European reference equations for CO and NO lung transfer

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ABSTRACT: **The aim of the present study was to calculate reference equations for carbon monoxide and nitric oxide transfer, measured in two distinct populations.**

The transfer factor of the lung for nitric oxide ($T_{L,NO}$) and carbon monoxide ($T_{L,CO}$) were measured in 303 people aged 18–94 yrs. Measurements were similarly made in two distant cities, using the single-breath technique. Capillary lung volume (V_c) and membrane conductance, the diffusing capacity of the membrane (D_m), for carbon monoxide ($D_{m,CO}$) were derived.

The transfer of both gases appeared to depend upon age, height, sex and localisation. The rate of decrease in both transfers increased after the age of 59 yrs. $T_{L,NO}$/alveolar volume (V_A) and $T_{L,CO}/V_A$ were only age-dependent. The mean $T_{L,NO}/T_{L,CO}$ was 4.75 and the mean D_m/V_c was 6.17 $min^{-1} \cdot kPa^{-1}$; these parameters were independent of any covariate. V_c and $D_{m,CO}$ calculations depend upon the choice of coefficients included in the Roughton–Forster equation. Values of 1.97 for $D_{m,NO}/D_{m,CO}$ ratio and 12.86 $min \cdot kPa^{-1}$ for 1/red cell CO conductance are recommended.

The scatter of transfer reference values in the literature, including the current study, is wide. The present results suggest that differences might be due to the populations themselves and not the methods alone.

KEYWORDS: Ageing, capillary lung volume, carbon monoxide, diffusion, nitric oxide, pollution

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STATEMENT OF INTEREST
None declared.

The measurement of the transfer of gases through the lung is one of the few tests aimed at investigating alveolar function. The 1957 model and equation of ROUGHTON and FORSTER [1] permitted the transfer of carbon monoxide through the aveolocapillary structure to be split into two resistances, one for the alveolar membrane (1/membrane conductance, the diffusing capacity of the membrane (D_m), for carbon monoxide ($D_{m,CO}$)) and the other for the blood reacting with the gas ($1/\Theta_{CO}V_c$), where Θ_{CO} is the red cell conductance at a concentration, set by the pioneers of the method, of 14.9 $g \cdot dL^{-1}$ [2] and V_c the capillary lung volume:

$$1/T_{L,CO}=(1/D_m) + (1/\Theta_{CO}V_c) \qquad (1)$$

where $T_{L,CO}$ is the transfer factor of the lung for carbon monoxide. The first technique used to solve this equation with two unknowns, D_m and V_c, was to measure two transfers of CO, one under conditions of normoxia the other under hyperoxia. Breathing O_2, by reducing Θ_{CO}, lowers the $T_{L,CO}$. GUENARD et al. [3] first published measurements of D_m and V_c using transfer factor of the lung for nitric oxide ($T_{L,NO}$) and $T_{L,CO}$ and assuming Θ_{NO} to be infinity, i.e. $T_{L,NO}=D_{m,NO}$.

The transfer of CO is dependant upon both D_m and V_c with Θ_{CO} as a finite value.

The relationship between D_m for nitric oxide ($D_{m,NO}$) and $D_{m,CO}$ introduces a constant a: $D_{m,NO}=aD_{m,CO}$. Therefore, the measurement of NO transfer alone permits the calculation of $D_{m,CO}$ and, by introducing the latter into the CO transfer equation, of V_c.

Most published reference values for D_m and V_c have been derived from the first two-step technique; one used the NO/CO method in a population of 127 healthy adults with a mean±SD age of ~40±12 yrs [4] and another focused on NO transfer in a population of 130 adults, including 17 subjects aged >60 yrs [5]. In the present study, the reference values for NO and CO transfer are derived from a larger population with a broad age range. FORSTER and co-workers' [6, 7] 1983 values for Θ_{CO}, rather than the 1957 values, are used here, and an a of 1.97.

