The Mechanisms of Airway Narrowing in Asthma

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To my mam and dad

who encouraged and supported me from the beginning

Abstract

In healthy subjects Deep Inspiration (DI) transiently dilates the airways, while many asthmatics show bronchoconstriction by a mechanism which is incompletely understood. I investigated how the method of assessment affects the response. The response as measured by specific airway conductance (SGaw) appeared to contradict that measured by forced expiration. This led to the formulation of a novel hypothesis to explain the asthmatic bronchoconstrictor response: That the negative intra-thoracic pressure associated with DI may temporarily increase airway oedema and thus reduce lumenal diameter. This was tested by comparing the effects of non-forced with forced inspiration (through resistance). In the asthmatic group, forced inspiration produced significantly more bronchoconstriction.

Airway hyperresponsiveness in asthma has been attributed to impaired ability of DI to stretch airway smooth muscle. The seminal study 'confirming' this, I argue, is flawed. I have re-tested the hypothesis. The asthmatic response was significantly greater than the control response even when DI was prohibited. Asthmatic hyperresponsiveness is therefore not attributable entirely to an abnormal asthmatic response to DI.

Many asthmatics display an apparent capacity for unlimited airway narrowing in response to bronchial challenge; most healthy subjects demonstrate a maximal (limited) response. The maximal response measured by a DI independent index represented a greater % change from baseline than the maximal established by a DI dependent index. This suggested some bronchoprotection resulting from DI but also the existence of a distinct mechanism which ultimately limited narrowing.

I reasoned that the capacity for unlimited airway narrowing is most likely a function of smaller airways. I investigated indices of small airway function and found they

predicted the ultimate response much earlier in challenge than FEV1, suggesting a possible practical test of the capacity for unlimited narrowing.

I postulate that the clearly established but limited relationship between the responses to DI and bronchial challenge may reflect the dependence of the response to DI on the degree of inflammation within the airway wall whereas the response to challenge may be determined by its overall thickness.

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Chapter 1

Background

1.1 The response to Deep Inspiration

In 1961 Nadel and Tierney (1) observed, in the context of pre-induced bronchoconstriction in healthy subjects, a bronchodilator response to deep inspiration (DI). In 1968 Froeb and Mead (2) studying healthy subjects, found an increase in anatomical dead space, suggesting dilatation of the airways, following DI. Subsequent studies have used a number of methods to assess the response to DI. The most commonly used method is to compare expiratory flow during a forced expiration following a partial (P) inspiration with the expiratory flow at the same lung volume during a forced expiration following maximum (M) inspiration, the M/P ratio (3-11). Using this method, in 1978, Fish et al (12) reported the response to DI in asthmatic and control subjects. They demonstrated a reduction in forced expiratory flow post DI with no significant effect being observed in the control group. This finding of a difference between asthmatic and healthy subjects suggested that the phenomenon might have an important clinical relevance. In 1981 Fish et al (13) again compared the response to DI (in the context of induced bronchoconstriction) between asthmatics and healthy controls, this time by comparing changes in airway resistance. They showed that the reduction in airway resistance after DI seen in healthy subjects was diminished or absent in asthmatics. These differences fuelled interest. Numerous studies have since reported the response to DI in healthy subjects (2, 3, 14), asthmatics (4-6, 11, 15, 16) and both asthmatics and healthy controls (7-10, 17-21)

which have demonstrated that the functional response to deep inspiration is different between the two groups.

It is the difference in the asthmatic and healthy response to DI that has generated much of the interest in this phenomenon. It suggests that an understanding of the mechanism underlying the response to DI might reveal something fundamental about the pathophysiology of asthma. Studies in healthy subjects have generally shown a bronchodilating response to DI (7-10, 14, 18, 22). The bronchodilatation post DI in healthy subjects is largely attributable to a reduction in airway smooth muscle tone. This explanation is consistent with the enhanced bronchodilating effect of DI seen in the context of methacholine induced bronchoconstriction in both asthmatic (4-6, 21) and healthy subjects (1, 8, 21) and with diminution of the bronchodilating effect of DI following administration of ß2 sympathetic agonists (6, 16, 23).

In asthma the reported response has been variable (4-6, 11, 15, 16). While milder asthmatics simply display a more limited bronchodilator response, subjects with more severe asthma show bronchoconstriction post DI. Indeed some studies have identified an inverse relationship between the bronchodilating effect of DI and severity (5, 16, 17, 20). The mechanism underlying the abnormal asthmatic response is not fully established. A diminution of the bronchodilating effect of DI can be explained by a reduction in the degree of stretch imposed on smooth muscle by DI in asthmatic subjects because of the relative unlinking of the airway from the retractile force of the surrounding parenchyma due to the increased adventitial thickness of the airway wall (24). An additional mechanism however is required to explain the bronchoconstrictor response observed in some asthmatics.

The time course to restitution of baseline after the effects of DI may shed some light on the underlying mechanisms responsible for the different responses to DI observed.

Green and Mead (25) measuring forced expiratory flow at various time intervals after deep inspiration in healthy subjects found most of the bronchodilating effect of DI had worn off after only 5 seconds. Lim et al (5) looked at this issue in two groups: asthmatics (who demonstrated bronchoconstriction after DI) and a group in which equally severe obstruction was induced by a bronchoconstrictor (who demonstrated bronchodilatation after DI). The time course for restitution of baseline airway calibre differed between the two groups. The group that demonstrated a bronchoconstrictive response to DI returned to baseline with a time constant of 30 seconds, in the bronchodilator group the time constant was 10 seconds. This difference has been reproduced in subsequent studies (26, 27). The difference seems to suggest a fundamentally different mechanism may be responsible for the changes post DI in the two groups. The time course to restitution of baseline has implications not only in studies of the effects of a deep inspiration but also in the practical application of lung function testing clinically. In a study by Malmberg et al (28) healthy subjects performed two stepwise methacholine tests, with either 6 or 3 min between dose steps. The percentage decrease in FEV1 per mg of inhaled methacholine decreased from 2.6 (1.9-5.2) to 1.7 (0.8-2.3) (median, interquartile-range) when the time interval was shortened. The results suggest that the deep inhalation associated with the FEV1 manoeuvre decreases the bronchial tone in airways constricted by methacholine for more than 3 minutes. In the same paper (28) a separate study was reported in which the response to methacholine was measured in healthy subjects who performed an FEV1 manoeuvre (and the preceding deep inhalation) **before** inhalation of methacholine. When an FEV1 manoeuvre was performed immediately before methacholine, the first FEV1 measured 3 min after provocation was higher (77% of basal FEV1) than if a pre-methacholine FEV1 manoeuvre was not performed (64% of

basal FEV1). This effect of a pre-methacholine FEV1 manoeuvre was also demonstrated at 2, 4 and 6, but not at 10 min after the start of methacholine inhalation. If an FEV1 manoeuvre was not performed before methacholine, the second and subsequent FEV1 measured in constricted airways was higher than the first, and of similar magnitude to the first FEV1 in tests where a pre-challenge FEV1 manoeuvre was performed. Implying the 'protective' effect of DI occurred whether it was performed either before or after administration of methacholine.

Thus far the discussion has focussed on a putative mechanism within the airways causing an alteration in their function after DI, (after the pre DI lung volume has been restored) – 'hysteresis of the airway'. One popular hypothesis considers the possibility of the same or a similar mechanism having a direct effect on the lung parenchyma – 'parenchymal hysteresis'. Given the close interdependent relationship between the airways and parenchyma the two mechanisms could not function entirely independently. Hysteresis within the parenchyma could result in lower lung recoil pressure post DI and a diminished retractile force on the airway wall, leading to a narrowing of the airways post DI. The net effect on the airways would therefore be a balance between airway and parenchymal hysteresis- the 'relative hysteresis hypothesis' (4, 7, 14, 23, 29). The origin of this putative 'parenchymal hysteresis' is unclear, it may result from the inherent hysteresis of surfactant, it may be due to a differences in the number of alveoli contributing to volume during inspiration and expiration, or it may be related to the behaviour of the respiratory bronchioles including the contractile Kapanci cells (4). In support of this hypothesis, appropriate changes in hysteresis of the quasi-static transpulmonary pressure-volume (Ptp-V) curve of the lungs (taken as an index of parenchymal hysteresis) have been demonstrated following induced bronchoconstriction (7) or bronchodilatation (23) but

the numbers studied were very small and the relevance to spontaneous asthma is unclear. There are also several theoretical reservations about the use of the area within a (Ptp-V) curve as a relevant index of parenchymal hysteresis:

1. The constraints of in vivo testing mean that the pressures are measured in quasi-static conditions, not actually static. I.e. flow exists. The force involved in overcoming the flow resistance will cause inspiratory pressures to be more negative and expiratory pressures to be more positive, thus causing an over-estimation of static pressure volume hysteresis. In those with more severe airflow obstruction (more severe asthmatics with lower M/P ratios) the over-estimation will be greater. Depending on flow rates, measurements on anaesthetised dogs (30) show this over-estimation to be up to 5x that seen in true static Ptp-V curves. In other words changes in the airway alone could account for some of the apparent increases in 'parenchymal hysteresis' seen accompanying low M/P ratios.

2. The hysteresis demonstrated in Ptp-V curves relates to the difference in recoil pressure at isovolumic points on the inspiratory and expiratory phases between TLC and RV. In the assessment of an M/P ratio the relevant difference in recoil pressure is that between isovolumic points on the expiratory limb of a full curve (i.e. when expiration has been preceded by a full inspiration) and the recoil pressure during an expiration begun at around end tidal inspiration. The expiratory limb of the tidal curve probably most closely represents such a pressure. Such differences are very small in comparison to the inspiratory and expiratory phases of the full cycle. The full curve hysteresis has been used as a surrogate for the hysteresis we are interested in. There is no evidence that it is a good surrogate.

3. Even if we accept the principle of 'parenchymal hysteresis' and its effect on the airway, there is no reason why this should be relatively greater in asthmatic compared

with normal subjects. Evidence that it actually is greater is sparse. This evidence relates to changes in the Ptp-V curve in response to bronchoconstrictors and bronchodilators, as outlined above (4, 7, 8, 23). Explanations employed in these papers suggest a more peripheral distribution of receptors in asthmatic subjects. This may or may not be the case but there is no evidence to back it. Even if true, it does not explain why M/P ratios are less than 1 in many asthmatic subjects. It also does not explain why the M/P ratio rises as asthma improves. This phenomenon is observed both longitudinally (16) and in cross section (5).

Although the relative hysteresis hypothesis only relies on an abnormal balance between airway and parenchymal hysteresis the assumption of an increased parenchymal hysteresis is often implicit in a number of the papers that invoke it. The marked hyperinflation seen in asthma is in part due to the loss of lung recoil pressure, 'reduced tone' (31, 32). Where there is reduced tone in a system there is often reduced hysteresis.

Furthermore, the relative hysteresis hypothesis does not explain all empirical findings, for example, the finding in spontaneous asthma of an increase in M/P ratio following a bronchodilator (16). Also, after methacholine challenge, M/P can increase markedly, suggesting dominant airway hysteresis. This would imply wider airways during deflation but no corresponding DI-induced change in anatomical dead space was seen (4). This finding was attributed to a possible difference in the generations of airways contributing to M/P ratio and those contributing to the measurement of anatomical dead space. This may or may not be the case but the finding in the same study, in asthmatic subjects, of an increase in isovolumic forced expiratory flow post DI (M/P > 1) in conjunction with a reduction in SGaw is more difficult to explain and certainly cannot be accounted for by the relative hysteresis hypothesis.

If this finding were to be reproduced any new alternative hypothesis would clearly have to account for it. Before the development of any novel hypothesis to explain the bronchoconstricting response to DI in asthmatic subjects can be considered, we first need to take into account the variability in reported responses both in asthma and healthy subjects.

The majority of studies in healthy subjects report a bronchodilating response to DI (7-10, 14, 18), indeed it is this 'typical' response that various hypotheses have attempted to explain. However, a number of studies have reported either no response or even bronchoconstriction in response to DI (1-3, 17, 19, 20). In asthmatic subjects the reported response to DI has been even more variable, with results varying from bronchoconstriction (15, 18, 19) to bronchodilatation (7-10) with most studies reporting a variable response within this subject group (4-6, 11, 16, 17, 20). Clearly a large part of the observed variability in asthmatic subjects can be explained by the variability in severity of asthma, with some studies identifying an inverse relationship between the bronchodilating effect of DI and severity (5, 16, 17, 20). One further source of apparent variability in response, which has not been formally explored, is variability in the method used to assess that response. The most commonly used method uses forced expiratory flow in the M/P ratio (3-11). Even within this method there remains considerable room for variability. The lung volume, as a percentage of vital capacity (% VC), at which the flow rates are compared has not been standardised, volumes used have ranged from 25% to 50%VC. Other methods to assess the response to DI include a comparison of airway resistance (Raw) or specific airway conductance (SGaw) before and after a deep breath. (1, 4, 5, 14-16, 18-21), and the volume of the dead space before and after DI (2). Some authors have reported the response to DI as assessed by two different indices in the same group of subjects.

Lim et al (5) found that the changes in SGaw were consistent with the changes in max flow (M/P ratio) in two groups of asthmatics, one demonstrating bronchoconstriction, the other bronchodilatation post DI. Pellegrino et al (21) found, in the context of induced bronchoconstriction, that in asthmatic individuals, DI had a significantly smaller effect on flow but not on Raw compared with normal individuals. And Burns et al (4) found disagreement between these two indices in a group of asthmatics, with M/P > 1 but a reduced SGaw post DI. This disagreement may simply be an anomaly but we note that many studies in asthmatics using M/P report an apparent bronchodilator response to DI (M/P >1)(6-11) whilst those studies using SGaw in asthmatics suggest DI has a bronchoconstricting effect. (5, 15, 16, 18, 19).

In chapter 4 we assess the dependence of the apparent response to DI on the method used to assess it. The findings appear to shed some light on the underlying mechanisms and an alternative hypothesis is suggested. This hypothesis is then tested in the study reported in chapter 8.

1.2 The Ultimate Response to Methacholine Challenge – a maximal (plateau) response or unlimited narrowing.

The most commonly used index of bronchial responsiveness is PD_{20}(FEV1). A single index however, cannot reflect all facets of the response to incremental bronchial challenge. The presence of a maximal (plateau) response or the converse, the capacity for unlimited airway narrowing shown by many asthmatics is a less familiar facet of the response to bronchial challenge than PD_{20}(FEV1) but potentially far more important. The obvious clinical correlate would appear to be the susceptibility to severe, potentially fatal, exacerbations of asthma.

In 1984 Woolcock et al (33) described the shape of the dose response curve to bronchial challenge in asthmatic and healthy subjects. Bronchial challenge (with histamine) was continued until either a 60% fall in FEV1 or a maximal 'plateau' response occurred. The dose response curves demonstrated a close fit to a sigmoidal equation and values for alpha (the position constant) and beta (the slope constant) could be determined. PD_{20}(FEV1). The most commonly used index of bronchial responsiveness, is a hybrid index in that it is determined by both the position and the slope of the curve. Its usefulness lies in its robustness and repeatability. However a single index cannot reflect all facets of response. Perhaps the most important finding in the Woolcock study was that the normal and 2 mildly asthmatic subjects demonstrated a plateau response. I.e. in these subjects a point was reached beyond which no further airway narrowing occurred despite increased doses of histamine. The other subjects demonstrated at least a 60% fall in FEV1 in response to challenge (at which point the challenge was terminated) apparently demonstrating the capacity for unlimited narrowing. It could be argued that the presence or absence of this capacity for apparent unlimited narrowing is a more important facet of response than PD_{20}(FEV1). It would appear to have significant implications in acute severe asthma and the potential for fatality. The phenomenon described by Woolcock (33) is now well established with most healthy subjects and some mild asthmatics demonstrating a plateau response (34). The proportion of subjects demonstrating the phenomenon has varied between studies. Much of this variability will be due to subject selection but of course the value to which FEV1 is allowed to fall before the presence or absence of a plateau response is determined will have a significant bearing on it. Intuitively it seems likely that the maximal fall in FEV1 at plateau will vary, with some being below the 40% or even 60% threshold. Those who do not plateau prior to the given

'safety' threshold in a particular study probably fall into two categories: Those in whom a plateau would be established at a lower value of FEV1 and those who have the capacity for true unlimited narrowing. In an absolute sense therefore it could be argued that any study may be mis-positioning the dividing line in this dichotomy, however in a practical sense those who do plateau below such threshold (particularly 60%) are probably more appropriately categorised as being susceptible to fatal exacerbations of asthma.

Some work has been done to try and understand the 'protective mechanism' that healthy and some asthmatic subjects seem to possess which limits airway narrowing. Sterk et al (35) assessed the effects of propranolol hexamethonium and indomethacin on the maximal response plateau. No difference from placebo was noted. They concluded that limitation to airway narrowing to methacholine in non-asthmatics is not due to a change in adrenergic, cholinergic, or ganglion-transmitted-nonadrenergic inhibitory activity nor to the release of prostaglandins. De Jongste et al (36) compared maximal bronchoconstriction in vivo and airway smooth muscle responses in vitro found no significant correlation. Suggesting that maximal bronchoconstriction in vivo is not limited by the maximal contractility of airway smooth muscle. This was consistent with the findings of Sterk et al (37) who found the response to combined histamine and methacholine was not significantly larger than the maximal response to histamine alone. They argued that this suggests that the plateau is due to factors other than limited smooth muscle activation.

Ding et al (38) used inspiratory pulmonary resistance (RL) during tidal breathing as the index of airway narrowing during challenge. In subjects who demonstrated a plateau response there was no correlation between the concentration of methacholine required to double RL and the maximum value of RL.

Boulet et al (39) reported in a group of non-asthmatics with seasonal allergic rhinitis (who demonstrated an increase in airway responsiveness in the pollen season) no correlation between the change in PC_{20}(FEV1) and the change in maximal response to challenge (Δ FEV1, max) in the pollen season.

Clearly therefore, the factors that determine the response to lower and higher dose MCh may be different. Aerts et al (40) used complex mathematical modelling (Cumulative Gaussian Distribution function and the Hofstee equation) to extrapolate the 'early' part of FEV1 dose response curve to estimate the plateau value. Although the data entered into the models was from the entire curve except the final 4 data points (which define the maximal response), these complex models were still rather inaccurate in their estimation of the plateau actually achieved. This would seem to suggest that the early changes in FEV1 did not actually contain the information required to estimate ultimate outcome i.e. the factors that determine the early changes in FEV1 may be different to those which determine plateau.

Ding et al (38) also investigated the effect of lung volume on maximal airway narrowing in healthy subjects. Inspiratory pulmonary resistance (RL) was measured at functional residual capacity (FRC) and FRC -0.5 liter or FRC +0.5 liter. The maximum response to methacholine was markedly altered by changing lung volume. The maximum fall of RL was greater at FRC - 0.5 liter; and less at FRC + 0.5 liter. The authors conclude that changes in lung volume act to alter the forces of interdependence between airways and parenchyma that oppose airway smooth muscle contraction. One might argue further that it seems likely that the site at which this airway-parenchymal interdependence is most important in terms of maintaining airway opening is where the airways are otherwise not supported by cartilage. By the

same argument it could be inferred that it is here, in the small airways, where the maximum response to challenge may be determined.

Brusasco et al (7) examined the relationship between quasi-static pulmonary hysteresis and maximal airway narrowing in healthy and asthmatic subjects. The quasi-static transpulmonary pressure-volume (Ptp-V) area was similar in the two groups at baseline. At MCh end point it was increased significantly in the group demonstrating an apparent capacity for unlimited narrowing yet remained unchanged in the group demonstrating a plateau in response. The hysteresis observed in the separation of the inspiratory and expiratory limbs of the Ptp-V curve is largely due to the closure of small airways towards the end of expiration which then, due to surface forces, require a greater force to re-open. I would argue therefore that the increased Ptp-V area seen in the first group could be accounted for entirely by an increase in the propensity for small airways to close completely as the lung empties. It seems likely therefore that subjects who display this increased propensity for complete (small airway) closure are more likely to display a capacity for unlimited airway narrowing i.e. will not display a plateau response to MCh.

Moore et al (41) described the shape and position of the complete dose-response curve for inhaled methacholine in 73 normal subjects. Amongst a number of findings it was noted that in those who achieved a plateau in terms of the maximal flow (Vm) for a given lung volume (% baseline VC), the maximal fall was greater at the lower lung volume (30% versus 50% of VC), suggesting the greater proportionate change had occurred in airways of smaller caliber. It would seem therefore that the presence or absence of the capacity for 'unlimited' narrowing is more likely to be determined by these airways. An in vitro study by Mitchell et al (42) reported changes in response to acetylcholine (ACh) with concurrent measurement of smooth muscle shortening,

lumenal narrowing and flow in large and small porcine bronchi. Maximum muscle shortening and lumen narrowing was greater in small than large bronchi. Small airways were 250-times more sensitive to ACh than large airways, for all measurements. But perhaps most interestingly high doses of ACh stopped flow in small bronchi, but produced a plateau in large bronchi. The cartilaginous support of the large airways may have protected them from unlimited narrowing, even in the context of maximal smooth muscle stimulation.

The protective effect of cartilage preventing complete airway closure was more formally explored in an animal study (43) in which pulmonary resistance was significantly increased by intravenous treatment with papain in a concentration that produced generalized cartilage softening. Papain pretreatment also resulted in a substantial alteration in the pulmonary resistance-dose relationship to intravenously administered acetylcholine.

The conclusions from these studies would seem to be that: (1) The ultimate outcome to challenge: a plateau in response or apparent unlimited narrowing is not evident from the responses in the early stages of challenge – at least using the standard indices such as FEV1. (2) There is some evidence to suggest that the capacity or otherwise for unlimited narrowing is determined by the small airways.

