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The inspired sine-wave technique: A novel method to measure lung volume and ventilatory heterogeneity

Richard M. Bruce[1] | **Phi Anh Phan**[2] | **Edmund Pacpaco**[3] | **Najib M. Rahman**[3] | **Andrew D. Farmery**[2]

[1]Centre for Human and Applied Physiological Sciences, School of Basic and Medical Biosciences, Shepard's House, Guy's Campus, King's College London, London, UK

[2]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK

[3]Oxford University Hospitals NHS Foundation Trust, Oxford Centre for Respiratory Medicine, Oxford, UK

Correspondence
Richard M. Bruce, School of Basic and Medical Sciences, Shepard's House, Guy's Campus, Kings College London, London SE1 1UL, UK.
Email: richard.bruce@kcl.ac.uk

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Abstract

The inspired sine-wave technique (IST) is a new method that can provide simple, non-invasive cardiopulmonary measurements. Over successive tidal breaths, the concentration of a tracer gas (i.e. nitrous oxide, N_2O) is sinusoidally modulated in inspired air. Using a single-compartment tidal-ventilation lung model, the resulting amplitude/phase of the expired sine wave allows estimation of end-expired lung volume (ELV), pulmonary blood flow and three indices for ventilatory heterogeneity (VH; ELV_{180}/FRC_{pleth}, ELV_{180}/FRC_{pred} and ELV_{60}/ELV_{180}). This investigation aimed to determine the repeatability and agreement of ELV with FRC_{pleth} and, as normal ageing results in well-established changes in pulmonary structure and function, whether the IST estimates of ELV and VH are age dependent. Forty-eight healthy never-smoker participants (20–86 years) underwent traditional pulmonary function testing (e.g. spirometry, body plethysmography) and the IST test, which consisted of 4 min of quiet breathing through a face mask while inspired N_2O concentrations were oscillated in a sine-wave pattern with a fixed mean (4%) and amplitude (3%) and a period of either 180 or 60 s. The ELV_{180}/FRC_{pleth} and ELV_{180}/FRC_{pred} were age dependent (average decreases of 0.58 and 0.48% $year^{-1}$), suggesting an increase in VH with advancing age. The ELV showed a mean bias of −1.09 litres *versus* FRC_{pleth}, but when normalized for the effects of age this bias reduced to −0.35 litres. The IST test has potential to provide clinically useful information necessitating further study (e.g. for mechanically ventilated or obstructive lung disease patients), but these findings suggest that the increases in VH with healthy ageing must be taken into account in clinical investigations.

KEYWORDS
ageing, pulmonary function testing, respiratory physiology

1 | INTRODUCTION

Traditional pulmonary function tests, such as spirometry and whole-body plethysmography, are a crucial aspect of the diagnosis, characterization and management of lung disease (Pellegrino et al., 2005). However, they require specific and often unfamiliar respiratory manoeuvres, which make them unsuitable for critically ill or sedated patients, and their reproducibility is reduced in both elderly and paediatric populations (Bellia et al., 2000; Beydon et al., 2007). The inspired sine-wave technique (IST) is a new method of measuring or continuously monitoring cardiopulmonary function, which requires only passive patient cooperation (Clifton, Clifton, Hahn, & Farmery, 2013; Farmery, 2008; Phan, Farmery, & Hahn, 2015). Therefore, the technology can provide simple, non-invasive cardiopulmonary measurements in both the outpatient and critical care settings.

Over successive tidal breaths, the concentration of a tracer gas (e.g. nitrous oxide, N_2O) is sinusoidally modulated in inspired air. The amplitude and phase of the expired sine wave are altered by the pulmonary ventilation and blood flow (if the tracer gas is soluble) and distorted further by ventilatory heterogeneity (VH). A mathematical model of the lung is used to process flow and concentration data and recover values for cardiorespiratory variables, such as end-expired lung volume (ELV) and pulmonary blood flow ($\dot{Q}_P$), and, in addition, indices for VH can be calculated.

The technique originated from the work of Zwart and colleagues (Zwart, Bogaard, Jansen, & Versprille, 1978; Zwart, Seagrave, & Van

New Findings

- **What is the central question of this study?**
  We present a new non-invasive medical technology, the inspired sine-wave technique, which involves inhalation of sinusoidally fluctuating concentrations of a tracer gas. The technique requires only passive patient cooperation and can monitor different cardiorespiratory variables, such as end-expired lung volume, ventilatory heterogeneity and pulmonary blood flow.
- **What is the main finding and its importance?**
  In this article, we demonstrate that the measurements of end-expired lung volume are repeatable and accurate, in comparison to whole-body plethysmography, and the technique is sensitive to the changes in ventilatory heterogeneity associated with advancing age. As such, it has the potential to provide clinically valuable information.

Dieren, 1976) and was extended by Hahn, 1996, Hahn, Black, Barton, & Scott (1993) by using patient-safe gases, such as O_2 and low concentrations of N_2O, and developing a more realistic model of tidal ventilation of the lung (Gavaghan & Hahn, 1996; Whiteley, Gavaghan, & Hahn, 2001). Preliminary investigations in animals and human particpants have since shown that the technique has the potential to provide useful measures of cardiorespiratory function (Clifton et al., 2013; Farmery, 2008; Williams et al., 1998). In this investigation, we initially aimed to determine the accuracy of the IST measurment of ELV in healthy particpants, by examing its agreement with functional residual capacity (FRC) measured via whole-body plethysmography (FRC_{pleth}), and to examine the repeatability of the ELV measurement. Moreover, as healthy ageing results in several alterations in pulmonary structure and function, such as increases in lung compliance, the enlargement of airspaces and increases in VH (Gillooly & Lamb, 1993; Turner, Mead, & Wohl, 1968; Verbanck et al., 2012), in this investigation we also aimed to assess whether the IST estimates of ELV and VH are age dependent.