METHODS

Subjects

Nonsmoking persons (n=303) without cardiovascular, pulmonary or systemic diseases, aged >18 yrs and of both sexes (142 females and 161 males) were included. The subjects were from

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two French cities; the main cohort was from Grenoble, in the Alps (altitude ~300 m), and comprised 233 subjects aged 18–94 yrs; the other was from Bordeaux, near the Atlantic Ocean (altitude ~30 m), and comprised 70 subjects, aged 20–70 yrs. These distant locations were chosen to check the reproducibility of the reference equations between different places. All of the subjects were middle-class workers, the youngest were students and the oldest retired. All were advised of the aim of the protocol and gave their informed consent. The protocol was approved by the ethics committee of the University Hospital of Bordeaux (Bordeaux, France). Ex-smokers were not included if aged <40 yrs. Subjects aged >40 yrs were included if they had stopped smoking for $\geqslant$10 yrs and had had a cigarette consumption of <5 packs·yr^{-1}. They were either sedentary or practised a sports activity (or heavy work) for >3 h·week^{-1} (32% in Bordeaux and 28% in Grenoble). Individuals that were classified as obese class I (30<body mass index (BMI)<34.9 kg·m^{-2}) and without disease were also included. Obesity class II (34.9<BMI<39.9 kg·m^{-2}) and III (BMI >40 kg·m^{-2}) individuals were excluded from participating in the present study.

Measurement of NO and CO transfer

The procedures and materials used were similar in both cities. $T_{L,NO}$ and $T_{L,CO}$ were measured simultaneously during a single-breath manoeuvre using an automated apparatus (Medisoft, Dinant, Belgium). Subjects were in the sitting position and wore a nose-clip. A mixture containing 0.28% CO, 14% He and 21% O_2 balanced with N_2 was mixed with an NO/N_2 mixture (450 ppm NO in N_2; Air Liquide Santé, Paris, France). The final concentration of NO in the bag inspired was 40 ppm and that of O_2 was 19.1%. The apparatus was calibrated for gas fractions using automated procedures. The linearity of the analysers was factory checked. Linearity on site could be checked by dilution procedures, *i.e.* dilution of the NO mixture with the CO/He/O_2 mixture to check NO analyser linearity, or dilution of the CO/He/O_2 mixture with air to check CO analyser linearity. The screen pneumotachograph was calibrated daily using a 2-L syringe. The subject breathed through a mouthpiece and a filter connected to the pneumotachograph. When needed, they were requested to make a deep expiration. Then, at the onset of the following inspiration, a valve opened, allowing the subject to inspire the mixture during a rapid deep inspiration. A breath-hold of 4 s was then requested, followed by a rapid expiration. The first 0.8 L of expired gas was rejected and the following 0.6 L was sampled in a bag, which was automatically analysed for NO, CO and He. The delay in analysis of the sample of expired gas was constant at 35 s. Alveolar volume (V_A) during the apnoea was calculated using the He-dilution technique.

The measurements of $T_{L,NO}$ and $T_{L,CO}$ were accepted if two successive measurements of $T_{L,NO}$ and $T_{L,CO}$ were within 10% of each other. A third measurement was made if the two measurements were not within 10%. If this last measurement did not fit with one of the two previous measurements, the subject was considered unable to perform reproducible measurements and excluded from the cohort. The set of values from the NO and CO transfers with the greater $T_{L,CO}$ was retained. D_m and V_c were calculated according to the equation of ROUGHTON and FORSTER [1]:

$$1/T_{L,CO}=1/D_{m,CO}+1/\Theta_{CO}V_c \qquad (2)$$

$1/\Theta_{NO}$ was assumed to be negligible [3], *i.e.* $T_{L,NO}=D_{m,NO}$. $D_{m,CO}$ was calculated as $D_{m,NO}/a$, where $a=1.97$ following Graham's law. In order to calculate V_c, the Θ_{CO} reference values of FORSTER and co-workers [6, 7] were selected. These choices are considered in more detail in the Discussion section. The mean capillary oxygen tension was estimated to be 13.3 kPa (100 mmHg), taking into account the slightly lower than normal oxygen fraction of the inspired mixture. The haemoglobin concentration was not measured [8] but set at 13.4 g·dL^{-1} for females and 14.6 g·dL^{-1} for males [9], *i.e.* $1/\Theta_{CO}$ was multiplied by 14.9/13.4 and 14.9/14.6, respectively, since 14.9 g·dL^{-1} was the haemoglobin concentration in the work of ROUGHTON *et al.* [2], in agreement with the maximal O_2 concentration in the oxyhaemoglobin form of 0.2 mL·mL blood^{-1} cited in the associated article [1]. $1/\Theta_{CO}$ was 14.29 min^{-1}·kPa^{-1} for females and 13.16 min^{-1}·kPa^{-1} for males. As suggested by STAM *et al.* [8], the slight differences in haemoglobin concentration in a healthy population did not justify its measurement, since the recovered mean V_c was not significantly altered by the introduction of pre-set values for females and males. The total breath-holding time was calculated according to the method of GRAHAM *et al.* [10], *i.e.* from the beginning of inspiration less the first 30% of the inspiratory time to the middle of the expiratory sample time.