1.3 The Relationship Between the Response to Deep Inspiration and the Response to Bronchial Challenge

The relationship of the response to DI and the response to challenge can be considered in either direction. It is well established that the bronchodilating effects of a DI are augmented in the context of induced bronchoconstriction (1, 4-6, 8) when the airway

narrowing is induced by smooth muscle constriction. Interestingly in the context of the late response to an allergen challenge, when the principal mechanism underlying airway narrowing is thought to be airway inflammation, the response to DI becomes more bronchoconstricting compared with pre challenge responses (44). Thus the response to challenge does influence the response to DI. The reverse relationship has also been investigated.

Fish et al in 1978 (12) reported the difference in response to DI between asthmatic and control subjects. Asthmatic subjects demonstrated a reduction in forced expiratory flow post DI with no significant effect being observed in the control group. In 1981 Fish et al (13) again compared the response to DI between asthmatics and healthy controls this time in the context of induced bronchoconstriction, on this occasion by comparing changes in airway resistance. The reduction of airway resistance following DI in controls was less or absent in asthmatic subjects. The results were attributed to a failure, in asthmatic subjects, of DI to stretch (and relax) airway smooth muscle. This led the authors to hypothesise that hyperresponsiveness in asthma may be caused by impaired ability of inspiration to stretch airway smooth muscle. Skloot et al (9) reasoned that if this hypothesis were true, then the sensitivity to inhaled methacholine of normal and asthmatic subjects should be the same if the challenge was carried out under conditions where deep inspiration was prohibited. It is noteworthy that studies (45) (46) (47) using specific airway conductance (SGaw) as the index of airway function have consistently shown hyperresponsiveness in asthmatic subjects. In principle such measurements are independent of DI but it is not clear from these papers to what extent, if any, DI was prohibited prior to the measurement of SGaw. The influence of any preceding DI therefore could not be excluded.

Skloot (9) performed methacholine challenge in asthmatic and control subjects under conditions where the prohibition of deep inspiration was formally documented. They found no apparent difference in the responses of the two groups. The concluded that: 'hyperresponsiveness in asthma is caused by an impairment in the ability of inspiration to stretch airway smooth muscle'. This was reported widely in the medical literature at the time and was even noted by the lay media.

However I have a number of reservations about this study and its conclusions:

(1) As discussed above, a diminution of the bronchodilating effect of DI can be explained by a reduction in the degree of stretch imposed on smooth muscle by DI in asthmatic subjects (perhaps due to an unlinking of airway and parenchyma (24)). This could not however account for the bronchoconstrictor response seen in some asthmatics, for which an additional mechanism is required.

(2) Whatever the mechanism, the diminution (or reversal) of the bronchodilating effect of DI on induced bronchoconstriction in asthmatics implies that if DI is prohibited the difference in responsiveness between asthmatic and normal subjects is inevitably reduced. It is therefore important to determine whether, in the absence of DI, there is complete loss of asthmatic hyperresponsiveness or only the relative diminution that would have been predicted by studies published prior to 1995 (and indeed since (48)). Only complete loss would support the revised (and more limited) hypothesis that: hyperresponsiveness in asthma can be accounted for entirely by the altered response to DI.

(3) Further, there are serious theoretical reservations that the index of bronchoconstriction ('τ') used in the Skloot study might obscure differences between the responses of the two groups. The 'τ' index is derived from 'partial' forced expiratory manoeuvres. Since the end inspiratory lung volume (EILV) at which such

expiratory manoeuvres is initiated varies, τ, an index in the time domain was used. τ equals the forced expiratory time between 25% and 75% of the partial expiration divided by the natural log of 3. Skloot et al showed that the response to methacholine as measured by τ was similar in asthmatic and control subjects and concluded that asthmatic hyperresponsiveness was attributable to a lack of smooth muscle relaxation with deep inspiration. The volume independence of the index relies on the assumption that the volume-time relation during forced expiration approximates to a monoexponential function. Under this assumption Skloot et al argue that the τ index is 'equal to the reciprocal of the mean slope of the flow volume curve between 25% and 75% of the forced expiration'. In fact, although not stated by Skloot, the assumption implies that the descending limb of the flow volume curve is rectilinear and that τ is equal to the reciprocal of the gradient of the entire slope of the flow volume curve. Whilst this is a reasonable assumption in healthy young subjects, with airway narrowing the flow volume curve is characteristically concave and therefore the volume time curve is not mono-exponential. The more severe the airway narrowing, the further the deviation from this assumed curve. This implies that τ is not independent of volume, rather it will decrease if tidal breathing occurs over a higher volume range. For a given degree of bronchoconstriction therefore, an increase in EILV (or RV) will result in a lower value of τ suggesting less bronchoconstriction. The rise in EILV as bronchoconstriction progresses therefore would tend to mask the change in τ. If the rise in EILV was greater in asthmatic subjects than in healthy subjects, the masking effect would be greater in that group. The net effect would therefore be to underestimate the difference in responsiveness between the two groups.

The discussions in the papers by Fish et al (13) and Skloot et al (9) focus on asthmatic bronchial hyperresponsiveness as a generic entity. As we discussed above no single index of response is likely to be able to reflect all aspects of response. The relationship of the response to deep inspiration and the perhaps more important facet of responsiveness, the ultimate outcome to challenge (maximal, plateau response or unlimited narrowing) seems to have received less attention in the literature. In non-asthmatic subjects, particularly in the context of induced bronchoconstriction, deep inspiration induces airway dilation. The degree of 'bronchoprotection' afforded by this mechanism and whether, it alone could account for the limitation to airway narrowing seen in some subjects has been addressed by some authors.

Sterk et al (49) measured the responses to challenge in non-asthmatic subjects using 'partial' flow (Vp) - from a forced expiration not preceded by a deep inspiration, as well as the 'maximum' flow (Vm) – from a forced expiration preceded by a deep inspiration, derived at 40% of control vital capacity. A maximal response plateau was demonstrated in all subjects based on Vp. The plateau for Vp occurred after a greater percentage fall than in the case of Vm. Similar findings were reported by Moore et al (41). Both indices Vp and Vm were determined at 30% and 50% of VC. At each lung volume in those who achieved a plateau the mean maximal decrease was greater in Vp than Vm. Maximal fall at the plateau was greater at lower lung volumes (30% versus 50% of VC). The difference in the level of the plateau for Vp and Vm merely reflects previously reported data on the increased bronchodilating effect of deep inspiration in the context of induced bronchoconstriction.

Pellegrino (10) reported a novel and rather complex approach to the same question. The effect of DI was quantified as the linear regression coefficient of the percent decrements of maximal (Vm) versus partial (Vp) forced expiratory flow at 50% of

FVC over the initial steps of challenge (= 'MP slope'). Airway sensitivity was inferred from the MCh provoking dose (PD) causing Vm50 or Vp50 ('maximal' and 'partial' flows at 50% VC) to decrease by 40% or FEV1 by 15% (PD_{15} FEV1). The absence of a limit to bronchoconstriction was predicted by either MP slope or any PD with accuracies between 71 and 81%, but with an accuracy of 87% by a combined function including MP slope and PD_{40}Vp50. The complexity of the model, I believe, probably hides some simple and previously established relationships. Although not absolute, the correlation between PD_{20}(FEV1) and the presence or absence of a plateau is reported. The prediction of the ultimate outcome of challenge given information about a response beyond the initial stages of challenge is perhaps no surprise, although PD_{15}(FEV1) will of course occur earlier in challenge than PD_{20}(FEV1) the principle I believe is the same. Also, although the MP slope appears to be telling us about the response to DI the index does of course contain information about the response of the small airways over the initial steps of challenge. It may be this component of the index that contains the predictive power. Nevertheless, It is clear that DI does offer some degree of bronchoprotection in the context of induced bronchoconstriction. Indeed it my well be that a large bronchodilating response to DI could cause a plateau to occur above rather than below whatever safety threshold is set (say 40% or 60% decline in FEV1) thus re-classifying the subject as one with limited as opposed to apparent unlimited narrowing. The response to DI is therefore clearly an important factor at least in terms of limiting the maximal plateau response. However the existence of a plateau in a partial flow response to challenge implies that some 'mechanism' limiting airway narrowing in response to challenge would appear to be operating independently of the effects of deep inspiration.

1.4 Other Factors Which May Influence the Response to Bronchial Challenge – Abnormal Smooth Muscle Function and The Thickness of the Airway Wall

1.4.1 Abnormal Smooth Muscle Function

The functional hallmark of asthma is the variability of its airway obstruction. By definition the severity of the airway obstruction will vary over short periods of time either spontaneously or in response to stimuli. Such stimuli may be either bronchodilating e.g. β_2 agonists or bronchoconstricting e.g. methacholine. The magnitude of response in terms of the bronchodilatation is often used as a diagnostic tool clinically. The magnitude of response, measured as the dose of methacholine required to achieve a 20% fall from a baseline in the value of FEV1, is essential to the definition of bronchial hyperresponsiveness. It is nevertheless the swiftness of the response that also characterizes asthma clinically.

It would seem almost intuitively obvious that in comparison to non asthmatic subjects a swift and exaggerated bronchodilating response to a drug known to relax bronchial smooth muscle and a similarly swift and exaggerated bronchoconstricting response to a substance known to constrict bronchial smooth muscle would seem to imply that the abnormality in asthma lies in the behavior of the smooth muscle. It is perhaps surprising therefore that the correlation between in vivo responsiveness and in vitro reactivity of smooth muscle whenever examined has been found to be very weak. De Jongste et al (36) compared maximal bronchoconstriction in vivo and airway smooth muscle responses in vitro and found no significant correlation. Vincenc et al (50) compared in vivo responses to histamine in 14 patients prior to thoracotomy with in vitro responses to histamine of both parenchymal and bronchial tissue. Although a

wide range of responsiveness occurred in vivo, as measured by the histamine inhalation test, the variation in the in vitro dose-response curves was negligible. There was no correlation between the dose of histamine that resulted in a 20% reduction in forced expiratory volume in one second and the concentration of histamine producing 50% of the maximal response in vitro. The authors concluded that the findings raised the possibility that airway hyperresponsiveness may not result from an intrinsic abnormality of airway smooth muscle. The following year Armour et al (51) measured the airway responsiveness of a group of 25 patients scheduled for lung resection. 10 of 25 patients had a greater than or equal to 20% fall in FEV1 in response to inhaled methacholine (responders), with PD_{20} FEV1 values ranging from 0.6 to 7.3 μmol. The sensitivity to carbachol and histamine of the bronchial smooth muscle resected from these patients was similar in tissue from responders and non-responders. There was no correlation between in vivo responsiveness to methacholine and in vitro sensitivity to carbachol or histamine. The volume of smooth muscle in some of these airway preparations was quantitated. There was a significant correlation between the maximum tension change in response to histamine and the volume of smooth muscle in each airway. There was no similar correlation for carbachol. Again the authors concluded that in vivo responsiveness could not be explained in terms of smooth muscle sensitivity. Cerrina et al (52) measured human bronchial muscle responses to histamine and isoproteronol in vitro, similar conclusions were reached. Nagai (53) reported results from 41 patients who had been enrolled in a larger (mortality) study who died, came to autopsy, and provided adequate tissue to quantitate lesions. All subjects had moderate to severe chronic airflow obstruction and a broad range of responses to 250 micrograms isoproterenol inhalation. Thus although not exclusively asthmatic some subjects had clear positive responses to the inhaled

drug. The relationships between pulmonary lesions and both bronchodilator response and variability of FEV1 were investigated. Airway responsiveness to isoproterenol in vivo was positively correlated with bronchial eosinophilia, bronchial inflammation, and bronchiolar fibrosis ('re-modeling'). However airway smooth muscle was not related to airway responsiveness or variability.

Despite these negative findings latterly there has been some renewed interest in smooth muscle function in relation to asthmatic hyperresponsiveness (27). This seems to have been born out of the noted abnormal response to deep inspiration. Much of this work has focused on the importance for normal functioning of periodic stretch and relaxation of airway smooth muscle inherent in the normal breathing cycle (54). It has been argued that the reduced mechanical load on airway smooth muscle in asthma, due to the unlinking of airway and parenchyma (55, 56) may itself have a secondary effect on smooth muscle function (57). Given the known effects of stretch on reducing smooth muscle tone (28, 58), and the cyclical stretch of smooth muscle inherent in tidal breathing, it seems plausible to argue that if the unlinking of the airway and parenchyma (due to adventitial thickening in asthma) were to lead to a reduced degree of stretch during every inspiration of a tidal breath then that may itself lead to an abnormal development of tone, with smooth muscle in a more 'latched' state i.e. a 'semi-permanent' state of contraction. The functional importance of this possible 'semi-permanent' state as opposed to the simple loss or diminution in asthma of the transient relaxation in tone following a deep inspiration discussed above is not clear. Whether real or not and whether functionally important or not, these argued changes in smooth muscle behavior are not primary but secondary to geometrical changes in the airway wall. Moore et al (59) found the prohibition of DI during methacholine challenge in healthy subjects led to a greater decline in FEV1 (measured

at the end point of challenge and itself necessitating a preceding DI). The authors argued that failure of periodic inflation may interfere with the bronchodilating effect of DI, and this may be fundamental to the difference in bronchodilatation caused by DI in asthmatics and normal subjects. The implication that the abnormality resides in the behavior of the smooth muscle however would appear to be founded on a significant number of assumptions.

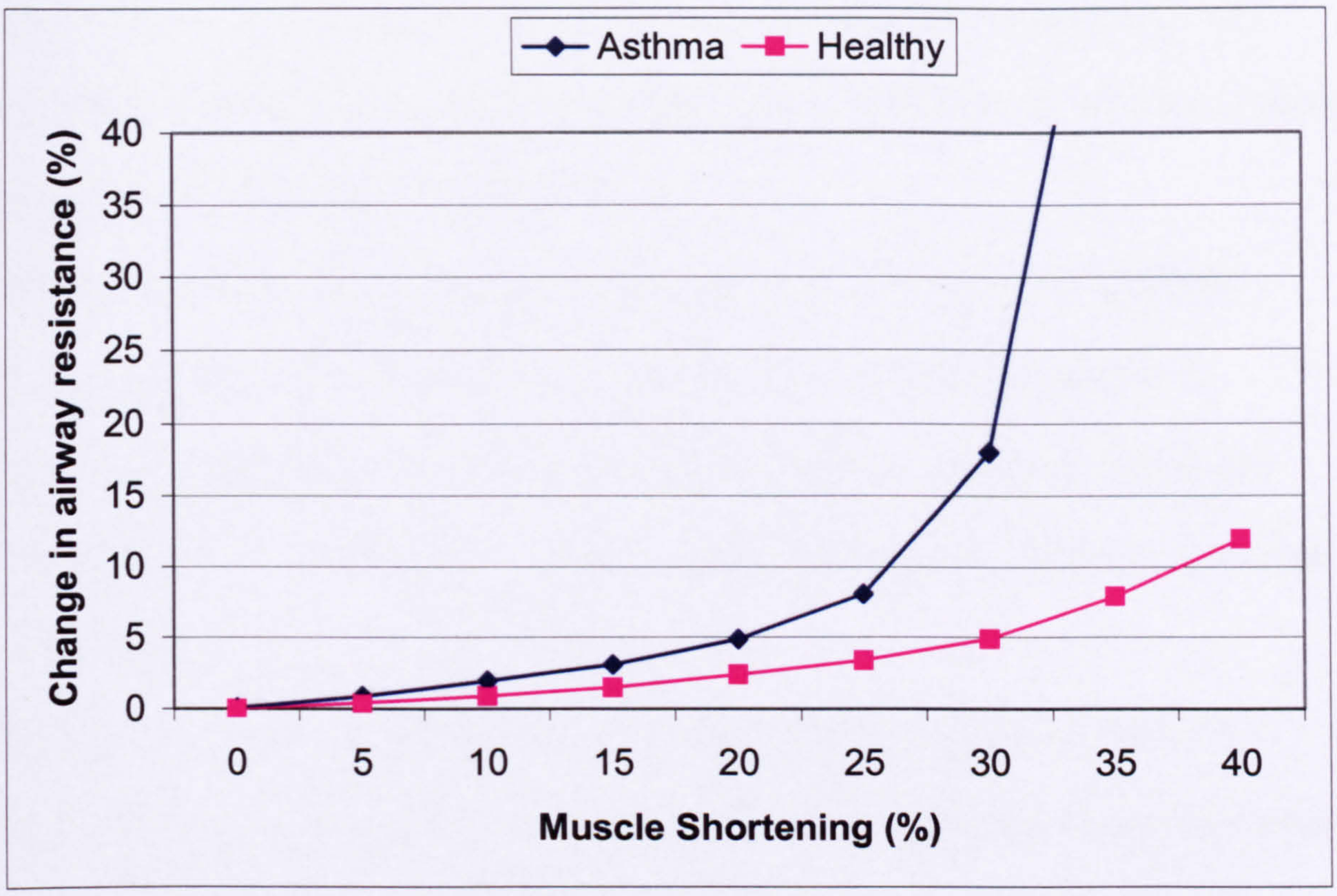
In a computational model (60) based on an earlier model by Wiggs et al. (61) the effects of the morphologically determined increased airway smooth muscle mass, adventitial mass, and submucosal mass observed in patients with asthma and chronic obstructive pulmonary disease (COPD) on the increase in airway resistance in response to a bronchoconstricting stimulus were investigated. Although increased adventitial thickness was found to increase constriction by reducing airway and parenchymal interdependence and increased submucosal thickness also led to greater lumenal occlusion for any degree of smooth muscle shortening, the authors concluded that the increased muscle mass was likely to be the most important abnormality responsible for the increased resistance observed in response to bronchoconstricting stimuli. Two assumptions however were implicit in this model: (i) The increased muscle thickness in asthma led to a proportionate increase in maximal muscle tension. Whilst an increase in muscle thickness would seem likely to be associated with some increase in maximal muscle tension, it is worth remembering that this re-modeling is 'pathological' and evidence that the increase in tension would be *proportionate* to the increase in thickness is lacking. (ii) That airway wall thickness remains constant during bronchoconstriction. In fact a later study by Mitchell et al (62) using isolated bronchi from pigs demonstrated that in fact it increases. This increase in wall

thickness would of course narrow the airway lumen – a significant contributory factor to increased resistance during bronchoconstriction not taken account of in the model. In summary therefore evidence that abnormal smooth muscle function is responsible for the abnormal behavior of asthmatic airways in general and hyperresponsiveness in particular is far from conclusive. As hinted at by some of the modeling above the effect of geometric changes such as the thickened airway wall, particularly in the context of induced bronchoconstriction needs further exploration.

1.4.2 Thickness of the airway wall

Let us consider another mathematical model. This model is considered for illustrative purposes only.

Using a basic mathematical model published by Moreno et al (63) a later study (64) used morphometric data from postmortem specimens of lung obtained from both asthmatics and non-asthmatics. The internal and external perimeter of the airways and the submucosal and mucosal thicknesses were measured in small and large, asthmatic and non-asthmatic airways. The increased wall area in asthma was found to be due to increased areas of epithelium, muscle, and submucosa. These data were fed into the Moreno model, which calculated the change in airway resistance in a typical asthmatic and healthy airway for a given degree of smooth muscle shortening. The results are represented schematically here.



From this model the modest differences in airway wall thickness had little effect on airways resistance at base line. However very large increases of airways resistance are observed in the asthmatic airway at degrees of smooth muscle shortening that would produce only small increases in resistance in the normal airway. Let us now consider the converse, bronchodilatation in response to $ß_2$ agonists in asthma. From the graph we see that if such treatment relaxed smooth muscle to reduce the degree of contraction from say 30% to 15% shortening for example, then the reduction in resistance in the asthmatic airway is large and probably symptomatically detectable, whereas the corresponding change in resistance in the normal airway is barely perceptible. I reproduce these data and this model here not as proof of the overriding importance of airway wall thickness in determining airway resistance but merely to illustrate that variations in wall thickness could theoretically account for many of the observed features in asthma.

We need to review the empirical evidence for a relationship between airway wall thickness and changes in resistance in order to assess its relative contribution. Several papers have reported on this (24, 65-70).

Tiddens et al (68) studied lung tissue specimens from 72 patients with different degrees of COPD who were operated on for a solitary peripheral lung lesion. Maximum expiratory flow and the reversibility of airflow obstruction determined preoperatively were significantly related to the airway wall area but not to the smooth muscle area.

Okazawa et al (67) studied freshly excised dog lung lobes and concluded that the magnitude and variability of airway smooth muscle shortening and airway narrowing in response to maximal constricting stimuli are influenced by mechanical factors related to airway wall geometry.

A post mortem study (66) found the adventitial, submucosal, and muscle area of the asthmatic airways were greater than those of COPD and control subjects. These parameters were also greater in the 8 patients with fatal asthma compared with the 7 patients with non-fatal asthma.

In another mathematical model using post mortem morphometric data Wiggs et al (71) concluded that airway wall thickening and a loss of lung recoil can partially explain the airway hyperresponsiveness observed in patients with chronic obstructive lung disease and asthma.

As discussed earlier Macklem (24) described how increased wall thickness, this time with the focus on the adventitial layer can affect airway constriction in response to stimuli. This would lead to a decreased load on the smooth muscle due to unlinking of the interdependence between the airway and the surrounding parenchyma.

