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TECHNICAL NOTE

Standardization of single-breath transfer factor (TLCO); derivation of breathholding time

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Standardization of single-breath transfer factor (TLCO); derivation of breathholding time. D.J. Chinn, R. Harkawat, J.E. Cotes.
ABSTRACT: Breathholding time is usually defined using the procedure of Ogilvie *et al.* or of Jones and Meade; these procedures depend on knowing when inspiration begins and, for the latter, when it ends. Some alternative procedures do not require this information. With a view to standardizing the measurement, transfer factor of the lungs for carbon monoxide (TLCO) was measured in 18 adults with labile airflow obstruction, who were assessed before and after inhalation of salbutamol. The inflection points defining the start and end of inspiration were defined visually (method 1), and by extrapolation of the linear part of the inspiratory limb of the single-breath trace (method 2). The spirograms met recognized standards of quality and were rated by experienced observers.

Compared with method 1 the TLCO and related indices by method 2 were lower using the procedure of Jones and Meade and higher using the procedure of Ogilvie *et al.* in each case by, on average, 1.0%. The within- and between-day variabilities were independent of the method used. Thus, the extrapolation and visual methods yielded interchangeable results when applied by experienced operators. However, extrapolation may be easier for inexperienced operators.
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The effective breathholding time used in the calculation of transfer factor reflects the mean time available for gas exchange during the test procedure. The latter comprises inspiration of a mixture of carbon monoxide and other gases, breathholding for approximately 10 s, then expiration, during which a post-deadspace gas sample is taken for analysis. The inspiration should be both rapid and complete, with the beginning and end occurring as abrupt events. However, this is seldom the case in practice, as the start of inspiration can be subject to hesitation and the inspiratory rate often falls as full inflation is approached (fig. 1).

Two conventions for calculating the breathholding time are widely used. In the modified procedure of Ogilvie *et al.* [1], all of the time of inspiration is included but the time of sample collection is omitted. In the procedure of Jones and Meade [2], the time is the sum of two thirds of the time of inspiration, the times of breathholding and deadspace washout, and half of the time of sample collection. The European Coal and Steel Community [3] recommends the Jones and Meade procedure and the American Thoracic Society recommends that one or other of the procedures should be used [4]. Both procedures entail identification of the start of inspiration; the Jones and Meade procedure also requires definition of the start of breathholding. The times can be represented as points on the volume/time record of the breathholding test (fig. 1). The points can be identified visually. Using this technique, we have previously reported the within- and between-day variabilities for breathholding time in patients with labile airflow limitation, before and after inhalation of salbutamol [5]. The between-day reproducibilities were, before and after salbutamol, for the Jones and Meade procedure 4.5 and 2.1%, and for the Ogilvie procedure 3.1 and 2.3%, respectively. The variability of the Jones and Meade times was reduced significantly after salbutamol but the improved reproducibility did not extend to the resulting measurements of transfer factor [5]. The discrepancy was due to interdependence between the components of the transfer measurement [6]. The visual method used in that study was applied by experienced operators who interpreted the tracings empirically. This approach might not be optimal for inexperienced operators. In addition, it did not conform to a precise definition such as is needed for automation of the measurement procedure. The inflection points can be identified positively by assuming a constant rate of change of volume with time, the rate being obtained either arithmetically or graphically. Using the latter method, a line is drawn to represent the maximal flow rate during the test inspiration and then extrapolated at both ends (fig. 1). In the present paper, the transfer factors obtained by this method are compared with those by the visual method used previously.

