

# Reference Values for the Diffusing Capacity Determined by the Single-Breath Technique at Different Altitudes: The Latin American Single-Breath Diffusing Capacity Reference Project

Juan Carlos Vázquez-García, Rogelio Pérez-Padilla, Alejandro Casas, Patricia Schönffeldt-Guerrero, Jonatan Pereira, Claudia Vargas-Domínguez, Mónica Velázquez-Uncal, David Martínez-Briseño, Luis Torre-Bouscoulet, and Laura Gochicoa-Rangel

**BACKGROUND:** The lung diffusion capacity ( $D_{LCO}$ ) determined by the single-breath technique greatly helps in the differential diagnosis and classification of severity of common lung diseases. However, widespread use of single-breath  $D_{LCO}$  tests in Latin America has been limited, in part, by the lack of appropriate reference values. Our objective was to derive robust reference equations for single-breath  $D_{LCO}$  from healthy Hispanic adults, using the most recent guidelines and taking into account altitude above sea level and hemoglobin. **METHODS:** We recruited healthy adults from Caracas (690 m), Santiago (650 m), Mexico City (2,240 m), and Bogota (2,640 m).  $D_{LCO}$  testing was completed using an instrument that exceeds American Thoracic Society/European Respiratory Society 2005 guidelines for spirometry and single-breath  $D_{LCO}$  and provided centralized training and a quality assurance program. **RESULTS:** We included 480 healthy Hispanic adults (58.3% women) with a mean age of 46 y (range 22–83 y). Their mean  $\pm$  SD single-breath  $D_{LCO}$  was  $30.4 \pm 9.2$  mL/min/mm Hg. Results as a percentage of predicted by Crapo's reference values (the closest to obtained values) were  $83 \pm 10\%$  (Caracas),  $91 \pm 10\%$  (Santiago),  $104 \pm 17\%$  (Mexico City), and  $118 \pm 19\%$  (Bogota), and current suggested adjustments by hemoglobin or altitude did not correct differences, especially in Santiago and Caracas. **CONCLUSIONS:** We recommend these new single-breath  $D_{LCO}$  reference equations to predict single-breath  $D_{LCO}$  in Latin America performed with current instruments and procedures and including as a predictor altitude above sea level. *Key words:*  $D_{LCO}$ ; reference values; altitude; hemoglobin. [Respir Care 2016;61(9):1217–1223. © 2016 Daedalus Enterprises]

## Introduction

The combination of spirometry and the lung diffusion capacity ( $D_{LCO}$ ) determined by single-breath technique tests is widely available in pulmonary function testing (PFT)

laboratories, since it assists in the differential diagnosis of patients with dyspnea, assesses the severity of both obstructive and restrictive types of lung disease, and provides objective measurements of treatment efficacy.<sup>1</sup> The guidelines for PFT were updated by the American Thoracic Society (ATS) and European Respiratory Society (ERS) in

---

Dr Vázquez-García, Dr Pérez-Padilla, Dr Vargas-Domínguez, Dr Velázquez-Uncal, Mr Martínez-Briseño, Dr Torre-Bouscoulet, and Dr Gochicoa-Rangel are affiliated with the Departamento de Fisiología Respiratoria. Instituto Nacional de Enfermedades Respiratorias, "Ismael Cosío Villegas," Tlalpan, Distrito Federal, Mexico. Dr Casas is affiliated with the Fundación Neumológica Colombiana, Bogotá, Colombia. Dr Schönffeldt-Guerrero is affiliated with the Instituto Nacional del Tórax y Universidad de Chile, Santiago de Chile, Chile. Mr Pereira is affiliated with the Universidad Central de Venezuela, Caracas, Venezuela.

---

This study was supported by each participant institution. nnd Medical provided equipment for pulmonary function testing, but there was no participation in the design, analysis, or writing of the paper. The authors have disclosed no conflicts of interest.

Dr Vázquez-García presented a version of this paper at the IX Congress, Asociación Latinoamericana de Tórax (ALAT), held July 31 to August 2, 2014, in Medellín, Colombia.<sup>10</sup>

2005<sup>2</sup> and are widely recognized as standards of practice by other pulmonary subspecialty societies worldwide.

The selection of appropriate reference values is crucial to adequately interpret PFT results, to ascertain the correct classification of functional patterns, and to more accurately estimate disease severity. However, the 2005 ATS/ERS PFT interpretation guidelines<sup>2</sup> did not recommend a single set of single-breath  $D_{LCO}$  reference equations. In fact, few reference value studies have been reported since 2000.<sup>3-6</sup>

Most PFT laboratories in Latin America currently use single-breath  $D_{LCO}$  reference equations from studies of non-Hispanic adults in Europe or the United States,<sup>7-9</sup> but these give substantially different percent-of-predicted single-breath  $D_{LCO}$  values for many patients, increasing misclassification rates.