Uhlig et al (72) reported a study in which airway vascular engorgement was induced in piglets by placing a balloon catheter in the left atrium. The effect on baseline Raw was modest but engorgement lead to a significantly enhanced response to methacholine. After engorgement, methacholine lead to a 67.8% increase in Raw at a dose that produced only a 10.8% increase in Raw without engorgement. Perhaps most interestingly the change in airway wall thickness from this process of 'engorgement' appeared rather modest. In airways with a mean size >3mm, for example, the total cross-sectional area of the airway in animals with engorgement only was 3.2 mm^2 versus 2.8 mm^2. The proportionate changes in smaller airways were similar.

In a study on anaesthetized dogs Brown et al (69) investigated whether increasing airway wall thickness could potentiate the lumenal narrowing effect of histamine. High-resolution computed tomography was used to directly measure the changes in the caliber and wall thickness of conducting airways after histamine challenge, before and after airway wall thickness was increased with a normal saline volume load. Histamine alone reduced lumenal area to 71% of control value, while after volume loading, which was noted to increase wall area, histamine reduced lumenal area to 54% of control value. Quantitative modeling in this study indicated that the oedema in the airway wall was mostly outside the smooth muscle and that the smooth muscle shortening with histamine was similar before and after volume loading, indicating that the difference after volume loading was not due to enhanced smooth muscle constriction. The authors concluded that a moderate degree of acute airway wall thickening could potentiate the constrictor response to histamine.

In a second study by the same group (70) the effects of a similar volume load using either normal saline or homologous blood were compared. On this occasion no smooth muscle constriction was induced, in fact the measurements were made after

administration of atropine. The normal saline increased wall thickness 150% and reduced lumenal area by 68%. The comparative changes following volume loading with blood were 108% and 81%. This suggests that rather than vascular engorgement it is the presence of oedema fluid in or immediately surrounding the airway wall acts to decrease the airway lumen in this situation. The authors go on to argue that since the degree of airway narrowing was only moderate, it seems unlikely that airway wall thickening or oedema could be a primary cause of conducting airway obstruction in patients with asthma or impaired left ventricular function. However Brown's earlier study and the model by Moreno et al (63) above clearly demonstrate that the effect of an increased wall thickness is primarily to potentiate the effect of smooth muscle shortening rather than to have a major effect on lumenal area at 'baseline'.

1.5 The relationship of the concentration of exhaled nitric oxide (NO) to the mechanical function of small airways and the response to bronchial challenge

Nitric oxide (NO) plays an important role as an inflammatory mediator in the airways. Exhaled nitric oxide is known to be elevated in asthma (73-78) and its role as a marker of severity has been extensively investigated in a variety of clinical settings. The relationships reported to various clinico-pathological markers of asthma however have been somewhat variable. Many studies, in a variety of clinical contexts, have identified a correlation between exhaled NO and bronchial hyper-responsiveness, including: steroid naïve asthma (76, 78) and asthma associated with seasonal allergic rhinitis (74) as well as chronic stable asthma (79). In the context of steroid naïve asthma exhaled [NO] has also been shown to correlate with diurnal variability in peak expiratory flow (PEF) (76). Although in one study NO concentration was found to

correlate with FEV1(% predicted) in a large group across a broad spectrum of severity ranging from subjects attending the emergency department with acute severe asthma through chronic stable asthma to non-asthmatic controls(75), most studies show no significant correlation with FEV1(% predicted) (76-78, 80) other functional markers of severity such as symptom scores (76) or beta-agonist use (76). The reason for this disparity between the presence of a correlation between NO and bronchial responsiveness (PD_{20}(FEV1)) yet the absence of correlation between either of these indices and baseline FEV1 is not clear.

The possible importance of the function of the small airways in determining the 'ultimate' outcome of bronchial challenge (plateau or unlimited narrowing) is discussed above, as is the relatively weak relationship between this facet of response and baseline FEV1. Although the PD_{20}(FEV1) and the ultimate outcome of challenge are different facets of the response, the two are correlated. A relationship may therefore exist between the baseline function of the small airways and PD_{20}(FEV1). If so and if as reported exhaled NO is principally derived from the terminal airways (74, 81) then this may go some way towards explaining the said disparity. I.e. exhaled NO may be reflecting inflammation (and thus function) of the small airways only, this in turn would be expected to correlate with the response to bronchial challenge yet may be only loosely related to indices such as FEV1. FEV1, in the absence of significant airway obstruction, is probably determined principally by the function of the more central airways.

It has been suggested that another pertinent factor could be the bronchoprotective role of NO in the context of induced bronchoconstriction. Several studies have reported the effect of endogenous NO on airway hyperresponsiveness when induced by a variety of mediators in animal models: histamine (82), bradykinin, citric acid,

tachykinin NK_1 selective antagonist and protease activated receptor 2 (83-86). Other studies have reported that acute bronchoconstriction induced by allergen inhalations is potentiated by Nitric Oxide Synthase inhibitiors (87-89). It is interesting to note that these molecules are primarily inflammatory mediators whose effect on smooth muscle constriction may indirect. Based on these in vitro and animal studies clinical researchers have examined the role of NO in asthma. In a randomised placebo controlled study Ricciardolo et al (90) found potentiation of bradykinin and methacholine induced bronchoconstriction after pre treatment with the NOS inhibitor, suggesting a bronchoprotective role for endogenous NO in mild asthma. However they found the effect greater with bradykinin than methacholine induced bronchoconstriction, which also would seem to suggest that the protective role of NO is mediator specific and not a direct inhibition of smooth muscle constriction. Further, inhaled NO has also been shown to have a small bronchodilator effect in asthma (91) outwith the context of induced bronchoconstriction. It would seem difficult therefore to explain the correlation between airway responsiveness to methacholine and exhaled NO together with the absence of a strong correlation between exhaled NO and baseline FEV1 in terms its 'bronchoprotective' properties. Also, and perhaps most fundamentally, the direction of the correlation between exhaled NO with airway responsiveness would seem to run contrary to the argument that its protective role would explain that correlation (increased concentration of exhaled NO is associated with increased responsiveness).

Thus whether the exhaled NO is simply acting as a marker of airway inflammation or is produced as a protective response to the airway narrowing consequent upon that inflammation may not be relevant to the above hypothesis. Site of production rather than function of NO would seem to be the crucial characteristic.

The relationship between baseline function of the small airways, NO, the response to challenge and baseline FEV1 needs to be more formally explored.

1.6 Summary

The response to deep inspiration is an interesting physiological phenomenon. The difference in the response to DI between asthmatic and healthy subjects suggests that an understanding of its underlying mechanism may have some clinical importance. There is a general consensus that a bronchodilating response is probably mediated by stretch and relaxation of airway smooth muscle. The mechanism underlying the abnormal asthmatic response is controversial. Diminished stretch of the smooth muscle during DI could account for a diminished bronchodilator response however an additional and separate mechanism is required to explain the bronchoconstrictor response.

Responsiveness to bronchial challenge is normally measured by the index PD_{20}(FEV1), the dose of stimulant required to induce a 20% fall from baseline in FEV1. A potentially more important aspect of the response to bronchoconstricting stimulants however is the presence of a maximal (plateau) response or the potential for unlimited airway narrowing. Whilst there is some correlation between the two indices, the ultimate outcome of challenge cannot be predicted with certainty from the changes early in challenge of FEV1. This may reflect the different generations of airways involved in these two indices. A priori reasoning and some evidence suggest that the capacity for unlimited narrowing may be a function of the small airways. The relationship between the response to deep inspiration and the capacity for unlimited narrowing is interesting. The bronchodilating effect of DI in the context of

induced bronchoconstriction will clearly have a bronchoprotective effect. The maximum constriction in terms of indices of airway function not dependent on the effect of a deep breath (Vp, say) seems to be greater than the maximum effect as measured by indices dependent on the effects of DI (Vm, say). However the presence in some individuals of a maximal, plateau response in terms of indices such as Vp suggest that in these individuals at least whatever protective mechanism is limiting airway constriction, it is operating independently of the effects of DI.

Of the other factors that may influence the abnormal asthmatic response to bronchial challenge, perhaps the most intuitively appealing is an abnormality of smooth muscle function. The evidence for such however is scant. Although perhaps intuitively less obvious, simple mathematical modeling and some evidence suggests that airway wall thickness may have a greater influence than previously thought.

Aims

1. To determine to what degree the measured response to DI is dependent on the method used to assess it and to describe the relationship of that dependence.

In chapter 4 the response to DI is assessed in terms of its effect on SGaw and forced expiration, the latter is reported over a range of lung volumes.

2. To develop and test a hypothesis to explain the abnormal asthmatic response to deep inspiration.

The hypothesis is developed in chapter 4 and tested in chapter 8.

3. To re-test the hypothesis that asthmatic hyperresponsiveness is entirely due to impairment in the ability of inspiration to stretch airway smooth muscle.

In chapter 5 the hypothesis tested by Skloot is re-tested using an index of airway function independent of both the effect of DI and the changes in EILV that are shown to occur as challenge progresses.

4. To identify 'the mechanism' which imposes a limit on airway narrowing in healthy subjects and mild asthmatics in response to bronchial challenge.

Assuming that, in the absence of significant bronchoconstriction, FEV1 is determined principally by the larger airways we reasoned that early in challenge changes in FEV1 probably reflect changes in calibre of the larger airways. Given their cartilaginous support however, narrowing of the central airways will be limited and their behaviour will not determine the capacity for unlimited narrowing. Thus early changes in FEV1 may not predict ultimate outcome. This is more likely to be a function of the smaller

airways. In the study reported in chapter 6 this hypothesis is tested in relation to the ultimate outcome of challenge. The predictive power of the response of a number of indices of small airway function early in challenge is examined, including the index PD_{20}(Vm20), the dose causing a 20% fall in the maximal flow at 20% (remaining) VC. The response to challenge is measured in terms of DI dependent and independent indices.

5. To explore and characterize the relationship between the maximum response to methacholine challenge – a plateau or unlimited narrowing and:

a. The response to DI

b. The baseline function of the smaller airways

c. The concentration of exhaled nitric oxide

In chapter 7 the relationship between the response to challenge and airway function at baseline (pre methacholine) is examined. The response to DI, The maximum flow at 50% FVC (Vm50) and the concentration of exhaled NO pre challenge are all compared with the outcome of challenge.

The role in airway function, including the response to DI and bronchial challenge, of airway wall thickness and in particular the distinction between airway wall inflammation/oedema and the features of more chronic re-modelling are discussed in light of the findings in chapters 4 – 8.

Chapter 3

Subjects and Generic Methods

3.1 Subjects

In total 34 control subjects and 40 asthmatic subjects were involved in the different studies as detailed in tables 3.1 and 3.2. All asthmatic subjects had previously received a diagnosis of asthma from a physician. A careful history was taken by a second physician at recruitment ensuring a corroborating opinion. Withdrawal of inhaled steroids to establish 15% bronchodilator reversibility off treatment was not considered necessary to the studies and was not performed. The asthmatic subjects in the study in chapter 7 were all steroid naive. Difficulty in recruiting such a rare subset of asthmatic subjects precluded such for all studies. None of the asthmatic subjects were taking more than 400μg of inhaled steroid (beclomethasone) per day. Normal subjects were all hospital employees. They reported no symptoms of asthma, had never received a diagnosis of asthma from a physician and had normal responses to a standard methacholine challenge. All subjects were non-smokers and had had no recent upper respiratory tract infection at the time of study. Atopic status was not established. Approval was obtained from the local Ethics Committee and written informed consent was obtained.

Table 3.1 Control Subjects

subject	Age	sex	chapter 4	chapter 5	chapter 6	chapter 7	chapter 8
1c	33	m	●	●	●		
2c	31	f		●	●		
3c	38	f			●		
4c	19	f	●	●	●	●	
5c	22	m	●	●	●		●
6c	34	m	●	●	●		
7c	21	m	●	●	●		
8c	26	m	●	●	●		●
9c	43	m		●	●		
10c	35	f			●		
11c	31	m		●	x	●	●
12c	30	m	●	●			
13c	32	m	●				
14c	38	f	●			●	●
15c	24	m	●				
16c	33	m	●				
17c	28	f	●				
18c	30	f	●				
19c	26	m	●				
20c	25	m	●				
21c	24	m	●				
22c	34	m				●	
23c	31	m				●	●
24c	19	f				●	
25c	18	f				●	
26c	29	m				●	
27c	25	m				●	
28c	38	m				●	
29c	30	f					●
30c	28	f					●
31c	27	m					●
32c	35	m					●
33c	35	f					●
34c	25	f					●

In chapter 6 'x' denotes those not included in the final analysis

Table 3.2 Asthmatic Subjects

subject	Age	sex	chapter 4	Chapter 5	Chapter 6	Chapter 7	Chapter 8
1a	25	m	•	•	•		
2a	20	f	•	•	•		
3a	31	f			•		
4a	28	m	•	•	•		
5a	21	m	•	•	•		
6a	18	f	•	•	•		
7a	30	f	•	•	•	•	
8a	40	m		•	•		
9a	19	f			•		
10a	41	f		•	•		
11a	29	m			x		
12a	28	m			x		
13a	33	f		•	x		
14a	19	f	•	•			
15a	19	f	•				
16a	28	m	•			•	
17a	28	m	•			•	
18a	27	f	•				•
19a	30	f	•			•	
20a	28	m	•			•	
21a	30	m	•			•	
22a	41	f	•				
23a	34	m	•			•	
24a	28	m				•	
25a	19	m				•	
26a	29	m				•	
27a	27	m				•	
28a	20	f				•	
29a	20	f				•	
30a	32	m				•	
31a	29	f				•	
32a	28	m					•
33a	31	m					•
34a	26	f					•
35a	34	f					•
36a	36	m					•
37a	39	f					•
38a	35	m					•
39a	41	f					•
40a	31	m					•

In chapter 6 'x' denotes those not included in the final analysis

3.2 Spirometric Values

FEV1, FVC and all derivatives of the flow volume curve (92) were measured and derived using the same flow sensor and software used to monitor expiratory flows during methacholine challenge (Sensormedics, 'Vmax'). In each case the mean of three technically good and reproducible values was calculated

3.3 Maximal / Partial (M/P) Ratio

All respiratory function tests were performed with the subject seated and breathing via a mouthpiece attached to a flow sensor connected to specially designed software (Sensormedics, 'Vmax'). Volume expired was derived by integration of the flow signal.

The M/P manoeuvre began with tidal breathing for one minute. The volume time record was monitored on screen to ensure no deep breaths occurred. At the end of a normal tidal inspiration, (at end inspiratory lung volume, EILV) subjects performed the 'partial' (P) manoeuvre, by forced expiration to RV. This was followed by a maximal inspiration to TLC. Then, without pause, the 'maximal' (M) expiration, a forced expiration from TLC to RV, was performed. M and P flows at various isovolumic points were derived using software designed for that purpose (Sensormedics 'Vmax'). Three technically good manoeuvres were obtained in each subject. The mean M/P ratio at each volume was calculated.

3.4 Specific Airway Conductance (SGaw) and SGaw ratio (pre : post deep inspiration)

The measurements were performed with the subject seated and breathing via a mouthpiece attached to a flow sensor connected to specially designed software (Sensormedics, 'Vmax'). Volume expired was derived by integration of this signal. SGaw was measured during panting at functional residual capacity (FRC) by body plethysmography (93, 94). After panting (at a rate of between 60 and 180 breaths per minute), subjects performed a full inspiration followed by a full expiration for measurement of total lung capacity (TLC), residual volume (RV) and vital capacity (VC). Slope measurements were performed automatically by the 'Vmax' software using the central portion (± 0.5 litres per second) of the flow-pressure curve. Mean SGaw from three technically good manoeuvres were calculated.

The SGaw Ratio (pre : post DI)

During tidal breathing for one minute, the volume time trace was monitored to ensure that no deep breaths occurred. After this DI free period SGaw, TLC, FRC and RV were measured. To measure 'post DI' SGaw, subjects initially performed three to four tidal breaths in order to establish FRC. From FRC subjects then performed a maximal inspiration to TLC. Without pausing subjects returned to FRC as quickly as a non-forced expiration would permit (< 1 second) and SGaw was again measured. Mean SGaw from three technically good 'pre DI' and 'post DI' manoeuvres were calculated.

These two means were used to calculate the (post: pre) SGaw ratio.

Caveat. A very few volunteers were unable to perform the panting manoeuvre necessary for the measurement of SGaw. These volunteers were withdrawn immediately and took no part in any study that required the measurement of SGaw.

3.5 Calibration of the Vmax System

All calibration procedures were carried out daily before any measurements were made. Calibration protocols were those recommended by the manufacturer (Sensormedics) for the 'Vmax' system. On screen instructions for calibration were followed.

a. Flow –Volume Calibration

The calibration procedure consisted of two measurements combined in one continuous procedure:

1.Calibration sequence: a calibrated volume syringe (6 litres) was connected to the mass flow sensor and stroked 5 times to measure the volume inspired and expired by the syringe. The strokes were performed as smoothly and consistently as possible. The inspiratory and expiratory flow rates (displayed on screen) were between 3-6 l/s (0.5 – 1.0 seconds stroke duration). Correction factors were then calculated automatically by the software to fine-tune the volume measurement. This ensured the mass flow sensor measured within $\pm$ 3% of the known volume of the syringe

2.Verification Sequence: the syringe was stroked five more times and the inspired and expired volumes were measured using the newly calculated correction factors.

Differing flow rates for these strokes was permissible (between 20-720 l/s). To meet the American Thoracic Society for high and low flow criteria for volume calibration at last one of the last 4 expiratory strokes had to have an average flow rate less than 05 l/s and at least one had an average flow rate greater than 3.0 l/s These measurements made up the calibration results and were closely checked for accuracy against the known syringe volume.

b. Plethysmographic Pressure Calibration

With the cabin door closed, the procedure consisted of energising an internal 50ml calibration syringe that simultaneously pumped air in and out of the cabin and a small fixed volume chamber, thereby exposing two pressure transducers (Vbox – box pressure transducer and Pm – mouth pressure transducer) to known pressure /volume signals. Two series of sixteen strokes were displayed on screen for each transducer. The first stroke series was used to calculate the correction factors, the second was used to verify the newly calculated factors. The % targets for Vbox and Pm were checked to be in the range 97 –103%. If not the calibration was repeated. In addition to displaying the % target values, the computer evaluated the correction factors calculated and displayed a warning message if the factors were out of range. The acceptable range for Vbox was 0.7 – 1.3 (at sea level) and 0.7 – 1.3 for Pm (irrespective of atmospheric pressure).

3.6 Calibration of the Mefar Dosimeter

The principal argument for dosimetry as a mode of delivery of drug lies in its precision. A dosimeter is designed to deliver air at a known (and fixed) pressure for a

precise period of time (controllable). The air pressure is used to activate one of a series of nebulisers. Nebuliser output may be varied by adjusting the time of activation. Accurate calibration of such a system is therefore crucial.

We purchased the commercially available Mefar MB3 dosimeter (Mefar s.r.l., Bovezz, Italy) supplied with 10 Mefar jet nebulisers. Each nebuliser is individually calibrated by the manufacturer. Results of this calibration are supplied in the form of a graph relating duration of activation to output, as measured by weight loss. However measurement of the weight loss of a nebuliser following activation does not truly reflect its output of aerosolized solute. Weight lost through jet nebulisation is known to contain two distinct components: aerosol (the reservoir drug solution suspended as respirable particles) and water vapor (which contains no drug solute) (95-98). Of these only aerosol output is of clinical relevance. Calibration based on weight loss will necessarily over estimate the dose of delivered drug (96). In order to calibrate the dosimeter we used the method designed by Dennis (96), a chemical tracer technique which measures true aerosol output.

Measurement of Aerosol Output

Aerosol output (AO) from each nebuliser was assessed using a fluoride tracer method (96). Four milliliters of 1% NaF solution was added to the nebuliser reservoir. The nebuliser was then activated through the Mefar dosimeter. The pattern of activation used was five 2-second bursts with a five second pause between each (a total of 10 seconds activation). This was chosen to mimic the activation pattern that would be used in subsequent trials. During activation of the nebuliser, ambient air was drawn at 15 litres / min through a fitted T- piece over the nebuliser by means of a vacuum pump. This entrained and impacted aerosol onto a 25mm Whatman glass fibre (GF/A)

filter (BDH Chemicals ltd) held within a metal cassette, held within 5cm from the nebuliser head. After collection filters were removed and placed in 25ml universal bottles. Fluoride residues were subsequently dissolved in an appropriate buffer and quantified electronically as described below. Each nebuliser was activated by this method three times, producing three filters for separate analysis.

Analysis of Aerosol Output

Total Ionic Strength Adjustment Buffer (TISAB; BDH Chemicals ltd.) was prepared as a 50% solution in distilled water. 20ml was then added to each Whatman filter within 25ml plastic universal bottles. The bottles were then sealed and flouride was allowed to desorb overnight. The recovery of fluoride from filters was complete (>98%) and no fluoride was detected in unused filters. Fluoride analysis followed well established protocols (99). Fluoride standards were prepared by microlite injections of 20.0, 50.0, 75.0 and 100.0 micro-litres of 1.00% NaF into 20ml aliquots of 50% TISAB, resulting in 2.38 $x10^{-4}$M, 5.95 $x10^{-4}$M, 8.93 $x10^{-4}$M, 1.19 $x10^{-3}$M fluoride solutions. Both standard and test solutions were equilibrated to 25°c in a water bath. Flouride concentrations within the buffered solutions were then measured electrochemically with a fluoride specific ion electrode (Corning Ltd, Halstead) on a Corning 225pH / ion meter with a calomel reference electrode. This electro-chemical system had a log linear relation between concentration and activity (mV) from 10^{-1} M to 10^{-6} M. All solutions were continually agitated during analysis with an electromagnetic stirrer. An internal four-point calibration was established. The standard curve was used to quantify all test solutions and reported directly the microlite of aerosol fluoride impacted on and desorbed the test filters. The error of fluoride determination was within 2%.