2 | METHODS

2.1 | Ethical approval

All participants received verbal and written information regarding the protocol and experimental procedures before giving their written informed consent. The protocol for the study was approved by an NHS ethical committee (16/SC/0057, ethics protocol 1.0) and conforms to the *Declaration of Helsinki, 2013*, except for registration in a database. Forty-eight never-smoker participants volunteered for the study; characteristics and pulmonary function test data are shown in Table 1. The age of participating subjects ranged between 20 and 86 years, and all were defined as healthy through clinical screening with the following criteria: no childhood/past medical history of respiratory disease, no history of respiratory symptoms suggestive of disease, no upper respiratory tract infections in the previous 8 weeks, and no history of smoking.

TABLE 1 Participant characteristics and pulmonary function test measurements ($n = 48$; 26 men and 22 women)

	Mean (SD)	Percentage of predicted (SD)
Age (years)	53.5 (22)	—
Height (m)	1.7 (0.1)	—
Weight (kg)	71.5 (12)	—
BMI (kg m^{-2})	24.9 (4.2)	—
FEV_1 (litres)	3.3 (1)	102.4 (17.1)
FVC (litres)	4.3 (1.3)	105.6 (18.5)
FEV_1%FVC	77.8 (6.9)	96.5 (7.5)
TLC (litres)	6.3 (1.3)	105.4 (13.2)
RV (litres)	2.2 (0.8)	104.4 (20.7)
FRC (litres)	3.4 (0.8)	107.5 (16.6)
T_{LCO}	7.8 (2.6)	98.7 (15.5)
K_{CO}	1.5 (0.3)	94.6 (16)
VA/TLC	0.87 (0.05)	—
ELV_{180}	2.3 (0.8)	—
ELV_{60}	1.5 (0.5)	—
$\dot{Q}_P$	4.9 (1.7)	—

Abbreviations: BMI, body mass index; ELV_{180} and ELV_{60}, inspired sine-wave technique estimate of end-expired lung volume measured using a sine-wave with a 180 or a 60 s period, respectively; FEV_1, forced expiratory volume in 1 s; FRC, functional residual capacity; FVC, forced vital capacity; K_{CO}, transfer coefficient for carbon monoxide; $\dot{Q}_P$, inspired sine-wave technique estimate of pulmonary blood flow; RV, residual volume; TLC, total lung capacity; T_{LCO}, transfer factor for carbon monoxide; and VA/TLC, ratio between a single-breath helium-dilution TLC measurement (VA) and TLC from whole-body plethysmography. The percentage predicted values were obtained via reference values (Quanjer et al., 1993, 2012).

2.2 | Study protocol

All procedures for each participant were performed on the same day within the Respiratory Medicine Department of the Churchill Hospital, Oxford University Hospitals NHS Foundation Trust. Before testing, a medical history and anthropometric data were recorded. Participants then underwent traditional pulmonary function testing: spirometry, body plethysmography and the single-breath test of carbon monoxide uptake (Jaeger MasterScope Body; Carefusion, Höchberg, Germany); followed by the IST test.

2.3 | Inspired sine-wave technique test

Participants were seated and asked to breathe quietly through a face mask connected to a mainstream infrared N_2O and CO_2 sensor (IRMA, Danderyd, Sweden) and an ultrasonic flowmeter (VenThor – 22/2A, Budapest, Hungary); see Figure 1 for a schematic representation of the set-up. At the start of each inhalation, a small quantity of N_2O was injected into the participant's inspired gas by a mass flow controller (Alicat Scientific, Inc., Tucson, AZ, USA). The volume injected was

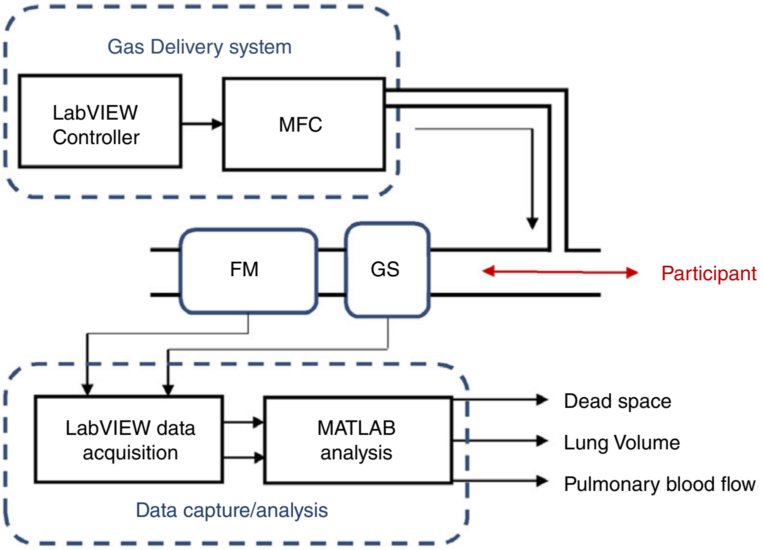

FIGURE 1 Schematic representation of the inspired sine-wave device. The participant inhales and exhales air through a flowmeter (FM) and gas sensor (GS). At the start of each inspiration, a small quantity of N_2O is delivered into the participant's inspired gas via a mass flow controller (MFC). A mathematical model of the lung processes the flow and concentration data and recovers values for cardiorespiratory variables, such as dead space, alveolar volume and pulmonary blood flow, and, in addition, an index for ventilation heterogeneity can be calculated

proportional to each breath's inspiratory flow, and over successive breaths the concentration of inspired N_2O oscillates in a sine-wave pattern around a set mean (4%) with a predetermined amplitude (3%) and frequency (60 or 180 s period). The inspired N_2O fraction can therefore be defined as follows:

$$F_I(t) = \bar{F}_I + \Delta F_I \sin\left(\frac{2\pi}{T}t + \Phi\right) \quad (1)$$

where $\bar{F}_I$ and ΔF_I are the mean and amplitude of the N_2O sinusoid, respectively, T is the period, and Φ is the phase.

