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Original research

Multiple breath washout (MBW) testing using sulfur hexafluoride: reference values and influence of anthropometric parameters

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► Additional material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/thoraxjnl-2020-214717>).

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An abstract containing preliminary analysis of the underlying data was presented at the online 2020 annual congress of the American Thoracic Society.

Received 27 February 2020

Revised 7 January 2021

Accepted 20 January 2021

Published Online First

16 February 2021

ABSTRACT

Background Multiple breath washout (MBW) using sulfur hexafluoride (SF_6) has the potential to reveal ventilation heterogeneity which is frequent in patients with obstructive lung disease and associated small airway dysfunction. However, reference data are scarce for this technique and mostly restricted to younger cohorts. We therefore set out to evaluate the influence of anthropometric parameters on SF_6 -MBW reference values in pulmonary healthy adults.

Methods We evaluated cross-sectional data from 100 pulmonary healthy never-smokers and smokers (mean 51 (SD 20), range 20–88 years). Lung clearance index (LCI), acinar (S_{acin}) and conductive (S_{cond}) ventilation heterogeneity were derived from triplicate SF_6 -MBW measurements. Global ventilation heterogeneity was calculated for the 2.5% ($\text{LCI}_{2.5}$) and 5% (LCI_5) stopping points. Upper limit of normal (ULN) was defined as the 95th percentile.

Results Age was the only meaningful parameter influencing SF_6 -MBW parameters, explaining 47% (CI 33% to 59%) of the variance in LCI, 32% (CI 18% to 47%) in S_{acin} and 10% (CI 2% to 22%) in S_{cond} . Mean LCI increases from 6.3 (ULN 7.4) to 8.8 (ULN 9.9) in subjects between 20 and 90 years. Smoking accounted for 2% (CI 0% to 8%) of the variability in LCI, 4% (CI 0% to 13%) in S_{acin} and 3% (CI 0% to 13%) in S_{cond} .

Conclusion SF_6 -MBW outcome parameters showed an age-dependent increase from early adulthood to old age. The effect was most pronounced for global and acinar ventilation heterogeneity and smaller for conductive ventilation heterogeneity. No influence of height, weight and sex was seen. Reference values can now be provided for all important SF_6 -MBW outcome parameters over the whole age range.

Trial registration number NCT04099225.

INTRODUCTION

Multiple breath washout (MBW) has the potential to reveal ventilation heterogeneity which is frequently present in patients with obstructive lung disease and small airway dysfunction. However, it is often missed by commonly used tests.^{1,2} Spirometry was shown to be insensitive to changes in patients with asthma that can be made visible using MBW.^{3,4} These changes are associated with symptoms, most notably dyspnoea, cough and phlegm in COPD.⁵

Key messages

What is the key question?

- Reference data for multiple breath washout (MBW) testing are scarce, with available studies being mostly restricted to younger cohorts and using nitrogen as tracer gas.

What is the bottom line?

- Sulfur hexafluoride-MBW-derived parameters show an age-dependent increase from early adulthood to old age, being most pronounced for global and acinar ventilation heterogeneity.

Why read on?

- For the first time, reference values can now be provided in an adult setting over a wide age range and for all common parameters.

Dyspnoea may be a direct expression of hyperinflation,⁶ which was shown to be addressable therapeutically.⁷ The impact of peripheral airways is yet not restricted to late stages. Instead small airway dysfunction occurs early in the natural course of the disease. In bronchial asthma, changes in small airways are also a hallmark and present in more than 90% of patients. Moreover, clinical subtypes of small airway disease were identified using different tests including MBW.⁸ Lung clearance index (LCI) is the most frequently used MBW outcome parameter and refers to global ventilation heterogeneity. Phase III slopes represent local ventilation heterogeneity in the acinar (S_{acin}) and conductive (S_{cond}) airways. Open wash-in MBW using sulfur hexafluoride (SF_6) and mass spectrometry are considered the gold standard⁹ while being associated with high costs and effort. Hence, nitrogen (N_2) has been frequently used as tracer gas despite suffering from several methodological drawbacks such as back diffusion,^{10,11} measurement inaccuracies¹² and leaks.¹³ Introduction of a photo-magneto-acoustic multigas analyser allowed the direct measurement of SF_6 concentrations with high accuracy.¹⁴ Recently, this led to construction of a closed-circuit SF_6 -MBW set-up considerably facilitating application and reducing costs.¹⁵ Meaningful differences in MBW outcome measures depending on the test gases have been described.^{16,17} In general, N_2 -based