Results

Output of each nebuliser unit (µl) of solution for each '10 second activation' in a series of 3.

nebuliser	**1**	**2**	**3**	**average**
1	61.3	64.9	61.4	62.53
2	65.7	64.6	67.2	65.83
3	57.9	78	79.5	71.8
4	68.8	63.7	72.1	68.2
5	56.1	80	76.7	70.93
6	85.6	56	66.4	69.33
7	74.5	72.6	66.6	71.23
8	63.4	63.3	63.6	63.43
9	66.6	67.7	67.1	67.13
10	80.3	77	78.8	78.7
average				**68.91**

Calculation

Using these results one could calculate the total activation time in seconds (usually delivered in 5 equal activations) to deliver 50µl of solute (10µl per activation) for each nebuliser unit individually. To fully utilize the precision in this data requires a software driven nebuliser capable of being programmed with different activation times for each stage of challenge. Alternatively the average may be used to calculate

an average activation time to be used for all stages of challenge.

Methacholine Stock and Diluents

Methacholine Chloride was obtained in chrystalline form from ACIC (Canada) Inc., in quantities of 100g. The crystals are hygroscopic and so were kept in a sealed container in a freezer. A stock solution of 64mg/ml (with phenol 0.4% as a preservative) was supplied by the hospitals pharmacy. It was kept in a sealed container in a refrigerator in the lab. At this dilution it has a shelf life of 4 months. The pharmacy also supplied 2 diluents: Diluent A (sodium bicarbonate 0.275% and phenol 0.4%), Diluent B (sodium chloride0.8%, sodium bicarbonate 0.275% and phenol 0.4%)

Preparation of Methacholine Solutions

The standard test requires the preparation of 10 doubling concentrations of methacholine chloride from 0.0625mg/ml to 32 mg/ml, in 10 separate test tubes labelled 1-10, in volumes of 4ml each, using the stock solution of methacholine with diluents A and B. The following protocol was used:

1. Ensure the test tubes are clean and dry before use.
2. Place test tubes in rack labelled 1-10 left to right.
3. Replace filter in 'Pipetteman' auto pipette.
4. Check pipette ends are clean and dry
5. Set pipette to desired number, usually 4ml (this should be checked repeatedly during the procedure).
6. Put 4ml methacholine stock solution in tube 10. Change pipette end.

7. Put 4ml diluent A in tube 10 (this reduces acidity and osmolality towards physiological levels). Cap and mix well. Change pipette end.
8. Put 4ml diluent B in tubes 1-9 (this maintains pH and osmolality near to physiological levels). Check volumes. Change pipette end.
9. Take 4 ml from tube 10 and place in tube 9. Cap and mix well.
10. Take 4ml from tube 9. Fill and empty pipette 2 or 3 times to flush pipette end, then place 4ml in tube 8.
11. Repeat step 10 to provide serial 2-fold dilutions in the remaining tubes.
12. Check volumes of solutions in tubes. If there is an obvious difference between tubes start again.
13. Label test tube rack with date and check test tubes are labelled correctly.
14. Unused solution at the end of that day was discarded.

Bronchial Challenge

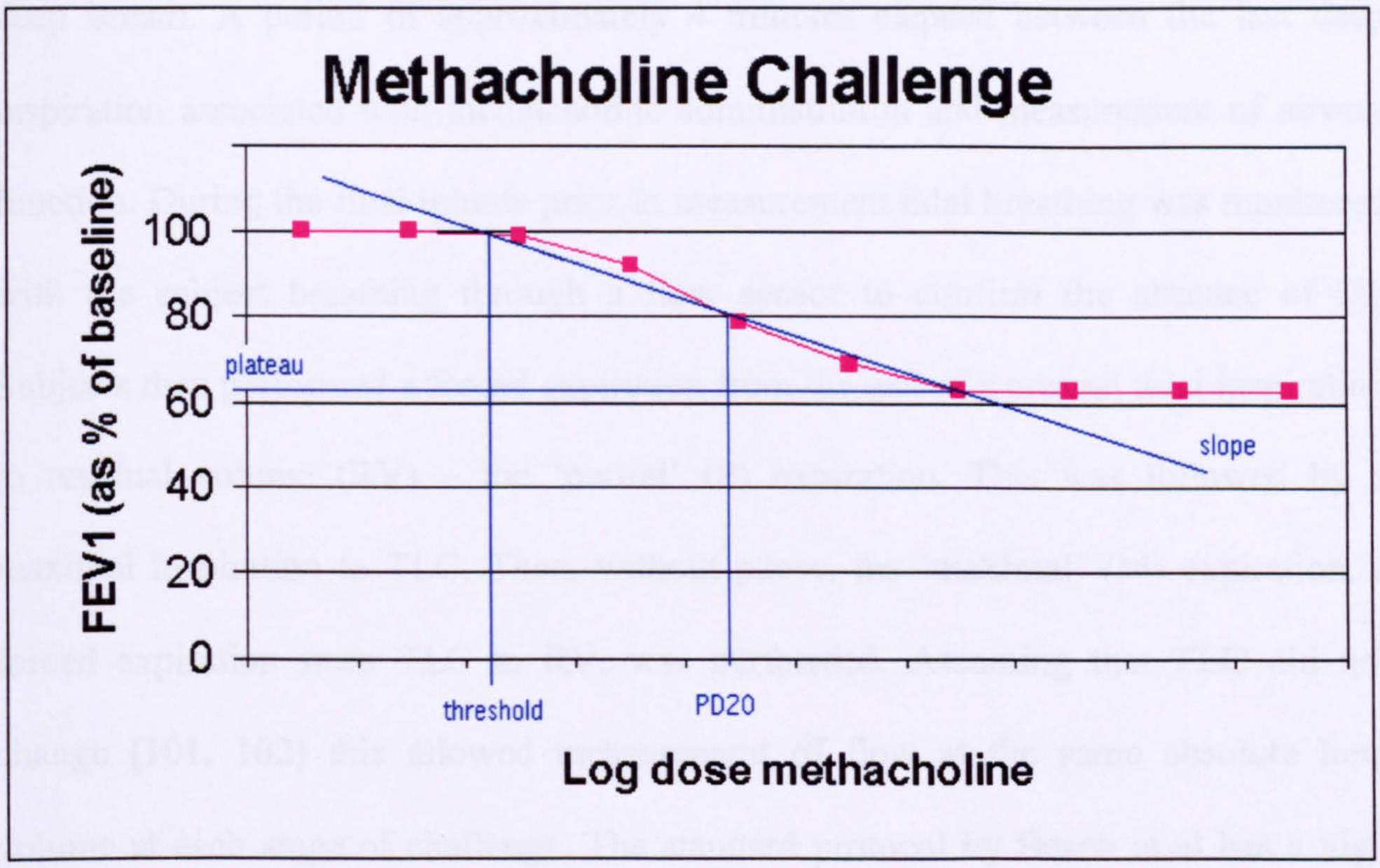
Bronchial challenge is an important diagnostic test finding increasing use in clinical practice. It is a measure of 'airway responsiveness'. The test involves doubling incremental doses of the bronchoconstricting drug, methacholine (or histamine) sequentially delivered as aerosol. The standard regimen starts with an initial dose of 3.125mcg and proceeds to a possible standard maximum of 6,400mcg.

Pre test baseline respiratory function is established (usually FEV1). After each dose is delivered, this measure is repeated. A graph of response (fall in FEV1) vs. dose of methacholine can then be plotted (graph 3.1).

The most commonly used index of bronchial reactivity is the PD_{20}, the dose of drug producing a 20% fall in respiratory function (FEV1), a value interpolated from the

above graph. Other specific points of note however would include: (i) threshold of response, (ii) slope of graph, (iii) level of plateau of response (if present).

Figure 3.1



3.7 Methacholine Challenge (dosimeter method).

As used in chapters 5 and 6

Subjects performed a modified version of a standard methacholine challenge (100). Methacholine aerosol was administered in doubling cumulative doses (3.125 to 6,400 μg) from a Mefar dosimeter at 5-minute intervals until a 40% decrement in FEV_1 was recorded or the dose sequence completed. The aerosol was released electronically (using a thermistor) in 10 μl (± 10%) aliquots over 1.5 seconds as the subject began to inhale from end tidal expiration. He/she continued to inhale for a further 4 seconds after aerosol delivery. If full inspiration was achieved earlier, breath holding

completed this 5.5 second period. Five aliquots inhaled in rapid succession comprised a single challenge dose, further doses being administered at 5-minute intervals. Each dose was administered immediately on completion of the measurements following the previous dose. After each dose of methacholine subjects were asked to avoid taking a deep breath. A period of approximately 4 minutes elapsed between the last deep inspiration associated with methacholine administration and measurement of airway function. During the final minute prior to measurement tidal breathing was monitored with the subject breathing through a flow sensor to confirm the absence of DI. Subjects then performed a forced expiration from the end of a normal tidal inspiration to residual volume (RV) – the 'partial' (P) expiration. This was followed by a maximal inspiration to TLC. Then, without pause, the 'maximal' (M) expiration, a forced expiration from TLC to RV, was performed. Assuming that TLC did not change (101, 102) this allowed measurement of flow at the same absolute lung volume at each stage of challenge. The standard protocol by Beach et el has a high degree of precision with the co-efficient of repeatability of 3.0 (using the statistical method described by Bland and Altman (103)). The requirement for a consistent DI free period and time interval from delivery of methacholine to a 'partial' expiration limited the number of 'M/P' manoeuvres to one at each stage of challenge. This limitation is likely to reduce the reproducibility of the test. For each index of lung function the cumulative dose provoking a 20% fall from its baseline value (PD_{20}) could be calculated.

Dose Regimen

Nebuliser	No. of inhalations	Concentration (mg/ml)	Individual dose (mcg)	Cumulative dose (mcg)
1	5	0.0625	3.125	3.125
1	5	0.0625	3.125	6.25
2	5	0.125	6.25	12.5
3	5	0.25	12.5	25
4	5	0.5	25	50
5	5	1	50	100
6	5	2	100	200
7	5	4	200	400
8	5	8	400	800
9	5	16	800	1600
10	5	32	1600	3200
10	10	32	3200	6400

3.8 Methacholine Challenge (tidal breathing method).

As used in Chapter 7

Although careful monitoring of what were felt to be appropriate DI free periods had occurred in the studies in chapters 5 and 6, the involvement of DI in the administration of methacholine in a study in part designed to assess the response to DI seemed illogical. Thus, at the suggestion of my supervisor, for the study in chapter 7 an alternative (tidal breathing) protocol was adopted.

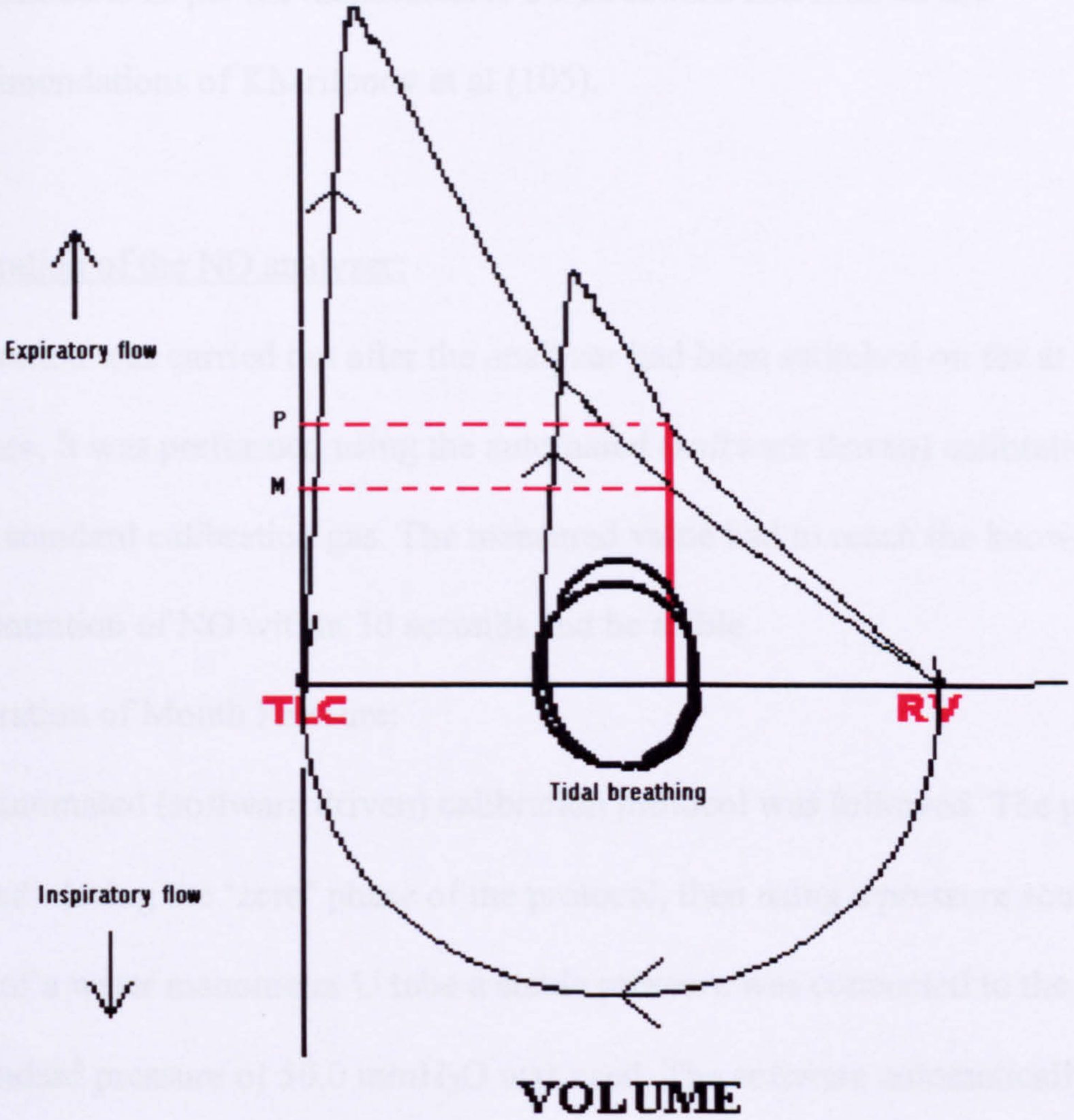
The method used was modified from the protocol by Juniper et al. (104). This method is reported to be less precise, i.e. has a higher co-efficient of repeatability, than the dosimeter method (10.9 vs. 3.0) (100, 103). But the difference is thought to be due to the difference in quantifying FEV1 in the standard protocols. In the modified protocol used, as in the dosimeter protocol, the requirement for a consistent DI free period and time interval from delivery of methacholine to a 'partial' expiration limited the number of manoeuvres to one at each stage of challenge. This factor is likely to be most important in determining reproducibility.

Methacholine aerosol was administered in doubling concentrations (from 0.03125 to 16mg/ml) from a nebuliser unit (Mefar) with mouth piece, driven by a 'Pari TurboBoy' Compressor delivering compressed air at 40psi. Subjects wore nose clips throughout the challenge protocol. For the delivery of each concentration subjects were instructed to relax and breathe quietly (tidal breathing) and to avoid any deep breaths. A respiratory inductance plethysmograph ('respitrace', Studley Data Systems, Oxford. Model 250), which was worn throughout the challenge protocol, was used to monitor the absence of deep breaths.

Subjects breathed each concentration for precisely 2 minutes. After methacholine delivery subjects transferred to the mouth piece and flow sensor (monitoring flow and volume derived by integration). Subjects first performed a further 90 seconds tidal breathing, the avoidance of deep breaths being monitored on screen. Thus ensuring a total of 3.5 – 4 minutes without DI. At the end of a normal tidal inspiration, (at end inspiratory lung volume, EILV) subjects performed the 'partial' (P) manoeuvre, by forced expiration to RV. This was followed by a maximal inspiration to TLC. Then, without pause, the 'maximal' (M) expiration, a forced expiration from TLC to RV, was performed. M and P flows could then be extracted at any lung volume using software designed for that purpose (Sensormedics 'Vmax') (figure 3.2). Assuming that TLC did not change (101, 102) this allowed measurement of M and P flow at the same absolute lung volume at each stage of challenge. Finally two further FEV1 manoeuvres were performed allowing measurement of the response to challenge by this standard index.

Subjects then return immediately to inhalation of Methacholine at the next concentration. Challenge continued until a 20% fall in FEV1 or the dose sequence was completed. For each index of lung function the concentration of methacholine provoking a 20% fall from its baseline value (PC_{20}) could be calculated.

Figure 3.2



3.9 Exhaled Nitric Oxide Concentration (NO)

Using a commercially available (Logan Research LR 2000 series) chemiluminescence analyser, subjects exhaled slowly from total lung capacity at a constant flow rate. This is determined by the inherent resistance in the system and a voluntarily maintained mouth pressure of 40mmH_2O monitored visually on screen by the subject. This pressure ensures closure of the soft palate and precludes nasal air leak. The concentration of exhaled CO_2 was also monitored on the same time plot on screen. The concentration of exhaled NO (ppb) corresponding to the start of the plateau of

exhaled CO_2 was recorded. The mean of 3 technically good values was calculated. The method is as per the manufacturer's instructions and follows the recommendations of Kharitonov et al (105).

Calibration of the NO analyzer:

Calibration was carried out after the analyzer had been switched on for at least 30 minutes. It was performed using the automated (software driven) calibration protocol and a standard calibration gas. The measured value had to reach the known concentration of NO within 30 seconds and be stable.

Calibration of Mouth Pressure:

The automated (software driven) calibration protocol was followed. The pressure was 'zeroed' during the 'zero' phase of the protocol, then using a pressure source in the form of a water manometer U tube a stable pressure was connected to the transducer. A standard pressure of 50.0 mmH_2O was used. The software automatically adjusted the calibration factor. The pressure was then removed from the positive pressure port.

3.10 Statistical Analysis

All statistical analysis was performed using a commercially available software package 'SPSS 10.0 for Windows'. All between group comparisons were made using either a paired or unpaired (as appropriate) two-tailed Student's t test. Comparisons with unity, as in the case of M/P ratio at a given lung volume in chapter 4, was done using a one sample Student's t test.

All correlations were performed using Pearson's Correlation Coefficient.

In the case of methacholine challenge results, all 'provoking dose' indices were logarithmically transformed before inter group comparisons were performed.

Chapter 4

The Apparent Response of Airway Function to Deep Inspiration Depends on the Method of Assessment

INTRODUCTION

The response to DI in asthmatic and non-asthmatic subjects is reported to be different in most published studies. However there is also considerable variability in the reported response to DI within each of these subject groups. A variety of methods have been used to assess the response to DI. The purpose of this study is to determine to what degree the measured response to DI is dependent on the method used to assess it and to describe the nature of that dependence.

In the study the response to DI is assessed in terms of its effect on SGaw and forced expiration, the latter over a range of lung volumes. The findings are discussed and the implications for any putative mechanism to explain the 'bronchoconstrictor' response to DI in asthmatic subjects are considered.

METHODS

Subjects and methods

We studied 16 mildly asthmatic and 16 normal subjects of similar age (tables 3.1 – 3.4 and summarised in table 4.1).

All respiratory function tests were performed with the subject seated and breathing via a mouthpiece attached to a flow sensor (Sensormedics). M/P ratio, SGaw and SGaw ratio (post:preDI) were measured by the methods described in chapter 3 (sections 3.3 & 3.4).

Protocol

After initial consent and measurement of FEV1 and VC, all tests were performed the following day. All subjects followed an identical protocol in the order: (1) M/P ratio, (2) SGaw (pre DI) and (3) SGaw (post DI) according to the methods described in chapter 3.

In each of these three indices the mean of three technically good values was recorded. There was a 5-minute time interval between the final measurement of M/P and the first measurement of SGaw. After the final measurement of SGaw (pre DI) subjects proceeded immediately to the measurement of SGaw (post DI).

The five minute interval between the measurement of M/P and the start of the SGaw (pre DI) manoeuvre plus the one-minute monitored 'DI free' period integral to this measurement I felt minimised the potential for any residual effect from the preceding manoeuvre on the measurement of SGaw. Lest there be however, the order was identical in all subjects so that any (theoretical) order bias would not differ between the asthmatic and healthy groups.

Analysis

M/P

The M/P ratios were compared to unity at each lung volume (40,35,...,15%VC and FRC) in each group using, one sample Student's t test. The M/P ratios in the asthmatic and control groups were compared at each lung volume using unpaired t tests. The mean M/P ratio at each lung volume was plotted against lung volume (%VC) for asthmatic and healthy subjects separately. The association between lung volume and M/P ratio was assessed by Pearson's Correlation Coefficient.

SGaw Ratio

The SGaw (post: pre DI) ratio in both the asthmatic and control groups was compared with unity using one-sample t tests. A comparison between the two groups was performed using an unpaired t test.

M/P compared with SGaw ratio.

Correlations were examined between the SGaw ratio and M/P ratio at each lung volume (40,35,...,15%VC and FRC), in each group and the two groups combined.

Caveat

The relative novelty of the indices used in this study and the lack of clear previously published data on repeatability made power calculations prior to study design difficult. I did not set out to formally establish an index of reproducibility thus this remains essentially unknown. The natural volatility in any index based on a ratio suggests the inherent 'noise' in the signals may be high. The study was therefore probably prone to 'type two' statistical errors. Thus, although any statistically

significant finding is likely to be valid, the finding of the absence of a statistically significant relationship should be interpreted with caution.

RESULTS

The relationships of M/P ratios to lung volume (Fig 4.1) show the following features:

(i) M/P ratio increased significantly and systematically as lung volume decreased in both subject groups.

