North-African Reference Values of Alveolar Membrane Diffusion Capacity and Pulmonary Capillary Blood Volume

Sonia Rouatbi^{a, b} Helmi Ben Saad^{a, b} Imed Latiri^b Zouhair Tabka^{a, b}
Hervé Guénard^c

^aDepartment of Physiology and Functional Explorations, Farhat Hached Hospital, and ^bDepartment of Physiology, Faculty of Medicine Ibn El Jazzar, Sousse, Tunisia; ^cLaboratory of Physiology, Faculty of Medicine Victor Pachon, Bordeaux, France

Key Words

Carbon monoxide • Diffusing capacity • Gas exchange • Nitric oxide • North-African reference values • Predicted pulmonary function • Respiratory function • Transfer factor

Abstract

Background: In North-African adults, location-specific reference values for membrane diffusion capacity (D_m) and pulmonary capillary blood volume (V_c) were needed. **Objectives:** To verify the applicability of previously published reference equations for D_m and V_c in North-African healthy adults (age >18 years) and to determine specific reference equations for North Africa. **Methods:** The study was designed as a prospective cross-sectional study. Anthropometric data (age, height, weight and body mass index) and D_m and V_c were assessed in 85 healthy Tunisian adults. Univariate and multiple linear regression analyses were used to determine reference equations and to calculate the lower limit of the normal range (LLN). **Results:** The mean ages $\pm$ SD (minimum – maximum) for male and female adults were 53 ± 21 (21–85) and 42 ± 16 (18–72) years, respectively. Previously published reference equations did not reliably predict measured D_m and V_c . The reference equation ($r^2 = 47\%$) for D_m was $-36.16 + 45.37 \times \text{height} - 0.34 \times \text{age} + 0.39 \times$

$\text{weight} + 7.41 \times \text{gender}$ (0 = female and 1 = male). To calculate the D_m LLN subtract 24.36 from the reference value. The reference equation ($r^2 = 30\%$) for female V_c was $94.70 - 0.57 \times \text{age}$, and the reference equation ($r^2 = 52\%$) for male V_c was $0.82 - 0.48 \times \text{age} + 52.47 \times \text{height} + 0.16 \times \text{weight}$. To calculate the V_c LLN subtract 28.52 and 26.54 from these reference values for females and males, respectively. **Conclusion:** These V_c and D_m reference equations supplement the international World Bank of reference equations.

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Introduction

The measurement of gas transfer in the lung is one of the few tests aimed at investigating deep lung function. According to the classic model of pulmonary diffusion proposed by Roughton and Forster [1] lung diffusing capacity (D_l) for carbon monoxide ($D_l\text{CO}$) is composed of two resistances arranged in series:

$$\frac{1}{D_l\text{CO}} = \frac{1}{D_m\text{CO}} + \frac{1}{\theta_{\text{CO}} \cdot V_c},$$

where pulmonary membrane diffusing capacity (D_m) for CO ($D_m\text{CO}$) is the CO conductance across the alveolar-

capillary tissue membrane and plasma barrier; θCO is the rate of CO uptake by whole blood and combination with hemoglobin (Hb) measured in vitro, and V_c is the pulmonary capillary blood volume. In normal subjects, resistance of the membrane ($1/D_m\text{CO}$) and erythrocytes [$1/(\theta\text{CO} \cdot V_c)$] contributes almost equally to the overall diffusive resistance across the lung. In order to estimate D_m and V_c from CO uptake, $D_m\text{CO}$ must be measured at two alveolar oxygen tension ($P_{A\text{O}_2}$) levels, with the assumption that $P_{A\text{O}_2}$ does not alter $D_m\text{CO}$ [1].

D_l estimated using nitric oxide as the tracer gas ($D_l\text{NO}$) has been proposed as a direct measure of the conductance of the alveolar membrane ($D_m\text{NO}$) [2]. Because the reaction rate of NO binding to Hb is some 280 times faster than that of CO [3], the rate of NO uptake by blood (θNO) is extremely large and $1/(\theta\text{CO} \cdot V_c)$ becomes negligible, i.e. $D_l\text{NO}$ is approximately equal to $D_m\text{NO}$ [2, 3].