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To cite: Trinkmann F, Maros M, Roth K, et al. *Thorax* 2021;**76**:380–386.

systems reproducibly yield higher absolute LCI readings than SF₆-MBW.^{18–20} To date, much clinical data are available in patients with cystic fibrosis using N₂-based MBW systems. Reference data are scarce and mostly restricted to younger cohorts. Therefore, we set out to (1) evaluate the influence of anthropometric parameters on SF₆-MBW outcome measures and (2) generate reference values in pulmonary healthy controls.

METHODS

Subjects

We evaluated cross-sectional data from pulmonary healthy never-smokers and smokers. Participants were included prospectively using convenience sampling. Subjects had normal lung function testing and no respiratory symptoms. Normal lung function required a combination of normal spirometry, whole-body plethysmography, impulse oscillometry and Krogh factor (K_{CO}) in both qualitative and quantitative analyses. Details are given in the online supplemental appendix 1. The study was registered at ClinicalTrials.gov. Written informed consent was obtained from all participants prior to inclusion.

Study protocol

All subjects underwent three consecutive MBW tests in upright position followed by impulse oscillometry (MasterScreen IOS, CareFusion 234, Höchberg, Germany), spirometry and whole-body plethysmography (MasterScreen Body). Oscillometry was performed during tidal breathing. Parameters were averaged over multiple respiratory cycles within 20 s. Transfer factor for carbon monoxide (TLCO) was derived from K_{CO} and ventilated alveolar volume as measured in single breath technique. All lung function measurements were taken cross-sectionally at a single time point and independently assessed by two experienced investigators. Reference values for spirometry, whole-body plethysmography and transfer factors were derived from the revised 1993 version of the European Community for Steel and Coal (ECSC) equations.²¹ For impulse oscillometry, reference values as provided by Vogel and Smidt²² were used.

Multiple breath washout

A commercially available closed-circuit system (Innocor, PulmoTrace ApS, Glamsbjerg, Denmark) was used for MBW measurements.¹⁵ The device consists of a 3 L rebreathing bag filled with a mixture of room air and test gas (94% O₂, 1% SF₆ and 5% N₂O; PulmoTrace ApS) from a commercially available on-board gas cylinder (bolus fraction 20%). Subjects breathed through the device using a mouthpiece and wearing a nose clip. A scrubber was serially placed between the rebreathing bag and the patient to reduce carbon dioxide concentrations during rebreathing. A standard bag volume of 2.5 L was used. Adjustments were made

upfront in steps of 0.25 to 0.5 L, if functional residual capacity (FRC) was known or suspected to considerably deviate. Likewise, adaptations became necessary during measurements with the subject emptying the bag early during rebreathing. Maximal slow inspirations up to the bag volume followed by slow expirations were performed for the first six breaths in order to achieve a complete wash-in. Inspiratory gas concentrations were closely monitored and held below 3% for carbon dioxide and above 18% for oxygen. The further manoeuvre was performed during relaxed tidal breathing. Washout was then started by switching between the bag and room air using a fast-operating pneumatic valve. This was automatically triggered at the end of expiration under the direction of the operator. We stopped the test when end tidal SF₆ had fallen below 1/40 of the starting concentration for three consecutive breaths. Detailed analysis and quality control were performed offline using proprietary software provided by the manufacturer (V8.0 beta 1 software). Mean FRC and LCI were derived from three consecutive washouts during tidal breathing. LCI is defined as the number of FRC turnovers to reduce tracer gas to a predefined level. This is referred to as stopping point and was calculated for the traditional 2.5% (LCI_{2.5}) as well as 5% (LCI₅) of the initial tracer gas concentration. Additionally, phase III slopes were analysed from multiple breaths yielding parameters of acinar (S_{acin}) and conductive (S_{cond}) ventilation heterogeneity. We used a semiautomated approach plotting slopes against FRC turnovers. All breaths were visually analysed and excluded if no clear linear phase III portion could be identified. S_{cond} was then derived by linear regression in the range between 1.5 and 6 turnovers. S_{acin} was calculated as the mean slope of the three first breaths minus S_{cond} contribution.⁹ Only subjects with at least two technically acceptable MBW measurements²³ based on slightly modified American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria (online supplemental appendix 1) were included in the final analysis.

