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A small amount of inhaled nitric oxide does not increase lung diffusing capacity

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ABSTRACT: The aim of the present study was to determine: 1) whether 40–50 ppm nitric oxide (NO) increases diffusing capacity of the lung for NO ($D_{L,NO}$) and carbon monoxide ($D_{L,CO}$), membrane diffusing capacity for CO ($D_{m,CO}$) and pulmonary capillary blood volume (V_c); 2) the actual number of tests required to provide a reasonable estimate of $D_{L,NO}$, $D_{L,CO}$, $D_{m,CO}$ and V_c; and 3) repeatability of these parameters using the single-breath $D_{L,NO}$–$D_{L,CO}$ method.

In total, 31 subjects performed five single-breath hold manoeuvres at rest, inhaling 43±3 ppm NO together with a standard diffusion mixture.

$D_{L,NO}$ ($D_{m,CO}$) remained unchanged from the first to fifth trial. However, compared with the first trial, $D_{L,CO}$ and V_c had decreased by the fourth (-4±5%; 95% confidence interval (CI)=-5– -2%) and third trial (-5±7%; 95% CI=-7– -2%), respectively. Repeatability over five trials was 17, 3 and 7 mL·min^{-1}·mmHg^{-1} for $D_{L,NO}$, $D_{L,CO}$ and $D_{m,CO}$, respectively, and 13 mL for V_c when $D_{m,CO}=D_{L,NO}/2.42$.

In conclusion, nitric oxide inhaled during sequential single-breath manoeuvres has no effect on diffusing capacity of the lung for nitric oxide and, thus, membrane diffusing capacity for carbon monoxide. Since more than two and three trials will lower pulmonary capillary blood volume and diffusing capacity of the lung for carbon monoxide, respectively, the average value of only two properly performed trials is suggested.

KEYWORDS: Diffusing capacity, nitric oxide, repeatability

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The equation of the diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) has been classically described as:

$$1/D_{L,CO} = (1/D_{m,CO}) + (1/\Theta CO \cdot V_c) \quad (1)$$

where $D_{m,CO}$ represents pulmonary membrane diffusing capacity for carbon monoxide (CO) and ΘCO is the specific blood transfer conductance for CO. V_c represents pulmonary capillary blood volume [1]. Membrane resistance ($1/D_{m,CO}$) and red cell resistance ($1/\Theta CO \cdot V_c$) usually contribute equally to the overall diffusive resistance across the lung [2], although this has been debated [3]. To obtain $D_{m,CO}$ and V_c, $D_{L,CO}$ has been traditionally measured at two different levels of alveolar oxygen tension (P_{A,O_2}), ~13.3–16.0 kPa (~100–120 mmHg) and ~79.8 kPa (~600 mmHg). For each level of P_{A,O_2}, $1/D_{L,CO}$ is then plotted on the y-axis and $1/\Theta CO$ is plotted on the x-axis. A line is then drawn through two points and the x-intercept ($1/D_{m,CO}$) and slope ($1/V_c$) can be solved. This two-step method can be time consuming and uncomfortable to perform, especially during exercise.

However, over the past 15 yrs, the measurement of diffusing capacity of the lung using the transfer gases nitric oxide (NO) and CO together permits one to obtain $D_{m,CO}$ and V_c in a single-breath manoeuvre, thus allowing a similar distribution of the two gases and reducing the number of measurements and testing time by half [4, 5]. The velocity constant of the combination of NO with haemoglobin is about 280 times faster than that of CO [6], and thus the specific blood transfer conductance for NO (ΘNO) is so large that the red cell resistance to NO ($1/\Theta NO$) approaches zero [7]. Therefore, diffusing capacity of the lung for nitric oxide ($D_{L,NO}$) equals the membrane diffusing capacity for NO ($D_{m,NO}$), and is independent of V_c and haemoglobin concentration [8]. Others have made the same assumption that ($1/\Theta NO$) is negligible [9–14], and it was recently determined that a nonzero $1/\Theta NO$ would not be able to explain their experimental data [12]. Therefore, these data suggest that $D_{L,NO}$ is a good measure of $D_{m,NO}$. Given that the molecular weight of CO and NO are 28 and 30 g·M^{-1}, respectively, and solubilities (Bunsen coefficients) of CO and NO in plasma at 37°C are 0.0215 and 0.0439 [15], respectively, the diffusivity of NO, which is the solubility of NO divided by the square root of the molecular weight (MW) of NO ($\sqrt{MW_{NO}}$), is ~1.97 times