The aim of this study was to generate robust single-breath  $D_{LCO}$  reference equations from a group of healthy Hispanic adults from 4 large Latin American cities with the rationale that testing with standardized methods would improve current suggested adjustments by hemoglobin and altitude. Some of the results have been reported previously in the form of an abstract.<sup>10</sup>

## Methods

### Subjects

Previous studies showed that adults living at high altitude have higher single-breath  $D_{LCO}$  values,<sup>11</sup> so we chose cities located at different altitudes above sea level for our study, including Santiago de Chile (650 m), Caracas (960 m), Mexico City (2,240 m), and Bogota (2,640 m). The study was approved by the ethics committees of each institution (see the supplementary materials at <http://www.rcjournal.com>), and participants gave their written informed consent. We adopted standardized instrumentation, training, quality control, and systematic review of results as described previously.<sup>12</sup> PFT technologists for this study underwent a standardized, 1-week training in Mexico City on proper use of equipment, quality assurance, and practical testing. Men and women  $\geq 25$  y old were recruited by announcements in all institutions and related hospitals that invited students, employees, and the

## QUICK LOOK

### Current knowledge

The lung diffusion capacity ( $D_{LCO}$ ) determined by the single-breath technique is commonly essential in the process of diagnosis and evaluation of severity of chronic lung diseases. This testing should be performed under the current guidelines (American Thoracic Society/European Respiratory Society 2005), widely recognized as standards of practice. However, widespread use of single-breath  $D_{LCO}$  tests in Latin America has been limited, in part, by the lack of appropriate reference values.

### What this paper contributes to our knowledge

This is a multinational study regarding reference values of  $D_{LCO}$  obtained in an adult population from Latin America at different altitudes. The main results were obtained using current guidelines, modern instruments, and good test quality, giving comparable results across the 4 participant cities and with substantial advantages over previous studies. Reference equations properly adjust for age, height, sex, altitude up to 2,600 m, and hemoglobin.

relatives of patients from a variety of socioeconomic levels to participate. Similar announcements were also placed in the vicinity of the hospitals. We used a brief interview and a standardized questionnaire<sup>13</sup> to exclude those who reported smoking  $>400$  cigarettes in their lifetime; who had undergone previous upper abdominal or thoracic surgery; who had received a physician diagnosis of diabetes mellitus, respiratory disease, or cardiovascular disease; or who had recently experienced respiratory symptoms. We also excluded pregnant women, those with a heart rate  $>110$  beats/min, and those with surgery of the eyes or ears in the 3 months before testing. Data from subjects who were unable to perform acceptable single-breath  $D_{LCO}$  test were not included in the final analysis.

### Testing

All study sites used the EasyOnePro (firmware V04b, ndd Medical Technologies, Zurich, Switzerland), a small portable instrument for spirometry and single-breath  $D_{LCO}$  tests. The test gas was 21% oxygen (not adjusted for altitude), 10% helium, 0.3% carbon monoxide, and the remaining gas was nitrogen. Unacceptable maneuvers were not used for data analysis. We used standard venipuncture techniques to draw blood for hemoglobin analysis. Test methods closely followed 2005 ATS/ERS guidelines,<sup>14</sup> and the quality assurance program followed its recommenda-

---

Supplementary material related to this paper is available at <http://www.rcjournal.com>.

Correspondence: Juan Carlos Vázquez-García MD MSc, Departamento de Fisiología Respiratoria, Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas", Calzada de Tlalpan 4502, Colonia Sección XVI, C.P. 14080, Tlalpan, Distrito Federal, Mexico. E-mail: drjcvazquez@gmail.com.

DOI: 10.4187/respcare.04590

tions and included biological controls in all sites and simulators at the beginning of the study in Mexico City and Bogota, the cities with highest altitude, as described with more detail in the supplementary material.

### Statistical Analyses

At the end of the study, averages and SD values or means and intervals were used to describe the variables according to their distribution. Reference equations for single-breath  $D_{LCO}$ , alveolar volume ( $V_A$ ) (single-breath total lung capacity), and single-breath  $D_{LCO}/V_A$  (dependent variables) were generated separately for men and women, using robust multiple regression models, including as independent variables linear and quadratic terms for weight, height, age, and altitude above sea level, with and without measured hemoglobin (since the latter was often not available at the time of testing). Squared predictors were tested as well as interaction between relevant variables. The transformed variables remained in the final equation only if they significantly improved the  $r^2$  of the equation. We estimated directly the lower limit of normal (5th percentile) separately for men and women, utilizing the semi-parametric quantile regression,<sup>15</sup> including predictors utilized for prediction of mean single-breath  $D_{LCO}$ . We also estimated the residual volume as  $V_A$ -FVC and generated predicting equations for residual volume/single-breath total lung capacity.

### Results

We recruited 480 healthy subjects (58.3% women) who fulfilled the selection criteria and completed tests of good quality. Most non-eligible individuals were current or previous smokers or were discovered in a brief interview to have respiratory symptoms or asthma. After testing was done, we excluded 11 individuals because of body mass index  $>35$  kg/m<sup>2</sup>, 5 because of air flow obstruction found in response to bronchodilator and atopy suggestive of asthma, 3 because of diabetes, 3 because of hypertension, and 4 because of claustrophobia that would not allow them to enter the body box used for additional testing.