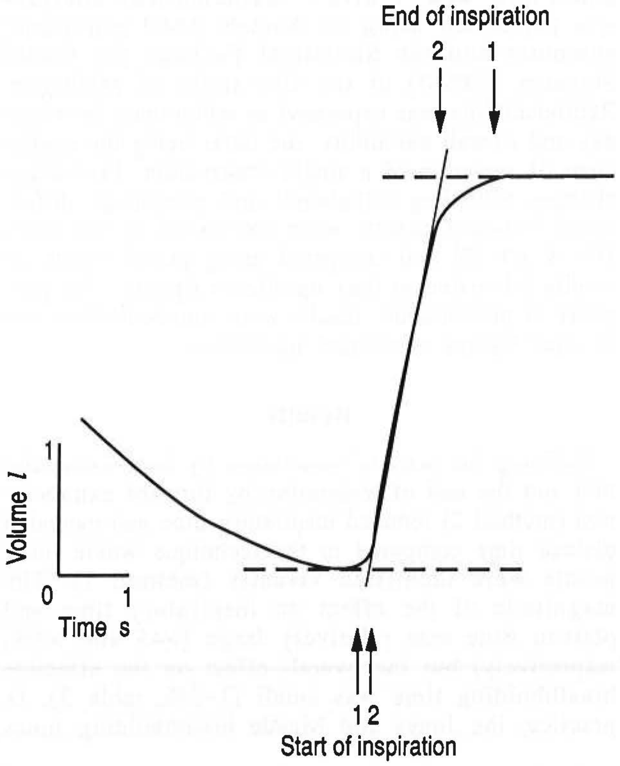

Fig. 1. – Comparison of visual (method 1) and extrapolation technique (method 2) for deriving time of inspiration. In this instance the times of inspiration by the two methods were, respectively, 2.3 and 1.1 s.

Methods

The subjects were 17 male and one female patient volunteers, who were referred for assessment of confirmed or suspected occupational lung disease. They were selected for the present study on account of having labile airflow limitation (increase in forced expiratory volume in one second (FEV_1) after salbutamol >10%) and of spanning a range of pulmonary transfer factors (TL) from very high to very low (mean TL 82% predicted [7], range 26–148%). Lung function and other features are summarized in table 1. Transfer factor was measured by the single breath carbon monoxide method of Forster *et al.* [8]; it was the product of the carbon monoxide transfer coefficient (Kco = TL/VA) and the alveolar volume (VA) during breathholding. The latter was the sum of the volume inspired and the residual volume measured by the closed circuit method with helium as the indicator gas. The alveolar volume, and hence the transfer factor, were also calculated from the dilution in the lung of the helium present in the test breath, allowance being made for the absorption of carbon dioxide prior to analysis of helium and for the instrument and anatomical deadspaces (VA' and TL' respectively) [7].

Table 1. – Details of subjects; mean values (with range or SD in parenthesis) and % change after salbutamol

	Mean	Range or SD	% change
Age yrs	63.3	(44–77)	-
Stature m	1.70	(1.54–1.84)	-
Body mass kg	67.2	(51–85)	-
Forced expiratory volume l	1.43	(0.54)	16.5
Forced vital capacity l	3.26	(0.79)	15.1
Total lung capacity l	6.67	(1.41)	NS
Residual volume l	3.08	(0.97)	-12.6
Transfer factor* $mmol \cdot min^{-1} \cdot kPa^{-1}$	6.99	(2.31)	NS

*: method of Jones and Meade; NS: no significant change.

Transfer factor was measured using transfer test apparatus (Morgan model B). The initial alveolar carbon monoxide (CO) concentration was estimated from the inspired CO concentration (0.28%) adjusted for the dilution in the lung of helium in the test inspirate (14% He in air). The final CO concentration was that in an alveolar sample of 0.7 l collected after exhalation of a washout volume of 0.9 l. Prior to sample collection, the bag was flushed with room air and emptied with standard suction. Allowance was made for the dilution of the collected sample which this entailed. The breathholding manoeuvre comprised exhalation to residual volume, inhalation to total lung capacity, breathholding for approximately 8 s, then exhalation to residual volume: inhalation and exhalation were required to be as rapid as possible, with inhalation taking less than 4 s. Helium was analysed using a katharometer and carbon monoxide by an infrared analyser. The transfer test spirometer was calibrated using a gas syringe (Mercury Electronics Ltd), the analysers by serial dilution of test gas in the closed circuit apparatus and the speed of the kymograph using a stopwatch. The kymograph speed ($1\ cm \cdot s^{-1}$) was accurate to within 2% and the other measurements to within 1%.

The start and finish of inspiration, deadspace washout and sample collection were located on the spirogram by eye (method 1); the inspiratory component was also estimated by extrapolation as described in the introduction (method 2). The tracings were read independently by two experienced observers. Times were measured in duplicate to 0.05 s. The effective breathholding time included the plateau time together with parts of the times of inspiration and expiration as follows.

Procedure 1 (Ogilvie *et al.*) - the inspiratory time and the time of deadspace washout.

Procedure 2 (Jones and Meade) - two-thirds of the inspiratory time and the expiratory time up to halfway through the period of sample collection.

Procedure 3 (American Thoracic Society's Epidemiological Standardization Project) - the midpoint of inspiration by volume to the end of deadspace washout.

Procedure 4 (Morgan automatic apparatus) - the time during inspiration when the preset deadspace washout volume (0.9 *l*) had been inhaled, to the time after breathholding when the washout volume plus half the preset sample volume (0.7 *l*) had been expired.

Procedures 1 and 2 entailed defining the start and, for procedure 2, the end of inspiration, whereas procedures 3 and 4 did not.

