



Official ERS technical standards: Global Lung Function Initiative reference values for the carbon monoxide transfer factor for Caucasians

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This is the largest collection of normative T_{LCO} data and represents a step towards standardised interpretation <http://ow.ly/4PcZ30dB1tn>

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ABSTRACT There are numerous reference equations available for the single-breath transfer factor of the lung for carbon monoxide (T_{LCO}); however, it is not always clear which reference set should be used in clinical practice. The aim of the study was to develop the Global Lung Function Initiative (GLI) all-age reference values for T_{LCO} .

Data from 19 centres in 14 countries were collected to define T_{LCO} reference values. Similar to the GLI spirometry project, reference values were derived using the LMS (lambda, mu, sigma) method and the GAMLSS (generalised additive models for location, scale and shape) programme in R.

12 660 T_{LCO} measurements from asymptomatic, lifetime nonsmokers were submitted; 85% of the submitted data were from Caucasians. All data were uncorrected for haemoglobin concentration. Following adjustments for elevation above sea level, gas concentration and assumptions used for calculating the anatomic dead space volume, there was a high degree of overlap between the datasets. Reference values for Caucasians aged 5–85 years were derived for T_{LCO} , transfer coefficient of the lung for carbon monoxide and alveolar volume.

This is the largest collection of normative T_{LCO} data, and the first global reference values available for T_{LCO} .

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Background

Lung function tests (LFTs) are important tools in the evaluation of the respiratory system. The correct interpretation of LFT results relies on the availability of appropriate reference values to help distinguish between health and disease and to assess the severity and nature of any functional impairment. Global Lung Function Initiative (GLI) multiethnic all-age reference values are available for spirometry [1]. However, there are no standardised reference values available for the second most clinically used LFT, the single-breath transfer factor of the lung for carbon monoxide ($TLCO$, or diffusing capacity of the lung for carbon monoxide ($DLCO$)). $TLCO$ is a strong indicator of the efficiency of gas exchange in the lung, and is frequently used to inform diagnosis and monitor patients.

The European Respiratory Society (ERS) and American Thoracic Society (ATS) standards for the measurement of carbon monoxide gas transfer in the lungs were recently updated [2] and additional guidelines for interpretation of the technique are available [3]. There are several methodological aspects that may affect the interpretation of the results, with details presented in the documents. The interpretation guidelines provide a list of $TLCO$ reference values; however, no consensus was reached, nor recommendations provided, regarding which equations were best for children, adults or those in the various ethnic groups other than to advise that laboratory directors should thoughtfully select reference values that match the values obtained from healthy individuals of appropriate background tested in their own laboratories. Changes in equipment, software and measurement techniques, combined with shifts in population characteristics, mean that some of the previously published reference values for $TLCO$ may no longer be appropriate. The purpose of this study was to collate contemporary $TLCO$ data from healthy individuals and derive GLI reference values for $TLCO$ measurements.

Methods

An application was approved for an ERS task force to develop global $TLCO$ reference values. Task force co-chairs were approved by the ERS. Task force members were scientists with experience in international guidelines, clinical experience of routine lung function testing and knowledge of gas transfer, including research publications. Potential conflicts of interest were disclosed and vetted.

Data sources

The authors of papers that published $TLCO$ data in healthy individuals after the year 2000 were contacted and invited to share their data with the GLI $TLCO$ task force. Of the 17 studies identified, 70% submitted data. Details about the equipment and methodology used were collected from the published papers, or from the authors or manufacturers directly, to confirm that methods were compatible with those currently available to customers. In addition, information about the task force was circulated through international and local respiratory societies to solicit unpublished data or published studies that had not been identified. All contributing authors provided explicit permission for data to be shared with the GLI group. An online, secure data portal was developed to capture de-identified data (www.gligastransfer.org.au). Data contributors signed a data-sharing agreement, submitted details about their study population, equipment, settings and research ethics. All data were submitted using a standard data template; initial data queries were performed and contributors were asked to correct errors before data were accepted. Inclusion criteria include nonsmokers without a history of respiratory disease. All data were uncorrected for haemoglobin (Hb) concentration. Outliers were identified using *a priori* criteria: forced expiratory volume in 1 s (FEV_1) z-scores >5 or <-5 and height z-scores >5 or <-5 in children (aged ≤ 18 years). These limits were used to identify data discrepancies and exclude subjects at the extremes of the healthy population. In addition, observations were considered to be outliers if the alveolar volume (V_A) was smaller than the forced vital capacity (FVC). Sensitivity analyses were performed excluding individuals who were obese, where obesity was defined as body mass index (BMI) centile $>85\%$ in children [4] and BMI $>30 \text{ kg}\cdot\text{m}^{-2}$ in adults (aged >19 years). The z-scores derived for individuals in the full dataset and the “normal weight” dataset were compared using a paired t-test.

