The assessment of airflow obstruction from tidal breathing expiratory flow recordings in patients with chronic obstructive pulmonary disease.

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by

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ABSTRACT

The research presented in this thesis focuses on the identification and utilisation of novel indices measured from tidal breathing expiratory flow patterns to assess airflow obstruction in adults. The work is in two parts, firstly the development of the data collection protocol and the identification of suitable indices and secondly the evaluation of the reproducibility of these indices and their comparison with conventional indices and reported symptoms.

The current routine method used to assess airflow obstruction is the measurement of FEV_1, however this test requires patient understanding of the test procedure and maximal effort to produce satisfactory results. Furthermore, forced expiration is not a breathing manoeuvre that people perform in every day life. The usefulness of FEV_1 in assessing reversibility to bronchodilation was studied in a retrospective study which showed that 41% of patients who were identified as non-reversible by FEV_1 criteria showed reversibility of forced or relaxed vital capacity, indicating that FEV_1 does not detect all the physiological effects of bronchodilators. If tidal breathing is measured, a number of the disadvantages of FEV_1 are overcome. It is effort-independent, requires no learning of special breathing manoeuvres on the patient's part and so can be performed even in acutely breathless patients.

A method of recording tidal breathing patterns was developed in which the type of equipment and the measurement protocol was comfortable for the subject, applicable to the routine clinical setting and impacted minimally on the tidal breathing pattern. A five-minute collection time was chosen, recorded after a two-minute acclimatisation period. A method of producing an averaged breath was developed from which a number of novel tidal indices could be measured. After analysis of these indices in pilot studies, three indices, ($TBEV_1$ (volume of air exhaled in the first second of a tidal breath), TPEF (peak tidal expiratory flow rate) and EF_{25} (expiratory flow at end tidal volume plus 25% end tidal volume) were identified which changed consistently following bronchodilators and these were subsequently studied in more depth. Each of these indices correlated with specific conductance (sGaw) at baseline and showed significant increases after bronchodilator but not after placebo treatment in patients known to have reversible airflow obstruction.

For the tidal breathing method to be clinical acceptable it had to be shown to be reproducible and ideally to correlate with patient perceived breathlessness. The final section of the thesis reports studies of reversibility of these measures. Reproducibility was similar to conventional indices of airway obstruction.

In summary this thesis describes development of a method for the measurement and analysis of tidal breathing expiratory flow patterns, which yields consistent and physiologically plausible changes in defined tidal indices following bronchodilator treatment.

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ABBREVIATIONS

Abbreviation	Description
FEV_1	Volume expired in the first second of a forced expiration
FVC	Forced vital capacity
RVC	Relaxed vital capacity
PEFR	Peak expiratory flow rate
MEFV	Maximum expiratory flow volume
MEFT	Maximum expiratory flow time
V_E	Minute ventilation
T_E	Expiratory time
T_I	Inspiratory time
T_{TOT}	Total breath duration
V_T	Tidal volume
f	Breathing frequency
TC	Breathing cycle duration ($T_E + T_I$)
V_T/T_I	Mean inspiratory flow rate
V_T/T_E	Mean expiratory flow rate
V_A	Alveolar ventilation
V_D	Physiological dead space
TLC	Total lung capacity
RV	Residual volume
FRC	Functional residual capacity
Vr	Relaxation volume
MBD	Mean breath duration

Abbreviation	Description
DL_{CO}	Transfer factor of carbon monoxide
sGaw	Specific conductance of the airways
Raw	Airways resistance
Vtg	Thoracic gas volume
CO_2	Carbon dioxide
O_2	Oxygen
$PaCO_2$	Arterial carbon dioxide pressure
PaO_2	Arterial oxygen pressure
COPD	Chronic obstructive pulmonary disease
ASTER	Harmonic analysis of the entire airflow profile
R.I.P.	Respiratory inductance plethysmography
V_A/Q	Ventilation / perfusion ratio
MIGET	Multiple inert gas elimination technique
Pdi	Transdiaphragmatic pressure
TBFVL	Tidal breathing flow volume loops
T_{PTEF}/T_E	Ratio of time needed to reach maximum expiratory flow to total expiratory time
dV/V_T	Ratio of the volume expired before peak expiratory flow achieved to expiratory volume
NEP	Negative expiratory pressure
$TBEV_1$	The volume exhaled in the first second of a tidal breath
TPEF	The peak tidal expiratory flow rate
$TEEV_1$	The volume exhaled in the last second of a tidal breath
EF_{25}	Expiratory flow at end tidal volume plus 25% end tidal volume
Ve max	Maximal expiratory flow
HPV	Hypoxic pulmonary vasoconstriction

Abbreviation	Description
Trs	The time constant of the respiratory system.
EV	Extrapolated volume (the volume of dynamic hyperinflation)
PD_{20}	Provocative dose which causes a 20% drop in FEV_1

STATEMENT

I have been principal investigator in all the work presented in this Thesis, being involved in the planning, execution and analysis of all studies.

Some of the results of the studies described in this Thesis have been previously presented elsewhere; a list of publications is presented.

Joanne M. Montgomery

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PUBLICATIONS ARISING FROM WORK

Work included in this thesis has been presented at the British Thoracic Society meeting in 1996 and 1997 (poster discussion sessions) and at the American Thoracic Society meeting in 1998 (poster session).

1. Reversibility to bronchodilators: Are forced expiratory manoeuvres the best? Samways JM and Innes JA. *Thorax* 1996; 51(suppl 3): A46.

2. Use of tidal breathing expiratory flow profiles to assess airflow obstruction. Samways JM and Innes JA. *Am. J. Respir. Crit Care Med.* 1998; 157: A44.

3. Use of tidal breathing expiratory flow profiles to assess airflow obstruction. Samways JM and Innes JA. *Thorax* 1997; 52(suppl 3): A78.

SECTION 1

Chapter 1: Introduction.

1.1: Overview.

Airflow obstruction is of importance in medicine because of the morbidity and mortality associated with it. A survey by the Lung and Asthma Information Agency showed that in the UK in males and females over 65 years old the crude mortality rates from COPD were 48.2 and 22.5 per 10,000 respectively [1].

This thesis is concerned with the methods used to quantify airflow obstruction with particular reference to novel methods of assessing obstruction from recordings of tidal breathing.

1.2: Causes of Airflow Obstruction.

Chronic airflow obstruction occurs in a number of diseases including asthma, emphysema, chronic bronchitis and cystic fibrosis, and it is therefore obvious that it is caused by a number of different mechanisms, Comroe et al [2] list the main reasons that airways become obstructed. These are: -

- Constriction of bronchiolar smooth muscle.

- Mucosal congestion or inflammation.
- Oedema of bronchiolar tissues.
- Plugging of the lumen by mucus.
- Cohesion of mucosal surfaces by surface tension forces.
- Infiltration, compression or fibrosis of bronchioles.
- Collapse or kinking of bronchioles due to loss of radial traction and/or regional variations in airway resistance.

One or more of the above may cause each disease state. However, whatever the underlying cause of the obstruction the end results are: -

- A decrease in dynamic compliance due to an increase in total airways resistance.
- Hyperinflation due to air trapping and loss of elastic lung recoil, which leads to an increase in the work of breathing.

1.3: Why look at Spontaneous Breathing?: Problems with Forced Expiration.

Since the 1960s the maximum expiratory flow volume (MEFV) curve has been used as a method of quantifying of airflow obstruction [3]. The MEFV and maximum expiratory flow time (MEFT) curves allow measurement of several variables, the main ones being FEV_1, FVC and PEFR. As these measurements have been used for many years they are well understood and the normal ranges are well defined, however many groups e.g. Burrows [4] and Mahler [5] have reported that these lung function tests correlate only weakly or not at all [6] with the degree of dyspnoea experienced by a patient. It has also

been reported by Smith [7] that patients tested for reversibility to bronchodilators sometimes report symptomatic benefit but show no improvement in the conventional forced expiratory variables.

The forced expiratory procedure can cause cough, wheeze and dynamic airway collapse and is effort dependent and so false low results may be obtained.

It has also been shown that the "volume history" of the expiratory manoeuvre can alter the results. Brusasco [8] studied the effect of inhalation to TLC in normal subjects who performed maximal expiratory manoeuvres and partial expiratory manoeuvres before and after inhalation of methacholine. They found that the maximal expiratory manoeuvre showed significantly reduced RV compared to the partial manoeuvre both before and after methacholine inhalation. In addition the flow rates were greater for the partial expiratory manoeuvre with the differences increased after methacholine. They concluded that the inspiration to TLC was causing stress relaxation in the airways.

Lim et al [9] examined the effects of volume history on asthmatics with both spontaneous obstruction and acutely induced obstruction. They found that with spontaneous obstruction Ve max (maximal expiratory airflow) was decreased after a deep inhalation and that this decrease was directly related to the severity of the obstruction. When acute obstruction was induced, either by cold air bronchoprovocation or doubling doses of nebulised histamine Ve max was increased after a deep inhalation. They suggested that the difference in the response in the two groups was due to differences in the site of the obstruction.

Other studies have shown either no change [10], bronchoconstriction [11] or bronchodilation [12] following inspiration to TLC in asthmatics. Fairshter [13] studied airway hysteresis in normal subjects and patients with chronic airflow obstruction and found that specific conductance (sGaw) increased after an inspiration to TLC and that the increase was reduced as the time between the deep inspiration and sGaw measurement increased. In patients with COPD the sGaw decreased after inspiration to TLC i.e. the deep

inspiration caused bronchoconstriction. D'Angelo [14] found that in COPD patients the type of inspiration prior to the forced expiration was important, they looked at the effect of two different inspirations and found that expiratory flows were 20-40 % larger when preceded by a rapid inspiration with no end inspiratory pause.

These unpredictable bronchoconstrictor or bronchodilator effects of volume history will complicate the interpretation of FEV_1 and FVC measurements.

These limitations of forced expiration raise the question: Can useful measurements be made of airflow obstruction from spontaneous (tidal) breathing?

1.4: Spontaneous Ventilation in Normal Subjects.

By convention the breathing pattern is described by the minute ventilation (V_E) and its components tidal volume (V_T) and respiratory frequency (f). Other commonly used descriptors of breathing pattern are time to expire (T_E), time to inhale (T_I) and total breath duration (T_{TOT}). In a study during which Priban [15] looked at different resting breathing patterns in man he observed that although the breathing pattern at rest may seem regular, close inspection showed small changes in the depth and rate of the breathing. In a later study [16] he looked more closely at these changes and found that in 15/20 subjects V_T and f were negatively correlated at rest and, therefore, that the magnitude of the fluctuations in ventilation were less than those of its components. He stated that the fluctuations occurring in V_T and f were too fast to be associated with a feedback loop involving the blood gas tensions and more likely to be due to a behavioural control mechanism which maintains a tidal volume and frequency at a level which minimises the work of breathing.

Newsom Davis and Stagg [17] went on to investigate the volume and time components of the breathing pattern. They confirmed Priban's findings that V_T and T_{TOT}

(reciprocal of f) were positively correlated, but found that there was an even stronger positive correlation between V_T and T_I which implies that the mean rate of inspiration for each breath (V_I) is held relatively constant. The correlation between V_T and T_E was found to be weaker than the V_T - T_I correlation; this larger variation in T_E was partly due to swallowing and expiratory pauses. They stated that the tendency for ventilation to remain constant depends primarily on the tendency for the V_I to be held constant and that variability in ventilation was due to uncorrelated scatter of T_E with respect to V_T. They also observed subjects' breathing pattern during periods of CO_2 rebreathe and found that minute ventilation was increased by a increase in V_T and shortening of T_E, while the T_I stayed relatively unchanged and that V_I increased as inhaled CO_2 level was raised.

Kay [18] also studied the relationship between V_T, T_I and T_E during rest and found correlation between both V_T and T_I and between V_T and T_E.

This early work on breathing pattern lead to the conclusion that mean inspiratory flow (V_T/T_I) is set by the respiratory drive, and that mean expiratory flow (V_T/T_E) reflects the elastic recoil of the lungs.

In 1987 studies by Shea and Benchetrict showed that individuals possess a characteristic breathing pattern not only in terms of the standard respiratory variables [19], but also in terms of the shape of the entire airflow profile as quantified by harmonic analysis (ASTER) [20] and that the breathing pattern is highly conserved over time [21]. Further to this work in 1989 Shea et al [22] studied the breathing pattern of adult identical twins and found that there were highly significant similarities within twin pairs compared to random pairs of two individuals from the same population.

1.5: Spontaneous Ventilation in Patients with Airflow Obstruction.

A few groups have looked at the minute ventilation and its components in COPD patients compared with normals [23-25], and at the effect of severity of COPD [26] with different findings which are summarised in table 1.1. The differences in the results have been attributed mainly to differences in the measuring method.

Sorli et al [23] studied 2 groups of COPD patients, one eucapnic and the other hypercapnic; and compared their minute ventilation and its components to a normal control group. Flow was measured using a mouthpiece and Fleisch pneumotach and mouth occlusion pressure was measured along with the other breathing pattern components. They found that ventilation was increased in both COPD patient groups compared to the normal control group but that only in eucapnic COPD patients did this reach significance at the 5% level. This increase was due to small non-significant changes in frequency and V_T.

Tobin et al [24] studied the breathing pattern in normal subjects and in patients with a variety of disease states using respiratory inductance plethysmography (R.I.P.). In those with COPD who were not hypercapnic ($PaCO_2 < 44$ mmHg) they found that the V_T and f was moderately increased leading to a raised V_E. The T_I was shorter by close to 1 second compared to the normal subjects, the T_I/T_{TOT} was reduced, and the V_T/T_I was elevated. Findings were similar in COPD subjects who were hypercapnic.

Loveridge et al [25] looked at the breathing pattern of COPD patients compared to normals using RIP. The patients were categorised as having moderate to severe airflow obstruction and were all eucapnic. They found that the breathing pattern in the patients with COPD was significantly different from the age matched normal controls. The rate of breathing was increased in the COPD group that led to an increase in V_I. Both T_I and T_E were shorter in the COPD group but the V_T was unchanged between groups leading to the V_T/T_I being increased in COPD group. They also found that the variability of these

Group	Ventilation	V_T	Frequency	T_I	T_I/T_{TOT}	V_T/T_I
Sorli [23]	↑	NS	NS	NS	NS	↓
Tobin [24]	↑	↑	↑	↓	↓	↑
Loveridge [25, 26]	↑	NS	↑	↓	NS	↑

Table 1.1: Change in breathing pattern in eucapnic patients with COPD compared to that in normal subjects.
(NS = no significant change)

variables was less in the COPD group. These findings were duplicated in a later paper by Loveridge [26] in which he compared the breathing pattern in three groups of COPD patients, a moderate group a severe one and one in respiratory failure, he found no significant difference between the moderate and severe groups.

Therefore overall the studies show that the minute ventilation in COPD patients is increased as compared to normal subjects. This is due to small increases in tidal volume and breathing frequency, which are mainly caused by a decrease in T_I with either no change or a small decrease in T_E. The large changes in T_I and small changes in V_T means that ventilation is increased due to an increase in the mean inspiratory flow, and in some cases to mean expiratory flow.

1.6: Causes of Increased Minute Ventilation in COPD.

The cause of the increase in minute ventilation seen in patients with COPD is not yet fully understood. There are a number of different processes in the lung, which are affected by airway obstruction and may play a part in the increase.

1.6.1: V_A/Q Mismatch.

In the normal lung there is a distribution of V_A/Q ratios due to ventilation changing less as you go from the apices to the bases than perfusion, as shown by West & Dollery in 1960 [27]. In patients with COPD the changes in resistance to airflow and compliance occur non-uniformly throughout the lung and lead to an increase in the unevenness of

ventilation within the lungs. Wagner, using the MIGET technique [28], found three distinct patterns of V_A/Q with the lungs of patients with COPD [29] (figure 1.1).

Burrows [30] described two groups of patients with COPD with distinct clinical presentations. Type A or "pink puffer" have a normal or low $PaCO_2$ a mildly decreased PaO_2 and a low carbon dioxide diffusing capacity (TL_{CO}) and Krogh coefficient (K_{CO}). Type B or "blue bloaters" present with cough and sputum production, fluid retention, recurrent cor pulmonale, polycythemia and are more likely to be hypoxemic and hypercapnic.

Wagner also showed that patients with the Type A presentation, mainly had type H pattern while Type B patients were equally likely to have either type H, L or HL pattern and mixed Type A and B patients were found to have type H or HL patterns (figure 1.1). It must be noted however, that an increase in non-uniformity of ventilation may not correlate to the increase in V_A/Q mismatch as the body has a number of mechanisms that tend to compensate for the mismatch. The first occurs in regions where the V_A/Q is decreased, in these regions perfusion is reduced thereby raising the local V_A/Q. West [31] attributes this to hypoxic pulmonary vasoconstriction (HPV). Von Euler and Liljestrand first described this in 1946 [32] and a review of the subsequent research into HPV was reported in 1986 by Voelkel [33]. Kato and Stubbs [34] and Nagasaka [35] have shown that it is the arteries of the terminal respiratory units in the hypoxic lung region that constrict. However it is not yet clear whether HPV occurs directly in a localised vascular site with distinct biochemistry or if it is mediated by a local hormone [33].

The second occurs within the areas of the lung that have a high V_A/Q, it is thought that the fall in PCO_2 in the airways in these regions causes an increase in their resistance thereby decreasing ventilation to these regions and reducing the V_A/Q [31].

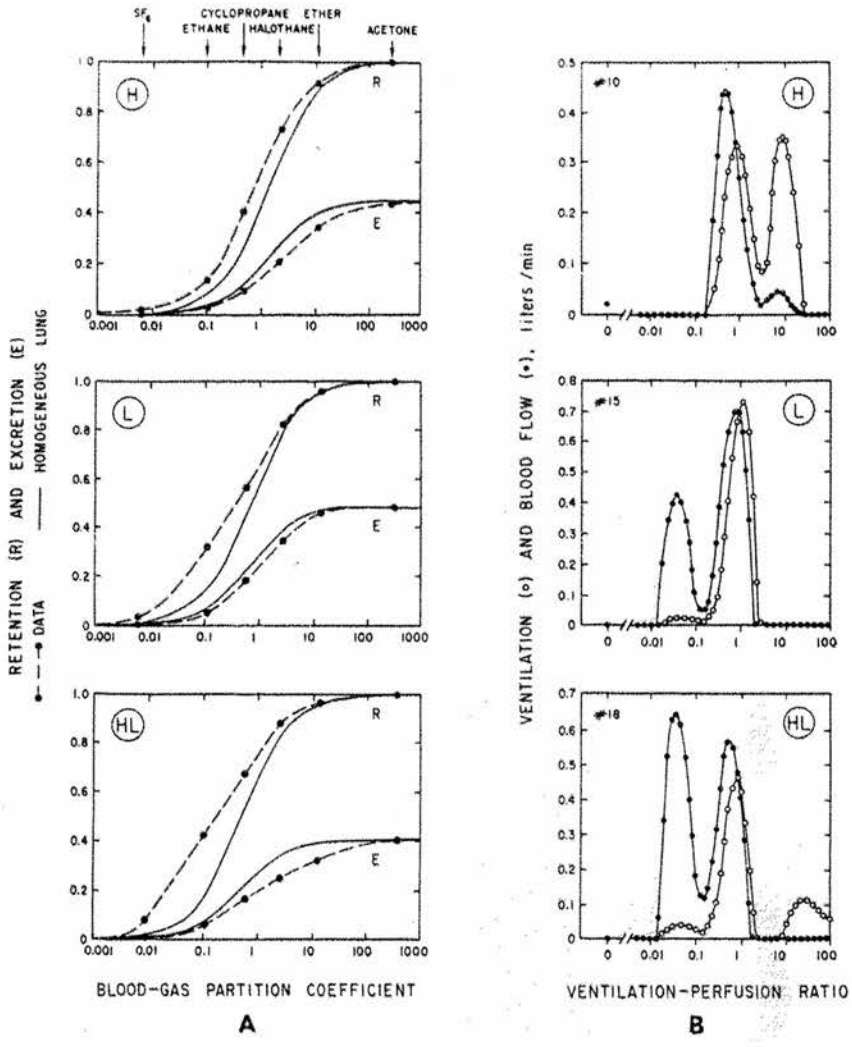


Figure 1.1: Examples of different VA/Q patterns obtained from MIGET [29]. Type H is characterised by a mode of high V_A/Q units, type L is characterised by a mode of low V_A/Q and type HL has additional modes both above and below the main body.

These disorders of V_A/Q matching may lead to increased ventilation in several ways: -

a) Increased physiological dead space. Areas of high V_A/Q will receive ventilation but not participate in gas exchange. The result will be an effective increase in dead space ventilation occurring alongside alveolar ventilation, so total Vi will be increased. If maldistribution of ventilation is sufficiently severe hypercapnia may result, further stimulating ventilation.

b) Low V_A/Q areas classically result in hypoxia, as underventilated units contribute desaturated blood to the pulmonary veins. If sufficient low V_A/Q areas are present arterial hypoxaemia will result and may stimulate ventilation via the chemoreceptors.

1.6.2: ABNORMAL AFFERENT TRAFFIC FROM LUNG RECEPTORS.

However there is a group of patients with COPD that do not consistently exhibit hypoxia, the "pink puffers", and so hypoxia cannot be the only cause for increased ventilation. A second possible mechanism is abnormal afferent traffic from the lung sensory and chemoreceptors. In humans regulation of breathing is dependent on a complex system with three main components. One of these components, the control centre, is acted upon by afferent neural signals from lung receptors, among other inputs.

Bleecker et al [36] showed that it was the stimulation of the receptors whose afferent pathways were in the vagus nerves that caused the change in breathing pattern observed in dogs after inhalation of histamine. This was supported by a study by De Jonste et al [37] who found that tissue from the lungs of patients with COPD had increased histamine responsiveness to that from the lungs of patients without COPD. Cazzola et al [38] showed that pirenzepine (an anti-cholinergic) acted as a bronchodilator in patients with

COPD by decreasing the sensitivity of the vagal sensory endings and causing a vagal afferent blockade.

Therefore it can be hypothesised that, in patients with COPD, abnormal afferent traffic from overly sensitive sensory receptors may act upon the respiratory control centre via the vagus nerve leading to changes in the ventilatory pattern.

1.6.3: Perception of increased respiratory work in the chest wall.

A third possible cause of the increased minute ventilation in patients with lung diseases associated with airflow obstruction is changes in the perception of the respiratory work of the chest wall. It has been observed that asthmatics tend to overbreathe, Kelsen [39] observed that it was bronchospasm induced by methacholine inhalation that increased minute ventilation. They found that the perception of the effort involved in breathing was heightened and suggested that inputs from the mechanoreceptors in the lungs and greater stimulation of the chest wall mechanoreceptors as a result of increases in lung elastance may explain this response.

1.7: Lung Volume and Functional Residual Capacity in Airflow Obstruction.

In most patients with airflow obstruction FRC and RV are increased, also TLC is frequently raised especially in those patients with chronic obstruction. Both static and dynamic mechanisms are involved in the increase in FRC and RV.

The mechanisms by which FRC is increased are complex and believed to be due to both the airways' mechanical properties and the control of breathing. One reason is that the loss in lung recoil pressure results in an increased relaxation volume (V_r) of the lungs (the volumes at which the lung recoil pressure is balanced by the chest wall recoil pressure).

Another cause is dynamic pulmonary hyperinflation, when FRC occurs above Vr, due to slowing of expiration to such an extent that expiration is not completed before inspiration is initiated. The reasons for this slowing of expiration include: -

- The increased resistance to expiratory airflow delaying lung emptying.
- Dynamic collapse of small airways, because of lack of support, causing dynamic narrowing of the airways and airflow limitation.

These together lead to the inability to increase flow above the prevailing tidal breathing flow rate (expiratory flow limitation) as described by Pride and Macklem [40]. As airway resistance is lower at higher lung volumes, due to increased lung recoil and increased radial traction on collapsible airways, patients breathe at higher lung volumes so that they can increase their expiratory flow rates.

The increase in residual volume (RV) is also thought to be due to a combination of static and dynamic factors.

The loss of elastic lung recoil results in the airway closure occurring at higher lung volumes. For the reasons outlined above, expiratory flow is decreased, increasing the time taken to empty the lungs to such an extent that ERV may be limited by the breath-holding ability of the patients, also due to lack of support of the airways dynamic airway collapse may occur at higher lung volumes resulting in gas trapping, with truncation of expiratory airflow.

1.8: Respiratory Muscle Activity in Normal Subjects and Patients with COPD.

The tidal breath flow time curve in normal subjects is sinusoidal in shape with peak expiratory flow occurring near the middle of the breath. Agostoni [41] hypothesised that the delay in peak tidal expiratory flow is produced by continued activity of the inspiratory muscles after the end of inspiration (post inspiratory braking), and that abolition of this braking would cause a rapid rise in pleural pressure. This was confirmed by studies from McIlroy [42] and Pierce [43] who looked at the flow and pressure curves of normal subjects during voluntarily relaxed expirations.

It has also been shown [44-45] that in patients with COPD the diaphragm is flattened and its fibres shortened, primarily because of hyperinflation. As a result it operates at a sub-optimal portion of its length-tension curve and is unable to act as an effective inspiratory muscle and the transdiaphragmatic pressure generated during inspiration is less than normal. Brennan et al [46] studied the thoracoabdominal mechanics during tidal breathing in patients with emphysema while sitting. He found that in most patients the rib cage motion was greater than in normal subjects, and concluded that the patients are using their intercostal and accessory muscles during inspiration. They also observed that some patients had paradoxical inward movement of the lower rib cage during inspiration (Hoover's sign, believed to result from inward tension exerted by the flattened diaphragm). The same authors also observed that some patients showed abnormal abdominal motion with the abdomen diameter being smaller during expiration than during inspiration at the same lung volume; and concluded that the abdominal muscles were contributing to expiration. This they suggested could happen in two ways: -

1. To enable expiration to be completed before an inspiration is triggered.
2. To aid diaphragmatic function by exerting an upward force which will increase the diaphragm length and curvature before the next inspiration.

Respiratory motion of the rib cage and abdomen in this group of patients is a complex function of muscle activity, chest wall mechanics and lung mechanics, and apart from concluding that the diaphragm is disadvantaged during inspiration by the hyperinflated lungs, fuller understanding of the relative contribution of the accessory muscles to this movement would require pleural and transdiaphragmatic pressure measurements supported by EMG recordings and is outwith the scope of this thesis.

A study by Ninane [47] found that the transverse abdominis was the principal muscle of active expiration and was active before the external oblique and rectus abdominis. They also found that the expiratory contraction of the transverse abdominis was significantly correlated with FEV_1 both expressed as percent predicted and absolute value. Because the transverse abdominis is a deep muscle, surface electrodes may give a false negative when trying to detect expiratory muscle activity. Grimby et al [48] have shown that using abdominal volume, measured by respiratory inductive plethysmography, and gastric pressure is a more sensitive method of determining expiratory muscle activity.

1.9: Previous Work Examining Spontaneous Breathing in Airflow Obstruction.

The use of tidal breathing flow volume loops (TBFVL) to assess airflow obstruction when the subject is unable to perform a forced expiration has been examined in animals and infants.

1.9.1: Animal Studies.

McKiernan et al [49] found that cats suffering from chronic bronchial disease could be distinguished from healthy cats by a number of variables measured from TBFVL (Table 1.2).

The main differences in the TBFVL were seen during expiration. In the bronchitic cats peak expiratory flow occurred later in the breath compared to healthy cats, (approximately end expiration plus 60% of V_T in bronchitic cats compared to plus 70% in healthy ones). Overall the flow volume loop was flatter in the bronchitic cats throughout expiration with decreased expiratory flows. The timing of the breath was also found to be different in bronchitic cats with T_E being longer than T_I compared to them being the same in healthy cats.

Petsche et al [50] reported the use of TBFVL in identifying horses with recurrent airway obstruction (heaves) before it became so severe that clinical signs could identify it. They found that respiratory frequency was increased primarily due to a shortening in inspiratory time and an increase in mean inspiratory flow. The pattern of the TBFVL changed from a biphasic one, to one in which the expiratory peak flow occurred earlier in the expiration and was of a greater magnitude.

Variable	Normal (n=9)	Bronchitic (n=7)
Area under the expiratory curve from peak expiratory flow to end tidal volume (mm^2)	3795 ± 1986	1661 ± 1075
Area under the total expiratory curve (mm^2)	5499 ± 2893	3021 ± 1736
Volume exhaled 0.5 seconds after the start of a tidal breathing exhalation (ml)	44.8 ± 12.2	29.5 ± 9.0
Peak expiratory flow divided by peak inspiratory flow	1.04 ± 0.18	0.78 ± 0.10
Expiratory flow at midtidal volume divided by inspiratory flow at midtidal volume	1.16 ± 0.22	0.82 ± 0.10
Expiratory flow at end tidal volume plus 25% V_T divided by inspiratory flow at end tidal volume plus 25% V_T	1.06 ± 0.19	0.81 ± 0.17
Expiratory flow at end tidal volume plus 25% V_T divided by expiratory flow at end tidal volume plus 12.5% V_T	1.27 ± 0.09	1.17 ± 0.11
Expiratory time divided by inspiratory time	1.00 ± 0.15	1.31 ± 0.14
Inspiratory time divided by total breath time	0.5 ± 0.04	0.43 ± 0.03
Peak expiratory flow (ml/sec)	113.7 ± 29.1	79.8 ± 19.8
Expiratory flow at end tidal volume plus 25% VT (ml/sec)	86.32 ± 26.5	61.39 ± 21.7

Table 1.2: TBFVL variables which distinguish bronchitic cats from healthy ones [49].

