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Reference equations for DLNO and DLCO in Mexican Hispanics: influence of altitude and race

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ABSTRACT

Objectives This study aimed to evaluate pulmonary diffusing capacity for nitric oxide (DLNO) and pulmonary diffusing capacity for carbon monoxide (DLCO) in Mexican Hispanics born and raised at 2240 m altitude (midlanders) compared with those born and raised at sea level (lowlanders). It also aimed to assess the effectiveness of race-specific reference equations for pulmonary diffusing capacity (white people vs Mexican Hispanics) in minimising root mean square errors (RMSE) compared with race-neutral equations.

Methods DLNO, DLCO, alveolar volume (VA) and gas transfer coefficients (KNO and KCO) were measured in 392 Mexican Hispanics (5 to 78 years) and compared with 1056 white subjects (5 to 95 years). Reference equations were developed using segmented linear regression (DLNO, DLCO and VA) and multiple linear regression (KNO and KCO) and validated with Least Absolute Shrinkage and Selection Operator. RMSE comparisons between race-specific and race-neutral models were conducted using repeated k-fold cross-validation and random forests.

Results Midlanders exhibited higher DLCO (mean difference: +4 mL/min/mm Hg), DLNO (mean difference: +7 mL/min/mm Hg) and VA (mean difference: +0.17 L) compared with lowlanders. The Bayesian information criterion favoured race-specific models and excluding race as a covariate increased RMSE by 61% (DLNO), 18% (DLCO) and 4% (KNO). RMSE values for VA and KCO were comparable between race-specific and race-neutral models. For DLCO and DLNO, race-neutral equations resulted in 3% to 6% false positive rates (FPRs) in Mexican Hispanics and 20% to 49% false negative rates (FNRs) in white subjects compared with race-specific equations.

Conclusions Mexican Hispanics born and raised at 2240 m exhibit higher DLCO and DLNO compared with lowlanders. Including race as a covariate in reference equations lowers the RMSE for DLNO, DLCO and KNO and reduces FPR and FNR compared with race-neutral models. This study highlights the need for altitude-specific and race-specific reference equations to improve pulmonary function assessments across diverse populations.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ There is an ongoing discussion in the field of pulmonary medicine and public health about the variables to be included in predicting lung function. This debate extends beyond academic circles to practical implications in healthcare. For lung diffusing capacity, several concerns exist on the accuracy of reference equations when geographical and racial/ethnic factors are overlooked, potentially leading to misdiagnoses and inappropriate clinical interventions, thereby jeopardising patient health.

WHAT THIS STUDY ADDS

⇒ This study provides a significant contribution to the field by creating reference equations for DLCO and DLNO among Mexican Hispanics moderate-altitude residents (2240 m, midlanders), as well as sea level residents (lowlanders). The findings, which show that midlanders show higher DLCO, DLNO and alveolar volume compared with lowlanders, underscore the importance of altitude consideration in reference models for accurate lung function assessment. Furthermore, as in other pulmonary function tests, adding race/ethnicity as a covariate to reference equations lowers the root mean square error for DLNO, DLCO and KNO, along with a reduced false positive and false negative rate compared with race-neutral models.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings of this study open new avenues for future research. It highlights the importance of creating more robust reference equations, developed prospectively, that include altitude and different populations from various geographic locations, considering the type of device used in the measurements and well-standardised manoeuvres. This study may influence future research directions, clinical protocols and health policies, promoting more correct diagnoses and improved health outcomes across diverse populations.

INTRODUCTION

There is significant debate over including variables like altitude and race in lung function prediction. Some experts argue that race lacks biological justification and may perpetuate health disparities.[1] Currently, no

comprehensive reference equations exist for individuals from diverse geographic areas and ethnic backgrounds. Using race-specific equations can disadvantage underrepresented groups. The American Thoracic Society (ATS) recommends pulmonary function testing (PFT) laboratories adopt a race-neutral approach using the Global Lung Function Initiative (GLI) global reference equations, which pool data across all races and ethnicities.[2] While race-neutral equations are available for spirometry,[3] similar approaches for lung volumes and pulmonary diffusing capacity are expected soon.

Several studies show that lung function measurements are influenced by race/ethnicity.[4] For instance, race/ethnicity accounts for 15% of the variance in vital capacity (VC), with black people having ~15% lower VC than age, height and sex-matched white people.[5] Pulmonary diffusing capacity for carbon monoxide (DLCO) is also approximately 2 to 4 mL/min/mm Hg lower in black people compared with white people, even when adjusted for age, height, sex, vital capacity, and haemoglobin (Hb) concentration.[6 7] Using DLCO prediction equations for white people in the black population incorrectly overestimates the lower limit of normal (LLN) by ~12%,[8] mislabelling many healthy black individuals as having abnormal DLCO values.

The simultaneous measurement of lung diffusing capacity for nitric oxide (DLNO) and lung diffusing capacity for carbon monoxide (DLCO) using the NO–CO double diffusion technique[9] has recently gained popularity. The European Respiratory Society's (ERS) 2017 standards provide universal guidelines for this technique, likely increasing its use among pulmonologists. DLNO is recommended for routine PFT due to its technical advantages over CO[10] and its patient-friendliness compared with the multistep Roughton and Forster technique.[11] Given the racial disparities observed in DLNO, particularly among African Americans, it is crucial to assess if race-neutral reference equations for DLNO might be more effective than race-specific reference equations.