All $TLCO$ data (and consequently transfer coefficient of the lung for carbon monoxide (KCO) data) were adjusted to the inspiratory oxygen partial pressure at standard barometric pressure (P_B ; 760 mmHg or 101.3 kPa) using the following equations [2, 5]:

$$\begin{aligned} \text{For SI units (mmol; kPa): } TLCO[P_{B,\text{adjusted}}] &= TLCO \cdot (0.505 + 0.00488 \cdot P_B) \\ \text{For traditional units (mL; mmHg): } TLCO[P_{B,\text{adjusted}}] &= TLCO \cdot (0.505 + 0.00065 \cdot P_B) \end{aligned}$$

For $TLCO$ datasets that did not provide P_B data ($n=11$), the altitude of the centre in which the reference values were obtained was used to estimate P_B , using the following equation [2, 6] where h is the altitude

above sea level in m:

$$P_B(\text{kPa}) = 101.3 \cdot (1 - 2.25577 \cdot 10^{-5} \cdot h)^{5.25588}$$

$$P_B(\text{mmHg}) = 760 \cdot (1 - 2.25577 \cdot 10^{-5} \cdot h)^{5.25588}$$

In addition, we corrected corrected values in centres that used a fixed dead space correction of 150 mL ($V_{D,\text{an, fixed}}$) such that the anatomic dead space was calculated as 2.2 mL·kg⁻¹ ($V_{D,\text{an, est}}$) [7]:

$$T_{LCO}' = T_{LCO} \times (V_1 - V_{D, \text{equip}} - V_{D, \text{an, est}}) / (V_1 - V_{D, \text{equip}} - V_{D, \text{an, fixed}})$$

Complete details of the calculations can be found in the online supplementary material.

In addition, the following methodological considerations were investigated before the submitted data were combined: equipment type, breath-hold calculation, size and timing of alveolar sample collection and the year during which data were collected.

Statistical analyses

The complex nature of the relationship between body size, age, sex and lung function, particularly during periods of rapid growth, means that traditional linear regression analyses are not sufficient to derive appropriate reference values for lung function outcomes [8]. More flexible statistical modelling techniques allow the complexity of the relationship to be explained and to reflect biologically plausible relationships of lung function with age, sex and height. In the case of T_{LCO} outcomes, we investigated body surface area as an independent predictor. Body surface area was defined as $0.007184 \cdot (\text{weight}^{0.425}) \cdot (\text{height}^{0.725})$ [9]. We have previously shown that the GAMLSS (generalised additive models of location shape and scale) [10] modelling approach is highly suitable to derive reference values for lung function outcomes [1, 8, 11]. The lambda, mu, sigma (LMS) method is an extension of regression analysis which includes three components: 1) the skewness (λ), which models the departure of the variables from normality using a Box-Cox transformation; 2) the median (μ); and 3) the coefficient of variation (σ), which models the spread of values around the median and adjusts for any nonuniform dispersion [12]. The three quantities (LMS) are allowed to change with height and/or age, to reflect changes in the distribution as people grow. We applied the LMS method using the GAMLSS package in the statistical programme R [10]. Goodness of fit was assessed using the Schwarz Bayesian criterion, Q-Q plots and worm plots [8].

Results

Study population

19 centres contributed data from 14 countries. Data from 12659 individuals between the ages of 4 and 91 years were collected, of which 12639 (99.8%) had valid T_{LCO} data available. All T_{LCO} values that were collected using traditional units (mL·min⁻¹·mmHg⁻¹) were converted to SI units (mmol·min⁻¹·kPa⁻¹), (T_{LCO} traditional units = $2.986421 \times T_{LCO}$ SI units). Overall, the mean±SD FEV₁ z-score for 11473 individuals with spirometry data was 0.1 ± 1.1 , indicating a good fit with the GLI spirometry population [1]. 85% of the study population was Caucasian, with the remaining non-Caucasian population (n=1874) both from single sites (e.g. Japan (10%) and Hong Kong (4.5%)) and individuals where ethnic group was indicated as not Caucasian. Due to the lack of non-Caucasian data, T_{LCO} reference values were developed for Caucasians only (table 1).

One centre was excluded because the breath-hold time was 5 s (n=211). 58 observations were excluded because the V_A was smaller than the FVC. 11 observations were excluded because FEV₁ values were >5 z-scores or <-5 z-scores. 775 observations were excluded because of missing height, weight or age. Correcting T_{LCO} for barometric pressure, such that T_{LCO} was standardised to sea level ($P_B=760$ mmHg or 101.3 kPa), on average (95% CI) corrected T_{LCO} values by -1.5 (-1.54 – -1.51) SI units (online supplementary figure S1). Adjusting the anatomic dead space decreased the T_{LCO} on average by 0.02 (0.01–0.02) SI units (online supplementary figure S2); the correction resulted in greater relative changes in T_{LCO} in children (1.5%) compared with adults (0.7%). As expected, since females weigh less than males (average 65 kg in females; 78 kg in males) the anatomic dead space correction was negative in adult males and positive in adult females.

Reference values

The population used to derive reference equations for T_{LCO} outcomes (n=9710), ranged in age from 4.5 to 91 years (median (interquartile range) 45 (26–57) years) (online supplementary figure S5); half of whom were male. Findings from preliminary modelling identified significant differences in predicted values between males (n=4859) and females (n=4851), therefore sex-specific equations were created for T_{LCO} , V_A

TABLE 1 Summary of Caucasian data included in the Global Lung Function Initiative transfer factor of the lung for carbon monoxide reference values