Statistical analysis

Mean values are given as $\pm$ SD unless stated otherwise. Student's t-test or χ^2 test was used to compare continuous or categorical variables, respectively. Cohen's d with 95% CI is given as absolute value to quantify the effect size (ES). The coefficient of variation (CV) was calculated as SD/mean from the valid MBW measurements. An alpha error of less than 5% in two-sided testing was considered statistically significant. R statistical software (V3.5.1, Foundation for Statistical Computing, Vienna, Austria) was used for all data analyses.²⁴ First, the influence of anthropometric parameters was assessed. Age, height, weight and sex were included into an elastic-net generalised linear model assessed at lambda plus 1 SE. L1 regularisation was used

Table 1 Baseline characteristics

	Unit	Overall (N=100)		Never-smoker (n=68)		Smoker (n=32)		P value	ES
		Value	Range	Value	Range	Value	Range		
Age	Years	51 $\pm$ 20	20–88	49 $\pm$ 21	20–88	54 $\pm$ 16	22–81	0.22	0.24 (0.19 to 0.66)
Height	cm	171 $\pm$ 9	152–198	172 $\pm$ 9	153–198	169 $\pm$ 9	152–193	0.22	0.26 (–0.16 to 0.69)
Weight	kg	76 $\pm$ 17	45–132	75 $\pm$ 16	45–132	79 $\pm$ 20	51–120	0.33	0.23 (–0.19 to 0.65)
BMI	kg/m ²	26.2 $\pm$ 5.4	16.3–49.1	25.6 $\pm$ 5.2	16.3–49.1	27.5 $\pm$ 5.5	19.9–37.8	0.11	0.35 (–0.08 to 0.78)
Male	n (%)	54 (54)		37 (54)		17 (53)		1.0	0.03 (–0.39 to 0.45)

Effect size (ES) as measured by Cohen's d with 95% CI.

BMI, body mass index.

for variable selection based on a k-fold cross-validation approach to find optimal hyperparameters. In order to quantify the influence of these anthropometric variables and smoking status on MBW parameters, a standard generalised linear model with Gaussian link function without regularisation was used. Their corresponding CIs of the explained variances were estimated based on 10 000 bootstrap samples with replacement for sample sizes of 100. Second, reference values were calculated based on a cubic splines generalised additive model for location scale and shape (GAMLSS) approach. Separate models were fitted (1) independently of smoking status and (2) for never-smokers only.²⁵ Upper limit of normal (ULN) and lower limit of normal (LLN) were defined as the 95th and 5th percentiles, respectively.

