Transfer factor, lung volumes, resistance and ventilation distribution in healthy adults

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ABSTRACT Monitoring of chronic lung disease requires reference values of lung function indices, including putative markers of small airway function, spanning a wide age range.

We measured spirometry, transfer factor of the lung for carbon monoxide (T_{LCO}), static lung volume, resistance and ventilation distribution in a healthy population, studying at least 20 subjects per sex and per decade between the ages of 20 and 80 years.

With respect to the Global Lung Function Initiative reference data, our subjects had average z-scores for forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and FEV_1/FVC of −0.12, 0.04 and −0.32, respectively. Reference equations were obtained which could account for a potential dependence of index variability on age and height. This was done for (but not limited to) indices that are pertinent to asthma and chronic obstructive pulmonary disease studies: forced expired volume in 6 s, forced expiratory flow, T_{LCO}, specific airway conductance, residual volume (RV)/total lung capacity (TLC), and ventilation heterogeneity in acinar and conductive lung zones.

Deterioration in acinar ventilation heterogeneity and lung clearance index with age were more marked beyond 60 years, and conductive ventilation heterogeneity showed the greatest increase in variability with age. The most clinically relevant deviation from published reference values concerned RV/TLC values, which were considerably smaller than American Thoracic Society/European Respiratory Society-endorsed reference values.

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We present reference equations for median and variability of most lung function indices, in the age range 20–80 years http://ow.ly/SsZFW

Introduction

Studies in patients with asthma or smoking-induced lung disease require appropriate reference values to determine lung function abnormality, especially in the early stages of the disease process. While asthma can arise at any age, chronic obstructive pulmonary disease (COPD) usually develops later in life, yet both chronic conditions lead to follow-up periods of lung function potentially extending through old age. Both conditions are usually marked by a spirometric deficit which can now be set against reference equations that are valid worldwide [1, 2]. However, the characterisation of asthma and smoking-induced lung disease can also benefit from lung function and ventilation distribution indices that are thought to be sensitive to two distinct but sometimes concomitant phenomena: peripheral airway abnormality and abnormal heterogeneity of airway function at all lung depths. Hence, clinical research studies regularly include indices dedicated to represent peripheral and heterogeneous airway function [3]. Abnormality of these lung function indices can sometimes be assessed by comparison with reference values, either obtained for a particular index obtained in one study population [4, 5] or based on reference equations constructed from the summary of previous equations [6]. To date, the most complete set of reference values acquired on a well-defined population (n=179; 18–86 years) is by ROBERTS et al. [7], since it includes spirometry, plethysmography, transfer factor of the lung for carbon monoxide (T_{LCO}) and single-breath washout indices (phase III slope) on nonsmoking adults.

The main objective of this work was to provide easy-to-use reference equations on all lung function and ventilation indices that have proven to be relevant to asthma, COPD or cystic fibrosis (CF) to some extent in the past. On the lung function side, T_{LCO} and the ratio of residual volume (RV) over total lung capacity (TLC) are particularly relevant to COPD and asthma. On the ventilation distribution side, indices of acinar and conductive (S_{acin}, S_{cond}) ventilation heterogeneity derived from the normalised phase III slope analysis of the multiple-breath washout (MBW) have been used to characterise bronchial hyperresponsiveness in asthma [8] or asthma control [9], or to pick up different stages of smoking-induced lung disease [10]. In more severe lung disease such as in patients with COPD or patients with CF, the lung clearance index (LCI) [11] and the curvilinearity (Curv) derived from the MBW concentration curve [12] may suffice to quantify degree of heterogeneity. Airway resistance is also seen to be increased in asthma and COPD, with values depending on the method (forced oscillation or plethysmography) [13] or the equipment used [14]. Even if most indices are bound to be partly apparatus- and method-dependent, the dependence of any physiological index on age and/or height will likely be similar across laboratories. Here, we aimed to obtain all the above indices on the same population, in the same lung function laboratory of the university hospital and coached by the same well-trained lung function technician.

Materials and methods

The study protocol was approved by the local UZ Brussel research ethics committee (B14320097554 and B143201112421 pertaining to data covering ⩽65 and >65 years age groups, respectively); selected spirometric and MBW indices from our centre have been published previously for the 25–65 years group [15]. Lung function and MBW tests were collected on healthy nonsmoking subjects with <3 pack-years (in case of ex-smokers) in the age range 20–80 years. All participating subjects were Caucasian, were required to have a body mass index <33 kg·m^{-2} and were defined as healthy through clinical screening, with no medical history of respiratory disease. At least 40 subjects (20 males/20 females) were recruited per decade in the age range 20–80 years, based on the recommended number of subjects described by QUANJER et al. [16]. We adapted the numbers needed to the age range under study here (20–80 years) where age dependency of most indices was expected to be monotonous, in contrast to what occurs in the age range <20 years [1]. However, because we anticipated different variability around the median in different age ranges for at least some indices, we applied generalised additive models for location, scale and shape method proposed by the Global Lung Function Initiative (GLI) [1, 2] to obtain LambdaMuSigma (LMS) model fits. From the resulting parameters L, M and S, predicted values and lower and upper limit of normal (LLN, ULN) can be readily computed; see example in online supplementary material.