Country	Subjects	Equipment	Altitude m	Anatomic V _D	Breath-hold calculation	Reported values	Caucasian %	Obese %	FEV ₁ z-score
New Zealand	71	SensorMedics Vmax Encore	20	Fixed	Jones–Meade	Average	100	18.30	−0.31±0.9
Australia	605	Other	8	Fixed	Jones–Meade	Average	100	18.40	0.56±1.0
Australia	120	SensorMedics Vmax Encore	10	Fixed	Jones–Meade	Average	100	8.30	0.25±0.9
Spain	430	Jaeger MasterScreen	710	Body size adjusted	Jones–Meade	Average	100	27.90	−1.0±1.3
The Netherlands	543	Jaeger MasterScreen	13	Fixed	Unknown	Automated	100	3.00	NR
USA	300	SensorMedics Vmax Encore	222	Body size adjusted	Jones–Meade	Average	100	9.00	0.03±0.9
USA	1302	Collins	50	Body size adjusted	Jones–Meade	Automated	100	18.10	0.14±0.9
Bulgaria	547	Jaeger MasterScreen	150	Body size adjusted	Jones–Meade	Largest	100	8.00	0.3±0.8
Bulgaria	335	Jaeger MasterScreen	150	Body size adjusted	Jones–Meade	Largest	100	10.20	NR
Mexico	191	Other	2240	Fixed	Jones–Meade	Average	100	14.10	0.04±1.2
Greece	942	Jaeger MasterScreen	460	Fixed	Unknown	Largest	100	27.60	0.3±0.9
New Zealand	151	SensorMedics Vmax Encore	10	Fixed	Jones–Meade	Average	100	4.60	−0.5±1.0
Italy	80	Other	100	Unknown	Unknown	Unknown	100	NR	0.06±0.9
Italy	3552	SensorMedics Vmax Encore	904	Unknown	Unknown	Average	100	25.70	0.2±1.0
Canada	541	Other	Various	Unknown	Unknown	Unknown	100	7.80	0.4±1.6

Data are presented as n or mean±SD, unless otherwise stated. V_D: dead space volume; FEV₁: forced expiratory volume in 1 s; NR: not reported.

and K_{CO} (figure 1). Height and age were both independent predictors of T_{LCO}, where natural logarithmic transformation of height and a spline function for age were necessary. As body surface area is correlated with alveolar surface area in children [13], we investigated body surface area as an independent predictor variable in the models. However, body surface area was highly correlated with height and therefore not included as an independent predictor.

The between-individual variability of T_{LCO} values was age dependent, with greater variability observed in children and older individuals (figure 2). On average, the variability of T_{LCO} was greater than that observed for FEV₁. Together with the median predicted values, the between-individual variability and skewness adjustment derived from the LMS method allowed for the calculation of a lower limit of normal (LLN), as well as the calculation of z-scores (figure 1 and table 2). The resulting z-scores had a mean of zero, and a standard deviation of one, indicating good fit to the data.

Sensitivity analyses

To test whether the inclusion of overweight individuals (n=2630; 27% of total population) affects the interpretation of the results, we created reference values limiting the sample to adults with a BMI <30 kg·m^{−2} or children with a BMI <85% percentile (n=7771). The difference in z-scores for an individual, whether overweight individuals were included or not, was −0.05 units (95% CI −0.050–0.048). Since including overweight individuals did not bias the prediction models, we chose to include these in the final models to maximise the sample size and generalisability of the final reference values.

Physiologically relevant differences

Based on the observed variability of the T_{LCO} we identified 0.5 z-scores as a threshold for a physiologically relevant difference. This equates to ~0.3–0.8 mmol·min^{−1}·kPa^{−1} or 10% relative change in T_{LCO}, which was higher in older individuals.

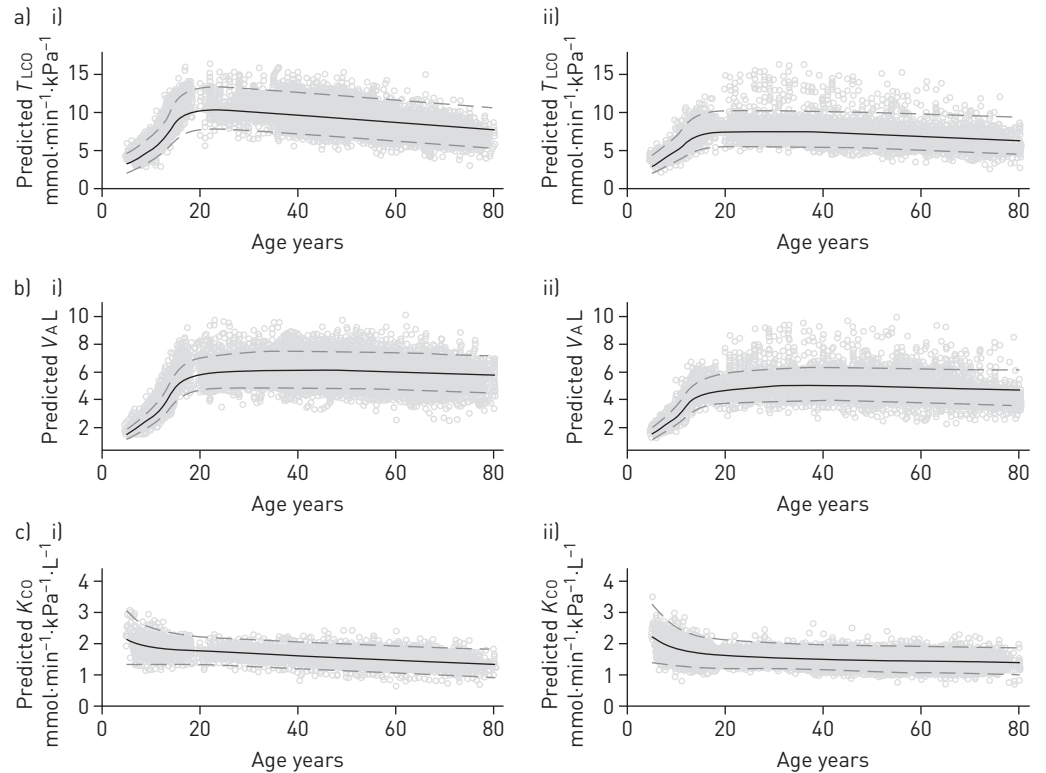


FIGURE 1 a) Predicted transfer factor of the lung for carbon monoxide (T_{LCO}) in i) males $n=4859$ and ii) females $n=4851$; b) alveolar volume (V_A) (at standard temperature, pressure and dry conditions) in i) males $n=4793$ and ii) females $n=4837$; and c) transfer coefficient of the lung for carbon monoxide (K_{CO}) in i) males $n=4793$ and ii) females $n=4837$. Data are presented as the predicted values for age (assuming an average height at each) and 95% confidence limits. Prediction equations are overlaid on observed values. The average height used in children was the 50th height-for-age centile from Centers for Disease Control and Prevention growth charts [4], whereas in adults, the average height observed in the study population was used (172 cm in males and 162 cm in females).