The difference in where peak expiratory flow (PEF) occurs in cats and horses may be explained by the fact that in bronchitic cats the T_E was increased whereas in horses with heaves the T_E was decreased and it was suggested that increasing the PEF may be the only way the horses could exhale their V_T in the time available. Horses were also observed to have reduced inspiratory muscle activity during early expiration so that the unopposed elastic recoil of the lung and thoracic wall causes the high and early peak expiratory flow rate.

1.9.2: Infant Studies.

Over the last decade two methods have been developed to allow measurement of forced expiratory flows in infants. However the first, forced deflation technique, requires endotracheal intubation and the second, rapid thoracoabdominal compression, only produces a partial expiratory flow-volume loop and it is not known if flow limitation is achieved used this technique [51], therefore studies have been carried out looking at the use of tidal breathing expiratory flow rate in assessing airflow obstruction by a number of different groups [52-58]. In the majority of studies the main variables that have been measured are T_{PTEF}/T_E (the ratio of the time needed to reach maximum expiratory flow to total expiratory time) and dV/V_T (the ratio of the volume expired before peak expiratory flow achieved to expiratory volume).

The results of these studies have been mixed. Carlsen [53] found that both T_{PTEF}/T_E and dV/V_T discriminated asthmatic children from healthy ones in terms of baseline values and reversibility to bronchodilators, whereas both Cutrera et al [52] and Van der Ent [57] reported that dV/V_T and T_{PTEF}/T_E did not distinguish healthy children from those with

asthma or cystic fibrosis. However, they both reported that the tidal breathing variables correlated to maximum expiratory flow volume variables both at baseline and following bronchodilation or histamine challenge.

However, Aston [54] who studied T_{PTEF}/T_E during bronchial challenge showed no change post challenge and concluded that it was an insensitive measure of airflow obstruction. Clarke [55] also reported that T_{PTEF}/T_E was an insensitive indicator of airway obstruction and was only useful in extreme cases when expiratory airflow limitation was present even during tidal breathing.

Seddon [58] studied intubated and non-intubated premature infants and found that T_{PTEF}/T_E correlated to lung compliance but not to airways resistance and concluded that T_{PTEF}/T_E maybe influenced by elastic rather than flow-resistive properties.

The difference in the results between the groups could be due to different methodologies, such as equipment used, i.e. facemask versus mouthpiece, instrumental dead space; shape of the thorax, position of the subject; state of arousal; post natal age and the number of breaths sampled and analysed. For example, Cutrera [52] and Van der Ent [57] recruited school age children (mean age 10.5 yrs. range 5.3 - 17.5 yrs. and mean age 7 yrs. range 3 - 16 yrs. respectively) studied sitting and awake whereas Aston [54] and Clarke [55] recruited infants (aged 6 - 12 months and 1 week to 23 months respectively) studied supine and sedated. The mechanical properties of the infant chest wall, which is highly compliant, are known to differ greatly from those found in older children and adults. Van der Ent [57] stated that further studies are needed to define that possible sources of measurement variability and develop a standard method for measuring tidal breathing variables.

1.9.3: ADULT STUDIES.

In 1981 Morris et al [59] looked at flow against time in normal subjects and patients with a variety of respiratory disease states. They showed that patients with COPD did not show the sinusoidal flow pattern observed in normal subjects and patients with restrictive respiratory diseases but a 3 phase expiratory pattern consisting of i) a rapid rise to maximum flow, ii) a slow decline in flow throughout most of the expiration and iii) an abrupt ending of expiration where the flow drops suddenly through zero. Representative tracings of tidal flow against time in the different patient groups studies are shown below (figure 1.2). They studied the timing variables derived from the tidal expiratory flow (which had previous been identified in infant studies i.e. T_{PTEF}/T_E and dV/V_T) to quantify this difference and found them to correlate with other variables of airflow obstruction.

In 1990 Morris et al [60] studied the causes of the 3 phase expiratory pattern observed in COPD patients. They observed that the pattern of flow and pressure seen in these patients with airflow obstruction was the same as that seen by McIlroy et al [42] and Pierce [43] in their studies looking at removal of the normal "braking" effect of continued inspiratory muscle activity in early expiration. This loss of inspiratory muscle braking was confirmed when they found that inspiratory muscle activity, measured by surface electrodes at the 7th right intercostal space, stopped abruptly at the end of inspiration (figure 1.3). They also found that phasic inspiratory activity started just before the flow reversal and opposed the alveolar pressure (intrinsic peep) still available to drive expiration. The patients probably sense this as work associated with inspiration and therefore perceive inspiration as being more difficult.

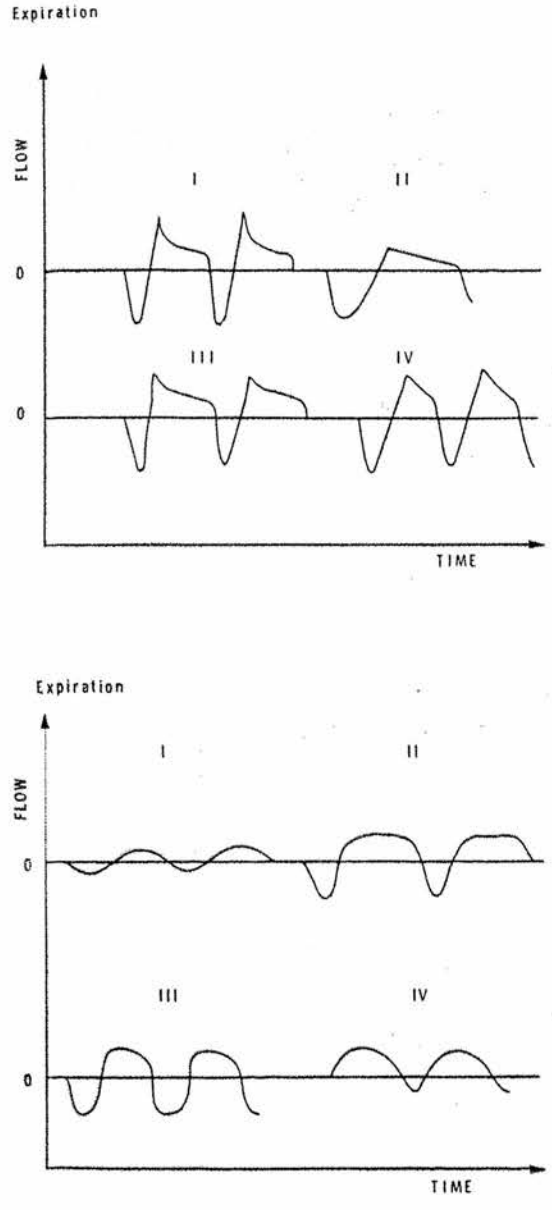


Figure 1.2: Tracings of tidal flow against time in four patients with airflow obstruction (upper panel), in three normal subjects (lower panel I,II and III) and one patient with restrictive lung disease (lower panel IV). Taken from Morris et al [59].

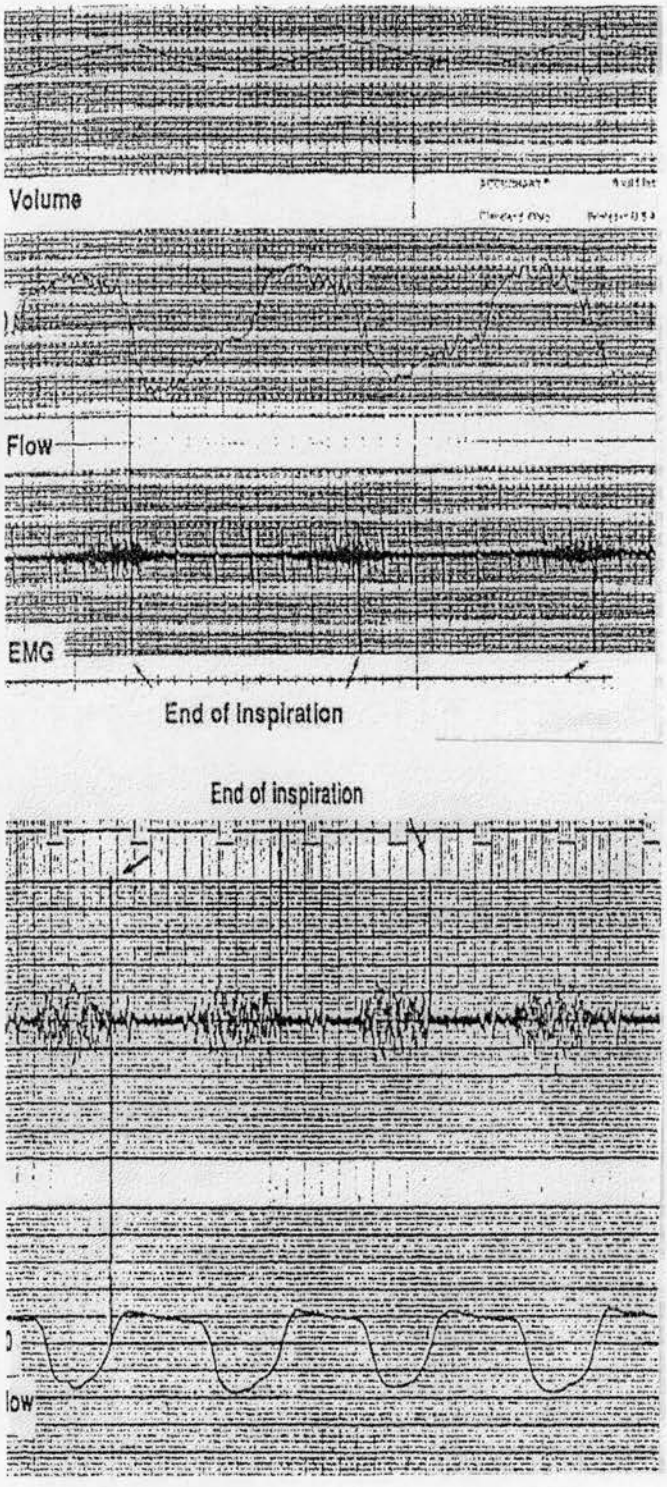


Figure 1.3: Typical EMG tracings from a normal subject (A) and a representative patient with chronic airflow obstruction (B)
during tidal breathing showing loss of post inspiratory inspiratory braking. EMG from surface electrodes in 7th right intercostal space. This figure is modified from Morris et al [60].

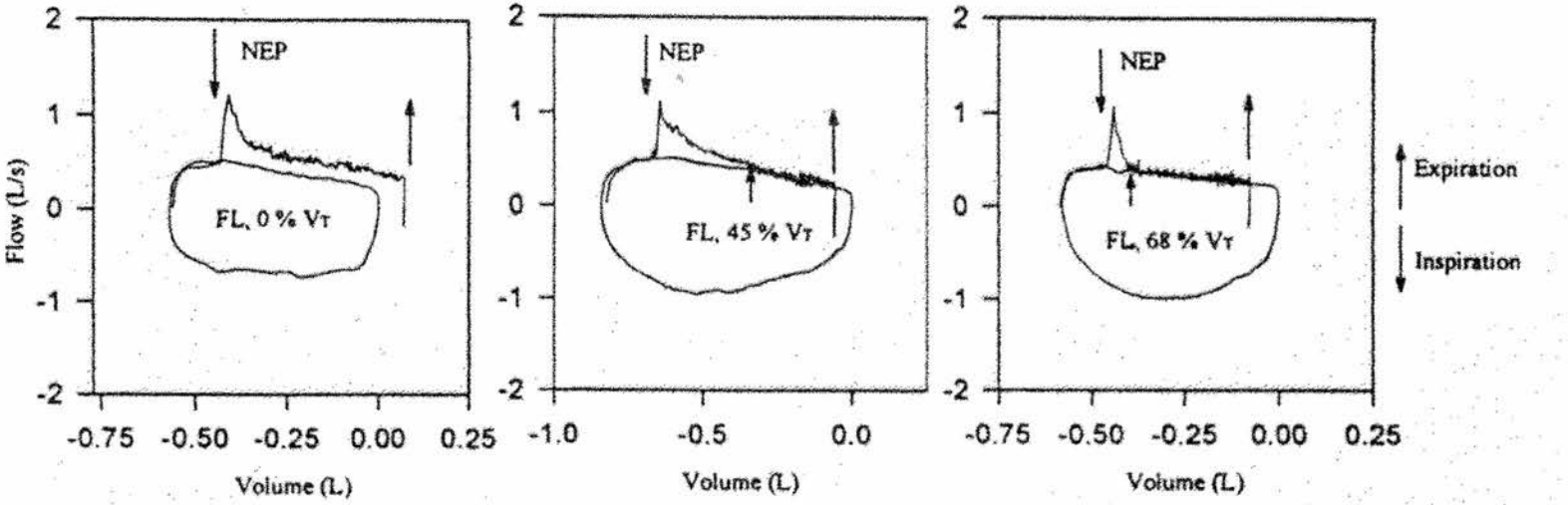


Figure 1.4: Flow-volume loops of test breaths and preceding control breaths of three representative patients with different degrees of expiratory flow-limitation (FL)(panel A), FL over less than 50% of control expired tidal volume (VT) (panel B), FL over more than 50% VT (panel C). Long arrows indicate points at which negative expiratory pressure was applied and removed. Short arrows indicate onset of flow limitation. Zero volume is end expiratory lung volume of control breaths [64].

In 1998 two studies by Morris [61] and Williams [62] described three further variables derived from tidal expiratory flow but this time from the portion of expiratory flow after peak flow had been achieved. In these cross-sectional studies they found that these tidal breathing variables correlated with conventional measures of respiratory function.

Koulouris et al [63] have reported a technique known as negative expiratory pressure (NEP), which detects expiratory airflow limitation during spontaneous breathing (figure 1.4). They looked at a group of COPD patients and found that they could classify the degree of flow limitation depending on whether flow limitation was present when the patient was supine or when sitting. Eltayare et al [64] have used this technique to correlate expiratory flow limitation to dyspnoea score.

1.10: HYPOTHESIS.

Since tidal expiration is predominantly a passive event in patients with COPD, it will be possible to deduce information about the degree of airflow limitation and the effects of bronchodilators from averaged expiratory tidal flow profiles

SECTION 2

Chapter 2: Is FEV_1 the Best Measure of Bronchodilator Response in Patients with Airflow Obstruction?

2.1: Introduction.

As an introduction to the topic of clinical measurements from other than forced maximal expirations the opportunity arose to study retrospectively a large number of RVC measurements gathered during routine clinical respiratory function testing. These data were examined to determine if, during routine spirometry, FEV_1 picked up all patients with a reversible component to their airflow obstruction or if FVC or RVC increased the number of patients termed reversible to bronchodilators.

The hypothesis was that bronchodilators have measurable beneficial effects on lung function that are not evident on standard FEV_1 testing. This would lead to patients who may benefit from bronchodilators being classified inappropriately as non-reversible if tested in compliance with current guidelines.

The most commonly used test of respiratory function is forced spirometry, which was first described as early as 1947 by Tiffeneau and Pinelli [65]. This test is well understood and the normal ranges for the variables measured are well defined. However, there are also a number of disadvantages associated with the forced

expiration. It is unphysiological, (i.e. not a manoeuvre patients perform in everyday life), shows some effort dependence and only correlates weakly, if at all, with dyspnoea [4-6]. The inspiration to TLC performed prior to the forced expiration can itself alter the degree of airflow obstruction [8-14].

The 1997 BTS guidelines for the management of chronic obstructive pulmonary disease [66] stated that when assessing reversibility to bronchodilators the preferred outcome measure is change in FEV_1. Smith et al [7] studied additional variables in a group of 100 consecutive patients sent for evaluation of reversible airways obstruction. They measured reversibility in terms of spirometric variables and plethysmographic variables and reported that 82% of patients were identified as reversible by spirometric variables and 15/18 of the remaining patients were identified as reversible by plethysmographic variables. They concluded that the patients missed by spirometry probably had volume related changes to bronchodilators and so a combination of available tests should be used to test for reversible airflow obstruction. Plethysmography is however expensive and not universally available.

It has been reported [67] that patients with airflow obstruction often have a relaxed vital capacity (RVC) which exceeds the forced vital capacity (FVC), the latter often being limited by cough or dynamic airway collapse.

2.2: METHODS.

2.2.1: SUBJECTS.

378 randomly selected records from patients who had attended the Respiratory Function Laboratory at the Western General Hospital, Edinburgh for bronchodilator reversibility testing between 1993 and 1996 were studied. Randomisation was achieved by 40 records being picked from each drawer of the filing cabinets holding the results for these years. Results were included for analysis if the patient had been referred with a clinical diagnosis of obstructive lung disease, had a baseline FEV_1 of less than 80% predicted and a FEV_1/FVC ratio of less than 75% and underwent reversibility testing to either salbutamol or ipratropium bromide. Clinical details on the request forms indicated that the majority of these patients had a clinical diagnosis of chronic obstructive pulmonary disease (298/378, 79%), and the majority of the remainder had a clinical diagnosis of asthma (76/378, 20%).

2.2.2: DATA COLLECTION.

Data for FEV_1, FVC and RVC were available both before and after either 2.5 mg salbutamol and/or 250 μg ipratropium bromide nebulised via a disposable sidestream nebuliser (Medic-aid Ltd, West Sussex, England) and CR50 compressor (Medic-aid Ltd, West Sussex, England) in all patients. FEV_1 and FVC were performed as per British Thoracic Society (BTS) and Association of Respiratory Technology and Physiology (ARTP) guidelines [68].

2.2.3: Measurement of RVC.

RVC is measured as a part of routine spirometry by the pulmonary laboratory at the Western General Hospital. There is no agreed standard technique for performing an RVC manoeuvre, patients are simply instructed to "Take a big breath in and then gently blow all the way out". They start by performing one RVC manoeuvre, go on to have FEV_1/FVC measurements to the standard BTS / ARTP criteria [68] and then RVC is repeated if 1) RVC was less than FVC, 2) FVC was unobtainable due to coughing on repeated attempts or 3) FVC had changed more than 10% since a previous laboratory visit.

2.2.4: Analysis of Data.

The records were analysed to determine those that showed FEV_1, FVC and / or RVC reversibility. Reversibility was defined as 160 ml or greater increase in FEV_1 and a 330 ml or greater increase in FVC. RVC reversibility was also taken as an increase of 330 ml or greater [69].

2.2.5: Statistical Analysis.

To investigate the influence of disease severity on the reversibility measurements, correlations of reversibility indices with baseline FEV_1 was performed. These were evaluated using Pearson's product moment correlation (r) on log-normalised data. A p value of less than 0.05 was considered significant.

2.3: RESULTS.

378 patients (201 males) were studied, aged 64 ±12 (mean ± SD) years with baseline FEV_1 57 ± 28% predicted. 48% of patients were treated with nebulised salbutamol (2.5 mg) only, 4% were given nebulised ipratropium bromide (250 μg) only and 48% had both. As can be seen from the Venn diagram (Figure 2.5), 48% of this group of patients had FEV_1 reversibility and 52% did not. Of those that did not demonstrate FEV_1 reversibility 41% could be identified as reversible by FVC or RVC criteria, 12% by FVC reversibility alone and 12% by RVC reversibility alone.

The correlations between change in the variables following bronchodilator and baseline FEV_1 (Figure 2.6) show that, whereas the baseline FEV_1 did not correlate with change in FEV_1 ($r = 0.10$, $p = NS$); there was a significant negative correlation between change in FVC and baseline FEV_1 ($r = -0.27$, $p < 0.0001$) and change in RVC and baseline FEV_1 ($r = -0.21$, $p < 0.0001$). This means that patients with a low baseline FEV_1 tend to have larger increases in FVC and RVC than those with higher baseline FEV1's.

298 (166 males) of the patients studied had a referring diagnosis of COPD, (aged 67 ±10 (mean ± SD) years with baseline FEV_1 48 ± 20% predicted). As can be seen from the Venn diagram (Figure 2.7), 47% of this group of patients had FEV_1 reversibility and 53% did not. Of those that did not demonstrate FEV_1 reversibility 47% could be identified as reversible by FVC or RVC criteria, 13% by FVC reversibility alone and 13% by RVC reversibility alone.

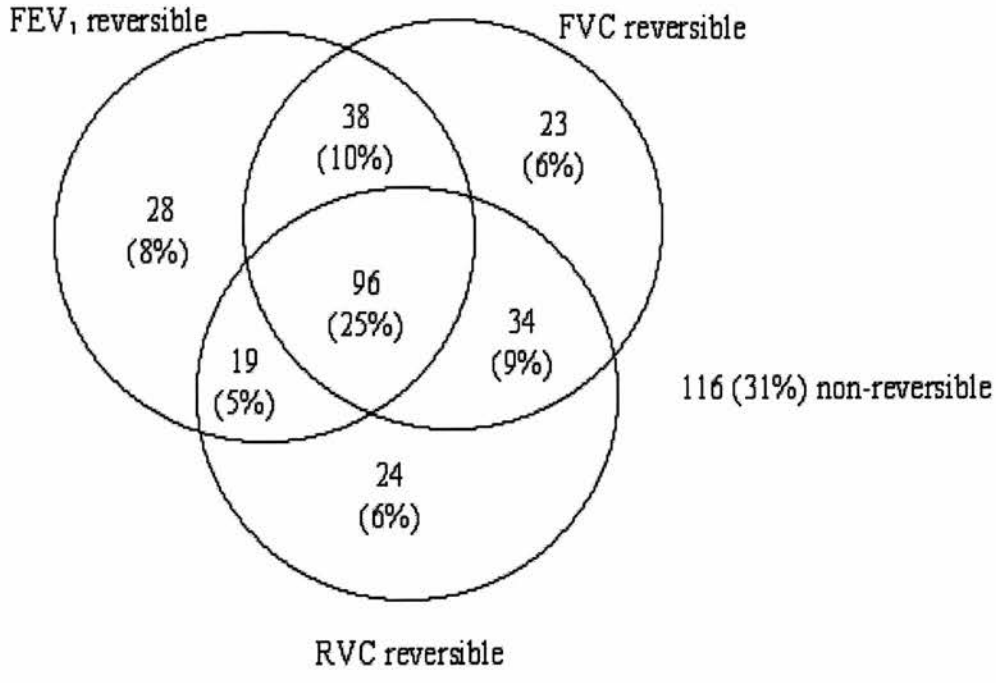


Figure 2.5: Venn diagram representation of results.
Numbers inside circles represent number of patients who demonstrated reversibility in the variables. Data in brackets represent the percentage of the total number of patients studied.

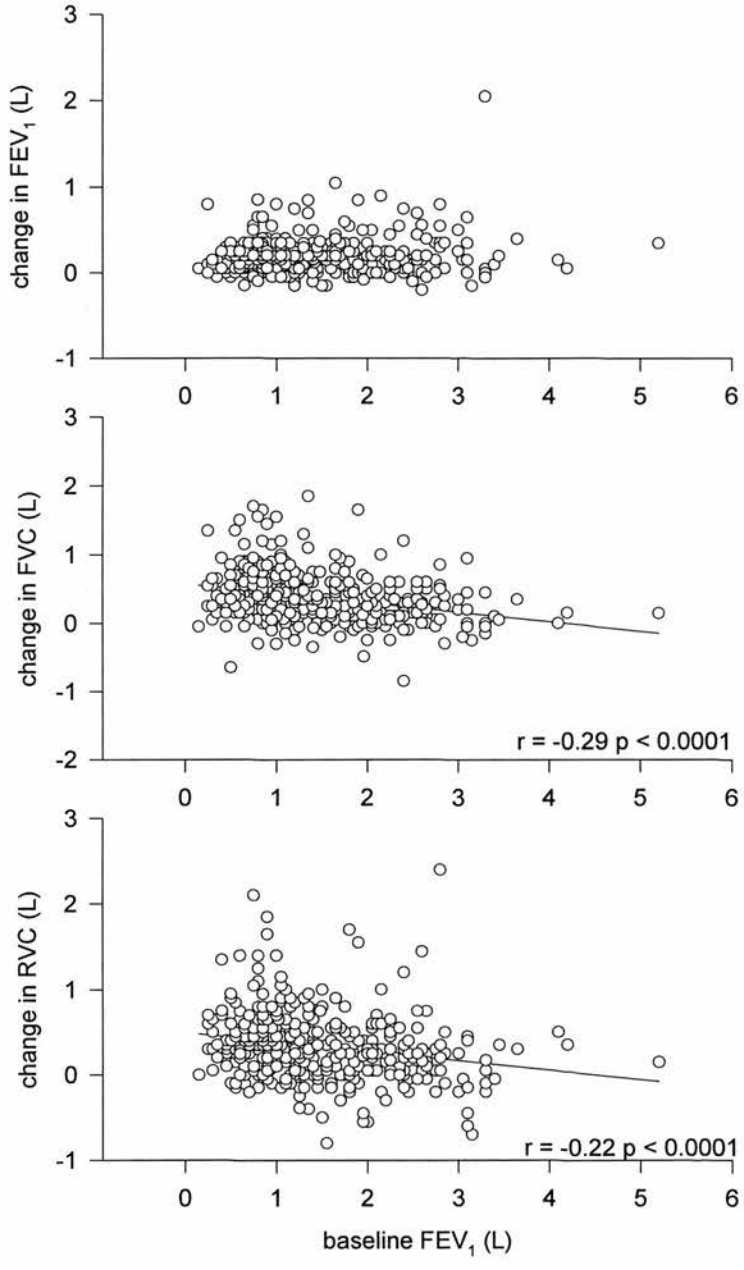


Figure 2.6: Effect of severity of lung disease on reversibility variables. Correlations of baseline FEV_1 values with absolute change in respiratory variables after bronchodilation. Pearson product moment correlation and p values are shown calculated from log normalised data, N=378.

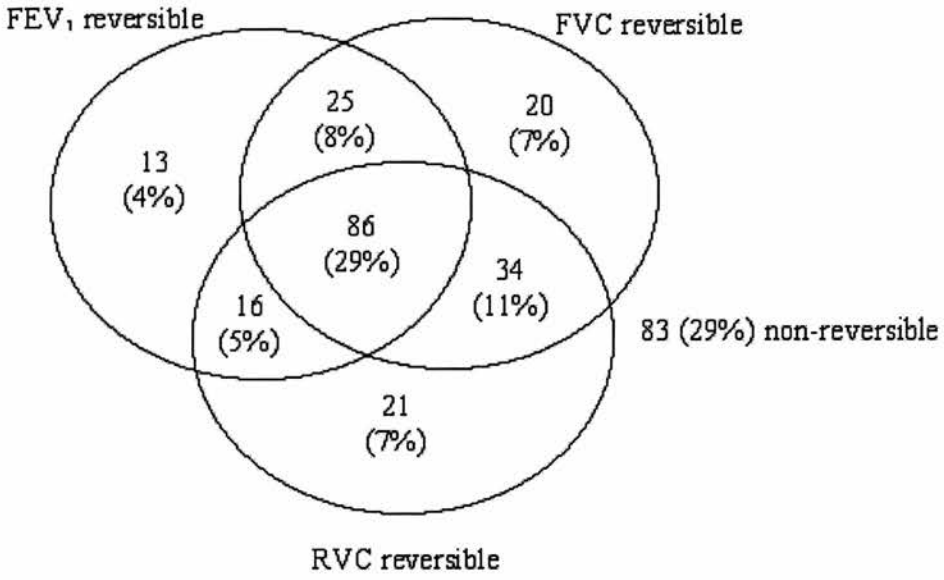


Figure 2.7: Venn diagram representation of results for patients with referring diagnosis of COPD only.
Numbers inside circles represent number of patients who demonstrated reversibility in the variables. Data in brackets represent the percentage of the total number of patients studied.

2.4: Discussion.

This retrospective study of routinely collected lung function data has shown that 48% of this patient group have demonstrated FEV_1 reversibility, but an additional 21% had reversibility in RVC and / or FVC but not in FEV_1. These FVC and RVC changes are greater than the within-patient test-to-test variability and therefore represent physiological change following bronchodilators that is missed if only FEV_1 criteria are used.

The BTS guidelines [66] focus entirely on FEV_1 and state that an increase in FEV_1 should be used to define reversibility to bronchodilators and that the post-bronchodilator FEV_1 can be used as a marker against which to assess future treatment. A negative FEV_1 response does not preclude benefit from bronchodilators in terms of improved walking distance or a reduction in the perception of breathlessness.

Thus they state, in line with common clinical experience, that some patients have an unexplained benefit from bronchodilators that is not reflected in FEV_1 reversibility. Does this benefit correlate with, and is it predictable from, bronchodilator changes in FVC and RVC? To answer this would require a prospective study of subjective and objective benefit from bronchodilators, comparing FEV_1 responders with FVC and RVC responders and with non-responders.

