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Reference values for alveolar membrane diffusion capacity and pulmonary capillary blood volume

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ABSTRACT: The reference values for diffusion capacity of the alveolar capillary membrane ($T_{m,CO}$) and pulmonary capillary volume (Q_c) are scarce, while the standard deviations of the equations are large. New equations and residual standard deviations (RSDs) were determined in a sample of healthy subjects.

$T_{m,CO}$ and Q_c values were measured in 117 (72 females, 45 males) nonsmoking healthy subjects. The carbon monoxide transfer factor ($T_{L,CO}$) was determined when the volunteer was breathing room air and subsequently, when the volunteer was breathing 100% oxygen. From these data, $T_{m,CO}$ and Q_c values were calculated.

The females' $T_{L,CO}$ was 3.15 mmol·min^{-1}·kPa^{-1} lower than the males', apparently caused by lower female lung volume. $T_{m,CO}$ and Q_c were lower in females, but correction for lung volume eliminated this difference. Q_c^{-1} reference equations for females and males, respectively, are 4.375×10^{-2}-$1.085\times10^{-2}\times$height and 4.455×10^{-2}-$1.085\times10^{-2}\times$height (RSD for both sexes: 2.544×10^{-3}). $T_{m,Co}^{-1}$ reference equations for females and males, respectively, are $0.111+3.304\times10^{-4}\times$age-$4.753\times10^{-2}\times$height and $0.127+3.304\times10^{-4}\times$age-$4.753\times10^{-2}\times$height (RSD for both sexes: 1.085×10^{-2}). The general character of these equations complies with earlier publications, the only difference being that the RSDs are 1.18–2.76 times lower.

New reference equations for diffusion capacity of the alveolar capillary membrane and pulmonary capillary volume are available with considerably smaller residual standard deviations.

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Measurement of the single-breath carbon monoxide (CO) transfer factor ($T_{L,CO}$) to determine the quality of gas transfer is presently a standard procedure. The transfer of CO over the alveolar capillary membrane is governed by two resistances in series: the resistance of the membrane itself (the physical resistance (R_m)) and the capillary resistance (R_{cap}): $R=R_m+R_{Cap}$. The latter resistance is believed to be influenced by the pulmonary capillary blood volume (Q_c), through the binding of CO to haemoglobin and the transfer/transport of CO into the red blood cell (the latter two presented by the chemical resistance, the CO reaction rate coefficient in red blood cells (θ_{CO})). The chemical resistance depends on the red blood cell oxygen (O_2) concentration. It is customary to present the terms in $R=R_m+R_{cap}$ by their reciprocals (conductances) and arrive at the well-known Roughton-Forster equation [1], which describes the two-resistor model for CO transfer:

$$\frac{1}{T_{L,CO}} = \frac{1}{T_{m,CO}} + \frac{1}{\theta_{CO} Q_c} \quad (1)$$

where $T_{m,CO}$ denotes the diffusion capacity of the alveolocapillary membrane. By modifying the inspired O_2 concentration, the terms $T_{m,CO}$ and Q_c in Equation 1 can be determined.

In order to put measurements of these variables into clinical use, a comparison between the measured values and reference values is needed to determine the severity of the disease process [2]. When comparing actual with predicted values, the use of standardized residuals is recommended. References values for the two components of the transfer factor and their standard deviations (SD) are scarce; only the values of COTES [3] and FRANS [4] are widely available, but the derivation of these values is not extensively described (the equations of COTES [3] are listed in his book as "not published"). The reference values of CRAPO *et al.* [5] were determined using a rebreathing technique, which differs from the more commonly used single-breath technique. The aim of the present study is to provide reference values and the corresponding residual standard deviations (RSDs) for $1/T_m$ and $1/Q_c$, derived from measurements in a cohort of healthy volunteers.

Materials and methods

Subjects

One-hundred and seventeen (72 females, 45 males) nonsmoking healthy subjects were invited to participate in this study. They were recruited from the

nursing, administrative and laboratory staff of the hospital; all had sedentary jobs without physical strain and lived in an area without heavy traffic and/or air pollution. All subjects gave verbal and written consent to participate. The Medical Ethical Committee of the hospital approved the study.