The main characteristics of the participants by city are depicted in Table 1. The mean age was 46 y (range 22–83 y). Age distribution from 25 to 65 y was similar in the 4 cities, but the Caracas center recruited only a few adults  $>65$  y old. On average, recruited individuals were  $162 \pm 10$  cm tall and had a weight of  $66.8 \pm 11.5$  kg (only 4.2% had a body mass index  $>30$  kg/m<sup>2</sup>), a single-breath  $D_{LCO}$  of  $30.4 \pm 9.3$  mL/min/mm Hg, and a vital capacity of  $3.9 \pm 1.0$  L. The mean difference between the 2 acceptable single-breath  $D_{LCO}$  tests was  $0.98 \pm 0.76$  mL/min/mm Hg.

From all individuals, 455 (94.8%) had 2 acceptable maneuvers, whereas 7 had 1, and 18 had none, and single-breath  $D_{LCO}$  repeatability was better than 3 units for subjects. The intratest coefficient of variability for single-breath  $D_{LCO}$  was  $2.45 \pm 1.99\%$  (SD), and that for single-breath  $D_{LCO}/V_A$  was  $3.25 \pm 3.7\%$ . The mean intratest coefficient of variability for  $V_A$  in the studied population was  $2.79 \pm 3.1\%$  for the 2 selected maneuvers. The coefficient of variability was consistently higher for single-breath  $D_{LCO}/V_A$  and for  $V_A$  than for single-breath  $D_{LCO}$ . The number of  $D_{LCO}$  maneuvers performed was 2 for 76% of participants, 3 for 19%, 4 for 3%, and 5 for 2%.

Independent predictors of a higher single-breath  $D_{LCO}$  included male sex, height, altitude, and hemoglobin (see Figs. S1 and S2 in the supplementary material), whereas single-breath  $D_{LCO}$  declined with aging (Figs. 1 and 2). Hemoglobin was available for 169 men and 250 women (87% of all subjects). Adding squared age, height, or altitude terms or interaction terms to the equation increased  $r^2$  significantly in some models. Parsimonious reference equations are given in Table 2 with and without hemoglobin. Similar prediction equations using only linear terms are shown in Table 3, with slightly less variability explained by the models (lower  $r^2$ ).

Variance of single-breath  $D_{LCO}$  increased with altitude or barometric pressure with significant Breusch-Pagan and Cook/Weisberg heteroskedasticity tests and was eliminated if regression was done with log single-breath  $D_{LCO}$  instead of single-breath  $D_{LCO}$ , but prediction of single-breath  $D_{LCO}$  did not improve over the untransformed term. The Breusch-Pagan test was also not significant if altitude was eliminated from the equation, but  $r^2$  decreased significantly (see Table 2). Using the daily barometric pressure available from the instrument (or mean barometric pressure for the city) did not increase the  $r^2$  when compared with use of the mean altitude above sea level (data not shown). The concordance correlation coefficient between the internally predicting equation using hemoglobin and altitude was 0.89 (95% CI 0.87–0.91) and was 0.88 for the equation without hemoglobin (95% CI 0.86–0.90) but was only 0.77 (95% 0.74–0.81) for the equation without altitude or hemoglobin (based only on sex, age, and height).

The healthy adults in this study had single-breath  $D_{LCO}$  values on average above (5–17%; see Table 1) those of previous studies,<sup>8,9,16</sup> with mean values closest to the study of Crapo and Morris,<sup>7</sup> done at 1,420 m above sea level and using an  $F_{IO_2}$  of 0.25, simulating sea level (see Table 1 and Fig. 3). We observed heterogeneous results for the different cities, with an overall concordance correlation coefficient  $\rho = 0.69$  (95% CI 0.65–0.73) between predicted and observed results. Adjusting single-breath  $D_{LCO}$  by hemoglobin and altitude<sup>14,17</sup> improved the prediction in Mexico and Bogota, but overestimation remained for Santiago and Caracas: overall  $\rho = 0.75$  (95% CI 0.71–0.78) ad-