One main problem when looking at the response to bronchodilators is the way in which the change in values is expressed. Anthonisen et al [70] showed that in patients with COPD the percentage change in FEV_1 was greatly influenced by the initial FEV_1 value. Absolute change was not significantly related to the initial FEV_1 although a large absolute change in FEV_1 was most commonly found in patients with a

high initial FEV_1. They concluded that using just one definition of response would introduce bias and so two variables (absolute change and percentage change) showing concordant changes should be used to interpret a negative or positive response. Our data distinguishes responders from non-responders on the basis of absolute change only, this was chosen as there is no agreed value for a significant increase in FVC and RVC in terms of percentage change. However when looking at FEV_1 response alone for this patient population if significant reversibility was defined as greater than 160 ml and 15% change then a further 40 patients would be deemed unresponsive on FEV_1 criteria or if the BTS criteria for expressing significant change in FEV_1 was used (i.e. 200 ml and 15% increase post bronchodilator) the percentage of patients that would be defined non-responsive would be increased from 52% to 240/378 (63%). Thus using either of these alternative definitions of reversibility would result in even fewer patients being classified as responders despite their showing bronchodilator induced changes in FVC and RVC greater than the natural variability.

This study also showed a statistically significant negative correlation between baseline FEV_1 measurement and the absolute change in FVC and RVC post bronchodilator. This is not seen with the change in FEV_1 post bronchodilator. This means that patients with more severe airflow obstruction are more likely to show improvement in FVC and RVC following bronchodilators. This may be because functional effects of bronchodilation are occurring which are only picked up by the RVC and FVC and remain undetected by FEV_1. One such bronchodilator effect could be on the increased closing volume that is often seen in patients with chronic airflow obstruction. This increase results from a loss in elastic lung recoil and from narrowed airways and results in an increased residual volume (RV), the amplitude of which is related to the severity of the lung disease. If after bronchodilation the airway calibre is

increased, airway closure will occur at a lower lung volume and RV will be decreased. This would lead to an increase in FVC and RVC that is related to the severity of the airflow obstruction. However as FEV_1 is largely unaffected by closing volume these changes in FVC and RVC may occur in the absence of a change in FEV_1.

Another reason for the lack in correlation between baseline FEV_1 and change in FEV_1 may be the way the data are expressed. A study by Brand et al [71] examined the best way of expressing bronchodilator response. They confirmed the conclusions of Anthonisen [70] that using the percentage change could lead to the belief that patients with a low initial FEV_1 were more responsive to bronchodilators. They stated that if absolute change was used a true bronchodilator response could be distinguished from random variation in FEV_1, but this does not correct for differing lung sizes; such corrections may be made by relating the absolute change in FEV_1 to the initial FEV_1 % predicted. If this approach is taken with the present study data then the absolute change in FEV_1 is still not correlated with the pre FEV_1 %predicted and the absolute change in FVC and RVC are still negatively correlated with initial FEV_1 % predicted ($r=0.41$ $p<0.0001$ and $r=0.30$ $p<0.0001$ respectively).

This study was retrospective and the following factors should be borne in mind when interpreting the results. There is no agreed standard technique for performing an RVC manoeuvre. Attempting to standardise flow rate (for example by incentive spirometry) was tried but found to be inappropriate because the wide range of vital capacity in the population results in widely varying expiratory time. Instead the instruction to patients was simply to "Take a big breath in and the gently blow all the way out". The range of resulting expiratory flow is therefore large with some patients, on the first attempt, virtually performing an FVC manoeuvre whilst others blowing so slowly that the exhaled volume was determined by the breath holding

ability of the patient. Where these patterns were seen repeat measures were obtained. Ideally for future studies an optimal standardised method for recording an RVC should be defined possibly by setting a target flow rate to achieve exhalation of the vital capacity in a defined time.

The protocol for RVC measurement currently requires a repeat RVC attempt if the first RVC was less than FVC. While this may have introduced a selection bias towards finding more RVC > FVC results, it is unlikely that this is an important source of bias. This is because most measurement errors in FVC and RVC will lead to underestimation, i.e. it is difficult to get an erroneously high value of RVC or FVC, so the finding of RVC > FVC, when FVC has been performed to standard criteria of reproducibility is likely to be a genuine result rather than an artefact of measurement.

In this retrospective study, the precise diagnosis for the patients was not known, as the investigator was entirely dependent on the clinical details entered by the requesting physician on the request form. The inclusion criteria were that the patients were referred with clinical details indicating obstructive lung disease, showed obstructive spirometry on baseline testing and went on to have bronchodilator responses tested. The majority had a clinical diagnosis of COPD, many of the remainder had asthma and a few had miscellaneous diagnoses such as bronchiectasis. The data for those patients who had a referring diagnosis of COPD only were analysed and the percentage of non-responders in respective to FEV_1 only increased by 1% to 53%. All other values were similar to those from the data from all referring diagnoses. Therefore although each of these diseases leads to airway obstruction via different processes, as the aim of this retrospective study was to show that in some patients reversibility to bronchodilators was only shown in vital capacity measurements and not

in FEV_1 it was felt acceptable to include all the data regardless of the referring diagnosis.

Further studies are needed to answer several questions that could not be addressed by the present retrospective study. Firstly the reproducibility of FVC and RVC reversibility remains to be determined. Some work has been done studying the short-term variability of VC by Tweeddale et al [69]. They found that an increase of 330ml or more was required to show a significant response in vital capacity to bronchodilators. This figure excludes the natural variability in the measurement with 95% confidence. However, to date, no studies have been reported which investigate the reproducibility of the response of FVC and RVC to bronchodilators. Secondly the correlation of these variables with clinical or symptomatic benefit should be assessed. The aim of reversibility testing is to target bronchodilator therapy to patients who will receive clinical benefit from them. The study by Smith et al [7] found that a portion of patients who showed no FEV_1 reversibility on spirometry did show significant improvement in plethysmographic volume related variables, however they did not measure any score of the patient's subjective breathlessness although this reported subjective improvement was the basis for their study. The present retrospective study also involved no subjective measure of clinical improvement.

Therefore, in summary, this study showed that 1) a significant proportion of this group of patients are being identified as non-responders to bronchodilators on FEV_1 criteria when they may, in fact, have measurable physiological improvement from them; and 2) that FVC and RVC measurements appear to detect a group of patients who show a functional effect of bronchodilation which is undetectable by FEV_1. This study supports Smith et al's [7] statement that there is no one 'best test'

for reversibility but that FEV_1, FVC and RVC should be used together to assess reversibility especially in those patients with more severe airflow obstruction.

In light of the results from this retrospective study it was felt that alternative methods of assessing airflow obstruction were needed that would be more sensitive than FEV_1 whilst still being easily applicable to everyday clinical testing. The rest of this thesis examines one possible approach of using tidal breathing expiratory flow profiles to provide information on the presence and severity of airflow obstruction.

Chapter 3: Measurement of Tidal Breathing in Man: Methods.

3.1: Determinants of normal tidal flow patterns.

3.1.1: Wakefulness drive.

In 1961 Fink [72] proposed the presence of a wakefulness drive to breathe. In this study patients were subjected to either passive (mechanical ventilated) or active (voluntary) hyperventilation so that $PaCO_2$ was reduced by at least 10 mmHg and the PaO_2 was raised. It was reported that even though any chemical drive to breathe was assumed absent the patients who stayed awake all continued to breathe rhythmically at a normal frequency. However one patient who became drowsy stopped breathing. Fink therefore asserted that "Cerebral activity associated with wakefulness is a component of the normal respiratory drive".

This apnoeic response reported by Fink has now been repeatedly observed in many studies in both anaesthetised and sleeping humans [73-76] made hypocapnic by either passive mechanical hyperventilation or hypoxia-induced hyperventilation.

3.2: Critique of Spontaneous Breathing Measurements.

There are a number of problems associated with the use of spontaneous breathing in the assessment of airflow obstruction. These include: -

3.2.1: Behavioural influences.

As stated above the breathing pattern is influenced by the arterial blood gas concentrations that change in relation to changing metabolic demands. During wakefulness the breathing pattern is also influenced by behavioural demands and these two control systems are in competition with one another.

Shea [77] describes the effects of different behavioural stimuli on the breathing pattern. He stated that the behavioural control causes inhibitory and/or excitatory inputs to respiratory motor neurons that affect V_T, breathing frequency, airflow pattern and the recruitment profile of different respiratory muscles. Behavioural influences include those under explicit voluntary respiratory control e.g. speech, singing, sniffing or swimming; and those where there is no explicit voluntary control e.g. mental activity, coughing, swallowing and exercise.

3.2.2: Equipment effects.

Gilbert et al [78] were the first to show that breathing through a mouthpiece, with noseclips on, into respiratory apparatus results in a higher V_T, a lower breathing frequency and an unchanged or increased minute ventilation compared to unimpeded

breathing. These changes were attributed to the irritating effects of the instrumentation on the nasal and oral mucosa. However Douglas et al [79] suggested that the change in breathing route (i.e. a shift from nasal breathing to oral), rather than the instrumentation, was responsible for the changes in breathing pattern. This theory was supported by studies by Rodenstein et al [80]. Perez et al [81] postulated a number of causes for the change in breathing pattern when using a mouthpiece and noseclip. These included a change in resistance to airflow, change in dead space, decreased stimulation of nasal flow receptors and bypassing the humidifying and temperature adjustment capabilities of the nasal passage. Cole et al [82] observed that the use of a mouthpiece decreased airflow resistance and stated this could be one reason for the increase in minute ventilation, this was supported by Weissman et al [83] who found that decreasing the mouthpiece diameter and thus increasing airflow resistance prevented the changes in minute ventilation.

Drummond [84] suggested that the use of a mouthpiece and hence oral breathing was also associated with a smaller FRC and RV than when a mask is used, possibly via the activation of inspiratory muscles during mask breathing.

3.2.3: Effects of intervention with regard to any changes in FRC or breathing pattern.

In a study by Rusconi et al [85] the effects of changes in respiratory rate post histamine challenge on tidal expiratory flow variables in infants were investigated. They found that the change in T_{PTEF}/T_E was positively correlated to respiratory rate. When looked at further they found that with small increases in respiratory rate T_E did not change but T_{PTEF} and T_{PTEF}/T_E did. However in infants who had large changes in

respiratory rate post histamine, T_E was shortened and masked the simultaneous change in T_{PTEF}, such that T_{PTEF}/T_E did not change. They concluded that T_{PTEF}/T_E could not be used reliably to evaluate airways obstruction when concomitant changes in respiratory rate occurred.

Western and Patrick [86] found that focusing attention on breathing, even without a facemask or mouthpiece caused the breathing pattern to become significantly slower and deeper than normal.

It is therefore important to be consistent in the type of measuring apparatus used when recording tidal breathing in order to decrease any changes in the breathing pattern due to the apparatus. It is also important to control the external environment so that stimuli do not affect the breathing pattern during recording of tidal breathing. Ingvar [87] and Phelps [88] defined a "resting unstimulated state" for their studies. They stated that the subject should be relaxed with a minimum of sensory input motor output and cognitive activity whilst avoiding fluctuations in the level of alertness. Shea et al [77] found that this was impossible to achieve in most people for long periods without causing drowsiness. For studies lasting longer than about 10 minutes Shea aimed at making measurements in a steady state, with the subject reading or listening to a taped story in order to control the level of arousal.

3.3: Collection of Tidal Breathing Data.

3.3.1: Introduction.

The studies described in the following chapters rely upon the reproducible collection of tidal breathing data during a steady resting state. Consideration of the points outlined below was important whilst developing the collection methodology.

- Auditory and visual stimuli.
- Effect of differing levels of arousal.
- Equipment effects.

The collection method has evolved over time and only the final protocol is described here.

3.3.2: Choice of Equipment.

3.3.2.1: Flowmeter.

Accurate flow measurements are central to the examination of tidal flow patterns, it was therefore important that the flowmeter used fitted the following criteria: -

- Light weight. As the flowmeter would be attached to the subject via a facemask with no other support it was important that it was as light as possible to avoid discomfort to the subject.
- Add minimal external resistance. In order that the effects of the measuring equipment on the breathing pattern be minimised as much as possible it is important that the flowmeter should not add an external resistance. ATS criteria [89] are that the resistance should be less than 1.5 cm H_2O/L/s.
- Minimal dead space. Sackner et al [90] reported that increasing the dead space of the recording equipment increased ventilation by increasing V_T, therefore to minimise equipment effects, the dead space of the flowmeter should be as small as possible.
- Linear response over the physiological range of resting flows.
- Stable zero flow over long periods of time.

The flowmeter used throughout this work is the Birmingham Research and Development Limited (BRDL) ultrasonic flowmeter, which has the following specifications: -

- Weight 100 g.
- Linearity, quoted by manufacturer as linear over 60 L/sec, tested over a range of flows from –2 L/sec to 2 L/sec (see chapter 3.3.2).
- Acceptably low dead space (175ml).
- Minimal resistance to airflow.
- Stable zero over time periods of at least 20 minutes. Not adversely affected by condensation, as zero flow does not drift after it has been breathed through for at least 20 minutes (see Appendix C).

For a detailed explanation of the principle of operation and technical specifications see Appendix A.

3.3.2.2: Face mask.

Studies [78-83] have shown that breathing through a mouthpiece increases V_T and V_E and may lower frequency. Later studies [79-81] have suggested that this change is due to the change in breathing route from nasal to oral, rather than the actual instrumentation. Drummond [84] has also shown that FRC and RV are reduced during mouthpiece breathing compared to mask breathing. To overcome this effect on breathing pattern we used a face mask during tidal breathing flow collection, so that each subject could breathe through their nose, mouth or a combination of the two, thereby not altering their normal breathing route. The self–sealing adult facemask (System 22, Medic-Aid Ltd, West Sussex, England) used has an inflatable balloon seal and is lightweight, 50 g (figure 3.8).

During the studies it is necessary to change from recording flow to delivering inhaled therapy. Removal of the facemask flow meter assembly to allow therapy to be

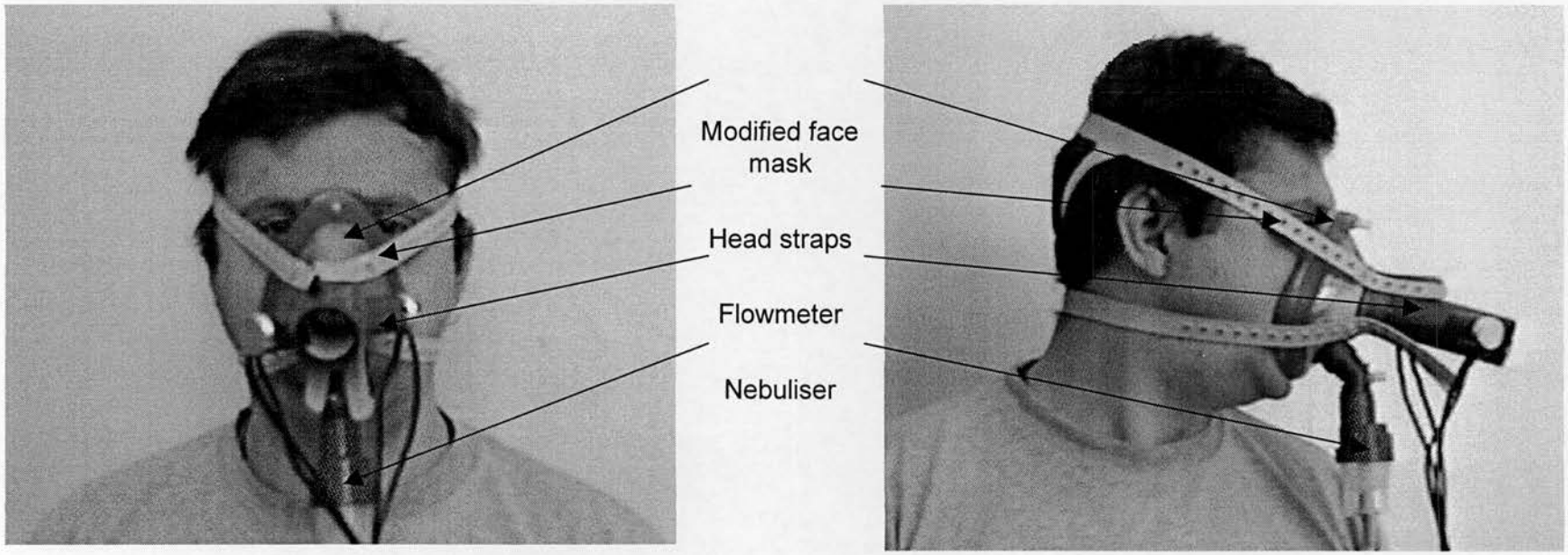


Figure 3.8: Photographs of the modified face mask used for tidal breathing flow data recording along with attached sidestream nebuliser and ultrasonic flowmeter.

given would cause disturbance to the subject and necessitate a second acclimatisation period. To overcome this a second port was added to allow the flow meter and a nebuliser pot to be attached concurrently (figure 3.8). The disadvantage of this arrangement is that the dead space of the equipment was increased from 175 ml to 215 ml (the equipment dead space of the mask was measured by fitting it to a simple model of a face and filling with water, the dead space of the nebuliser chamber was also measured by water fill). However the advantage of not needing to disturb the subject outweighs this increase in equipment dead space.

3.3.2.3: Computer.

To facilitate averaging and analysis, data were captured directly to a computer and stored in digital form. The output from the flow channel of the flowmeter box was connected to channel one of an Amplicon PC26AT analogue to digital converter, via a connector box. An in-house computer program (Appendix B) sampled the flow data from the ADC card at 100 Hz. This frequency was chosen, as it is sufficiently high to reproduce the frequency content of the physiological flow signal. The data were converted into mV, using a computer algorithm, and then into L/sec and the result stored on disk. The mV to L/sec conversion factor was calculated by using a rotameter to blow air through the flowmeter at known flow rates (see section 3.3.2).

3.3.3: CONTROL OF VISUAL AND AUDITORY STIMULI.

3.3.3.1: Blindfold.

In 1996 Shea et al [91] reported the effects of visual and auditory stimuli on resting ventilation. They found that adding either auditory or visual stimuli to a baseline condition of relaxed wakefulness increased V_E, and decreased frequency and concluded that when measurements of breathing at rest are made the environmental and behavioural variables need to be controlled. To achieve this all studies were carried out in a quiet dimmed room with the subjects blindfolded.

3.3.3.2: Tape player and headphones.

Ingvar [87] and Phelps [88] have defined a 'resting unstimulated state' for making respiratory measurements, stating that the subjects should be relaxed with a minimum of sensory input, motor output and cognitive activity. However due to the length of time that subjects' breathing was recorded (between 15 and 40 minutes), it was possible that subjects would become drowsy or even fall asleep, as a result of controlling the environmental and behavioural conditions, thereby changing the "wakefulness drive to breathe" [77] component of their breathing pattern throughout the study. Studies were therefore performed under conditions of resting steady state as outlined by Shea [77] by playing the subjects a taped story through headphones to control the level of arousal. This had the added benefit of providing a distraction to the subjects so that they did not think about their breathing and change their breathing pattern.

Patients were observed during the recording periods for any evidence that they had fallen asleep, such as loss of small body movements or slowing of the respiratory rate. They were also required to perform maximum inspiration at 5 minute intervals during the recording periods allowing the level of wakefulness to be assessed and asked at the end of the recording period if they had fallen asleep.

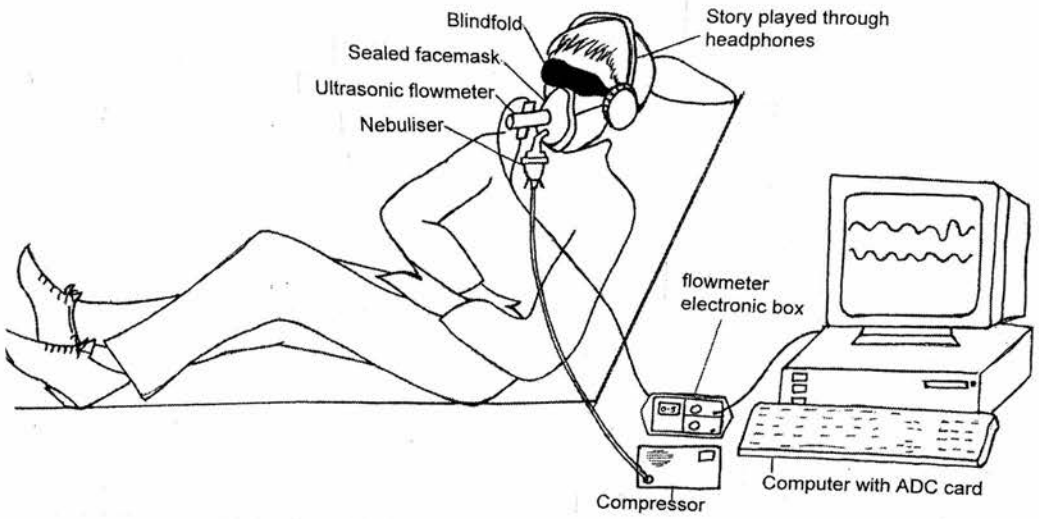
3.3.4: PROTOCOL.

Subjects for all studies described in this thesis were recruited from the population of patients who attended the respiratory function laboratory for routine reversibility testing. They were told that the purpose of the studies was to look at different ways of assessing breathing problems, but were not told that it was tidal breathing that was being primarily studied.

Chairman's approval from the local Ethics Committee was obtained prior to commencement of the studies and informed consent was obtained, in writing, from subjects at the time of their enrolment into the studies.

Subjects were fitted with the self-sealing facemask with a rubber head strap and the mask checked for leaks. The ultrasonic flowmeter was connected to the top port on the facemask and the nebuliser pot attached, via an angled connector, to the lower port (figure 3.8).

Subjects were asked to sit on a couch in a semi recumbent position with their head resting on a pillow, so that they were as comfortable as possible and asked not to move or speak during the study period, so that breathing artefacts were not introduced, and to listen to the taped story (figure 3.9). Ramirez-Venegas et al [92] observed that



Flow rate was sampled by the PC at 100 Hz.

Figure 3.9: Diagrammatic representation of flow recording and nebulisation equipment.

salbutamol reduces dynamic hyperinflation therefore to monitor the lung volume at which subjects were breathing, the inspiratory capacity (IC) was measured. Subjects were told that they would be asked to breathe in until their lungs are full and then hold their breath for 1-2 seconds approximately every 5 minutes. The breath hold period also allowed monitoring of zero flow to ensure no drift had occurred during the study period. They were not told that their spontaneous breathing pattern was being recorded, again to limit how much they thought about their breathing.

The subject was left for at least 5 minutes to rest and then once the equipment was attached and the subject was comfortable they were left for a further 2 minute acclimatisation period so that they had time to get used to breathing with the facemask on and to relax so that a steady state was achieved. These times were judged adequate after analysis of much longer periods of tidal breathing under these circumstances in pilot studies (see chapter 3.3.1). No subjects were unable to tolerate the recording equipment and none admitted to falling asleep on questioning after the study.

After this time for acclimatisation, sampling of airflow was started and once every 5 minutes the subject was asked to inhale to total lung capacity and then breath-hold for 2-3 seconds at TLC.

If nebulised treatment was being given, flow sampling was stopped and a three way tap (placed between the compressor and the inlet of the nebuliser pot) opened to connect the compressor to the nebuliser pot and allow nebulisation to begin.

3.4: Analyses Of Tidal Breathing Data.

3.4.1: Introduction.

In house computer programs were developed for the analysis of the flow data. This allowed for fast analysis of a large number of breaths, which would have been impossible if the analysis was done by hand.

There were a number of problems associated with the recording of tidal breathing and its analysis that had to be overcome. The main one was the breath-to-breath variability observed during spontaneous tidal breathing. Priban [15] observed that there were breath-to-breath variations in the rate and depth of spontaneous breathing pattern in normal subjects. Newsom Davis [17] looked at volume and time components of breathing pattern and again found breath-to-breath variability in normal subjects at rest. Loveridge [25] studied the spontaneous breathing pattern at rest in subjects with chronic airflow obstruction and observed that although the breath-to-breath variability was less than in normal subjects it was still present. Therefore if only one or a few breaths were analysed per subject per study period any change observed could be due to breath-to-breath variability and not a true change.

To overcome the problem of breath-to-breath variability it was decided to sample and record 5 minutes of tidal breathing for each set of data required. This equated to 13 $\pm$ 5 breaths per minute. From this large number of breaths average breaths were calculated for each study period that could be compared before and after treatment and with conventional respiratory variables. The creation of averaged breath profiles results in very significant reduction in the random and cardiac-related flow artefacts seen in single-breath recordings. However there were still a number of

problems inherent in calculating an average breath from a large number of variable breaths. These included: -

- Aberrant breaths due to body movement or behavioural influences under explicit voluntary respiratory control e.g. speech, sniffing and sighing; and those not under explicit voluntary control e.g. coughing and swallowing.
- Varying breath length. As stated above breath length can vary breath-to-breath meaning that averaging is not a simple as it first appears.

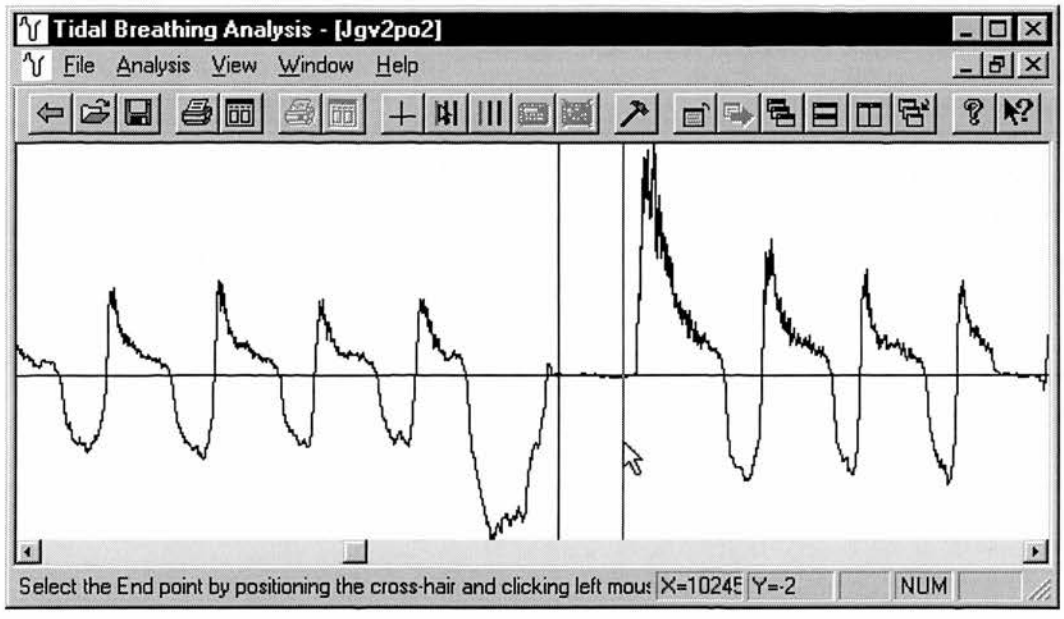
Various steps in the calculation of averaged breaths were performed to overcome these problems. As with the collection protocol the analysis procedure has evolved and only the final analysis method is outlined here. A copy of the program can be found in Appendix B.

3.4.2: Tidal Breathing Analysis Program.

Each period of breathing was checked for zero flow drift, and re-zeroed if necessary (figure 3.10). Algorithms were then applied to determine the start of each expiration and inspiration and, in order to exclude expiratory pauses, the end of expiration (figure 3.10). These algorithms were developed by visual inspection of large amounts of raw flow data to determine the equations which best picked up the start of expiration and inspiration. The end of expiration was defined as the point at which the flow rate falls below a threshold value of 5 ml/sec (see Appendix B).