One of the most promising aspects of DLNO measurement is its relative insensitivity to inspired oxygen concentration. Unlike DLCO, where an ~1% decrease in inspired oxygen concentration correlates with an ~1.3% increase in DLCO,[12–14] the change in DLNO remains negligible.[15] However, it remains unclear whether DLNO is influenced in individuals who were born and raised at moderate altitudes since birth (2240 m, herewith called midlanders), where arterial oxygen pressure typically averages around 70 mm Hg.[16] Is there an adaptation to the alveolar-capillary membrane that enhances DLNO among individuals accustomed to such altitudes throughout their lives?

This study has two primary aims: first, to investigate differences in DLNO, DLCO, alveolar volume (VA) and the rate of change of NO or CO from alveolar gas (KNO, KCO) between Mexican Hispanics from Mexico City and those from sea level (lowlanders) and second to examine racial disparities in diffusing capacity between white people of Eastern European origin and Mexican Hispanics. By merging datasets of Eastern European descent[10] with Mexican Hispanics, we assessed racial/ethnic differences in pulmonary diffusing capacity, allowing for a comparison between race-specific and race-neutral approaches in developing reference equations. We hypothesised that race/ethnicity would independently predict DLCO, DLNO, VA, KCO and KNO, potentially interacting with factors like height, height^2, age or age^2. Additionally, we proposed that being born and raised at 2240 m altitude would induce adaptations to the alveolar-capillary membrane, enhancing DLNO, VA and KNO compared with individuals raised at sea level, after controlling for other covariates.

MATERIALS AND METHODS

Participants

Non-diseased Mexican Hispanics were recruited in Mexico City (2240 m) between September 2017 and June 2019 and in Boca Del Río, Veracruz (10 m) from September to October 2019. These cities have a poverty rate of about 33% (Mexico City) and 58% (Veracruz), in 2021.[17]

Data sources

Reference equations for DLNO in non-diseased white subjects from five previous studies were combined.[18–22] No additional IRB approval was needed, as the data were deidentified and previously published.[23]

Measurements

Participants completed spirometry following the 2005 ATS/ERS standards,[24] with DLCO and DLNO measured according to the 2017 ERS guidelines using a preferred 10 s breath-hold time.[9] For the Mexican Hispanic participants, measurements were taken with the Jaeger MasterScreen Pro PFT device, averaging a 10.9±0.4 s breath-hold time. In contrast, the white subjects' dataset used three different PFT devices, with shorter breath-hold times averaging 6.2±1.4 s.[23] The variability in PFT device models and brands can affect diffusing capacity,[23 25] making it crucial to consider. Repeatability criteria for DLCO and DLNO were set at <3 and <17 mL/min/mm Hg, respectively.[26]

Least Absolute Shrinkage and Selection Operator (LASSO) regression

The LASSO was used to pinpoint key predictors for DLCO, DLNO, VA, KCO and KNO, aiming to reduce overfitting. LASSO regression applies variable selection and regularisation to improve both the accuracy of predictions and the clarity of reference equations.

Segmented linear regression

Segmented linear regression was used to create reference equations with predictors identified by LASSO. The age

breakpoint was initially estimated visually and refined iteratively.[27] However, for KCO and KNO, traditional multiple linear regression provided a better fit and was used instead. The LLN was set at the fifth percentile, calculated as LLN=predicted value −1.645·residual SD. Visual representations of the predicted values and LLN were created using the US median height at a specific age (online supplemental table S1) and input into the models.

Model comparison

Models were compared using the Bayesian information criterion (BIC) for fit[28] and evaluated for multicollinearity, outliers and adherence to assumptions. The necessity of having 'altitude' and 'race' as covariates in the model was assessed using BIC and root mean square error (RMSE). Models with a lower BIC fit better than those with higher BICs.[29] The online supplemental provides further details on how model differences in BIC are interpreted.

Prediction accuracy

To assess prediction accuracy, a repeated 10-fold cross-validation procedure was used, repeated 100 times to reduce variability and provide a robust RMSE estimate. For the Mexican Hispanic dataset, 10 subsets of approximately 40 subjects were created. Each iteration involved training segmented regression models (DLNO, DLCO and VA) and multiple linear regression models (KCO and KNO) on 90% of the data, validating with the remaining 10%. This was repeated 100 times, ensuring each subset was validated once. The median, minimum, maximum and 95%CI of the RMSE were reported, along with the median and 95% CI of correlation coefficients between predicted and actual values.

For the combined dataset of 1456 subjects (1064 white Eastern European descendants, 392 Mexican Hispanics), the same cross-validation process was applied. The data was split into 10 subsets of ~146 subjects. Each model was trained on 90% of the data and validated on 10%, repeated 100 times. The median, minimum, maximum and 95% CI of the RMSE were reported, along with the median and 95% CI of the correlation coefficients between predicted and actual values.

Agreement

To assess the agreement between predicted and observed values, we used the concordance correlation coefficient (see online supplemental file). Additionally, the weighted *kappa* statistic was presented as an alternative measure of agreement,[30] comparing the LLN from race-specific models to the LLN from race-neutral models.

Variable importance

Random forests plots were used to determine the most important predictors in rank order according to variable importance. Each variable's contribution to model's predictive accuracy or the ability to reduce impurity was ranked ordered. The most important predictor was the variable that was most influential in making predictions.