In the absence of venous recirculation of the N_2O sine wave, which is known to be negligible at the periods used in the present study, i.e. <5 min (Gavaghan & Hahn, 1995; Hahn et al., 1993), forcing sinusoidal inspired N_2O concentrations results in the end-tidal (i.e. alveolar) N_2O concentrations also oscillating sinusoidally. Figure 2 shows a typical data set recorded from the IST test.

Using a single-compartment tidal ventilation model of the lung, the resulting amplitude and phase of the expired sine wave allow the estimation of $\dot{Q}_P$ and alveolar volume (V_A). The sum of V_A and airways dead space (V_D), as measured via the Bohr technique (Phan, Hahn, & Farmery, 2017), from the IST estimate of ELV. The period (in seconds) of the inspired N_2O sine wave used to estimate ELV is signified by the subscript, i.e. ELV_{180} or ELV_{60}. Modelling and preliminary empirical data have shown that ELV_{180} has the closest agreement with FRC_{pleth}. The lung model and estimation steps have been described elsewhere (Clifton et al., 2013; Gavaghan & Hahn, 1996; Phan et al., 2015), a summary of which is given in the Appendix.

2.4 | Indices of ventilation heterogeneity (VH)

Three potential indices are proposed: (i) ELV_{180}/FRC_{pleth}; (ii) ELV_{180}/FRC_{pred}; and (iii) ELV_{60}/ELV_{180}. Lower values suggest greater VH, and whereas values close to one suggest homogeneity.

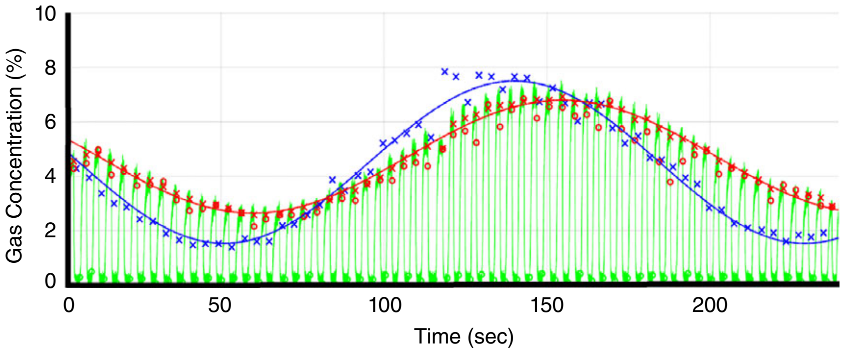

FIGURE 2 A typical data set collected from one participant during a 4 min inspired sine-wave technique test. The green line is the expired N_2O concentration measured by the mainstream infrared gas sensor. The blue and red crosses are the N_2O concentrations in inspired gas and end-tidal gas, respectively. The red open circles denote the beginning of inspiration, when the gas is delivered. The blue and red lines are the inspired and expired N_2O sine waves, respectively

The first two indices rely on the nature of single-compartment models used to estimate ELV. For the IST, ELV measurements in a unicompartmental lung are a predicable function of ventilation, the sine-wave period and the attenuation of the expired sine wave, as described by Equation (1), and errors (underestimations) in the calculated volume suggests a degree of VH (Whiteley et al., 2001). As such, increasing heterogeneity will result in greater disparity between ELV_{180} and FRC_{pleth}, or the FRC estimated via prediction equations (FRC_{pred}):

$$\text{Male } FRC_{pred} = 2.34 \times H + 0.01 \times A - 1.09$$

$$\text{Female } FRC_{pred} = 2.24 \times H + 0.001 \times A - 1$$

where H is standing height (in metres) and A is age (in years; Quanjer et al., 1993).

The third index relies on ELV measurements becoming more dependent on sine-wave period with increasing VH. In a homogeneous (unicompartmental) lung, the ELV measurement should be unaffected by the period used. However, preliminary modelling and experimentation has uncovered that where VH exists, the estimated cardiopulmonary variables become period dependent (Whiteley et al., 2001). Therefore, the ratio of two ELV measurements made using different sine-wave periods (ELV_{60} and ELV_{180}) might estimate the degree of VH.

In addition, a further simple index (VA/TLC) of gas mixing inefficiency and VH has been calculated, which is the ratio between a single-breath helium-dilution total lung capacity (TLC) measurement (VA) and a TLC measurement from whole-body plethysmography (Cotes, Chinn, & Miller, 2006).

2.5 | Inspired sine-wave technique test protocol

Participants were seated and rested for 5 min before each IST test. For the duration of the IST test, participants were asked to breathe quietly through a face mask, sealed with no leaks, held up in front of them by an adjustable articulating arm. All participants performed two IST tests; 3 min of inhaling a constant concentration of N_2O (4.2%) followed by 4 min of forcing an inspired N_2O sine wave (mean = 4%, amplitude = 3%) at periods of 180 and 60 s. Participants then repeated these two tests after a 15 min interval.

2.6 | Statistical analysis

All statistical analysis was conducted using Microsoft Excel (2016) and a standard statistical package (SPSS, Chicago, IL, USA). The agreement between FRC_{pleth} and ELV_{180} was assessed using Bland–Altman analysis. The repeatability of duplicate ELV_{180} measurements was assessed using linear regression analysis, Bland–Altman analysis and the coefficient of variation (standard deviation/mean × 100). The first and second ELV_{180} measurements were also compared using Student's paired t test, with statistical significance taken as $P < 0.05$. Multiple stepwise regression analysis was performed on the three VH indices (ELV/FRC_{pleth}, ELV/FRC_{pred} and ELV_{60}/ELV_{180}), and independent variables included age, sex, height and FRC_{pleth}. Multiple stepwise regression analysis was also performed for ELV_{180} and FRC_{pleth}, and independent variables included age, sex and height. The statistical significance level for retention was set at $P < 0.05$. When sex was uncovered as a significant factor for the ELV_{180} and FRC_{pleth} measurements, multiple regression analysis was repeated for each sex. Pearson's correlation coefficient was also calculated for the three IST indices of VH and pulmonary function test measurements: forced expiratory volume in 1 s, forced expiratory volume in 1 s/forced vital capacity and VA/TLC.