Raw flow data were displayed visually, on the computer screen, as a flow / time graph along with the automatically generated breath markers. A dialog box was

A



B

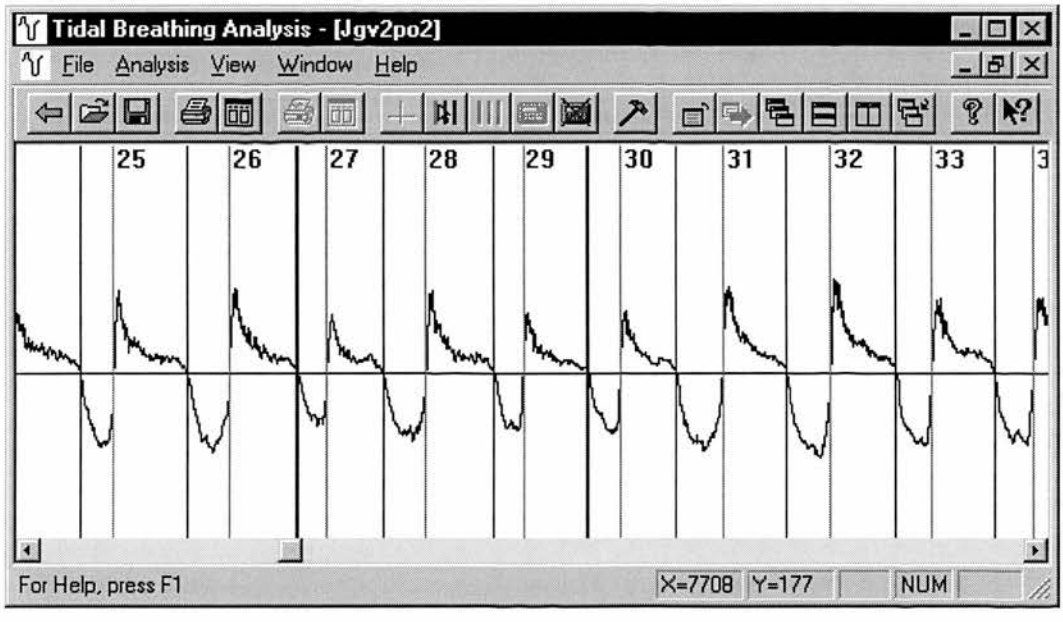


Figure 3.10: Screen shot of tidal breathing analysis program showing A) re-zeroing of flow and B) sample flow data with breath markers inserted.

then activated which allowed the operator to select breaths for analysis. All available breaths were included with the following exceptions: -

- Aberrant breaths due to sighing, coughing, speaking or swallowing were visually identified and manually excluded from further analysis along with one breath either side.
- Breaths that had not been detected by the breath detection algorithms.
- Exceptionally long or short duration breaths (see below).

Because of the way that the averaging is performed for subsequent analysis (see above) any abnormally short or long breaths would affect the average breath. Including short breaths would mean that the average breath would be short, as averaging is stopped when the end of the shortest breath is reached. Similarly, including very long breath in the averaging would mean that the averaged breath would include data from only the first portion of a long breath and therefore artificially increase the flow rate of the later part of the averaged breath. Therefore the analysis program excluded any abnormally short or long breaths from the averaging, to do this the mean breath duration (MBD) of the selected breaths was calculated and any breaths outside of ± 15% of the MBD were excluded. The value of 15% was determined by looking at frequency histograms of expiratory time for 5 minutes of tidal breathing data for 4 subjects and determining the percentage of MBD that would exclude "outsider" breaths (figure 3.11).

The remaining breaths were then used to calculate the standard tidal breathing variables as summarised in table 3.3. The novel tidal breathing variables were calculated from averaged breath data. Because of variable breath lengths a simple average breath for a complete inspiration and expiration could not be produced.

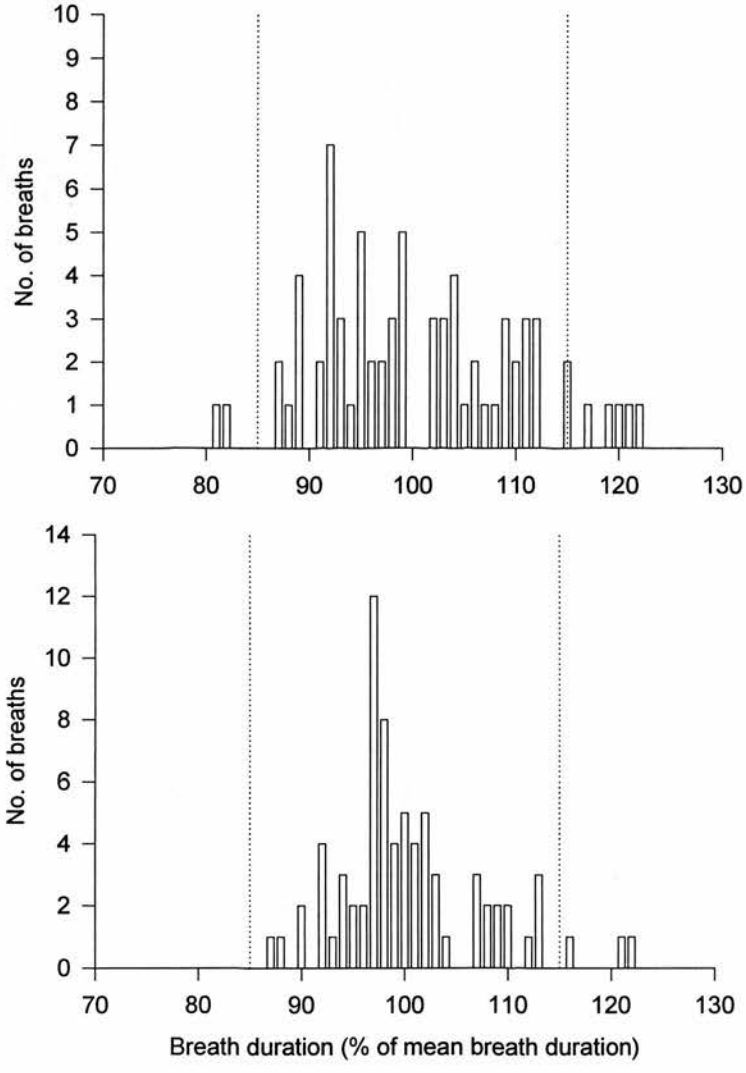


Figure 3.11: Data from two representative patients showing the number of breaths to be excluded if those outside of the mean breath duration (MBD) ± 15% were omitted from analysis.

Variable	Units	Description
V_T	L	Tidal volume
F	Bpm	Frequency
V_E	L/min	Minute ventilation
T_E	Sec	Expiratory time
T_I	Sec	Inspiratory time
T_{ET}	Sec	Expiratory time to flow threshold (excluding expiratory pauses)
T_{TOT}	Sec	Total breath duration

Table 3.3: Conventional tidal breathing variables calculated from raw tidal flow data.

There were a number of possible ways to overcome the problem of varying breath duration. Benchetrit [21] and Williams [62] et al used a method of normalising the breath duration. However as the mechanical relaxation properties of lung tissue are time-dependent, normalising breath length would arbitrarily distort the data in the time dimension, potentially obscuring important but small changes in the flow pattern. Therefore two different averaged breaths were produced for each time period (figure 3.12). The first, to examine the early part of the expiratory flow profile, was created by breaths being aligned at the onset of expiration and averaged up to the end of the shortest breath (S_{EXP}). The second, to examine late expiration, was created by breaths being aligned at end expiration and averaged back to the onset of the shortest breath (E_{EXP}). For each method, flow data were averaged in 10ms time bins. A number of variables could then be measured from these "bin-averaged" breaths.

A summary of the different methods that could be used to generate representative tidal flow profiles along with advantages and disadvantages of each method is shown in table 3.4

3.5: Validation of Data Collection Protocol.

3.5.1: Optimisation of the data collection time.

3.5.1.1: Introduction.

Not only must any new test of airflow obstruction be reliable and reproducible it must also be acceptable to the patients to perform. During the collection of tidal

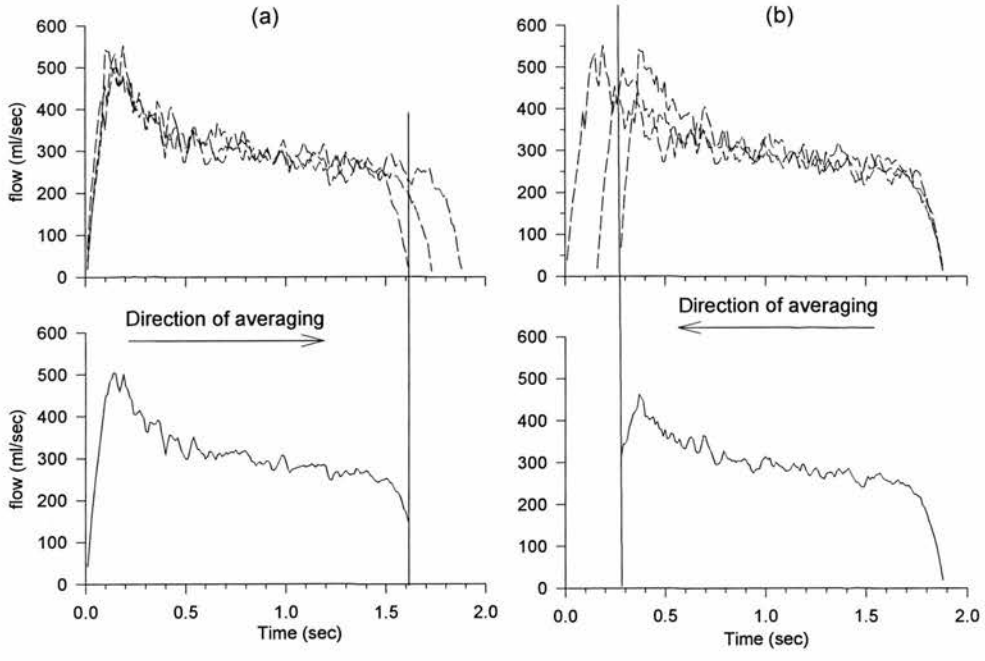


Figure 3.12: Diagrammatic representation of time bin averaging used to construct average breaths from tidal breathing flow data.
(a) To examine the early part of the flow profile, breaths were aligned at the onset of expiration and averaged up to the end of the shortest breath. (b) To examine late expiration breaths were aligned at end expiration and averaged back to the onset of the shortest breath.

Method	Advantages	Disadvantages
Analyse single breaths and average results	No distortion of data	Extremely laborious therefore limited to only a few (?representative) breaths Results influenced by random and cardiac artefact (noise)
"Normalise" all breaths to the same breath duration then average flow data	One way to overcome differing breath length	Distorts data in time dimension, affecting time-dependent relaxation data Each breath needs separately scaled to a standard length – laborious
Average data in time bins, treating start and end of breath separately	Easily automated for large numbers of breaths No distortion of flow data in the time dimension	Cannot generate a single complete averaged breath (but can still compute mean Ti, Te and Ttot)

Table 3.4: Summary: methods of generating averaged "representative" tidal flow profiles

breathing data the most common comment from subjects was that it took a long time and that wearing the face mask could get slightly claustrophobic towards the end of the collection period. Also the longer the collection time the larger the probability that the patient would become drowsy and any change in wakefulness could cause breathing pattern changes as discussed earlier. In pilot studies, 15 minutes of resting tidal data were collected and this made possible the comparison of values obtained from the first 5 minutes of data with those obtained from the full 15 minutes of data. This analysis was performed to determine the minimum time needed for collection of acceptable data.

3.5.1.2: Method.

Data collected from pilot studies were analysed. Briefly, the subjects were allowed to relax for at least 5 minutes whilst the equipment was prepared. Then to allow them to acclimatise to the flow recording equipment they were left for a further 2 minutes, once the equipment was attached (as described in chapter 3.1.4), before airflow sampling and recording was started. 15 minutes of airflow data from spontaneous tidal breathing were then sampled, displayed and stored on computer.

The 15 minutes of data were divided into 3 sets of 5 minutes and average breaths and tidal breathing variables calculated on both the full 15 minutes of data and on the first 5 minutes of data. Paired t-tests were performed to determine if there were any significant differences between the results obtained from 5 and 15 minutes periods of data.

Results were considered to be statistically significant if the probability was < 0.05.

3.5.1.3: Results.

Data were collected from 25 patients (4 studies per patients). There were no significant differences between the tidal breathing variables obtained from 5 minutes of data compared with those obtained from 15 minutes of data (Table 3.5) Bland and Altman plots [93] were constructed for V_T. Freq, V_E, EF_{25}, $TBEV_1$ and TPEF showing the results graphically (figure 3.13), and these confirm that there is no systematic difference between the results obtained by collecting 5 minutes and 15 minutes of data.

3.5.1.4: Discussion.

In order to optimise the methodology for patient compliance and acceptability and to make the test applicable for every-day use it was necessary to decrease the collection time. A sampling period of 5 minutes was used because at a normal breathing frequency of 12 breaths per minute this gave approximately 60 breaths to be used in the construction of the averaged breaths. This could only be done if the results calculated from the 5 minutes of data were not significantly different from that obtained from the original 15 minutes of data. An analysis of data has shown that there were no statistically significant differences between the two collection times for any of the tidal breathing variables, whether calculated from the original data (e.g. V_T, freq and V_E) or calculated from the averaged breaths (e.g. EF_{25}, $TBEV_1$ and TPEF (see chapter 5.1). Therefore all subsequent study protocols involved data collection over 5 minutes instead of 15 minutes.

Variables	Median value		Significance
	15 minutes	5 minutes	
V_T	0.63	0.63	NS
Freq	17.0	17.2	NS
V_E	10.5	10.6	NS
T_E	2.22	2.21	NS
T_I	1.33	1.30	NS
T_{ET}	2.22	2.20	NS
T_{TOT}	3.52	3.47	NS
EF_{25}	0.25	0.25	NS
$TBEV_1$	0.37	0.37	NS
TPEF	0.49	0.49	NS

Table 3.5: Comparison of values calculated from 5 and 15 minutes worth of data.
P values from Wilcoxon Signed Rank Test. NS = not significant.

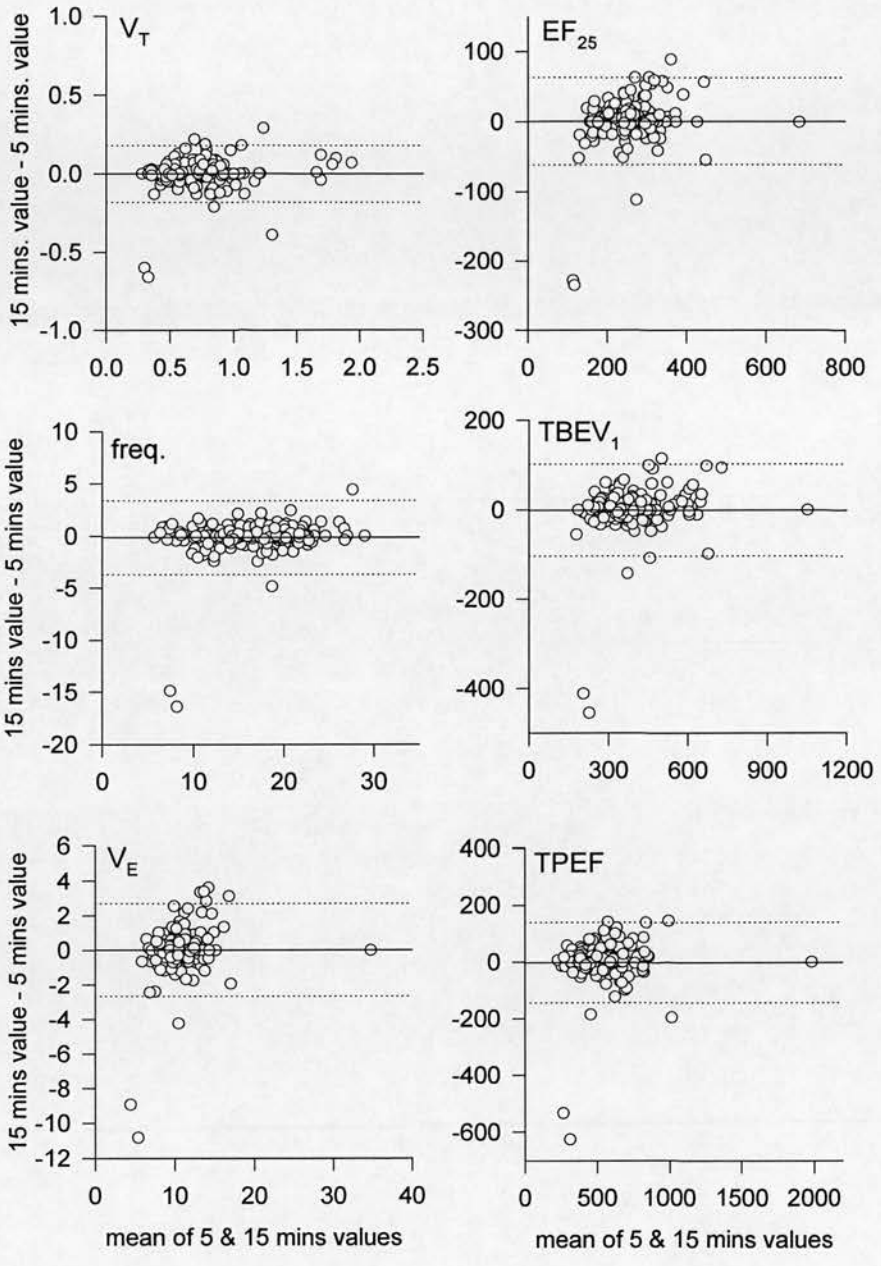


Figure 3.13: Bland and Altman plots showing comparison of 5 and 15 minutes data analysis.
Solid line is the mean difference and dashed lines the 95% confidence limits of the difference between results from 5 and 15 minutes data.

3.5.2: VERIFYING THE SAMPLING RATE AND DETERMINING THE FLOW RATE CORRECTION FACTOR.

3.5.2.1: Introduction.

Before the data collection protocol could be used in any study, the sample rate had to be checked to ensure that it was 100 Hz. Also the correction factor had to be determined to convert the voltage output from the analogue to digital converter to L/sec for the flow rate channel.

3.5.2.2: Method.

To check the sampling frequency a signal generator was connected to the flow rate channel of the connector box. This was then used to deliver a square wave pulse at three different frequencies (10 Hz, 1 Hz and 0.1 Hz).

The voltage output from the signal generator was recorded using the data collection programme and the data subsequently analysed to check that the collection programme was sampling at 100 Hz.

To calculate the correction factor for the flow rate a rotameter was connected to ultrasonic flowmeter and flow was recorded at a range of rates between –2 L/sec and 2 L/sec. The resulting voltage output from the ADC recorded at each flow rate were plotted against the flow rate and linear regression used to calculate a conversion equation.

Once the correction factor had been determined, it was checked by putting 3 litres of air through the flowmeter at different stroke rates using a Hans Rudolph

calibrating syringe. The resulting volumes calculated from delivering 3L at differing flow rates were recorded and the variability calculated.

3.5.2.3: Results.

The number of samples in each square wave was counted for each of the three frequencies. Figure 3.14 shows that for 10 Hz each square wave consisted of 10 samples, for 1 Hz each square wave consisted of 100 samples and for 0.1 Hz each square wave consisted of 1000 samples. This confirmed that the programme was sampling at 100 Hz, i.e. 100 samples every second.

Figure 3.15 shows the raw data recorded by the data collection programme at the different flow rates applied to the ultrasonic flowmeter by the rotameter.

Figure 3.16 shows the linear relationship between the voltage output from the ADC and the actual flow rate. The equation of the fitted line was hard coded into the analysis program so that all resulting flow data were recorded in L/sec.

Regression line equation

Flow (L/sec) = 0.00529 + (0.00486 x ADC voltage (mV))

3L Syringe Calibration

Table 3.6 shows that the average volume measured was 3.03 litres. This is within 1% of the actual volume of 3.00 litres that is within the ATS guidelines for volume measurements of ± 3% [89].

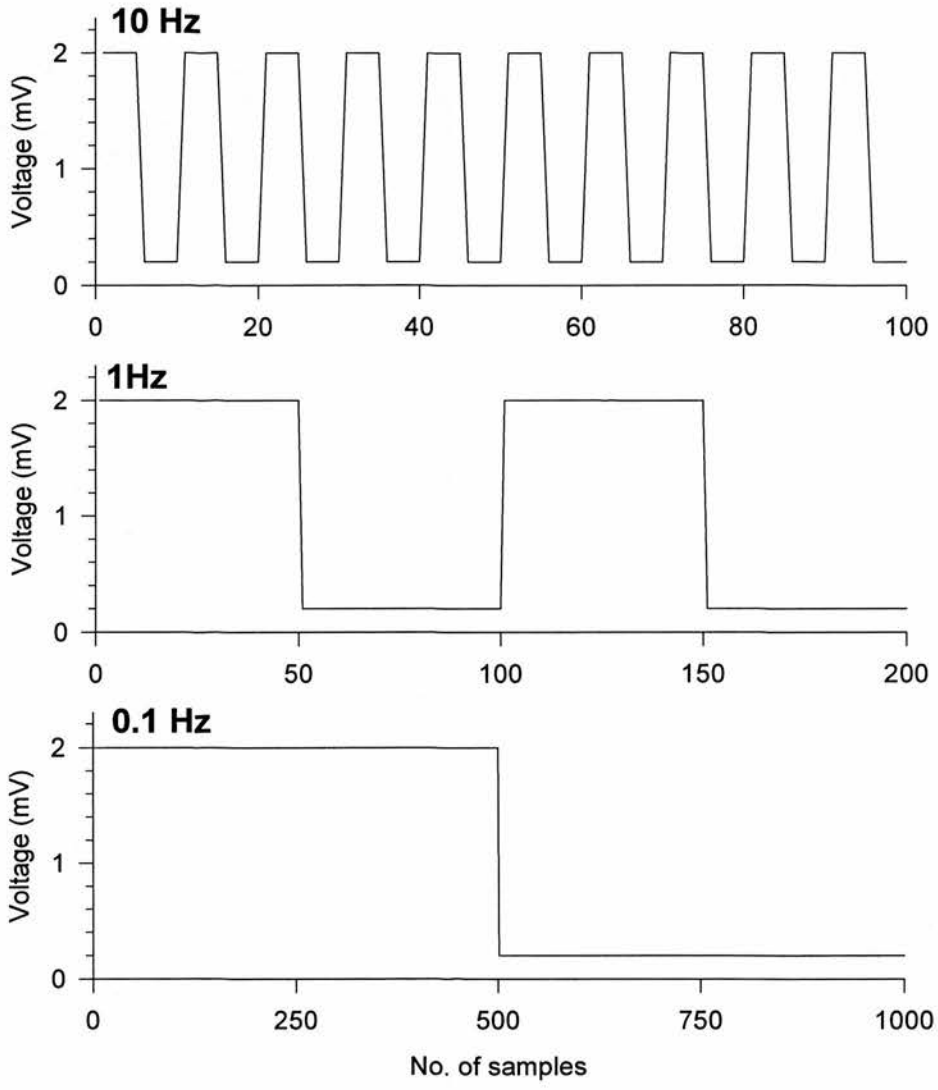


Figure 3.14: Output from ADC from application of square wave pulses at 10, 1 and 0.1 Hz showing number of samples per pulse.

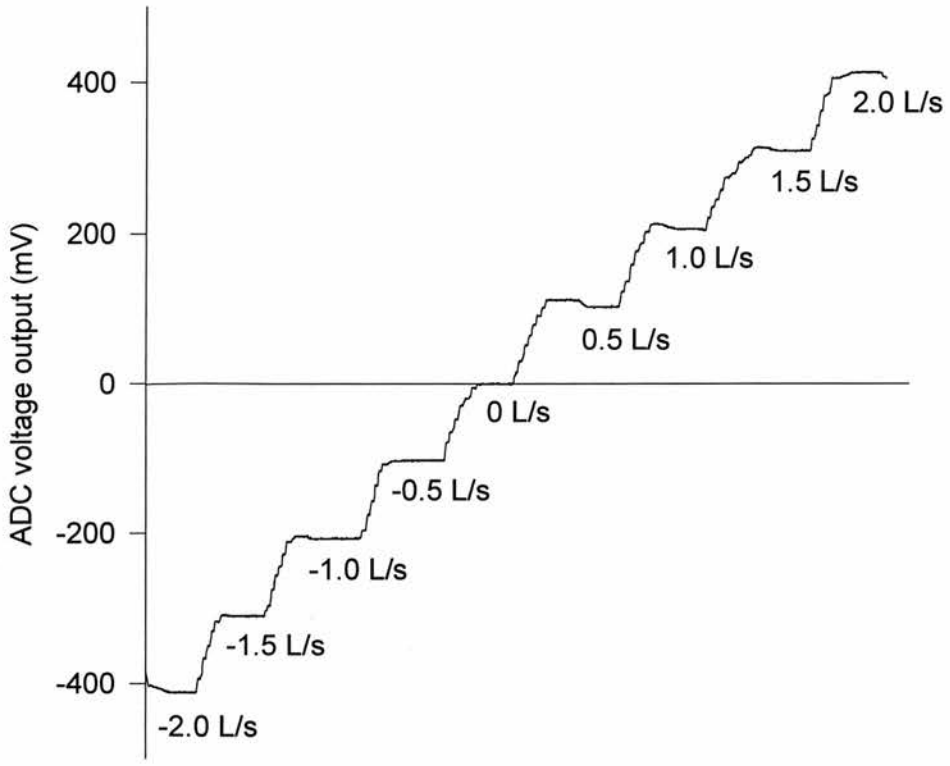


Figure 3.15: Raw data voltage output from ADC at a range of flow rates delivered by the rotameter.

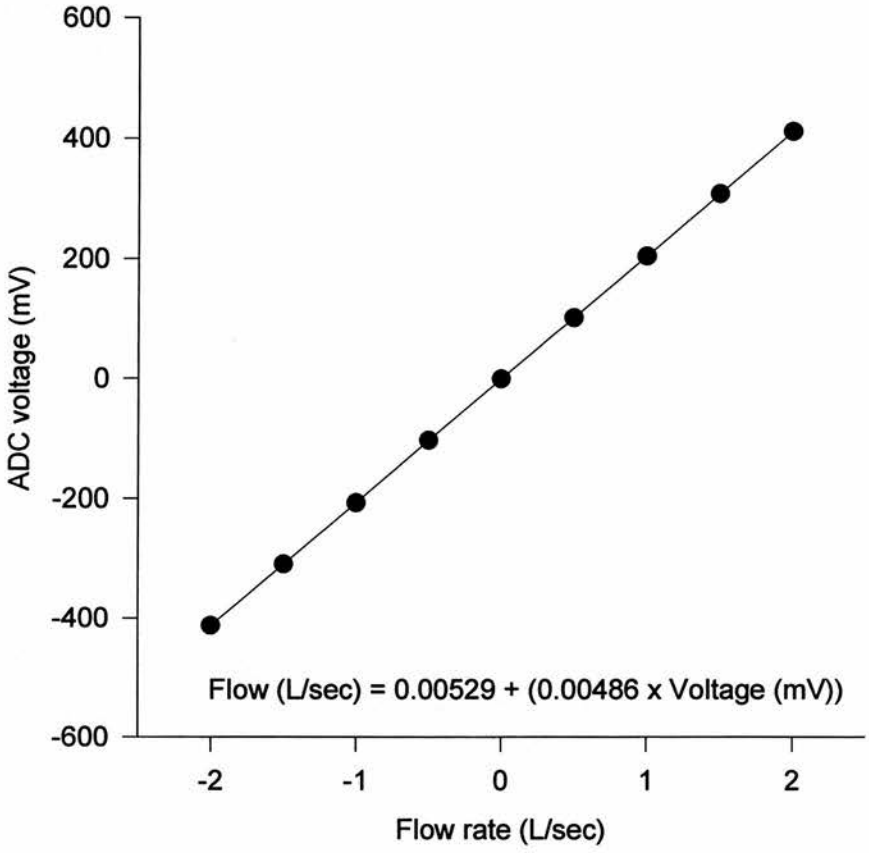


Figure 3.16: Scatter plot of actual flow rate and recorded voltage output from the ADC along with the fitted linear regression line and regression equation.

Stroke Speed	Fast	Medium	Slow
VC(L)	3.02	2.98	3.05
	2.96	3.08	3.03
	3.07	3.05	3.08
	3.00	2.98	2.98
	2.96	2.97	2.99
	3.07	2.90	3.07
	3.05	3.09	2.96
	3.11	2.94	3.07
	2.97	3.03	3.11
	3.09	3.15	3.09
Mean (L)	3.03	3.02	3.04
SD	0.06	0.08	0.05

Table 3.6: Volumes recorded from multiple strokes of a 3.00 L calibrating syringe at 3 different stroke speeds.

3.5.2.4: Conclusion.

These data confirm that the apparatus had the necessary accuracy, linearity and frequency response required for the intended range of physiological measurements.

3.6: CONVENTIONAL RESPIRATORY FUNCTION VARIABLES.

3.6.1: INTRODUCTION.

Conventional respiratory function variables were measured to allow comparison of the tidal breathing variables. There is no agreed gold standard for measuring airflow obstruction; we therefore recorded both spirometry (FEV_1 and FVC), which are the most commonly used tests and airways resistance that a number of groups believe to be the gold standard. Lung volumes were measured to monitor the level of FRC.

3.6.2: SPIROMETRY.

Spirometry was performed using either a Fleisch pneumotach as part of the Jaeger whole body plethysmograph or a Vitalograph wedge bellows spirometer. Both pieces of equipment are routinely used within the pulmonary function department for spirometry and were calibrated each study day by J. Montgomery and quality controlled weekly by a qualified respiratory function technician. The plethysmograph was also calibrated before each study using the equipment's standard calibration

program. Forced expiratory manoeuvres were performed to BTS and ARTP standards until 3 technically satisfactory results were achieved. Relaxed vital capacity was also measured on all patients (see chapter 2.2.3).

3.6.3: Airways resistance and Lung Volumes.