Statistical analysis

A linear multiple regression model for each of the variables was tested for V_c, $T_{L,CO}$, $T_{L,NO}$ (or $D_{m,CO}$), V_c/V_A, D_m/V_A and $T_{L,CO}/V_A$. $T_{L,NO}$ and $D_{m,CO}$ were proportional; both values are nevertheless reported in order to compare the present $D_{m,CO}$ with others. The independent variables were sex (zero female, one male), age (in yrs), height (in m) and weight (in kg) or BMI (kg per m squared), which takes into account both of the previous variables. This analysis was performed on the whole population (Grenoble and Bordeaux). A further analysis was performed taking into account the city of origin of the subjects as an independent variable. The variables whose significance levels were >0.05 were rejected from the model. For a new observation of the independent variables, an interval of prediction at the level of 1–α for the dependent variables was introduced. The prediction was directly obtained using the regression equation and estimates of its parameters.

RESULTS

The age distributions of females and males are shown in figure 1. The number of people included per decade was steady in the age range 20–70 yrs, and decreased in older people. The mean (range) total breath-hold time was 5.5 (4.9–6.1) s.

$T_{L,CO}$, $T_{L,NO}$, $D_{m,CO}$ and V_c were each best fitted for age using two linear equations. The first one, for young adults, had a shallow slope, and the second, for older adults, had a steep slope. The slope for people aged >59 yrs (β_o) was derived from the addition of the slope of the young population (β_y) and an additive value (β_{ad}) multiplied by the difference between the effective age in yrs (A) and 59:

$$\beta_o=\beta_y+\beta_{ad}(A-59) \qquad (3)$$

The age threshold between the young and old populations was chosen to maximise the F-statistic of the model. BMI and
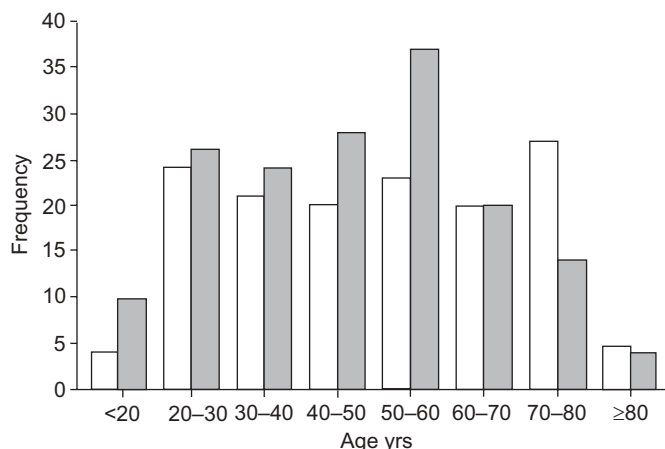
FIGURE 1. Age distributions of females (□) and males (■) in the sample population. The youngest subjects were ⩾18 yrs of age; the ⩾80-yrs category included a few subjects aged >90 yrs.

weight were rejected as significant covariates; age, height and sex were retained. Males gave greater values than females of the same age and height. The three selected independent variables explained ~70% of the variability. Figure 2 illustrates the biphasic effect of age on V_c in males. Table 1 gives the coefficient estimates of the bilinear age relationship for all variables in the whole population, and table 2 gives the estimates, taking the city of origin of the subjects into account. The city of origin exerted slight but significant effects on $T_{L,CO}$ and $T_{L,NO}$, which were lower in Grenoble than Bordeaux (mean differences 8.5 and 13.2%, respectively). The intervals of prediction can be calculated taking into account all of the covariates (see Appendix; an Excel program for the calculation of these intervals is available on request).