(ii) The M/P ratio was greater than one at most lung volumes in both groups. However only at lower lung volumes (25, 20 and 15%VC in asthmatics, 20 and 15%VC in healthy controls) was the difference from unity statistically significant.

(iii) At each of the lung volumes 40,35,...,15%VC (fig.4.1) and FRC (fig.4.2) the mean M/P ratio was (non-significantly) greater in the asthmatic than the healthy subjects.

In contrast to the M/P ratios the mean SGaw ratio (fig. 4.2) was greater in the healthy than the asthmatic subjects ($p=0.005$). Indeed in both asthmatic and healthy groups the SGaw (post:pre DI) ratio contrasted with the M/P ratio measured at the same lung volume (FRC). In the asthmatic group SGaw ratio was less than 1 ($p = 0.049$), suggesting bronchoconstriction in response to DI, mean (sem) = 0.938 (0.029). In healthy controls the SGaw ratio was greater than 1 ($p = 0.048$) suggesting bronchodilatation in response to DI, mean (sem) = 1.063 (0.029).

Relation between SGaw ratio and M/P ratio (Table 4.2, Figs. 4.3a & 4.3b)

In healthy subjects the correlations between SGaw ratio and M/P ratio at each lung volume as well as with the mean M/P ratio over the various lung volumes investigated

(40,...,15%VC) were statistically significant (Table 4.2 and fig.3b). At FRC the correlation just failed to reach conventional significance. In asthmatic subjects by contrast, no significant correlation was found at any lung volume or with mean M/P (40,...,15%VC) (Table 4.2 & fig 3a).

Table 4.1

Baseline Characteristics of Subjects

	Age	FEV1	FEV1 % predicted	SGaw‡	%VC remaining at FRC†
	Mean (sd)*	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Asthmatic	27.9 (8.3)	3.63 (0.91)	94 (12.8)	0.10 (0.03)	45.2 (10.3)
Healthy	27.5 (5.9)	4.35 (0.87)	102 (8.6)	0.15 (0.05)	42.8 (8.1)
p value	0.87	0.03	0.03	0.008	0.46

* standard deviation; ‡ SGaw (pre DI); † functional residual capacity.

Table 4.2

Correlation between SGaw ratio and M/P at various lung volumes.

	Pearson's Correlation	40%VC	35%VC	30%VC	25%VC	20%VC	15%VC	Mean (40-15%)	FRC*
Asthmatic	r	0.28	0.25	0.25	0.22	0.26	0.17	0.23	0.39
	p	0.29	0.36	0.36	0.41	0.33	0.67	0.40	0.14
Healthy	r	0.76	0.75	0.73	0.75	0.63	0.57	0.73	0.48
	p	0.0006	0.0008	0.0012	0.0009	0.0082	0.022	0.001	0.060

* functional residual capacity

Figure 4.1
M/P ratio vs Lung Volume in Asthmatic and Healthy subjects

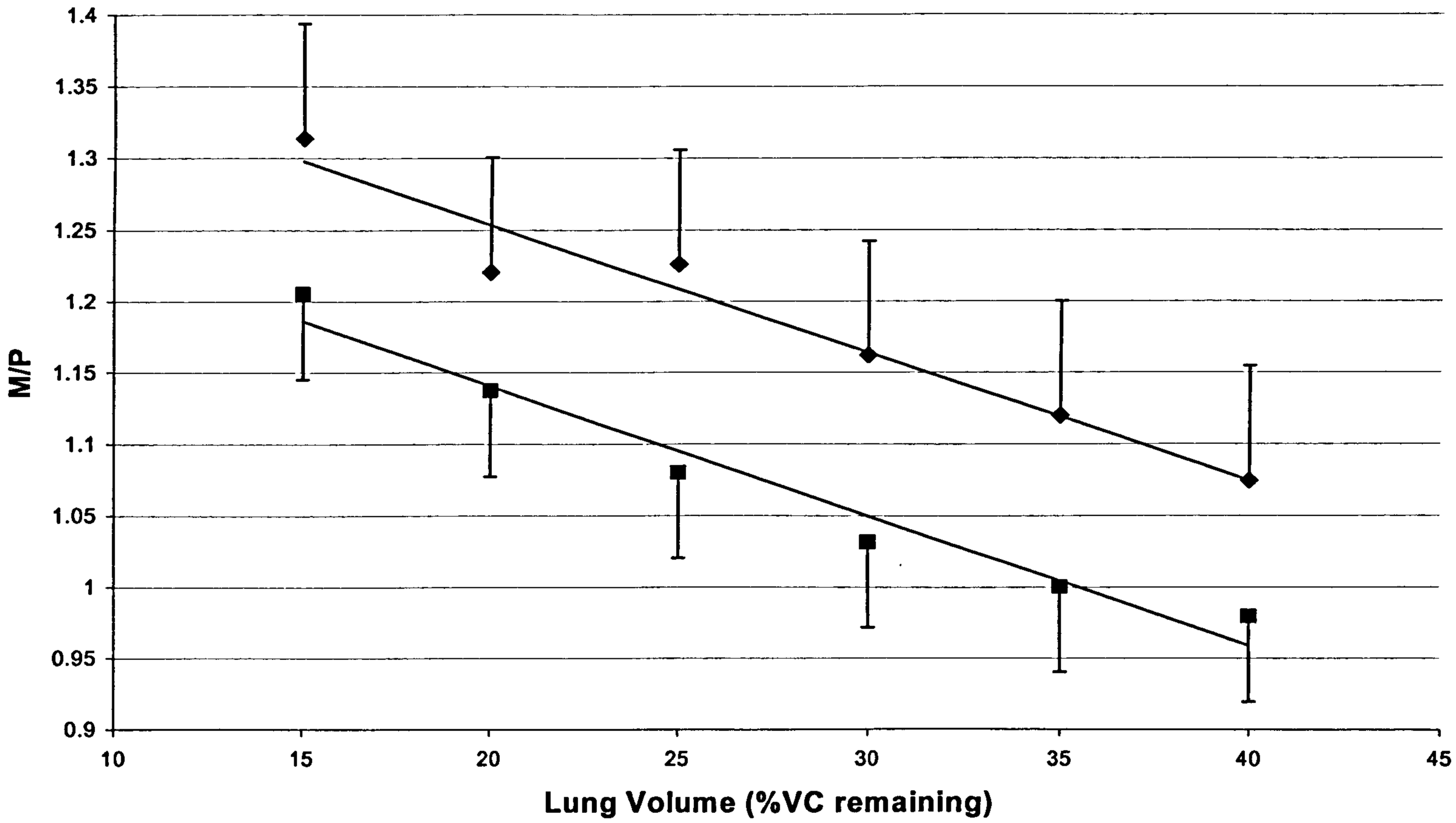
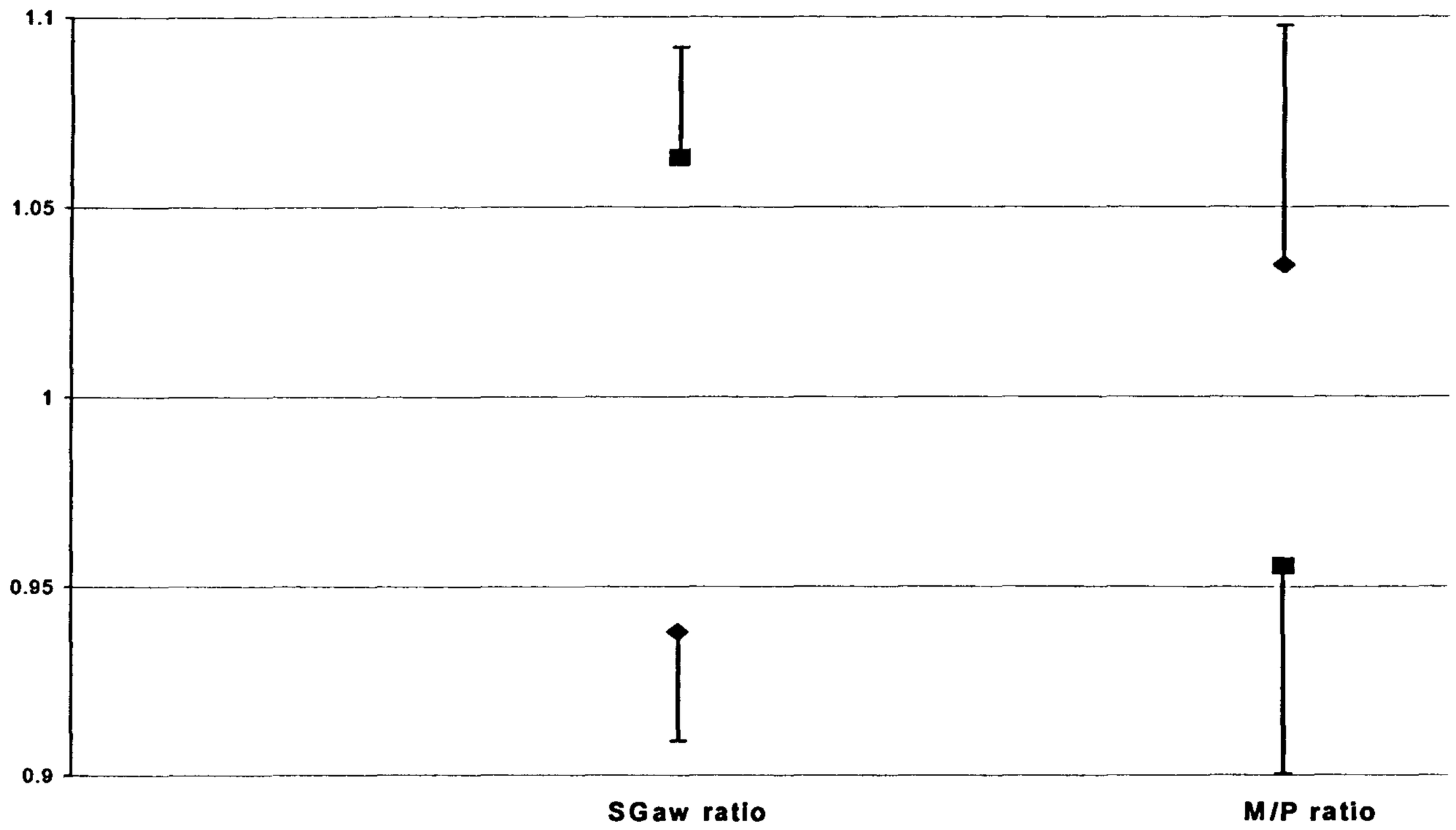


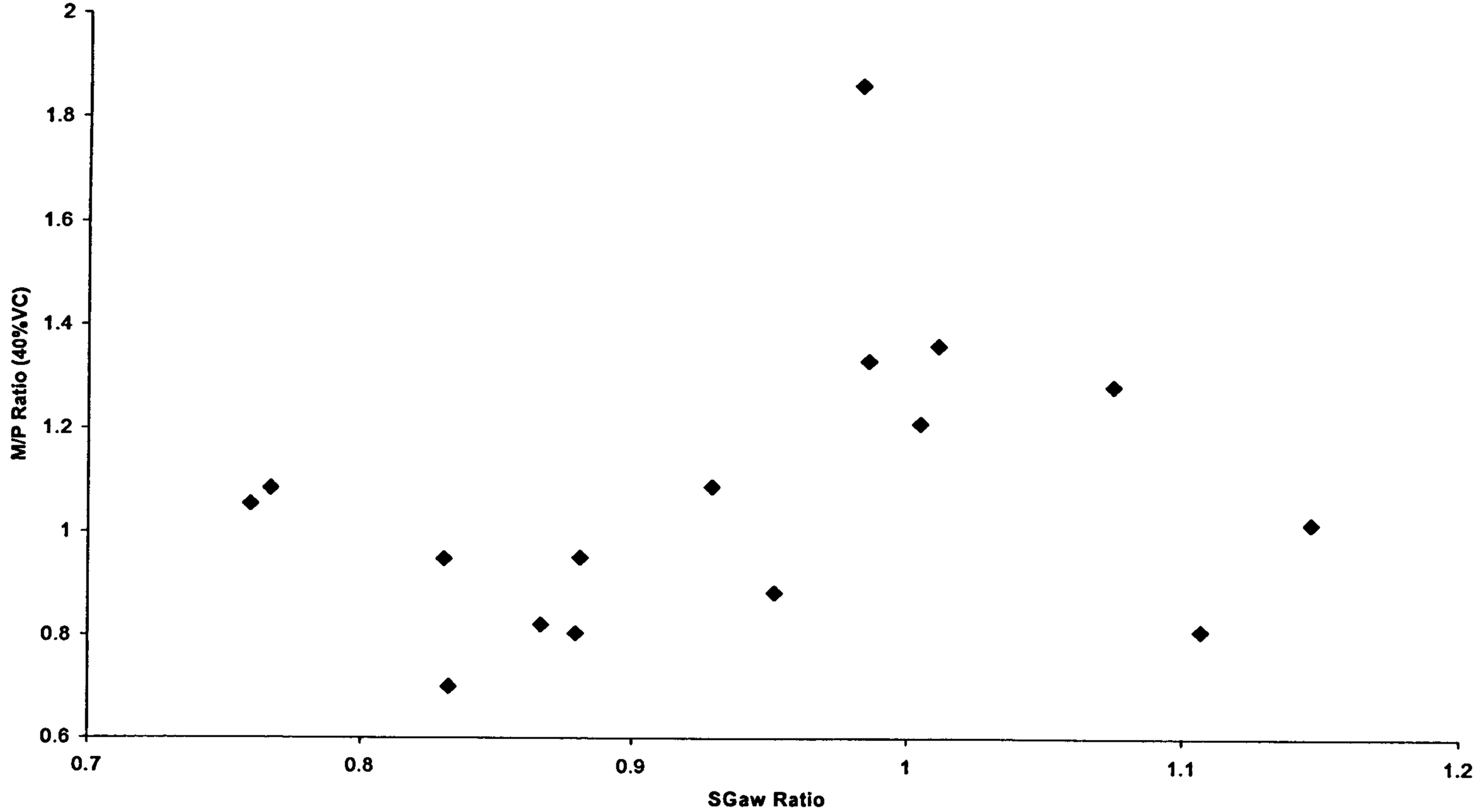
Fig.4.1 Mean (+/- sem) M/P ratio vs Lung Volume in Asthmatic (υ) and Healthy subjects (ν). This demonstrates the dependence of M/P ratio on lung volume in the two subject groups. In each group the relationship was highly significant. ($r = -0.98$, $p = 0.0005$ and $r = -0.98$, $p = 0.0008$ in healthy and asthmatic subjects respectively).

Figure 4.2
Mean SGaw ratio and M/P ratio in Asthmatic and Healthy subjects



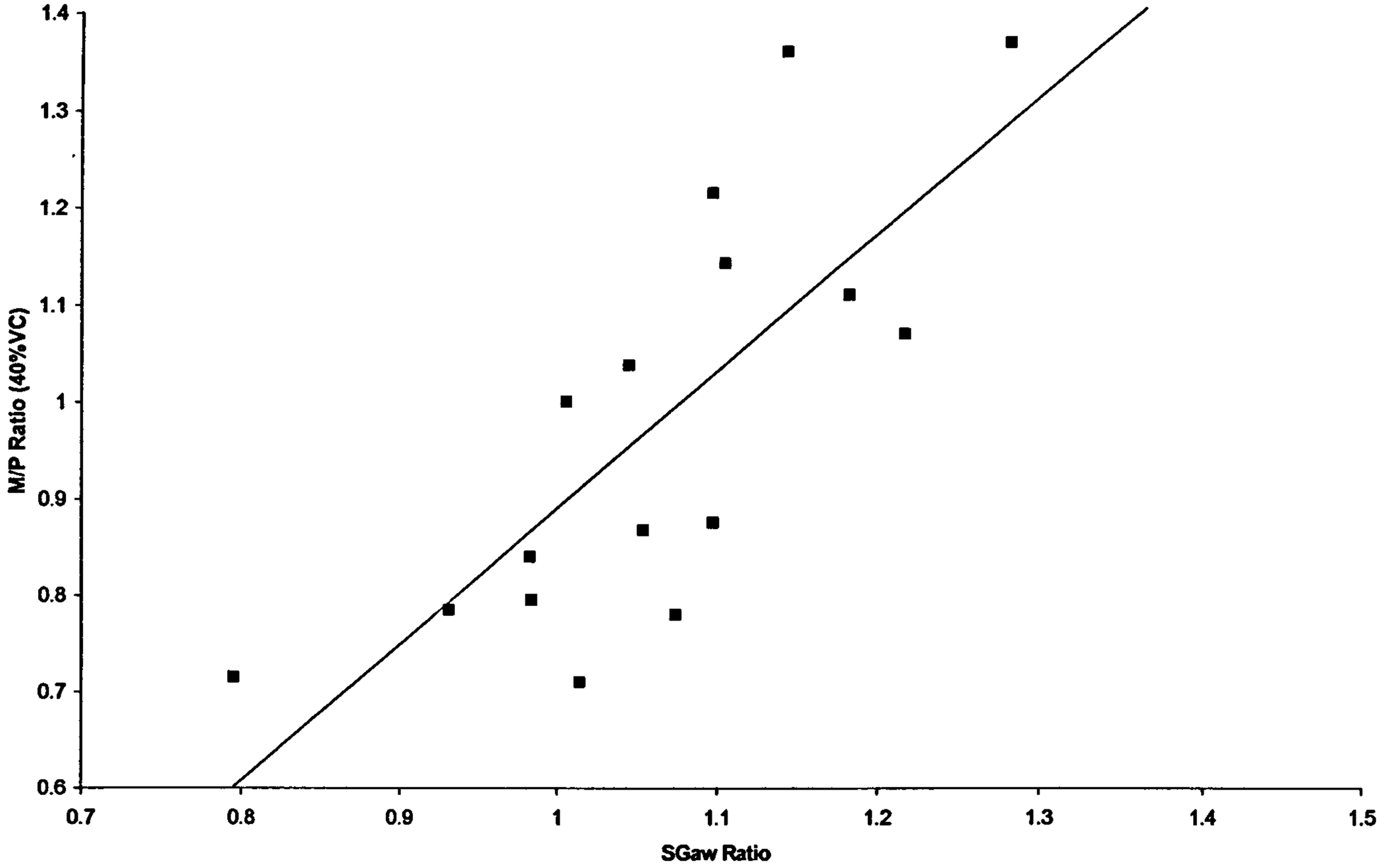
Mean (+/- sem) SGaw ratio and M/P ratio in Asthmatic (υ) and Healthy subjects (ν). Note SGaw ratio > 1 in healthy subjects, and Sgaw ratio <1 in asthmatic subjects. For M/P ratio the reverse relationship applies; M/P >1 in asthmatic subjects and M/P < 1 in healthy subjects.

Figure 4.3a
M/P Ratio at 40%VC vs SGaw Ratio in Asthmatic Subjects.



M/P Ratio at 40%VC vs SGaw Ratio in Asthmatic Subjects. ($r = 0.28$, $p = 0.29$). Demonstrating a lack of correlation between SGaw ratio and M/P ratio in asthmatic subjects.

Figure 4.3b
M/P Ratio at 40%VC vs SGaw Ratio in Healthy Subjects.



M/P Ratio at 40%VC vs SGaw Ratio in Healthy Subjects. (r = 0.76, p = 0.0006). Demonstrating a clear correlation between SGaw ratio and M/P ratio in healthy subjects.

DISCUSSION

In assessing the airway response to DI several authors have used SGaw ratio (post : pre DI) (1, 4, 5, 14-16, 18-20) while others have used M/P ratio. In addition the latter has been reported at different lung volumes (25 to 50 %VC remaining) in different studies (3-11). In two studies the response to DI has been assessed by both indices in the same subjects but with conflicting results. Lim et al (5) found that changes in SGaw were qualitatively consistent with changes in maximum flow (M/P ratio) in asthmatic subjects. By contrast Burns et al (4), also studying asthmatic subjects, found divergence between these two indices, with M/P > 1 but reduced SGaw post DI. Furthermore it is noteworthy that most studies in asthmatics using M/P report an apparent bronchodilator response to DI (M/P >1) (6-11), whilst those using SGaw suggest that DI has a bronchoconstricting effect. (5, 15, 16, 18, 19). Similarly in the present study the asthmatic subjects showed apparently paradoxical findings. Mean SGaw ratio suggested bronchoconstriction post DI, yet mean M/P ratio, at the same lung volume, suggested bronchodilatation. The healthy subjects in this study showed the converse pattern, with SGaw ratio suggesting bronchodilatation and M/P ratio suggesting bronchoconstriction.

The M/P Ratio

These results clearly show that M/P ratio varies in a systematic way with the volume at which it is measured, increasing as lung volume decreases both in asthmatic and healthy subjects. This is consistent with the findings of Pellegrino et al (106) who measured M/P at three lung volumes (30, 40 & 50%VC) and found that it increased as volume (remaining) declined.

I have considered three possible explanations for this finding:

(i) Gas compression artefact.