This was measured by the Jaeger whole body plethysmograph using a modification of the DuBois method [94]. As the plethysmograph software corrected for changes in temperature and humidity within the box, measurements of airways resistance (Raw) and thoracic gas volume (Vtg) could be made from tidal breathing without the patient having to pant. Airways resistance was calculated from pressure volume loops and Vtg calculated from pressure volume loops recorded during periods of no flow (respiratory effort against a closed shutter) and sGaw calculated from the Raw and Vtg. Subjects then performed a slow vital capacity manoeuvre to allow other lung capacities and volumes to be calculated. All measurements were made to BTS and ARTP standards [68] and three technically satisfactory sets of results with less than 10% variation in total lung capacity (TLC) were obtained for each measurement.

3.7: Statistical Analysis.

All statistical analysis of the results was carried out using commercially available software (SigmaStat for Windows V 1.0, Jandel Corporation) on a personal computer. Data were tested for normality and then where appropriate parametric or

non-parametric statistical methods applied. Results were considered to be statistically significant if the probability was < 0.05.

SECTION 3

Chapter 4: Measurement of Tidal Breathing: Outline of Studies.

The following outlines the programme of studies undertaken to investigate the usefulness of variables measured from tidal breathing expiratory flow profiles in assessing airflow obstruction. Each study was set up to examine a different set of questions.

Many variables can be derived from the expiratory flow profile. The following questions were therefore asked:

- What variables can be measured and used in the assessment of airflow obstruction (Section 5.1)?

- How do tidal variables correlate with conventional variables between subjects (section5.2 & 5.3)?

- What is the measure-to-measure variability of the novel tidal breathing variables and the conventional variables (Chapter 6)

- How reproducible were the baseline values in the short and long term (chapter 7)?

- How reproducible were the responses in tidal breathing variables following bronchodilation in the short and long term (chapter 7)?

- How does the change in tidal variables and conventional variables correlate with subjective changes in breathlessness following bronchodilation (chapter 8)?

Chapter 5: Defining and Validating Tidal Breathing Variables in Patients with COPD.

5.1: Introduction.

Once the protocols for the collection of tidal breathing flow data and for the average breath calculation had been finalised it was necessary to identify variables measured from the averaged breath that reflected airflow obstruction. There were two sources for these novel variables (table 5.7).

5.1.1: Existing Variables.

Tidal breathing has been studied as a way of identifying airflow obstruction in animals [49,50], infants [51-58] and adults [59-64], and a number of variables of obstruction have been identified.

The variables chosen to be investigated using data collected as described in chapter 3 were those that could be measured directly from the flow data. They consisted of standard descriptors of breathing pattern (V_T, T_E and T_I), other time components of tidal breathing (T_{TOT} and T_E/T_I) and flow related variables (PEF/PIF, EF_{25}, dV/V_T, T_{PTEF} and T_{PTEF}/T_E).

5.1.2: NEW SELF DEVELOPED VARIABLES.

From review of the literature, the following hypothesis was formed. Since tidal expiration is predominantly a passive event in patients with COPD, it will be possible to deduce information about the degree of airflow limitation and the effects of bronchodilators from averaged expiratory tidal flow profiles. This implies that the initial phase of expiratory tidal flow will be determined principally by elastic recoil and the degree of airflow obstruction. Therefore if airflow obstruction is reduced, following bronchodilation, the flow rates and volume expired at the start of expiration should increase.

Variables were therefore sought which should be sensitive to this increase in flow rate. As the time course of any changes was not known data were examined from both the beginning and the end of the expiration. A number of variables were tested: $TBEV_{0.5}$, $TBEV_1$ and $TBEV_2$ (the volume of air exhaled in the first half a second, second and two seconds of a tidal breath), TPEF (the peak tidal expiratory flow rate) and $TEEV_1$ (the volume of air exhaled in the last second of a tidal breath, excluding any expiratory pause).

5.1.3: VARIABLES SELECTED FOR STUDY.

Pilot studies were carried out in 17 patients with COPD to determine which of the variables detailed above might be sensitive indicators of changes in airflow obstruction following a therapeutic dose of salbutamol. Those variables that changed most, and most consistently, in a biologically plausible direction were then selected for further study. These variables are listed in table 5.7 and described graphically in

Variable	Definition
Existing	
V_T	Tidal volume
EF25	Expiratory flow at end tidal volume plus 25% end tidal volume [50].
T_{PTEF}	Time to peak expiratory flow
T_{PTEF}/T_E	Time to peak expiratory flow / expiratory time [59].
Novel	
$TBEV_1$	The volume of air exhaled in the first second of a tidal breath.
TPEF	The peak tidal expiratory flow rate.
$TEEV_1$	The volume of air exhaled in the last second of a tidal breath, (excluding any expiratory pause).

Table 5.7: Definition of variables selected for studies of tidal breathing.

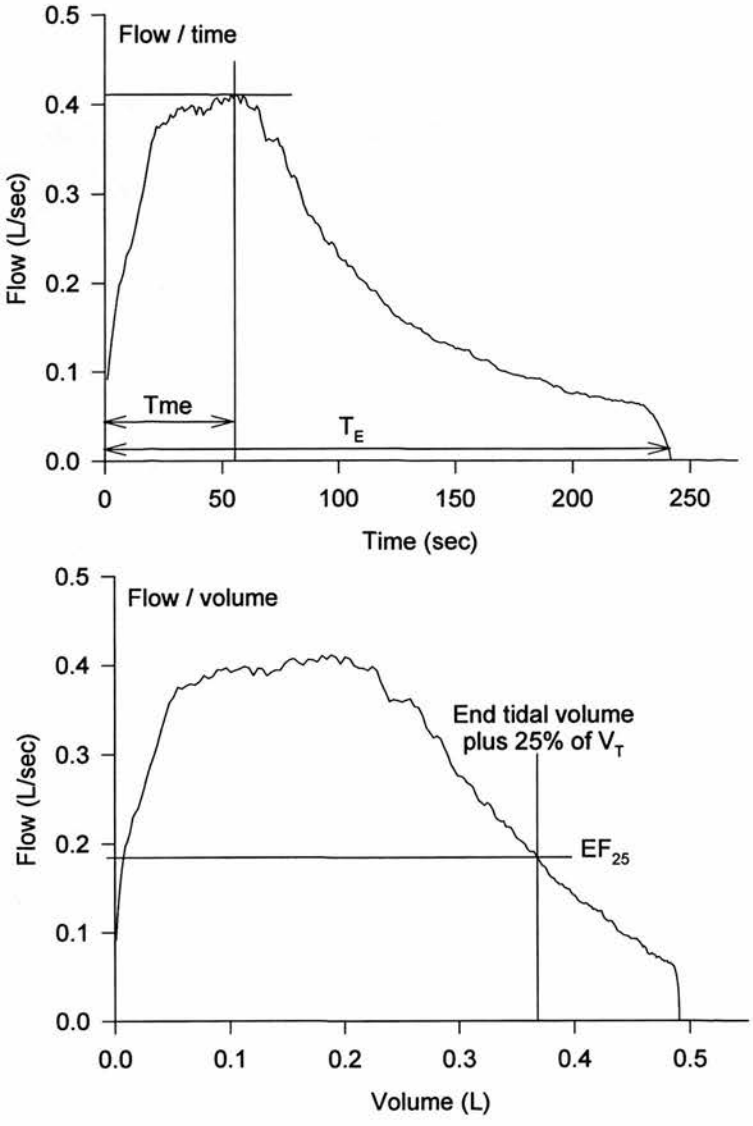


Figure 5.17: Diagrammatic representation of tidal breathing variables studied by previous groups.

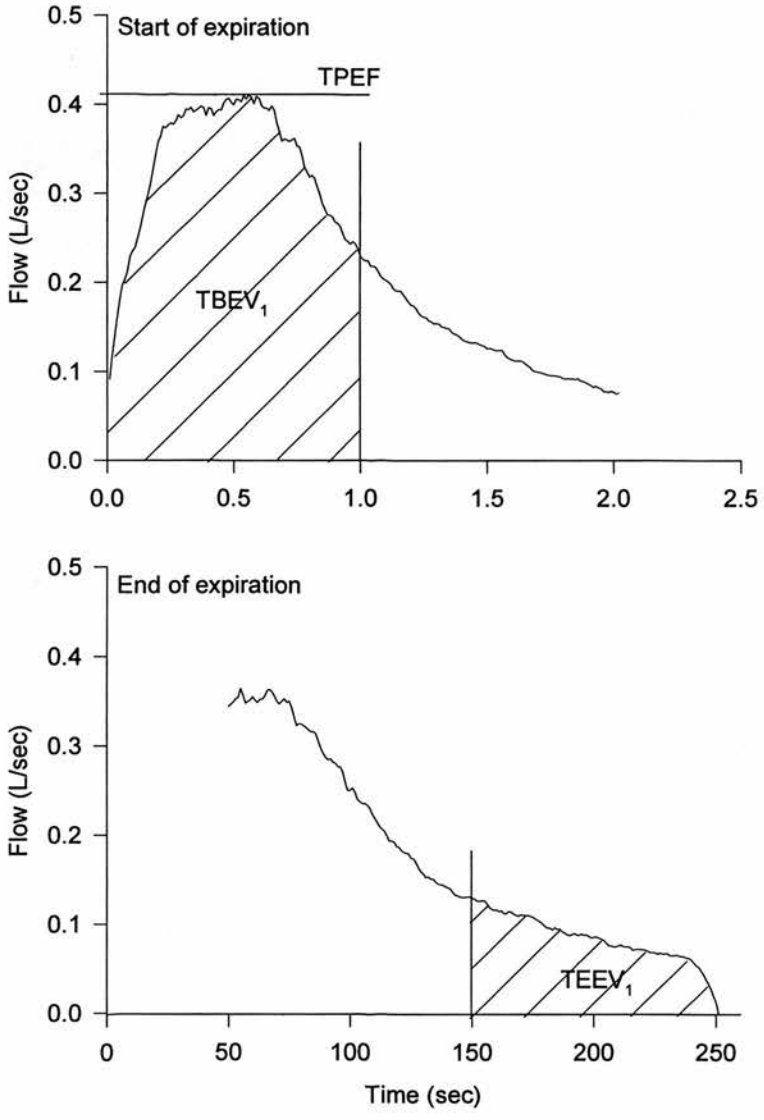


Figure 5.18: Diagrammatic representation of self developed tidal breathing variables.

figures 5.17 and 5.18. The remaining variables, listed in table 5.8, were excluded from further study.

This approach was chosen in an attempt to minimise the effect of mechanistic preconceptions on the choice of new measures.

In order to examine the usefulness of each of these variables in assessing the degree of airflow obstruction present it was necessary to obtain values of the tidal breathing variables at various levels of airflow obstruction. One possible approach for this would be to look at a large number of subjects with varying degrees of airflow obstruction, however this approach introduces between subject variability into the analysis. An alternative approach was therefore chosen; of looking at a smaller number of subjects with variable airflow obstruction studied at different levels of severity so that each subject acted as their own control. To achieve the different levels of airflow obstruction subjects were studied after they had withheld their bronchodilators and after bronchodilation, in order to confirm that any effects where due to the bronchodilation the subjects were also studied before and after nebulisation with saline, which acted as a placebo control.

Variable	Definition
Existing	
T_E	Expiratory time
T_I	Inspiratory time
T_{TOT}	Total breath duration
T_E/T_I	Ratio of expiratory time to inspiratory time
PEF/PIF	Ratio of peak expiratory to peak inspiratory flow rate
D_V/V_T	Ratio of the volume expired before peak expiratory flow achieved to expiratory volume
Novel	
$TBEV_{0.5}$	The volume of air exhaled in the first half second of a tidal breath.
$TBEV_2$	The volume of air exhaled in the first two seconds of a tidal breath.

Table 5.8: Definition of variables excluded from further studies of tidal breathing.

5.2: METHODS.

5.2.1: SUBJECTS.

28 patients (21 males), with a diagnosis of COPD, were studied on two different occasions with different treatments (random order blinded to subject and investigator). The mean (SD) age of the patients was 63 (11) years; they had mild to severe airflow obstruction (45.0% (20.8%) mean (SD) FEV_1 %predicted) and previously demonstrated partial reversibility to salbutamol and/or ipratropium bromide (>160ml increase in FEV_1 post [86]). A further 21 patients were studied on a single occasion with active treatment. The mean (SD) age of these patients was 70 (7) and they had similar severity of disease as the previous group (43.0% (15.5%) mean (SD) FEV_1 %predicted) and had also previously demonstrated partial reversibility to salbutamol and/or ipratropium bromide. Patients were recruited from the population who had attended the pulmonary function laboratory for reversibility testing within the past 4 years.

Subjects were requested to withhold their short acting bronchodilators for 4 hours prior to testing on the 2 study days.

All subjects were free from upper or lower respiratory tract infections for at least 2 weeks prior to the study.

Written informed consent was obtained from each patient prior to commencement of the study.

5.2.2: PROTOCOL.

The equipment and experimental set up are described in detail in chapter 3.1.1 and 3.1.2.

The time line diagram (figure 5.19) shows the sequence of tests and study medication. Briefly, spirometry and plethysmography were performed according to BTS and ARTP guidelines [68]. To allow the subjects time to settle they were allowed to relax for at least 5 minutes whilst the equipment was prepared. Then to allow them to acclimatise to the flow recording equipment they were left for a further 2 minutes, once the equipment was attached, before airflow sampling and recording was started. 5 minutes of airflow data from spontaneous tidal breathing were then sampled, displayed and stored on computer. Without disturbing the patient or mask in any way the mask-mounted nebuliser (sidestream nebuliser and CR50 compressor, Medic-Aid, West Sussex, England.) was activated to deliver the study medication. A further 5 minutes of tidal breathing flow data were then sampled, displayed and stored and then spirometry and plethysmography repeated.

Twenty eight patients performed two separate studies on different days. On one occasion they received 2.5 mg of salbutamol and 250 μg of ipratropium bromide by nebuliser and on the other nebulised normal saline. The order was randomised and both the patient and investigator were blinded to the order of the medication, as an independent member of the Respiratory Function Laboratory staff loaded the nebuliser. A further 21 patients attended for one study day (n=21) and received 2.5 mg

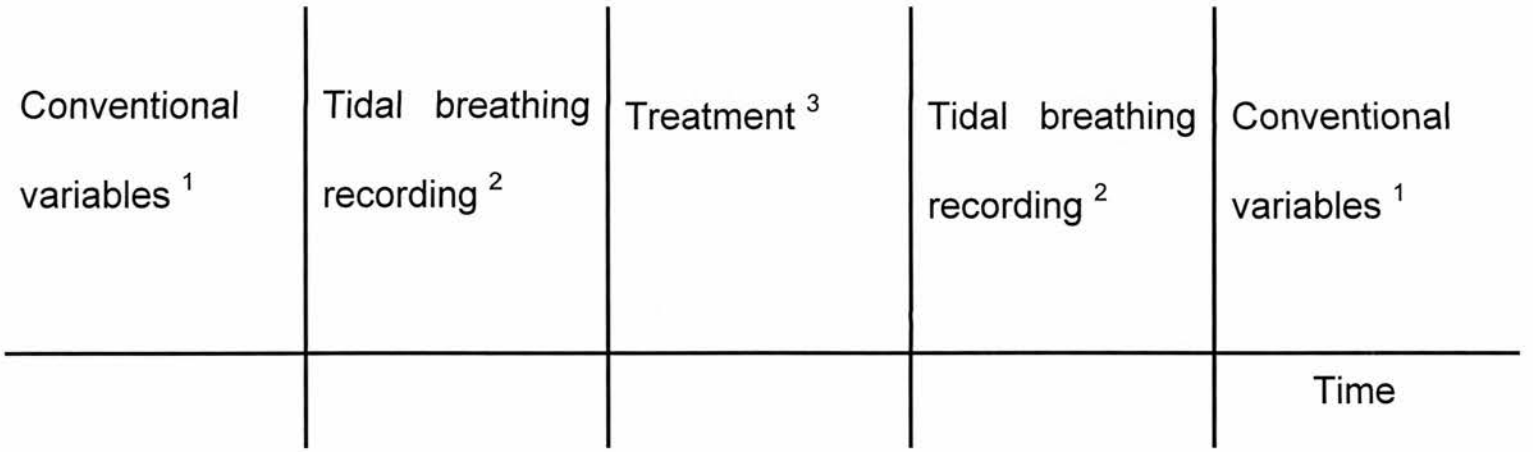


Figure 5.19: Time line of the sequence of testing for a single study day.

key

[1] 3 technically satisfactory FEV_1, FVC, ($Raw_{0.5}$, Vtg in a subset).

[2] 15 mins. spontaneous tidal breathing with an inspiration to TLC and 2-3 second breath-hold every 5 mins.

[3] nebulised 2.5 mg salbutamol $\pm$ 250 μg ipratropium bromide, or saline placebo (random order, 2 study days).

of salbutamol and 250 μg of ipratropium bromide by nebuliser, with no placebo (saline) limb.

5.3: Results.

5.3.1: Correlation of tidal variables with conventional variables of airflow obstruction.

There were significant positive linear relationships between FEV_1 and $TBEV_1$ ($r = 0.33$, $p = 0.02$) and EF_{25} ($r = 0.40$, $p = 0.004$). TPEF was not significantly correlated with FEV_1. There were significant linear relationships between sGaw and $TBEV_1$ ($r = 0.46$, $p = 0.003$), TPEF ($r = 0.35$, $p = 0.03$) and EF_{25} ($r = 0.40$, $p = 0.01$) (figure 5.20). Thus patients with worse airway obstruction as measured by conventional variables had lower values for $TBEV_1$, TPEF and EF_{25} and the correlations were generally stronger for sGaw than with FEV_1.

5.3.2: Effect of bronchodilation.

Mean (SD) values pre and post treatment in the active and placebo groups are presented in tables 5.9 and 5.10 for both conventional and novel tidal breathing variables. The values pre treatment were compared with those post (table 5.9, 5.10, figure 5.21). Bronchodilation was accompanied by a rise in resting ventilation due to

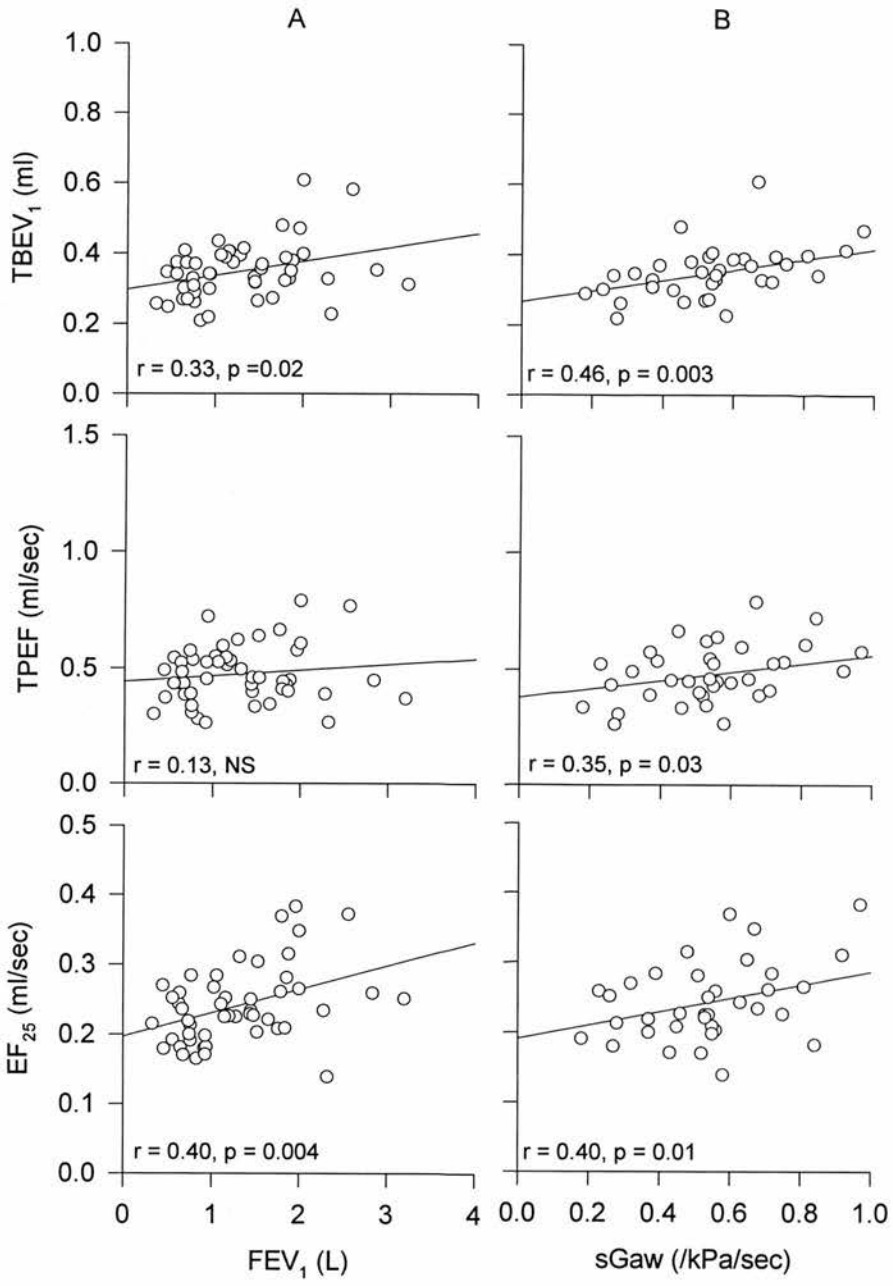


Figure 5.20: Correlation of baseline tidal breathing variables ($TBEV_1$, TPEF and EF_{25}) with baseline A) FEV_1 (n = 49) and B) sGaw (n = 39). Pearson's product moment correlation (r) and p values are shown.

VARIABLE (units)	Abbrev^n	Active treatment		
		Pre	Post	P value
Forced expired volume in 1sec (L)	FEV_1	1.33 (0.66)	1.62 (0.68)	< 0.05
Forced vital capacity (L)	FVC	2.63 (0.89)	3.31 (0.95)	< 0.05
Airways resistance (kPa.sec/L)	$Raw_{0.5}$	0.45 (0.15)	0.27 (0.11)	< 0.05
Specific airways conductance (/kPa/sec)	sGaw	0.56 (0.45–0.75)	0.95 (0.71-1.37)	< 0.05
Residual volume (L)	RV	3.91 (1.32)	3.49 (1.22)	< 0.05
Tidal volume (L)	V_T	0.59 (0.49-0.80)	0.70 (0.53-0.88)	NS
Minute ventilation (L/min)	V_E	9.6 (1.66)	11.1 (1.87)	< 0.05
Expiratory time (s)	T_E	2.31 (1.90-3.33)	2.21 (1.96-3.03)	NS
Inspiratory time (s)	T_I	1.42 (1.19-1.73)	1.32 (1.18-1.60)	NS
Mean inspiratory flow rate	V_T/T_I	0.41 (0.10)	0.52 (0.12)	< 0.05
Time to max. expiratory. flow (s)	T_{PTEF}	0.33 (0.24-0.56)	0.32 (0.18-0.47)	NS
Ratio of T_{PTEF} to exp. time	T_{PTEF}/T_E	0.16 (0.08)	0.14 (0.09)	NS
Flow at 75% of tidal volume (L/s)	EF_{25}	0.24 (0.05)	0.28 (0.06)	< 0.05
Volume expired in last sec. Of tidal breath (L)	$TEEV_1$	0.16 (0.06)	0.18 (0.06)	NS
Volume expired in first sec. of tidal breath (L)	$TBEV_1$	0.35 (0.08)	0.41 (0.10)	< 0.05
Tidal peak expiratory flow rate (L/s)	TPEF	0.48 (0.12)	0.56 (0.14)	< 0.05
Inspiratory capacity (L)	IC	2.80 (0.83)	2.84 (0.82)	NS

Table 5.9: Conventional and tidal breathing variables. Mean (SD) (or Median (interquartile range) for those data not normally distributed) values for spirometry, whole body plethysmography and tidal breathing variables pre and post active treatment.
Pre treatment values compared with post treatment values by 2 way RM ANOVA with pairwise comparisons using Student-Newman-Keuls test.

VARIABLE (units)	Abbrev^n	Placebo		
		Pre	Post	P value
Forced expired volume in 1sec (L)	FEV_1	1.29 (0.75)	1.32 (0.77)	NS
Forced vital capacity (L)	FVC	2.60 (0.96)	2.52 (0.97)	NS
Airways resistance (kPa.sec/L)	$Raw_{0.5}$	0.44 (0.17)	0.36 (0.13)	NS
Specific airways conductance (/kPa/sec)	sGaw	0.58 (0.41-0.74)	0.63 (0.49-0.84)	NS
Residual volume (L)	RV	3.79 (1.38)	3.72 (1.21)	NS
Tidal volume (L)	V_T	0.57 (0.49-0.75)	0.59 (0.48-0.73)	NS
Minute ventilation (L/min)	V_E	10.1 (2.30)	9.5 (2.13)	NS
Expiratory time (s)	T_E	2.19 (1.84-2.87)	2.48 (1.96-3.15)	NS
Inspiratory time (s)	T_I	1.32 (1.10-1.44)	1.35 (1.13-1.51)	NS
Mean inspiratory flow rate	V_T/T_I	0.47 (0.13)	0.46 (0.11)	NS
Time to max. expiratory. flow (s)	T_{PTEF}	0.28 (0.20-0.38)	0.34 (0.22-0.44)	NS
Ratio of T_{PTEF} to exp. Time	T_{PTEF}/T_E	0.13 (0.07)	0.14 (0.07)	NS
Flow at 75% of tidal volume (L/s)	EF_{25}	0.24 (0.06)	0.22 (0.05)	NS
Volume expired in last sec. Of tidal breath (L)	$TEEV_1$	0.17 (0.14-0.21))	0.15 (0.12-0.17))	NS
Volume expired in first sec. of tidal breath (L)	$TBEV_1$	0.34 (0.09)	0.34 (0.11)	NS
Tidal peak expiratory flow rate (L/s)	TPEF	0.49 (0.13)	0.30 (0.15)	NS
Inspiratory capacity (L)	IC	2.22 (1.02)	2.36 (0.96)	NS

Table 5.10: Conventional and tidal breathing variables. Mean (SD) (or Median (interquartile range) for data not normally distributed) values for spirometry, whole body plethysmography and tidal breathing variables pre and post placebo treatment.
Pre treatment values compared with post treatment values by 2 way RM ANOVA with pairwise comparisons using Student-Newman-Kuels test.

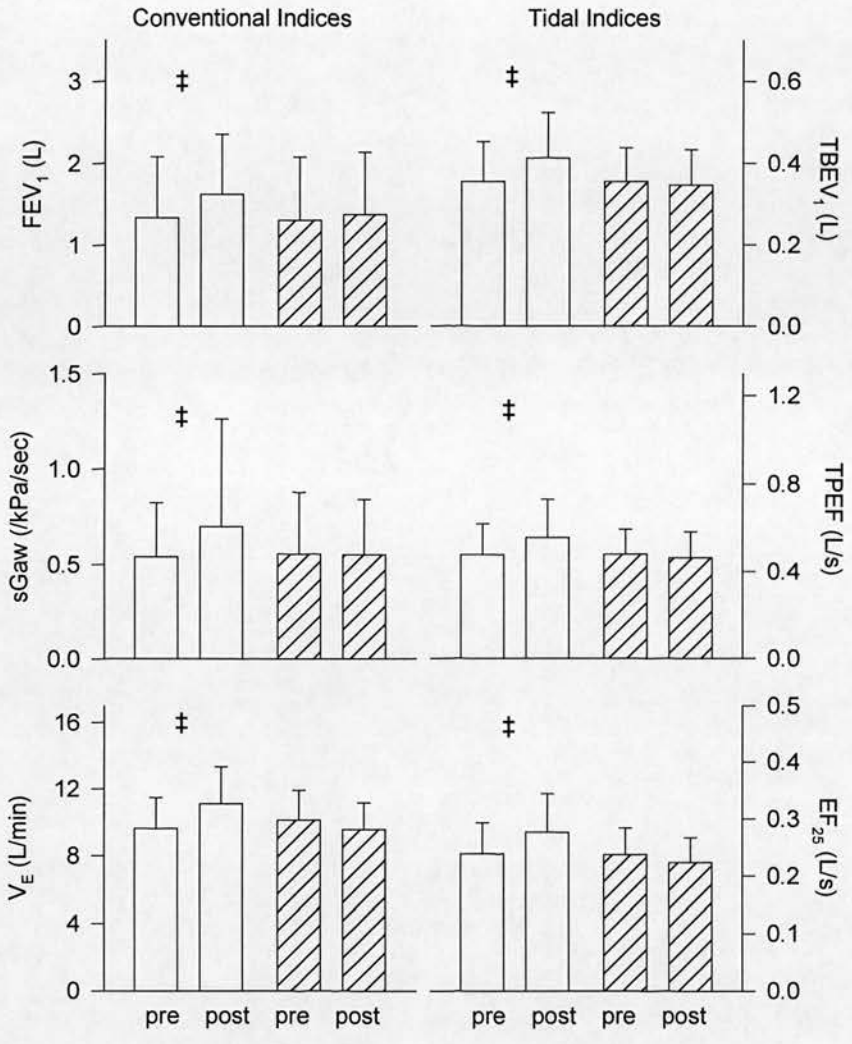


Figure 5.21: Changes with treatment. Data for conventional and tidal breathing variables pre and post bronchodilation (open) or placebo treatment (hatched).
Columns represent the mean group measurement with standard deviation bar. n = 28 (sGaw n= 18). ‡ = $p < 0.05$ for 2 way RM ANOVA.

non significant rises in V_T and frequency. The increase in V_T (mean V_T 0.67 L pre and 0.73 L post bronchodilation), along with the decrease in T_I (mean T_I 1.55 s pre and 1.45 s post bronchodilation), resulted in V_T/T_I (a measure of respiratory drive) increasing significantly after bronchodilator treatment (median value 0.41 L/s pre and0.52 L/s post bronchodilation). Three of the novel tidal breathing variables (EF_{25}, $TBEV_1$ and TPEF) showed statistically significant increases post active treatment but not post placebo.