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greater than that of CO and, thus, the theoretical factor between membrane diffusing capacities for NO and CO is:

$$(\text{NO solubility}/\sqrt{\text{MWNO}})/(\text{CO solubility}/\sqrt{\text{MWCO}}) = 1.97 \quad (2)$$

Indeed, the solubility of either gas will depend upon the composition of the fluid that the gas has to diffuse through. If the fluid changes composition, the relative solubilities may well be different pre- and post-exercise. Nevertheless, data obtained from sick and healthy patients performing rebreathing manoeuvres suggest that the actual ratio of $D_{L,NO}$ to $D_{m,CO}$ is ~2.42 [11, 12]. The larger ratio may be due to a higher than assumed NO solubility in plasma as well as NO facilitated diffusion [12].

Several researchers have previously obtained $D_{L,NO}$ from single-breath [4, 5, 9, 10, 13, 14, 16–19] or rebreathing manoeuvres [11, 12], along with the simultaneous measurement of $D_{L,CO}$ to obtain $D_{m,CO}$ and V_c. Since brief exposure to NO does not interfere with physiological function [11, 12, 20, 21], it seems pertinent to use NO as a test gas to assess lung diffusion capacity. However, there is still debate as to whether inhalations of high NO concentrations (~40–50 ppm) during sequential single-breath manoeuvres can affect the pulmonary microcirculation. At those NO concentrations, pulmonary vasodilation may occur, increasing $D_{L,CO}$, V_c and perhaps $D_{L,NO}$ ($D_{m,CO}$) [22]. Therefore, the first objective of the present study was to determine whether five sequential single breath-hold manoeuvres inhaling ~40 ppm of NO increase $D_{L,CO}$, $D_{L,NO}$ (and thus $D_{m,CO}$) and V_c. The current hypothesis was that five repeated inhalations of 40–50 ppm of NO would not increase $D_{L,CO}$, $D_{L,NO}$ ($D_{m,CO}$) or V_c.

Furthermore, the recent 2005 American Thoracic Society (ATS)/European Respiratory Society (ERS) task force guidelines of standardisation of the single-breath determination of CO uptake in the lung [23] mentioned that more research is needed to determine the actual number of tests required to provide a reasonable estimate of the average $D_{L,CO}$. Therefore, the second objective of the current study was to determine the actual number of tests required to provide a reasonable estimate of not only $D_{L,CO}$, but $D_{L,NO}$ ($D_{m,CO}$) and V_c for a given person. The present authors' hypothesis was that an average of three measurements would be needed to obtain a reasonable estimate of all those parameters.

The third objective was to determine the repeatability of lung diffusing capacity and its components over five trials in a given patient using the newer single-breath method of CO and NO. This would help to decide whether a change in an observation of $D_{L,NO}$, $D_{L,CO}$, $D_{m,CO}$ and V_c represents a real clinical change in the pulmonary system or just measurement error. The current authors' hypothesis was that the repeatability for $D_{L,NO}$, $D_{L,CO}$ and $D_{m,CO}$ would be 10, 2 and 6 mL·min^{-1}·mmHg^{-1}, respectively, and 10 mL for V_c.

METHODS

Subjects

In total, 31 healthy subjects were recruited (15 females, 16 males) and all completed the study (aged 33±9 yrs; weight 68.6±12.5 kg; height 169.9±9 cm). All subjects were non-smokers. These subjects had normal resting spirometry function (forced expiratory volume in one second (FEV_1) >80% predicted, and FEV_1/forced vital capacity (FVC) >0.70) and no history of cardiopulmonary disease. Each subject was required to come into the laboratory on one occasion only.