Methodological differences

We only included data where breath-hold time was reported to be 10 s. 13 centres reported having used the Jones–Meade calculations; five reported that the calculation method was unknown. There was a minimal difference in T_{LCO} z-scores (mean difference 0.04, 95% CI 0.0005–0.08; $n=9630$) between those that used the Jones–Meade method and those that did not report a method; these differences were not considered to be clinically or physiologically relevant. Most data were collected on commercial equipment (SensorMedics (29.5%; five centres), Jaeger (29.4%; five centres) and Collins (11.8%; two centres)), while

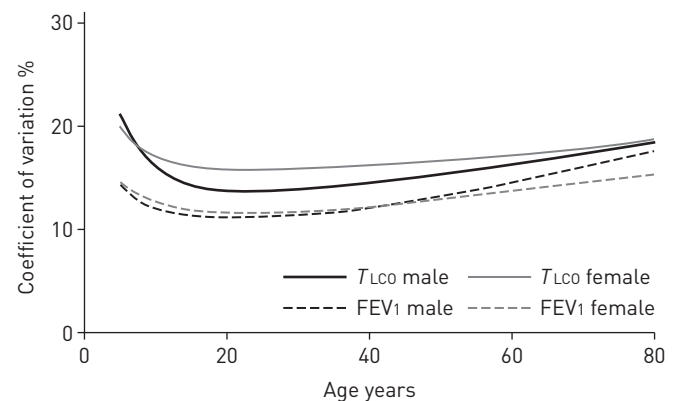


FIGURE 2 Between-subject variability of transfer factor of the lung for carbon monoxide (T_{LCO}) across age; the variability of forced expiratory volume in 1 s (FEV_1) in males and females is included as a comparator.

TABLE 2 Summary of equations for predicted values for the median (M), the variability around the median (S) and the skewness (L) for each of the transfer factor of the lung for carbon monoxide (TLCO) test outcomes (TLCO, transfer coefficient of the lung for carbon monoxide (Kco) and alveolar volume (VA))

	M	S	L
Male			
TLCO mmol·min ⁻¹ ·kPa ⁻¹	exp(-8.758548+2.151173·ln(height)-0.027927·ln(age)+Mspline)	exp(-1.98249+0.03430·ln(age)+Sspline)	0.38713
DLCO mL·min ⁻¹ ·mmHg ⁻¹	exp(-7.664278+2.151173·ln(height)-0.027927·ln(age)+Mspline)		
Kco (SI) mmol·min ⁻¹ ·kPa ⁻¹ ·L ⁻¹	exp(2.47708-0.30924·ln(height)-0.12173·ln(age)+Mspline)	exp(-1.97633+0.01536·ln(age)+Sspline)	0.64731
Kco (trad) mL·min ⁻¹ ·mmHg ⁻¹ ·L ⁻¹	exp(3.57135-0.30924·ln(height)-0.12173·ln(age)+Mspline)		
VA L	exp(-11.175544+2.450697·ln(height)+0.092353·ln(age)+Mspline)	exp(-2.24731+0.03069·ln(age)+Sspline)	0.69230
Female			
TLCO mmol·min ⁻¹ ·kPa ⁻¹	exp(-9.008743+2.171106·ln(height)-0.025634·ln(age)+Mspline)	exp(-1.84162+0.01379·ln(age)+Sspline)	-0.27064
DLCO mL·min ⁻¹ ·mmHg ⁻¹	exp(-7.914474+2.171106·ln(height)-0.025634·ln(age)+Mspline)		
Kco (SI) mmol·min ⁻¹ ·kPa ⁻¹ ·L ⁻¹	exp(3.009809-0.437869·ln(height)-0.102958·ln(age)+Mspline)	exp(-1.63864-0.07180·ln(age)+Sspline)	0.41767
Kco (trad) mL·min ⁻¹ ·mmHg ⁻¹ ·L ⁻¹	exp(4.104078-0.437869·ln(height)-0.102958·ln(age)+Mspline)		
VA L	exp(-12.205809+2.649906·ln(height)+0.074487·ln(age)+Mspline)	exp(-2.11477+0.01328·ln(age)+Sspline)	0.11093

Height and age are expressed as cm and years, respectively. Lower limit of normal (5th percentile): exp(ln(M)+ln(1-1.645·L·S)/L); upper limit of normal (5th percentile): exp(ln(M)+ln(1+1.645·L·S)/L); z-score: ((measured/M)^L-1)/(L·S); % predicted: (measured/M)·100; exp(): natural exponential; ln(): natural logarithm. Mspline and Sspline correspond to the age-varying coefficients provided in the online supplementary material.

