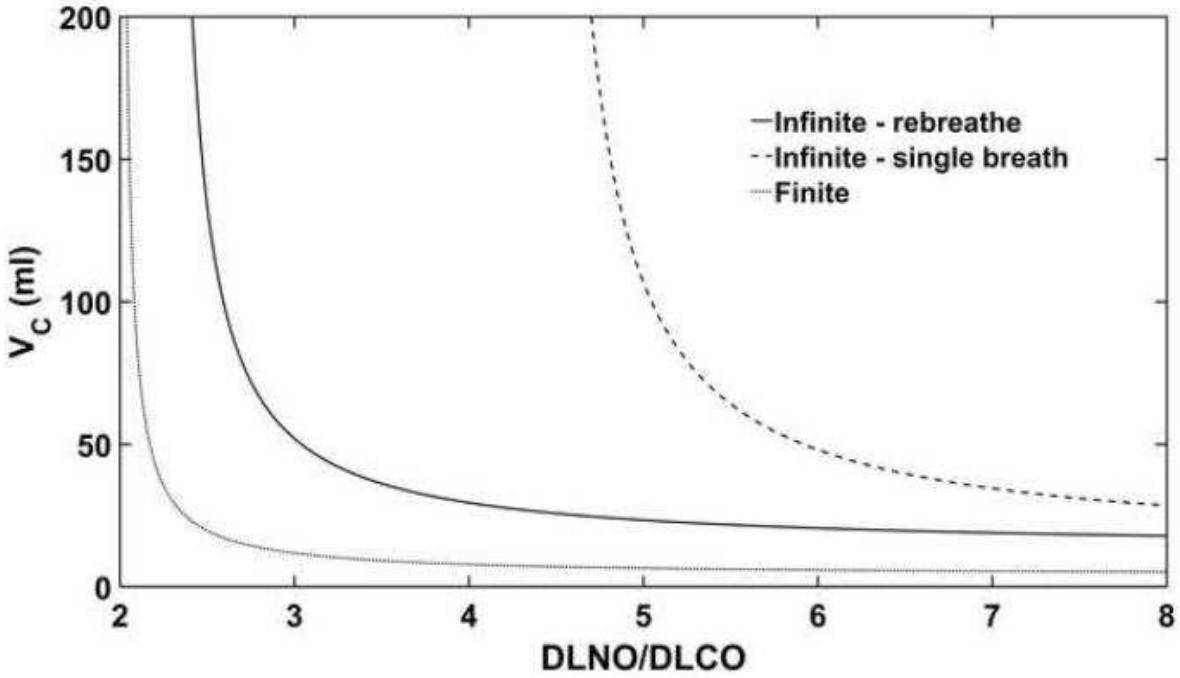


Figure 6: Theoretical V_C values over a range of DLNO/DLCO ratios. V_C was calculated under both the infinite and finite θ_{NO} assumptions using DLCO = 20 ml/min/mmHg. The infinite calculation of V_C is dependent on the technique used in our laboratory; hence, both rebreathe and single breath are shown here. Under both assumptions, calculation of V_C is increases rapidly when the DLNO/DLCO ratio is equal to the α ratio/Krogh coefficient used. DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; V_C, pulmonary capillary blood volume.