A previous study by Rouatbi et al. [4] showed the following: gender had no effect on D_m and V_c ; D_m and V_c declined significantly with age, D_m was significantly correlated with height in the adult group and with weight in the elderly group, and V_c was significantly correlated with height in the adult group. Therefore, the authors concluded that the knowledge of these physiological changes increases the understanding of pathological changes by establishing specific reference equations [4]. Reference values are important to diagnose the effect of various pathologies on lung function, e.g. sarcoidosis, heart failure and transplantation, and subclinical lung damage in bleomycin-treated patients [5–7]. In order to put lung function measurements into clinical use, a comparison between the measured values and the values predicted from reference equations is needed to determine the severity of the disease [8]. Comparisons are based on reference ranges in non-Tunisian adults, since V_c and D_m reference equations in Tunisian or North-African adults have not been published to date [8–11]. However, this use is questionable since no verification of their reliability has been made in this population whereas some specificity has been noted in several points, e.g. ethnicity, anthropometric data, lifestyle and socioeconomic levels [12, 13].

In North-African adults, the need for specific local reference values has been shown at least for spirometric and walking test data [12–14]. Therefore, the applicability and reliability of previously published V_c and D_m reference equations in adults have to be assessed in this population, in order to exclude erroneous clinical interpretation of V_c and D_m values based on non-North-African reference equations. Also, and as it is recommended by the American Thoracic (ATS) and European Respiratory Society

(ERS), ideally, pulmonary reference values should be derived from a population similar to the individual subject using the same kind of instrument and testing procedure [15].

Therefore, as the ATS/ERS [15] encourage the continuing interest of researchers to derive and apply reference equations worldwide, the aims of the present study were to verify the applicability and reliability of the previously published reference equations of D_m and V_c in a population of healthy North-African adults aged >18 years [9, 10], and, if needed, to establish new reference equations and their corresponding residual standard deviations (RSDs).

Methods

We performed this prospective study over a 7-month period (from January to June 2009) in the Department of Physiology and Functional Exploration at the Farhat Hached Hospital (Sousse, Tunisia, altitude <100 m).

Study Design

Subjects were recruited among hospital workers ($n = 80$) and the parents of medical school students ($n = 30$). Data from each volunteer adult included: gender, age, height, weight, smoking history, medication use, medical history, physical examination and lung function testing. All subjects received a copy of their exploration results and when an unsuspected dysfunction was discovered, they were sent to a specialist. The study protocol was approved by the local ethics committee, and written informed consent was obtained from each study participant prior to the study.

Sample Size

A predictive equation of the sample size was calculated [16]: $n = (Z^2 \times p \times q / \Delta^2)$, where n was the number of subjects required, Z was the 95% confidence level, q was equal to $1 - p$, Δ was the precision ($= 8\%$), and p was the estimation of the D_m decline induced by age. According to Butler and Kleinerman [17], capillary density decreases 15% ($p = 0.15$) between 20 and 57 years of age. The sample size was thus 77 subjects. Therefore, to determine reference equations, we included an initial group of 110 subjects.

Subjects

Healthy volunteers aged >18 years were included. All subjects were non-smoking, had sedentary jobs without physical strain and lived in an area without heavy traffic and/or air pollution. No athletes were included. Upon questioning and physical examination, all volunteers were found to be without complaints or diseases. Marked and extreme obesity or underweight were criteria for exclusion. Pregnancy was also an exclusion criterion because of possible changes in pulmonary hemodynamics. No special attention was paid to the menstrual cycle.

Medical Questionnaire

A medical questionnaire recommended for epidemiological research was used to assess subject characteristics [18].

Physical Examination

Age was verified by the identity card. Height (± 0.01 m) was measured with a height gauge with participants standing back straight, heels joined, but without shoes. Body weight (± 1 kg) was measured without heavy clothes and the body mass index (BMI) was calculated ($\text{BMI} = \text{weight}/\text{height}^2$, kg/m^2). The following definitions were adopted [19]: underweight ($\text{BMI} < 18.5$), normal weight ($18.5 \leq \text{BMI} \leq 24.9$), overweight ($25.0 \leq \text{BMI} \leq 29.9$), moderate obesity ($30.0 \leq \text{BMI} \leq 34.9$); marked obesity ($35.0 \leq \text{BMI} \leq 39.9$) and severe obesity ($\text{BMI} \geq 40$).