Upon questioning and physical examination, all volunteers were found to be without complaints or disease. Pregnancy was an exclusion criterion because of the possible changes in (pulmonary) haemodynamics. No special attention was paid to the menstrual cycle. A stringent inclusion criterion was a normal haemoglobin level. The normal values in this hospital ranged from 7.7–9.6 mM for females and from 8.6–10.7 mM for males. The demographic data and mean lung function are presented in table 1.

Measurements

First, the single breath $T_{L,CO}$ was determined when the volunteer was breathing room air with 21% O_2 ($T_{L,low}$) and, subsequently, the same was done when the volunteer was breathing 100% O_2 ($T_{L,high}$) [6]. Using the equation:

$$\frac{1}{\theta_{CO}} = (0.001 + 0.000134 P_{c,O_2}) \quad (2)$$

the θ_{CO} value for the 21% and 100% O_2 situation was calculated [3], where P_{c,O_2} is the mean capillary O_2 tension, which is estimated from the alveolar O_2 tension (P_{A,O_2}), the O_2 consumption and diffusing capacity (rendering P_{c,O_2} slightly lower than P_{A,O_2}). The P_{A,O_2} was measured in the exhaled alveolar sample and not by using the alveolar air equation. The latter could produce incorrect results because the inhaled fraction O_2 is changed due to the presence of helium (He) and CO [3, 6]. The term 0.001 in Equation 2 is based on a partition coefficient of 2.5. The following equations used to calculate Q_c and T_m can be derived from Equation 1 by algebraic manipulation of the high and low O_2 expressions of this equation:

$$Q_c = \frac{\frac{1}{\theta_{high}} - \frac{1}{\theta_{low}}}{\frac{1}{T_{L,high}} - \frac{1}{T_{L,low}}} \quad (3)$$

Table 1. – Demographic and lung function data on the male and female subjects

Variable	Males	Females
Age yrs	40.1±10.8	38.3±11.5
Weight kg	82.6±11.8	66.0±8.8
BMI	24.7±2.9	23.1±3.1
Height m	1.83±0.07	1.69±0.06
FEV_1 % pred	112.0±12.7	111.2±13.8
TLC % pred	108.3±10.8	110.3±12.3
V_A/TLC %	97±5	94±4

Data are presented as mean±SD. BMI: body mass index; FEV_1: forced expiratory volume in one second; TLC: total lung capacity; V_A: alveolar volume. V_A was obtained using single-breath helium dilution and TLC *via* body plethysmography.

and

$$T_m = \frac{1}{\frac{1}{T_{L,low}} - \frac{1}{\theta_{low} Q_c}} \quad (4)$$

where θ_{high} and θ_{low} are the CO reaction rate coefficients under 100% and 21% O_2 conditions, respectively.

Estimations of $1/Q_c$ and $1/T_{m,CO}$ were made using a Jaeger Compact Lab Transfer system (Erich Jaeger GmbH, Wuerzburg, Germany).

After a rest (in which the questioning and physical examination took place), all volunteers inhaled a test gas containing 0.25% CO, 9% He and balance air, while their lungs were filled with room air. They inhaled the test gas from residual volume up to total lung capacity (TLC) level in the shortest possible time (<2.5 s), and subsequently held their breath for 10 s after which they exhaled quickly into a sample bag. The breath-hold period was calculated starting from two-thirds of the way through inspiration time and ending half way through the sample collection. The first portion of the exhaled volume (800 mL) containing the dead space (300 mL in total) was discarded and only the alveolar fraction was sampled (sample volume 800 mL). From this sample bag, the exhaled fraction of CO and He was determined. This procedure was performed in triplicate.

The next step was to inhale 100% O_2 for a period long enough to stabilize the exhaled O_2 at the high level of 95%. The exhaled O_2 fraction was monitored breath-by-breath and the $T_{L,CO}$ measurement only started when the exhaled fraction became stable for $\geqslant$60 s. ROTHEN *et al.* [7] found that after 40 min of 100% O_2 breathing, the total atelectasis volume was 4.2±4.5 cm^2, compared to 1.6±1.6 cm^2 when breathing room air. As a result of this, and because the repeated vital capacity manoeuvres inhibit atelectasis formations, significant atelectasis is improbable here. Without disconnecting the volunteer from the apparatus, a test gas containing 0.25% CO, 7.7% He and balance 100% O_2 was inhaled from residual volume up to TLC level. The rest of this procedure was identical to the one described above and was also performed in triplicate. Thus, in total six determinations took place, which did not result in a build-up of significant carboxyhaemoglobin (COHb) levels (±3% COHb).