Binary classification of the LLN

Binary classification compared the LLN in race-neutral models to race-specific models. True positives, true negatives, false positives and false negatives were obtained for the race-neutral models and compared with the race-specific equations. The Matthews correlation coefficient was calculated to provide an overall score for model classification.[31] Statistical significance was set at $p<0.05$. Additional methodological details are in the online supplemental file.

RESULTS

In the process of developing reference equations, about 4% of the pooled dataset was omitted. Initially, 15 Mexican Hispanics with a body mass index of 35 kg/m^2 or higher were excluded, followed by the exclusion of an additional 28 Mexican Hispanics and 20 white subjects who had studentised residuals of 3.00 or greater. The final pooled dataset consisted of 1456 participants, including 392 Mexican Hispanics aged 5 to 78 years and 1064 white people aged 5 to 95 years. Later, after having developed segmented and linear models solely from the Mexican Hispanic data, segmented and linear regression models were developed using the pooled data from Mexican Hispanics and white people. This move aimed to streamline equations for future applications. Online supplemental table S1 illustrates the anthropometric data that was used in the Mexican Hispanics subjects to illustrate graphically changes in diffusing capacity with age and height. It is worth noting that only the Mexican Hispanics dataset had age recorded to the first decimal place. Even after controlling for the false discovery rate,[32] there were differences between the Mexican Hispanics born and raised at altitude compared with the Mexican Hispanics born and raised at sea level (online supplemental table S2). The raw data from the Mexican Hispanic dataset are graphically displayed in the online supplement (online supplemental figure S1). The anthropometric characteristics of the white participants are presented elsewhere.[23]

Mexican Hispanic reference equations

Reference equations for the Mexican Hispanic dataset are presented in table 1 and their fitted z-scores are provided (online supplemental table S3). The fitted z-scores were evenly dispersed across the lifespan (online supplemental figure S2). Prediction accuracies for the Mexican Hispanic reference equations are found in online supplemental table S4. Prediction equations for the DLNO/DLCO ratio had suboptimal fit, with only 8% of variance explained. Percentiles were derived from raw data (online supplemental table S5). Online

Table 1 Segmented (piecewise) reference equations for DLCO, DLNO and VA in Mexican Hispanics and multiple linear regression equations for KCO and KNO in Mexican Hispanics

DLCO (n=392) (mL/min/mm Hg) (not adjusted for altitude prior to analysis), breakpoint=21.0 years old, AIC=2195. BIC=2231.	**R^2 Change**	**Estimate**	**SE**	**95% CI**	**Adjusted R^2**	**RSE**
$Intercept_1$ (for 5.0–21.0 years old)		–4.786	3.84	–12.32, 2.74	0.54	3.51_1
$Intercept_2$ (for 21.1–78.0 years old)		–6.326				4.23_2
Age^2_1 (for 5.0–21.0 years old)	5%	–0.006471	0.0051	–0.0164, 0.0034		
Age^2_2 (for 21.1–78.0 years old)	5%	–0.0029790	0.0008	–0.0044, –0.0015		
Height (cm)	25%	0.1471	0.0321	0.084, 0.21		
Age×$Height^2$	10%	0.000009163	0.0000	0.0000, 0.0000		
Altitude (m)	9%	0.001739	0.0003	0.0012, 0.0022		
Sex (1=male; 0=female)	6%	1.894	0.48	0.95, 2.84		
DLNO (n=392) (mL/min/mm Hg) Breakpoint=17.4 years old, AIC=2802. BIC=2834.	**R^2 Change**	**Estimate**	**SE**	**95% CI**	**Adjusted R^2**	**RSE**
$Intercept_1$ (for 5.0–17.4 years old)		–45.91	6.51	–56.67, –33.16	0.73	8.46_1
$Intercept_2$ (for 17.5–78.0 years old)		–39.87				8.69_2
Age^2_1 (for 5.0–17.4 years old)	1%	0.0181250	0.0178	–0.0167, 0.0530		
Age^2_2 (for 17.5–78.0 years old)	1%	–0.0017467	0.0004	–0.0026, –0.0009		
Height (cm)	56%	0.7295	0.0564	0.619, 0.84		
Sex (1=male; 0=female)	9%	7.4062	1.05	5.35, 9.46		
Altitude (m)	8%	0.002966	0.0006	0.0019, 0.0041		
VA (n=392) (L). Breakpoint=18.0 years old, AIC=513. BIC=545.	**R^2 Change**	**Estimate**	**SE**	**95% CI**	**Adjusted R^2**	**RSE**
$Intercept_1$ (for 5.0–18.0 years old)		–0.863	0.15	–1.15, –0.57	0.86	0.38_1
$Intercept_2$ (for 18.1–78.0 years old)		–0.220				0.51_2
Age^2_1 (for 5.0–18.0 years old)	5%	0.002057	0.0008	0.0005, 0.0037		
Age^2_2 (for 18.1–78.0 years old)	5%	0.0000721	0.000024	0.00003, 0.0001		
$Height^2$ (cm)	67%	0.0001875	0.0000	0.0002, 0.0002		
Sex (1=male; 0=female)	8%	0.5043	0.057	0.39, 0.62		
Altitude (m)	5%	0.00007543	0.0000	0.0000, 0.0002		
KCO (n=392) (mL/min/mm Hg/L) AIC=1026. BIC=1050.	**R^2 Change**	**Estimate**	**SE**	**95% CI**	**Adjusted R^2**	**RSE**
Intercept		12.43	0.76	10.94, 13.93	0.34	0.89
Height (cm)	16%	–0.0471	0.0051	–0.058, –0.037		
Age	7%	–0.07873	0.016	–0.11, –0.05		
Age×$Height^2$	8%	0.000002558	0.000	0.0000, 0.0000		
Altitude (m)	4%	0.0003688	0.0001	0.0003, 0.0005		
KNO (n=392) (mL/min/mm Hg/L) AIC=1615. BIC=1639.	**R^2 Change**	**Estimate**	**SE**	**95% CI**	**Adjusted R^2**	**RSE**
Intercept		26.35	0.873	24.63, 28.07	0.46	1.88
$Height^2$ (cm)	17%	–0.0003335	0.00004	–0.0004, –0.0003		
Age×Height	14%	0.001461	0.00048	0.0005, 0.0024		
Age	13%	–0.2869	0.075	–0.4344, –0.1394		
Altitude (m)	1%	0.0003982	0.00018	0.0002, 0.0006		