Single-breath apparatus and technique

Volume and gas calibration of the Ergocard and the Hyp'Air lung diffusion system (Medisoft, Dinant, Belgium) were performed prior to each testing session. The subjects breathed through a three-way pneumatic valve developed by Medisoft. A dead space washout volume of 900 mL was allowed, and an expired sample volume of 900 mL was collected. The instrument dead space was measured at 140 mL (including the mouthpiece, valve and filter dead spaces). Anatomical dead space (mL) was estimated as bodyweight in kg × 2.2 [23]. The concentrations of inspiratory gases were 0.295% CO, 9.96% He, 20.98% O_2 and balance N_2 for gas mixture one, and 1,000 ppm NO and balance N_2 for gas mixture two. A third mixture of 77 ppm NO and balance N_2 was used for calibration purposes only. For the single-breath manoeuvre, an inspiratory bag was filled with 5–8 L depending on the subject's FVC using the first two gas mixtures. Once the mixtures were injected into the inspiratory bag, the concentration of CO, He, NO, and O_2 were analysed simultaneously over 30 s by gas analysers. The injection of the various gas mixtures into the inspiratory bag resulted in approximate inspired concentrations of CO, He, NO and O_2 at 0.30%, 9%, 40 ppm and 20%, respectively. The types of gas analysers used for measuring inspired and expired gas mixtures have been reported elsewhere [13]. Inspired volume was measured, corrected for instrument and anatomical dead space, and converted to standard temperature, pressure and dry conditions. Breath-holding time was calculated using the method of JONES and MEADE [24].

Calculation of diffusion capacities, D_m and V_c

Diffusion capacities for NO and CO were calculated simultaneously from the exponential disappearance rate of each gas with respect to He using the method by JONES and MEADE [24]. The formulae for calculating $D_{L,CO}$ can be found in the 2005 ATS/ERS guidelines [23]. All results were standardised to a haemoglobin (Hb) concentration of 14.6 g·dL^{-1} for males and 12.0 g·dL^{-1} for females, and a P_{A,O_2} of 13.3 kPa (100 mmHg) by inserting these values into the following formula by ROUGHTON and FORSTER [1]:

$$(1/\Theta CO) = (0.73 + 0.0058 \times P_{O_2}) \times (14.6/[\text{Hb}]) \quad (3)$$

where $1/\Theta CO$ was 1.426 and ΘCO was 0.701 mL·min^{-1}·mmHg^{-1} for males, $1/\Theta CO$ was 1.594 and ΘCO was 0.627 mL·min^{-1}·mmHg^{-1} for females and P_{O_2} was partial pressure of oxygen. A $D_{L,NO}$ to $D_{m,CO}$ ratio of 2.42 [11, 12, 14] and 1.97 [5, 9, 10, 13, 19] was used as the theoretical ratio of $D_{L,NO}$ to $D_{m,CO}$ during single-breath manoeuvres since those ratios have both been used in the past. The ratio of 2.42 has been determined recently during rebreathing manoeuvres at rest and during exercise [11, 12], which can result in $D_{m,CO}$ values that are more in line with the current normative values [25]. Therefore, due to the two different $D_{L,NO}$ to $D_{m,CO}$ ratios reported in the literature, the current authors reported two different $D_{m,CO}$ values and two different V_c values.

A breath-holding time of 4–5 s was chosen because it has been shown that $D_{L,CO}$ values were not different with a 3- or 5-s

breath hold compared with a 7- and 10-s breath hold [26] and a breath-holding time of ~9 s would result in a less than detectable amount of expired NO [4]. The present authors did not account for NO back pressure in the calculations since exhaled NO concentrations at rest are negligible, ranging 11–66 ppb (0.011–0.066 ppm) [27], which tend to decrease during exercise [28]. The amount of NO back pressure then would minimally affect $D_{L,NO}$ calculations as the measurements were carried out in the ppm range, which is ~75–500 times larger than the exhaled NO concentration at rest or during exercise after a single-breath inspiration of 40–60 ppm NO. Accounting for CO back pressure is also negligible as it has been shown that 2 min between tests virtually eliminates all the CO gas from the lungs in healthy subjects [29]. As the recent ATS/ERS guidelines recommend a minimum of 4 min between $D_{L,CO}$ measurements [23], subjects performed the single-breath manoeuvre with a minimum of 4.5 min between tests. Five sequential diffusion capacity tests were performed.

Statistical analyses

A one-way repeated measures ANOVA was used to determine if there were significant differences in $D_{L,CO}$, $D_{L,NO}$, $D_{m,CO}$ and V_c between the five trials. A Tukeys/Kramer *post hoc* comparison test was used to see where the differences in the five trials occurred. In addition, the variables height, age and weight were examined by forward stepwise multiple regression to determine which and what combination most predicted $D_{L,NO}$ values at rest. The measurement error for $D_{L,NO}$, $D_{m,CO}$, $D_{L,CO}$ and V_c was calculated as the square root of the residual mean square error (which is also called the within-subject standard deviation) obtained from the one-way repeated measures ANOVA [30]. The repeatability of each variable was then obtained multiplying the within-subject standard deviation by 2.77 [30]. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 31 subjects, 29 had normal spirometry function (table 1) based on the fact that FEV_1 was >80% pred for all subjects, and the FEV_1/FVC was >0.7 for all but two subjects (FEV_1/FVC 0.67 and 0.68, respectively). The single breath-hold manoeuvres, on the whole, were performed adequately (table 2). Breath-hold time was consistent within 0.3 s for all five trials, and the average inspired volume was always >90% of the FVC. The average alveolar volume was also maintained within 0.2 L for all trials.