3 | RESULTS

3.1 | Indices of ventilation heterogeneity

Figure 3a, b shows that ELV_{180}/FRC_{pleth} and ELV_{180}/FRC_{pred} decrease as a function of age, suggesting an increase in VH. The other assessed independent variables (sex, height and FRC_{pleth}) did not

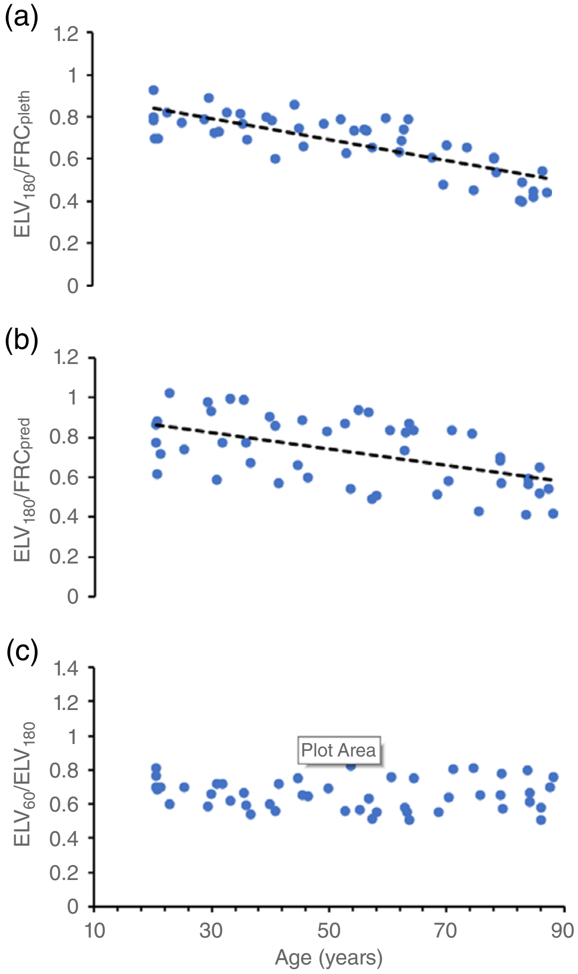

FIGURE 3 (a–c) Scatterplot of ELV_{180}/FRC_{pleth}, ELV_{180}/FRC_{pred}, and ELV_{60}/ELV_{180} *versus* age with a linear regression line. Abbreviations: ELV_{180} and ELV_{60}, inspired sine-wave technique estimate of end-expired lung volume measured using a sine-wave with a 180 or a 60 s period, respectively; and FRC_{pleth}, functional residual capacity measured via whole-body plethysmography

TABLE 2 Regression equations for inspired sine-wave technique indices of ventilatory heterogeneity (ELV$_{180}$/FRC$_{\text{pleth}}$, ELV$_{180}$/FRC$_{\text{pred}}$ and ELV$_{60}$/ ELV$_{180}$) and for ELV$_{180}$ and FRC$_{\text{pleth}}$

	Regression equations	Adjusted *R*2
Indices of ventilatory heterogeneity		
ELV$_{180}$/FRC$_{\text{pleth}}$	$-0\ 0048 \times A + 0.9427$	0.62
ELV$_{180}$/FRC$_{\text{pred}}$	$-0\ 0041 \times A + 0.9478$	0.28
ELV$_{60}$/ELV$_{180}$	–	–
Lung volumes		
ELV$_{180}$		
Men	$4.956 \times H - 0.012 \times A - 5.325$	0.71
Women	$4.292 \times H - 0.007 \times A - 4.794$	0.79
FRC$_{\text{pleth}}$		
Men	$5.635 \times H + 0.008 \times A - 4.989$	0.71
Women	$4.989 \times \text{H} + 0.013 \times A - 5.928$	0.69

Abbreviations: ELV$_{180}$ and ELV$_{60}$, inspired sine-wave technique estimate of end-expired lung volume measured using a sine-wave with a 180 or a 60 s period, respectively; FRC$_{\text{pleth}}$, functional residual capacity measured via whole-body plethysmography; *H*, height (in metres); *A*, age (in years).

TABLE 3 Pearson's correlation coefficients (*r*) for inspired sine-wave technique indices of ventilatory heterogeneity (ELV$_{180}$/FRC$_{\text{pleth}}$, ELV$_{180}$/FRC$_{\text{pred}}$ and ELV$_{60}$/ ELV$_{180}$), with FEV$_1$, FEV$_1$/FVC and VA/TLC

Indices of ventilatory heterogeneity	FEV$_1$	FEV$_1$/FVC	VA/TLC
ELV$_{180}$/FRC$_{\text{pleth}}$	0.63*	0.34*	0.56*
ELV$_{180}$/FRC$_{\text{pred}}$	0.64*	0.15	0.57*
ELV$_{60}$/ELV$_{180}$	0.08	–0.02	0.04

Abbreviations: ELV$_{180}$ and ELV$_{60}$, inspired sine-wave technique estimate of end-expired lung volume measured using a sine-wave with a 180 or a 60 s period, respectively; FEV$_1$, forced expiratory volume in 1 s; FRC$_{\text{pleth}}$, functional residual capacity measured via whole-body plethysmography; FVC, forced vital capacity; and VA/TLC, ratio between a single-breath helium-dilution TLC measurement (VA) and TLC from whole-body plethysmography.