The values for $T_{L,CO}$ under room air and 100% O_2 conditions were averaged and used for the calculation of $1/Q_c$, and $(1/T_{m,CO})\times(1/Q_c)$ is expressed mL^{-1} and $1/T_{m,CO}$ as $mmol^{-1}\cdot min\cdot kPa$. No correction was made for haemoglobin (Hb) levels. STAM *et al.* [8] showed that in healthy volunteers with normal Hb levels, correction has only a very limited effect. The distribution of the Hb levels is narrower than that of $1/Q_c$ and $1/T_{m,CO}$ and no variance reduction of the latter two is possible.

CO gas was analysed by means of infrared absorbance and He by thermal conductivity (He analysis was corrected for high O_2 tensions). Before each measurement, both analysers were calibrated using certified gas mixtures.

Analysis

Biological data are frequently lognormally distributed, so the measured $1/Q_c$ and $1/T_{m,CO}$ values were

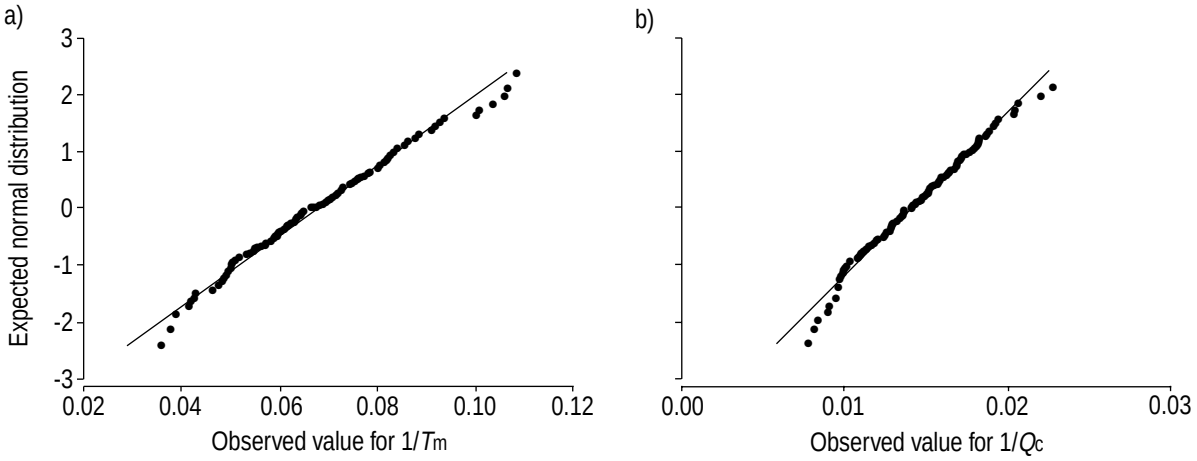

Fig. 1. – Distribution of the a) $1/T_{m,CO}$ and b) $1/Q_c$ values. The straight lines in these graphs represent the normal distribution. The values show a close overlap with the normal distribution in both cases. $T_{m,CO}$: diffusion capacity of the alveolar capillary membrane; Q_c: pulmonary capillary volume.

subjected to a Kolmogorov-Smirnov test to examine deviations from the normal distribution. The $1/Q_c$ and $1/T_{m,CO}$ values were averaged and the ±2 SD range was calculated. To estimate possible differences between males and females, the data were subjected to analysis of (co)variance to explain the influence of covariates.

The $1/Q_c$ and $1/T_{m,CO}$ values were incorporated into a general linear model analysis of (co)variance, which has the same outcome as the classical multiple regression analysis approach. The independent variables were sex, age, height and the interaction between sex and age/height. The latter two test for parallelism in regression slopes and determine whether the male/female regression slopes for age and height differ. If they do not differ, the slopes can be pooled. Incorporation of a multitude of (irrelevant) variables weakens the predictive power of any regression model; useful variables show high correlations with $1/Q_c$ and $1/T_{m,CO}$, but low intercorrelations.

Spearmann's correlation coefficients were calculated to determine the relationships between the independent variables. Significance levels were set at α=0.05 and all data are represented as mean±SD.