The data show that $D_{L,NO}$ and, thus, $D_{m,CO}$ remained unaltered from trial one to trial five (table 3). However, compared with the first trial, $D_{L,CO}$ and V_c significantly decreased by the fourth (-4±5%; 95% confidence interval (CI) -5– -2%; $p<0.05$) and third trial (-5±7%; 95% CI -7– -2%; $p<0.05$), respectively. When $D_{m,CO}=D_{L,NO}/2.42$, the per cent predicted based on age, height and sex was 111±31% (range 62–164%; $p<0.05$), but when $D_{m,CO}=D_{L,NO}/1.97$, the per cent predicted was 136±39% (72–202%; $p<0.05$). For V_c, when $D_{m,CO}=D_{L,NO}/2.42$ the per cent predicted based on height and sex was 116±19% (76–155%; $p<0.05$), but when $D_{m,CO}=D_{L,NO}/1.97$ the per cent predicted for V_c was 100±16% (66–131%; $p>0.05$). Therefore, when $D_{m,CO}=D_{L,NO}/2.42$, the $D_{m,CO}$ is reduced closer to normative values and the V_c is increased above normative values. The opposite occurs when $D_{m,CO}=D_{L,NO}/1.97$ as the $D_{m,CO}$ is largely increased above the predicted value, but V_c is reduced to 100% pred.

The measurement error and repeatability of five trials are presented in table 4. The repeatability represents the critical value in which a clinically measurable change in a given patient occurs between sessions. From the repeated measures ANOVA, a real clinical change in a patient's pulmonary diffusion capacity and its components was 17.2 and 3.2 $mL \cdot min^{-1} \cdot mmHg^{-1}$ for $D_{L,NO}$ and $D_{L,CO}$, respectively.

TABLE 1 Subject characteristics and resting spirometry

	Mean±SD (range)	Predicted	% predicted
Females n	15		
Males n	16		
Age yrs	33±9 (18–56)		
Weight kg	68.6±12.5 (49.4–98.8)		
Height cm	169.9±9.0 (155–189.5)		
BMI# $kg \cdot m^{-2}$	23.6±3.1 (18.1–30.2)		
BSA¶ m^2	1.77±0.19 (1.44–2.17)		
FEV_1 L	3.93±0.67 (2.77–5.67)	3.73±0.61 (2.73–5.00)	106±11 (89–126)
FVC L	4.89±0.84 (3.28–6.57)	4.54±0.81 (3.43–6.23)	109±12 (87–134)
FEV_1/FVC	0.81±0.06 (0.67–0.92)		
PEF $L \cdot s^{-1}$	9.06±2.14 (5.71–13.90)	8.52±1.54 (6.49–11.33)	106±13 (84–140)
FEF_{25-75} $L \cdot s^{-1}$	4.86±1.19 (2.81–8.03)	3.81±0.53 (2.74–4.77)	127±23 (87–177)*

BMI: body mass index; BSA: body surface area; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; PEF: peak expiratory flow; FEF_{25-75}: mean forced expiratory flow between 25 and 75% FVC. #: calculated at weight (kg) divided by height (m); ¶: calculated as 0.0097 × (height in cm+weight in kg)-0.545. *: predicted value significantly different than measured value ($p<0.05$). Pulmonary function values in males calculated as a percentage of normal values predicted for males and females of same height and age from HANKINSON *et al.* [31].