$^*P < 0.05$.

reach statistical significance in the multiple regression analysis for all three heterogeneity indices, and although age was a significant contributor to the regressions of ELV$_{180}$/FRC$_{\text{pleth}}$ and ELV$_{180}$/FRC$_{\text{pred}}$ ($P < 0.05$), it did not reach significance for ELV$_{60}$/ELV$_{180}$ (Figure 3c). Regression equations for these VH indices are shown in Table 2, and their correlation with pulmonary function test measurements is shown in Table 3.

3.2 | Lung volume (ELV$_{180}$): repeatability and agreement with FRC$_{\text{pleth}}$

The mean measured ELV$_{180}$ was 2.31 litres (±0.75; range: 1.01–3.55). Bland–Altman analysis (Figure 4) shows that the mean difference between repeated measurements was –0.05 litres (±0.2), 1.29% of the mean, and the 95% limits of agreement were between –0.42 and 0.31 litres. The coefficient of variation for repeated ELV$_{180}$ measurements was 4.8%. Linear regression analysis between these measurements produced an R^2 of 0.94, $y = 0.983 + 0.0871$ (Figure 5). The first and second measurement were not statistically different ($P = 0.76$).

The mean measured FRC$_{\text{pleth}}$ was 3.4 litres (0.85; range 2.11–5.19), with an average percentage predicted value of 107.5% (16.6; range 67.4–120). Bland–Altman analysis (Figure 6) shows that the mean differences between FRC$_{\text{pleth}}$ and ELV$_{180}$ measurements was −1.08 litres (±0.53), and the 95% limits of agreement were between −2.1 and –0.37 litres. Regression equations for ELV$_{180}$ and FRC$_{\text{pleth}}$ from the present cohort of participants are shown in Table 2. An age-normalized Bland–Altman plot is shown in Figure 7.

4 | DISCUSSION

4.1 | Lung volume

If we accept FRC$_{\text{pleth}}$ as the gold-standard measure of end-expiratory lung volume, the present study suggests that the IST test underestimates lung volume by ~1.09 litres. Underestimations are expected, because body plethysmography measures the volume of compressible gas in the thorax and inevitably includes volumes that are either located in regions that are poorly ventilated or that do not communicate with airways, including that within the abdominal cavity. This volume cannot be quantified by the IST test or traditional dilution tests (Schaanning & Gulsvik, 1973) and thus contributes to an inevitable bias.

However, the underestimation of ELV$_{180}$ relative to FRC$_{\text{pleth}}$ clearly has a component of age dependence. Multivariate linear regression analysis on the present cohort uncovered that FRC$_{\text{pleth}}$ increases by ~0.01 litres year^{-1} of age and, interesting, ELV decreases by approximately –0.01 litres year^{-1} (Table 2). This implies that the accuracy of the IST estimate of lung volume reduces with age (and probably reflects increasing VH; see section 4.2 Ageing and ventilatory heterogeneity). However, the agreement of ELV$_{180}$ with FRC$_{\text{pleth}}$ can be improved. Using the regression equations from the multivariate analysis, ELV$_{180}$ and FRC$_{\text{pleth}}$ can be normalized to that predicted of a 20-year-old for a given height and sex. Bland–Altman analysis of age-normalized ELV$_{180}$ and FRC$_{\text{pleth}}$ (Figure 7) shows a much smaller mean bias of –0.35 litres (with 95% limits of agreement at ±0.73 litres). This underestimation of ELV$_{180}$ is similar to that of helium-dilution measurement of FRC$_{\text{pleth}}$ (e.g. –0.3 litres; Schaanning & Gulsvik, 1973). As such, simply normalizing the ELV$_{180}$ measurement for age provides a more accurate measurement of end-expiratory lung volume.

The ELV measurement was very repeatable between tests separated by a 15 min period, with a coefficient of variation of 4.77%, i.e. similar that of whole-body plethysmography and helium-dilution measurements of end-expired lung volume, which range from approximately 4 to 7% and from 5 to 10%, respectively (Hankinson, Stocks, & Peslin, 1998). There was no effect of test order on the duplicated measurements, as shown by Bland–Altman analysis of the first and second ELV measurements (mean bias of 0.05 litres, 95% limits of agreement of 0.36) and the failure of their means to reach a statistically significant difference. The mean absolute percentage difference between the duplicated measurements was 7.3% (±7.6).

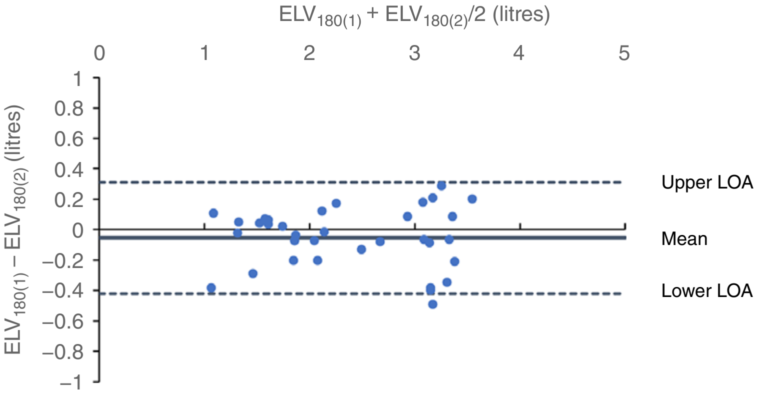

FIGURE 4 Bland–Altman plot showing the agreement of two repeated inspired sine-wave technique measurements of end-expired lung volume ($ELV_{180(1)}$ *versus* $ELV_{180(2)}$). Abbreviation: LOA, limits of agreement