Breath-hold time ranged from 10.3 to 12.0 s. For a worked example, please see the online supplemental file. When adding up the R^2 change, add Age^2 once only due to its use as the breakpoint. The Jeager MasterScreen Pro lung function testing device was used for developing these models. If the Hyp'Air Compact device is used, then add 3.9 (95% CI 3.3 to 4.6) mL/min/mm Hg to the DLCO, 14.6 (95%CI 12.3 to 16.9) mL/min/mm Hg for DLNO, 0.44 (95% CI 0.35 to 0.53) L for VA, 0.31 (95% CI 0.19 to 0.43) mL/min/mm Hg/L for KCO and nothing for KNO.
For KCO and KNO, regular multiple linear regression was used, as the BIC was lower compared with a segmented model.
AIC, Akaike information criterion; BIC, Bayesian information criterion; DLCO, diffusing capacity of the lung for carbon monoxide; DLNO, diffusing capacity of the lung for nitric oxide; KCO, The rate of change of CO from alveolar gas, per unit pressure of CO; KNO, The rate of change of NO from alveolar gas, per unit pressure of NO; RSE, residual standard error; VA, alveolar volume.

supplemental file visually depicts various model assumptions for the Mexican Hispanic Reference equations (online supplemental figures S3–S7). The concordance correlation coefficient for DLCO, DLNO and VA ranged between 0.71 and 0.93 (online supplemental figures S8–S10), while for KCO and KNO it was 0.52 and 0.63, respectively (online supplemental figures S11 and S12).

Hb concentration had minimal influence on DLCO or DLNO in the pooled dataset, so no adjustment was made. The RMSE for DLCO was 3.67 mL/min/mm Hg when Hb was added as a covariate in the DLCO reference equation presented in online supplemental table S6, and 3.72 mL/min/mm Hg, when Hb was removed as a covariate, indicating a 1.4% difference in RMSE when Hb was added or excluded. The RMSE for DLNO was 13.3 mL/min/mm Hg when Hb was added as a covariate in the DLNO reference equation presented in online supplemental table S6, and 13.4 mL/min/mm Hg, when Hb was removed as a covariate, indicating a 0.7% difference in RMSE when Hb was added or excluded.

In general, habitual residence at 2240 m increased DLCO, DLNO and VA in Mexican Hispanics. DLCO increased by 3.9 mL/min/mm Hg with an upper bound of ~4.9 mL/min/mm Hg at 2240 m compared with being born and raised at sea level when all the other variables were controlled (table 1). Similarly, DLNO increased by 6.6 mL/min/mm Hg with an upper bound of ~9.2 mL/min/mm Hg at 2240 m compared with sea level when all the other variables were controlled (table 1). Altitude increased VA by 0.17 L (170 mL), with an upper bound of ~0.224 L (224 mL) in midlanders compared with lowlanders.

Furthermore, there were sex differences in diffusing capacity. When all other variables were controlled, being a Mexican Hispanic male increased DLCO, DLNO and VA by ~2 mL/min/mm Hg, ~7 mL/min/mm Hg and ~0.50 L compared with being a Mexican Hispanic female.

Race-specific and race-neutral reference equations

The race-specific and race-neutral equations for all 1456 subjects are found in online supplemental tables S6–S9. The number of subjects in the pooled dataset—divided among race, age group and sex—is presented in online supplemental figure S13. Race-specific models for DLNO, DLCO, VA, KCO and KNO exhibited significantly lower BIC values (more than 10 units) compared with race-neutral models (online supplemental table S10), indicating a greater than 99% likelihood that models that include race as a covariate in reference equations are more correct compared with those that omit race. However, based on the results of repeated 10-fold cross validation, the median RMSE for VA and KCO was not different between race-specific and race-neutral models (online supplemental table S11). Prediction equations for the DLNO/DLCO ratio had suboptimal fit in white subjects, with only 10% of variance explained. Thus, percentiles were derived from raw data (online supplemental table S12).

Random forest analyses reveal that the mean RMSE increases for DLCO, DLNO and KNO by 18% (3.71 vs 4.38 mL/min/mm Hg), 61% (13.4 vs 21.5 mL/min/mm Hg) and 4% (2.44 vs 2.53 mL/min/mm Hg/L), respectively, when racial variables and their interaction effects are excluded from the models (online supplemental figures S14–S16). Conversely, VA and KCO show similar RMSE values whether race variables and interactions are included or not (online supplemental figures S17 and S18). The predictor 'sex' (0=females, 1=male) significantly influences the increase in RMSE for DLCO, DLNO and VA when removed from these models (online supplemental figures S14, S15 and S17).