TABLE 2 Characteristics of the five sequential single breath-hold manoeuvres in 31 subjects

Variables	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average of 5 trials
Breath-hold time s	5.5±0.6 (4.7–6.7)	5.3±0.4 (4.7–6.0)	5.3±0.5 (4.6–6.2)	5.2±0.4 (4.6–6.0)*	5.3±0.5 (4.6–6.5)	5.3±0.4 (4.7–6.0)
Inspired volume L	4.8±0.8 (3.0–6.8)	4.7±0.8 (3.0–6.5)	4.7±0.9 (2.9–6.4)	4.8±0.9 (3.0–6.8)	4.7±0.8 (3.0–6.4)	4.7±0.8 (3.0–6.6)
Inspired volume % of FVC	97.4±6.5 (80.7–108.9)	97.0±6.2 (80.1–108.7)	96.0±7.9 (77.5–109.3)	97.9±6.4 (78.0–109.8)	96.6±6.3 (76.5–109.5)	97.0±6.0 (78.6–109.2)
Alveolar volume L	6.6±1.1 (4.5–9.3)	6.5±1.1 (4.5–9.4)	6.4±1.1 (4.3–9.5)*	6.5±1.2 (4.4–9.7)	6.4±1.1 (4.4–9.4)*	6.5±1.1 (4.4–9.4)
Inspired NO concentration ppm	39.4±8.0 (11.8–53.1)	41.2±5.9 (21.3–49.5)	44.3±3.4 (38.5–50.9)*	44.4±3.7 (31.9–49.8)*	44.9±2.7 (38.8–50.0)*	42.8±3.3 (31.7–49.4)
Expired NO concentration ppm	3.7±1.4 (1.4–1.0)	4.0±1.2 (1.2–1.8)	4.2±1.2 (1.2–2.3)*	4.4±1.0 (1.0–2.7)*	4.4±1.1 (1.1–2.0)*	4.2±1.1 (1.1–2.2)
Inspired CO concentration %	0.28±0.00 (0.27–0.28)	0.28±0.00 (0.27–0.28)*	0.28±0.0 (0.27–0.28)*	0.28±0.00 (0.27–0.28)*	0.28±0.00 (0.27–0.28)*	0.28±0.00 (0.27–0.28)
Expired CO concentration %	0.13±0.01 (0.10–0.15)	0.13±0.01 (0.10–0.15)	0.13±0.01 (0.10–0.16)	0.13±0.01 (0.12–0.15)*	0.13±0.01 (0.11–0.16)*	0.13±0.01 (0.11–0.15)
Inspired O_2 concentration %	19.0±0.3 (18.2–19.4)	19.1±0.1 (18.9–19.3)	19.1±0.1 (18.6–19.3)	19.1±0.1 (18.9–19.3)	19.1±0.1 (18.9–19.3)	19.1±0.1 (18.8–19.3)
Expired O_2 concentration %	15.0±0.7 (13.2–16.0)	15.0±1.0 (11.3–16.7)	15.2±0.9 (12.6–16.8)	15.2±0.9 (12.7–16.7)	15.3±0.7 (13.7–16.7)	15.1±0.7 (13.3–16.5)
Inspired He concentration %	9.4±0.1 (9.1–9.6)	9.4±0.1 (9.3–9.6)*	9.4±0.1 (9.3–9.6)*	9.4±0.1 (9.2–9.6)*	9.4±0.1 (9.3–9.6)*	9.4±0.1 (9.2–9.6)
Expired He concentration %	6.3±0.4 (5.5–7.1)	6.4±0.4 (5.6–7.2)	6.4±0.4 (5.7–7.2)	6.5±0.4 (5.6–7.2)	6.5±0.4 (5.6–7.2)*	6.4±0.4 (5.7–7.1)

Data are presented as mean±SD (range). FVC: forced vital capacity; NO: nitric oxide; CO: carbon monoxide; O_2: oxygen; He: helium. *: significantly different from trial one ($p<0.05$). The breath-hold time includes inspiration time plus apnoea time.

When $D_{m,CO}=D_{L,NO}/2.42$, a real clinical change for $D_{m,CO}$ and V_c were 7.1 mm·min^{-1}·Hg^{-1} and 13 mL, respectively. When $D_{m,CO}=D_{L,NO}/1.97$, a real clinical change for $D_{m,CO}$ and V_c was any change >8.7 mL·min^{-1}·mmHg^{-1} and 9.8 mL, respectively.

The variables age, weight and height were examined by forward stepwise multiple regression to predict $D_{L,NO}$ values at rest. The only variable that appreciably affected $D_{L,NO}$ was height. Predicted $D_{L,NO}$ in mL·min^{-1}·mmHg^{-1}=2.0164×height in cm–175.63 (r^2=0.39; SEE=23.3). Adding the other two variables to the equation did not increase the coefficient of determination significantly. Therefore, $D_{L,NO}$ at rest was best predicted by height.