Plethysmographic Measurements

Plethysmographic measurements (Zan 500 plethysmographie; Zan, Germany) were performed according to the ATS/ERS recommendations [20, 21]. All volumes (residual volume, tidal volume and thoracic gas volume) and capacities (total lung capacity and slow vital capacity) were measured. The results of forced expiratory volume in 1 s, forced vital capacity and all lung data were compared with local age- and gender-matched reference values [12, 13].

Diffusion Capacities, D_m and V_c Measurements

Single-Breath Apparatus and Technique

Volume and gas calibrations of the Hyp'Air lung diffusion system (Medisoft; Dinant, Belgium) were performed prior to each testing session. Roughton and Forster [1] demonstrated that CO diffusion measured by the $D_L\text{CO}$ method reflected both D_m and reaction with V_c [1, 22]. Consequently, the double-transfer technique enables to determine with precision if the defect is in the alveolar membrane or in the pulmonary capillary blood.

Pulmonary Membrane Diffusing Capacity

D_m and V_c were measured simultaneously during a single-breath maneuver using the automated apparatus. Subjects were in the sitting position with a nose clip. A mixture containing 0.28% CO, 14% helium (He) and 21% O₂ balanced with N₂ was mixed with NO/N₂ (450 ppm NO/N₂; Air Liquide Santé, Tunisia). The final concentration of NO in the inspired bag was 40 ppm and that of O₂ was 19.1%. The apparatus was calibrated for gas fractions using automated procedures. The linearity of the analyzers was factory checked. Linearity on site could be checked by dilution procedures, i.e. dilution of the NO mixture with the CO/He/O₂ mixture to check NO analyzer linearity, or dilution of the CO/He/O₂ mixture with air to check CO analyzer linearity [10, 22]. The screen pneumotachograph was calibrated daily with a 2-liter syringe. The subject breathed normally and then a deep expiration was followed by a rapid deep inspiration of the mixture. A breath hold of 4 s was then requested followed by a rapid expiration. The first 0.8 liters of gas expired were rejected as the further 0.6 liters were sampled in a bag which was automatically analyzed for NO, CO and He. The delay to analyze the sample of expired gas was constant (35 s). The alveolar volume (VA) was determined during apnea and calculated using the He dilution technique.

According to the recent international guidelines, subjects performed $D_L\text{CO}$ measurements with a minimum of 4.5 min between tests. Five sequential diffusion capacity tests were performed. The average value of two trials with difference in diffusing capacity being within 10% of each other is considered acceptable [23].

Calculation of Diffusion Capacities, D_m and V_c

Values of D_m and V_c were calculated according to the model of Roughton and Forster [1]. '1/ θNO ' was assumed negligible [24], therefore $D_L\text{NO} = D_m\text{NO}$. $D_m\text{CO}$ was calculated as $D_m\text{NO}/a$ where $a = 1.97$ following Graham's law [1, 24]. To calculate V_c , the θCO reference values recommended by Forster [25, 26] were chosen. Mean capillary O₂ pressure was estimated at 100 mm Hg taking the slightly lower than normal O₂ fraction in the inspired mixture into account.

Data Analysis

Preliminary descriptive analyses included frequencies for categorical variables (gender) and means and SD for continuous ones (age, weight, height, BMI, VA, D_m and V_c).

Univariate Analysis

The dependent variables (D_m and V_c) were normally distributed according to the Kolmogorov-Smirnov normality test [16].

t tests were used to evaluate associations between D_m or V_c and gender, and Pearson product moment correlation coefficients for associations between D_m or V_c and continuous measures. Linearity of associations between D_m or V_c and continuous measures was checked graphically by plotting each regressor against D_m or V_c .

Comparison with Published Regression Equations

Comparison was made by two ways: (1) Our D_m or V_c values were compared with those predicted from published reference equations [9, 10] using scatter plots and paired t tests. (2) As proposed by Bland and Altman [27], comparisons between measured and predicted D_m and V_c [9, 10] were performed by means of the limits of agreement (LOA), where individual differences (measured minus predicted D_m or V_c) were plotted against the corresponding mean value. From these data, LOA were then calculated [27]. The reference equation that provides the LOA closest to zero will be the most appropriate for our population.