# REFERENCE VALUES FOR $D_{LCO}$ IN LATIN AMERICA

Table 1. Characteristics of Healthy Hispanic Adult Study Subjects

| Characteristic                         | Santiago<br>(520 m, $n = 60$ ) | Caracas<br>(900 m, $n = 78$ ) | Mexico City<br>(2,240 m, $n = 149$ ) | Bogota<br>(2,625 m, $n = 193$ ) | Total<br>( $N = 480$ ) |
|--|--------------------------------|-------------------------------|--------------------------------------|---------------------------------|------------------------|
| Age, y                                 | 44.8 $\pm$ 12.7                | 39.9 $\pm$ 11.5               | 43.8 $\pm$ 15                        | 50.3 $\pm$ 16                   | 46.1 $\pm$ 15*         |
| Height, cm                             | 167.2 $\pm$ 9                  | 162.2 $\pm$ 9                 | 161.5 $\pm$ 10                       | 160.4 $\pm$ 9                   | 162.1 $\pm$ 10*        |
| Weight, kg                             | 73 $\pm$ 12.6                  | 67.1 $\pm$ 11.7               | 65.2 $\pm$ 12.2                      | 65.4 $\pm$ 9.8                  | 66.8 $\pm$ 11.6*       |
| $D_{LCO_{sb}}$ , mL/min/mm Hg          | 28.3 $\pm$ 6.7                 | 23.9 $\pm$ 4.9                | 30.6 $\pm$ 9.8                       | 33.2 $\pm$ 9.6                  | 30.4 $\pm$ 9.3*        |
| Inspiratory time, s                    | 1.2 $\pm$ 0.3                  | 1.2 $\pm$ 0.5                 | 1.3 $\pm$ 0.5                        | 1.1 $\pm$ 0.3                   | 1.2 $\pm$ 0.4*         |
| Vital capacity, L                      | 4.3 $\pm$ 1.1                  | 3.7 $\pm$ 0.8                 | 4.0 $\pm$ 1.1                        | 3.9 $\pm$ 1                     | 3.9 $\pm$ 1*           |
| Breath hold time, s                    | 10.5 $\pm$ 0.3                 | 10.1 $\pm$ 0.3                | 10.4 $\pm$ 0.3                       | 10.1 $\pm$ 0.3                  | 10.3 $\pm$ 0.3*        |
| Hemoglobin, mg/dL                      | 14.6 $\pm$ 1.5                 | 13.7 $\pm$ 1.3                | 14.5 $\pm$ 1.7                       | 15.6 $\pm$ 1.6                  | 14.9 $\pm$ 1.7*        |
| BMI, kg/m <sup>2</sup>                 | 26 $\pm$ 3.1                   | 25.4 $\pm$ 3.5                | 24.9 $\pm$ 3                         | 25.4 $\pm$ 2.7                  | 25.3 $\pm$ 3           |
| Obese (BMI >30 kg/m <sup>2</sup> )     | 13.3 $\pm$ 4.4                 | 7.7 $\pm$ 3.0                 | 2.0 $\pm$ 1.1                        | 1.5 $\pm$ 0.9                   | 4.2 $\pm$ 0.9*         |
| $D_{LCO_{sb}}/V_A$ , mL/min/mm Hg/L    | 5.2 $\pm$ 0.6                  | 5.1 $\pm$ 0.7                 | 6.1 $\pm$ 1                          | 6.3 $\pm$ 1.2                   | 5.9 $\pm$ 1.1*         |
| $V_A$ , L                              | 5.52 $\pm$ 1.2                 | 4.7 $\pm$ 0.8                 | 4.9 $\pm$ 1.1                        | 5.28 $\pm$ 1.1                  | 5.14 $\pm$ 1.1*        |
| CoV $D_{LCO}$ , %                      | 2.46 $\pm$ 1.9                 | 2.9 $\pm$ 2.3                 | 2.4 $\pm$ 2                          | 2.32 $\pm$ 1.9                  | 2.44 $\pm$ 2           |
| CoV $D_{LCO}/V_A$ , %                  | 3.35 $\pm$ 3.8                 | 3.7 $\pm$ 2.2                 | 2.9 $\pm$ 3.2                        | 3.33 $\pm$ 3.2                  | 3.18 $\pm$ 3.2         |
| CoV $V_A$ , %                          | 2.4 $\pm$ 3.6                  | 2.8 $\pm$ 2.2                 | 2.8 $\pm$ 3.4                        | 3 $\pm$ 2.9                     | 2.8 $\pm$ 3.1          |
| $D_{LCO_{sb}}$ % Crapo                 | 91 $\pm$ 10                    | 83 $\pm$ 10                   | 104 $\pm$ 17                         | 118 $\pm$ 19                    | 105 $\pm$ 21*          |
| $D_{LCO_{sb}}$ % Roca                  | 98 $\pm$ 11                    | 89 $\pm$ 11                   | 113 $\pm$ 18                         | 128 $\pm$ 20                    | 113 $\pm$ 22*          |
| $D_{LCO_{sb}}$ % ERS                   | 102 $\pm$ 13                   | 91 $\pm$ 11                   | 116 $\pm$ 21                         | 133 $\pm$ 23                    | 117 $\pm$ 25*          |
| $D_{LCO_{sb}}$ % NHANES                | 98 $\pm$ 11                    | 91 $\pm$ 11                   | 112 $\pm$ 17                         | 122 $\pm$ 19                    | 111 $\pm$ 20*          |
| $D_{LCO_{sb}}$ % this study with Hb    | 104.7 $\pm$ 12.5               | 95.5 $\pm$ 12.5               | 100.5 $\pm$ 13.7                     | 100.1 $\pm$ 13.7                | 100.2 $\pm$ 13.5*      |
| $D_{LCO_{sb}}$ % this study without Hb | 101.9 $\pm$ 11.9               | 96.9 $\pm$ 12.2               | 101.3 $\pm$ 15.0                     | 99.2 $\pm$ 14.2                 | 100.0 $\pm$ 13.9       |
| Residuals equation with Hb             | 1.2 $\pm$ 4.0                  | -1.4 $\pm$ 3.1                | 0.2 $\pm$ 4.0                        | 0.1 $\pm$ 4.6                   | 0.06 $\pm$ 4.1*        |
| Standardized residuals with Hb         | 0.29 $\pm$ 0.8                 | -0.32 $\pm$ 0.8               | 0.04 $\pm$ 1.0                       | 0.02 $\pm$ 1.0                  | 0.02 $\pm$ 1.0*        |
| Residuals without Hb                   | 0.42 $\pm$ 3.0                 | -0.85 $\pm$ 3.0               | 0.51 $\pm$ 4.4                       | -0.21 $\pm$ 4.8                 | 0.03 $\pm$ 4.3         |
| Standardized residuals without Hb      | 0.09 $\pm$ 0.7                 | -0.18 $\pm$ 0.8               | 0.12 $\pm$ 1.0                       | -0.05 $\pm$ 1.1                 | 0.01 $\pm$ 1.0         |