DISCUSSION

The present study showed that repeated inhalations of 40–50 ppm NO during single breath-hold manoeuvres does not increase $D_{L,NO}$, $D_{m,CO}$, $D_{L,CO}$ or V_c. In fact, five sequential breath-hold manoeuvres did not change $D_{L,NO}$ and, thus, $D_{m,CO}$. However, $D_{L,CO}$ significantly decreased by the fourth trial, and V_c significantly decreased by the third trial. The drop in $D_{L,CO}$ by the fourth trial due to progressive increase in carboxyhaemoglobin was similar to that reported elsewhere [33]. As such, the data show that the actual number of tests required to provide a reasonable estimate of $D_{L,NO}$, $D_{L,CO}$, $D_{m,CO}$ and V_c during properly performed manoeuvres is two trials. The average of the two trials should then be reported. Any more than two properly performed manoeuvres will lower V_c, and more than three will lower $D_{L,CO}$. Therefore, two properly performed manoeuvres are sufficient to obtain all the components of lung diffusion capacity using the $D_{L,NO}$–$D_{L,CO}$ method.

There has been some concern that NO may affect the pulmonary microcirculation due to its vasoactive properties

TABLE 3 Lung diffusing capacity and its components over five sequential trials in 31 subjects

Variables	Predicted value	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average of 5 trials
$D_{L,NO}$ mL·min^{-1}·mmHg^{-1}	167±18 (137–206)	166±29 (115–214)	169±29 (114–222)	167±31 (112–214)	168±30 (110–226)	165±30 (108–217)	167±29 (112–217)
$D_{m,CO}$ mL·min^{-1}·mmHg^{-1}#	62.4±10.6 (49.1–84.8)**	68.8±12.0 (47.3–88.6)	69.6±12.1 (47.0–91.9)	69.0±12.6 (46.3–88.4)	69.3±12.5 (45.4–93.6)	68.2±12.4 (44.8–89.8)	69.0±12.1 (46.4–89.6)
$D_{m,CO}$ mL·min^{-1}·mmHg^{-1}¶	62.4±10.6 (49.1–84.8)**	84.5±14.7 (58.1–108.8)	85.5±14.8 (57.8–112.9)	84.8±15.5 (56.9–108.6)	85.2±15.3 (55.7–114.9)	83.8±15.3 (55.0–110.3)	84.8±14.9 (57.0–110.0)
$D_{L,CO}$ mL·min^{-1}·mmHg^{-1}	36.5±4.1 (29–44.5)**	32.9±6.0 (23.4–49.6)	32.4±5.6 (22.1–43.7)	32.1±6.2 (21.2–47.6)	31.7±5.8 (21.2–44.1)*	31.4±5.9 (20.8–41.9)*	32.1±5.8 (21.7–45.4)
V_C mL^{+}	75±8 (63–97)**	91±16 (59–149)	87±13 (56–120)	87±16 (59–143)*	84±12 (57–116)*	83±13 (50–109)*	86±13 (58–127)
V_C mL§	75±8 (63–97)	77±12 (51–120)	75±10 (49–100)	74±12 (51–115)*	73±10 (50–98)*	72±10 (52–92)*	74±11 (50–105)

Data are presented as mean±SD (range). $D_{L,NO}$: lung diffusing capacity for nitric oxide; $D_{m,CO}$: alveolar membrane diffusing capacity for carbon monoxide; $D_{L,CO}$: lung diffusing capacity for carbon monoxide; V_C: pulmonary capillary blood volume. #: when $D_{m,CO}=D_{L,NO}/2.42$; ¶: when $D_{m,CO}=D_{L,NO}/1.97$; +: when $D_{m,CO}=D_{L,NO}/2.42$; §: when $D_{m,CO}=D_{L,NO}/1.97$. *: significantly different from trial one ($p<0.05$); **: predicted value significantly different from the measured value averaged over five trials ($p<0.05$). Predicted $D_{m,CO}$ values are from ZANEN *et al.* [25] and are reported as mL·min^{-1}·mmHg^{-1}. For males, $D_{m,CO}=3\times1/(0.127+0.0003304\times age-0.04753\times height$ in m); for females, $D_{m,CO}=3\times1/(0.111+0.0003304\times age-0.04753\times$ height in m). Predicted $D_{L,CO}=0.41\times$height in cm$-0.21\times$age-26.31 ($r^2=0.60$; SEE=4.8) from CRAPO and MORRIS [32]. V_C values are from ZANEN *et al.* [25]. For males $V_C=1/(0.04455-0.01805\times$height in m); for females $V_C=1/(0.04375-0.01805\times$height in m). Predicted $D_{L,NO}$ in mL·min^{-1}·mmHg$^{-1}=2.0164\times$height in cm-175.63 ($r^2=0.39$; SEE=23.3) from the current study. The units reported are traditional (mL·min^{-1}·mmHg^{-1}). For SI units (mmol·min^{-1}·kPa^{-1}), divide by 3.