The regression equations for the variables $T_{L,CO}/V_A$, $D_{m,CO}/V_A$ and V_c/V_A are given in table 3. The models for $T_{L,CO}/V_A$ and $D_{m,CO}/V_A$ involve only the covariate age. Height, weight
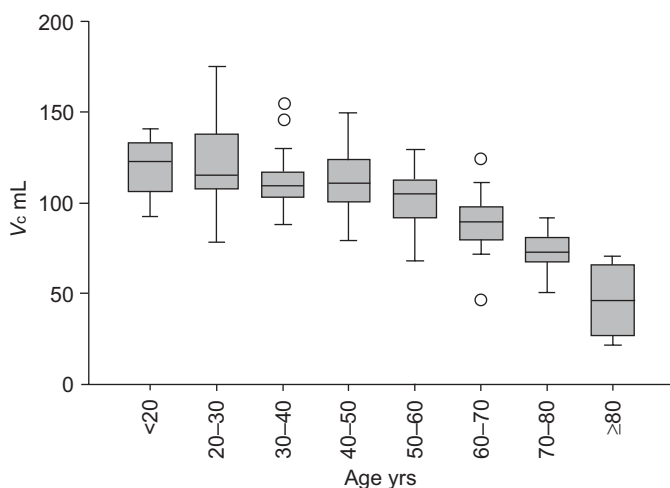
FIGURE 2. Boxplot showing capillary lung volume (V_c) as a function of age in males. Boxes represent median and interquartile range; vertical bars represent range (○: outliers). There is a biphasic decrease in V_c as a function of age.

and BMI increased r^2 by no more than 2%. The computation of the 95% confidence interval involved the covariance matrix of the estimates. No difference in $T_{L,CO}/V_A$ between underweight (17<BMI<20 kg·m^{-2}; n=29) and class I obese people (35>BMI>30 kg·m^{-2}; n=10) was observed (41.4±5.3 and 39.8±7.5 mL·min^{-1}·kPa^{-1}·L^{-1}, respectively). It is worth noting that the models explain ~50% of the variability in $T_{L,CO}/V_A$ and $D_{m,CO}/V_A$, but only 37% in V_c/V_A. D_m/V_c and $T_{L,NO}/T_{L,CO}$ were independent of any covariate (age, weight, height, V_A, BMI and sex). Their means were 0.82 min^{-1}·mmHg^{-1} and 4.75, respectively. The intervals between 5th and 95th percentiles of their univariate distributions were 0.63–1.04 min^{-1}·mmHg^{-1} and 4.10–5.44, respectively. D_m was linearly related to V_c: $D_m=7.3+0.73V_c$ (units as in tables 1 and 2). The constant 7.3, however small, was significantly different from 0.

DISCUSSION

Apart from providing reference values for $T_{L,NO}$, $T_{L,CO}$ and their derived variables, the present data show that variables divided by V_A produce figures dependent on age alone, and that $D_{m,CO}/V_c$ and $T_{L,NO}/T_{L,CO}$ are independent variables.

$T_{L,NO}$, $T_{L,CO}$, V_c and $D_{m,CO}$ are strongly dependent upon anthropometric variables; among them, age appears to be the most important. The changes in these variables are by-products of lung ageing. It is noteworthy that the loss in membrane conductance is nearly proportionate to that in V_c, since the ratio $D_{m,CO}/V_c$ is independent of age.

Ageing decreases the performance of all organs and functions. The decay in lung transfer of both NO and CO appeared biphasic, relatively slow in young adults and then faster in the elderly, suggesting a reduction in the surface of the lung available for gas exchange. This biphasic decline was described by GEORGES et al. [11]. The loss of lung surface is due to the coalescence of alveoli along with loss of alveolar walls [12]. This loss is suggested to be accompanied by loss of pulmonary capillaries. Morphometric evidence on this point is scarce, apart from a study showing a 15% decrease in capillary density between the ages of 20 and 57 yrs [13].

The variables $T_{L,NO}$, $T_{L,CO}$, V_c and $D_{m,CO}$ divided by V_A are independent of anthropometric variables except age. Large lungs at a given age have a greater exchange surface and a greater number of capillaries than small ones; thus, ratios of $T_{L,NO}$, $T_{L,CO}$, V_c and $D_{m,CO}$ to V_A reduce the link to sized anthropometric variables. For example, V_c/V_A ratio decreases from 15 mL·L V_A^{-1} at 26 yrs to 10 mL·L V_A^{-1} at 73 yrs. Another advantage of relating the variables to V_A is to avoid sex differences, which are not related to lung tissue structural differences but to differences in the size of the lung for a given height and age.