The RMSE differences between race-specific and race-neutral models, analysed through Random Forests, underscore the impact of excluding race and its interaction effects while maintaining other covariates constant. In contrast, when comparing the best covariates in race-specific models to those in race-neutral models, the RMSEs are more comparable. For example, the median RMSE difference in the best fit for race-specific versus best fit for race-neutral models for DLCO, DLNO and KNO is 0.10 mL/min/mm Hg, 1.7 mL/min/mm Hg and 0.2 mL/min/mm Hg, respectively (online supplemental table S11).

Nonetheless, the density plots illustrate that the distribution of RMSE values for the best fit race-specific equations for DLCO, DLNO and KNO is shifted left compared with those for race-neutral models, with correlation coefficients moving right (online supplemental figures S19–S21). This suggests a superior model fit and more consistent correlation performance in race-specific models over race-neutral models for these measurements. However, for VA and KCO, the density plots overlap, indicating similar RMSE and correlation results between race-specific and race-neutral models (online supplemental figures S22 and S23). This implies that for these measurements, the impact of race/ethnicity is less pronounced.

The type of PFT device also affected the outcome, as DLCO, DLNO and VA were increased by ~4 mL/min/mm Hg, ~15 mL/min/mm Hg and ~0.4 L, respectively, when the Hyp'Air Compact device was used compared with the other PFT devices (online supplemental table S6).

Race-specific equations for DLCO and DLNO were used to determine the LLN for approximately 1 to 6% of the pooled dataset, in contrast to the LLN derived from race-neutral equations presented in table 2. When these two sets of equations were compared, they exhibited false discovery rates ranging from 3% to 88% and false negative rates (FNRs) from 20% to 49% (table 2). The overall discordance rates for subjects classified as either below the LLN (labelled 1) or above (labelled 0) were 1.8%, 3.4%, 0.5%, 1% and 2% for DLCO, DLNO, VA, KCO and KNO, respectively. Additionally, the weighted

Table 2 Evidence that race-specific reference equations reduce the false negative and false discovery rates while improving precision, compared with race-neutral reference equations

	When using DLCO race-neutral compared with race-specific equations		When using DLNO race-neutral compared with race-specific equations	
Estimate	white people (n=1064)	Mexican Hispanics (n=392)	white people (n=1064)	Mexican Hispanics (n=392)
Prevalence of <LLN	4%	6%	5%	1%
MCC	0.88	0.67	0.71	0.28
AUC	0.90	0.87	0.76	0.85
Sensitivity (probability of detection)	0.80	0.76	0.51	0.75
Specificity (true negative rate)	1.00	0.97	1.00	0.94
False positive rate (probability of a false alarm)	0.00	0.03	0.00	0.06
False negative rate (miss rate)	0.20	0.24	0.49	0.25
False omission rate	0.01	0.02	0.02	0.00
False discovery rate	0.03	0.37	0.00	0.88
Positive predictive value (precision)	0.97	0.63	1.00	0.12
Negative predictive value	0.99	0.98	0.98	1.00
F_1 score (harmonic mean of precision and sensitivity)	0.88	0.69	0.68	0.20
Jaccard index (threat score)	0.79	0.53	0.51	0.11

Online supplemental tables S6 to S9 were used to generate these results.

AUC, area under the curve; DLCO, lung diffusing capacity for carbon monoxide; DLNO, lung diffusing capacity for nitric oxide; LLN, lower limit of normal; MCC, Matthews correlation coefficient.

kappa statistic, indicating agreement between the race-specific subjects below the LLN, and the race-neutral subjects below the LLN, was 0.79 (95% CI=0.71 to 0.87) for DLCO, 0.51 (0.39 to 0.63) for DLNO, 0.95 (0.90 to 0.99) for VA, 0.87 (0.81 to 0.94) for KCO and 0.81 (0.74 to 0.88) for KNO. This implies that there was 'strong' to 'almost perfect' agreement for identifying subjects below the LLN for VA and KCO, 'moderate-to-strong' agreement for identifying subjects below the LLN for DLCO and KNO and 'weak-to-moderate' agreement for identifying subjects below the LLN for DLNO.[30]

From the equations in table 1, predicted DLCO, DLNO and VA were plotted across all ages (figure 1, online supplemental figure S24 and S25). A negative exponential smoother was used to make the segmented regression lines more aesthetically pleasing. The US Department of Health median height and weight values at each age were used to calculate the predicted values (online supplemental table S1).[33] We standardised a breath-hold time of 10 s and the use of the Jaeger MasterScreen Pro PFT device (figures 1–3). The DLCO was compared against the GLI reference equations for DLCO,[34] van der Lee's prediction equation[20] and other established DLCO reference equations for Mexican Hispanic children residing in Mexico City (2240 m),[35] including adult Hispanics from Central and South America[36] (figure 2). Again, the Jaeger MasterScreen PFT device was used to standardise figure 2. The prediction equations from Central and South America were much different compared with the other prediction equations for DLCO[35 36] (figure 2). Prediction equations for the DLNO/DLCO ratio in white people had suboptimal fit. So the percentiles were calculated from the raw data (online supplemental table S12).