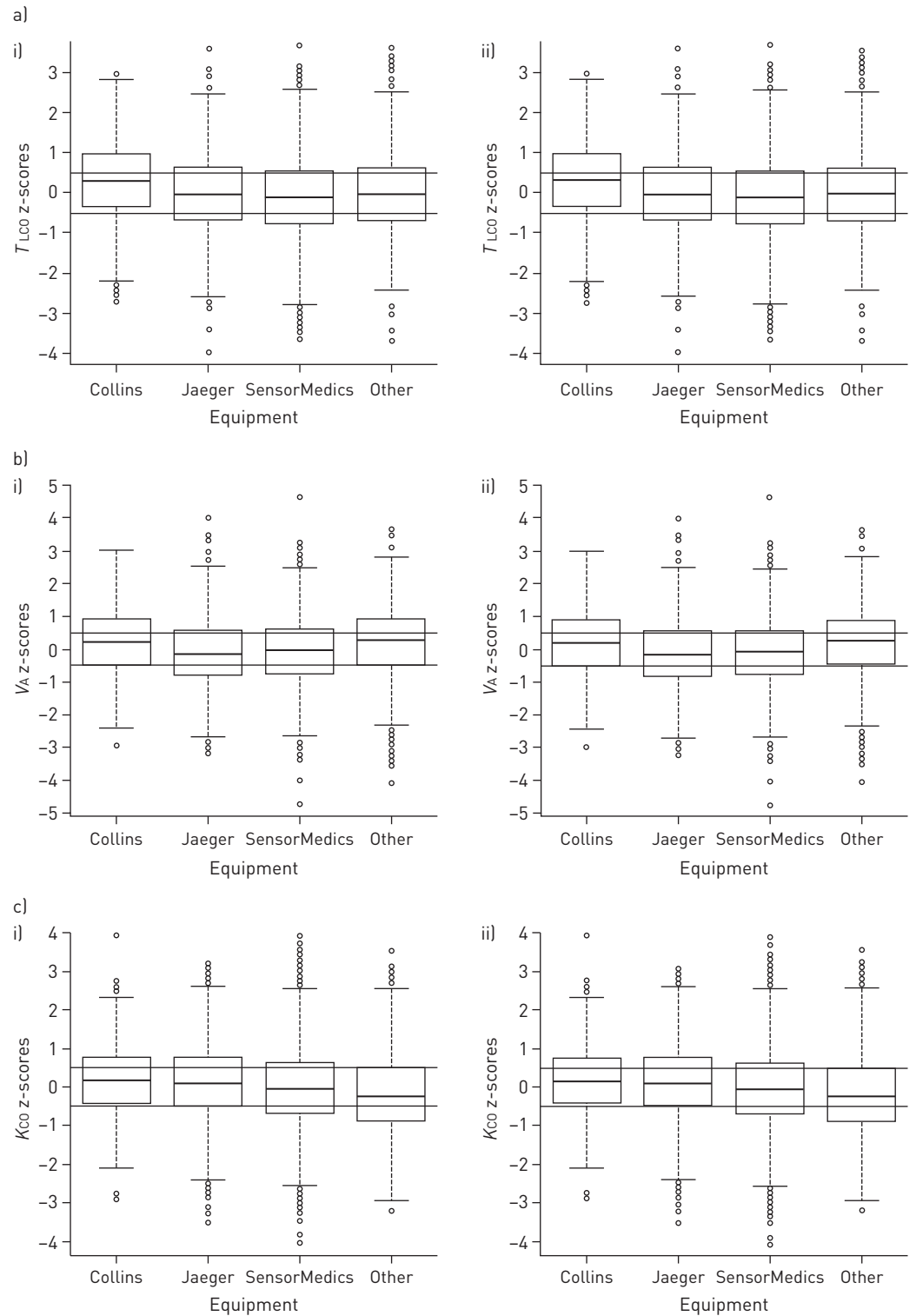


FIGURE 3 Differences in a) transfer factor of the lung for carbon monoxide (T_{LCO}) in i) males $n=4859$ and ii) females $n=4851$; b) alveolar volume (V_A) in i) males $n=4793$ and ii) females $n=4837$; and c) transfer coefficient of the lung for carbon monoxide (K_{CO}) z-scores in i) males $n=4793$ and ii) females $n=4837$ according to equipment in Caucasian subjects. Boxplots indicate the median value (centre line); interquartile range (box) and minimum and maximum values, excluding outliers greater than three times the lower quartile (error bars).

26.7% (six centres) reported “other” equipment not listed on our predefined list of commercial devices and one centre did not report the equipment type. There were minimal differences in T_{LCO} between different equipment types, which was consistent between males and females (figure 3). Four centres reported using