V_c and D_m Reference Equations

Reference equations were determined using anthropometric data [age, height, weight, BMI and gender (0 = female and 1 = male)] as predictors of V_c or D_m in a stepwise linear regression model. The applicability of the reference equation is evaluated by the correlation (r) and determination (r^2) coefficients and by the standard error (SE).

The 95% confidence interval (CI) was calculated as follows [16]: $95\% \text{ CI} = 1.64 \times \text{RSD}$. A measured V_c or D_m lower than the LLN (= predicted value - $1.64 \times \text{RSD}$) is considered as abnormal. Analyses were carried out using Statistica (Statistica Kernel version 6; StatSoft, France). Significance was set at the 0.05 level.

Results

An initial sample of 170 volunteer adults (80 women) was examined; 60 subjects did not meet inclusion criteria.

Anthropometric and Pulmonary Data

Figure 1 shows the age distribution of the 110 subjects (58 women). Twenty-seven subjects (25%) were >60 years.

Fig. 1. Age distribution in the study cohort (n = 110).

Table 1. Anthropometric and pulmonary data

	Female (n = 58)	Male (n = 52)	Total sample (n = 110)
Age, years	42 ± 16	53 ± 21*	47 ± 19
Height, m	1.60 ± 0.09	1.68 ± 0.09*	1.64 ± 0.10
Weight, kg	64 ± 9	69 ± 13	67 ± 12
BMI	25 ± 5	24 ± 4	25 ± 5
VA, liters	4.80 ± 0.86	5.76 ± 1.28*	5.25 ± 1.18
D _m , ml/min/mm Hg	46.75 ± 11.34	56.37 ± 20.27*	51.30 ± 16.82
V _c , ml	70.44 ± 17.22	74.99 ± 19.04	72.59 ± 18.16

Data are means ± SD. * p < 0.05 (male vs. female).

Six (4 men), 51 (25 men), 34 (15 men) and 19 (8 men) subjects were underweight, of normal weight, overweight and moderate obesity.

All study participants were healthy. The hospital workers represented a major part (73%). Their medical records were consulted and their recent Hb values were noted. All these workers, known to be free of anemia, had normal Hb values (>12 g/dl for males and >13 g/dl for females) according to their medical records. According to the medical questionnaire, remaining subjects (27%) were not known to have anemia.

Plethysmographic data expressed as percentage of predicted values were normal.

In comparison with women, men were significantly older and taller, and had a higher weight, a greater VA and D_m (table 1). Substantial variability in the D_m and V_c was present in these healthy subjects, with a range of 40.30–118.80 ml for V_c and 20.90–97.00 ml/min/mm Hg for D_m.

D_m and V_c Univariate Analysis

Gender affected significantly D_m but not V_c (table 1).

Table 2 exposes the univariate correlation coefficients between the subjects' data and D_m or V_c. In brief, in the total sample, V_c was significantly correlated with age, height, BMI and VA, and D_m was significantly correlated with age, height, weight and VA.

Comparisons with Published Reference Equations

Figure 2a, b shows a comparison of measured D_m values and those predicted from reference equations from the Netherlands [9] and France [10], respectively. Figure 3 illustrates Bland and Altman [26] comparisons between measured D_m values and those predicted from reference equations from the Netherlands [9] and France [10], respectively. As can be deduced from figure 2a, b, there was a systematic bias between the measured D_m values and those predicted from the published reference equations

Fig. 2. Comparison of measured ($n = 110$) and predicted D_m determined from Dutch [9] (a), French [10] (b) and North-African (c) reference equations with the line of identity. r = Correlation coefficient; regression equation (y = measured value; x = predicted value); ----- = linear regression line.

Table 2. Univariate correlation coefficients between subject data and V_c or D_m

	Female ($n = 58$)		Male ($n = 52$)		Total sample ($n = 110$)	
	D_m	V_c	D_m	V_c	D_m	V_c
Age	-0.45*	-0.55*	-0.57*	-0.66*	-0.40*	-0.55*
Height	0.61*	0.36*	0.55*	0.57*	0.60*	0.47*
Weight	0.12	-0.07	0.36*	0.21	0.33*	0.12
BMI	-0.27*	-0.28*	0.05	-0.11	-0.10	-0.21*
VA	0.72*	0.41*	0.72*	0.63*	0.74*	0.54*

* $p < 0.05$.