DISCUSSION

This study had two main objectives. First, we examined the physiological differences in DLNO, DLCO, VA, KNO and KCO between Mexican Hispanics from Mexico City (2240 m altitude) and those from sea level. Living at 2240 m resulted in increased DLCO (~4 mL/min/mm Hg), DLNO (~7 mL/min/mm Hg) and VA (~0.2 L) compared with sea level residents. Also, KCO and KNO values were 0.8 to 0.9 mL/min/mm Hg/L higher at 2240 m compared with sea level.

Second, we identified racial differences in diffusing capacity between white people of Eastern European descent and Mexican Hispanics. Removing racial factors from models increased RMSE by 18%, 61% and 4% for DLCO, DLNO and KNO, respectively. Race-specific models showed stronger correlations, with lower RMSE and higher correlation coefficients, than race-neutral models.

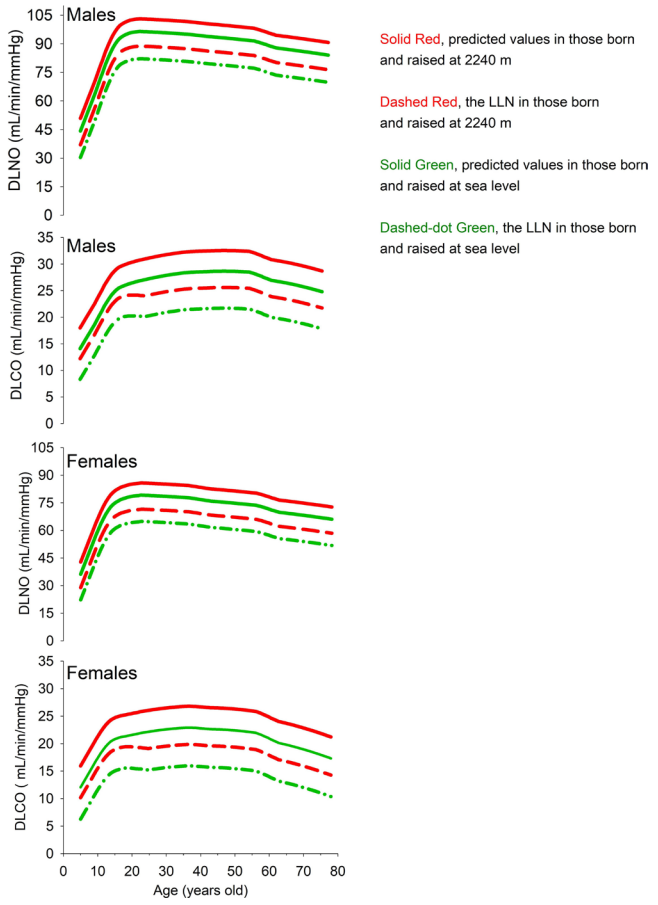

Figure 1 Predicted DLNO and DLCO and the LLN versus age in Mexican Hispanic males and Mexican Hispanic females. Mexican Hispanic highlanders were those who were born and raised in Mexico City (2240 m). Mexican Hispanic lowlanders were those who were born and raised at sea level (1.4 m). The various fitted curves are based on the median height from 5 to 78 years of age in the US population.[33] Breath-hold times were standardised to 10 s. Other covariates controlled in the model were: the Jaeger MasterScreen Pro PFT device. The LLN was the z-score of –1.645, 5th percentile. A negative exponential function was used to smooth the data. DLCO, diffusing capacity of the lung for carbon monoxide; DLNO, diffusing capacity of the lung for nitric oxide; LLN, lower limit of normal at the 5th percentile.

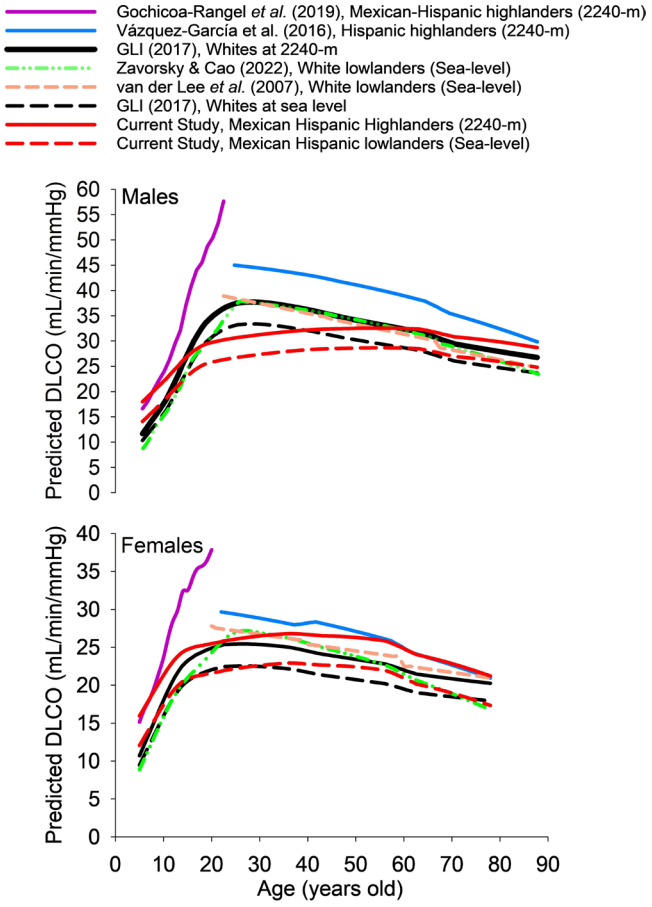

Figure 2 Predicted DLCO in Mexican Hispanics, other Hispanics and white people from other articles.[20 23 34–36] Notice how the predicted DLCO values from Central and South America[35 36] are much higher in males and for males and females≤20 years of age than the current model or any other model presented. DLCO, diffusing capacity of the lung for carbon monoxide.