RESULTS

Baseline characteristics and lung function

Data were acquired in a total of 104 subjects. The prespecified exclusion criteria were met by four subjects, resulting in 100 subjects (54 men) undergoing final analysis. The baseline characteristics of 68 never-smokers and 32 smokers (5 active

smokers, 27 former smokers) are summarised with between-group differences in table 1. Lung function data are given in table 2. Differences between smokers and never-smokers were only found for maximum expiratory flow at 25% (MEF₂₅) of FVC and S_{acin}. The overall CV was 3.2% for LCI_{2.5} and 3.3% for LCI₅. At least two valid phase III slope measurements could not be acquired in 10 subjects. These were slightly older (55±20 vs 50±20 years, p=0.46, ES=0.26, CI −0.40 to 0.91) as compared with subjects with successful measurements. Additionally, phase III slopes showed larger variability with a CV of 50% for S_{acin} and 124% for S_{cond}. The mean washout duration was 104±37 s for LCI_{2.5} and 79±27 s for LCI₅ (p<0.0001, ES=0.8, CI 0.5 to 1.1).

Influence of anthropometric parameters

Age was the only meaningful contributor to LCI and S_{acin} in the regularised model, whereas no association with S_{cond} was found in the overall collective. In a non-penalised model, age explained 47% (CI 33% to 59%) of the variance in LCI, 32% (CI 18% to 47%) in S_{acin} and 10% (CI 2% to 22%) in S_{cond}. Smoking accounted for 2% (CI 0% to 8%) of the variability in LCI, 4%

Table 2 Lung function

		Overall (N=100)		Never-smoker (n=68)		Smoker (n=32)		P value	ES
	Unit	Value	Range	Value	Range	Value	Range		
Spirometry									
FEV ₁ /FVC	%	83±6	70–107	83±6	70–107	82±5	71–93	0.58	0.11 (−0.31 to 0.53)
FEV ₁	%pred	103±14	65–135	103±14	73–135	103±13	65–124	0.87	0.04 (−0.38 to 0.46)
VC	%pred	104±14	64–138	103±15	64–138	105±13	73–131	0.54	0.13 (−0.30 to 0.55)
MEF ₇₅	%pred	98±23	48–164	98±24	49–164	97±21	48–138	0.83	0.04 (−0.38 to 0.46)
MEF ₅₀	%pred	90±29	35–170	92±30	37–170	86±26	35–151	0.33	0.20 (−0.22 to 0.62)
MEF ₂₅	%pred	65±26	13–141	68±26	18–141	58±25	13–111	<0.05*	0.43 (−0.01 to 0.86)
Body plethysmography									
TLC	%pred	110±15	81–157	109±14	81–140	111±16	84–157	0.48	0.16 (−0.27 to 0.58)
RV	%pred	126±36	13–219	125±36	15–219	127±39	13–213	0.80	0.06 (−0.36 to 0.48)
RV/TLC	%	39±9	20–57	38±9	20–57	40±8	20–54	0.28	0.22 (−0.20 to 0.65)
sR _{tot}	%pred	73±32	22–178	72±31	22–178	75±33	26–154	0.65	0.10 (−0.32 to 0.52)
Gas transfer									
K _{co}	%pred	96±11	80–135	97±12	80–135	93±9	80–114	0.12	0.31 (−0.12 to 0.73)
TLCO	%pred	87±12	58–134	87±13	65–134	85±10	58–106	0.34	0.19 (−0.24 to 0.61)
Impulse oscillometry									
R5	%pred	96±20	53–144	95±20	53–137	98±19	57–144	0.43	0.17 (−0.26 to 0.59)
D5–20	%	15±14	0–57	14±14	0–57	17±12	0–52	0.21	0.26 (−0.17 to 0.68)
Ax	kPa/L	0.32±0.30	0.0–1.42	0.31±0.33	0.0–1.42	0.32±0.24	0.03–1.35	0.96	0.01 (−0.42 to 0.44)
F _{res}	Hz	12±4	3–23	11±4	3–23	12±3	7–19	0.27	0.22 (−0.21 to 0.64)
Multiple breath washout									
LCI _{2.5}	–	7.2±1.0	5.7–10.2	7.1±0.9	5.7–10.2	7.4±1.0	5.9–9.9	0.07	0.41 (−0.02 to 0.84)
LCI ₅	–	5.6±0.7	4.5–7.4	5.6±0.7	4.5–7.4	5.8±0.6	4.7–7.2	0.08	0.39 (−0.05 to 0.81)
FRC _{MBW}	L	2.9±0.7	1.6–4.6	3.0±0.7	1.6–4.6	2.8±0.7	1.9–4.3	0.37	0.19 (−0.61 to 0.24)
S _{acin}	L ^{−1}	0.09±0.07	−0.04 to 0.36	0.08±0.06	−0.04 to 0.28	0.11±0.08	−0.04 to 0.36	<0.05*	0.54 (0.07 to 1.00)
S _{cond}	L ^{−1}	0.04±0.04	−0.07 to 0.2	0.04±0.04	−0.05 to 0.2	0.04±0.05	−0.07 to 0.16	0.78	0.07 (−0.38 to 0.51)