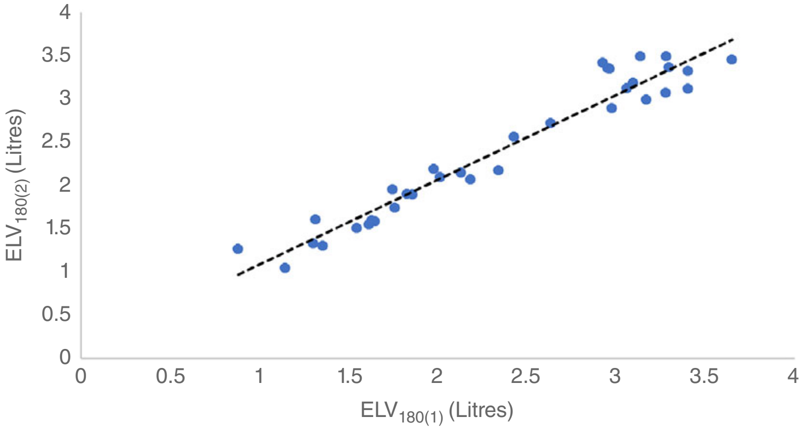

FIGURE 5 Linear regression analysis of the two repeated inspired sine-wave technique measurements of ELV_{180} ($ELV_{180(1)}$ *versus* $ELV_{180(2)}$)

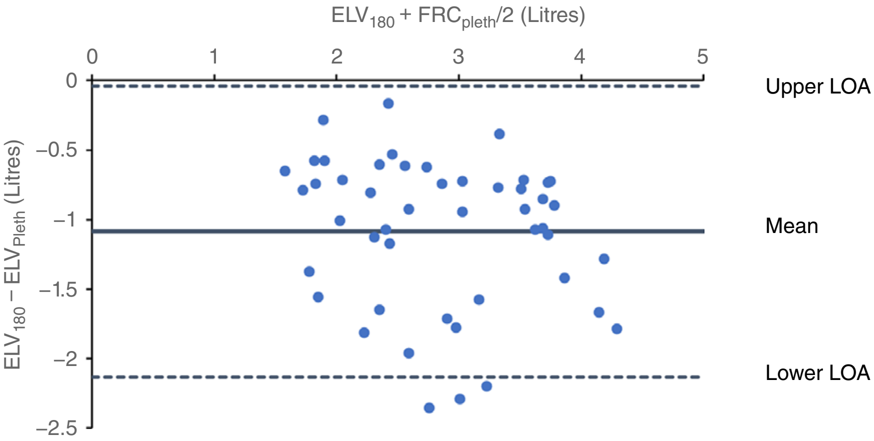

FIGURE 6 Bland–Altman plot showing the agreement between ELV_{180} and FRC_{pleth}. Abbreviation: LOA, limits of agreement

This complies with the current standard guidelines for acceptable differences (10%) between duplicated helium-dilution and nitrogen-washout measurements of lung volumes (Wanger et al., 2005).

Accurate and precise measurements of lung volume offer valuable information to respiratory clinicians, because alterations in lung mechanics occur in several pathologies (Pride & Macklem, 2011). In addition, the IST test has potential applications in the critical care setting, because continuous monitoring of ELV could be of great value in ventilated patients where positive end-expiratory pressures have been applied, by reducing the risk of ventilator-induced lung injury via volutrauma or atelectrauma (Slutsky & Ranieri, 2013). Further study in these specific patient groups is both warranted and needed.

However, although volume estimations are based upon a single-compartment lung model, significant respiratory disease and VH will probably impair the accuracy of the technique. Indeed, multicompartment lung models could be used to provide better

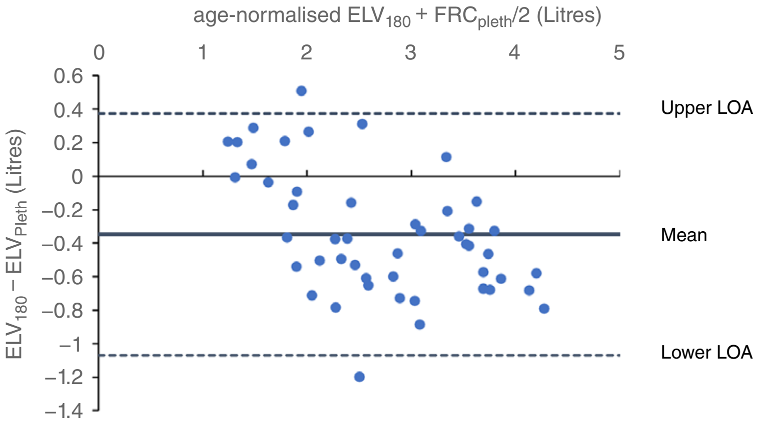

FIGURE 7 Bland–Altman plot showing the agreement between age-normalized ELV_{180} and FRC_{pleth}. Using the regression equations from multivariate analysis, ELV_{180} and FRC_{pleth} are normalized to that predicted of a 20-year-old for a given height and sex. Abbreviation: LOA, limits of agreement

estimates of lung volume where VH exists (Whiteley et al., 2001), and further work is required to examine this. However, an advantage of using unicompartmental models is that they can be more easily ‘inverted’, whereby recorded physiological data (e.g. the attenuation of the expired sine wave) can be inserted into them and recover estimations of cardiorespiratory parameters (e.g. ELV or $\dot{Q}_P$; Hahn & Farmery, 2003). Indeed, finding inverse solutions for these variables in the heterogeneous lung can be problematic when using more complex and mathematically flexible multicompartment models (Whiteley et al., 2001). In addition, the physiological interpretation of such models can be challenging for physiologists and clinicians. Overall, the practicality and value of using single-compartment models have ensured their continued use (Farmery, 2008; Hahn & Farmery 2003).