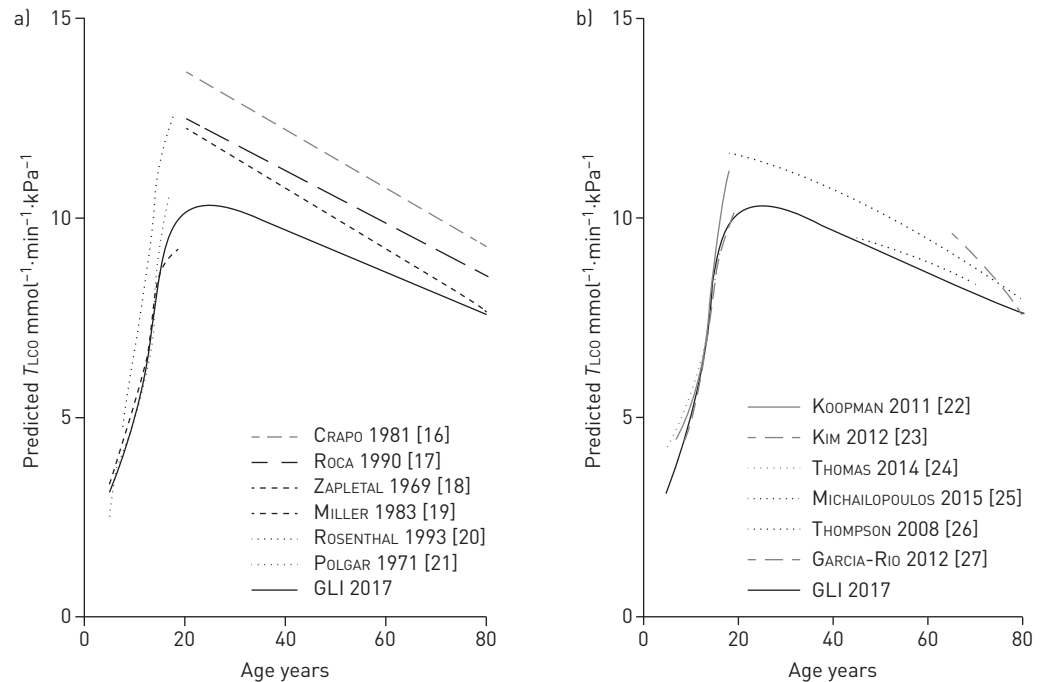


FIGURE 4 Comparison of transfer factor of the lung for carbon monoxide (T_{LCO}) reference equations to the current Global Lung Function Initiative (GLI) equations. Equations found in a) most commercially available equipment and b) more recently published studies.

19% oxygen, and T_{LCO} values were corrected using the equation by KANNER and CRAPO [14] (online supplementary figure S3). In the majority of centres, T_{LCO} values were reported as an average of acceptable tests (eight centres, 47.1%). Others reported the largest value (three centres, 17.7%), values generated by equipment software (three centres, 17.7%), did not report the method used (two centres, 11.8%) or selected “other” (one centre, 5.9%). The method of reporting results did not lead to physiologically relevant differences in T_{LCO} . The reporting of values was equipment-specific, except for the case of Jaeger and “other”, where reporting of values was centre-specific. A summary of the original and final corrected T_{LCO} values used to derive the reference values is presented in online supplementary figure S4.

Haemoglobin correction of T_{LCO} outcomes

All of the T_{LCO} data included in this healthy population were uncorrected for Hb. However, T_{LCO} is dependent on the amount of Hb in the pulmonary capillary bed. To gauge the potential effect of variation

TABLE 3 Comparison of predicted values for individuals using available transfer factor of the lung for carbon monoxide (T_{LCO}) reference values

First author, year [reference]	T_{LCO} mmol·min $^{-1}$ ·kPa $^{-1}$		
	178-cm, 64-year-old male	178-cm, 20-year-old male	150-cm, 10-year-old male
GLI, 2017	9.2	10.9	6.4
CRAPO, 1981 [16]	11.3	14.5	
MILLER, 1983 [19]	9.2	12.6	
ROCA, 1990 [17]	10.3	13.3	
GUTIERREZ, 2004 [28]	8.3	10.5	
THOMPSON, 2008 [26]	9.3	10.8	
KOOPMAN, 2011 [22]			6.4
KIM, 2012 [23]			6.0

The list of equations is not meant to be comprehensive, rather it provides a range of differences that might be expected. GLI: Global Lung Function Initiative.

TABLE 4 Summary of the practical recommendations for applying Global Lung Function Initiative transfer factor of the lung for carbon monoxide (T_{LCO}) reference values

Barometric pressure (altitude)	T_{LCO} and K_{CO} values should be adjusted to standard pressure (101.3 kPa or 760 mmHg) prior to calculation of predicted values
Anatomic dead space	Anatomic dead space should be either measured from the tracer gas washout or estimated using body size
Hb	T_{LCO} values should be uncorrected for Hb; Hb levels should be considered in the interpretation

Hb: haemoglobin; K_{CO} : transfer coefficient of the lung for carbon monoxide.

from the standard reference values of $8.31 \text{ mmol}\cdot\text{L}^{-1}$ ($134 \text{ g}\cdot\text{L}^{-1}$) for females and children and $9.06 \text{ mmol}\cdot\text{L}^{-1}$ ($146 \text{ g}\cdot\text{L}^{-1}$) for adult males, we used the National Health and Nutrition Examination Survey (NHANES) III age-, sex- and ethnicity-specific reference values for Hb [15] to calculate an expected Hb level for all individuals and then calculated T_{LCO} adjusted for the predicted Hb level using $T_{LCO_{\text{Hb}}} = T_{LCO} \times (1.7 \times \text{Hb} / (0.7 \times \text{Hb}_{\text{reference}} + \text{Hb}))$ [2]. There was no difference in the z-scores calculated using the Hb-corrected T_{LCO} reference values *versus* the Hb-uncorrected T_{LCO} reference values (mean difference <0.0001). In addition, adjusting for Hb as a covariate in the prediction model did not improve the overall model fit, nor was age- and sex-predicted Hb an independent predictor of T_{LCO} .

Ethnic differences

85% of the data were from Caucasians, and there were insufficient data from any other ethnic group to derive all-age equations, therefore the final prediction equations are limited to Caucasians. The majority of the non-Caucasian individuals were adults from Japan (10%). T_{LCO} z-scores calculated based on Caucasian data were on average -0.1 ± 1.4 z-scores lower than for Caucasians, with a pattern of higher T_{LCO} z-score values in younger individuals. For V_A , the average z-score was 0.31 ± 1.1 units higher than that for Caucasians. T_{LCO} data collected in adult males from Hong Kong were -0.25 ± 1.2 z-scores lower than Caucasians, with lower values in older individuals.

Comparison with existing reference values

Compared with many earlier T_{LCO} reference values for adults, the GLI T_{LCO} reference values are noticeably lower (figure 4a); however, compared with more-recently published equations, many of which are included in the GLI dataset, the new GLI T_{LCO} equations are quite comparable (figure 4b). For an individual, interpretation of results can be quite different depending on which equation is used (table 3).