Results

The single-breath $T_{L,CO}$ values on room air are expressed as a percentage of predicted [9]. The female group showed a small, but significant departure from the expected value: 90±14.2% pred ($p<0.001$). No significant departure was found for males: 99±14.1% pred (p=0.638). Males showed a higher transfer factor than females (p=0.002), with a mean difference of 3.15 $mmol \cdot min^{-1} \cdot kPa^{-1}$ (95% confidence interval (CI) of the difference 3.77–2.52).

The Kolmogorov-Smirnov test indicated no significant deviation from the normal distribution for $1/Q_c$ and $1/T_{m,CO}$ values (p=0.2) (fig. 1).

The analysis of variance of $1/Q_c$ and $1/T_{m,CO}$ values showed significant sex differences (p=0.011 and $p<0.001$, respectively), even after correction for height and age. The mean and ±2 SD ranges for $1/Q_c$ and $1/T_{m,CO}$ in females and males are shown in table 2. The interactions between sex and height/age were nonsignificant ($p>0.063$ in all cases), indicating that the regression slopes for age and height do not differ between males and females.

In table 3, the correlation coefficients between $1/Q_c$, $1/T_{m,CO}$ and the other lung function parameters are shown. They are significantly correlated with height, forced expiratory volume in one second (FEV_1), TLC and residual volume. These variables are, therefore, possible candidates for use in regression analysis. However, the correlation between height on the one hand and FEV_1, TLC and residual volume on the other was also highly significant, thus rendering them less suitable [10]. As a result of this, and in order to be able to compare the outcome to earlier approaches [3, 4], only height and age were included in the analysis.

Table 2. – Descriptive summary of $1/Q_c$ and $1/T_{m,CO}$ levels in healthy males and females

Sex	$1/Q_c$ mL^{-1}	$1/T_{m,CO}$ $mmol^{-1} \cdot min \cdot kPa$
Females		
Mean±SD	$1.583\times10^{-2}\pm3.082\times10^{-3}$	$7.613\times10^{-2}\pm1.345\times10^{-2}$
±2 SD range	9.666×10^{-3}-2.199×10^{-2}	4.923×10^{-2}-1.030×10^{-1}
Males		
Mean±SD	$1.159\times10^{-2}\pm2.260\times10^{-3}$	$5.418\times10^{-2}\pm9.624\times10^{-3}$
±2 SD range	7.073×10^{-3}-1.611×10^{-2}	3.493×10^{-2}-7.343×10^{-2}

Q_c: pulmonary capillary blood volume; $T_{m,CO}$: diffusion capacity of the alveolar capillary membrane.

Table 3. – Correlation coefficient matrix of $1/T_{m,CO}$ and $1/Q_c$ with age, height, forced expiratory volume in one second (FEV_1), total lung capacity (TLC) and residual volume

	Age	Height	FEV_1	TLC	Residual volume
$1/T_{m,CO}$	0.208*	-0.678**	-0.773**	-0.743**	-0.264**
$1/Q_c$	0.055	-0.687**	-0.699**	-0.755**	-0.401**

$T_{m,CO}$: diffusion capacity of the alveolar capillary membrane; Q_c: pulmonary capillary blood volume. *: correlation is significant at 0.05 level; **: correlation is significant at 0.01 level.

The derived equations for $1/Q_c$ and $T_{m,CO}$ are listed in tables 4 and 5 together with those of COTES [3] and FRANS [4]. Significant predictors for $1/T_{m,CO}$ were age (p=0.001) and height (p=0.005), but only height was a significant predictor for $1/Q_c$ (p<0.001). The RSDs are 1.18–2.76 times lower than those of COTES [3] and FRANS [4], indicating a significantly higher power to detect deviations from the normal values.

COTES [3] listed a value for T_m/Q_c of 0.2. The present authors found a similar value of 0.21±0.035, while no significant difference was found between females and males (p=0.4).