5.4: Discussion.

These data show that tidal breathing recordings can be used to detect changes in expiratory airflow following bronchodilators and that these changes correlate with bronchodilation as judged by conventional plethysmographic techniques.

Our results show that although T_{PTEF}/T_E in our group of patients is similar to that in the group studied by Morris [59] it does not correlate with baseline FEV_1 or sGaw nor is the change post bronchodilation statistically significant. It was therefore concluded that T_{PTEF}/T_E is not a sensitive indicator of changes in airflow obstruction.

The variables T_{PTEF} and $TEEV_1$, did not show significant increases after bronchodilation nor did they correlate to our gold standard of airflow obstruction, airways resistance ($r = 0.19$, $p = 0.18$ and $r = 0.06$, $p = 0.68$ respectively), and so again it was concluded that these were not sensitive indicators of airflow obstruction. This left three novel tidal breathing variables that correlated to conventional measures of

airflow obstruction and increased significantly after bronchodilation but not after placebo treatment. It is these three variables ($TBEV_1$, TPEF and EF_{25}) that are further studied in the following chapters.

One method for comparing the sensitivity and specificity of the new variables for assessing airflow obstruction would be to construct receiver-operator curves [95], however sensitivity and specificity can only be calculated if there is an agreed gold standard for the novel variables to be measured against (i.e. something which tells you the "true positive" rate of bronchodilator response). As there is no agreed gold standard for measuring airflow obstruction this approach is difficult to apply here.

Due to the methodology chosen for breath averaging it was not possible to produce a complete averaged breath from which to measure the tidal breathing variables. Instead separate portions of each recorded breath are averaged in discrete "time bins" then the variables are measured from the bin-averaged breath. Alternative methods of analysis include the production of an averaged breath by normalising disparate breath lengths (method of Benchetrit and Shea [21, 22]) or calculating each variable for each breath and averaging the results (method of Morris and Williams [61, 62]). Each approach has advantages and disadvantages, as stated elsewhere in this thesis. Primarily the production of a whole averaged breath by normalisation of the breath length would lead to distortion of time-dependent changes, and the analysis of single breaths would be particularly sensitive to random noise and cardiac artefact. The bin-averaging method employed in this thesis has none of these disadvantages but does not yield a complete averaged breath. At present there is no one perfect method of averaging a time-varying signal and so the method chosen was the one that was felt to be least likely to distort the variables to be measured (i.e. none of the chosen variables required the complete expiratory profile for its measurement).

In the present study bronchodilators had a number of different effects on resting breathing. Minute ventilation increased significantly; this could be due to either an increase in respiratory drive or increased airway calibre or both. The results show that after bronchodilation mean inspiratory flow rate (an indirect measure of respiratory drive) was significantly increased, and airways resistance significantly decreased. This suggests that it may be a combination of both mechanisms that leads to the increase in tidal expiratory flow as reflected in the increase in the novel tidal breathing variables, $TBEV_1$, TPEF, and EF_{25}. However to distinguish fully between these two mechanisms requires reliable measures of intrabreath resistance and respiratory drive and is beyond the scope of this thesis.

In this group of patients inspiratory capacity did not change significantly after bronchodilation thereby indicating that FRC was unchanged. However there was a significant reduction in RV after bronchodilation in the subgroup of patients that had RV measurements. This was also found in a study by O'Donnell et al [96] who found that small but consistent changes in TLC led to IC and VC underestimating changes in FRC in 43% of their patient group. In their study, RV changed more than other volume measurements and was therefore better at detecting a reduction in air trapping after bronchodilation. It was not however practical using the open circuit required for the present studies to measure RV directly, so the compromise of deducing changes in FRC from IC was used.

The degree of correlation between the tidal breathing variables and FEV_1 was different than that between the tidal breathing variables and sGaw. This raises the question of what is the gold standard for measuring airway obstruction with which to compare these novel tidal breathing variables. Various groups [97-100] have studied the response to bronchodilators as measured by spirometry (FEV_1) and plethysmography (sGaw) and found that sGaw correlates better than FEV_1 with

subjective benefit [97, 98] and that increases post bronchodilator were larger for sGaw than for FEV_1 [99, 100]. This may be important when using FEV_1 to determine the reversibility of airflow obstruction to bronchodilation or steroid response as patients could be incorrectly categorised as non-responsive to treatment.

In conclusion, three novel tidal breathing variables have been identified which change in an expected and reproducible way following bronchodilation, and which correlate well with conventional variables of airflow resistance. These variables can be measured without the need for special breathing manoeuvres and with relatively inexpensive equipment.

Chapter 6: Measure to Measure Variability in Novel Tidal Breathing Variables and Conventional Variables.

6.1: Introduction.

All physiological measures have some degree of natural variability measure to measure. This variability is a result of changes in patient performance and equipment accuracy. In order for any change in variable to be attributed to an intervention of any kind the magnitude of change must exceed the natural variability associated with that variable. It is therefore important when defining and validating any new variable that the natural variability inherent in it is determined.

The standard index for describing the natural variability is the coefficient of repeatability determined from consecutive measurements made within a short time period with no intervention between them.

6.2: Method.

Data collected from the pilot studies described in chapter 5 were analysed to determine the natural variability in the novel tidal breathing variables.

The 15 minutes of data collected were divided into 3 sets of 5 minutes and the novel tidal breathing variables described in chapter 5.1 calculated for the second and third data sets, the first 5 minutes of data were not used as in these first studies there was no set acclimatisation time and so the first 5 minutes was used as the acclimatisation time (all subsequent studies had an acclimatisation time of 2 minutes as part of the protocol). These two sets of data were then used to calculate the coefficient of repeatability for each variable.

For the conventional variables of spirometry and airways resistance, the three technically satisfactory measurements obtained for the study described in chapter 5 were used. Again, for the reason stated above, the second and third measurements were used to calculate the coefficient of repeatability.

Once these coefficients were calculated for each variable, the data collected pre and post bronchodilation were reanalysed to determine if the magnitude of change was greater than the natural variability.

6.3: Results.

6.3.1: Calculation of 95% Confidence Intervals.

Data were collected in 25 patients for the novel tidal breathing variables, 17 subjects for airways resistance and 27 patients for FEV_1.

The paired data for the second and third periods is shown in table 6.11. The calculated 95% confidence interval (2 x Standard deviation of the differences between

Variable	FEV_1 (L)		sGaw (/kPa/sec)		EF_{25} (ml/sec)		$TBEV_1$ (ml)		TPEF (ml/sec)	
Period	2	3	2	3	2	3	2	3	2	3
Patients1	0.63	0.62	0.56	0.55	130	141	211	204	245	235
2	2.32	2.32	1.23	0.28	187	182	377	357	557	505
3	0.8	0.83	0.61	0.70	249	264	390	422	489	551
4	0.49	0.56	0.45	0.45	203	202	253	243	298	283
5	0.94	1.03	0.54	0.54	233	209	314	299	392	375
6	0.76	0.76	0.56	0.50	357	380	600	610	809	803
7	1.45	1.4	0.24	0.21	206	215	487	483	668	674
8	1.84	2	1.07	1.12	265	283	351	363	445	470
9	1.76	1.64	0.56	0.56	239	207	278	253	320	294
10	2.68	2.84	0.26	0.28	264	223	396	397	527	506
11	0.29	0.33	0.89	0.96	233	241	365	390	416	456
12	1.16	1.09	0.44	0.46	209	234	368	410	588	670
13	0.67	0.66	0.80	0.62	258	242	300	289	501	460
14	1.28	1.28	0.30	0.34	167	199	393	437	483	509
15	0.64	0.6	0.99	0.92	166	185	231	248	343	368
16	0.65	0.56	0.40	0.37	271	275	345	361	549	585
17	0.44	0.46	0.57	0.55	259	281	433	445	794	752
18	1.28	1.2			189	198	262	247	310	295
19	1.48	1.4			272	274	369	362	473	450
20	0.84	0.92			226	220	278	247	344	325
21	1.28	1.32			182	194	357	363	566	584
22	1.48	1.44			294	307	383	380	507	494
23	1.32	1.28			305	290	401	410	469	460
24	1.28	1.32			266	280	355	363	542	529
25	3.16	3.2			221	200	346	319	433	433
26	0.77	0.76								
27	1.6	1.84								

Table 6.11: Paired data for periods 2 and 3 used to calculate 95% confidence intervals for FEV_1, sGaw, EF_{25}, $TBEV_1$ and TPEF.

repeated measures) for FEV_1 was 170 ml, sGaw 0.13 /kPa/sec, $TBEV_1$ 42 ml, EF_{25} 39 ml/sec and for TPEF was 67 ml/sec.

6.3.2: Analysis of Effect of Bronchodilation.

Figure 6.22 shows the graphical representation of absolute change in novel tidal breathing variables ($TBEV_1$, TPEF and EF_{25}) post active treatment compared to absolute change in A) FEV_1 (n=49) and B) sGaw (n=39) post active treatment; along with the 95% confidence intervals for repeated measurement of each variable without intervention (dotted lines).

These graphs show that for each of the variables some of the patients have an increase in both the conventional variable and in the tidal breathing variable, the numbers of patients is shown in table 6.12a. There are however subsets of patients who show either an increase in the conventional measure but a decrease in the tidal breathing variable and vice versa. The graph also shows that there are a proportion of patients who do not demonstrate increases greater than natural measure to measure variability and that this proportion of patients is greater for the tidal breathing variables than for FEV_1 or sGaw.

A number of patients did not show changes in FEV_1 greater than the natural variability (16/49 33%) and of these a small proportion showed changes in the novel variables greater than the natural variability (table 6.12b). A number also did not show changes in sGaw greater than the natural variability (9/39 23%) and of these a proportion showed changes in the novel variables greater than the natural variability (table 6.12b).

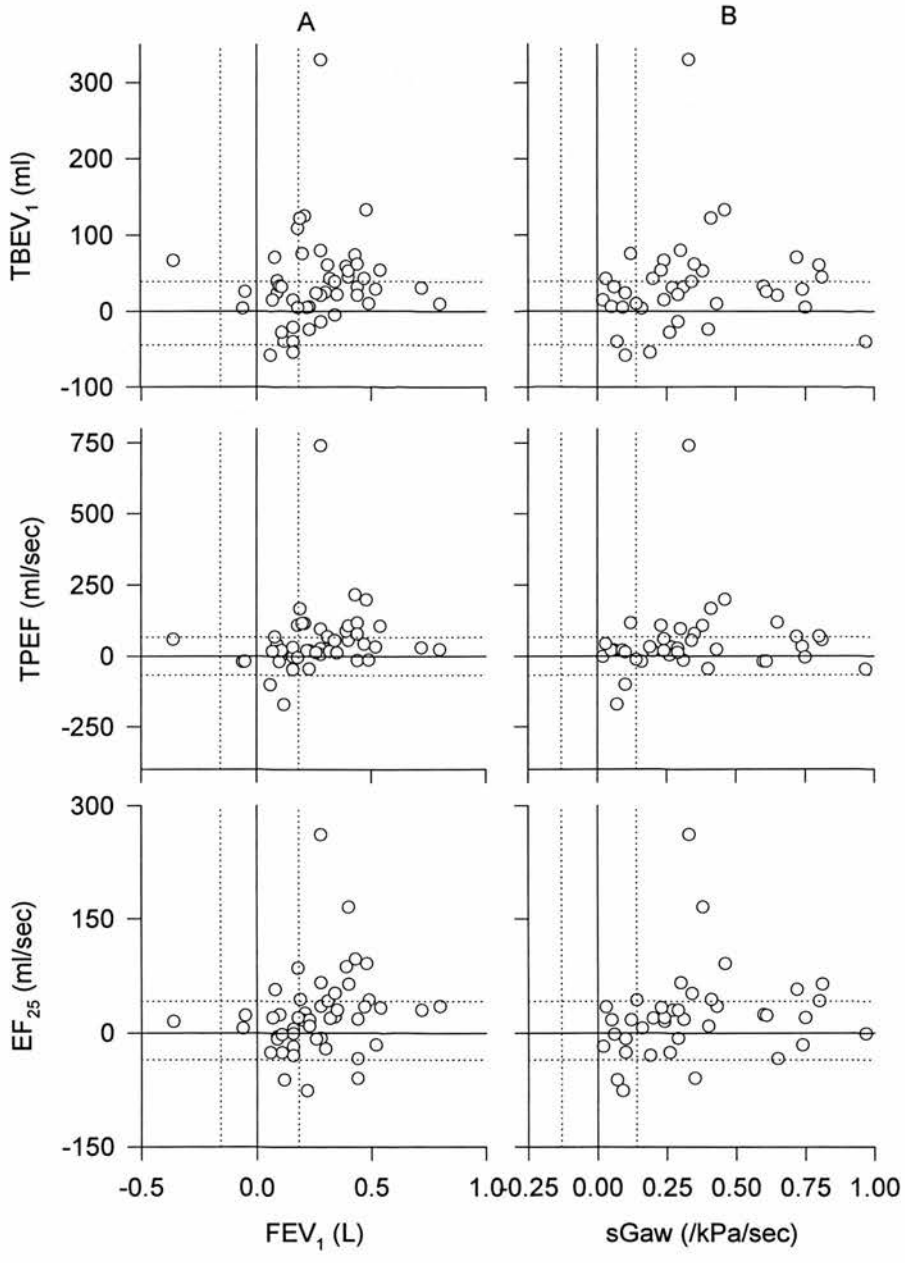


Figure 6.22: Changes in novel tidal breathing variables compared to conventional variables following bronchodilation.
The dotted lines represent the 95% confidence limits for repeated measurement of each variable without intervention.

(a)

Novel variable	(I) FEV_1 (N=33)	(ii) sGaw (N=30)
$TBEV_1$	16 (48%)	12 (40%)
TPEF	14 (42%)	10 (33%)
EF_{25}	12 (36%)	10 (33%)

(b)

Novel variable	(I) FEV_1 (N=16)	(ii) sGaw (N=9)
$TBEV_1$	2 (12%)	2 (22%)
TPEF	1 (6%)	1 (11%)
EF_{25}	1 (6%)	0 (0%)

Table 6.12: (a) The number (percentage) of patients who had a significant change in (i) FEV_1 or (ii) sGaw and in the novel tidal breathing variable. (b) The number (percentage) of patients who did not have a significant change in (i) FEV_1 or (ii) sGaw but did have a significant change in the novel tidal breathing variable.

6.4: Discussion.

In order to be able to interpret any change in respiratory variable after intervention the natural variability of the measure must be taken into account. Tweeddale et al [69] examined the short-term variability of FEV_1 in patients with obstructive ventilatory defects. They found that in order to exclude natural variability with 95% confidence FEV_1 had to increase by more than 160 ml. In the present study this value was calculated to be 170 ml. No study has been reported examining this value for sGaw but this study concludes that an increase of more than 0.13 /kPa/sec is needed to exclude natural variability.

The values for $TBEV_1$, EF_{25} and TPEF were calculated to be 42mls, 39ml/sec and 67 ml/sec respectively. However this does not allow any determination of what change is required for a clinically significant change in symptoms.

To examine the change in variables following bronchodilation in light of the natural variability the data collected in the study described in chapter 5 were plotted along with the 95% confidence interval calculated here (figure 6.22).

Although patients were recruited for the study had to have had previously demonstrated reversibility to bronchodilators by spirometry a proportion did not show changes greater than the natural variability in FEV_1 or in sGaw. However of these there was a proportion of patients who showed increases greater than variability in the novel tidal breathing variables (table 6.12b). This raises again the question of what is the gold standard measure of airflow obstruction.

Van Noord et al [101] looked at this when comparing the application of forced oscillation technique to variables of forced expiration and plethysmographic airways

resistance in assessing airflow obstruction. They performed multivariate analysis of differences between pre and post bronchodilator values and found that the effect of salbutamol was best described by a combination of Raw and FVC when looking at percentage change and by a combination of sGaw and FVC when looking at absolute change.

Specific conductance (sGaw) may be the better gold standard when looking at airflow obstruction because it measures only the conductance of the airways whereas FEV_1 and FEV_1/FVC integrate changes of dynamic airways function, lung recoil and the forces applied by the respiratory muscles into the one measure and do not allow for individual parts to be studied.

Chapter 7: Reproducibility of tidal breathing variables.

7.1: Introduction.

Chapter 6 examined the measure-to-measure (within day) variability of the novel tidal breathing variables. However before any new variable can be used routinely the reproducibility of the measure against time must be determined. The following study examined both short-term (within 2 weeks) and long-term (over 6 months) reproducibility.

7.2: Method.

To answer the question of the short-term reproducibility of the variables data from the group of patients who had attended for testing within two weeks were analysed.

For the long-term reproducibility patients, who had attended for the previous studies, were asked to repeat the study after 6 months.

The protocol for each study day was the same as described in chapter 5.2.

7.3: RESULTS.

7.3.1: SHORT-TERM REPRODUCIBILITY.

7.3.1.1: Baseline.

45 patients attended the laboratory on two separate occasions a mean (SD) 10 (10) days apart. Their mean (SD) age was 66 (10) years and they had a baseline %pred FEV_1 of 44% (19)%. Spirometric (FEV_1, FVC and RVC), airways resistance (Raw and sGaw), breathing pattern (V_T and V_E) and novel tidal breathing ($TBEV_1$, TPEF and EF_{25}) variables were looked at on both occasions. None of these variables showed any statistically significant difference between the two study days at baseline (table 7.13) and the 95% confidence limits of the difference between visits are of a similar magnitude for all variables when corrected for the different sizes of the actual variable value. Figure 7.23 shows the individual patient differences as Bland and Altman plots.

7.3.1.2: Percentage change after bronchodilation.

44% of the group of patients described above (20 patients) also had measurements made after nebulisation of ipratropium bromide and salbutamol on two occasions. The two study days were a mean (SD) 6(3) days apart and the patients' mean (SD) age was 70 (7) years and they had a baseline %pred FEV_1 of 44% (16)%. There was no significant difference in any of the variables studied between the two study days (Table 7.14). The 95% confidence limits of the difference between visits are of a similar value for all variables (table 7.14) although RVC and Raw show the

variable	Visit 1	Visit 2	Mean value	Paired t-test	95% CL of between visit difference	95% CL as a % of mean value
FEV_1 (L)	1.31	1.31	1.31	NS	0.37	28.2
FVC (L)	2.63	2.70	2.67	NS	0.75	28.1
RVC (L)	3.01	3.03	3.02	NS	0.96	31.8
Raw (kPa.sec/L)	0.42	0.42	0.42	NS	0.19	45.2
sGaw (/kPa/sec)	0.59	0.60	0.60	NS	0.20	33.3
V_T (L)	0.63	0.61	0.62	NS	0.45	24.2
V_E (L)	9.7	10.2	10.0	NS	4.06	40.6
EF_{25} (ml/sec)	236	249	242	NS	99.25	41.0
$TBEV_1$ (ml)	349	363	356	NS	149.63	41.2
TPEF (ml/sec)	467	494	480	NS	185.64	38.7

Table 7.13: Group mean values for baseline values for the two study days less than 2 weeks apart.
NS = not significant.

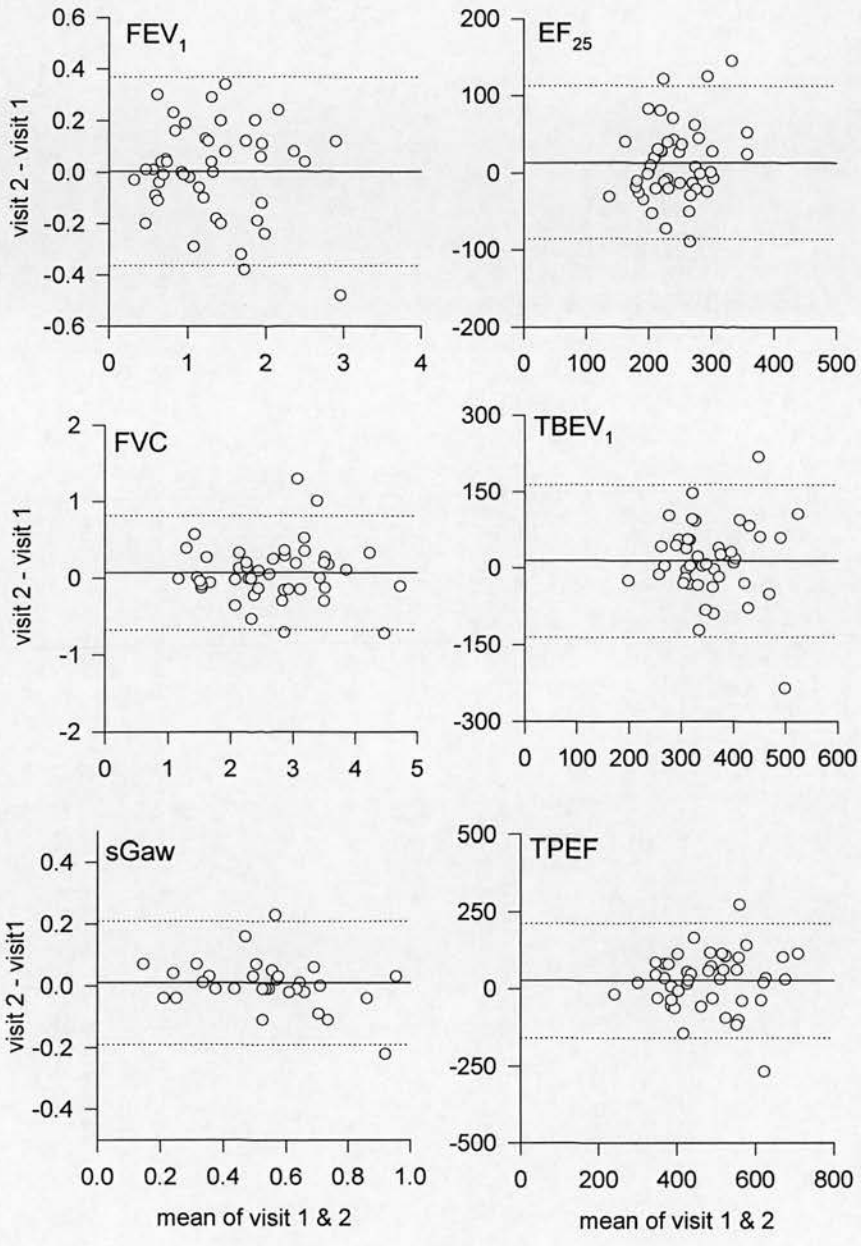


Figure 7.23: Bland and Altman plots showing comparison of visit 1 and visit 2 baseline data.
Solid line is the mean difference and dashed lines are the 95% confidence limits of the difference between visit 1 and visit 2 for short-term reproducibility.

variable	Visit 1	Visit 2	Mean value	Paired t-test	95% CL of between visit difference
FEV_1 (L)	21.37	19.79	20.58	NS	42.60
FVC (L)	21.10	20.66	20.88	NS	35.60
RVC (L)	12.41	11.37	11.89	NS	18.78
Raw (kPa.sec/L)	-25.68	-25.67	-25.68	NS	25.60
sGaw (/kPa/sec)	47.48	52.64	50.06	NS	62.18
V_T (L)	12.55	9.99	11.27	NS	46.98
V_E (L)	13.82	11.40	12.61	NS	56.20
EF_{25} (ml/sec)	10.78	3.96	7.37	NS	49.93
$TBEV_1$ (ml)	12.86	5.23	9.05	NS	41.63
TPEF (ml/sec)	10.66	4.77	7.72	NS	38.66

Table 7.14: Group mean values for percentage change after bronchodilation values for the two study days less than 2 weeks apart.
NS = not significant.

smallest dispersion around the mean (95% CL of 18.78 and 25.60 respectively).

7.3.2: LONG-TERM REPRODUCIBILITY.

7.3.2.1: Baseline.

14 patients attended the laboratory on two separate occasions a mean (SD) 364 (176) days apart. Their mean (SD) age was 63 (11) years and they had a baseline %pred FEV_1 of 50% (17%). The same variables were looked at as for short-term reproducibility and none of these showed statistically significant difference at baseline (table 7.15). The 95% confidence limits of the difference between visits were of a similar magnitude for all variables when corrected for the different sizes of the actual variable value (table 7.15) although all values were larger than for the short-term reproducibility. Figure 7.24 shows the individual patient differences as Bland and Altman plots.

7.3.2.2: Percentage change after bronchodilation.

13 patients attended the laboratory on two separate occasions a mean (SD) 338 (151) days apart. Their mean (SD) age was 63 (11) years and they had a baseline %pred FEV_1 of 50 (18)%. There was no statistically significant change of any of the variables between the two study days (table 7.16). The 95% confidence limits of the difference between visits were of a similar value for all variables (table 7.16) and like the baseline results were greater than for short-term reproducibility.

Variable	Visit 1	Visit 2	Mean value	Paired t-test	95% CL of between visit difference	95% CL as a % of mean value
FEV_1 (L)	1.49	1.67	1.58	NS	0.98	63.3%
FVC (L)	2.72	3.04	2.88	NS	1.65	57.3%
RVC (L)	2.90	3.09	5.99	NS	1.71	28.5%
Raw (kPa.sec/L)	0.32	0.35	0.34	NS	0.22	64.7%
sGaw (/kPa/sec)	0.87	0.82	0.85	NS	0.23	27.1%
V_T (L)	0.73	0.68	0.71	NS	0.33	46.5%
V_E (L)	11.32	10.39	10.86	NS	6.10	56.1%
EF_{25} (ml/sec)	269	246	258	NS	157.03	60.9%
$TBEV_1$ (ml)	404	389	397	NS	193.30	48.7%
TPEF (ml/sec)	534	549	542	NS	277.07	51.1%

Table 7.15: Group mean values for baseline values for the two study days more than 6 months apart.
NS = not significant.

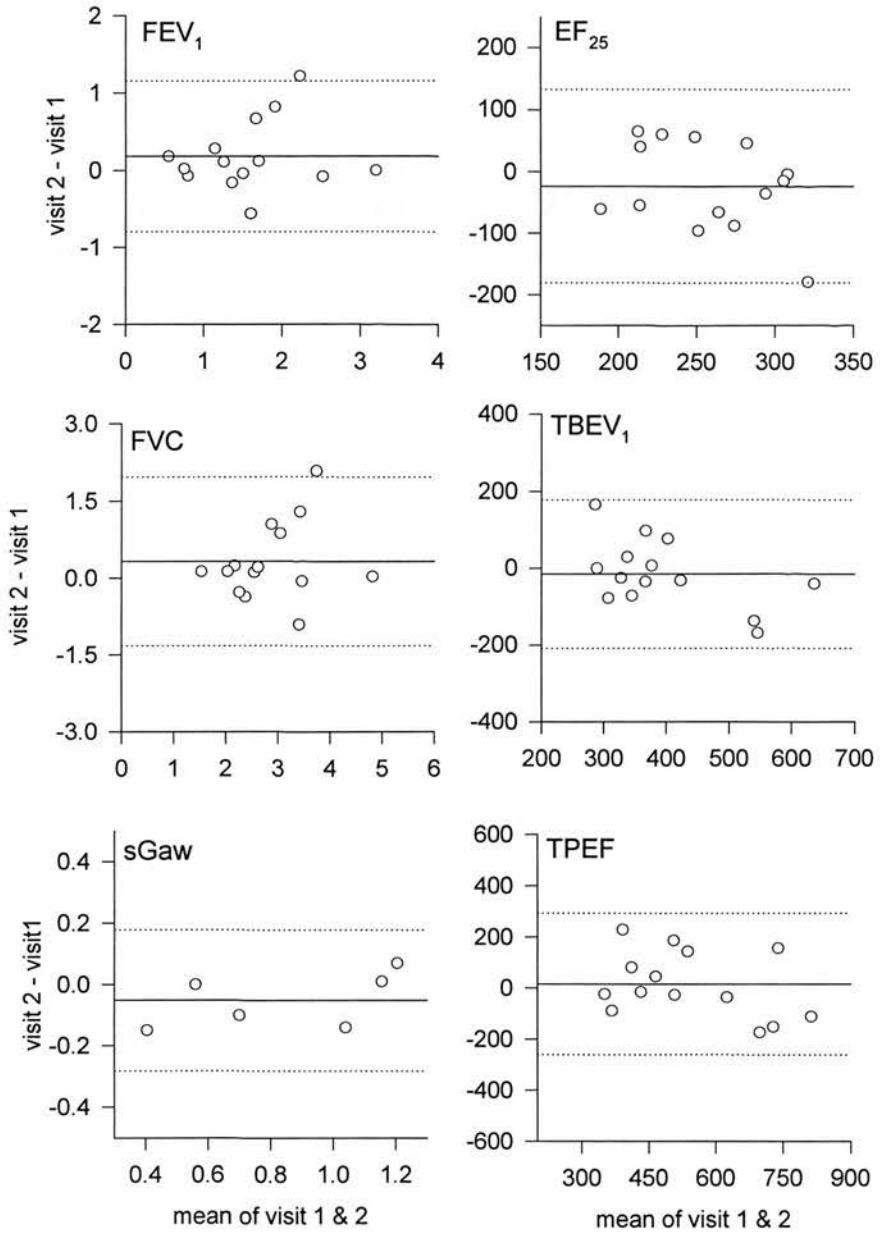


Figure 7.24: Bland and Altman plots showing comparison of visit 1 and visit 2 baseline data for visits more than 6 months apart.
Solid line is the mean difference and dashed lines are the 95% confidence limits of the difference between visit 1 and visit 2.