Two measurements of transfer factor were made on each occasion: to be acceptable the inspired volumes were required to agree to within 0.2 *l*, the alveolar volume (VA) had to be within 10% of total lung capacity and the measurements of TL' by procedure 4 had to agree to within 5% [3]. The latter values were calculated at the time using a Hewlett-Packard 9825 calculator. An allowance was made for the carbon monoxide back tension [5]. Other aspects including the equations for calculating transfer factor and the reference values are given elsewhere [7].

Subjects had not smoked for at least 2 h, or used their salbutamol inhalers for at least 4 h, before the tests, which were in the order dynamic spirometry, anthropometry, transfer factor and static lung volume. Subjects then inhaled 200μg of salbutamol, after which the physiological measurements were repeated in the same order. The average time between the first determination of TL before and after salbutamol was 54 min. The measurements were repeated at the same time of day two weeks later. The latter results did not differ significantly from those on the first occasion. The two sets were used to estimate the between-day variability. All flow rates and volumes were expressed at body temperature and pressure saturated with water vapour (BTPS) hence TL/VA (KCO) had the units $mmol \cdot min^{-1} \cdot kPa^{-1} \cdot l$ $BTPS^{-1}$. Mathematical analysis was performed using an Amdahl 5860 mainframe computer and the Statistical Package for Social Sciences (SPSS[x]) of the University of Michigan. Reproducibility was expressed as within-day, between-day and overall variability, the latter being the coefficient of variation of a single observation. Percentage changes following salbutamol and percentage differences between results were expressed in the form $100\ \Delta\ x/\bar{x}$ [5] and compared using paired t-tests on results calculated to four significant figures. For purposes of presentation, results were rounded-off to two or three figures as seemed appropriate.

Results

Defining the start of inspiration by back-extrapolation and the end of inspiration by forward extrapolation (method 2) reduced inspiratory time and extended plateau time compared to the technique where these points were identified visually (method 1). The magnitude of the effect on inspiratory time and plateau time was relatively large (>45 and >8%, respectively) but the overall effect on the effective breathholding time was small (1–2%, table 2). In practice, the Jones and Meade breathholding times

Table 2. – Mean (and SD) of inspiratory time, plateau time and effective breathholding time (s) for measurements by the two methods made before and after inhalation of salbutamol

	Inspiratory time		Plateau time		Breathholding time			
					Jones and Meade		Ogilvie *et al.*	
	Before	After	Before	After	Before	After	Before	After
Method 1	2.20 (0.74)	2.18 (0.56)	8.00 (0.57)	7.82 (0.51)	11.15 (1.00)	10.55 (0.60)	11.16 (0.48)	10.81* (0.29)
Method 2	1.36 (0.46)	1.32 (0.39)	8.68 (0.47)	8.58 (0.37)	11.26 (1.05)	10.73 (0.63)	11.00 (0.48)	10.70 (0.28)
Method 1-Method 2† Change %	-46.6	-49.3	8.3	9.4	1.0	1.7	-1.5	-1.0

*: significant difference after salbutamol compared with Jones and Meade method ($p<0.01$); †: $p<0.01$ comparing methods 1 and 2 (paired t-test).

Table 3. – Mean (SD) of transfer factor (TL) before and after salbutamol

	Jones and Meade					Ogilvie *et al.*			
	Before		After			Before		After	
Method 1	6.99	(2.31)	6.91	(2.23)	NS	6.93	(2.16)	6.71	(2.07)*†
Method 2	6.93	(2.33)	6.80	(2.19)	NS	7.04	(2.22)	6.78	(2.10)*
Difference	*		*			*		*	

*: $p<0.05$; NS: not significant; †: reduced compared with equivalent result by Jones and Meade method.

obtained using method 2 were significantly higher than those obtained using method 1; the average differences were, respectively, 1.0 and 1.7% for results obtained before and after salbutamol. By contrast, for deriving the Ogilvie *et al.* breathholding times method 2 gave significantly lower values than method 1, although again the differences were small (table 2). The values calculated for TL, TL' and Kco by the two methods differed by inversely proportional amounts. Comparing the results by the Jones and Meade procedure with those by the procedure of Ogilvie *et al.*, there were no significant differences before salbutamol by either method. After salbutamol the values for TL, TL' and Kco by the technique of Ogilvie *et al.* were significantly lower when method 1 was used; this difference was not present using method 2 (table 3). Thus, extrapolation reduced the difference between the techniques of Jones and Meade and Ogilvie *et al.* after salbutamol.