Discussion

In the present study, reference equations for $1/Q_c$ and $1/T_{m,CO}$ have been determined in a sample of healthy subjects. There are striking similarities with two previous approaches, but the major difference is the smaller RSDs and hence, the higher sensitivity to detect disease. Height has the same influence in all approaches; taller people show smaller $1/Q_c$ and $1/T_{m,CO}$ values, which means that Q_c and $T_{m,CO}$ values increase with increasing height. With regard to age, in the equations for $1/T_{m,CO}$, positive terms were found, meaning that $T_{m,CO}$ decreases as one gets older. It must be noted that in all equations, the influence of age is much smaller than that of height, so the significance of these differences is limited. A significant influence of age for $1/Q_c$ is lacking in all approaches. Systematic differences in the predicted values are present due to differences in the regression coefficients and constants. The predicted $1/Q_c$ value derived from this study is smaller in short females and larger in taller females. The "break-even point" is at a height of 1.85 m, so in the majority of female subjects the authors predict smaller values. For male $1/Q_c$, the authors always predict smaller values, so it can be estimated that Q_c is always larger than estimations of earlier equations (fig. 2).

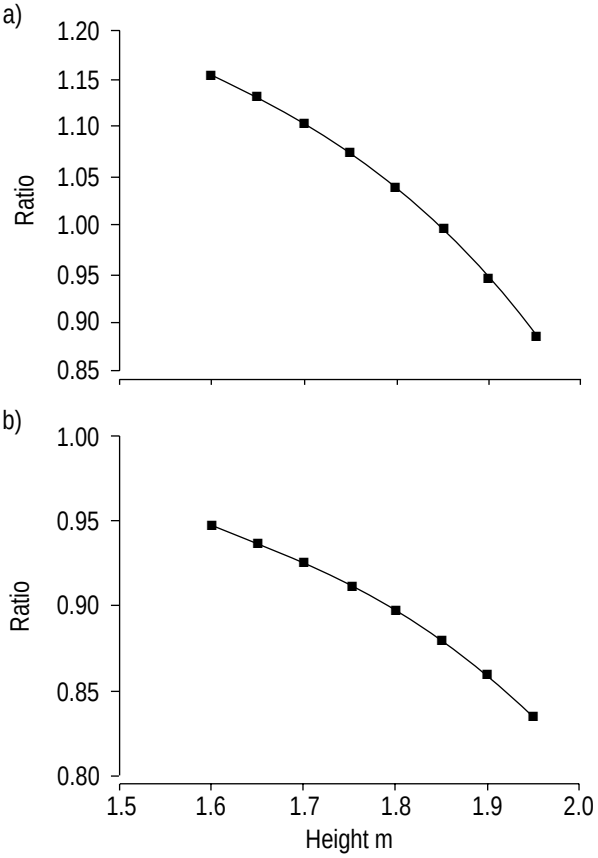

Fig. 2. – Ratio of predicted values for $1/Q_c$ in a) females and b) males derived from the present study, and those of COTES [3] and FRANS [4]. The ratio depicts the predicted values of COTES [3] and FRANS [4] divided by those of the current study. Only one line is present because an age factor is not included. Q_c: pulmonary capillary volume.

Table 4. – Regression equations for $1/Q_c$ in males and females

Sex	Regression equation	RSD
Females[#]	4.375×10^{-2}-$1.805\times10^{-2}\times$height	2.544×10^{-3}
Males[#]	4.455×10^{-2}-$1.805\times10^{-2}\times$height	
Females[¶]	6.1×10^{-2}-$2.74\times10^{-2}\times$height	6×10^{-3}
Males[¶]	4.7×10^{-2}-$2.01\times10^{-2}\times$height	3×10^{-3}

Q_c: pulmonary capillary volume; RSD: residual standard deviation. [#]: contains the equations derived in the present study; [¶]: contains equations derived in studies of COTES [3] (females) and FRANS [4] (males). Height is in metres.

Table 5. – Regression equations for $1/T_{m,CO}$ in males and females

Sex	Regression equation	RSD
Females[#]	$0.111+3.304\times10^{-4}\times$age-$4.753\times10^{-2}\times$height	1.085×10^{-2}
Males[#]	$0.127+3.304\times10^{-4}\times$age-$4.753\times10^{-2}\times$height	
Males and females[¶]	$0.135+3.6\times10^{-5}\times$age-$5.4\times10^{-2}\times$height	3×10^{-2}

$T_{m,CO}$: diffusion capacity of the alveolar capillary volume; RSD: residual standard deviation. [#]: contain equations derived in the present study; [¶]: contains equations derived in the study of COTES [3]. Height is in metres and age in years.