During forced expiration gas within the thorax undergoes compression. The measurement of expired volume during forced expiration can therefore underestimate the change in thoracic gas volume. In the M/P manoeuvre the 'M' and the 'P' expiratory traces are positioned in relation to each other and the volume axis on the basis of the expired gas volume. Given the compression already present within the thorax during 'maximal' expiration at the point which appears to be isovolumic with EILV – the lung volume at which the 'partial' expiratory manoeuvre was begun, the actual thoracic gas volumes on the two curves, at this point at least, are likely to be different. Also the degree of compression will depend on the volume of gas within the thorax and indeed the pressure generated. The degree of the underestimation will therefore vary as lung volume varies during the forced expiratory manoeuvres. Thus this 'error' in assessing lung volume using expired gas volume may result in a systematic error in the M/P ratio which itself may vary with lung volume. This 'compression artefact' may theoretically have explained our findings in relation to M/P and lung volume, however two previous studies (106, 107), which included values based on plethysmographic measurement of lung volume (TGV) as the reference as well as expired volume suggest that the findings are not attributable to gas compression artefact and though based on more limited data, the findings of plethysmographic measurements (107) in both healthy and asthmatic subjects suggest a similar inverse relationship between M/P and lung volume (TGV) to that reported in the present study.

(ii) A mathematical consequence of the change in RV.

Previous studies (9, 11, 106, 108) have shown, and I have confirmed, that when DI increases expiratory flows, FVC also increases, i.e. RV following the maximal expiratory manoeuvre is less than 'RV' following the partial manoeuvre. It is clear therefore, particularly in the case of healthy subjects when the descending limb of the flow-volume relationship is essentially straight, that it is a geometric impossibility for the M/P ratio to be constant at different volumes i.e. volume dependence of the M/P ratio inevitably accompanies a shift of the maximal expiratory curve to the right of the partial curve. This explanation however, merely tells us that M/P must be volume dependent; it offers no insight into the mechanism.

(iii) Generational differences in the airways.

Theory suggests that the lower the lung volume, the greater the dependence of maximum expiratory flow on peripheral airway calibre (109). Thus dependence of M/P on lung volume might reflect a differing effect of DI on different generations of airway, with the larger airways showing relatively less bronchodilatation. There is evidence that the stretch associated with DI causes a temporary reduction in smooth muscle tone. This effect is consistent with the enhanced bronchodilating effect of DI seen in the context of methacholine induced bronchoconstriction in both asthmatic (4-6, 17) and healthy subjects (1, 8) and with diminution of the bronchodilating effect of DI following administration of $ß_2$ sympathetic agonists (6, 16, 23). The greater cartilaginous support of the more central airways may result in less smooth muscle stretch during DI than would occur peripherally and thus less relaxation post DI. This

would result in relatively less dilatation of the larger airways and a lower M/P ratio at higher lung volumes.

SGaw Ratio

In healthy subjects mean SGaw increased after deep inspiration, while in the asthmatic subjects it decreased, findings that are in accord with most previous studies. DI may stretch asthmatic airways less effectively, either because of a change in the smooth muscle structure or behaviour or because airway wall inflammation and oedema uncouples the interdependence between the airway and the surrounding parenchyma (24). However, this would account only for a diminution in the bronchodilating effect of DI, and not for frank bronchoconstriction. Hysteresis of the lung parenchyma of relatively greater magnitude and acting in opposition to hysteresis of the airway has been proposed as the mechanism to explain bronchoconstriction post DI in asthmatic subjects (4, 7, 14, 23, 29). While this would account for a reduction in SGaw post DI in asthmatic subjects, it would not explain the apparently paradoxical increase in forced expiratory flow observed in the same subjects. Thus differential hysteresis of airways and parenchyma cannot account for all reported observations.

Comparison of the responses to DI as measured by M/P ratio and SGaw ratio.

At first sight the response of the airways to DI as measured on the one hand by SGaw ratio and on the other by the M/P ratio would appear to be contradictory in both the asthmatic and healthy groups. For comparison with SGaw ratio the most appropriate volume at which to measure M/P would seem to be FRC (the lung volume at which SGaw is calculated). In the asthmatic subjects the mean M/P at FRC was greater than

one, whereas the mean SGaw ratio was less than one. In the healthy subjects, the reverse situation applied, mean M/P ratio was less than one, yet mean SGaw ratio was greater than one. Clearly therefore the factors that determine the effect of DI on SGaw must differ from those which determine the change in forced expiratory flow. The different conditions under which airway function is assessed by these two methods may explain the apparent paradox. SGaw is a measurement of airway function in the 'unstressed' state, while forced expiratory flow depends on both unstressed airway dimensions and compliance of the airway wall, which determines its ability to withstand the large compressive forces occurring during forced expiration.

If as well as narrowing following DI, asthmatic airways were to become more rigid they would resist compression during forced expiration, which would *tend* to increase expiratory flow, particularly at lower lung volumes, despite a reduction in SGaw. The net result on forced expiratory flow would therefore depend on the relative contribution of these two factors. With a sufficient reduction in airway compliance post DI, forced expiratory flow could increase despite marginal narrowing of the unstressed airway.

In healthy subjects (where SGaw ratio >1 yet M/P was less than in the asthmatic subjects) increased compliance of the airway wall consequent on smooth muscle relaxation post DI would *tend* to reduce forced expiratory flow. The same mechanism may operate, albeit to a lesser degree in asthmatic airways but a further mechanism is required to explain the observed post DI bronchoconstriction (SGaw ratio <1) as well as the hypothesised increase in airway wall rigidity post DI (leading to M/P >1).

In healthy subjects the strong positive correlation between M/P and SGaw ratio (Table 4.2, fig 4.3) suggests the absence (or relative insignificance) of any such additional mechanism. On the other hand, a marked lack of correlation between M/P and SGaw

ratio was noted in the asthmatic group. This suggests an additional, DI-related mechanism, which varies independently of the DI induced change in smooth muscle tone. Both this hypothesised mechanism and the change in smooth muscle tone post DI may each affect unstressed airway calibre (and hence SGaw) and airway wall rigidity, but if so their respective contributions would have to differ quantitatively. In addition to smooth muscle stretching therefore, if a single further mechanism is to account for all of the observed effects of DI it must satisfy a number of conditions:

(i) It must account for bronchoconstriction post DI in asthmatics.

(ii) It must account for an increase in airway wall rigidity post DI in asthmatics.

(iii) It should be distinct from, and thus vary independently of, smooth muscle stretch/relaxation.

(iv) It should be absent or insignificant in healthy subjects.

According to the relative hysteresis hypothesis (4, 7, 14, 23, 29), greater hysteresis of the parenchyma than the airways in asthma results in bronchoconstriction post DI (SGaw<1). However parenchymal hysteresis results in lower lung recoil pressure post DI and would lessen the retractile force on the airway wall. This would render the airway more, rather than less, susceptible to compressive forces during forced expiration, thus condition (ii) is not satisfied.

As an alternative explanation of the disparate findings of the effects of DI in this and other studies I propose the following hypothesis:

A large proportion of the increased thickness of the airway wall in asthma is due to inflammation, which includes: increased vascularity, leaky capillaries, inflammatory exudate and oedema. Even in stable situations the equilibrium of intra /extra vascular

fluid flux is dynamic and delicately balanced. This equilibrium could clearly be altered by the large, negative intra thoracic pressure generated during a rapid deep inspiration. Such pressure applied to a leaky, low-pressure capillary bed would cause a net extravasation of fluid into the airway wall, increasing its thickness and reducing its lumen (and thus reducing SGaw). The increased interstitial fluid would also render the airway wall more turgid, reducing its compliance as recently reported with airway wall inflammation in vitro (110, 111). Consequently the airway would be less susceptible to compressive forces during the subsequent forced expiration. As this putative effect is distinct from DI induced smooth muscle stretch, it may account for the lack of correlation between the M/P and SGaw ratio in asthmatic subjects. In healthy subjects without inflammatory changes such a mechanism would be absent or insignificant.

In summary, therefore, DI associated reduction in smooth muscle tone due to stretching is likely to occur in both healthy and asthmatic subjects, although there is evidence that this effect may be diminished in asthma. A reduction in smooth muscle tone would tend to dilate the unstressed airway and reduce airway wall rigidity. This would increase SGaw but the effect on forced expiratory flow would be determined by the relative magnitude of these two opposing effects. I suggest that in inflamed asthmatic airways extravasation of fluid during DI would reduce lumenal diameter and therefore SGaw but at the same time it would increase airway wall rigidity and thus *tend* to increase forced expiratory flow post DI.

The response of maximal flow and SGaw to DI in any individual asthmatic subjects would then be the net result of the effects of the reduction of smooth muscle tone and fluid flux in the airway wall.

In conclusion the observed response to DI clearly depends on the method of assessment. Thus the simple descriptors 'bronchodilatation' and 'bronchoconstriction' are inadequate to describe that response fully. These findings are clearly of practical importance to future studies of the effect of deep inspiration on airway function. In addition, the dependence of airway function on both the lung volume and the method of assessment may be of broader interest as it may provide insight into the pathophysiology of the asthmatic airway.

Chapter 5

Airway Hyperresponsiveness in Asthma: Not just a problem of smooth muscle relaxation with Inspiration.

INTRODUCTION

The relative attenuation of the bronchodilating effect of DI seen in asthmatics led Fish et al (13) to hypothesise that hyperresponsiveness in asthma is caused by impaired ability of inspiration to stretch airway smooth muscle. Skloot et al (9) reasoned that if this hypothesis were true, the sensitivity to inhaled methacholine of normal and asthmatic subjects should be the same if the challenge was carried out under conditions where deep inspiration was prohibited. They performed methacholine challenge under such conditions in asthmatic and control subjects and found no apparent difference in the responses of the two groups. There are theoretical reservations that their index of bronchoconstriction may obscure differences between the responses of the two groups. The hypothesis is re-tested using an index not prone to such an error.

SUBJECTS AND METHODS

I studied 10 mildly asthmatic and 10 normal subjects (tables 3.1–3.2 and 5.1). Subjects performed a modified version of a standard methacholine challenge as described in chapter 3 (3.6 Methacholine Challenge - dosimeter method). As described earlier, the requirement for both a consistent DI free period and a standard time interval from delivery of methacholine to measurement of expiratory flow limited the number of 'partial' manoeuvres to one at each stage of challenge. As the volume at which subjects commenced their partial expiration inevitably varied, the lung volume (expressed as percentage **baseline** VC) at which 'partial flow' (Vp) was measured was selected such that in all subjects at all stages of challenge, the partial expiration was commenced above that point. The greatest volume that satisfied this condition was 35% baseline VC (65% expired). The expiratory flow at this volume during a partial expiration, Vp_{35} , was extracted using software linked to the flow sensor (Sensormedics, Vmax). In addition I calculated the index τ used by Skloot et al (9) (defined as: FET_{25-75} / ln3, where FET_{25-75} is the time to expire the middle 50% of the partial VC) from the same forced partial expiratory manoeuvres. The volume at the end of tidal inspiration (EILV) just prior to the forced partial expiration was determined as a percentage of baseline VC using the subsequent TLC as a reference point.

All controls completed the challenge sequence up to a total cumulative dose of 6,400μg methacholine. For a number of the asthmatic subjects the challenge was terminated by a 40% fall in FEV_1 before the dose sequence was complete. The highest delivered dose common to all subjects (asthmatics and controls) was 50 μg.

Comparison of response between the two groups was therefore made at this dose. All comparisons between the two groups were made using the two-tailed Student's t test.

(Table 5.2)

As expected, there was a clear difference in the standard response to challenge as measured by FEV1 between the two groups. In addition however, the highly significant difference in response as measured by Vp_{35} shows that even in the absence of DI the asthmatic subjects were more responsive. The difference in response as measured by the τ index, which had been derived from the same forced expiratory manoeuvres as Vp_{35}, failed to reach statistical significance. I found a greater rise in EILV and RV between baseline and 50μg methacholine in asthmatics than controls.

Table 5.1

Baseline Characteristics of Subjects

	Age Mean (sd)	FEV1(% pred) Mean (sd)	FEV1 PD_{20} Median
Asthmatic	28.4 (6)	96.6 (11.0)	128 μg
Control	29.0 (6)	101.2 (10.0)	>6400μg

Characteristics of subjects including response to methacholine challenge as measured by FEV1 PD_{20}, the provoking dose causing a 20% fall in FEV1 from baseline.

Table 5.2

Measurements pre challenge and after inhalation of 50 μg methacholine

	Asthmatic	**Control**	**p value**
FEV1 (l) baseline	3.53 (0.81)	4.21 (0.83)	0.082
FEV1 (l) post Mch	2.86 (0.92)	4.03 (0.71)	0.006
FEV1 post Mch, % baseline	80.6 (16.8)	96.5 (6.7)	0.018
Vp_{35} (ls^{-1}) baseline	2.25 (0.90)	3.40 (1.08)	0.019
Vp_{35} (ls^{-1}) post Mch	0.44 (0.41)	2.52 (1.33)	0.0008
Vp_{35} post Mch, % baseline	25.9 (27.8)	72.1 (21.8)	0.0007
τ (s) baseline	1.06 (0.43)	0.64 (0.20)	0.014
τ (s) post Mch	2.79 (1.44)	1.17 (0.65)	0.007
τ post Mch, % baseline	277 (139)	184 (60)	0.075
Δ EILV (% baseline VC)	15.0 (13.2)	2.4 (6.9)	0.019
Δ RV (% baseline VC)	15.1 (13.7)	1.49 (6.54)	0.015

Between group comparisons in terms of FEV1, the partial flow at 35% baseline VC (Vp_{35}) and τ. Baseline values, values after a dose of 50 μg Mch and *response* (post Mch values as a % of baseline value) are shown. Values are mean (sd). RV in this table relates to RV at the end of partial expiration.

DISCUSSION

The hypothesis under investigation is that 'asthmatic hyperresponsiveness is due to a problem of smooth muscle relaxation with deep inspiration' (9). This idea was first mooted by Fish et al in 1981(13). At the heart of the hypothesis is that the asthmatic and healthy responses to DI differ. Fish et al compared the effect of DI on methacholine induced bronchoconstriction in asthmatic and non-asthmatic subjects. The reduction of airway resistance following DI in controls was less or absent in asthmatic subjects. The results were attributed to a failure, in asthmatic subjects, of DI to stretch (and relax) airway smooth muscle. Subsequently, a number of authors have investigated the response to DI in asthmatic subjects. While attenuation of the bronchodilator effect in asthma has been a common finding (5, 6, 16, 17) several authors have found a bronchoconstrictor effect of DI in some asthmatics, particularly the more severe (3, 5, 6, 16, 17, 19, 112). Such findings do not contradict Fish's proposed mechanism but cannot be entirely explained by it, as an additional mechanism to account for the bronchoconstriction is required. The mechanism(s) involved have been the subject of much debate. No final consensus has been achieved though some data support a mechanism involving an altered balance between airway and parenchymal hysteresis (7). Whatever the mechanism, the diminution (or reversal) of the bronchodilating effect of DI on induced bronchoconstriction in asthmatics implies that if DI is prohibited the difference in responsiveness between asthmatic and normal subjects is inevitably reduced. It is therefore important to determine whether, in the absence of DI, there is complete loss of asthmatic hyperresponsiveness or only the relative diminution, which would be predicted by previous studies. Only complete loss would support the revised (and more limited)

hypothesis that: hyperresponsiveness in asthma can be accounted for entirely by the altered response to DI.

Studies (45-47) using specific airway conductance (SGaw) as the index of airway function have consistently shown hyperresponsiveness in asthmatic subjects. In principle such measurements are independent of DI but it is not clear from these papers to what extent, if any, DI was prohibited prior to the measurement of SGaw. The influence of any preceding DI therefore could not be excluded.

In the present study I have measured the responses of asthmatic and control subjects to methacholine in the absence of DI.

All controls completed the challenge sequence up to a total cumulative dose of 6,400μg methacholine. For a number of the asthmatic subjects the challenge was terminated by a 40% fall in FEV_1 before the dose sequence was complete. The highest delivered dose common to all subjects (asthmatics and controls) was 50 μg. At this dose the mean fall in FEV1 in the control group was only 3.5%. However even at this relatively early stage of challenge this was statistically different to the mean asthmatic response (19.4%). At issue is whether in the absence of the effect of DI the asthmatic and control responses would be the same.

To test this requires an index of bronchoconstriction that is independent of both the effects of DI and any change in EILV that occurs as bronchoconstriction progresses. My results show that the latter is relevant as a greater increase in EILV during challenge was seen in the asthmatic subjects. Independence from DI was achieved by prohibiting DI for approximately four minutes prior to measurement of the partial flow. Volume independence was ensured by measuring Vp at the same absolute lung volume at each stage of challenge, in this case 35% baseline VC. The assumption that Vp_{35} is measured at isovolume relies on the assumption that TLC remains unchanged

during induced bronchoconstriction. The data of Kirby et al (101) and Lougheed et al (102) support the validity of this assumption as both studies showed no change in TLC with methacholine induced airway narrowing.

Using partial flow I found a highly significant difference between the asthmatic and control responses to methacholine challenge, implying that even in the absence of DI, asthmatics are more responsive to methacholine than controls. This leads us to the conclusion that hyperresponsiveness in asthma cannot be attributed entirely to an abnormal response to DI. This conclusion would appear to contradict the results of Skloot et al (9) who, using a different protocol, found that in the absence of DI asthmatic and control responses were similar. There are however, two essential methodological differences between this study and that of Skloot et al.

I used isovolume flow as the main index of bronchoconstriction, whereas they derived an index in the time domain.

Skloot et al prohibited deep breaths for the entire duration of the challenge. The protocol in this study allowed DI during the challenge, although partial manoeuvres were preceded by a four-minute DI-free period.

Let us consider each of these in turn:

<u>1. The 'τ' index:</u>

Skloot et al (9) derived an index of bronchoconstriction from partial forced expiratory manoeuvres. Since the lung volume (EILV) at which forced expiration is initiated varies, they defined an index in the time domain, τ, which equals the forced expiratory time between 25% and 75% of the partial expiration divided by the natural log of 3. They showed that the response to methacholine as measured by τ was similar in

asthmatic and control subjects and concluded that asthmatic hyperresponsiveness was attributable to a lack of smooth muscle relaxation with deep inspiration. The volume independence of the index relies on the assumption that the volume-time relation during forced expiration approximates to a monoexponential function. Under this assumption Skloot et al argue that the τ index is 'equal to the reciprocal of the mean slope of the flow volume curve between 25% and 75% of the forced expiration' (9). In fact the assumption implies that the descending limb of the flow volume curve is rectilinear and that τ is equal to the reciprocal of the gradient of the entire slope of the flow volume curve. Whilst this is a reasonable assumption in healthy young subjects, with airway narrowing the flow volume curve is characteristically concave and therefore the volume time curve is not monoexponential. The more severe the airway narrowing, the further the deviation from this assumed curve. This implies that τ is not independent of volume, rather it will decrease if tidal breathing occurs over a higher volume range. For a given degree of bronchoconstriction therefore, an increase in EILV (or RV) will result in a lower value of τ suggesting less bronchoconstriction. The rise in EILV as bronchoconstriction progresses therefore tends to mask the change in τ. As the rise in EILV was greater in the asthmatic subjects than in the healthy subjects (mean 15% vs 2.4% of baseline FVC respectively), the masking effect would be greater in that group. The net effect would therefore be to underestimate the difference in responsiveness between the two groups. When assessed in a typical asthmatic subject, a 15% rise in EILV reduced the observed increase in τ over the course of challenge by 54%. A change in EILV in healthy subjects has less effect on τ because, as described above, the volume time relation of forced expiratory flow more closely approximates to a monoexponential, FET_{25-75} and τ are therefore less volume dependent. When assessed in a typical healthy subject a

2.4% increase in EILV at the end point of challenge was found to have no measurable effect on τ. (For a more detailed account of the mathematical arguments discussed here see appendix 2.)

I found a clear difference in the response as measured by Vp_{35} between the two groups. When τ was applied to the same partial expiratory manoeuvres, I found, as did Skloot et al (9), a difference in responsiveness, which was not statistically significant. This suggests that the absence of a significant difference in τ in both studies may be due to an inherent flaw in the index, rather than a true absence of difference.

In the current study the responses of the two groups were compared after a cumulative methacholine dose of 50μg, at which point the mean fractional changes in τ in asthmatic and healthy subjects were 1.77 and 0.84 respectively (a difference which did not achieve statistical significance, $p = 0.075$). At the point of comparison in the study by Skloot et al (9) the mean fractional changes in τ in both asthmatic and healthy subjects were considerably less at 0.263 and 0.245 respectively. I have therefore also compared the responses of the two subject groups in the current study after a dose of methacholine which in the healthy subjects produced a similar mean change in τ to that seen in the Skloot study. After 3.125μg of methacholine the mean fractional changes in τ in asthmatic and healthy subjects were 0.34 and 0.25 respectively ($p = 0.62$), ie as in the study by Skloot et al, at this stage of challenge the asthmatic and healthy responses are indistinct.

I would argue therefore, that the apparent difference between the results of the two studies (the current demonstrating a distinction between asthmatic and healthy responses while the Skloot study did not) is due to a difference in the index of airway function used rather than a difference in either subject selection or challenge protocol.

2. Duration of the DI free period.

It was my experience that prohibition of DI for the entire duration of the challenge was impracticable. Even if DI can be consciously resisted for prolonged periods the cough frequently provoked by methacholine effectively ends the DI-free period.