Effect size (ES) as measured by Cohen's d with 95% CI.

*Statistically significant at p<0.05 (never-smoker vs smoker).

Ax, area under reactance curve; D5–20, frequency dependence of resistance; FRC, functional residual capacity; F_{res}, resonance frequency; K_{co}, Krogh factor; LCI, lung clearance index at 2.5% and 5% stopping points; MBW, multiple breath washout; MEF, maximum expiratory flow at 75%, 50% and 25% of FVC; %pred, percent of predicted; R5, resistance at 5 Hertz; RV, residual volume; S_{acin}, acinar ventilation heterogeneity; S_{cond}, conductive ventilation heterogeneity; sR_{tot}, specific total airway resistance; TLC, total lung capacity; TLCO, transfer factor for carbon monoxide; VC, vital capacity.

Figure 1 LCI_{2.5} normal values. Age-dependent plot of predicted values (solid line) with ULN and LLN. (A) Never-smokers (n=68) provide overall lower predictions as compared with (B) additionally including former or active smokers (n=100). Dashed lines and lighter grey represent a model-based extrapolation of actually measured cross-sectional data. LCI_{2.5}, lung clearance index at 2.5% stopping point; LLN, lower limit of normal; ULN, upper limit of normal.

(CI 0% to 13%) in S_{acin} and 3% (CI 0% to 13%) in S_{cond} , respectively. We therefore calculated normal values with ULN and LLN corrected for age.

Reference values

Figure 1 gives age-dependent predictions for LCI_{2.5} in the overall collective and never-smokers only. Likewise, predicted values for phase III slopes are given in figure 2. The underlying models are available for download from GitHub (<https://github.com/ftrinkmann>). A summary of calculated reference values per decade is given in table 3. Age-dependent predictions for LCI₅ in the overall collective and never-smokers can be found in online supplemental e-Figure 1.

DISCUSSION

We found that age is the only anthropometric parameter contributing to SF₆-MBW outcome measures. The effect was most pronounced for global and acinar ventilation heterogeneity and smaller for conductive ventilation heterogeneity. No influence of height, weight and sex was seen in our adult population. On this basis, we were able to generate age-corrected reference values for SF₆-MBW ranging from early adulthood to old age.

Our results extend the data available for paediatric cohorts and are in accordance with previous findings. Lum and coworkers²⁶ could demonstrate that LCI decreases with height