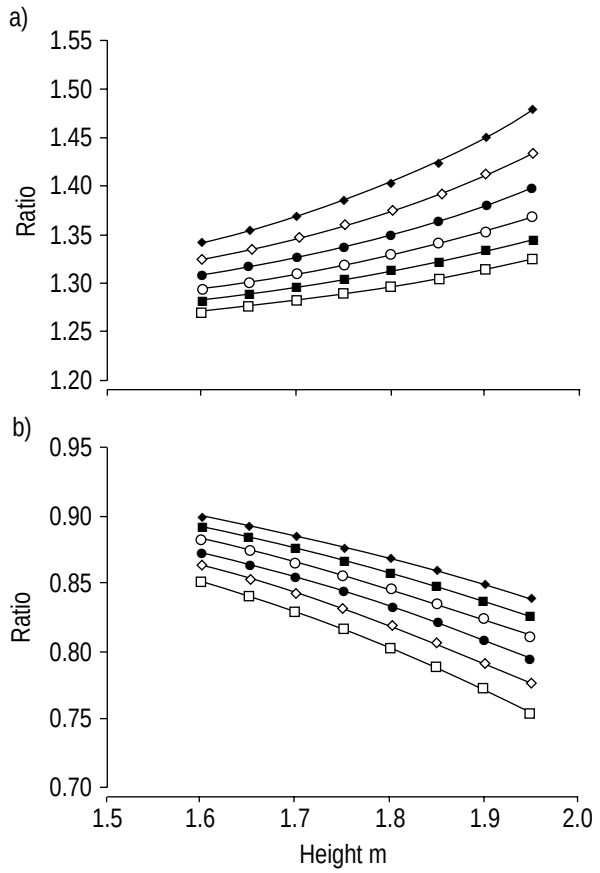

Fig. 3. – Ratio of predicted values for $1/T_{m,CO}$ in a) females and b) males derived from the present study and that of COTES [3]. The ratio depicts the predicted values of COTES [3] divided by those of the present study. The lines are broken down by age. ◆: 20 yrs; ◇: 30 yrs; ●: 40 yrs; ○: 50 yrs; ■: 60 yrs; □: 70 yrs. $T_{m,CO}$: diffusion capacity of the alveolar capillary membrane.

For $1/T_{m,CO}$, it is evident that the female constant in the equation of COTES [3] is larger, as it is in the present study, while this study's height factor is smaller. Thus, the authors predict smaller female estimates for $1/T_{m,CO}$ (=larger $T_{m,CO}$ values). In males, the picture is reversed; larger "Cotes" values for $1/T_{m,CO}$ (=smaller $T_{m,CO}$ values). The differences become stronger for taller male/female subjects (fig. 3).

Because information on the subjects and/or experimental set-up used by COTES [3] and FRANS [4] is only partially available, it is rather difficult to explain these differences. However, several factors can be ruled out. First, a systemic deviation due to the equipment or experimental procedures used is unlikely. When such systemic equipment differences were to blame, the deviations between the present equations and those of COTES [3] and FRANS [4] would be constant both in males and females, which is not the case. It is also very unlikely that physiological and/or anatomical differences are responsible. It is hard to argue that the alveolar capillary membrane structure in the volunteers in these studies would have been (very) different. This would imply that different types of people exist. To underline this argument, the authors point to the similar T_m/Q_c ratio, which must lead to the conclusion that the contribution of the alveolar and capillary resistance to the total transfer resistance is identical, indicating similar anatomy and/or physiology in all volunteers.

Alveolar volume (V_A) plays an important role, although the exact influence on $T_{L,CO}$ is still under debate [8, 11, 12]. It, therefore, seems plausible that when a sample of short subjects is selected, lower $T_{m,CO}$ and Q_c values will be measured and another set of regression equations will follow. In regression analysis, when the mean of a variable increases or decreases (keeping the width of the distribution the same), the constant of the regression equation will change but the coefficients will not. When the width of a distribution increases, it means that more extreme values are present, and regression analysis is sensitive to these values, especially when the sample is rather small. In theory, a few extreme values can profoundly change the entire picture. Thus, differences in V_A, or the distribution of it, might serve as an explanation for the differences between these and previous equations.