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

Table 1. Source of over 750 DLCO and DLNO observations

Study ID	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Exercise	Condition
			Rebreathe			
1.	66.5 ± 10.0	172.4 ± 10.3	82.2 ± 17.6	27.8 ± 4.4	--	HF + Control
2.	59.7 ± 10.6	174.5 ± 8.9	85.8 ± 17.6	28.1 ± 5.0	--	HF + Control
3.	25.9 ± 4.0	176.4 ± 11.7	71.3 ± 10.5	22.8 ± 2.1	Incremental to 70% W_{peak}	Healthy
4.	46.1 ± 20.6	176.2 ± 5.1	75.1 ± 7.4	24.2 ± 2.4	Incremental to 90% W_{peak}	Healthy
Average	49.6 ± 19.6	175.3 ± 8.2	78.7 ± 14.1	25.6 ± 4.1	--	--
			Single Breath			
5.	25.1 ± 2.4	180.7 ± 6.4	74.0 ± 10.1	22.6 ± 2.4	Constant @ 80 Watts	Healthy
6.	26.9 ± 3.3	173.5 ± 7.4	65.4 ± 6.8	21.7 ± 1.5	Constant @ 82 ± 27 Watts	Healthy
Average	25.9 ± 2.9	177.7 ± 7.7	70.6 ± 9.9	22.3 ± 2.1	--	--

Values are reported as mean ± SD. BMI, body mass index; HF, heart failure; W_{peak}, peak work rate.

ACCEPTED MANUSCRIPT

Table 2. DLCO/DLNO data and resulting Dm_{CO} and V_C values calculated via both the infinite and finite θ_{NO} assumptions

	Infinite Method	Finite Method	P-value
	Rebreathe		
DL_{CO}	26.5 ± 11.9		
Range	5.9 – 72.1		
DL_{NO}	93.1 ± 40.0		
Range	15.1 – 276.9		
DL_{NO}/DL_{CO}	3.58 ± 0.59		
Range	2.33 – 7.03		
Dm_{CO}	41.2 ± 17.7	63.8 ± 31.1	< 0.001
Range	6.7 – 122.5	8.33 – 319.8	
V_C	85.8 ± 46.3	89.8 ± 43.3	0.111
Range	18.1 – 497.2	20.1 – 253.3	
	Single Breath		
DL_{CO}	40.7 ± 8.2		
Range	29.3 – 68.6		
DL_{NO}	217.7 ± 36.8		
Range	165.4 – 335.4		
DL_{NO}/DL_{CO}	5.39 ± 0.43		
Range	4.59 – 6.62		
Dm_{CO}	49.5 ± 8.4	281.7 ± 81.5	< 0.001
Range	37.6 – 76.2	183.6 – 55.8	
V_C	223.2 ± 141.2	82.5 ± 18.7	< 0.001
Range	73.0 – 878.5	55.8 – 144.4	

Values are reported as mean ± SD. Values include all rest, submaximal, and incremental exercise observations as well as data from both healthy individuals and heart failure patients. DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; Dm_{CO}, alveolar capillary membrane conductance; V_C, pulmonary capillary blood volume.

ACCEPTED MANUSCRIPT

Table 3. Rest and submaximal exercise values for Dm_{CO} and V_C using the single breath technique calculated via both the infinite and finite θ_{NO} assumptions

		Rest	Exercise (80 ± 17 W)	Absolute Change	% Change	P-value
			Infinite Method			
Dm_{CO}		45.6 ± 6.5	53.7 ± 8.2	7.7 ± 3.6	16.9 ± 7.3	< 0.001
	Range	37.6 – 63.7	42.0 – 76.2	1.4 – 18.8	2.9 – 31.4	
V_C		162.6 ± 68.6	288.9 ± 168.9	129.0 ± 160.7	92.0 ± 112.2	< 0.001
	Range	73.0 – 427.8	109.7 – 878.5	–119.5 – 725.1	–52.1 – 472.4	
			Finite Method			
Dm_{CO}		279.1 ± 95.4	284.5 ± 64.2	0.1 ± 76.9	4.79 ± 27.0	0.498
	Range	185.4 – 608.0	183.6 – 422.7	–219.3 – 177.0	–37.9 – 95.5	
V_C		72.7 ± 12.9	93.1 ± 18.3	20.3 ± 10.9	28.5 ± 16.4	< 0.001
	Range	55.8 – 110.9	64.5 – 144.4	–3.6 – 39.9	–4.9 – 69.8	

Values are reported as mean ± SD. Dm_{CO}, alveolar capillary membrane conductance; V_C, pulmonary capillary blood volume.