All lung function measurements were performed in the same centre (centrally located in Belgium), by the same lung function technician, according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines for lung function measurements [17] using commercial equipment (Vmax Encore VE models 20c, 22 and 22d; Cardinal Health, Dublin, OH, USA). After standard lung function testing including spirometry, T_{LCO}, static lung volumes and airway resistance by body plethysmography, MBW tests were performed as previously described [15]. Volumes from all above-mentioned tests were reported at body temperature, ambient pressure, saturated with water vapour.

Spirometry

In each subject, three acceptable manoeuvres were performed that satisfied the reproducibility criteria of the ATS/ERS guidelines [18]. First, we selected the largest forced expired vital capacity (FVC), the largest

forced expired volume in 1 s (FEV1) and the largest forced peak expiratory flow (PEF) obtained over the three blows. Then, we selected the blow with the largest sum of FEV1 and FVC, from which we extracted forced expired volume in 6 s (FEV6), forced expiratory flow at 75% of FVC (FEF75%) and forced expiratory flow at 25–75% of FVC (FEF25–75%). Finally, derived indices FEV1/FVC and FEV1/FEV6 were considered for developing reference equations.

Carbon monoxide transfer measurement

Two acceptable tests were performed in each subject [19]. Using the single-breath technique and 21% O_2/0.3% CO/0.3% C_2H_2/0.3% CH_4 with balance N_2 as the inspiratory gas mixture, we obtained T_{LCO}, alveolar volume at total lung capacity (V_A) and transfer coefficient of the lung for carbon monoxide (K_{CO}) (*i.e.* T_{LCO}/V_A); all indices were reported as the mean of values obtained from both tests.

Static lung volumes and resistance using body plethysmography

Two acceptable tests were performed in each subject [20]. We retained the measurement of RV determined by whole-body plethysmography (RV_{pleth}), functional residual capacity determined by whole-body plethysmography (FRC_{pleth}), total lung capacity determined by whole-body plethysmography (TLC_{pleth}), airway resistance (R_{aw}) and specific airway conductance (sG_{aw}). All indices were reported as the mean of values obtained from both tests. To ensure extraction of proper FRC_{pleth} values with the body plethysmograph model that we used (Vmax Encore 22d), values labelled in the report as "FRC" and "V_{tg}" (thoracic gas volume) were examined to determine whether FRC corresponded to the actual FRC level observed on the traces. This had to be done because biological calibrators and tests with two independent plethysmograph boxes had indicated an artefact in FRC_{pleth} (despite proper RV_{pleth} and TLC_{pleth}). With our particular bodybox and its software settings this was due to an unpredictable volume drift upon closing the shutter valve, such that the relative levels corresponding to V_{tg} and FRC needed readjusting.

MBW test

A bag-in-box and valve system was used, with a re-inspired dead space (*i.e.* re-inspired volume in subsequent MBW inhalations) of 50 mL and a N_2 analyzer (PK-Morgan, Chatham, UK). The MBW test involved 1-L tidal breathing from FRC. Three acceptable tests were performed in each subject. Each MBW test was analysed with a semi-automated break-point analysis [21] from which phase III slope and mean expired concentration were obtained for every breath. FRC_{mbw} was obtained by mass balance including all breaths up to the one corresponding to mean expired concentration just under 1/0.40th of the initial N_2 concentration. From three acceptable tests, data were cumulated to obtain plots of mean expired concentration and phase III slope *versus* lung turnover (TO; cumulative expired volume/FRC_{mbw} for any given test). From the semi-log plot of mean expired concentration (as a percent of initial concentration) *versus* TO we derived: 1) LCI, as the TO value corresponding to the intercept between the semi-log plot of mean expired concentration and the 1/0.40th initial concentration level; 2) Curv, as unity minus the ratio of two regression slopes, where the regression slope in the denominator is that determined in the range TO=0 and TO=LCI/2 and in the numerator that between TO=LCI/2 and TO=LCI [12]. From the plot of normalised phase III slope (normalised by mean expired concentration) *versus* TO, we derived indices of acinar and conductive (S_{acin}, S_{cond}) ventilation heterogeneity, as well as two modified indices (S_{acin}*, S_{cond}*) for use in patient groups such as CF where ventilation heterogeneity is so marked that S_{cond} becomes invalid [22]. Finally, we determined Fowler dead space volume of the first breath (V_{DF}) in order to assess its potential impact on LCI when exploring the relationship between LCI, which is an overall index of ventilation heterogeneity, and its potential determinants (S_{acin}, S_{cond}).