In addition to the practical difficulties I had reservations as to whether the prohibition of DI for such an extended period was actually desirable. The available evidence on the duration of the effect of DI (5, 25, 26) suggests that this is brief. Using SGaw, Lim et al (5) attempted to quantify the duration of the effect of DI separately in two groups, one who showed bronchodilatation, the other bronchoconstriction after DI. The group showing bronchodilatation had a quicker recovery from the effect of DI than did the group demonstrating bronchoconstriction. Even with the slower recovery of the second group there was effectively complete restoration of baseline calibre well within a four-minute period. Pellegrino et al (26) also using SGaw, found almost complete restoration of baseline calibre 10 seconds and 60 seconds after DI in controls and asthmatics respectively. Green and Mead (25) measuring partial flow at various time intervals after deep inspiration in healthy subjects found most of the bronchodilating effect of DI had worn off after only 5 seconds. One study (28) demonstrated a measurable bronchodilating effect of DI on forced expiratory flow beyond four minutes (up to 6 minutes). This however was the apparent effect of a DI associated with an FEV1 manoeuvre performed before the administration of methacholine, its effect being measured after the administration of methacholine. This effect was demonstrated in healthy subjects only. There is no evidence that this small residual bronchoprotective effect, so long after DI, would be different in asthmatic and healthy subjects. Whilst it is theoretically possible that in this study a difference in the response (asthma vs. healthy) to the preceding DI may just have been

measurable at 4 minutes, most of the literature would seem to suggest it is either absent or negligible so late after the event. It would seem unlikely to account for the magnitude of the difference (asthma vs. healthy) in response to methacholine observed in this study.

The short term bronchodilating effect of DI is probably related to (though perhaps not entirely accounted for by) stretching of smooth muscle, as an enhanced bronchodilating effect to DI is seen in the presence of smooth muscle constrictors (1, 5, 6, 8) and a diminished bronchodilating effect of DI in the presence of beta 2 agonists (6, 16, 23). On the other hand prolonged inhibition of DI, particularly in the context of induced bronchoconstriction, may have other effects, in particular widespread micro-atelectasis. This in itself could affect expiratory flow. If we wish to study the effect of DI on smooth muscle tone such potentially confounding effects are better avoided.

On the basis of the evidence available I would suggest that the four minute DI free period used in this study is sufficient to identify the short term effects of DI, whilst not being so long as to induce unwanted effects, unrelated to smooth muscle stretching, on expiratory flow.

In summary, less difference in the responsiveness of asthmatic and control subjects to methacholine when DI is prohibited is implicit in the results of earlier studies. At issue is whether in the absence of DI, the asthmatic and control responses to methacholine are actually the same. My results suggest that the responses remain different even if DI is avoided, thus refuting the hypothesis that asthmatic hyperresponsiveness can be accounted for entirely by an altered response to DI. These results differ from those of Skloot et al. (9) and I suggest that the main reason for this difference is that the τ index does not allow valid comparison between asthmatic and

control responses when the volume time relation during forced expiration does not conform to a mono-exponential and the relative volume ranges of tidal breathing differ.

I conclude therefore that airway hyperresponsiveness in asthma cannot be accounted for solely by an abnormal response to deep inspiration.

Chapter 6

Prediction of the Ultimate Outcome to Methacholine Challenge (A plateau response or apparent unlimited narrowing) using Early Changes in Small Airway Function.

INTRODUCTION

In 1984 Woolcock at el (33) described the shape of the dose response curve to bronchial challenge in asthmatic and healthy subjects. Bronchial challenge (with Histamine) was continued until a 60% fall in FEV1 occurred. Perhaps the most important finding in the Woolcock study was that the normal and 2 mildly asthmatic subjects demonstrated a plateau response. I.e. in these subjects a point was reached beyond which no further airway narrowing occurred despite increased doses of Histamine. The other subjects demonstrated a 60% fall in FEV1 in response to challenge (at which point the challenge was terminated) apparently demonstrating the capacity for unlimited narrowing. It could be argued that the presence or absence of this capacity for apparent unlimited narrowing is a more important facet of response than PD_{20}(FEV1). It would appear to have significant implications in acute severe asthma and the potential for fatality. Its potential clinical usefulness is considerable. The practical difficulties in its measurement however, probably preclude its use as an everyday clinical tool.

Assuming that, in the absence of significant bronchoconstriction, FEV1 is determined principally by the larger airways I reasoned that early in challenge changes in FEV1 probably reflect changes in calibre of the larger airways. Given their cartilaginous support however, narrowing of the central airways will be limited and their behaviour

will not determine the capacity for unlimited narrowing. Therefore, early changes in FEV1 may not predict ultimate outcome. Unlimited narrowing is more likely a function of the smaller airways. To test this hypothesis the predictive power of the response of a number of indices of small airway function, early in challenge was examined, including the index PD_{20}(Vm20), the dose causing a 20% fall in the maximal flow at 20% (remaining) VC early in challenge.

METHOD

I studied 13 mildly asthmatic and 11 normal subjects (Tables 3.1, 3.2 and 6.1). Methacholine challenge was performed as described in chapter 3 (3.6 Methacholine Challenge - dosimeter method) to a maximum of 6,400 µg (asthmatics) or 51,200 µg (controls). Challenge continued until either: a 40% decrement in FEV_1 was recorded or the dose sequence completed or a maximal (plateau) response recorded. A plateau response was defined as a fall in FEV1 of <5% over three successive doses. Flow measurements at Vm and Vp at various volume points, on the maximal and partial curves respectively, were derived at the same absolute lung volumes at each stage of challenge. As the volume at which subjects commenced their partial expiration inevitably varied, There was no lung volume (expressed as percentage **baseline** VC) at which Vp could be measured in all subjects at all stages of challenge. The greatest lung volume which afforded us the capacity to measure the provoking dose required to produce a 20% fall in the partial flow (PD_{20}(Vp)) in a majority of subjects was 35% baseline VC. Thus Vp35 was measured and PD_{20}(Vp35) calculated. Response to challenge was also measured in terms of the provoking dose required to produce a fall of 20% in: FEV1, Vm20, and Vm35 and a 10% fall in Vm20; PD_{20}(FEV1), PD_{20}(Vm20), PD_{20}(Vm35), and PD_{10}(Vm20) respectively subjects were also categorised in terms of those who demonstrated a 40% fall in FEV1 and those who did not.

Protocol

Day1

All subjects underwent methacholine challenge (dose range 3.125μg to a maximum of 6,400 μg). Non-asthmatic subjects who did not demonstrate either a 40% fall or a maximal (plateau) response with this regimen returned for a second methacholine challenge on day 2.

Day2 (three weeks later)

Subjects returning underwent methacholine challenge with a dose range 25 μg to a maximum of 51,200 μg. Challenge continued until one of the following occurred: (1) The dose sequence was complete (2) A 40% fall in FEV1 occurred (3) The response curve developed a plateau (< 5% fall in FEV1 over final 3 doses) or (4) further doses were limited by systemic side effects of methacholine (Excessive sweating, salivation, pre-syncope or headache).

Those limited by systemic side effects after receiving doses in excess of 25,600μg without having demonstrated a 40 % fall in FEV1 were deemed not to have the capacity for unlimited narrowing.

In subjects who underwent the higher dose sequence challenge (day 2) responses to this challenge protocol are used in the analysis.

Analysis

All 'provoking dose' indices derived from the methacholine challenge were logarithmically transformed before inter group comparisons were performed. All comparisons between the two groups were made using a non-paired t test.

RESULTS

11 subjects (10 asthmatic, 1 control) demonstrated a 40% fall in FEV1 (group 1, '40% fall'). 9 control subjects demonstrated either a plateau response or less than a 40% fall in FEV1 at high dose (25,600μg or greater) methacholine (group 2, 'plateau'). (Table 6.1). One control and three asthmatic subjects failed to demonstrate either a 40% fall in FEV1 or a clear plateau at the maximum dose of 6,400μg in the case of the asthmatics and after withdrawing because of systemic side effects after 12,800μg in the case of the control subject, these 4 subjects were not included in the analysis.

In the case of PD_{20}(Vm35) two subjects who plateaued in response to challenge (in terms of FEV1) did not achieve a 20% fall in Vm35. For the purposes of statistical comparison their respective PD_{20} (Vm35) is recorded as the maximum dose given in each case (1,600μg and 51,200μg). This will tend to underestimate the difference in PD_{20} (Vm35) between the two groups.

In the case of PD_{20}(Vp35) the flow on the 'partial' expiration at 35% baseline vital capacity (Vp35) was only available at sufficient number of stages of challenge to allow determination of PD_{20} (Vp35) in 16 (9 asthmatics, 7 controls) of the 20 subjects. The lung volume at which the partial flow was commenced appeared almost random, though the theoretical systematic bias in only comparing partial flow in subjects who had a measurable partial flow at 35% VC would be to the selection of a more severe asthmatic group. This bias per se would not appear to undermine the finding of an absence of a statistically significant difference between the two groups in the PD_{20} (Vp35) index. However the reduced number of subjects available for comparison using this index does make a type 2 statistical error more likely.

Although values for baseline lung function (FEV1/FVC) were different between the two groups (p=0.04) there was considerable overlap between the two groups (fig 6.1). Baseline lung function could therefore not be used to predict the ultimate outcome of challenge with any degree of precision. In the subjects who failed to demonstrate a 40% fall in FEV1 none demonstrated a 20% fall either therefore in no subject in group 2 could a PD_{20}(FEV1) be determined. By definition all subjects in group 1 had a PD_{20}(FEV1), with median 77.5 μg. Clearly there were no overlapping values of this index between the two groups.

Log PD_{10}(Vm20) and Log PD_{20}(Vp35) were highly significantly different between the two groups ($p = 0.0001$ and 0.0007 respectively). Though in each case the ranges of values demonstrated some overlap (Table 6.1 and figs 6.2 & 6.3).

Log PD_{20}(Vm20) and Log PD_{20}(Vm35) were also highly significantly different between the two groups ($p < 0.000001$). In neither case did any of the values ranges overlap between the two groups (Table 6.1 and figs 6.4 & 6.5,).

The percentage change in FEV1 at the dose after which a 20% fall in Vm20 had been achieved was calculated for each subject. The mean value of this index was not different between the two groups ($p = 0.61$), (fig 6.6).

In subjects where a plateau, in terms of Vm35 and Vp35, could be established the maximum fall (mean of final 3 values) from baseline were: mean(sd)% 19.8(6.8)% and 63.5(11.2)% respectively.

Table 6.1

	Group 1 ('40% fall')	N	Group 2 ('plateau')	N	'p' value
FEV1/FVC	0.77(0.07)(0.65-0.86)	11	0.83(0.04)(0.78-0.88)	9	0.04
PD_{20}(FEV1)	166(230)(10.2-722.7)	11	No subject achieved a 20% reduction in FEV1	0	
Log PD_{20}(FEV1)	1.89(0.58)	11		0	
PD_{10}(Vm20)	6.89(10.6)(0.6-35.6)	11	3814(6810)(2.6-18920)	9	
Log PD_{10}(Vm20)	0.49(0.55)	11	2.54(1.24)	9	0.0001
PD_{20}(Vm20)	13.6(16.9)(1.1-48.4)	11	5398(7862)(94.5-23040)	9	
Log PD_{20}(Vm20)	0.79(0.59)	11	3.15(0.86)	9	<0.000001
PD_{20}(Vm35)	25.6(37.2)(1.5-127.1)	11	9435(16243)(181-51200)	7*	
Log PD_{20}(Vm35)	1.05(0.59)	11	3.46(0.77)	7*	<0.000001
PD_{20}(Vp35)	4.6(6.0)(1.1-19.8)	10	195(282)(5.6-688)	6	
Log PD_{20}(Vp35)	0.43(0.43)	10	1.75(0.81)	6	0.0007
FEV1 as % baseline after PD_{20}(Vm20)	94.3(4.0)(88.5-102.1)	11	93.4(3.7)(85.2-97.4)	9	0.61

For log data: mean(standard deviation)

For non-log data: mean(standard deviation)(range)

N: the number in each group contributing to the stated mean

* 2 subjects in group 2 had not achieved a 20% fall in Vm35 by the end of challenge. Thus only 7 subjects contribute to the calculated mean. The difference in the means therefore underestimates the difference between the two groups

Figure 6.1
FEV1/FVC in subjects who achieved a 40% fall in FEV1 and those who had a plateau in their response to methacholine challenge

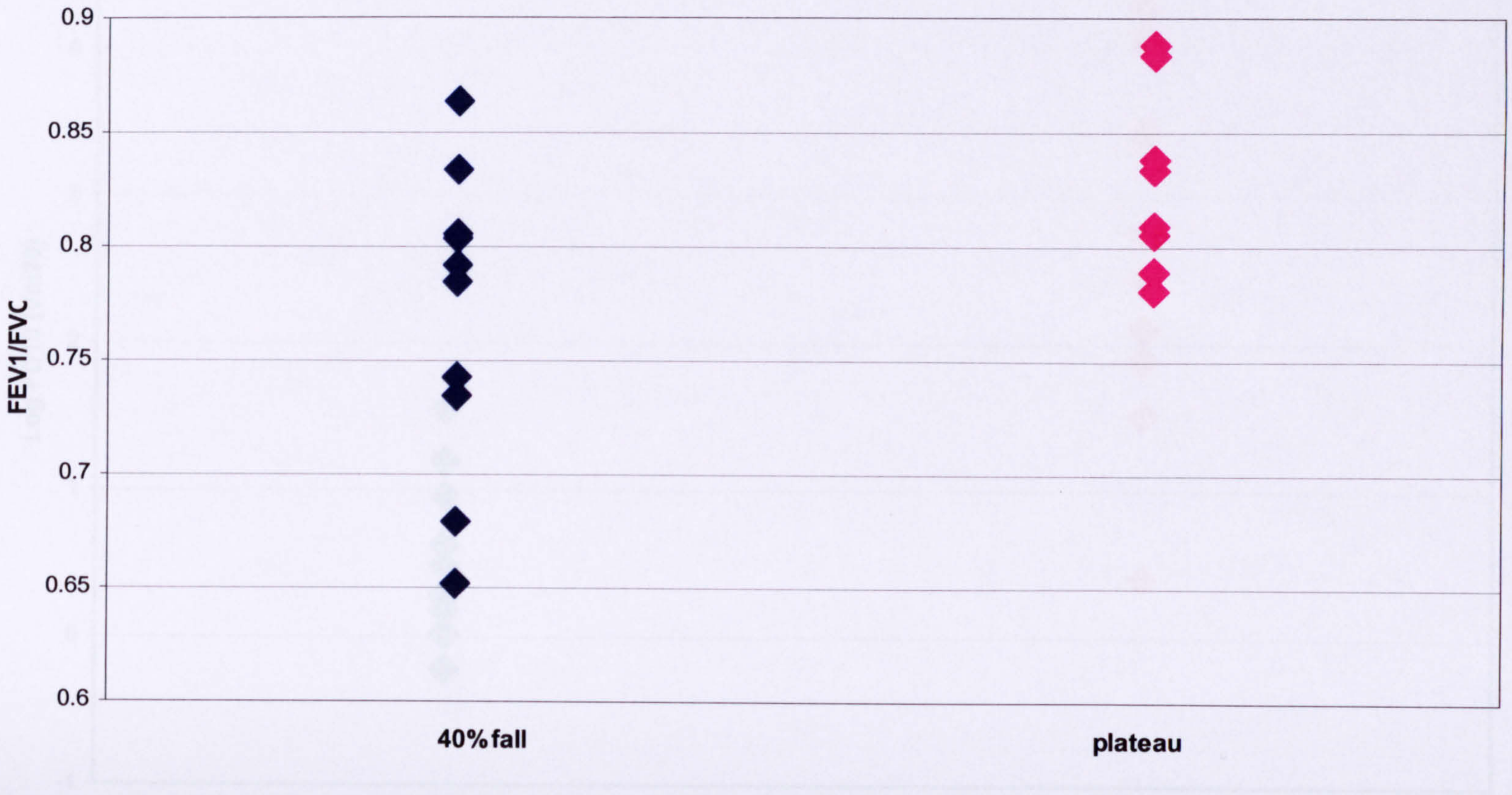


Figure 6.2
Log PD_{10} (Vm20) in those who achieved a 40 % fall in FEV1 and those who had a plateau in response to methacholine challenge

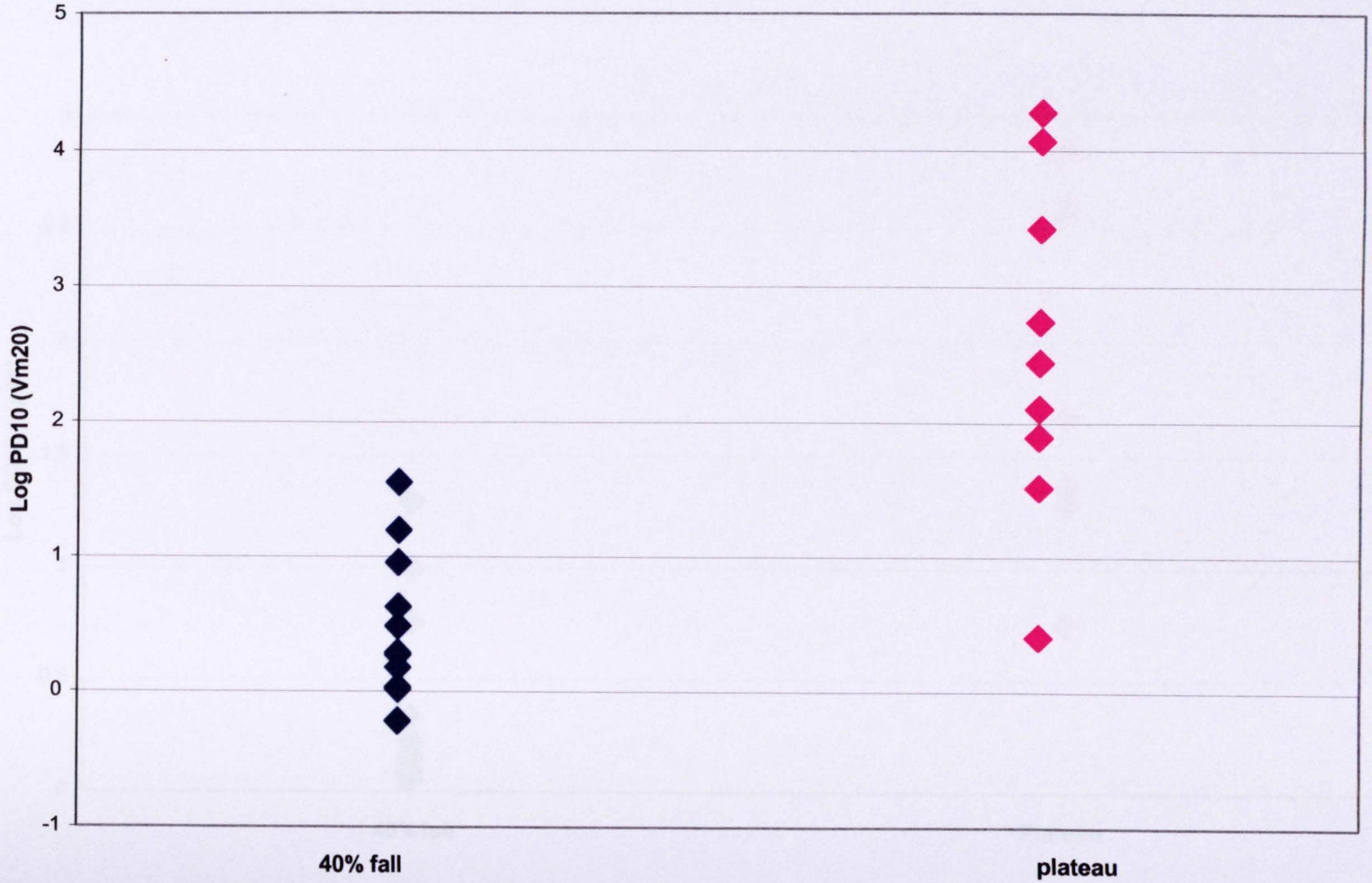


Figure 6.3
Log PD_{20} (Vp35) in subjects who achieved a 40% fall in FEV1 and subjects who had a plateau in response to methacholine challenge

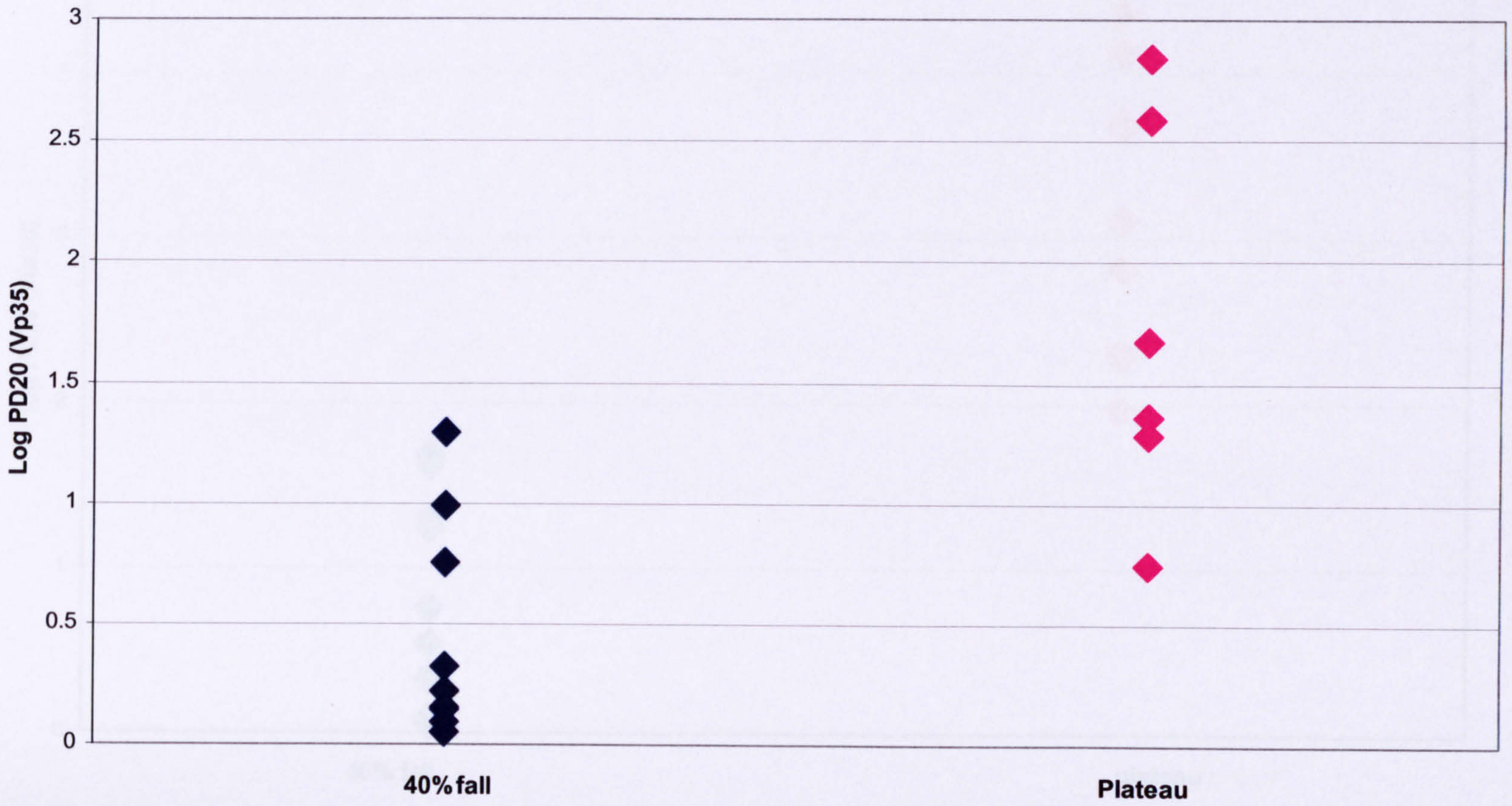


Figure 6.4
Log PD_{20}(Vm20) in those who achieved a 40% fall and those who had a plateau in response to methacholine challenge