during the first approximately 6 years of life and then remains rather stable after reaching around 115 cm. Hence, they proposed to use a constant ULN of 7.56 defined by the 97.5th percentile in subjects older than 6 years, which corresponds well to our model. No correlation between age and LCI was found when including younger collectives.^{27 28} With the normal range appearing to remain stable for the first four decades of life, a fixed ULN of 6.8 and 7.0 was proposed by Horsley *et al*²⁸ and Fuchs *et al*.²⁹ In patients older than 40 years a larger dispersion was seen. Despite all of these approaches being based on SF₆, small differences may be partly explained by system set-ups and tracer gas concentrations. Considerably larger differences can be attributed to tracer gases themselves as compared with set-up. It is well known that N₂-MBW reproducibly yields higher absolute LCI readings than SF₆-MBW.^{17 18} Much of this difference can be explained by physiological properties and technical aspects associated with the test gases.^{9 12 17 19} Concerning local ventilation heterogeneity, age was found to explain 7% and 16% of the variability in S_{acin} and S_{cond} derived from N₂-MBW, respectively.³⁰ Moreover, a significant influence of sex on S_{acin} as well as age on LCI was found. This corresponds to our data set where 10% of the variability in S_{cond} is explained by age. In contrast, contribution for LCI and S_{acin} is higher in our collective with 47% and 32%. Reference intervals for parameters of local ventilation heterogeneity using N₂ as tracer gas were higher than those found for SF₆ in our data at hand. Interestingly, mean LCI reference values were lower in their investigation than those for SF₆-MBW. This is contradictory to our findings and previously published data. While N₂ readings were found to be consistently higher in general as discussed above, Houlitz and coworkers³¹ could demonstrate higher mean predictions for LCI over a wide range between 7 and 70 years. Verbanck and coworkers found a 0.22-unit change in LCI per decade, while we could demonstrate an increasing rate with age between 0.2 and 0.5 units per decade.³⁰ It should be noted that including smokers led to a 0.3 increase in mean LCI, corresponding to a 10-year effect of ageing. In addition to differences in test gases and set-ups, parts of these differences may be explained by the sequence of lung function tests. Potential airway dilatation may lead to distortion of tidal breathing techniques. Therefore, we conducted all examinations requiring tidal breathing strictly before forced manoeuvres. Taken together, either test gas requires separate reference data and individual values should not be used interchangeably.

When interpreting our results, several aspects should be taken into consideration. For S_{acin} and MEF_{2.5}, significant differences were found between smokers and only never-smokers, respectively. For generating reference values, it would have been desirable to strictly include only never-smokers. However, this would have narrowed the age range investigated considerably as finding otherwise healthy, never-smoking septuagenarians and octogenarians is particularly difficult. We therefore applied an extremely strict definition for controls. This did not only include commonly used history, respiratory symptoms and spirometric findings. Additionally, we required parameters from whole-body plethysmography, oscillometry and gas transfer to be within the normal range for both never-smoking and smoking controls, respectively. As a consequence, smoking status explains a maximum of 4% of the variability in MBW parameters. Therefore, calculation of normal values itself is hardly affected by smoking status using our GAMLSS approach.

Further, it should be noted that a solid definition of normality is difficult especially in elderly patients. This is important in the context of the rather broad range of residual volume/total lung capacity (RV/TLC) (20%–57%) and TLCO (58%–134%

Figure 2 Phase III slope normal values. Age-dependent plot of predicted values (solid line) with ULN and LLN. (A) Never-smokers (n=68) provide overall lower predictions and less age dependency as compared with (B) additionally including former or active smokers for S_{acin} (n=100). (C) Marginal differences were found for S_{cond} in never-smokers (n=68) as compared with (D) additionally including former or active smokers (n=100). Dashed lines and lighter grey represent a model-based extrapolation of actually measured cross-sectional data. LLN, lower limit of normal; S_{acin} , acinar ventilation heterogeneity; S_{cond} , conductive ventilation heterogeneity; ULN, upper limit of normal.

of predicted) found in our study. An increased RV/TLC as well as differences between TLCO and K_{CO} are suggestive of ventilation heterogeneity and air-trapping. However, most of the values at the upper end (RV/TLC) or lower end (TLCO) were found in subjects well above 70 years of age. While reference data for body plethysmography are scarce in this age group, none of the subjects reported respiratory symptoms nor showed abnormal values in standard diagnostics. Moreover, we did not find disproportional deviations for ventilation heterogeneity, peripheral resistance or elastic properties. Taken together, this lets us assume that ageing effects within the physiological range

rather than unrecognised disease can be considered responsible for these changes.