Statistical analysis

Simple comparison between selected indices, linear and stepwise multiple regressions were performed using MedCalc (version 14.8.1; MedCalc, Mariakerke, Belgium). For the regression equations, we followed the guidelines and procedures described in QUANJER *et al.* [1] and LUM *et al.* [23], using the GAMLSS package (version 4.3-4) in R (version 2.15.2; R Foundation, Vienna, Austria). After Box–Cox–Cole–Green transformation of the data, an LMS model fit was obtained that allows the median and spread of the distribution to vary with height and age (or age squared). We also included sex and tested for interaction between age and sex in the initial estimate of the median. For Curv, which had some zero values, the Box–Cox–Cole–Green transformation required the addition of a very small positive value (+0.001) to the actual Curv measurement value. With the Box–Cox–Cole–Green transformation, the obtained coefficients for M are to be used in a linear expression to obtain the predicted value, whereas the obtained coefficients for S are to be used in an exponential expression to obtain the spread; L corresponds to nu estimated by a constant coefficient. The LLN and ULN (5th and 95th percentile) can then be computed as $M \times (1-1.645 \times L \times S)^{(1/L)}$ and $M \times (1+1.645 \times L \times S)^{(1/L)}$ [23]. Guided by the Schwarz Bayesian Criterion, the most suitable model with likely contributors was selected for each variable. In all the above analyses, statistical significance was accepted at $p < 0.05$.

Results

The total cohort comprised of 252 subjects, who were homogeneously distributed over the six decades between 20 and 80 years, and over both sexes (figure 1a) and with maximum BMI just under 32 kg·m^{-2}. To characterise our study population with respect to GLI2012, we determined z-scores for FEV1, FVC and FEV1/FVC based on the GLI2012 reference values for Caucasians [2]: mean (±SD) z-scores for FEV1, FVC and FEV1/FVC were −0.12 (±0.90), +0.04 (±0.85) and −0.32 (±0.71), respectively. Table 1 summarises the mean±SD of all variables studied here.

For most variables, reference equations were constructed, resulting in coefficients for sex, height, and age and/or age squared to obtain L, M and S (table 2); an example computation of predicted, ULN and LLN is provided in the online supplementary material. Figure 2 shows the raw data and their LMS fit (median, LLN, ULN) for two lung function indices that are particularly relevant for studies in asthma and COPD: T_{LCO} and RV/TLC. Figure 3 shows the raw data and their LMS fit for three relevant ventilation distribution indices: LCI, S_{acin} and S_{cond}. In figures 2 and 3, left and right panels are, respectively, female and male data drawn to the same vertical axis; in order to be able to also plot the LMS fit for those indices that depend on height, height was taken into account in each sex group by use of the height–age relationships of figure 1(b). The raw data plots with superimposed LMS fit for all indices of table 1 and their colour-coded comparison to other reference values, wherever available [4–7, 24–30], are in the online supplementary material.

No reference equations were drawn up for FRC_{pleth} and V_A due to methodological considerations (see Discussion section), but these indices were compared with FRC_{mbw} and TLC_{pleth}, respectively (figure 4). A Bland–Altman representation of FRC_{mbw} and FRC_{pleth} showed good overall agreement, while V_A consistently fell below TLC_{pleth} (by 0.46 L). Multilinear regression of LCI *versus* its potential contributors (S_{acin}, S_{cond}, V_{DF}/FRC and FRC) retained the first three indices, which together accounted for 58% of the LCI variability: partial correlation coefficients were +0.67 for S_{acin} (p<0.0001), +0.13 for S_{cond} (p=0.04) and +0.20 for V_{DF}/FRC (p=0.002).

Discussion

We have developed reference equations, LLN and ULN for indices derived from spirometry, T_{LCO}, static lung volume, resistance and ventilation distribution tests. The lung function indices studied are directly relevant to common respiratory illnesses, including asthma, COPD and CF.

Spirometry

As GLI2012 already offers worldwide valid reference equations [1], we used these to verify that our subjects obtained z-scores close to zero. The coefficients provided in table 2 for the most widely used indices already covered by the GLI, such as FEV1 and FVC, served to verify that the statistical methodology was sound and that the expected explanatory variables were obtained. We then produced reference equations for FEV6 and FEV1/FEV6 which have been suggested for use to screen smokers for restriction and obstruction in the primary care setting [31]. In the online supplementary figures S4 and S5, the LLN values for FEV6 and FEV1/FEV6 are seen to be close to those reported by Hankinson *et al.* [24]; they almost coincide in the age range 45–65 years, *i.e.* the age category where screening for smoking-induced lung disease is probably most relevant. The diagnostic value of FEF75% or FEF25–75% in adults has been questioned in recent years, *e.g.* in terms of their added value with respect to other spirometric indices when classifying patients [32].
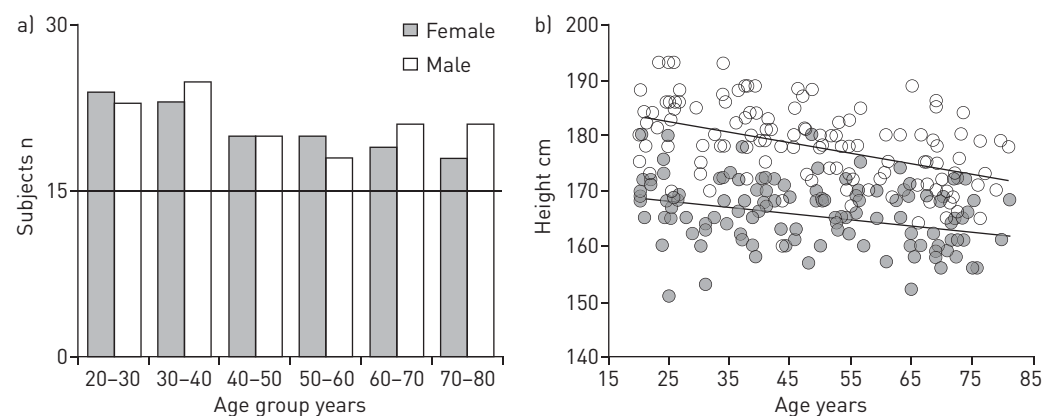

FIGURE 1 a) Histogram of subjects distributed across the different age categories in either sex. b) Scatterplots of height *versus* age in either sex group and their regression lines; corresponding regression equations for females: height (cm)= −0.1133×age (years)+171.16 and for males: height (cm)= −0.187×age (years)+186.97.