Discussion

These GLI reference values for T_{LCO} are the largest and first internationally representative collection of data from healthy Caucasian individuals for this commonly used pulmonary function test. Development of the GLI reference values has taken into consideration several of the methodological and equipment differences that are known to influence T_{LCO} values and presents a standardised way to interpret outcomes. Spirometry z-scores for the population used to derive the T_{LCO} equations fit the GLI 2012 spirometry population very well. However, the present T_{LCO} equations are limited to Caucasians, therefore additional data for non-Caucasians are urgently needed to increase the generalisability of these findings.

Similar to prediction equations for spirometry, sex, age and height were independent and significant predictors of T_{LCO} . The T_{LCO} equations are therefore sex-specific and describe a multiplicative relationship with age and height. Previous studies have used weight and/or surface area as predictor variables for T_{LCO} [26]. In our analysis, prediction equations were virtually identical whether overweight individuals were included or excluded from the dataset; we chose to use the more inclusive, larger dataset in the final prediction equations. Furthermore, height and surface area were highly correlated and therefore surface area was not included in the prediction model.

Previous studies have shown large differences in the predicted values between different prediction equations [23, 26]. Many older publications reporting T_{LCO} reference values were based on outdated equipment that is no longer available, and which often applied different assumptions and algorithms and using different gas concentrations. More recently, reference values have become available for T_{LCO} in children [22, 23], which are included in the GLI dataset. The GLI equations have the advantage of seamlessly continuing into adulthood, a significant advantage given the large discontinuity between previously published paediatric and adult equations (figure 4b).

There were insufficient data to create multiethnic reference values. The largest sample of non-Caucasian data were from Japanese adults, who had lower T_{LCO} z-scores on average and were biased by age. Older Japanese individuals had lower T_{LCO} z-scores, which may partially be explained by secular changes in socioeconomic and general health conditions that have affected body frames and leg length in Japan; this hypothesis requires further investigation [29].

The 2005 ATS/ERS standards on interpretation of lung function state that for each lung function index, values below the 5th percentile of the frequency distribution of values measured in the reference population are considered to be below the expected “normal range” [3]. This is often referred to as the LLN. Values at the upper end of the distribution are generally considered to be physiological variants, and as such, there is no upper limit of normal. It may be argued that an upper limit of normal is required for T_{LCO} , since conditions such as polycythaemia, left-to-right cardiac shunt or alveolar haemorrhage (e.g. Goodpasture’s syndrome) can result in higher than expected values. In addition, some authors state that asthma increases T_{LCO} [30], but not usually to a great extent. Other factors that increase pulmonary capillary blood volume, such as exercise or a decrease in intrathoracic pressure, such as during a Mueller manoeuvre, will also increase T_{LCO} . Cases of left-to-right cardiac shunt or acute alveolar haemorrhage are very rarely seen upon pulmonary function testing and T_{LCO} is not a standard test for their diagnosis. For these reasons, the LLN for T_{LCO} provided in these reference values is the 5th percentile. Similarly, the 5th percentile is used for VA.

Although the same arguments can be applied to reference values for KCO , there is a need to consider the effect of VA on KCO . Failure to inhale completely to total lung capacity will reduce T_{LCO} , but KCO will be increased. The KCO from a submaximal inhalation will be overestimated when compared to the reference value, yielding a normal or above-normal KCO when the T_{LCO} is actually reduced [31]. Thus, the reference value and the normal range for KCO are only valid when the VA is normal. The LLN reported here for KCO is the 5th percentile, but interpreters must use caution when the VA differs from the reference value. An individual with a low T_{LCO} and a low VA may have a KCO that erroneously lies within the normal range [3, 31].

As expected, the coefficient of variation is higher for T_{LCO} than FEV_1 , since T_{LCO} is dependent on several factors in addition to size of the lungs (figure 2). As seen in figure 2, the between-individual variability of T_{LCO} in females was greater than that observed in males. This sex-related difference in the coefficient of variation may be due to the previously observed mean changes of 13% in T_{LCO} in females during the menstrual cycle [32]. The highest value was observed just before the menses, and the lowest on the third day of menses. This mechanism is further supported by the GLI all-age analysis of the combined datasets which shows that the sex-related difference in T_{LCO} coefficient of variation is minimal in younger (aged <10 years) individuals, where the females were presumably prepubescent, and in the older (aged >55 years) individuals, where the females were presumably postmenopausal. For both males and females, alterations in lung structure and heterogeneity in ventilation that occur as the lung ages may reduce T_{LCO} in some persons, which in turn, may contribute to an increase in the variation of T_{LCO} in older adults [33].

Methodological differences

The GLI T_{LCO} were limited to studies that used modern equipment and standardised methodology, although we did not apply specific exclusions based on equipment or methodology other than the 5-s breath-hold. Where possible, we corrected data for altitude, oxygen concentration and anatomic dead space to standardise the interpretation of results between centres.

Within the data collected for the GLI task force, two key methodological differences between datasets were identified: 1) the method for correcting for dead space and 2) the altitude of the sites, both of which have a direct impact on T_{LCO} values. The updated standards [2, 34] note that the equipment dead space (filter, valve and mouthpiece) are not negligible (up to 350 mL) and should be considered in combination with anatomic dead space when determining T_{LCO} values. In adults, the combined dead space may be relatively small compared to the size of the lungs, but the likelihood of contamination of the sample volume is higher and may result in lower VA and T_{LCO} values. However, the effect of dead space could be larger in smaller individuals and especially in children where the combined equipment and anatomical dead space is relatively large compared to the size of their lungs. Within the GLI dataset, two different methods for estimating the anatomic dead space were used: 1) fixed volume of 150 mL and 2) estimated based on body size (see the online supplementary material for details). We observed that paediatric datasets that had a fixed dead space volume underestimated the T_{LCO} . When T_{LCO} values were adjusted based on estimated dead space relative to body weight, the differences between datasets was minimised. Secondly, we observed that T_{LCO} was higher for sites that were not at sea level. A contributory factor to the increase in T_{LCO} at these sites could be the lower alveolar oxygen tension due to the decreased P_B with increasing altitude. Using the altitude of the site as a proxy for P_B , we corrected all T_{LCO} data to 101.3 kPa (760 mmHg).