TABLE 4 Measurement error and repeatability of lung diffusing capacity and its components for 31 subjects

Variable	Measurement error#	Repeatability¶
$D_{L,NO}$ mL·min^{-1}·mmHg^{-1}	6.2	17.2
$D_{m,CO}$ mL·min^{-1}·mmHg^{-1+}	2.6	7.1
$D_{m,CO}$ mL·min^{-1}·mmHg$^{-1\S}$	3.2	8.7
$D_{L,CO}$ mL·min^{-1}·mmHg^{-1}	1.2	3.2
V_C mL^{+}	4.7	13.0
V_C mL§	3.5	9.7

$D_{L,NO}$: diffusing capacity of the lung for nitric oxide; $D_{m,CO}$: membrane diffusing capacity for carbon monoxide; $D_{L,CO}$: diffusing lung capacity for carbon monoxide. #: within-subject standard deviation for each variable was calculated as the square root of the mean squares error from the ANOVA summary table [30]; ¶: calculated as 2.77×within-subject standard deviation [30]; +: when $D_{m,CO}=D_{L,NO}/2.42$; §: when $D_{m,CO}=D_{L,NO}/1.97$. Each variable was reported to the nearest tenth decimal place.

[22]. One study showed that there is a small nonsignificant increase in $D_{L,CO}$ in the presence of NO [17]. However, the current authors have shown that brief exposure to NO does not interfere with physiological function. Other human [11, 12, 20, 21] and animal studies [34] are in agreement with the present study. Rebreathing 20–40 ppm NO over 16 s to 10 min resulted in no significant changes to oxygen uptake, arterial oxygen tension, alveolar–arterial oxygen tension difference, $D_{L,CO}$, D_m or V_c in sick and healthy subjects at rest or during exercise [11, 12, 21]. In mechanically ventilated rabbits, $D_{L,NO}$ values remained unchanged despite inspiratory NO concentrations varying from 10–800 ppm [34]. Furthermore, the pulmonary toxicity of inhaled NO at concentrations of ~40 ppm over a prolonged period of time is minimal [35].

There is concern that different P_{A,O_2} may slightly alter $D_{L,NO}$ [36]. However, more recent data has shown that varying the P_{A,O_2} in those with or without pulmonary disease does not affect $D_{L,NO}$ [11, 12], implying that the combined $D_{L,NO}$–$D_{L,CO}$ method can be used in hypoxaemic patients. Therefore, taken together, there is no reason to refrain from using NO and CO concurrently as a test gas to assess lung diffusion capacity.

The clinical implication of using both NO and CO concurrently in research and medical practice is that scientists and clinicians can immediately partition and quantify the components of lung diffusing capacity in a subject from a single 4-s breath-hold manoeuvre that requires minimal effort on the part of the patient, while simultaneously being able to pinpoint which component (V_c, D_m) is causing low (or high) total lung diffusion capacities. The ability to estimate $D_{m,CO}$ and V_c from one-step simultaneous measurement of $D_{L,NO}$ and $D_{L,CO}$ represents significant conceptual advantages. One conceptual advantage of the $D_{L,NO}$ technique is that with the standard Roughton–Forster method, cardiac output can vary between measurements of $D_{L,CO}$ at different O_2 tensions, which then have to be interpolated to obtain $D_{L,CO}$ at two O_2 tensions, but at the same cardiac output [12]. With the $D_{L,NO}$ method, all measurements are obtained at the same cardiac output and O_2 tension; no interpolation is necessary. Another conceptual

advantage is that with the traditional method, the distribution of CO gas in the lungs may be different at two different O_2 tensions, but with the $D_{L,NO}$ method only one inspiration is required, which results in a similar distribution of NO and CO gases. A third conceptual advantage is that with the traditional method, there is systematic underestimation of V_c and an overestimation of D_m since the inspiration at two different O_2 tensions affects alveolar–capillary membrane diffusion [37]. The $D_{L,NO}$–$D_{L,CO}$ method avoids this error altogether and should improve the accuracy of estimated $D_{m,CO}$ and V_c.