TABLE 1 Values of all parameters in the entire study population grouped by sex

	Female	Male
Subjects n	124	128
Height cm	165.7±6.1	177.8±7.2
BMI kg·m^{-2}	22.9±3.1	24.7±3.0
Spirometry		
FEV$_1$ L	2.8±0.6	3.9±0.8
FVC L	3.6±0.7	5.0±1.0
FEV$_1$/FVC	0.79±0.06	0.78±0.05
FEV$_6$ L	3.5±0.7	4.9±1.0
FEV$_1$/FEV$_6$	0.80±0.05	0.79±0.05
PEF L·s^{-1}	6.6±1.2	10.0±1.7
FEF$_{25-75\%}$ L·s^{-1}	2.7±0.9	3.6±1.2
FEF$_{75\%}$ L·s^{-1}	1.2±0.6	1.5±0.8
Carbon monoxide transfer		
T_{LCO} mmol·min^{-1}·kPa^{-1}	7.1±1.3	10.3±2.0
K_{CO} mmol·min^{-1}·kPa^{-1}·L^{-1}	1.52±0.22	1.56±0.23
V_A L	4.7±0.7	6.6±1.0
Body plethysmography		
RV$_{pleth}$ L	1.6±0.5	1.9±0.7
FRC$_{pleth}$ L	3.0±0.6	3.6±0.7
TLC$_{pleth}$ L	5.2±0.7	7.0±1.0
RV/TLC	0.31±0.09	0.27±0.09
R_{aw} kPa·s·L^{-1}	0.26±0.06	0.23±0.06
sG_{aw} L·s^{-1}·kPa^{-1}	1.23±0.25	1.23±0.28
Multiple-breath washout		
FRC$_{mbw}$ L	2.8±0.5	3.7±0.8
V_{DF}/FRC	0.051±0.010	0.049±0.010
LCI	6.5±0.6	6.5±0.6
Curv	0.22±0.09	0.21±0.09
S_{cond} L^{-1}	0.036±0.017	0.036±0.013
S_{acin} L^{-1}	0.10±0.05	0.12±0.05
S_{cond}* L^{-1}	0.046±0.022	0.046±0.017
S_{acin}* L^{-1}	0.10±0.05	0.11±0.05

Data are presented as mean±SD, unless otherwise stated. BMI: body mass index; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; FEV$_6$: forced expiratory volume in 6 s; PEF: peak expiratory flow; FEF$_{25-75\%}$: forced expiratory flow at 25–75% of FVC; FEF$_{75\%}$: forced expiratory flow at 75% of FVC; T_{LCO}: transfer factor of the lung for carbon monoxide; K_{CO}: transfer coefficient of the lung for carbon monoxide; V_A: alveolar volume; RV$_{pleth}$: residual volume determined by whole-body plethysmography; FRC$_{pleth}$: functional residual capacity determined by whole-body plethysmography; TLC$_{pleth}$: total lung capacity determined by whole-body plethysmography; R_{aw}: airway resistance; sG_{aw}: specific airway conductance; FRC$_{mbw}$: functional residual capacity determined by multiple-breath washout; V_{DF}: Fowler dead space volume of the first breath; LCI: lung clearance index; Curv: curvilinearity; S_{cond}: index of conductive ventilation; S_{acin}: index of acinar ventilation; S_{cond}*: modified index of conductive ventilation; S_{acin}*: modified index of acinar ventilation.

However, for completeness we also included FEF$_{75\%}$ and FEF$_{25-75\%}$ in the online supplementary material. Finally, the PEF (online supplementary figure S6) showed excellent agreement with both KUSTER et al. [26] and HANKINSON et al. [24], identifying similar independent contributors (sex, age, height) (table 2).

Transfer factor and body plethysmography

T_{LCO} and T_{LCO}/V_A show the expected dependence on sex, height and age consistent with fragmented data from the literature [4, 7, 25]. In figure 2 it can be appreciated that T_{LCO} shows a monotonous decrease over the entire age range with variability remaining almost constant throughout. Normal RV$_{pleth}$/TLC$_{pleth}$ values (figure 2b and online supplementary figure S13) were considerably smaller than those in the most recent ATS/ERS report [27]. The authors rightly pointed out that in the absence of more recent data, reference equations for lung volumes effectively refer to the ERS1983 reference equation for adults constructed from earlier equations resulting from plethysmographic and ventilation lung volume studies, and which potentially included smokers [6]. The RV$_{pleth}$/TLC$_{pleth}$ obtained here were closer to RV/TLC data obtained in 1991 by ROBERTS et al. [7] using helium dilution by closed-circuit rebreathing and obtained in nonsmokers.