After adjusting for other variables, Mexican Hispanics generally had lower DLCO, DLNO and VA than white people. The DLCO difference between white people and Mexican Hispanics remained consistent across the lifespan. For example, at 165 cm height, white people had a DLCO ~8 mL/min/mm Hg higher at both 25 and 78 years. At 185 cm height, the difference was ~10 mL/min/mm Hg.

For DLNO, the gap between white people and Mexican Hispanics decreased with age but widened with height. At 165 cm, the DLNO advantage for white people decreased from ~40 mL/min/mm Hg at 25 years to 11 mL/min/mm Hg at 78 years. At 185 cm, it decreased from ~60 mL/min/mm Hg at 25 years to 31 mL/min/mm Hg at 78 years. Thus, racial differences in DLNO widen with height but lessen with age.

However, differences in VA remain consistent regardless of height. At ages 25, white people have a higher VA by approximately 0.33 L compared with Mexican Hispanics, regardless of whether they are 165 cm or 185 cm tall. However, by age 70, these racial differences in VA disappear (regardless of height).

The brand of lung function testing equipment is essential in DLNO, VA and DLCO prediction equations. Previous work has demonstrated that the Hyp'Air Compact measures DLNO and VA that is ~18 to 24 mL/min/mm Hg and ~0.3 to 0.5 L higher than the Jaeger MasterScreen device.[23 25] The findings from this study showed an ~15 mL/min/mm Hg and ~0.4 L higher value in DLNO and VA, respectively, when Hyp'Air Compact device was used. The data of Mexican Hispanics that were added to the pooled data of Zavorsky and Cao[23] confirm these findings, but unlike those other two studies,[23 25] the Hyp'Air Compact device also increased DLCO independently by ~4 mL/min/mm Hg. Thus, pulmonary

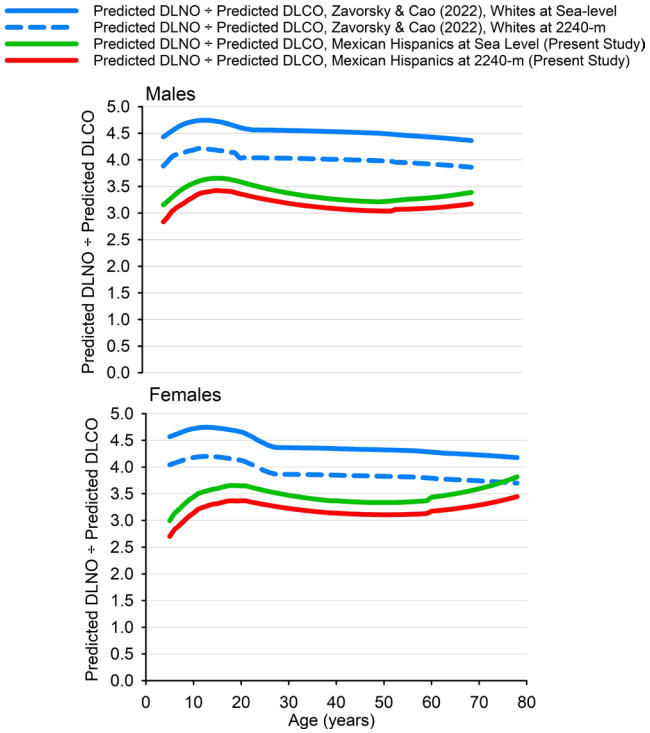

Figure 3 The DLNO/DLCO ratio cannot be predicted satisfactorily with regression models. However, the predicted DLNO value and the predicted DLCO value based on the median height and weight for males 5–95 years of age in the US population can be determined,[33] and from that, the Predicted DLNO ÷ the Predicted DLCO was plotted against age. A breath-hold time of 10 s, and the Jaeger MasterScreen Pro equipment was standardised. Regardless of altitude, white people has a predicted ratio larger in Mexican Hispanics. As well, regardless of race, habitual altitude residence at 2240 m lowers the predicted ratio compared with sea-level. DLCO, diffusing capacity of the lung for carbon monoxide; DLNO, diffusing capacity of the lung for nitric oxide.

function laboratories must correct for the testing device by using updated regression models for the complete lifespan[23] and race (when available).

While environmental and socioeconomic factors have been suggested as potential reasons for racial disparities in lung function variations,[37] some argue that socioeconomic factors have only a minimal impact, accounting for a maximum of 8% of these differences.[38] Our study, focusing on Mexican Hispanics residing in impoverished regions,[17] aimed to emphasise inherent racial (biological) characteristics rather than environmental or socioeconomic factors. Nevertheless, in our analysis of race-specific models across the combined dataset (DLCO, DLNO and KNO), we observed race, race×age and/or race×age^2 and/or race×height^2 interactions. These interactions suggest that as individuals age or as an individual's height increases, there is an increase in DLCO, DLNO and KNO by 23% to 30% in white subjects from 5 to approximately 30 years old that is due to race, and its interactions with age, age^2, height or height^2. This implies that socioeconomic and/or environmental influences may contribute to some of the observed racial/ethnic (biological) differences between white people and Mexican Hispanics. As well, lifestyle choices, occupational factors and other non-biological determinants of lung heath can offer a more holistic approach to understanding lung function.