Results are means  $\pm$  SD, except for obese (mean  $\pm$  SE).

\* Significant difference ( $P < .05$ ) among cities by analysis of variance.

BMI = body mass index

$V_A$  = alveolar volume

CoV = coefficient of variation of the acceptable maneuvers

Hb = hemoglobin

$D_{LCO}$  = diffusing capacity of the lung determined by the single-breath technique

$D_{LCO}\%$  = diffusing capacity of the lung determined by the single-breath technique expressed as percentage of predicted by several authors; predicted values were Crapo (3), Roca (5), ERS (11), NHANES (4)

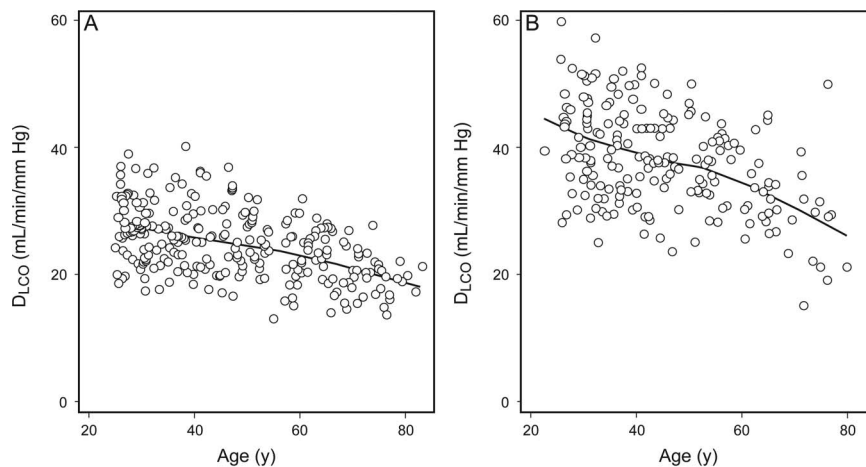


Fig. 1. Diffusing capacity of the lung ( $D_{LCO}$ ) determined by the single-breath technique as a function of age for women (A) and men (B). Variability decreased with age for women. Average values (lines) were obtained by locally weighted scatter-plot smoothing.

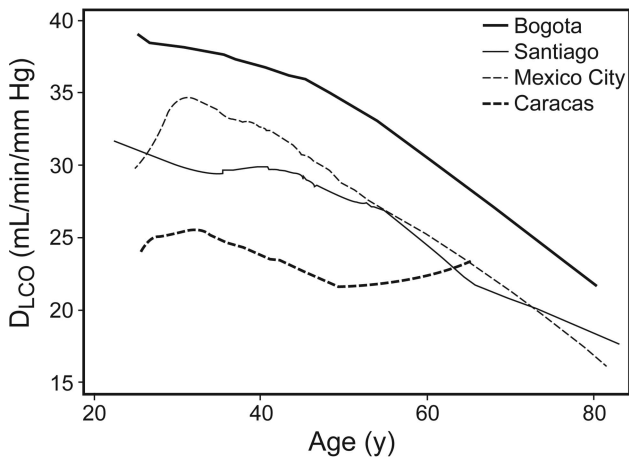


Fig. 2. Diffusing capacity of the lung ( $D_{LCO}$ ) determined by the single-breath technique as a function of age. Data smoothing was done using locally weighted scatter-plot smoothing.

justing only by hemoglobin and 0.81 (95% CI 0.78–0.84) adjusting by hemoglobin and altitude (see Fig. 3). The age coefficient from the study by Crapo and Morris<sup>7</sup> did not apply well for our data, as shown by a positive slope of single-breath percent-of-predicted  $D_{LCO}$  as a function of age in Figure 3. In the online supplementary material, we provide predicting equations for the single-breath  $D_{LCO}/V_A$  (KCO, e-Table 1), single-breath total lung capacity ( $V_A$ , e-Table 2), and residual volume/single-breath total lung capacity (e-Table 3) from the same participants.