Finally, V_A was consistently lower than TLC$_{pleth}$ and indeed paralleled TLC$_{pleth}$ over the entire range (figure 4), as could be expected [17]. It has been postulated that the ratio of TLC derived from a single- or

TABLE 2 Coefficients for L, M and S to obtain predicted values, upper limit of normal and lower limit of normal for all studied parameters

	R^2	M coefficients					S coefficients				L coefficient
		Sex	Height	Age	Age^2	Intercept	Height	Age	Age^2	Intercept	Intercept
Spirometry											
FEV1 L	0.80	−0.684	0.0295	−0.0249		−0.207	0.00937	0.00264		−3.87	−0.628
FVC L	0.79	−0.765	0.0501	−0.0217		−2.92	0.00377	0.00137		−2.86	−0.108
FEV1/FVC	0.32	−0.0195	−0.00229	−0.00199		1.28	0.00443	0.00350		−3.75	1.24
FEV6 L	0.80	−0.772	0.0476	−0.0237		−2.44	0.00504	0.000556		−3.05	−0.0563
FEV1/FEV6	0.28	−0.0202	−0.00224	−0.00162		1.26	0.00649	0.000341		−4.05	0.728
PEF L·s⁻¹	0.66	−2.98	0.0383		−0.000276	3.90	−0.00495		0.0000312	−1.07	0.741
FEF75% L·s⁻¹	0.67	−0.337		−0.0268		2.74		0.00502		−1.40	−0.323
FEF25–75% L·s⁻¹	0.60	−0.850		−0.0427		5.49		0.00785		−1.79	−0.253
Carbon monoxide transfer											
T_{LCO} mmol·min⁻¹·kPa⁻¹	0.79	−2.02	0.0947	−0.0465		−4.42	0.00310	0.00583		−2.90	−0.00441
K_{CO} mmol·min⁻¹·kPa⁻¹·L⁻¹	0.30	−0.0959	−0.00402	−0.00735		2.62	−0.00398	0.00423		−1.62	0.215
Body plethysmography											
RVpleth L	0.58		0.0192		0.000250	−2.23	0.0134		−0.0000771	−3.58	0.862
TLCpleth L	0.69	−0.993	0.0642			−4.45	0.00477			−3.02	0.437
RV/TLC	0.73	0.0355		0.0000405	0.0000423	0.157		−0.0539	0.000356	−0.137	1.11
Raw kPa·s·L⁻¹	0.27		−0.00271	−0.00634	0.0000656	0.837	0.00386	0.0108	−0.000115	−2.42	0.074
sGaw L·s⁻¹·kPa⁻¹	0.20			0.0282	−0.000330	0.730		−0.0154	0.000160	−1.31	0.587
Multiple-breath washout											
FRCmbw L	0.53	−0.251	0.0525			−5.67	0.00126			−1.99	0.136
VDF/FRC	0.18	−0.00513	−0.000635			0.161	−0.00152			−1.41	0.313
LCI	0.52			−0.0234	0.000480	6.34		−0.00130	0.000103	−3.02	−1.47
Curv	0.30			−0.00345	0.0000632	0.215		0.00970	−0.000170	−1.03	0.993
Scond L⁻¹	0.33			0.000331		0.0188		0.0166		−1.94	0.666
Sacin L⁻¹	0.45	−0.0153		−0.00296	0.0000471	0.132		0.00653	−0.0000486	−1.31	0.279
Scond* L⁻¹	0.31			0.000459		0.0216		0.0149		−1.74	0.389
Sacin* L⁻¹	0.44	−0.0156		−0.00286	0.0000454	0.128		0.00733	−0.0000625	−1.30	0.323