Although age was not associated with S_{cond} in the regularised model, we decided to also provide age-corrected reference values since it explains as much as 10% of the variance. S_{cond} is primarily subject to heterogeneity in pressure volume characteristics and bronchomotor tone of conductive airways.³² In contrast, S_{acin} is heavily depending on branching asymmetry as well as parallel variability of the acini.³³ This does not only explain differences between subjects, diseases or risk factors. Also, the different behaviour of ageing on S_{acin} and S_{cond} found in our investigation may be attributable to these factors. This is substantiated by previous findings. Considerable effects of ageing were described on airflow dynamics in a computational model.³⁴ These correspond to ventilation defects found on imaging in otherwise healthy elderly subjects.³⁵ However, no longitudinal data are currently available for functional tests and should therefore be subject of further investigation.

In contrast to the high intratest and intertest variability of traditional parameters of small airway obstruction such as MEF,^{36 37} LCI was shown to be highly reproducibly.²³ Variability of phase III slopes is larger as compared with the CV of up to 34% shown for MEF.³⁷ Clinical implications of these have not been conclusively investigated to date. Nevertheless, impairment of S_{acin} in patients with pulmonary hypertension allows to assume that early changes can already be detected and tracked today.³⁸ In smokers with normal spirometry, changes in S_{cond} were found and improvements to normal remained 12 months after smoking cessation.³⁹ These findings are supported by data in patients with cystic fibrosis⁴⁰ and adults with asthma.⁴¹ S_{cond} and S_{acin} may not represent all ventilation heterogeneities covered by LCI. Vice versa, LCI may not represent all ventilation heterogeneities

Age (years)	LCI _{2.5} (–)		LCI ₅ (–)		S_{acin} (L ^{–1})		S_{cond} (L ^{–1})	
	Mean	ULN	Mean	ULN	Mean	ULN	Mean	ULN
10	6.1	7.2	4.9	5.7	0.03	0.12	0.02	0.09
20	6.3	7.4	5.0	5.8	0.04	0.13	0.02	0.09
30	6.5	7.6	5.2	6.0	0.05	0.14	0.03	0.10
40	6.8	7.9	5.3	6.1	0.06	0.15	0.03	0.10
50	7.1	8.2	5.6	6.4	0.08	0.17	0.03	0.10
60	7.4	8.5	5.8	6.6	0.10	0.19	0.04	0.11
70	7.8	8.9	6.1	6.9	0.13	0.22	0.05	0.12
80	8.3	9.4	6.3	7.1	0.17	0.26	0.07	0.14
90	8.8	9.9	6.6	7.4	0.21	0.30	0.08	0.15
100	9.3	10.4	6.9	7.7	0.25	0.33	0.10	0.17

LCI, lung clearance index at 2.5% and 5% stopping points; S_{acin} , acinar ventilation heterogeneity; S_{cond} , conductive ventilation heterogeneity; ULN, upper limit of normal.

covered by either S_{cond} or S_{acin} .⁴² Therefore, a combined interpretation of parameters representing global and local ventilation heterogeneity seems promising. In contrast, MEF-derived parameters were shown to not contribute usefully to clinical decision making.⁴³

Total test duration is important in MBW testing. In general, washout is time-critical in N_2 -MBW, whereas wash-in and costs are relevant factors in SF_6 -MBW. Shorter washout durations are therefore an advantage of SF_6 allowing successful measurements also in patients with severe obstructive lung disease.^{44–47} Nevertheless, it should be noted that requiring a wash-in phase may neutralise some of the advantages of SF_6 . For both tracer gases, several adaptations to the measurement protocols have been proposed, including reduction of total measurements or earlier cut-offs for terminating the washout.^{44–47} Moreover, closed-circuit SF_6 -MBW was shown to effectively reduce wash-in times.¹⁵ We therefore calculated reference values for an earlier 5% stopping point reducing washout times considerably by 24%. Previously, an additional 34% reduction of total test time was achieved when only conducting two technically acceptable measurements.²³ Together these adaptations may increase success rates in general. However, it should be noted that a comparative evaluation of clinical endpoints and diagnostic performance is warranted especially concerning abbreviated washout.