Several studies have dealt with the subject of transfer coefficient of the lung for carbon monoxide (K_{CO}) and nitric oxide (K_{NO}) and their interpretation [14, 15]. It would be beyond the scope of the present study to comment in detail on this subject; however, one point is that the basic assumption in obtaining reference values using the single-breath method is that subjects, or patients, perform a full inspiration and reach their maximal V_A ($V_{A,max}$). $V_{A,max}$ depends upon the performance of inspiratory muscles and chest–lung mechanics. Subjects with the same anthropometric characteristics could

| TABLE 1 | Estimates of the regression equation parameters for dependent variables for the entire population |

	Intercept	Sex[#]	Height[¶]	Age yrs		r²
				≤59	>59	
$T_{L,CO}$ mL·min⁻¹·mmHg⁻¹	-12.954	5.044	29.291	-0.161	-0.262	0.728
$T_{L,NO}$ mL·min⁻¹·mmHg⁻¹	-54.687	27.346	136.493	-0.881	-0.900	0.716
V_c mL	-39.256	13.731	86.995	-0.435	0.886	0.656
$D_{m,CO}$ mL·min⁻¹·mmHg⁻¹	-27.760	13.881	69.286	-0.447	-0.457	0.716

$T_{L,CO}$: transfer factor of the lung for carbon monoxide; $T_{L,NO}$: transfer factor of the lung for nitric oxide; V_c: capillary lung volume; $D_{m,CO}$: diffusing capacity of the membrane for carbon monoxide. [#]: 0 female, 1 male; [¶]: in m. 1 mmHg=0.133 kPa.

have differing $V_{A,max}$. As a consequence, calculation of K_{CO} and K_{NO} by dividing $T_{L,CO}$ and $T_{L,NO}$ by $V_{A,max}$ introduces an additive relative scatter, as observed in the present study. For example, the confidence interval (2SD) of $T_{L,CO}$ for a male of 1.70 m in height and aged 50 yrs is 27%, whereas the mean $T_{L,CO}/V_A$ is 35%. Conversely, it seems logical to divide the $T_{L,CO}$ by a variable related to the amount of parenchyma in the lungs. Perhaps $V_{A,max}$ is not a good choice.

What would happen if a subject with healthy lungs did not reach $V_{A,max}$? Several authors have performed measurements at various percentages of $V_{A,max}$ [4, 8, 14–18]. In brief, K_{NO} and K_{CO} decrease as a function of percentage of $V_{A,max}$, i.e. both variables increase at low V_A. On the one hand, $D_{m,CO}$ and percentage $V_{A,max}$ are linearly related in the range 60–100% $V_{A,max}$ [4], and $D_{m,CO}$ reaches a maximum at 100% $V_{A,max}$. On the other hand, V_c decreases negligibly between $V_{A,max}$ and 60–80% $V_{A,max}$ [16, 18]. Therefore, the $T_{L,CO}$ versus V_A relationship, which takes into account both $D_{m,CO}$ and V_c, cannot be linear. If, nevertheless, a linear equation is fitted in a restricted range of percentages of $V_{A,max}$, a constant must be introduced into the equation [14], which is, in mathematics, an affine, not linear, function, i.e. for V_A=0, $T_{L,CO}$>0. An exponential fit has also been proposed [15]. Interpretation of K_{CO} and K_{NO} in patients unable to reach their $V_{A,max}$ should be cautious, taking into account the percentage of the predicted reduction in $V_{A,max}$ [14, 17].

Obese class I individuals with no significant medical history were included in the present study. Following this classification, the maximum BMI accepted was 34.9 kg·m⁻². Removing all obese class I individuals from the present study did not affect the results. Morbid (class III) obesity (BMI >40 kg·m⁻²) consistently alters lung diffusion by increasing V_c and decreasing D_m [19]. In moderate obesity, the alteration seems due to changes in V_A; the ratio $T_{L,CO}/V_A$ can be elevated in people with a BMI of >30 kg·m⁻² [20]. In the present study, subjects with abnormal V_A were not included. Furthermore, there was no difference in $T_{L,CO}/V_A$ ratio between underweight (BMI <18 kg·m⁻²) and obese class I individuals (30<BMI<35 kg·m⁻²); therefore, there was no objective reason for rejecting this population from the analysis. It is worth noting that these subjects, if clear from restrictive lung disease and sleep disturbances [21], should show normal $T_{L,CO}$ and K_{CO}. This point is of interest since class I obesity has a high prevalence in many countries.