[9, 10]. Indeed, measured D_m (mean $\pm$ SD) was significantly overestimated by 19.60 ± 13.74 ($p < 0.05$; fig. 3a) and 88.46 ± 32.32 ml/min/mm Hg ($p < 0.05$; fig. 3b) compared with the reference equations from France [10] and the Netherlands [9], respectively.

In figure 4a, b, measured V_c values were compared with those predicted from reference equations from the Netherlands [9] and France [10], respectively. Figure 5 depicts Bland and Altman [27] comparisons between mea-

sured V_c values and those predicted from the reference equations from the Netherlands [9] and France [10], respectively. As can be deduced from figure 4a, b, there was a difference between measured V_c values and those predicted from the published reference equations [9, 10]. Indeed, measured V_c (mean $\pm$ SD) was significantly over- and underestimated by 12.51 ± 16.75 ml ($p < 0.05$; fig. 5b) and 2.91 ± 15.91 ml (fig. 5a) by French [10] and Dutch [9] reference equations, respectively.

Fig. 3. Bland and Altman plot of measured and predicted D_m determined from the Dutch [9] (a) and French [10] (b) reference equations. r = Correlation coefficient; regression equation [y = (measured – predicted); x = (measured + predicted)/2]; ----- = linear regression line; = mean $\pm$ 1.96 SD.

Fig. 4. Comparison of measured ($n = 110$) and predicted V_c determined from Dutch [9] (a), French [11] (b) and North-African (c) reference equations with the line of identity. r = Correlation coefficient; regression equation (y = measured value; x = predicted value); ----- = linear regression line.

V_c and D_m Reference Equations

Due to the inadequacy of the published equations and, for practical reasons, daily interpretation, a reference equation should include only data which can be easily measured and are significantly associated with D_m and V_c. Therefore, we established equations with four common anthropometric parameters: gender, age, height and weight [15] (table 3). With respect to women, men and the total sample, anthropometric data appeared to be significant independent predictors.

D_m Reference Equation

The total sample D_m single model appeared to explain 47.4% of its variability. We thus used the following model as the D_m reference equation for our population (table 3):

$$D_m (\text{ml/min/mm Hg}) = -36.16 + 45.37 \times \text{height (m)} - 0.34 \times \text{age (years)} + 0.39 \times \text{weight (kg)} + 7.41 \times \text{gender (0 = female and 1 = male)}.$$

To calculate LLN, subtract 24.36 from the reference value. Compared with the published reference equations [9, 10] (fig. 3), the measured D_m values corresponded to 100 ± 24% of the predicted D_m (range: 50–170%; fig. 2c).

V_c Reference Equations

Due to the large differences between cumulative r² of male, female and total sample V_c reference equations (table 3), we had established two reference equations for females and males that explain 29.8 and 52.4% of the variability in V_c, respectively (table 3). Thus, we used the following reference equations:

$$V_c (\text{female}) (\text{ml}) = 94.70 - 0.57 \times \text{age (years)}.$$

To calculate LLN, subtract 28.52 from the reference value (see Appendix for examples).

$$V_c (\text{male}) (\text{ml}) = 0.82 - 0.48 \times \text{age (years)} + 52.47 \times \text{height (m)} + 0.16 \times \text{weight (kg)}.$$

To calculate LLN, subtract 26.54 from the reference value (see Appendix for examples).

Compared with the published reference equations [9, 10] (fig. 5), the measured V_c corresponded to 100 ± 20% of the predicted V_c (range: 65–165%; fig. 4c).