Since K_{CO} was calculated as T_{LCO}/VA , K_{CO} was also corrected for standard pressure. Although the use of a fixed P_B for a given site based on its altitude corrects for the mean effect of P_B , it does not correct for the day-to-day variations that occur in P_B due to high and low pressure cells, which are rarely outside the range of ± 3.33 kPa (± 25 mmHg). These pressure changes translate to a variation of up to $\pm 1.5\%$ in T_{LCO} , which contributes to between-individual variation in the combined datasets. The altitude correction is based on experimental evidence which measured the change in T_{LCO} with altitude, and thus does not assume any underlying physiological abnormality [5]. Finally, while we adjusted for altitude of the site, we could not adjust for the individual's Hb level, and there may be residual effects of higher altitude on Hb levels.

T_{LCO} is dependent on both the overall surface area and thickness of the alveolar–capillary membrane and the amount of Hb in the pulmonary capillary blood. As carbon monoxide competes with oxygen for binding with Hb, T_{LCO} is also dependent on the pulmonary capillary oxygen concentration. RUIZ-ARGÜELLES *et al.* [35] showed that Hb in an adult Mexican population living at an altitude of 2670 m was $5 \text{ g}\cdot\text{L}^{-1}$ higher in adult males and $15 \text{ g}\cdot\text{L}^{-1}$ higher in adult females compared to the Mexican population living at sea level. Laboratories at >1000 m above sea level need to consider Hb and be aware that further adjustments may be necessary. Ideally, individual T_{LCO} measurements should be corrected for the individual's Hb levels [3, 34], since Hb concentration will affect the rate of carbon monoxide uptake; but few clinical pulmonary function laboratories routinely make this correction. Only four of the available datasets provided Hb values, and therefore we could not derive Hb-corrected T_{LCO} reference values. The 2005 ATS/ERS statement recommends that predicted T_{LCO} values are corrected to standard Hb values using the equation derived by COTES and colleagues [36, 37]; however, the correction is dependent on the assumptions that the alveolar partial pressure of oxygen is 14.63 kPa (110 mmHg) and that the ratio of the membrane diffusing capacity to pulmonary capillary blood volume times the reaction rate of carbon monoxide with oxyhaemoglobin is $0.7 \text{ mL}^{-1}\cdot\text{min}^{-1}\cdot\text{mmHg}^{-1}\cdot\text{mL}\cdot\text{blood}$. While these equations provide a simple correction to $146 \text{ g}\cdot\text{L}^{-1}$ for males aged ≥ 15 years and $134 \text{ g}\cdot\text{L}^{-1}$ for females and children, measures of Hb levels in the general USA population (NHANES III) were substantially different from these fixed reference values, especially in children, males and non-Caucasians [15, 38]. White males have peak Hb of $155 \text{ g}\cdot\text{L}^{-1}$ at the age of 30 years, while both male and female African-American subjects have Hb levels ~ 8 – 10% lower than white subjects. Furthermore, the relationship between T_{LCO} and Hb in healthy individuals may not reflect that observed in disease groups, and thus there is a need to define clinically relevant correction factors for Hb.

Implementation

The updated T_{LCO} standards recommend that T_{LCO} is reported as the measured value, as well as the value adjusted to standard pressure [39]. Furthermore, table 4 summarises the additional adjustments that should be made by users prior to applying the GLI T_{LCO} reference values. The format of the T_{LCO} equations and look-up tables is identical to the GLI spirometry equations, which will facilitate implementation into many devices which already have the GLI spirometry equations programmed. The prediction equations (table 2) and look-up tables are provided in both SI and traditional units (www.lungfunction.org), and a worked example is included in the online supplementary material. Similar to previous GLI tools, researchers, clinicians and manufactures can access individual calculators, and other tools for applying these equations for large research datasets are also available at www.lungfunction.org.

Limitations

While the GLI T_{LCO} data represents the largest collection of normative data for T_{LCO} , the lack of data from non-Caucasians limits the generalisability. The extent to which ethnic differences for T_{LCO} occur is unclear and could not be explored in the current GLI dataset due to the limited sample of non-Caucasians. Some differences were observed between different equipment types and between centres, but these were generally within the limits of physiological variability. In a few cases, results were outside the physiologically defined limits and warrant further investigation, since it was not possible to ascertain whether differences within the current dataset were attributable to equipment, population or methodology. Since many of the causes of potential differences in T_{LCO} affect results in opposite directions, between-individual variability would be expected to increase, thereby underestimating the LLN, but this should not affect the predicted value. The adjustments traditionally used on T_{LCO} to correct for oxygen tension, barometric pressure and Hb levels have been challenged. The correction for barometric pressure (or altitude) is based on scant data [5] and may not be linear [39]. Further research on the effect of altitude on T_{LCO} is well warranted.

Conclusions

GLI reference values for T_{LCO} (2017) provide a generalisable reference to standardise the reporting and interpretation of T_{LCO} data for Caucasians. Data collection in non-Caucasians and future validation with

measurements made using contemporary equipment and updated ATS/ERS recommendations are necessary.

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