The independence of the $D_{m,CO}/V_c$ and $T_{L,NO}/T_{L,CO}$ ratios from any variable is noteworthy. The ratios $T_{L,NO}/T_{L,CO}$ and $D_{m,CO}/V_c$ are related:

$$T_{L,NO}/T_{L,CO}=a+(D_{m,CO}/V_c\Theta_{CO}) \qquad (4)$$

This independence is not unexpected since morphometric analysis of human lung has shown that the alveolar and endothelial surfaces are related [22], with their reported ratio being 0.88. From a theoretical point of view, the $D_{m,CO}$ to V_c relationship should intercept the axes at 0 as a significant value of 54.9 mL·min⁻¹·kPa⁻¹ was found, but not in a previous work from the same group [16]. However significant, this intercept value is small compared to $D_{m,CO}$ in adults and is probably due to the various assumptions made in the model (Θ_{CO} and haemoglobin concentration). GLÉNET et al. [16], in a theoretical and experimental study, gave an interpretation of the $T_{L,NO}/T_{L,CO}$ ratio, whose value would be inversely related to the product of the thicknesses of the alveolar membrane and blood capillary sheet. This ratio, being independent of anthropometric variables, itself appears as a morphometric characteristic of an individual. $T_{L,NO}/T_{L,CO}$ looks easier to interpret than $D_{m,CO}/V_c$ since it is dimensionless and does not require the introduction of the debatable values Θ_{CO} and a. It would be of interest to know whether the $T_{L,NO}/T_{L,CO}$ ratio remains stable throughout the life of a person.

As suggested by ZAVORSKY and MURIAS [23], two measurements of NO and CO transfer are sufficient to give reliable

| TABLE 2 | Estimates of the regression equation parameters for dependent variables taking city into account |

	Intercept	Sex[#]	Height[¶]	Age yrs		City[+]	r²
				≤59	≤59		
$T_{L,CO}$ mL·min⁻¹·mmHg⁻¹	-16.013	4.822	30.680	-0.159	-0.225	2.632	0.743
$T_{L,NO}$ mL·min⁻¹·mmHg⁻¹	-77.236	25.705	146.731	-0.869	-0.632	19.404	0.750
V_c mL	-44.715	13.334	89.474	-0.432	-0.821	4.698	0.681
$D_{m,CO}$ mL·min⁻¹·mmHg⁻¹	-39.206	13.048	74.483	-0.441	-0.321	9.850	0.750

$T_{L,CO}$: transfer factor of the lung for carbon monoxide; $T_{L,NO}$: transfer factor of the lung for nitric oxide; V_c: capillary lung volume; $D_{m,CO}$: diffusing capacity of the membrane for carbon monoxide. [#]: 0 female, 1 male; [¶]: in m; [+]: Grenoble × 0, Bordeaux × 1. 1 mmHg=0.133 kPa.

TABLE 3	Estimates of the linear model parameters for dependent variables		
	Intercept (95% CI)	Age (95% CI)	r²
$T_{L,CO}/V_A$	6.670 (6.464–6.876)	-0.034 (-0.038– -0.030)	0.486
V_c/V_A	19.808 (19.058–20.558)	-0.100 (-0.114– -0.086)	0.379
$D_{m,CO}/V_A$	16.071 (15.593–16.549)	-0.083 (-0.092– -0.074)	0.510

Units are as in tables 1 and 2; alveolar volume (V_A) is measured in L. $T_{L,CO}$: transfer factor of the lung for carbon monoxide; V_c: capillary lung volume; $D_{m,CO}$: diffusing capacity of the membrane for carbon monoxide.

values. Since they calculate the mean of these two measurements, the results provided by the test with the greater $T_{L,CO}$ were kept in the present study. The reference values for $T_{L,CO}$ in the literature are scattered; equations from CHINN et al. [15] give lower values than those from VAN DER LEE et al. [4], which, in turn, give lower values than those of the present study. ZANEN et al. [24] did not find a decrease in $T_{L,CO}$ with age, probably because their study was restricted to adults aged <60 yrs. Looking at K_{CO} and K_{NO}, the differences between studies appear maximal in young adults for K_{NO}. Compared with the results of VAN DER LEE et al. [4], the present study gave results 21 and 14% higher in females and males, respectively. K_{CO} differences are much less than for NO, 9 and 6% for females and males, respectively.