Discussion

In a large cohort of healthy North-African adults, V_c and D_m were prospectively measured by a double-transfer NO/CO method. The available published reference equa-

Table 3. Simplified reference equations (stepwise linear regression model)

Independent variables	B	Cumulative r ²	SE	RSD
<i>D_m</i>				
Female (n = 58)				
Constant	-104.88		32.28	9.04
Height	89.28	0.374		
BMI	0.35	0.388		
Male (n = 52)				
Constant	50.32		12.47	15.04
Age	-0.44	0.322		
Weight	1.00	0.452		
BMI	-1.65	0.482		
Total sample (n = 110)				
Constant	-36.16		28.81	12.43
Height	45.37	0.362		
Age	-0.34	0.392		
Weight	0.39	0.446		
Gender	7.41	0.474		
<i>V_c</i>				
Female (n = 58)				
Constant	94.70		5.33	14.55
Age	-0.57	0.298		
Male (n = 52)				
Constant	0.82		43.76	13.54
Age	-0.48	0.44		
Height	52.47	0.513		
Weight	0.16	0.524		
Total sample (n = 110)				
Constant	79.71		8.23	14.18
Age	-0.60	0.300		
Gender	9.68	0.382		
Weight	0.25	0.407		

B = Non-standardized regression coefficient; r² = determination coefficient. Retained reference equations: D_m (total sample) = 36.16 + 45.37 × height – 0.34 × age + 0.39 × weight + 7.41 × gender. To calculate LLN subtract 24.36 from the reference value. V_c (female) = 94.70 – 0.57 × age. To calculate LLN subtract 28.52 from the reference value. V_c (male) = 0.82 – 0.48 × age + 52.47 × height + 0.16 × weight. To calculate LLN subtract 26.54 from the reference value.

tions did not appear to reliably predict D_m or V_c in this population. Using only anthropometric data as independent predictors, we thus established new reference equations that explained 47, 30 and 52% of the variability in D_m, V_c (female) and V_c (male), respectively.

Study Procedure

In a large group of healthy subjects aged 18–85 years, we found the expected wide ranges in D_m and V_c, in line

Fig. 5. Bland and Altman plot of measured and predicted V_c determined from the Dutch [9] (a) and French [11] (b) reference equations. r = Correlation coefficient; regression equation [y = (measured – predicted); x = (measured + predicted)/2]; ----- = linear regression line; = mean $\pm$ 1.96 SD.

Table 4. Published reference equations

	Mean $\pm$ SD	Reference equation	r^2
<i>D_m</i> , ml/min/mm Hg			
The Netherlands (Zanen et al. [9])			
Males (n = 45; 40 $\pm$ 11 years old)	18.46 $\pm$ 103.91	$1/D_m = (0.127 + 3.304 \times 10^{-4} \times A - 4.753 \times 10^{-2} \times H)/7.5$	?
Females (n = 72; 38 $\pm$ 11 years old)	13.13 $\pm$ 74.35	$1/D_m = (0.111 + 3.304 \times 10^{-4} \times A - 4.753 \times 10^{-2} \times H)/7.5$	
France (Aguilaniu et al. [10])			
(163 males/144 females)			
≤ 59 years old	not mentioned	$D_m = -29.639 + 14.033 \times G - 0.434 \times A + 70.000 \times H$	0.70
> 59 years old		$D_m = -29.639 + 14.033 \times G - 0.444 \times A + 70.000 \times H$	
Present study			
Males (n = 52; 53 $\pm$ 21 years old)	20.90 $\pm$ 97.00	$D_m = -36.16 \times 45.37 \times H - 0.34 \times A + 0.39 \times W + 7.41 \times G$	0.47
Females (n = 58; 42 $\pm$ 16 years old)	21.90 $\pm$ 72.60		
<i>V_c</i> , ml			
The Netherlands (Zanen et al. [9])			
Males (n = 45; 40 $\pm$ 11 years old)	86.28 $\pm$ 442.48	$1/V_c = 10^{-2} \times (4.455 - 1.805 \times H)$	?
Females (n = 72; 38 $\pm$ 11 years old)	63.17 $\pm$ 324.46	$1/V_c = 10^{-2} \times (4.375 - 1.805 \times H)$	
France (Aguilaniu et al. [10])			
(163 males/144 females)			
≤ 59 years old	not mentioned	$V_c = -36.067 + 14.134 \times G - 0.434 \times A + 85.050 \times H$	0.68
> 59 years old		$V_c = -36.067 + 14.134 \times G - 0.910 \times A + 85.050 \times H$	
Present study			
Males (n = 52; 53 $\pm$ 21 years old)	42.86 $\pm$ 118.80	$V_c = -0.82 - 0.48 \times A + 52.47 \times H + 0.16 \times W$	0.52
Females (n = 58; 42 $\pm$ 16 years old)	40.30 $\pm$ 112.10	$V_c = 94.70 - 0.57 \times A$	0.30
H = Height (m); A = age (years); W = weight (kg); G = gender (0 = female; 1 = male).			