The value for sex is 0 for male and 1 for female subjects; height: cm; age: years. R^2: Cox–Snell generalised R^2; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEV6: forced expiratory volume in 6 s; PEF: peak expiratory flow; FEF75%: forced expiratory flow at 75% of FVC; FEF25–75%: forced expiratory flow at 25–75% of FVC; T_{LCO}: transfer factor of the lung for carbon monoxide; K_{CO}: transfer coefficient of the lung for carbon monoxide; RVpleth: residual volume determined by whole-body plethysmography; TLCpleth: total lung capacity determined by whole-body plethysmography; Raw: airway resistance; sGaw: specific airway conductance; FRCmbw: functional residual capacity determined by multiple-breath washout; VDF: Fowler dead space volume of the first breath; LCI: lung clearance index; Curv: curvilinearity; Scond: index of conductive ventilation; Sacin: index of acinar ventilation; Scond*: modified index of conductive ventilation; Sacin*: modified index of acinar ventilation.
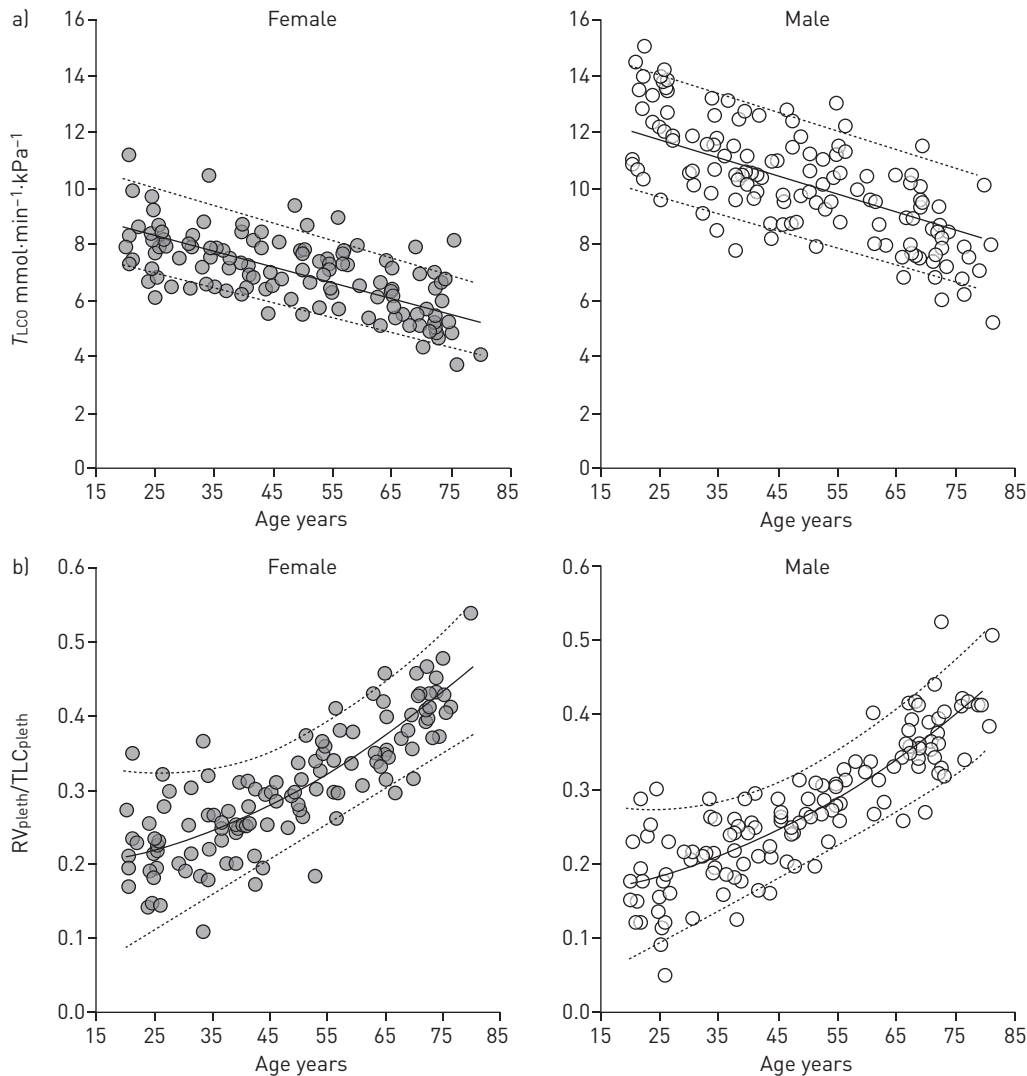
FIGURE 2 Scatterplots of a) transfer factor of the lung for carbon monoxide (T_{LCO}), and b) the ratio of plethysmographic residual volume and total lung capacity (RV_{pleth}/TLC_{pleth}) *versus* age. Median (solid line), and lower and upper limits of normal (dashed lines) produced by the LMS model fit are also shown. Colour-coded versions of these figure panels, comparing with reference values from the literature, are in the online supplementary material.

multiple-dilution test could be a marker of incomplete ventilation distribution [33], potentially making V_A/TLC a useful index in its own right. However, using TLC_{pleth} instead of TLC by dilution (multibreath or rebreathing) adds complexity to the interpretation of V_A/TLC_{pleth} as a surrogate index, especially in obstructive patients. As a result of manufacturer-implemented corrections in the plethysmographic bodybox and the way the so-called thoracic gas volume ("V_{tg}"; determined after the valve closure) is related to actual FRC, FRC_{mbw} was chosen over FRC_{pleth} as the preferred method to draw up reference equations for FRC. Nevertheless, when FRC_{pleth} was compared with FRC_{mbw} (figure 4), overall agreement was good, indicating general coherence of the data set. FRC_{mbw} slightly decreased with age, *via* its dependence on height (which decreases with age; figure 1b), but age was not an independent predictor. This agrees with the very small age dependency obtained from dilution FRC in ROBERTS *et al.* [7], which is the same in both sexes. This contrasts with the 10-fold greater age dependence in males *versus* females in the most used reference equations for FRC [27], probably due to a problem with summarised prior reference equations and the mixture of FRC values derived from ventilation and plethysmographic measurement [34].