Our data considerably extend the current knowledge in adult SF_6 -MBW testing. Reference values can now be provided over the whole age range. For the first time in adult MBW testing, this is not only restricted to the most common parameter LCI, but also includes phase III slopes S_{acin} and S_{cond} . While extrapolations of our model fit well to available data for subjects older than 6 years, normative data for phase III parameters that can be used for comparison are overall scarce. We were able to acquire local ventilation heterogeneity parameters in a considerable number of subjects but also encountered some difficulties. Analysis was not successful in 10 subjects. This was most often due to either not having sufficient phase III to compute slopes or an intermittently irregular breathing pattern. Together with the overall variability of these analyses, further research is warranted especially concerning clinical outcome studies. Our findings were acquired exclusively in white European adults sharing the lack of different ethnicities as a major drawback of most cohorts. Nevertheless, our model provides a sound basis for calculation of normal values for both research settings and clinical routine. Finally, two methodological considerations should also be taken into account. First, reference values for conventional lung function tests were derived from ECSC equations.²¹ Second, we used commercially available gas cylinders. These also contain N_2O , which is identical to the mixture used for cardiac output determination while differing in bolus fraction.^{14–48} Although N_2O is dispensable for MBW measurements, we were not able to use an N_2O -free mixture due to lack of availability.

CONCLUSIONS

SF_6 -MBW parameters showed an age-dependent increase from early adulthood to old age. The effect was most pronounced for global and acinar ventilation heterogeneity. A smaller yet still considerable influence was seen for conductive ventilation heterogeneity. No influence of other anthropometric parameters such as height, weight and sex was found. Together with previous findings, reference values for LCI can now be provided over the whole age range as well as for an earlier 5% stopping point. Moreover, our data are not restricted to global ventilation

heterogeneity but also comprise the parameters of local ventilation heterogeneities S_{acin} and S_{cond} .

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Acknowledgements We thank all subjects who agreed to take part in the study, as well as our technical assistants Maria Moritz and Sabrina Kraemer (1st Department of Medicine, University Medical Centre Mannheim) for their help in acquiring lung function data.

Contributors All authors read and approved the final version of the manuscript. FT: writing the first draft of the manuscript, study design, data management, data analysis. MM: data analysis, statistical advice. KR: data collection, data management. AH: data management, data collection. JuS: data management, data analysis. JG: data analysis, study design, data management. JoS: study design, data analysis. IA: study design, revision of the first draft of the manuscript. MB: study design, revision of the manuscript. FJFH: study design, data analysis, revision of the manuscript. TG: data analysis, data management, revision of the first draft of the manuscript.

Funding Parts of our work received external funding from Ms Ester Knorr (Germany, private donation) and Markedsmodningsfonden (Denmark, public grant). Internal financial support was given by Heidelberg University (MEAMEDMA, internal grant).

Competing interests The following financial activities outside the submitted work exist: FT received travel support from Actelion, Berlin Chemie, Boehringer Ingelheim, Chiesi, Novartis, Mundipharma and TEVA, as well as speaker or consultation fees from AstraZeneca, Berlin Chemie, Boehringer Ingelheim, Bristol-Myers Squibb, Chiesi, GlaxoSmithKline, Roche, Novartis and Sanofi-Aventis. JoS received travel support and speaker fees from Boehringer Ingelheim, GlaxoSmithKline, Novartis and Roche. MB received speaker or consultation fees from Bayer, Boehringer Ingelheim, Daiichi Sankyo, Impulse Dynamics and Zoll Medical. IA received travel support as well as speaker or consultation fees from Abiomed, Bayer, Boehringer Ingelheim and St Jude Medical.

Patient consent for publication Not required.

Ethics approval The study protocol was approved by the Medical Ethics Committee II of the Medical Faculty Mannheim (Heidelberg University), compliant with the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. The models generated and analysed during the current study are available for download from an online repository (<https://github.com/ftrinkmann>).

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