with previous studies [9, 10] summarized in table 4. Insofar as possible, the procedural factors that affect D_m or V_c variability were controlled with respect to non-inclusion criteria, examination at the same hour, the same altitude and the same examiner for all subjects.

Subject Group Composition

The sample size ($n = 110$) was smaller than that of the French study [10] ($n = 307$) and closer to that of the Dutch study [9] ($n = 117$; table 4). In a relatively large number of subjects (i.e. $n = 100$), spirometry data did not significantly differ between the published reference equations and the values from the local community [15]. Therefore, our sample size and our methodological precautions enabled us to obtain reliable results.

Our recruitment mode and subject age range were similar to those of previous studies with comparable objectives (table 4). Ideally, reference values are calculated with equations derived from measurements observed in a representative sample of healthy subjects in a general population [15]. Reference equations can also be derived from large groups of volunteers, provided that criteria for normal selection and proper distribution of anthropometric characteristics are satisfied [15]. In order to minimize the voluntary and selection bias [28], our subjects were free from chronic disease, although 19 (17%) subjects showed moderate obesity. We excluded subjects with marked or severe obesity due to known lung alterations [29–31].

NO-CO Technique

The clinical implication of using both NO and CO concurrently in research and medical practice is that scientists and clinicians can immediately quantify the components of D_l in a subject from a single 4-second breath hold maneuver that requires minimal effort on the part of the patient, while simultaneously being able to pinpoint which component (V_c or D_m) is causing low (or high) total D_l . The ability to estimate D_m and V_c from one-step simultaneous measurement of D_lNO and D_lCO represents significant conceptual advantages. One conceptual advantage of the double-transfer NO/CO method is that all measurements are obtained at the same cardiac output and O_2 tension; no interpolation is necessary [32, 33]. Another conceptual advantage is that using this method only one inspiration is required, which results in a similar distribution of NO and CO gases. A third conceptual advantage is that with the traditional method, there is a systematic underestimation of V_c and an overestimation of D_m since the inspiration at two different O_2

tensions affects alveolar-capillary membrane diffusion [34]. The double transfer NO/CO method avoids this error altogether and should improve the accuracy of estimated D_m and V_c [35]. To obtain reproducible and comparative measures, respiratory maneuvers were made in the sitting position for all subjects of the study. Indeed, Terzano et al. [36] demonstrated that in healthy subjects, there were postural variations in D_lCO that could be explained by a more homogeneous perfusion.

Limits of the Present Study

Hb concentration is another factor which could influence D_m and V_c values [15]. This variable was not measured, as suggested by Stam et al. [37]. In fact, the slight differences in this concentration in a healthy population did not justify Hb measurements as the recovered mean value of V_c was not significantly altered by introducing preset values for women and men [37].

In women, parity has not been identified. Indeed, Ben Saad et al. [14] did not find significant differences in spirometric and cardiovascular parameters between women having low and high parity.

Passive tobacco smoking, which is an important pulmonary risk factor, has not been specified in this study. Leitch et al. [38] concluded that the lapse of 1 h after cigarette smoking and the measurement of D_lCO exerted not mild but important influences on the expired CO level, but that passive smoking did not.

V_c - and D_m -Independent Contributors

Independent contributors of D_m and V_c in our total population sample were anthropometric data and VA (table 2). Height has the same effects in all approaches: V_c and D_m values increase with increasing height [9, 10]. Weight and BMI were two anthropometric factors that affected V_c and D_m [4]. Indeed, restrictive ventilatory defects were most markedly associated with obesity [39].