To the best of our knowledge, there are no reference equations for airway resistance (R_{aw}) obtained from body plethysmography and we can only compare the slight increase with age that we find in the age range >45 years with that obtained by 5 Hz forced oscillations [5]. The most noteworthy observation of our data set spanning 20–80 years is that there appears to be a minimum in R_{aw} around the age of 45 years (online supplementary figure S14). Specific conductance (sG_{aw}) is seen to remain relatively constant up to 50–55 years, after which it
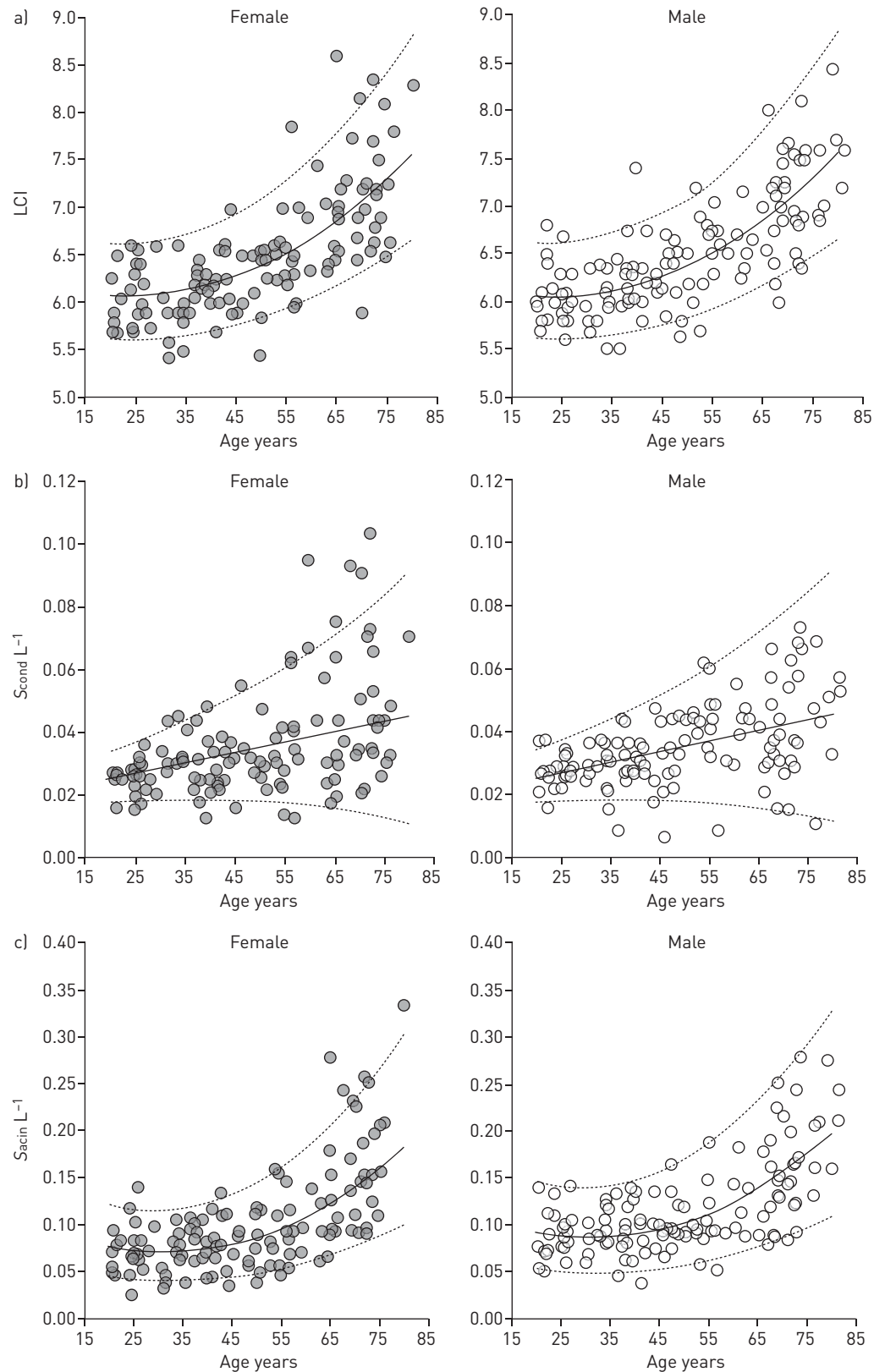
FIGURE 3 Scatterplots of standard indices derived from the multiple-breath washout test: a) lung clearance index (LCI), and b, c) indices of conductive and acinar ventilation heterogeneity (S_{cond}, S_{acin}). Median (solid line), and lower and upper limits of normal (dashed lines) produced by the LMS model fit are also shown.
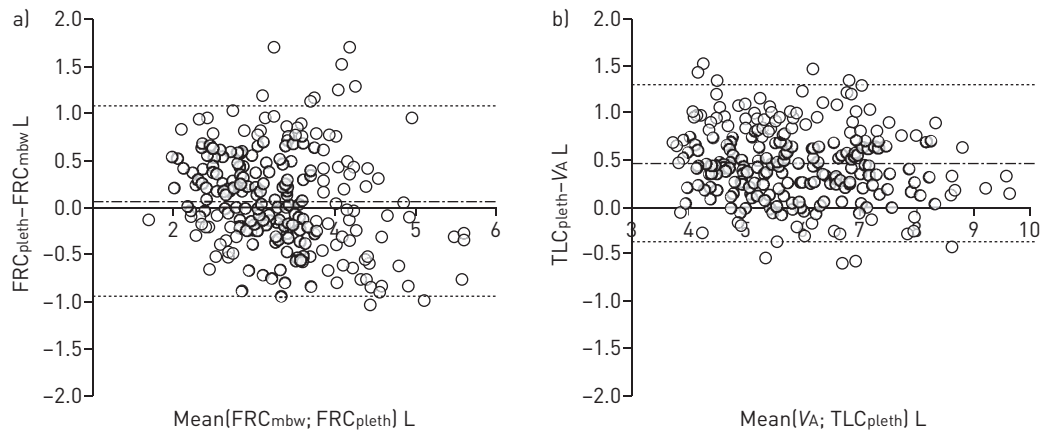
FIGURE 4 Bland–Altman plots of ventilated volumes obtained from a) the multiple-breath washout test (functional residual capacity (FRC_{mbw})) and b) the diffusing capacity test (alveolar volume (V_A)) and their counterparts obtained from plethysmography (total lung capacity (TLC_{pleth}) and FRC_{pleth})). Dashed-dotted and dashed lines indicate mean and ±1.96SD, respectively.