The ratio $D_{m,CO}=D_{L,NO}/2.42$ gives a better estimate of D_m. When the present measured values were compared against the published norms, using the traditional two-step Roughton–Forster method [25], the predicted $D_{m,CO}$ was 136% pred when $D_{m,CO}=D_{L,NO}/1.97$, but only 111% pred when $D_{m,CO}=D_{L,NO}/2.42$. However, the better per cent predicted values for V_c occurred when $D_{m,CO}=D_{L,NO}/1.97$. Nonetheless, since the traditional Roughton–Forster method underestimates V_c, the current authors feel that the predicted V_c values by ZANEN *et al.* [25] slightly underestimate V_c. Therefore, the best compromise is to estimate D_m and V_c from a single breath using the $D_{L,NO}$–$D_{L,CO}$ method and the formula $D_{m,CO}=D_{L,NO}/2.42$.

It was also important to clarify the repeatability of lung diffusion capacity and its components over five trials from the single-breath $D_{L,NO}$–$D_{L,CO}$ method. The repeatability allows clinicians to identify a true clinically meaningful change from measurement error. The difference between any two measurements for the same subject is expected to be <2.77 multiplied by the within-subject standard deviation; therefore, the repeatability was defined as 2.77 multiplied by the within-subject standard deviation obtained from the ANOVA [30]. Table 4 shows a true measurable clinical change in $D_{L,NO}$ and $D_{L,CO}$ as an absolute change of >17 and 3 mL·min^{-1}·mmHg^{-1}, respectively. In addition, a true clinical change in $D_{m,CO}$ and V_c is an absolute change of >7 mL·min^{-1}·mmHg^{-1} and 13 mL, respectively. Any change that is less than these values is considered a true measurement error. Indeed, $D_{m,CO}$ equals $D_{L,NO}$ divided by a fixed value, and V_c is derived from the $D_{L,NO}$ and $D_{L,CO}$; therefore the repeatability of D_m and V_c can be calculated from (or explained by) the repeatability of the $D_{L,NO}$.

Based on ATS and ERS criteria, the average value of two trials whose difference in diffusing capacity is within 10% of each other is considered acceptable. The present authors looked at the average $D_{L,NO}$ and $D_{L,CO}$ for all 31 subjects over five trials, and the repeatability data of this study are in agreement with the ATS/ERS guidelines as $D_{L,NO}$ (and thus $D_{m,CO}$) and $D_{L,CO}$ are found to be 10%. However, since the variability of the parameters $D_{L,NO}$ and $D_{L,CO}$ were independent of the magnitude of the measurement, the results invalidate the use of percentage value to describe the repeatability. Using a percentage will lead to underestimation of variability in low values and overestimation for high values. Others studies have also suggested using an absolute value rather than a percentage, since the diffusing capacity was also independent of the magnitude of the measurement [38, 39]. Therefore, the current authors recommend using an absolute difference rather than a percentage as alternative criteria for repeatability. It is also recommended to report the average of two trials when the absolute difference between the two measurements is within 17 mm·min^{-1}·mmHg^{-1} for $D_{L,NO}$, 3 mm·min^{-1}·mmHg^{-1} for $D_{L,CO}$, and 13 mL for V_c.

It is believed that the present paper is the first to actually quantify important measurable clinical changes in the parameters obtained from the single-breath $D_{L,NO}$–$D_{L,CO}$ method.

Conclusion

Small amounts of nitric oxide inhaled during sequential single-breath manoeuvres have no effect on lung diffusing capacity for nitric oxide and, thus, membrane diffusing capacity for carbon monoxide. The recommended ratio is membrane diffusing capacity for carbon monoxide=lung diffusing capacity for nitric oxide/2.42. As more than two and three single-breath manoeuvres will lower pulmonary capillary blood volume and lung diffusing capacity for carbon monoxide, respectively, the average value of the first two trials are recommended to provide a reasonable estimate of lung diffusing capacity for nitric oxide, lung diffusing capacity for carbon monoxide, membrane diffusing capacity for carbon monoxide and pulmonary capillary blood volume.

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