## Discussion

Single-breath  $D_{LCO}$  increased with altitude of residence, with body size (height or weight), and with hemoglobin and decreased with age, as described previously in various studies done at different times with heterogeneous equipment and procedures.<sup>3-6,8-10,14,16,18</sup> This study was done with current equipment and procedures and good test quality, giving comparable results across several cities of Latin America with substantial advantages over previous studies. Reference equations in use were obtained many years ago with varied equipment, procedures, and populations and according to our data give results with systematic bias for single-breath  $D_{LCO}$  and also with coefficients that do not adjust properly to changes with age, altitude, and height. Relevant additions from our study were: 1) comparison across different altitudes, and 2) inclusion of hemoglobin in the majority of the subjects. These allow simultaneous adjustments for independent variables indispensable in countries with significant populations residing at moderate or high altitudes. Current practice was to adjust obtained values for single-breath  $D_{LCO}$  by altitude and hemoglobin, with equations proceeding from different studies. With our reference values, altitude and hemoglobin can be taken into account simultaneously, but the most relevant variable was altitude, and once it was accounted for, the additional contribution of hemoglobin was moderate in the studied population, although statistically significant.

Table 2. Reference Equations for Diffusing Capacity of the Lung Determined by the Single-Breath Technique

| Variable                                | Including Hemoglobin |                       |         |                       | Without Hemoglobin |                       |          |                       |
|---|----------------------|-----------------------|---------|-----------------------|--------------------|-----------------------|----------|-----------------------|
|   | Men                  |                       | Women   |                       | Men                |                       | Women    |                       |
|   | Mean                 | Lower Limit of Normal | Mean    | Lower Limit of Normal | Mean               | Lower Limit of Normal | Mean     | Lower Limit of Normal |
| Age                                     |                      | −0.149*               | −0.168† |                       |                    | 0.648‡                | −0.0445  |                       |
| Age <sup>2</sup>                        | −0.00211*            |                       |         | −0.00114*             | −0.00249†          | −0.00838*             | −0.00114 | −0.00104‡             |
| Height, m                               | 392.1*               | 38.76*                | 19.65†  | 21.02                 | 243.5              |                       | 19.30†   | 24.33*                |
| Height <sup>2</sup>                     | −104.9*              |                       |         |                       | −59.85             | 10.87*                |          |                       |
| Weight                                  |                      |                       | 0.0765* |                       |                    |                       | 0.0906†  |                       |
| Altitude, km                            |                      |                       |         |                       | −9.907*            |                       | −7.218†  |                       |
| Altitude <sup>2</sup> , km <sup>2</sup> | 1.594†               | 0.852*                | 0.892†  | 0.831†                | 4.961†             | 0.931‡                | 3.295†   | 0.711*                |
| Hemoglobin                              | 1.151†               | 1.677†                | 0.967†  | 0.892‡                |                    |                       |          |                       |
| Constant                                | −346.1†              | −60.05*               | −19.91† | −26.47                | −200.3             | −16.57                | −6.464   | −19.49                |
| Observations                            | 169                  | 169                   | 250     | 250                   | 200                | 200                   | 280      | 280                   |
| MSE                                     | 5.067                |                       | 3.71    |                       | 5.153              |                       | 3.806    |                       |
| r <sup>2</sup>                          | 0.63                 | 0.44                  | 0.54    | 0.32                  | 0.60               | 0.39                  | 0.50     | 0.25                  |

Lower limit of normal was estimated by a quantilar regression, aiming for the 5th percentile. Adding body mass index offered no advantage over weight. A term for obesity (body mass index >30 kg/m<sup>2</sup>) was not significant. Robust regression was employed.

MSE = mean square error

\*  $P < .05$ .

†  $P < .01$ .

‡  $P < .1$ .

# REFERENCE VALUES FOR $D_{LCO}$ IN LATIN AMERICA

Table 3. Parsimonious Diffusing Capacity of the Lung Determined by the Single-Breath Technique Reference Equations Using Only Linear Terms

| Variable     | Including Hemoglobin |                       |         |                       | Without Hemoglobin |                       |         |                       |
|--------------|----------------------|-----------------------|---------|-----------------------|--------------------|-----------------------|---------|-----------------------|
|              | Men                  |                       | Women   |                       | Men                |                       | Women   |                       |
|              | Mean                 | Lower Limit of Normal | Mean    | Lower Limit of Normal | Mean               | Lower Limit of Normal | Mean    | Lower Limit of Normal |
| Age          | −0.200*              | −0.146†               | −0.167* | −0.123*               | −0.200*            | −0.141†               | −0.152* | −0.115†               |
| Height       | 38.30*               | 37.97†                | 19.60*  | 13.85                 | 41.61*             | 24.86                 | 18.92*  | 22.30‡                |
| Weight       |                      |                       | 0.0778† | 0.0574                |                    | 0.233‡                | 0.116*  |                       |
| Altitude, km | 4.843*               | 2.834†                | 2.779*  | 2.500*                | 5.527*             | 3.518*                | 3.333*  | 2.061†                |
| Hemoglobin   | 1.393*               | 1.755*                | 1.054*  | 1.074*                |                    |                       |         |                       |
| Constant     | −50.19*              | −61.81†               | −22.63* | −19.76                | −34.45*            | −31.02                | −11.11‡ | −14.43                |
| Observations | 169                  | 169                   | 250     | 250                   | 200                | 200                   | 280     | 280                   |
| MSE          | 5.37                 |                       | 3.769   |                       | 5.579              |                       | 3.946   |                       |
| $r^2$        | 0.583                | 0.4377                | 0.524   | 0.3219                | 0.531              | 0.3677                | 0.461   | 0.2414                |