Variable	Visit 1	Visit 2	Mean value	Paired t-test	95% CL of between visit difference
FEV_1 (L)	17.78	22.73	20.26	NS	44.13
FVC (L)	16.60	16.49	16.55	NS	61.78
RVC (L)	25.89	13.82	19.86	NS	59.35
Raw (kPa.sec/L)	-31.86	-33.00	-32.43	NS	27.38
sGaw (/kPa/sec)	60.60	72.37	66.49	NS	54.55
V_T (L)	-0.96	9.74	4.39	NS	42.46
V_E (L)	3.44	10.67	7.06	NS	51.36
EF_{25} (ml/sec)	6.87	18.25	12.56	NS	61.34
$TBEV_1$ (ml)	7.31	13.62	10.47	NS	42.62
TPEF (ml/sec)	9.74	13.70	11.72	NS	41.25

Table 7.16: Group mean values for percentage change after bronchodilation values for the two study days more than 6 months apart.
NS = not significant.

7.4: DISCUSSION.

The reproducibility of any new physiological variable is important for its acceptability for routine use.

In a study by Noseda et al [102] a number of different variables of lung function and exercise capacity were investigated for reproducibility and clinical relevance in patients with COPD. They found that FEV_1 was among four variables that showed the greatest clinical potential for functional assessment.

A year later Vollmer [103] reported that FEV_1 was more reproducible than single breathing nitrogen test variables and FEV_1/FVC when measured over a 9 to 11 year period. This would imply that FEV_1 was the most stable measure and so the best indicator of lung function, however it may also reflect that FEV_1 was not as sensitive to small changes in lung function as the other variables and failed to pick up slow declines or in lung function.

Enright et al [104] investigated the short-term variability of FEV_1 (mean 25 days apart). The mean difference between FEV_1 values was 10.2 ml with a mean intraindividual variability of 141 ml and a coefficient of variability (CV) of 5.8%.

In this study for FEV_1 the mean difference between values for short-term variability (mean of 10 days) was 2.8 ml. For long-term variability (mean of 364 days) the mean difference in FEV_1 values was 175 ml.

One difficulty in comparing the reproducibility of the novel variables to the conventional variables was that, for baseline values, the magnitudes of the values were different. An average FEV_1 was for this patient group was 1.4 L whilst the average $TBEV_1$ was 350 ml therefore absolute differences and 95% confidences limits (95% CL) of the data between two study days are of little value. To try to overcome this the

95% CL were corrected for the mean value size for the variable by reporting it as a percentage of the mean value. This showed that the reproducibility for novel tidal breathing variables and conventional variables were similar and that for all variables reproducibility was better for the short-term than the long-term time periods.

When looking at the percentage change after bronchodilation on different study days the magnitude of the response was similar for all variables and so the 95% CL could be compared directly. Again the values were similar for all variables and better for short-term reproducibility than long-term.

In summary this study has shown that the reproducibility of the novel tidal breathing variables (EF_{25}, $TBEV_1$ and TPEF) is similar to that for the conventional routinely used variables of FEV_1, FVC, RVC and sGaw.

Chapter 8: Correlation with Subjective Benefit.

8.1: Introduction.

The previous study has defined the minimum change that exceeds natural variability for each variable. However as Tweeddale et al [86] stated, in patients with severe obstruction smaller increases may be clinically relevant, whereas in patients with mild disease this minimum increase may not be noticeable. So not only must the natural variability be calculated for any new variables but also the increase necessary for clinical benefit to be felt.

For patients with chronic obstructive pulmonary disease the predominant complaint and one of the main reasons for seeking medical advice is dyspnoea [64]. In the early stages of the disease this is seen on exertion only, but in latter stages of COPD dyspnoea is also experienced at rest. Dyspnoea has been quoted [105] as being the main incapacitating symptom and the most common reason that exercise is curtailed.

Clinically the evaluation of the level of dyspnoea experienced by patients with COPD is important. Ramirez-Venegas [92] stated that the major reason for the prescription of bronchodilator medication in COPD was to improve symptoms, including dyspnoea. Therefore much work has been carried out to determine the best test of dyspnoea in patients with COPD [5, 106, 107].

One of the main criticisms of FEV_1 was that it correlates weakly [4,5] or not at all [6] with the degree of dyspnoea. It has also been reported [7] that patients tested

for reversibility to bronchodilators sometimes report symptomatic benefit but show no improvement in FEV_1.

It is therefore preferable that any new measure of airflow obstruction should correlate to the level of dyspnoea and to subjective benefit following treatment.

The aim of this part of the programme of studies was to determine if the novel tidal breathing variables of airflow obstruction described in chapter 5.1 correlated to dyspnoea; in both the baseline level and the improvement after nebulised bronchodilator.

The hypothesis was that patient's perception of changes in dyspnoea after nebulisation would correlate to the changes in physiological measures of airflow obstruction.

8.2: METHOD.

Dyspnoea scores were included prospectively in 35 of the tidal breathing recordings reported in chapter 5 for pre and post bronchodilation and in 11 patients pre and post placebo treatment.

The protocol for each study day was the same as described in chapter 5 section 2.2. In addition patients were asked to score their breathlessness on a 20 cm visual analogue scale (VAS) [108] (figure 8.25) at the start of testing, and after nebulisation (figure 8.26).

For baseline dyspnoea scores the subjects were asked to "Mark on the line how breathless you feel at the moment. The line goes from not at all breathless at one end to very breathless at the other". For the change in dyspnoea scoring the subjects

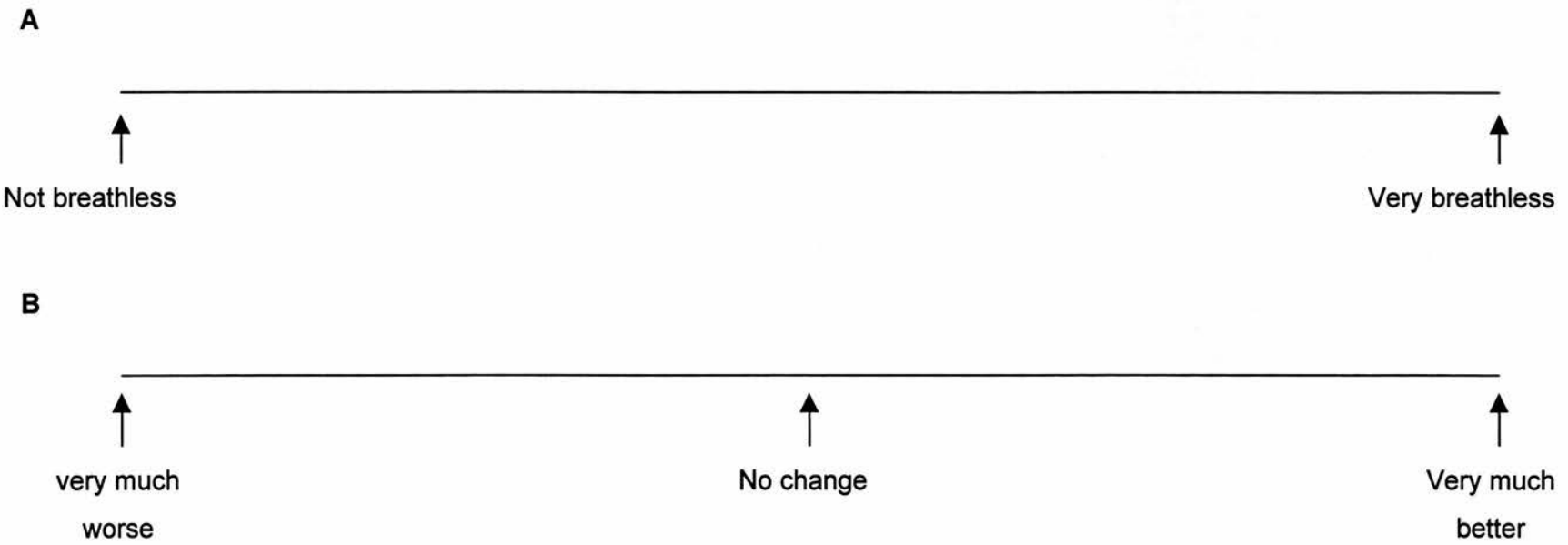


Figure 8.25: Visual analogue scale (VAS) for measuring (A) baseline breathless and (B) change in breathlessness intervention.

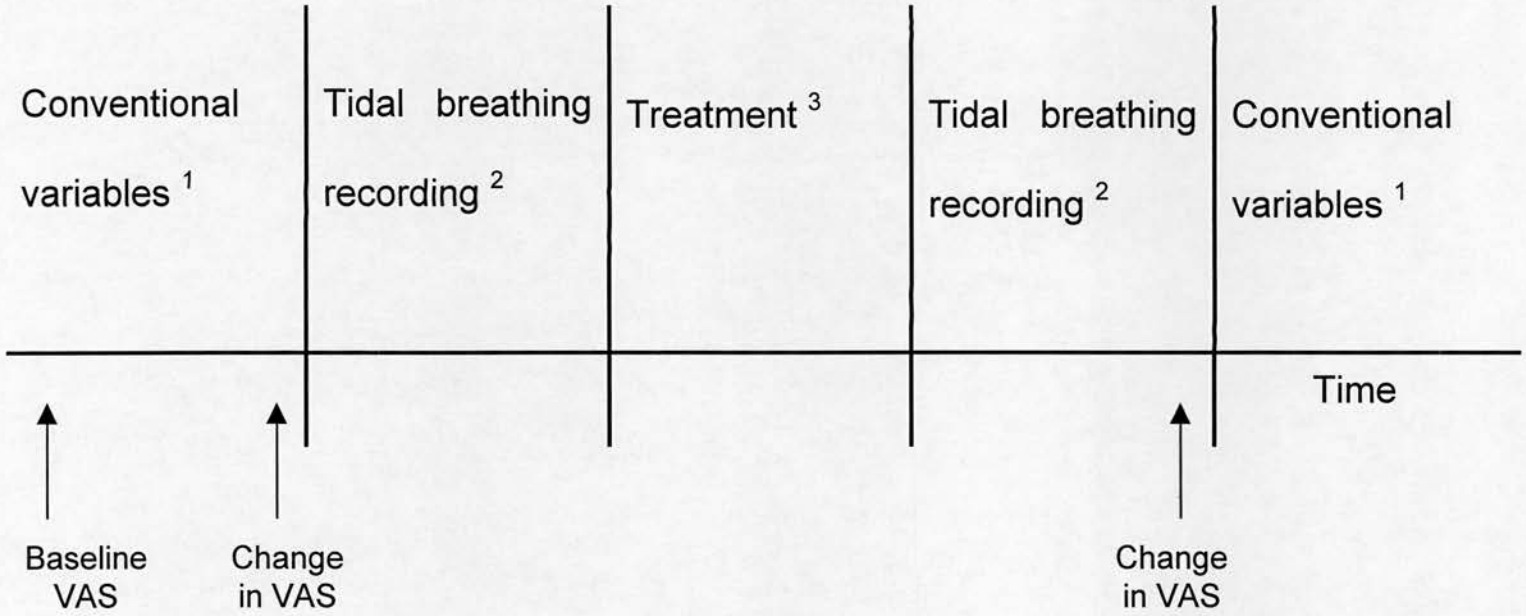


Figure 8.26: Time line of the sequence of testing for a single study day.

key

[1] 3 technically satisfactory FEV_1, FVC, ($Raw_{0.5}$, Vtg in a subset).

[2] 15 mins. spontaneous tidal breathing with an inspiration to TLC and 2-3 second breath-hold every 5 mins.

[3] nebulised 2.5 mg salbutamol ± 250 μg ipratropium bromide, or saline placebo (random order, 2 study days).

VAS breathlessness measured.

were asked to "Mark on the line how much your breathlessness has changed from the start of the study to now, if at all. The line goes from very much worse to very much better with no change in the middle".

In a subgroup of patients dyspnoea scores were also measured after the first set of spirometry was performed to assess if performing spirometry itself caused a change in patient perceived dyspnoea.

Twenty subjects performed the bronchodilator arm of the above protocol on two different occasions either within two weeks of each other, so that the reproducibility of the correlation of tidal breathing variables with dyspnoea could be examined.

8.3: Results.

8.3.1: Baseline.

Table 8.17 shows the Spearman's coefficient of correlation (r) and p values for spirometric, plethysmographic and tidal breathing variables with VAS score of breathlessness. No variable correlated with baseline breathlessness.

The range of scores was 1.8 to 19.1 with no patient scoring not breathless. There was no significant difference between patients who subsequently showed no FEV_1 response (less than 160 mls) to bronchodilators (VAS mean 10.1 range 1.8 to 15.9, N=9) and those who did show response in FEV_1 (160 ml or greater) (VAS mean 10.3 range 2.1 to 19.1, N=26).

Variable	Baseline	Change post		
		Placebo	Treatment	Placebo & treatment
FEV_1	NS	NS	NS	NS
FVC	NS	NS	NS	NS
RVC	NS	NS	NS	NS
Raw	NS	NS	NS	-0.45 / 0.002
SGaw	NS	NS	NS	0.46 / 0.001
V_T	NS	NS	0.33 / 0.049	NS
V_E	NS	NS	NS	0.46 / 0.001
EF_{25}	NS	NS	NS	NS
$TBEV_1$	NS	NS	0.40 / 0.02	0.37 / 0.010
TPEF	NS	NS	NS	0.29 / 0.049

Table 8.17: Correlation of baseline VAS dyspnoea score with respiratory variables and change in VAS score of dyspnoea with absolute change in respiratory variables post nebulisation.
Values represent Spearman's coefficient (Rs) / p value. NS = not significant.

8.3.2: CHANGE AFTER BRONCHODILATION AND PLACEBO.

The mean change in the dyspnoea score after spirometry but before bronchodilation was –0.5cm. A Mann-Whitney rank sum test showed that this small decrease was not statistically significant (p=0.76) and so all values quoted below are for the change in VAS score from baseline to after nebulisation.

The mean (SD) change in dyspnoea post nebulised bronchodilator was 4.9 (1.9) with a range of 0.8 to 8.1.

Table 8.17 shows the Spearman's coefficient of correlation (r) and p values for the absolute change of spirometric, plethysmographic and tidal breathing variables after bronchodilator and placebo nebulisation with subjective benefit as measured by change in VAS score. For all nebulised interventions (placebo and active) the change in physiological variables was plotted against change in dyspnoea in Figure 8.27.

Improvements in FEV_1, FVC and RVC were not correlated with improvements in dyspnoea whereas Raw, sGaw, and V_E were, along with the novel tidal breathing variables $TBEV_1$ and TPEF. The correlation between these variables and change in dyspnoea is shown (figure 8.27) along with the change in dyspnoea after either bronchodilator or placebo treatment for individual values and mean value (figure 8.28).

8.3.3: EFFECT OF SALINE.

The mean (SD) change in dyspnoea post nebulised saline was 1.08 (2.40) with a range of –4.2 to 4.3.

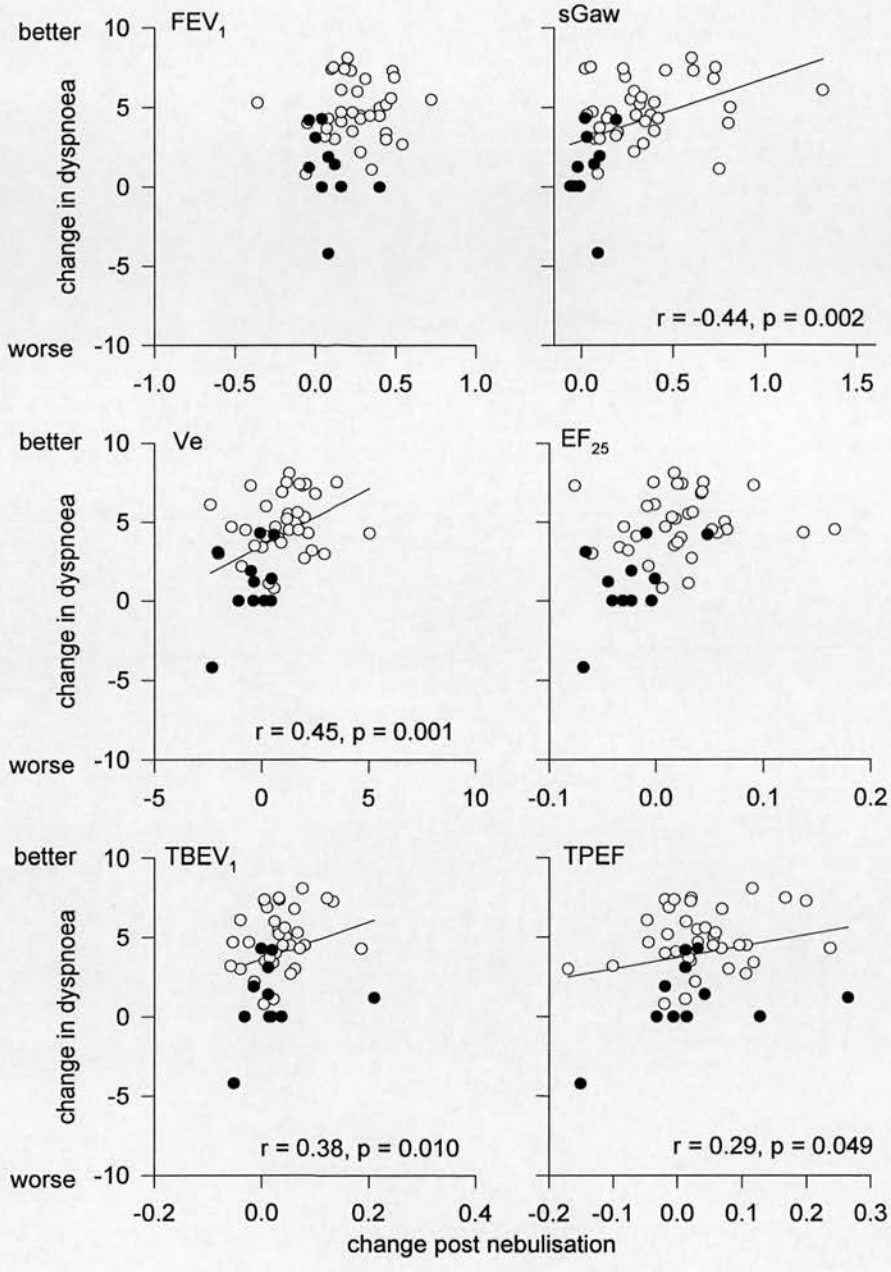


Figure 8.27: Correlation of change in dyspnoea with absolute change post active (○) and placebo (●) treatment for spirometric, plethysmographic and tidal breathing variables.
Regression lines and correlation coefficients refer to pooled (active and placebo) data.

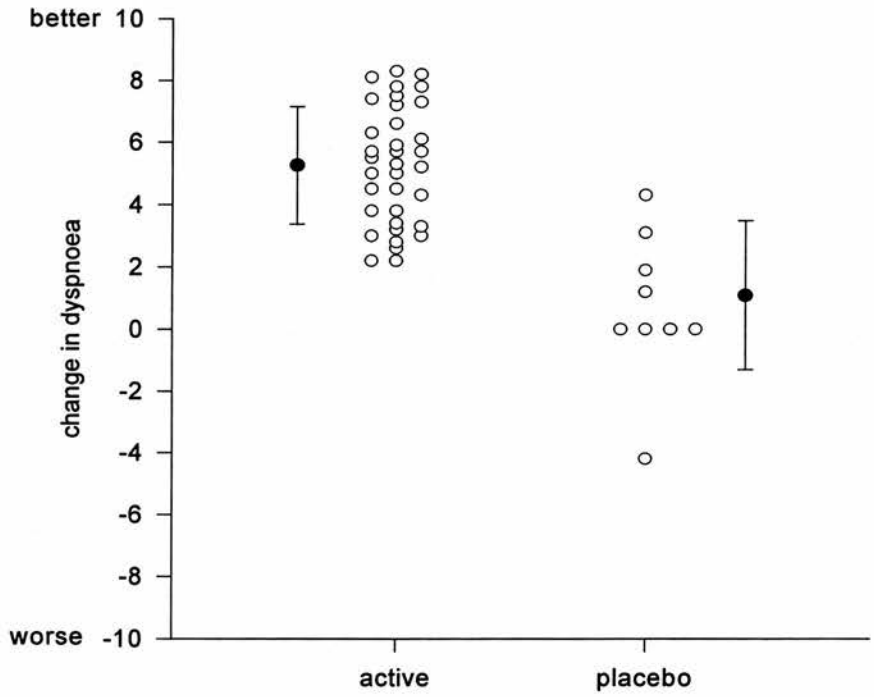


Figure 8.28: Change in VAS score dyspnoea after active treatment (salbutamol and ipratropium bromide) and placebo. Open circles represent individual data and closed circles represent mean value with standard deviation.

There was no correlation between change in breathlessness and absolute change after saline treatment for any variable (table 8.17).

8.3.4: Reproducibility of Dyspnoea Change.

20 patients performed the bronchodilator arm of the above protocol on two separate occasions. The mean (SD) number of days between the two visits was 6 (3) with a range of 2 to 15 days. Paired T-tests showed there was no significant difference between the change in dyspnoea score on the two visits. Figure 8.29 shows individual patient data for each visit along with the group mean (SD) for each visit.

8.4: Discussion.

Symptomatic benefit of treatment in COPD is recognised as an important outcome measure in both disease management [105, 107, 109, 110] and in trials of new treatment [106, 109].

The 1994 BTS guidelines for nebuliser use in COPD [66] states "After an assessment process the clinician must decide with the patient if the nebuliser treatment has produced subjective and objective benefit". The guidelines also state that if patients experience a subjective benefit from nebuliser treatment, even if no clear improvement peak flow clinicians should use their judgement as to whether nebulised therapy should be continued.

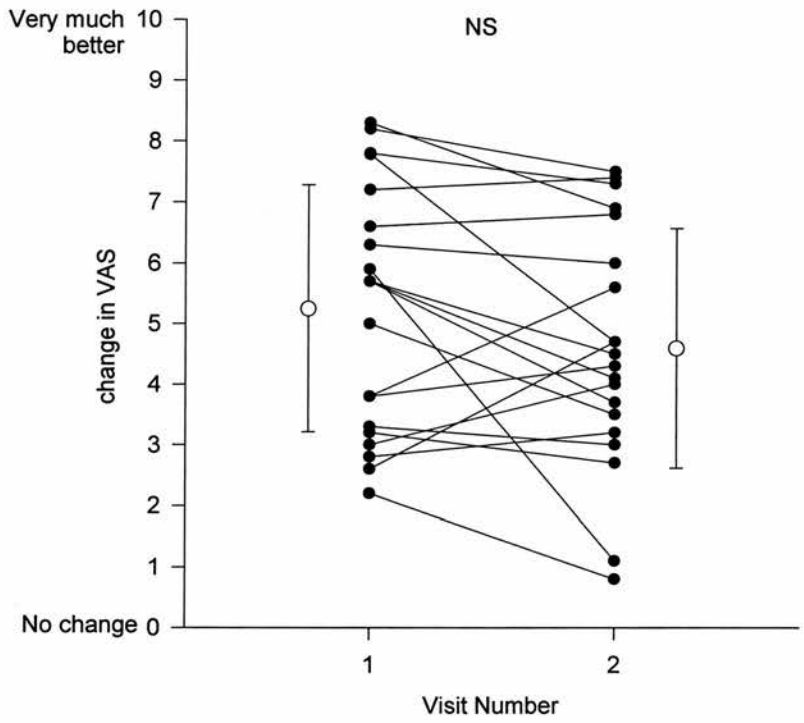


Figure 8.29: Change in dyspnoea score after bronchodilation on 2 separate occasions.
For individual patient data (•) and group mean and SD (○).
NS = no significant difference (t-test).

Subjective benefit in the absence of a change in conventional physiological variables may occur due to placebo effect or because physiological changes are occurring which are not detected by the conventional variables. In a study by Khan and O'Driscoll [111] it was found that nebulised isotonic saline caused a reduction in breathlessness at rest which was not present when an inactive nebuliser system was used, this was termed a placebo response. In this study they was no significant change in dyspnoea scores after placebo treatment. The possibility that undetectable (by conventional variables) physiological changes were occurring would be supported if new measures of airway obstruction, which are to be used to assess the efficacy of treatment, were correlated with subjective benefit.

The present study confirms the results of a previous study by Smith [7] that FEV_1, FVC and RVC do not correlate to change in dyspnoea. Further this study shows that the present gold standard of airway obstruction, sGaw does correlate to change in dyspnoea as reported previously [97, 98]. It has also shown that two of the novel tidal breathing variables described in chapter 5.1, $TBEV_1$ and TPEF, were correlated with changes in dyspnoea.

No variable showed any correlation with baseline dyspnoea. This may be because the patient perceived level of breathlessness is a very individual measure. Some patients with severe airflow obstruction score themselves as not breathless, whereas other patients with more mild disease score themselves as very breathless. This is likely to be because patients shift their scale of breathlessness depending on individual experience. This large intersubject variability in dyspnoea scoring will obscure the effects of airflow obstruction on dyspnoea scores in a population.

A limitation of the present study is that the measurement of breathlessness was made at rest and not all patients with COPD experience this. Therefore they may not

feel any benefit of bronchodilators at rest but only on exertion. However in the population study no patient scored 0 (not breathless) on the VAS, the lowest score being 1.8 (of a maximum score of 20) and only 5/35 (14%) had a score of less than 3. There was also no significant difference in resting breathlessness between those patients deemed responsive on FEV_1 criteria and those unresponsive.

A number of variables correlated with change in dyspnoea when looking at the response to treatment. This may be because although the actual level of dyspnoea is variable between patients the change after treatment is more consistently scored within patients.

As these measures are subjective patients were asked at the end of the final visit if they realised that they had been given different treatments on the different visits in order to determine if the blinding had been adequate. No patient said that at the time they thought they had been given different treatments but approximately 25% said that looking back it would explain why they had felt better after some study days than others.

In summary, in contrast to FEV_1, the absolute change in airways resistance, specific conductance, minute ventilation and the tidal breathing variables, $TBEV_1$ and TPEF correlate with subjective benefit after nebulised treatment.

Chapter 9: Discussion

The hypothesis addressed in this thesis is that novel variables measured from tidal breathing expiratory flow patterns can be used to assess airflow obstruction.

The studies reported in this thesis have shown that it has been possible to identify aspects of the tidal breathing expiratory profile which correlate with the degree of airflow obstruction present and which change in a physiologically plausible way in response to bronchodilator therapy.

This concluding chapter considers the success of developing the measurement and analysis protocols and the usefulness of the novel variables in a clinical setting.

The need for an alternative measurement of airflow obstruction to FEV_1 is becoming more accepted with groups looking at the use of other measures from spirometric manoeuvres to determine patients' response to inhaled treatment. This is confirmed in Chapter 2, which showed that if only FEV_1 was used to assess the response to bronchodilator therapy in patients with diseases involving airflow obstruction then potentially 21% of these patients would be denied treatment that may be beneficial for them.

The use of tidal breathing to assess changes in breathing due to disease has been explored for many years in animals and infants because of the inability of these groups to perform conscious breathing techniques such as the forced expiratory manoeuvre. Tidal breathing variables have been described that correlate with the presence and severity of obstructive lung disease in these groups.

9.1: IDENTIFICATION OF NOVEL VARIABLES AND PROTOCOL DEVELOPMENT.

The study described in chapter 5 showed that there are three novel tidal breathing variables which increased significantly after bronchodilation. These were $TBEV_1$, TPEF and EF_{25}.