With regard to age, in the equations for D_m and V_c of the total sample, negative terms were found (table 2), i.e. D_m and V_c decrease with increasing age. Indeed, the aging process is associated with degenerative changes [40, 41] which are associated with a decline in lung function. In previous studies, physiologic changes in the lungs occurred due to aging alone, e.g. aging lung or senile lung. In this study, the lungs revealed airspace enlargement without alveolar wall destruction [4, 40–42]. It must be noted that in all equations, the effect of age is much smaller than that of height, so the significance of these differences is limited [9, 10].

Choice of the Appropriate D_m and V_c Reference Equations

Selecting reference values for gas transfer is more problematic than selecting reference values for spirometry because interlaboratory differences are much larger [43, 44]. Some of these differences can be attributed to the calculating method and the adjustment for Hb concentration, carboxyhemoglobin concentration and altitude. Laboratory directors should thoughtfully select reference values that match the numbers produced in their laboratories. Optimally, it would require individual laboratories to measure V_c and D_m in a sample of healthy subjects and compare the results with several reference equations.

Due to the inadequacy of the published equations and for a pure and simple practical interest we established reference equations by including, as independent variables, height, age, weight and gender for D_m ; age, height and weight for male V_c and only age for female V_c . Inclusion of anthropometric data only in reference equations is justified by their easy method to assess their association with dependent variables. Although VA showed strong relationships with D_m and V_c in univariate analysis (table 2), it was not included in the reference equations. Indeed, VA is not found in published reference equations for V_c and D_m . Chinn et al. [42] showed that when VA is measured, the diffusion parameters corrected for VA can be obtained. The first of the two parameters serves the same purpose as the well-known KCO ($= D_lCO/VA$) [42]. It is not so much a question of whether VA is a better parameter than height or age, but its inclusion would result in a new and different parameter. D_mCO/VA , V_c/VA or $D_m/(VA \cdot \text{height}^{-2})$, $V_c/(VA \cdot \text{height}^{-2})$ are not routinely used at present [9, 42].

Equations of the present study still explain 47.4, 52.4 and 29.8% of the variability in D_m , and male and female V_c , respectively. It is still satisfying compared to other reference equations (table 4). In addition, compared to the published reference equations, we found significant differences between measured and predicted D_m and V_c (fig. 2–5). Several factors can be ruled out to explain these differences. A systemic deviation can be due to the equipment or experimental procedures employed. It is the case in the differences between the present equations and especially those of Zanen et al. [9] who calculated D_mCO and V_c from D_lCO measured at two levels of P_{AO_2} (25 and 100%) with the use of the θCO value of Roughton and Forster [1] whereas we used the more recent and accurate value of Forster [26]. D_m and V_c values are extremely sensitive to the value of θCO , as pointed by Hughes and Bates [45].

Physiological and/or anatomical differences may possibly explain this result [9], but it is hard to argue that the alveolar capillary membrane structure in the volunteers in these studies would have been different. This would imply that different types of people exist [9].

V_c and D_m Interpretative Strategy

As it is recommended by ATS/ERS [15], publications on reference equations should include explicit definitions of the upper and lower limits of the normal range, or provide information to allow the reader to calculate a lower range. For each 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' [15]. If the reference data have a normal distribution, the lower 5th percentile can be estimated as the 95% confidence interval using Gaussian statistics. If the distribution is skewed, the lower limit should be estimated with a nonparametric technique, such as the 95th percentile [15].

In the present study, and as D_m and V_c have a normal distribution, we have proposed to consider values lower than the difference between predicted and LLN values as lower values [9, 15, 46]. In accord with previous reports, the practice of using 80% of predicted as a fixed value for LLN can lead to important errors when interpreting V_c or D_m in adults [15]. In fact, using this fixed value of 80% of predicted, 20 (18%) and 15 (14%) subjects would have an abnormal D_m and V_c in our study, respectively. We would like to propose 65 and 50% of predicted for V_c and D_m , respectively, based on our study results.

Using our reference equations, extrapolation beyond the size and age of the subjects investigated should be avoided [47]. If a patient's age or height is outside the limits of our population, it should be stated that an extrapolation has been made.

In conclusion, we have established reference equations to interpret D_m and V_c values in healthy North-African adults. Our D_m and V_c reference equations supplement the World Bank of reference equations, in which physicians can choose according to the region or ethnic background of the patient.

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