Lower limit of normal was estimated by a quantilar regression, aiming for the 5th percentile. Adding body mass index offered no advantage over weight. A term for obesity (body mass index >30 kg/m<sup>2</sup>) was not significant. Robust regression was employed. Coefficients were obtained by robust regression.

MSE = mean square error

†  $P < .05$ .

\*  $P < .01$ .

‡  $P < .1$ .

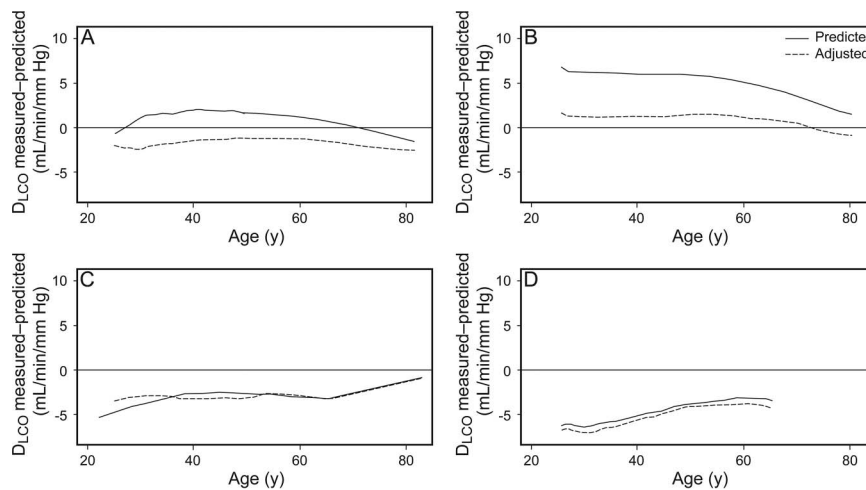


Fig. 3. Predicted diffusing capacity of the lung ( $D_{LCO}$ ) determined by the single-breath technique for the healthy Hispanic study subjects in Mexico City (A), Bogota (B), Santiago (C), and Caracas (D) (using the reference equations of Crapo and Morris<sup>7</sup>). Dashed lines show adjustment for hemoglobin and altitude. Graphs were plotted after smoothing of the residuals over age.

Limitations of the study included the following. Participant selection was not population-based (but nevertheless age distribution was adequate, including individuals of varying socioeconomic strata). Children were not included, and older adults (age >65) were 13% ( $n = 64$ ). More individuals were contributed by Mexico City and Bogota, and no city at sea level was included. Carboxyhemoglobin was not measured, but smokers were excluded. The usual level of carboxyhemoglobin in Mexico City was about 1.5% in the 1980s,<sup>19</sup> and average values continue the same (unpublished data from the PFT laboratory).

On the other hand, the uniform equipment, training, and methods and the varied altitude of residence provide, from

our point of view, significant advantages over current reference values. As done recently for spirometry by the Global Lung Function Initiative Group, it would be very useful to include younger individuals, including children during growth and development, to obtain continuous single-breath  $D_{LCO}$  equations from infancy to old age,<sup>20</sup> so we have shared our  $D_{LCO}$  data with the Global Lung Function Initiative Group.

Poverty is associated with low lung volumes for a given height,<sup>21,22</sup> and in developing countries, socio-economic status is often associated with ethnicity. Differences in the ratio of leg length to height can explain the majority of these differences in lung volumes, but we did not measure

sitting height or an index of socioeconomic status or ethnicity in our study subjects, which may have varied between study sites. Socio-economic status has rapidly improved during the past few decades in the urban areas of developing countries, leading to increases in lung volumes from one birth cohort to the next. These increases in lung volumes are probably associated with increases in single-breath  $D_{LCO}$  and  $V_A$ , but this has not yet been confirmed.

Single-breath  $D_{LCO}$  is a clinically important test underused in many parts of the world. Several factors have limited more widespread use: the cost of the equipment and its maintenance, the difficulty and cost of obtaining certified calibrating gas mixtures, a quality control considerably more demanding than spirometry, and the lack of appropriate reference values with adjustments for altitude and hemoglobin. The size and cost of single-breath  $D_{LCO}$  instruments has diminished during the past decade.

### Conclusions

We report reference values for single-breath  $D_{LCO}$  obtained in an adult population at different altitudes above sea level using modern guidelines and instruments. We suggest that these reference equations offer a significant advantage over existent equations when testing Hispanic patients at a high altitude or with hemoglobin outside of the normal range.