These differences could be due to the method used, as well as differences in the populations studied. VAN DER LEE et al. [4] used low inspiratory NO fractions (7–9 ppm) along with long breath-holding periods, leading to NO concentrations in the range 200 ppb, requiring a high-resolution chemiluminescent apparatus. As this technique is feasible in healthy nonallergic patients, it seems preferable to use high fractions of NO and short breath-holds in patients in whom the endogenous NO fraction can be elevated. If a fraction of 5% is taken as the maximum participation of endogenous NO to the expired concentration, it could be proposed as a safe procedure that the expiratory NO fraction in patients should be not less than 1 ppm in order to avoid contamination by endogenous NO. The NO transfer values reported by ZAVORSKY et al. [5] in a healthy population using the same material and protocol as in the present study, are close to those reported here; however, the rate decrease with age was considered constant in the work of ZAVORSKY et al. [5]. This slight difference could be due to the reduced number of old subjects in the latter study [5].

It has been shown, in healthy volunteers, that $T_{L,CO}$ [9, 24] and $T_{L,NO}$ [24] decrease slightly with breath-hold duration. In patients with distension, either chronic obstructive pulmonary disease or asthmatic, $T_{L,CO}$ increased with breath-hold duration [9, 25]. It is not proven that, even with a 10-s breath-hold, the $T_{L,CO}$ reached its maximal value [9]. Therefore, whatever the breath-hold duration, this fact should be integrated into the interpretation of $T_{L,CO}$ or $T_{L,NO}$ data in patients with distension. This lack of a stable value is probably due to a limitation of gas transport in the gas phase. Distension associated with poor ventilation in some part of the lung shifts the transition front between convection and diffusion of gases

toward the mouth [26], increasing the path length for diffusion of CO and NO. It is worth noting that the ratio $T_{L,NO}/T_{L,CO}$ is not altered by limitation of gas phase diffusion since both gases are involved in this limitation. In brief, it seems that a 40-ppm inspiratory NO fraction with a 4-s true breath-hold, i.e. a total breath-hold duration of 5–6 s, is a good compromise for routine applications, taking in to account the fact that pulmonary distension and/or heterogeneity in the distribution of ventilation might intervene, should be integrated into the interpretation of CO or NO transfer.

Methodological factors are often put forward to justify discrepancies between reference values; however, in the present study, the material and protocols used were the same in the two centres involved in the study. The differences in $T_{L,CO}$ and $T_{L,NO}$ between the two sites were, nevertheless, significant and permitted a partial reduction in the unexplained variability of the variables. Altitude might play a role as Grenoble is situated at an altitude of ~300 m and the altitude of Bordeaux is close to sea level. The effect of 300 m of altitude on pulmonary diffusing capacity measurements is slight as there is only a minor difference in arterial oxygen tension between Grenoble and Bordeaux.

Another factor causing discrepancy between reference values is heterogeneity in the physical activity status of the subjects. At rest, trained subjects exhibit $D_{m,CO}$ and V_c that are 20 and 25%, respectively, greater than in untrained subjects [27]. Therefore, mean reference values are higher in populations with a high prevalence of physically active subjects. Patients are supposed, in the main, to be sedentary; therefore, the lower limit of the reference values should be used. There was no difference in anthropometric characteristics or the physical activity status of the subjects between the two cities; therefore, these factors were not responsible for the location being a significant determinant of pulmonary diffusion.

The level of chronic air pollution could affect lung function; Grenoble is located in an industrial valley in the Alps, whereas Bordeaux is situated in a flat environment near the Atlantic Ocean. It has been shown that air pollution in areas in which people are living is a determining factor in cardiovascular disease [28]. Before reaching the blood, pollutants might damage the lung.

Before using reference values in a given city or place, it would be recommended to check the agreement of measurements made in some healthy representatives of that place with published reference values.

V_c and D_m were calculated using the Roughton–Forster model, with red cell CO conductance taken not from the early work of ROUGHTON et al. [2] but from the more recent work of FORSTER and co-workers [6, 7]. The reasons for this choice were twofold. First, according to FORSTER and co-workers [6, 7], the latter work was performed at pH 7.4, whereas the former was performed at pH 8; therefore, R.E Forster, who was involved in both studies, recommends the equation of the latter work [7]. Secondly, choosing the more recent equation gives $D_{m,CO}$ in agreement with the theoretical value of the coefficient a, i.e. a ratio of $D_{m,NO}$ (or $T_{L,NO}$) to $D_{m,CO}$ of ~2 [16], whereas using the early equation [2] requires empirically changing a to ~2.4–2.5 [29]. As pointed out by HUGHES and BATES [30], the use of

this more recent value for haemoglobin CO conductance leads to 15–20% smaller V_c and 15–20% greater $D_{m,CO}$. If consensus can be reached regarding the choice of a and haemoglobin CO conductance, all published reference values of V_c and $D_{m,CO}$ could be compared after appropriate correction.