4.2 | Ageing and ventilatory heterogeneity

It is well established that ageing results in several changes in pulmonary structure and function, including an enlargement of airspaces and increases in airflow obstruction (Janssens, Pache, & Nicod, 1999), both of which are likely to contribute to greater degrees of VH. Single-breath and multi-breath nitrogen-washout tests assess the efficiency of ventilation distribution, where greater VH is reflected in steeper phase III slopes and delayed N_2 clearance, respectively (Robinson et al., 2013). With advancing age, phase III of N_2 expirograms becomes steeper (Roberts, MacRae, Winning, Adams, & Seed, 1991; Sandqvist & Kjellmer, 1960), and the clearance of N_2 during a multi-breath nitrogen-washout test becomes delayed, as demonstrated by the positive linear associations between age and lung clearance index (LCI), indices of VH in conductive lung zone (S_{cond}) and indices of VH in acinar lung zone (S_{acin}) (Verbanck et al., 2012).

The degree of disparity between the IST measure of lung volume (ELV_{180}) and the true lung volume might also provide a useful index of VH. This is because ELV measurements are a perfectly predicable function of ventilation, the sine-wave period and its attenuation, and errors in the calculated volume suggests a degree of VH (Whiteley et al., 2001). This is comparable to a multi-breath nitrogen-washout test, where deviation in the N_2-washout curve from a perfectly exponential decay suggests a degree of VH. As such, the findings of the present study also suggest that healthy ageing is associated with increases in VH, because decreases in the ratio of ELV_{180} to FRC_{pleth} and ELV_{180} to FRC_{pred} with advancing age were observed. Indeed, there was a significant positive correlation between these IST indices of VH and VA/TLC, a simple measure of gas mixing inefficiency (Hansen, 2011), and also with measures of airflow obstruction (forced expiratory volume in 1 s and forced expiratory volume in 1 s/forced vital capacity; Table 3). The linear decrease in ELV_{180}/FRC_{pleth} occurred within the age range 20–85 years, and between these ages it declined by ~38% (an average of 0.58% $year^{-1}$). The ELV_{180}/FRC_{pred} also decreased linearly and between the ages of 20 and 85 years, where it reduced by ~31% (an average of 0.48% $year^{-1}$). These changes in ELV_{180}/FRC_{pleth} and ELV_{180}/FRC_{pred} with advancing age are comparable to extrapolated increases in lung clearance index (LCI), indices of VH in conductive lung zones (S_{cond}) and acinar (S_{acin}), which increase by 0.3–0.7% $year^{-1}$ between the ages of 20 and 85 years, assuming continued linearity (Verbanck et al., 2012).

The pulmonary changes associated with ageing have been collectively termed ‘senile-emphysema’ (Janssens et al., 1999; Knudson, 1991). However, there are clear histological distinctions from true emphysema, which also results in the destruction of alveolar walls and a more heterogenous increase in airspace size, suggesting significantly greater degrees of VH (Verbeken et al., 1992). Therefore, methods that quantify VH, such as the IST test, have potential as a clinically useful tool in the diagnosis and/or staging of chronic obstructive pulmonary disease, and further work is required to examine this possibility. However, our findings from the present investigation suggest that the physiological increases in VH with healthy ageing must be taken into account in any clinical investigation.

We found no change in the third IST index of VH (ELV_{60}/ELV_{180}) with age. Given the evidence that ageing can increase VH (Verbanck et al., 2012) and that our other indices (ELV_{180}/FRC_{pleth}, ELV_{180}/FRC_{pred} and VA/TLC) demonstrate this occurring in the present group of participants, it is likely that this index has failed to detect existing differences in VH between participants. This could either be a consequence of using a pair of unsuitable periods or this index is insensitive to the small changes in VH associated with age. From theoretical modelling (e.g. Whiteley et al., 2001), VH can be estimated from sine-wave periods that are sufficiently separated. This is analogous to how multiple inert gas elimination technique (MIGET) can achieve the same end by using gases of sufficiently dissimilar solubilities. The choice of sine-wave periods of 60 and 180 s was pragmatic and based upon what we have found to provide reliable signals. From modelling simulations and practical experience, periods <60 s can produce degraded data. This is because we obtain only a single data point per breath; therefore, as the period shortens the number of samples per cycle also reduces, decreasing reliability.

Conversely, longer periods give many data points per cycle, and the recovered lung volume is less affected by VH (Whiteley et al., 2001). The choice of 180 s was also based upon modelling and what we know from practical experience to give summative lung volumes less influenced by VH and most similar to 'true' lung volume. In addition, we wished to keep the duration of the test as short as possible for the convenience for patients, and longer periods would lengthen the procedure; and it is worth noting that, theoretically, as the period tends towards infinity, the influence of the lung time constants on the sine-wave signal tends to zero and so the signal-to-noise ratio declines. As such, these combined factors explain the choice of 60 and 180 s. However, despite the inability of the index to detect VH in healthy participants, its efficacy can be examined further by comparing healthy participants with patients who have obstructive lung diseases, a group known to have significantly increased VH (Aurora et al., 2004; Verbanck et al., 1998).

4.3 | Summary

The IST test is a new, non-invasive method of estimating cardio-pulmonary function that requires only passive patient cooperation. The present study has demonstrated that the IST indices of VH, ELV_{180}/FRC_{pleth} and ELV_{180}/FRC_{pred} are sensitive to age. The IST test's estimation of end-expired lung volume (ELV_{180}) was very repeatable and has close agreement with FRC_{pleth} when normalized for the effects of age. Overall, the IST test has the potential to provide clinically useful information; therefore, further work with mechanically ventilated patients and those with obstructive lung disease is warranted.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

Experiments were performed within the Respiratory Medicine Department of the Churchill Hospital, Oxford University Hospitals NHS Foundation Trust. R.M.B., P.P., N.R. and A.F. contributed to the conception and design of the work. R.M.B., P.P., E.P. and A.F. contributed to the acquisition, analysis and interpretation of the data. All authors contributed to the drafting of the work and/or revising it critically. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

APPENDIX

Summary of the inspired sine-wave technique

Figure A1 Illustrates the single-compartment model of the cardio-pulmonary system. The lung consists of one dead space compartment, V_D, and one lung compartment, $V_A(t)$, that expands and contracts with tidal volume. The body has one compartment, V_{body}, with $\dot{Q}_P$ flowing through the lung for gas exchange.