The response to salbutamol was independent of which method was used for the measurement of inspiratory time. Using the Jones and Meade procedure with either method 1 or method 2 the inhalation of salbutamol did not change the transfer factor (TL). By contrast using the procedure of Ogilvie *et al.* the transfer factor was reduced by salbutamol and this result was independent of which method was used to define the start of inspiration (table 3).

The within-day variability in transfer indices measured by the procedures by both Jones and Meade and Ogilvie *et al.* was independent of the timing method and whether or not salbutamol had been administered. Comparing the two procedures the within-day variability in Kco before salbutamol using method 2 was less for the Jones and Meade procedure than for that of Ogilvie *et al.* ($p<0.025$, table 4); for TL the difference in within-day variability was close to statistical significance ($p=0.053$).

The between-day variability in the transfer indices was independent of the procedure (Jones and Meade or Ogilvie *et al.*) and of the method used to identify the starting time.

Table 4. – Mean values for within-day (coefficient of variation) and between-day ($\Delta x/\bar{x}$) variability in Kco before and after salbutamol (%)

	Jones and Meade			Ogilvie *et al.*		
	Before	After		Before	After	
Within-day						
Method 1	4.2	3.3	NS	4.9	3.6	NS
Method 2	4.1	3.4	NS	4.9	3.6	NS†
	NS	NS		NS	NS	
Between-day						
Method 1	5.1	3.6	NS	5.8	4.3	NS
Method 2	4.9	3.8	NS	5.7	4.2	NS
	NS	NS		NS	NS	

†: before salbutamol variability of Kco was significantly less by Jones and Meade compared with Ogilvie *et al.* technique ($p<0.025$). Kco: carbon monoxide transfer coefficient.

Discussion

The choice of methods for defining the start of inspiration and of breathholding affects the Jones and Meade and modified Ogilvie *et al.* procedures which are those recommended for routine use. They are irrelevant for procedure 3 suggested by the ATS Epidemiological Standardisation Project (ESP) but the latter is now regarded as unsatisfactory [4, 9]. The present alternative methods for defining the beginning of inspiration and of breathholding do influence the absolute levels of the transfer indices but the differences between them are small (approximately 1%) unlike those with the ESP procedure, where the very different starting point (see methods) results in materially higher values for all transfer indices. This point was first noted by Crapo [9]. The present method 2 yields a better within-day reproducibility when used with the Jones and Meade procedure than with the Ogilvie *et al.* procedure. Thus, for maximal reproducibility the Jones and Meade procedure is preferable. This is fortunate as the procedure is also preferable on physiological grounds because, unlike the Ogilvie *et al.* and ESP techniques, it makes allowance for gas exchange continuing during sample collection [5] and the results obtained are closer to those using the three-compartment model [10], in which allowance is made for differences in CO uptake during the three phases of the manoeuvre.

The study was performed using subjects with labile airflow limitation who might be expected to perform the breathing manoeuvre, particularly the expiratory phase, relatively slowly. In some, but not all subjects, the inspiratory phase was also delayed and, on this account, the criterion for an acceptable inspiratory time was set at 4s, which conforms to that recommended by the ATS for patients with moderate to severe airways obstruction [4]. Hence, the findings are for inspiratory flow rates at the limit of acceptability when the difference between the two methods might be expected to be most marked. Smaller differences would be obtained in subjects without airflow limitation whose performance of the manoeuvre conformed more to a "square-wave".

The measurements were made by hand, so that the familiarity of the operators with the visual method could have affected the outcome. In this instance, it was applied and checked by two experienced observers (DJC, RH), whose reproducibility was little different from that by the extrapolation method. The latter might be expected to be more reproducible in the hands of an operator who was less experienced. This was not assessed in the present study but the expectation is supported by observation of technicians in training.

The present results are relevant for users of automatic apparatus, as these incorporate variations of one or other of the procedures for estimating breathholding time which have been discussed. The Morgan apparatus uses a volume-based version of the Jones and Meade procedure, in which the effective breathholding time is considered to have started after the inspiration of a predetermined volume (see methods). It ends after the expiration of half the collected gas sample. This procedure was previously found to have a better reproducibility than the Ogilvie *et al.* procedure incorporating method 1 ($p<0.05$) [5]. The use of method 2 did not reduce this discrepancy. Similar considerations are likely to apply to other apparatus which take into account the time of sample collection.

In conclusion, for measurements of transfer factor based on presently recommended criteria and procedures an extrapolation method for defining the beginnings of inspiration and of breathholding has little advantage over a visual method applied by experienced operators. Extrapolation lends itself to automation and is easier for inexperienced operators; the method can be recommended on this account.

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