A mean capillary oxygen tension of 13.3 kPa (100 mmHg) was chosen in both centres, leading to the hypothesis that the alveolar oxygen tension was 14.6 kPa (110 mmHg) and the mean difference between alveolar and capillary tension 1.33 kPa (10 mmHg). A small change in this oxygen tension has only a small effect on V_c, *e.g.* a capillary oxygen tension of 12.6 kPa (95 mmHg) instead of 13.3 kPa (100 mmHg) would reduce V_c by 1.2 mL.

In conclusion, simultaneous measurement of the transfer factor of the lung for nitric oxide and carbon monoxide permits calculation of the diffusing capacity of the membrane for carbon monoxide and capillary lung volume. The present study determined regression equations for the transfer factor of the lung for nitric oxide and carbon monoxide in normal subjects, as well as for the derived variables diffusing capacity of the membrane for carbon monoxide and capillary lung volume. All values decreased nonlinearly with age, and also varied with sex and height, but continued to fall with increased age. There were differences between the two populations tested. The role of chronic pollution in alveolar function requires further study.

APPENDIX

The interval of prediction of $T_{L,CO}$ for a new subject, based on their sex (S), age and height (H), is given by the formula:

$$E[T_{L,CO} \mid S;A;H] \pm t_{(303;1-\alpha/2)} \cdot \\ \sqrt{(C + C_A A + C_H H + C_{A^2} A^2 + C_{H^2} H^2 + C_{AH} AH)} \quad (5)$$

where $E[T_{L,CO}$ is the predicted value for $T_{L,CO}$, $t_{(303;1-\alpha/2)}$ is the $(1-\alpha/2)$ quantile of the distribution with 303 degrees of freedom and C is the constants associated with age and height. For instance, for $\alpha = 0.05$, $t = 1.968$. The values of C differ for males and females (table 4).

	S	C	A	H	A²	H²	AH
TABLE 4	Coefficients for the computation of the interval of prediction of dependent variables for the entire population						
$T_{L,CO}$	Male	82.261	-0, 123	-64.556	0.00026	17.670	0.056
	Female	74.651	-0, 118	-60.193	0.00026	17.670	0.056
$T_{L,NO}$	Male	1995.364	-2, 988	-1565.903	0.00637	428.600	1.364
	Female	1810.761	-2, 856	-1460.061	-0.00637	428.600	1.364
$D_{m,CO}$	Male	549.372	-0, 823	-431.131	0.00175	118.004	0.376
	Female	498.546	-0, 786	-401.990	0.00175	118.004	0.376
V_c	Male	901.623	-1, 350	-707.567	0.00288	193.667	0.617
	Female	818.209	-1, 290	-659.742	0.00288	193.667	0.617

S: sex; C: constant; A: age; H: height; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; $T_{L,NO}$: transfer factor of the lung for nitric oxide; V_c: capillary lung volume; $D_{m,CO}$: diffusing capacity of the membrane for carbon monoxide.

As for the previous variables, the interval of prediction for variables divided by V_A can be computed using the following equation:

$$Y^*(A) \pm t_{(303;1-\alpha/2)} \cdot \\ \sqrt{(C + C_A A + C_{A^2} A^2)} \quad (6)$$

where $Y^*(A)$ is the predicted value and $t_{(303;1-\alpha/2)}$ is the interval of prediction for the variables shown in table 5.

	C	C_A	C_{A_2}
TABLE 5 Coefficients for the computation of the interval of prediction of dependent variables divided by alveolar volume (V_A)			
$T_{L,CO}/V_A$	0.4536	-0.0004	4.21×10^{-6}
D_m/V_A	2.4074	-0.0022	2.24×10^{-5}
V_c/V_A	5.9971	-0.0054	5.57×10^{-5}

C: constant; A: age; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; D_m: diffusing capacity of the membrane; V_c: capillary lung volume.

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