After correcting for altitude and Hb concentration, there was still a difference in DLCO between Mexican Hispanic midlanders and lowlanders. Midlanders had a higher average DLCO by 1.8 (95% CI: 0.9 to 2.7 mL/min/mm Hg), about a 10% increase even after adjustments. Animal studies show pulmonary diffusing capacity can increase by ~20% when guinea pigs are exposed to high altitudes (3500 to 3800 m) for 3 to 24 weeks.[39 40] This adaptation is due to increased lung volume and alveolar surface area.[41] The ~7 mL/min/mm Hg (~5%) increase in DLNO in Mexican Hispanic midlanders supports this. With ≥85% shared variance between DLNO and DLCO in healthy subjects,[9 23] it is logical that both would increase in midlanders.

Reference equations specific to Mexican Hispanics residing at moderate elevations or sea level were developed (table 1). We employed repeated *k*-fold cross-validation to evaluate the accuracy of model predictions (online supplemental table S4), ensuring the generalisability of results for this demographic. The relevance of these models is underscored by the demographic significance of cities such as Mexico City. Neglecting racial/ethnic differences, whether biological or related to socioeconomic status, could similarly impact pulmonary function tests such as spirometry, lung volumes or maximal inspiratory/expiratory pressures. This oversight could lead to erroneous diagnoses, with significant medical and psychological consequences for patients.

To examine racial differences, we pooled data from white subjects from Zavorsky and Cao[23] with our current Mexican Hispanic dataset. If race/ethnicity were not included as a covariate, the false positive rate (FPR) for gas exchange impairment (DLNO below the 5th percentile) would be 6% for Mexican Hispanics under a race-neutral approach. The false discovery rate would be 88% among Mexican Hispanics using race-neutral reference equations instead of race-adjusted reference equations.

The study has a significant imbalance in sample sizes, with more Mexican Hispanic highlanders (319) than lowlanders (73), affecting generalisability. Different PFT devices introduced variability, requiring adjustments in the equations. The study highlighted issues with race-neutral equations, leading to higher FPRs among Mexican Hispanics and FNRs among white people compared with race-specific equations. While focusing on biological differences, the study did not address socioeconomic and environmental factors, which could also impact lung function disparities. Significant interactions between race, age and height suggest other unmeasured factors might influence results.

Incorrectly reporting DLNO as below the LLN can lead to unnecessary follow-up evaluations, additional diagnostic tests, increased time burden and financial strain, especially for those without comprehensive insurance. False positives divert medical attention from those who need it, delaying diagnosis and treatment of actual conditions and causing significant psychological distress.

DLNO is crucial for pulmonary function tests, enhancing cardiopulmonary disease classification when combined with DLCO.[42] Its technical superiority[10] underscores the need for widespread adoption of the NO–CO double diffusion method as outlined in the ERS technical standards,[9] to improve diagnostic accuracy and patient care globally.

CONCLUSION

Residing at 2240 metres from birth to adulthood significantly increases DLCO and DLNO (up to 5 and 9 mL/min/mm Hg, respectively) in Mexican Hispanics compared with those at sea level, with VA increasing by up to 0.45 L (450 mL). Race-specific equations for DLNO, DLCO and KNO show superior accuracy compared with race-neutral models. Highlanders exhibit a lower DLNO/DLCO ratio than lowlanders. We advocate for developing robust reference equations incorporating altitude and diverse racial populations, accounting for measurement device differences to enhance accuracy and reduce diagnostic errors.

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Contributors LG-R is responsible for the conception of the study, acquisition of the data and interpretation of the data and revising the manuscript for important intellectual content and approving the final manuscript version. GSZ is responsible for statistical analysis of the work, interpretation of the data, figure and table generation, writing the initial manuscript draft and revising the manuscript for important intellectual content and approving the final manuscript version. DM-B is responsible for statistical analysis of the work, interpretation of the data and revising the manuscript for important intellectual content and approving the final manuscript version. AD-L-S-M, AR-G, MHV, IL-T, CG-V and LT-B were responsible for data acquisition, revising the manuscript for important intellectual content and approving the final manuscript version. GSZ is responsible for the overall content as guarantor.

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Competing interests GSZ is a Global Lung Function Initiative (GLI) Network member. The GLI Network has published reference equations for spirometry, DLCO and static lung volumes using GAMLSS models. GSZ is the current cochair of the European Respiratory Society Task Force on interpreting pulmonary diffusing capacity for nitric oxide. LG-R is an executive member of the GLI Network and has coauthored the 2023 publication involving race-neutral spirometry equations developed using GAMLSS models.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by For the Mexican Hispanic dataset: informed consent was obtained from all participants (including parental consent for individuals < 18 years of age), and the study was approved by the science and bioethics committee of the Instituto Nacional de Enfermedades Respiratorias (C65-17) for Mexican Hispanic participants. For the white subject dataset: no additional IRB approval was needed, as the data were deidentified and previously published. Thus, this dats is not considered human subjects research. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement The data from Mexican Hispanics and/or the white subjects (Zavorsky & Cao (2022)) that were used in this study are available on reasonable request from GSZ. It is required that should any or part of the deidentified dataset be shared, then any abstract, conference proceedings or article that will be published related to this data here will have GSZ and LG-R as coauthors.

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