### ACKNOWLEDGMENTS

We thank additional collaborators in the single-breath  $D_{LCO}$  study group. Maria Montes de Oca (Caracas) participated in the final version of the protocol. Robert Jensen participated in the central PFT training. All tests and calibration checks were performed by Mónica Silva (Mexico), Patricia Sandoval (Santiago), Margarita Barrero, Maria Isabel Quintero (Bogota), and Jonatan Pereira (Caracas), who also served as biological controls. Monica Velazquez recruited normal subjects in Mexico City, and Elisa Sanchez-Gallen and Monica Silva received the tests done at all sites and verified quality. Paul L Enright MD reviewed the manuscript and gave thoughtful suggestions.

### REFERENCES

- Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. *Eur Respir J* 2005;26(5):948-968.
- Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. General considerations for lung function testing. *Eur Respir J* 2005;26(1):153-161.
- Garcia-Rio F, Dorgham A, Galera R, Casitas R, Martinez R, Alvarez-Sala R, Pino JM. Prediction equations for single-breath diffusing capacity in subjects aged 65 to 85 years. *Chest* 2012;142(1):175-184.
- Thompson BR, Johns DP, Bailey M, Raven J, Walters EH, Abramson MJ. Prediction equations for single breath diffusing capacity (Tlco) in a middle aged caucasian population. *Thorax* 2008;63(10):889-893.
- Piirilä P, Seikkula T, Valimäki P. Differences between Finnish and European reference values for pulmonary diffusing capacity. *Int J Circumpolar Health* 2007;66(5):449-457.
- Ip MS, Lam WK, Lai AY, Ko FW, Lau AC, Ling SO, et al. Reference values of diffusing capacity of non-smoking Chinese in Hong Kong. *Respirology* 2007;12(4):599-606.
- Crapo RO, Morris AH. Standardized single breath normal values for carbon monoxide diffusing capacity. *Am Rev Respir Dis* 1981;123(2):185-189.
- Neas LM, Schwartz J. The determinants of pulmonary diffusing capacity in a national sample of U.S. adults. *Am J Respir Crit Care Med* 1996;153(2):656-664.
- Roca J, Rodriguez-Roisin R, Cobo E, Burgos F, Perez J, Clausen JL. Single-breath carbon monoxide diffusing capacity prediction equations from a Mediterranean population. *Am Rev Respir Dis* 1990;141(4 Pt 1):1026-1032.
- Vázquez-García JC, Pérez-Padilla R, Casas A, Schönfeldt-Guerrero P, Pereira J, Tálamo C, et al. Proyecto Latinoamericano para generar valores de referencia de la prueba de difusión de monóxido de carbono. *Arch Bronconeumol* 2014;50 (Esp. Congreso\_2):78.
- Wagner PD, Araoz M, Boushel R, Calbet JA, Jessen B, Rådegran G, et al. Pulmonary gas exchange and acid-base state at 5,260 m in high-altitude Bolivians and acclimatized lowlanders. *J Appl Physiol* 2002;92(4):1393-1400.
- Wise RA, Teeter JG, Jensen RL, England RD, Schwartz PF, Giles DR, et al. Standardization of the single-breath diffusing capacity in a multicenter clinical trial. *Chest* 2007;132(4):1191-1197.
- Menezes AM, Vitoria CG, Perez-Padilla R. The Platino project: methodology of a multicenter prevalence survey of chronic obstructive pulmonary disease in major Latin American cities. *BMC Med Res Methodol* 2004;4:15.
- Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26(4):720-735.
- Koenker R. Quantile Regression. New York: Cambridge University Press; 2005.
- Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity): Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal Official Statement of the European Respiratory Society *Eur Respir J Suppl* 1993;16:41-52.
- Cotes JE, Dabbs JM, Elwood PC, Hall AM, McDonald A, Saunders MJ. Iron-deficiency anaemia: its effect on transfer factor for the lung (diffusing capacity) and ventilation and cardiac frequency during sub-maximal exercise. *Clin Sci* 1972;42(3):325-335.
- Marrades RM, Diaz O, Roca J, Campistol JM, Torregrosa JV, Barberà JA, et al. Adjustment of DLCO for hemoglobin concentration. *Am J Respir Crit Care Med* 1997;155(1):236-241.
- Cicero Sabido R, Becerril G, Ramírez E, Salinas F. [Levels of carboxyhemoglobin in smokers and non-smokers from 2 cities of the Mexican high plateaux]. *Rev Invest Clin* 1991;43(1):40-44.
- Stanojevic S, Stocks J, Bountziouka V, Aurora P, Kirkby J, Bourke S, et al. The impact of switching to the new global lung function initiative equations on spirometry results in the UK CF registry. *J Cyst Fibros* 2014;13(3):319-327.
- Hegewald MJ, Crapo RO. Socioeconomic status and lung function. *Chest* 2007;132(5):1608-1614.
- Menezes AM, Dumith SC, Perez-Padilla R, Noal RB, Wehrmeister FC, Martínez-Mesa J, et al. Socioeconomic trajectory from birth to adolescence and lung function: prospective birth cohort study. *BMC Public Health* 2011;11:596.