Two of these variables are measured from the early portion of the expiratory flow profile and one from near the end of the expiration. The reason that the majority of the variables were from the start of expiration may be explained from observation of the individual breaths. It was noted that the beginning of expiration was less variable than the end of expiration. Some patients started inspiration before they had reached FRC (no expiratory pause) whereas others showed expiratory pauses of up to 20 seconds in length.

This variability in the end of expiration makes the production of an averaged breath difficult. To try to overcome this, the computer algorithm used for breath analysis excluded expiratory pauses. This was achieved by defining a threshold level of flow rate at which the expiration was considered finished. It is unknown if small changes in this threshold would make a difference to the value of variables measured near the end of the breath or if small changes in the flow profile had been lost.

Another consideration in determining the best protocol for the collection of the data was the duration of the collection period and of any pre-collection acclimatisation period.

Early pilot studies showed that it was important to allow the patients time to acclimatise to the breathing equipment but this leads to the possibility that during this time the patients may have been altering their breathing pattern to suit breathing on the

equipment, or that the breathing may have changed whilst the equipment was being fitted and that during the acclimatisation period it was returning to normal.

As there is no way to test this, the equipment which was used in the studies was that which was thought likely to have the least effect on the breathing pattern, kept equipment dead space to a minimum, had minimum resistance to airflow and allowed the subjects the choice to mouth or nose breathe.

The collection time was initially chosen as 15 minutes, however some patients found themselves getting drowsy near the end of the collection period despite having a taped story to listen to.

To minimise the time taken for measurements, results taken from a shorter collection time were compared with those from a longer collection time. The study described in chapter 3.3.1 showed that data collected in 5 minutes did not differ significantly from data collected over 15 minutes. It was decided to still use the taped story even for this shorter collection time but this may not have been necessary and should be further studied. The reduced measurement period also meant that the total time for the data to be collected, including time to explain the procedure and to attach the equipment, was reduced to approximately 10 minutes. This is the same if not shorter than the time it takes to perform three forced expirations to BTS standards and makes the protocol more acceptable for everyday use.

All the analysis was performed by one investigator JMM, therefore it would be necessary for another investigator to analyse the data as well to determine how investigator specific the results are, e.g. do different investigators identify the same aberrant breaths for exclusion from analysis.

9.2: CORRELATION WITH CONVENTIONAL VARIABLES.

The three variables ($TBEV_1$, TPEF and EF_{25}) identified in chapter 5 to change post bronchodilation were also examined to determine if they correlated to the conventional variables of airflow obstruction. As there is no accepted gold standard for measuring airflow obstruction both FEV_1 and sGaw were studied.

$TBEV_1$ and EF_{25} correlated better with sGaw and FEV_1 than TPEF (figure 5.20) and the correlations of $TBEV_1$ and TPEF with sGaw were stronger than with FEV_1.

9.3: VARIABILITY.

The measure to measure variability was examined and reported in chapter 6. The 95% confidence interval for each variable was calculated but in order to compare these confidence interval between variables of different magnitudes the ratio of the confidence interval to the average value was calculated (table 9.18). This shows that the variability of the novel variables is comparable to that of FEV_1 and less than that for sGaw.

9.4: REPRODUCIBILITY.

The short and long term reproducibility of the variables were examined and reported in chapter 7. The data shows that there was no statistical difference between the two visits

variable	95% confidence interval (CI)	Average value (X)	CI:X
FEV_1	170 ml	1233 ml	0.14
sGaw	0.13 /kPa/sec	0.56 /kPa/sec	0.23
$TBEV_1$	42 ml	354 ml	0.12
TPEF	67 ml	483 ml	0.14
EF_{25}	39 ml	234 ml	0.17

Table 9.18: Comparison of the measure-to-measure variability for conventional and novel tidal breathing variables.

for either baseline or percentage change post bronchodilator values measured over short-time period (< 2 weeks) or a longer time period (> 6 months).

As with variability the 95% confidence interval of the between visit difference as a percentage of the mean value of the variable was calculated to allow comparison of the different variables.

For both short and long term reproducibility the values for the novel variables are similar to those for the conventional variables. For all values the longer term reproducibility was worse than for short term. This greater variability over long periods is a common feature of biological measurements.

9.5: PATIENTS WITH NO RESPONSE TO BRONCHODILATORS.

In all the studies described in this thesis one of the inclusion criteria for patient recruitment was that they had previously shown significant reversibility to bronchodilators on spirometry.

However, on the actual study day some patients did not show reversibility in the conventional variables. In order to determine if these patients who would have been described as unresponsive to bronchodilators on conventional testing showed increases in the novel variables this sub-group of patients was tested. The data in chapter 6 shows that of the 33% of patients who showed no significant change post bronchodilation by FEV_1 criteria 12% showed increases in $TBEV_1$, and 6% for TPEF and EF_{25}. For sGaw 23% did not show response to bronchodilators and of these for the novel variables $TBEV_1$, TPEF and EF_{25} the percentage who did show increase were 22%, 11% and 0% respectively.

Because the number of patients in this sub-group was small (N=16 for FEV_1 and N=15 for sGaw) it would have been better to include a group of patients who had not previously shown response to bronchodilation to study this in more detail.

9.6: COMPARISON OF PRESENT STUDY WITH PREVIOUS WORK.

The majority of previous studies investigating the possibility of using tidal breathing to assess airflow obstruction have been carried out in children. They have involved a variety of different protocols as outlined in chapter 1.9.2.

In the studies with younger children the data collection was performed with the subjects sedated, this is neither practical nor desirable when working with adults as it is not known what effect sedation would have on the breathing pattern.

In the study on adults by Morris and Lane [59] T_{PTEF}/T_E was examined in patients with airflow obstruction and normal subjects to see if this variable could distinguish between the two groups, which it did although on an individual basis there was overlap between the groups.

Morris and Lane [59] overcame the problem of breath variability by recording 10 consecutive breaths, measuring the value of the new variables for each breath and then averaging the results. This has the advantage of being able to use all of the breath. However it was not stated if any breaths were excluded due to swallowing, coughing, sighing etc. or if an acclimatisation period was allowed. They did however state that the variability of the breathing pattern was less for the obstructed group than for the normal subjects. The flow data were collected using a heated pneumotach and a mouthpiece, which in a later study they reported had a dead space of 120 ml. Therefore although

they imposed mouth breathing on the patients, with all the changes that this is reported to have on the breathing pattern [78-84] they have kept the dead space to a minimum. In the present study all flow data were collected using a facemask and ultrasonic flowmeter, thereby allowing the patients to breath either orally or nasally and hopefully decreasing the changes in breathing pattern due to the equipment, this meant an increase in the dead space to 175 ml but this was felt to be an acceptable compromise.

The study by Morris [59] also differed from the present study in that they looked at distinguishing patients with airflow obstruction from normal subjects, whereas the present study concentrated at looking at identifying novel variables that could pick up changes in airflow obstruction within an individual. The ages of the two groups in Morris study were not similar (34.2 ± 9.6 yrs for the normal group compared to 54.8 ± 14.7 yrs) in the obstructed group and again it is not known what changes may occur in the tidal breathing pattern with age.

As the present study did not include any normal subjects we are unable to state if the variables described here can distinguish between normal subjects and patients with COPD. This is a short coming of the present study which could easily be addressed in future studies.

Although the previous studies had identified T_{PTEF}/T_E as being able to distinguish normal subjects from patients with airflow obstruction and although we found similar baseline values for this variable in our patients, there was no change in this variable following bronchodilation. This is because the timing within breaths does not appear to change significantly after bronchodilation. Therefore it would not be expected for time related variables to change as the flow related ones did.

In a later study by Morris [61] the group looked at whether two variables derived from the end of the expiration could be used in assessing airflow obstruction. This work was based on the theory that the later stages of the expiration occurred by passive relaxation with no active muscle activity and that therefore the decay of flow with time reflects the compliance and resistance of the respiratory system. The slope of the linear portion of this decay was calculated (Trs). A second variable, extrapolated volume (EV), was also calculated by extrapolating the slope to zero flow and measuring the area under the extrapolated line.

This approach is valid as long as the expiration is relaxed, however some patients with more advanced disease utilise their abdominal muscles to help drive expiration [46-47] therefore making it active not passive.

Also from the present study we have observed that not all patients show the abrupt flow reversal that Morris [59-60] describes, some show exponential flow decay to zero flow and others show long expiratory pauses which would complicate their analysis.

In a third study by Williams [62] the breath to breath variability of the breathing pattern was examined. As with the previous study they again used a mouthpiece and heated pneumotach, there was no acclimatisation period and 12 ± 3 breaths were analysed per subject.

To overcome the difference in breath length and maximum flow rate each breath was scaled in respect of both the flow and time axis. The scaled breath was then divided into two portions, pre-T_{PTEF} (before the tidal peak expiratory flow rate) and post-T_{PTEF}. A linear regression line was the fitted to the decay slope in portion two.

The disadvantage of scaling the data in this way is that small changes in flow or time related variables will be lost and the fitting of a linear regression line will again

obscure small changes in the flow decay profile especially in the obstructed group as this tends to be more concave than linear.

The group reported that scaling the data in this way rendered the pre-T_{PTEF} portion of the breath useless for estimation of airflow obstruction. They did find though that the scaling enhanced the distinction between the different flow patterns for the post-T_{PTEF} data and they stated that the fit of the linear regression line to this portion of data was not important but that the slope of the line was, with a shallower slope reflecting worse airflow obstruction.

The advantage of this approach is that in trying to describe the shape of the flow profile a large portion of the breath is analysed and not one time point as with the present studies variables of EF_{25}. It maybe that a variable from the tidal breath similar to that of FEF_{25-75} measured from the MEFV curve maybe more sensitive having the advantage of describing a larger portion of the breath but not needing any scaling of the breath.

In the previous work by both Morris and Williams a small number of breaths have been analysed (10 – 15) with no acclimatisation period therefore making the testing time very short. More work is needed to determine if this is sufficient to give stable representative data (It maybe that the present study is causing itself problems with aberrant breaths and possible drowsiness of the patients by measuring for 7 minutes when this is not needed).

Shortcomings of the present method of tidal breathing analysis include: -

- The need for defined resting conditions to achieve steady state breathing pattern. Breathless patients may be unable to relax sufficiently to produce a steady breathing pattern or be able to tolerate the facemask.

- The bin averaging employed to produce the averaged breath depends on the accuracy of the breath detection and breath alignment.
- The manual selection of breaths to exclude from analysis is still required and this introduces an element of subjectivity in the analysis.
- The technique is not applicable to patients with very erratic breathing patterns (highly variable flow patterns between breaths), such as Cheyne Stokes breathing.

9.7: PRACTICALITY OF THE PROPOSED TIDAL BREATHING MEASUREMENT METHOD IN THE CLINICAL SETTING.

9.7.1: ADVANTAGES.

The measurement protocol developed is simple to implement and the analysis is straightforward and quick. Apart from the selection of breaths to exclude, all the analysis is automated.

The protocol is also simple for the patients to perform, it involves no complex breathing manoeuvres, other than a 2-3 second breath hold and does not require much effort. It would therefore be potentially applicable to the investigation of patients who are acutely ill or frail and unable to perform forced manoeuvres and in those in whom the forced expiration causes coughing, which leads to falsely low FVC and falsely large FEV_1/FVC ratios.

9.7.2: DISADVANTAGES.

The protocol requires that the patient be tested in a quiet room, which is not always available in a busy department with limited space.

The patients tested in these studies were clinically stable. More unstable patients may be unable to relax enough to give a steady breathing pattern or to tolerate the facemask.

9.8: CONCLUSION.

In conclusion, the initial hypothesis that tidal breathing flow profiles reflect airflow obstruction and would show changes in response to bronchodilation has been confirmed by these studies. This work has identified three novel variables, $TBEV_1$, TPEF and EF_{25} that can be measured from tidal breathing expiratory flow profiles that can be used to assess airflow obstruction in patients with COPD.

These three variables correlate with conventional variables, change in a physiologically plausible way after bronchodilation, have measure-to-measure variability and reproducibility comparable with conventional variables and correlate with the subjective decrease in dyspnoea after bronchodilation.

The present method overcomes many but not all of the problems associated with the measurement of forced expiration and previous attempts to quantify airflow obstruction from tidal breathing. With careful control of measurement conditions, it could be applied with little modification to clinical practice, and measurements are achievable in little more time than is taken to perform conventional spirometry.

Chapter 10: FUTURE WORK.

10.1: MECHANISMS BEHIND THE TIDAL FLOW PROFILE CHANGE.

The observations of tidal breathing presented in this thesis do not themselves contribute to a greater understanding of the underlying determinants of the tidal flow profile. In particular the relative importance of passive mechanical relaxation processes compared to respiratory muscle activity in determining instantaneous expiratory flow cannot be deduced from these recordings. Others [60] have emphasised the importance of post inspiratory braking of early expiratory flow by sustained inspiratory muscle activity as a determinant of early expiratory flow. This mechanism is said to be reduced in COPD, with mechanical relaxation being instead the dominant determinant of expiratory flow, however further studies of the patterns of activation of respiratory muscle groups during spontaneous breathing in COPD are needed to confirm or refute this concept. Such studies would necessarily involve the measurement of additional variables during tidal breathing including: -

- Respiratory muscle EMG, both inspiratory and expiratory.
- Intrabreath airways resistance.
- Measurement of the relative contributions of the chest wall and abdomen during tidal breathing.

10.2: OTHER PATIENT GROUPS.

The work described in this thesis was carried out on patients with COPD who had previously demonstrated partial reversibility to inhaled bronchodilators. Three other groups which would be interesting to investigate would be: -

- Patients who had symptomatic benefit from bronchodilators but no significant response as measured by spirometric variables, to investigate if the tidal breathing variables changed after bronchodilation.
- Patients with asthma who had significant reversibility to bronchodilators to see if the technique was applicable to a more generalised patient population.
- Normal subjects to ensure that the technique is not too sensitive and diagnoses reversibility in everyone.

10.3: OTHER STUDY SITUATIONS.

The ability to assess airflow obstruction during tidal breathing would be of benefit in a number of situations including: -

10.3.1: EXERCISE.

Much research is being undertaken looking at bronchoconstriction during exercise, and it is difficult for subjects to perform forced expiration whilst exercising.

Comparison of tidal breathing profiles at matched levels of ventilation could be used to study airway calibre during exercise.

10.3.2: BRONCHIAL CHALLENGE.

At present the response of subjects to bronchial challenge with substances such as methacholine and histamine is measured by changes in FEV_1. As the measurement of FEV_1 can cause coughing and coughing can blow the challenge drug from the airway it can lead to a falsely high PD_{20} (provocative dose which causes 20% drop in FEV_1). Tidal breathing measurements, on the other hand, do not cause coughing and would overcome this problem. However changes in the analysis of the variables may be needed to speed up the analysis time, which for the study in this thesis was done off line and took approximately 2 minutes per 5 minutes period.

10.4: CONCLUSION.

The method of tidal breathing analysis presented in this thesis opens up the possibility of generating new insights into the pathophysiology of airflow obstruction and the effects of treatment. Such methods observe the respiratory system operating under conditions which more closely resemble normal breathing than techniques which depend on forced expiration. Results are therefore arguably more relevant to the clinical condition and the limitations it imposes on patients' lives. Further studies are needed to elucidate more clearly the underlying determinants of tidal expiratory flow yet despite an incomplete understanding of the underlying processes, a relatively simple method of measuring, recording and averaging tidal flow has been found to be capable

of demonstrating significant and reproducible changes following the administration of bronchodilators. With appropriate development the technique could be applied to study airflow obstruction in other areas such as exercise and bronchial challenge.

Chapter 11: APPENDICES.

APPENDIX A. EQUIPMENT SPECIFICATIONS.

11.1: MODEL FR-41S ULTRASONIC PHASE-SHIFT FLOWMETER.

BRDL LTD, BIRMINGHAM, UK.

11.1.1: PRINCIPLE OF OPERATION.

The ultrasonic phase-shift transducer operates on the principle of the distortion of a sound wave by moving air. Two transducers Ta and Tb are mounted diagonally across a tube, through with air moves, at an angle θ.

Both transducer send and receive sound waves. If transducer Ta is driven at a frequency f, then the time taken, Δt, for the sound to reach the receiving transducer T2, positioned d cm away is: -

$$\Delta t_{1-2} = \frac{d}{C+V\cos\theta}$$

where: $V\cos\theta$ = the component of mean air flow along the path d

C = the velocity of sound in air

This represents a phase-shift, θ, of the transmitted signal, where: -

$$\phi_{1-2} = \frac{2\pi fd}{C+V\cos\theta}$$

If the functions of the two transducers are reversed this becomes: -

$$\phi_{2-1} = \frac{2\pi fd}{C - V\cos\theta}$$

and the difference between the two phase shifts, $\Delta\phi$, equals: -

$$\Delta\phi = 2\pi fd\left(\frac{2V\cos\theta}{C^2 - V^2\cos^2\theta}\right)$$

If C2 >> V2cos2ϕ this simplifies to: -

$$\Delta\phi = 4\pi fd\left(\frac{V\cos\theta}{C^2}\right)$$

Therefore the difference between the phase-shifts found with the ultrasound travelling in each direction is linearly related to the mean velocity of air in the tube. Because the velocity of sound in a gas is proportional to the square root of the temperature and independent of pressure, the relationship above is inversely proportional to temperature. The resulting variation in gain of the instrument corrects the volume flow rate for changes in temperature.

11.1.2: SPECIFICATIONS.

Linearity:	maximum deviation from true flow <2% of reading.
Baseline stability:	
Inhale : exhale	< 0.2% fsd.
Temperature (ambient)	< 0.2% fsd /°C.
Noise, zero flow	< 0.07% fsd, rms (0-60Hz).

Gain stability:	
Ambient : exhalate (20°C)	+ 0.16% gain change.
Temperature (23 – 35 oC	- 0.27 % /°C.
Response time:	Half of the flow is sampled at 160 Hz, giving a 50% response time of 6 ms and a 100% in 12 ms.
All output impedances:	100Ω.
Flow range:	± 20 L/sec.
Dead space:	70 ml.

APPENDIX B: COMPUTER PROGRAMS.

11.2: DATA COLLECTION COMPUTER PROGRAM.

A working copy of this program can be found on the enclosed CDROM.

11.3: DATA ANALYSIS COMPUTER PROGRAM.

A working copy of this program can be found on the enclosed CDROM.

11.4: BREATH MARKER ALGORITHMS.

The data analysis program looks for breath markers using the following algorithm: -

11.4.1: START OF EXPIRATION.

The program first looks for a set of 3 flow data samples that are increasing in value. It then checks to see if this data set is passing trough zero flow. The point where this occurs is deemed as the start of expiration.

11.4.2: START OF EXPIRATORY PAUSE.

After the start of expiration has been found the program skips 50 flow samples and then checks each sample until one is found with a flow value less than 5 ml/sec. This point is deemed the end of expiration and the start of an expiratory pause.

11.4.3: START OF INSPIRATION.

The program then looks for the flow to cross zero by looking for four monotonic decreasing flow samples each one 5 samples from the preceding one. The first sample in such a sequence is deemed the start of inspiration (figure 11.30).

The algorithm then goes back to looking for a start of expiration and loops through this routine until the end of the flow data set.

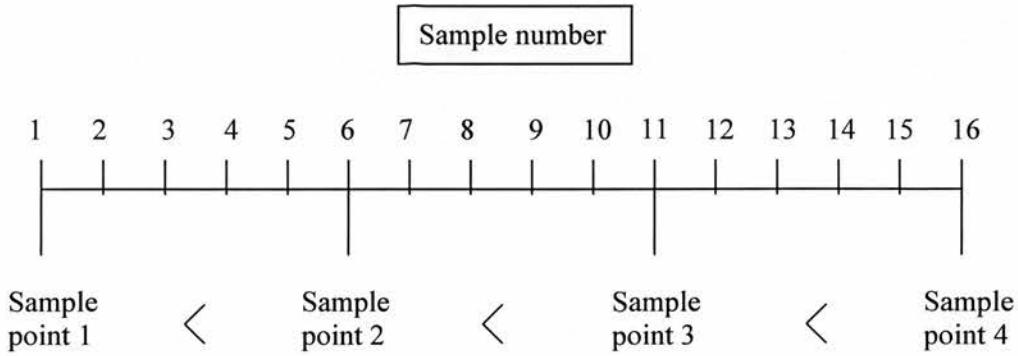


Figure 11.30: Diagrammatic representation of start of inspiration algorithm.

APPENDIX C: FLOW METER STABILITY.

11.5: DRIFT IN ZERO FLOW.

When measuring flow it is important that the flowmeter is stable. The easiest way to determine the stability of a piece of equipment is to check that the zero does not drift. For the ultrasonic flowmeter the amount of drift in zero flow was determined during three different situations :-

11.5.1: DRIFT OVER TIME.

The zero flow value was measured at the start and end of a 10 minute period to determine if the flowmeter was stable over time with no airflow through it (figure 11.31).

11.5.2: WITH SYRINGE STROKES.

Zero flow was measured before and after repeated inspirations and expirations via a 3 litre calibrating syringe. To determine if zero drift occurred to the flow of air (figure 11.32).

11.5.3: WITH TIDAL BREATHING.

Zero flow was measured at the start and end of a 15 minute period of tidal breathing. Zero flow was measured during 2-3 second breath holds. This was to determine if the change in temperature that occurs throughout inspiration and expiration caused drift in the flow meter (figure 11.33).

The data showed that no drift in zero occurred during any of the situations investigated. Thus indicating that the ultrasonic flowmeter would be stable for the measurement of tidal breathing.

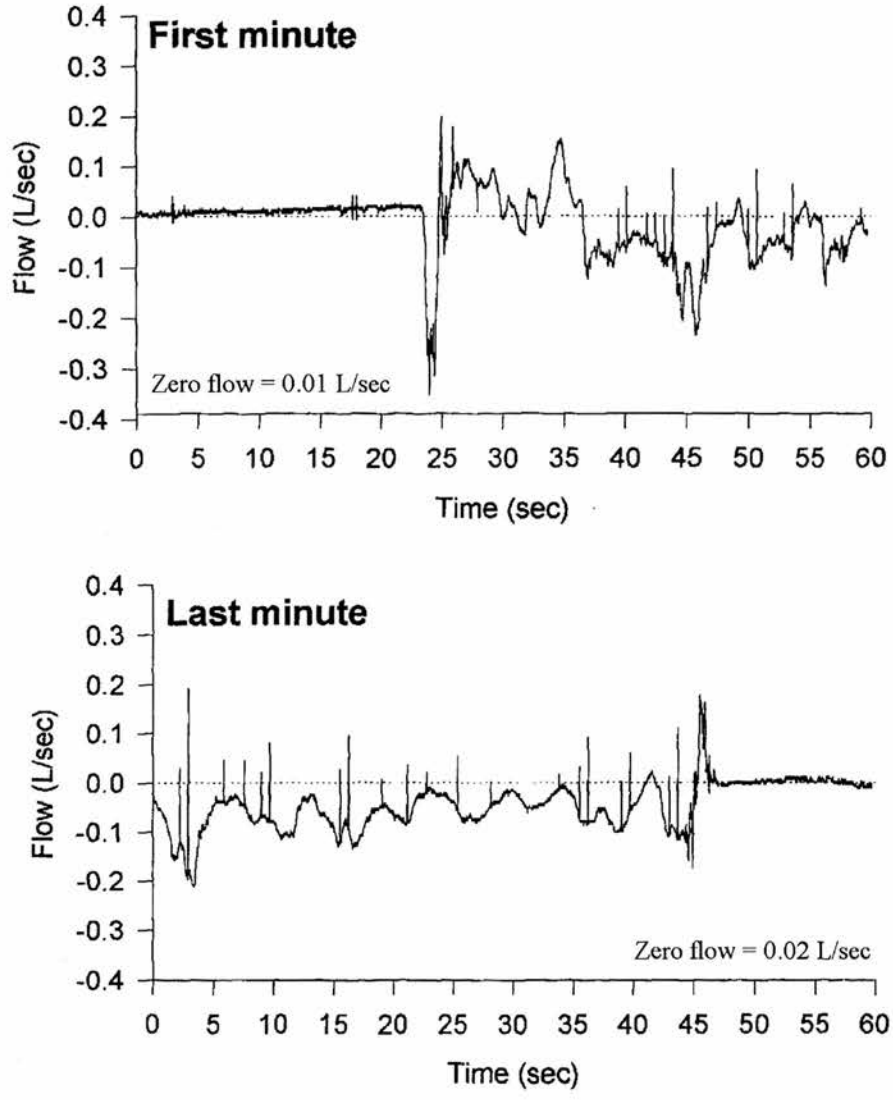


Figure 11.31: Drift over time zero flow measured at start and end of 10 minute period.

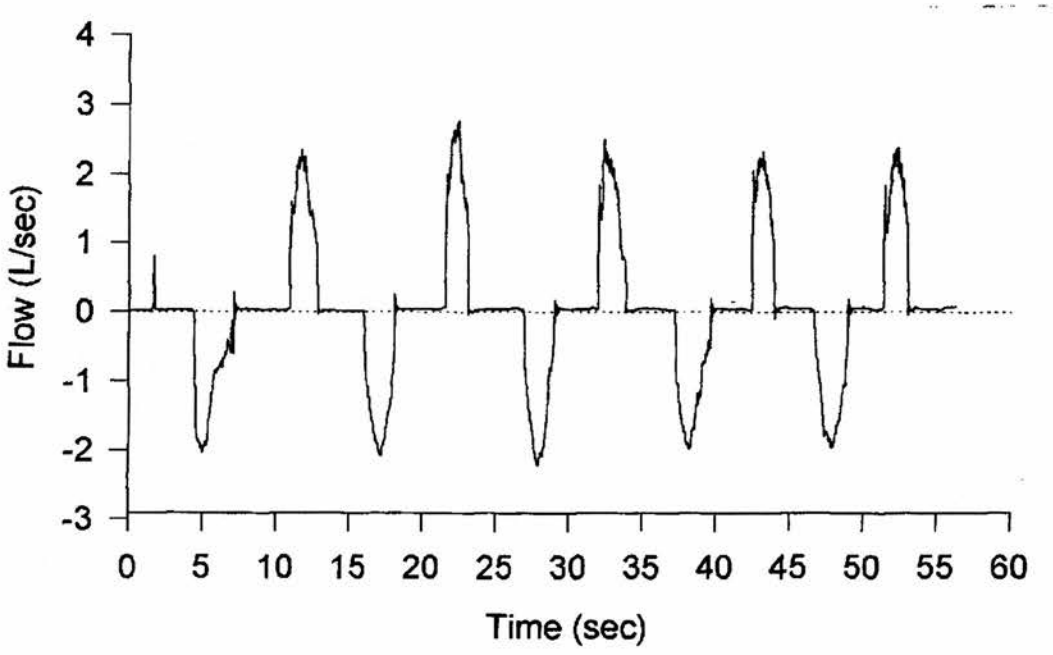


Figure 11.32: Zero flow measured at before and after 3L syringe strokes.

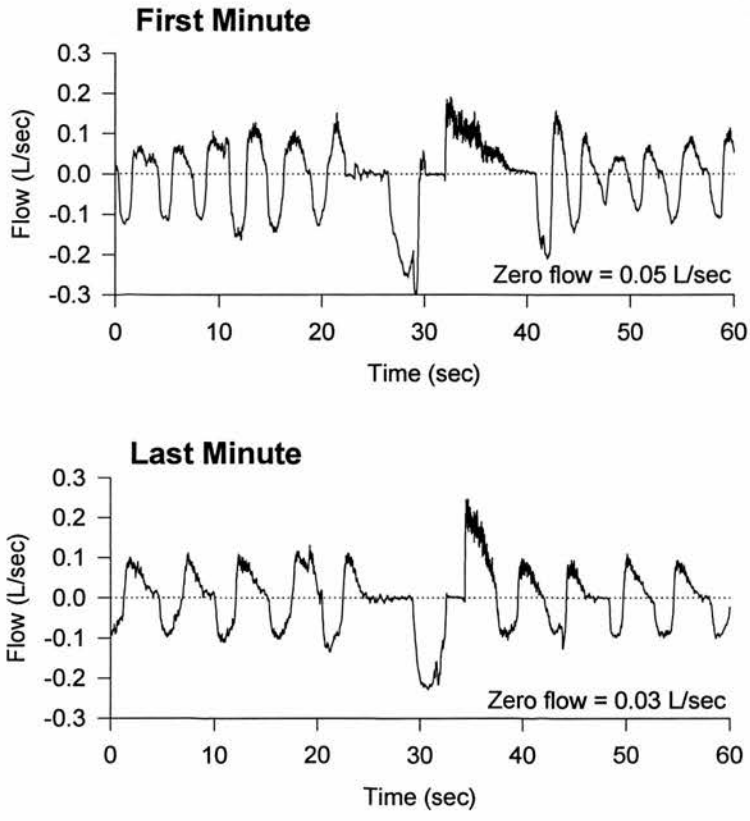


Figure 11.33: Flow data during the first and last minute of a 15 minute period of tidal breathing.
Zero flow measured during 2-3 second breath hold.

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