A major difference between the equations from this and previous studies is the magnitude of the RSD, which is a factor 1.18–2.76 lower. The latter is used to calculate the 95% CIs, which are used to decide whether or not a measured value is within the normal range. It is clear that the reduction of the RSDs will render the assessment of measured values more sensitive because the lower or upper end of the 95% CI lies closer to the predicted value. The smaller SD values are the direct result of a reduced heterogeneity in the sample, they probably result from a lack of extreme values and/or experimental errors. The SD estimate in a smaller sample, however, is more sensitive to a (few) outliers than a larger sample. As previously mentioned, regression analysis is particularly sensitive for outliers. The authors checked for and found no evidence of influencing outliers (data not shown), hence the equations and RSDs are trusted not to be biased by outliers. The role of experimental error will be clear.

The $1/T_{m,CO}$ values measured show a sex difference that is not present in the equations of COTES [3]. However, a sex difference should be expected. There is a general consensus that $T_{L,CO}$ differs in females and males and reference equations reflect this. $T_{L,CO}$ is influenced by the membrane and capillary resistance and when $T_{m,CO}$ does not exert a sex effect, the $T_{L,CO}$ sex difference must be caused solely by the capillary resistance. When variance due to a sex difference is not accounted for, it will be added to nonexplained variance and the RSD will increase. In the present study's equations, the sex difference is present as different regression constants and it could be argued that because they are rather close they should be pooled. Regression equations must accurately reflect the characteristics of the subjects (*e.g.* a significant sex difference in the present study). Pooling estimated variables when small or nonsignificant differences are found is not a good option because it is a decision influenced by the accuracy of the regression process. Taking this option may cause differences that are still clinically important to be ignored.

Because V_A plays an important role, the diffusing capacity makes it an attractive covariate to include in

reference equations. CHINN *et al.* [11] showed that the inclusion of V_A divided by height (H) squared ($V_A \cdot H^{-2}$) lowers RSDs considerably. However, when a V_A term appears in a reference equation, the diffusion parameters corrected for V_A, which results in $T_{m,CO}$ or Q_c per litre V_A can be obtained. The first of the two parameters serves the same purpose as the well-known K_{CO} ($=T_{L,CO}/V_A$). It is not so much a question of whether V_A is a better parameter than height or age, but its inclusion would result in a new and different parameter. $T_{m,CO}/V_A$, Q_c/V_A or $T_{m,CO}/V_A \cdot H^{-2}$, $Q_c/V_A \cdot H^{-2}$ are not, at present, routinely used. More research is needed to select the best parameter and to design a proper evaluation scheme (especially for Q_c).

The Roughton-Forster equation [1] depends on a correct calculation of the θ_{CO} parameter, and the proper value of this parameter still raises much discussion. FORSTER [13] highlighted the basic assumption that the P_{A,O_2} should at least be >20 kPa (>150 mmHg) to obtain a proper θ_{CO} value, although at the same time admitted that the errors introduced by using lower O_2 levels are small. Mistakes made in the determination of θ_{CO} will render $1/Q_c$ and $1/T_{m,CO}$ estimates invalid. This study was not designed to validate the approach of ROUGHTON and FORSTER [1], but experiments in which the nitric oxide (NO)-CO method was used to determine $1/Q_c$ and $1/T_{m,CO}$ show highly similar estimates when compared to their approach. As a test gas, NO has the advantage that it binds much better to Hb than CO does, in fact so good that R_{cap} can be set at zero. This relieves the researcher from estimating θ_{CO} because it is very large, and the term $1/\theta_{CO}Q_c$ becomes zero. MOINARD and GUENARD [14] derived equations to obtain $1/Q_c$ and $1/T_{m,CO}$ using the single-breath NO-CO diffusing capacity approach and compared the outcome to the classical approach of ROUGHTON and FORSTER [1]. The first method is not hampered by estimating θ_{CO} and the two methods produce similar values: Q_c was 85.5± 17 mL for the NO-CO method and 81.9±14.5 mL for the Roughton and Forster approach. Therefore, this must mean that the current estimates of θ_{CO} are not altogether highly flawed.

In summary, the authors have determined new reference equations for $1/Q_c$ and $1/T_{m,CO}$, which show similar relationships with height, age and $1/Q_c$ and $1/T_{m,CO}$ as previous ones. The major differences are the lower residual standard deviations.

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