slightly deteriorates (online supplementary figure S15). No published reference values exist for R_{aw} or sG_{aw} covering the 20–80 years range.

MBW test

First, using the raw data, we examined whether there was a link between LCI and S_{acin} and S_{cond}, as was found to be the case in asthma patients [35]. In the present study, the "disease" driving S_{acin} and S_{cond} values would be ageing, and indeed both appeared to correlate independently with LCI. An independent contribution from V_{DF} as a fraction of FRC also contributed to an increased LCI. In this case, ageing per se was probably not the driving factor since FRC and V_{DF}/FRC (online supplementary figures S16 and S17) do not show a significant dependence on age (table 2).

The reference equations for LCI, S_{cond} and S_{acin} (figure 3 and table 2) essentially extend those previously obtained across two laboratories in a narrower age range (25–65 years) [15]. The greatest difference between that study and the present one is that in the age range up to 80 years, LCI and S_{acin} no longer vary linearly with age, but both increase more rapidly as age increases, resulting in an age-squared dependence. This implies that in the case of smoking-induced lung disease, which often appears later in life, increased acinar ventilation heterogeneity previously reported by our group may have been partly due to ageing [36]. Nevertheless, the average S_{acin} values observed in the COPD patient groups without emphysema (0.295 L^{-1}; mean age 60 years) and with emphysema (0.444 L^{-1}; mean age 68 years) still largely exceeded the ULN obtained here in the corresponding 60–68 years range [36].

All three MBW indices of figure 3 are also characterised by a greater variability in the advanced age range, which is most marked for S_{cond} (and its modified version S_{cond}*). The increase of average S_{cond}, as well as intersubject variability in S_{cond}, with age is probably due to a greater intersubject heterogeneity of elastic properties of lung units larger than acini. The most likely explanation is loss of elastic recoil and changes in the elastic fibre network that are suggested to occur with ageing [37], bringing about a slight enlargement of respiratory bronchioles and alveolar ducts, without clear destruction of the alveolar walls. Probably, such structural changes are heterogeneously distributed over the lungs and do not occur to the same extent in all subjects. A recent computed tomography study also demonstrated age-dependent air trapping and airway remodelling in normal subjects aged 60–90 years, and the authors identified this to be a heterogeneous process [38].

Limitations of the study

Scaling down the number of subjects required, based on the n=150 per sex group proposed by QUANJER et al. [16] to obtain data in the range 5–90 years, we would have needed to include 15 subjects per decade and sex or 180 subjects in total. For lung volumes, the recommended number by STOCKS et al. [27] to obtain adult reference equations is 100 for men and women. One could argue that the recommended numbers may not hold for all other indices, yet the reasoning behind the recommended sample size in QUANJER et al. [16] was based on z-scores, which should in principle apply to all indices measured on normal subjects. Nevertheless, we accept that there is something arbitrary about the choice of a criterion for the number of subjects. Here, we refer to our data set (with a homogeneous distribution over the entire age range and a low number of outliers) and the overall agreement with predictions from other studies wherever available,

thus attesting to the adequacy of the number of subjects studied here. Another limitation concerns the equipment used, which mainly pertains to indices derived from MBW. While MBW indices S_{cond} and S_{acin} are less sensitive to equipment dead space than LCI [15], all three indices are sensitive to the gas used. This implies that the absolute values provided by the reference equations may not be blindly applied to other devices or laboratories elsewhere. However, the observed sex or age dependence of these indices and their variability should be similar, and can be borne in mind when selecting a group of healthy subjects locally in the validation phase of a newly installed MBW device in any given laboratory. A final limitation of the study is that the reference equations cannot be readily used for subjects of ethnicity other than Caucasian or for Caucasian subjects outside the normal length and weight ranges.

In conclusion, we obtained reference equations for most lung function indices currently implemented in a lung function laboratory with a keen interest in pulmonary research. For some variables (R_{aw}, sG_{aw}, V_{DF}/FRC), no such reference data existed; for others (RV/TLC, FRC), existing reference equations were judged inadequate, and for ventilation distribution data, these showed an accelerated deterioration (LCI, S_{acin}) and increased variability (S_{cond}) beyond 60 years.

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