When a tracer gas (e.g. N_2O) is inhaled, it passes through the dead space into the lung. It then diffuses into arterial blood and distributes to different parts of the body. After some transit time, the tracer gas travels back to the lung in mixed venous blood. Some will cross the blood–gas barrier into the lung, before eventually being exhaled in expired gas.

During an IST test, over successive breaths the inspired concentration of the tracer gas, $F_I(t)$, follows a sine-wave pattern. Consequently, after a short transient time the alveolar [$F_A(t)$], arterial

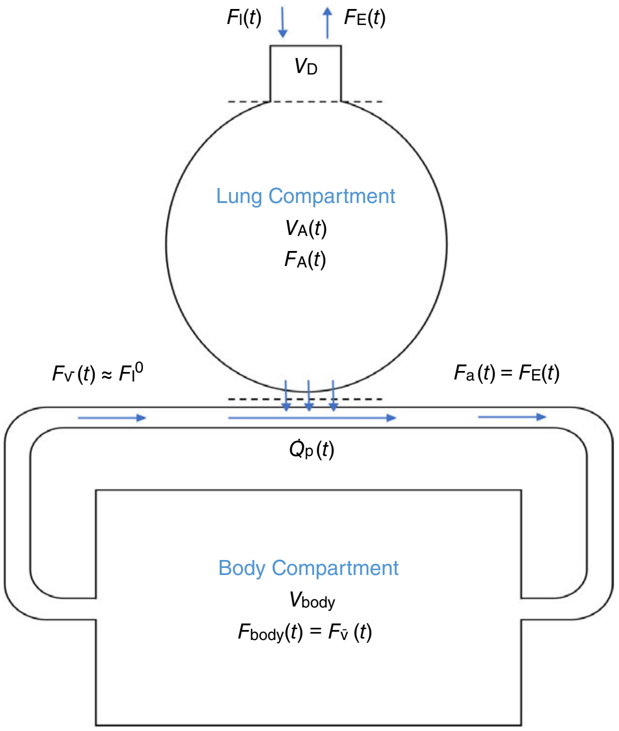

FIGURE A1 The single-compartment model of the lung and circulatory system. For definitions of terms, see the Appendix

$[F_a(t)]$ and mixed venous $[F_{\bar{v}}(t)]$ concentrations will all follow sine-wave patterns.

The body compartment acts as a low-pass filter to diminish the amplitude of the mixed venous sine wave, $F_{\bar{v}}(t)$. When the sine-wave period is short enough ($\leq$5 min), the amplitude of $F_{\bar{v}}(t)$ is small enough to assume that it is constant, and therefore $F_{\bar{v}}(t) = F_I^0$, in which F_I^0 is the mean of inspired sine wave $F_I(t)$.

The mass balance of the lung compartment for the *n*th breath can therefore can be written as follows:

$$F_{E,n-1} \times V_A + F_{\bar{I},n} \times \left(V_{T,n} - V_D\right) + F_{E,n-1} \times V_D - \lambda \times \dot{Q}_P \times \left(F_{E,n} - F_{\bar{v}}\right) \times \Delta t_n = V_A \times F_{E,n} + V_{T,n} \times F_{E,n} \tag{2}$$

$$\Leftrightarrow V_A \times \left(F_{E,n} - F_{E,n-1}\right) + \lambda \times \dot{Q}_P \times \left(F_{E,n} - F_I^0\right) \times \Delta t_n = V_D \times \left(F_{E,n-1} - F_{\bar{I},n}\right) + V_{T,n} \times \left(F_{\bar{I},n} - F_{E,n}\right)$$

in which $F_{E,n-1}$ and $F_{E,n}$ are the end-expired concentrations of breath $(n - 1)$ and n, respectively; V_A is the alveolar lung volume; V_D is the dead space, estimated from the Bohr method; Δt_n is the duration of the *n*th breath; $F_{\bar{I},n}$ is the mean inspired concentration of the *n*th breath; $F_{\bar{v}}$ is the mixed venous concentration, assumed to be equal to the mean of the inspired sine-wave concentration at steady state F_I^0; $\dot{Q}_P$ is the pulmonary blood flow; λ is the solubility of N_2O, 0.47; and $V_{T,n}$ is the tidal volume of the *n*th breath.

First, V_D is estimated using the Bohr method applied to an N_2O signal, as follows:

$$V_D = V_T \frac{F_E - F_{\bar{E}}}{F_E - F_{\bar{I}}}$$

in which $F_{\bar{E}}$ is the mean expired concentration. To compensate for the error associated with the non-uniform inspired concentration, a modified Bohr method has been proposed, improving the accuracy of the airway dead space estimation (Phan et al., 2017). For a series of breaths, a set of linear equations can be established from Equation 2 and solved for V_A and $\dot{Q}_P$. The ELV can then be calculated as the sum of V_D and V_A. Theoretically, three consecutive breaths would be sufficient to construct the linear equations to find solutions for V_A and $\dot{Q}_P$. In practice, the use of all breaths within a complete sine-wave period is recommended to improve repeatability. Interested readers can refer elsewhere (Phan et al., 2015) for further detail.

ORCID

Richard M. Bruce http://orcid.org/0000-0002-8941-3519

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