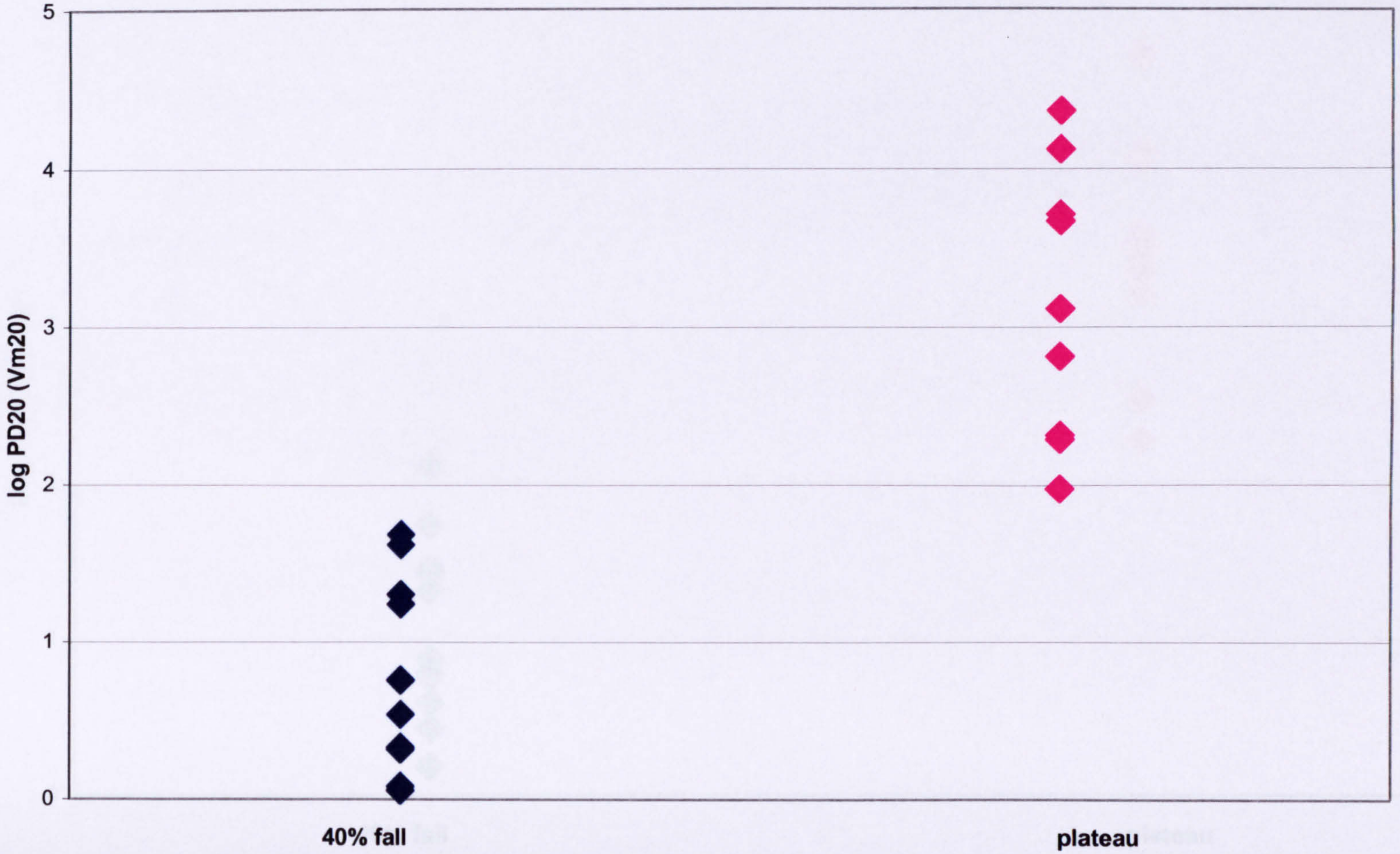


Figure 6.5
Log PD_{20} (Vm35) in subjects who achieved a 40% fall in FEV1 and those who had a plateau in response to methacholine challenge

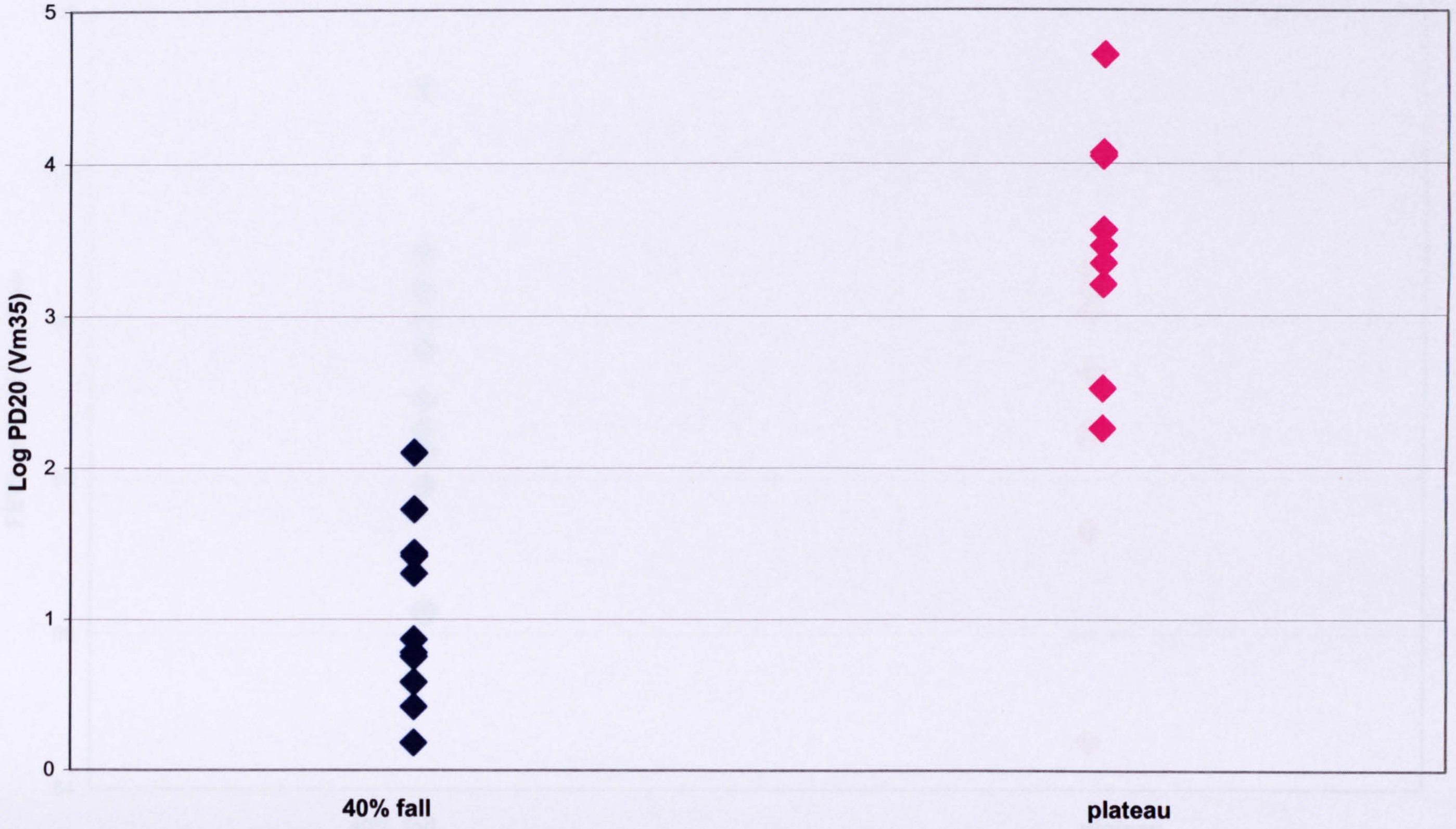


Figure 6.6
% change in FEV1 at dose which achieved 20% fall in Vm20 in subjects who demonstrated a 40% fall in FEV1 and subjects who had a plateau in response to methacholine challenge.

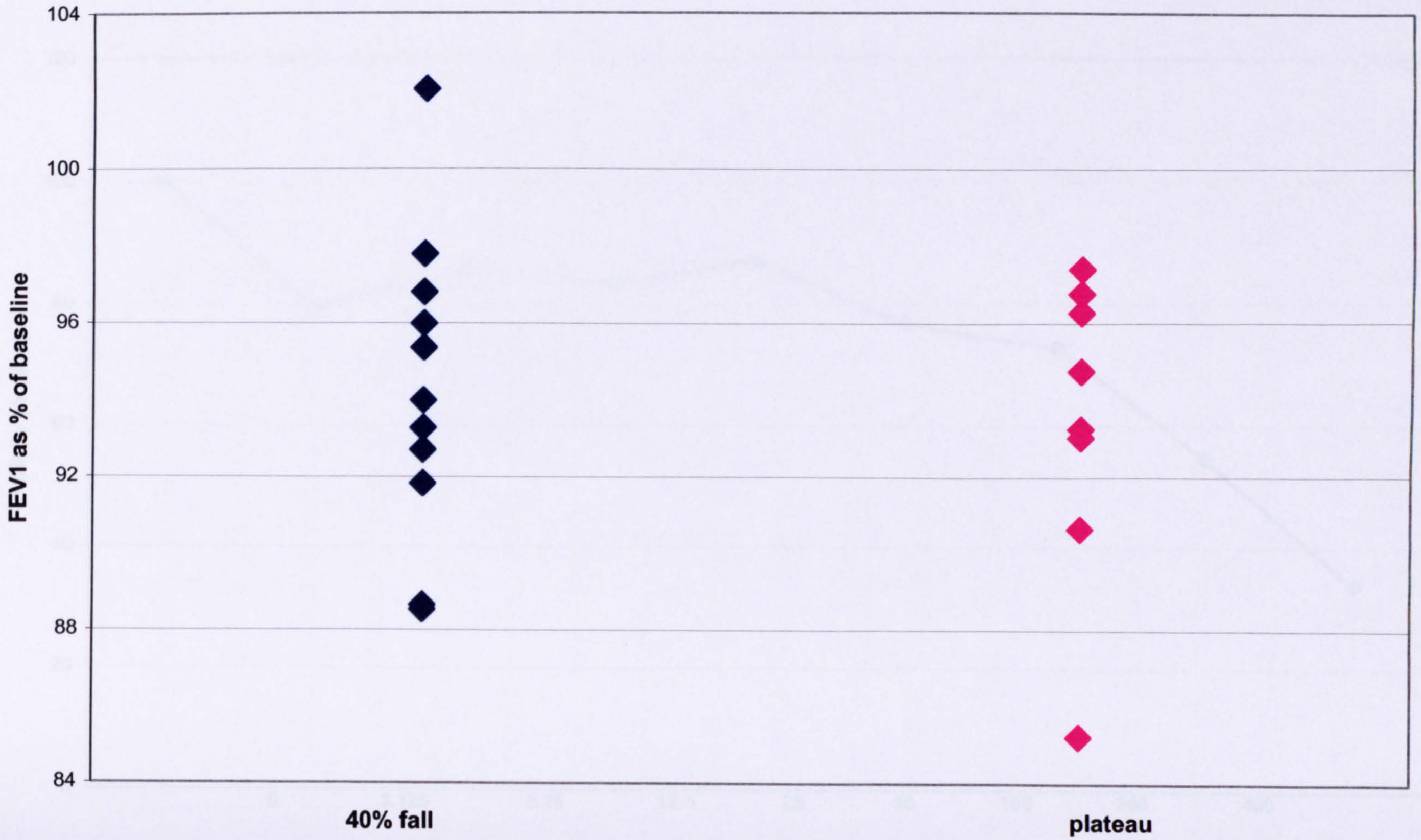
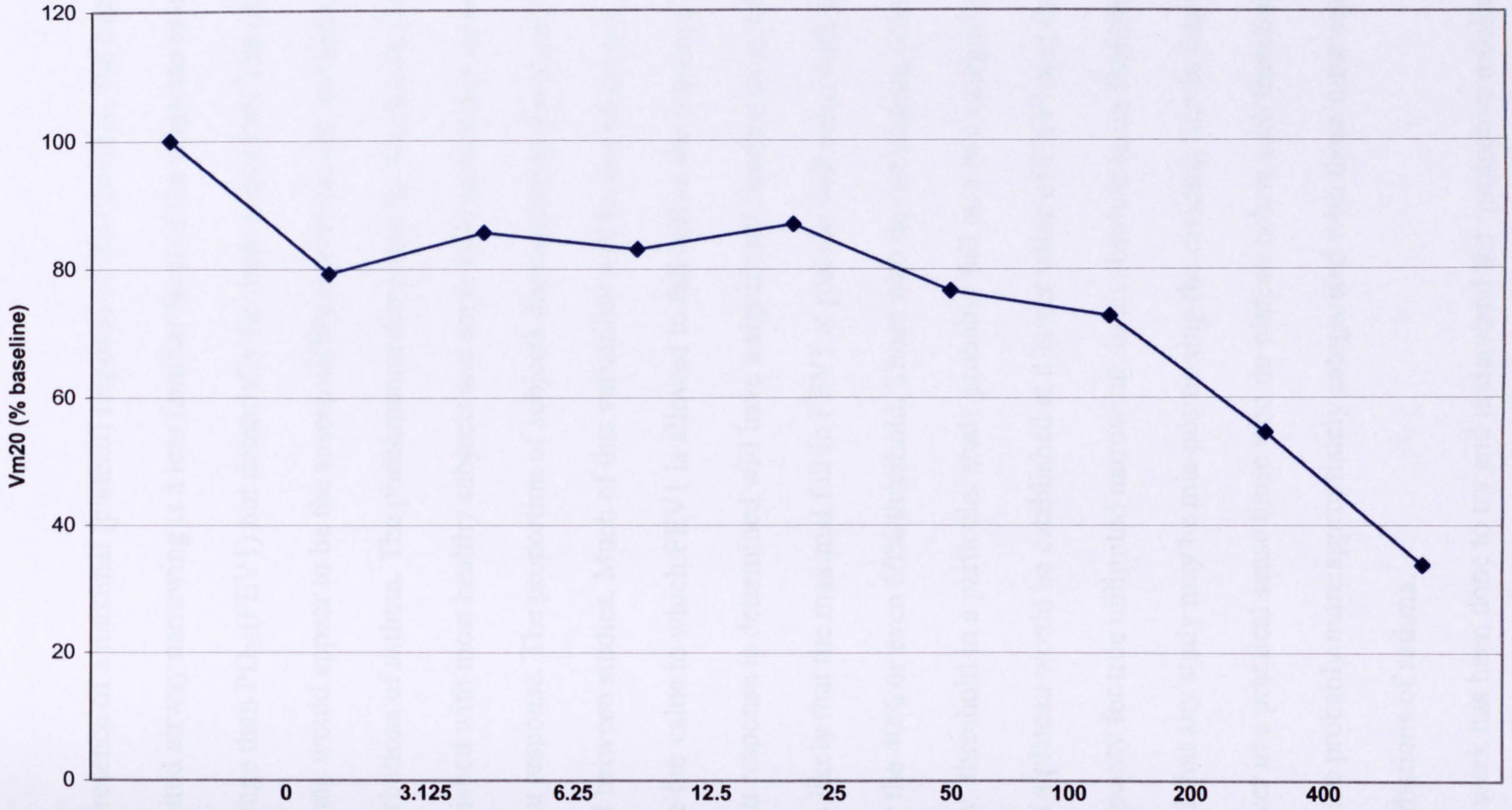


Figure 6.7
Change in Vm20 during methacholine challenge in subject 1a



DISCUSSION

The presence of a maximal (plateau) response or the converse, the capacity for unlimited airway narrowing is a less familiar facet of the response to bronchial challenge than PD_{20}(FEV1) but potentially far more important. The obvious clinical correlate would appear to be the susceptibility to very severe, potentially fatal, exacerbations of asthma. The phenomenon described by Woolcock (33) is now well established with most healthy subjects and some mild asthmatics demonstrating a plateau response. The proportion of subjects demonstrating the phenomenon has varied between studies. Much of this variability will be due to subject selection but of course the value to which FEV1 is allowed to fall before the presence or absence of a plateau response is determined will have a significant bearing on it. Intuitively it seems likely that the maximal fall in FEV1 at plateau will vary, with some being below the 40% or even 60% threshold. Those who do not plateau prior to the given 'safety' threshold in a particular study probably fall into two categories: Those in whom a plateau would be established at a lower value of FEV1 and those who have the capacity for true unlimited narrowing. In an absolute sense therefore it could be argued that any study may be mis-positioning the dividing line in this dichotomy, however in a practical sense those who do plateau below such threshold (particularly 60%) are probably more appropriately categorised with those most susceptible to fatal exacerbations of asthma.

Some work has been done to try and understand the 'protective mechanism' that healthy and some asthmatic subjects seem to possess which limits airway narrowing. Sterk et al (35) found that limited maximal airway narrowing to methacholine in nonasthmatics is not due to a change in adrenergic, cholinergic, or ganglion-

transmitted-nonadrenergic inhibitory activity nor to the release of prostaglandins. A study by De Jongste et al (36) which compared maximal bronchoconstriction in vivo and airway smooth muscle responses in vitro found no significant correlation. Suggesting that maximal bronchoconstriction in vivo is not limited by the maximal contractility of airway smooth muscle. This was consistent with the findings of Sterk et al (37) who found the response to combined histamine and methacholine was not significantly larger than the maximal response to histamine alone, suggesting that the plateau is due to factors other than limited smooth muscle activation.

I considered whether the plateau of the dose-response to methacholine, could be explained by the airway dilation that follows deep inspiration (DI) in non-asthmatics, a bronchoprotective factor known to differ between asthmatic and healthy subjects. In common with a previous study by Sterk et al (49) in the subjects in whom plateaus could be established I found the maximum fall in Vp35 to be greater than the maximum fall in Vm35. This, of course, confirms previously reported data on the greater bronchodilating effect of deep inspiration in the context of induced bronchoconstriction. It is clear therefore that DI had offered some degree of bronchoprotection in these subjects. The response to DI is therefore clearly an important factor at least in terms of limiting the maximal plateau response. However the existence of a plateau in a partial flow response to challenge implies that a 'mechanism' limiting airway narrowing in response to challenge would appear to be operating independent of the effects of deep inspiration.

In an animal model Mink et al (109) demonstrated that the maximum expiratory flow at a low lung volume (50% FVC) was a sensitive index of small airway narrowing. On this premise, the studies which compare the degree of maximal narrowing at

different lung volumes (38, 41) would seem to suggest that the capacity for unlimited narrowing, where it exists, lies within the small airways.

An in vitro study by Mitchell et al (42) reported changes in response to acetylcholine (ACh) with concurrent measurement of smooth muscle shortening, lumen narrowing and flow in large and small porcine bronchi. Maximum muscle shortening and lumen narrowing was greater in small than large bronchi. Small airways were 250-times more sensitive to ACh than large airways, for all measurements. But perhaps most interestingly high doses of ACh stopped flow in small bronchi, but produced a plateau in large bronchi, the cartilaginous support of the large airways perhaps protecting them from unlimited narrowing, even in the context of maximal smooth muscle stimulation.

Whatever the underlying mechanism and whatever the site within the airways the presence or absence of the capacity for unlimited narrowing is clearly important with potentially important clinical ramifications. To challenge subjects to a maximal 60% or even 40% maximal fall in FEV1 would not seem to be an appropriate investigation for routine clinical practice.

Even the complex mathematical modelling used by Aerts et al (40) failed accurately predict to the plateau even when many of the data points from the challenge were fed into the model. It would seem that the early changes in FEV1 may not actually contain the information required to estimate ultimate outcome of challenge.

I reasoned that if the small airways were indeed the site where the capacity for unlimited narrowing resided then early changes of small airway function during challenge may predict the ultimate outcome. If so this would provide further support for small airways as the site of this facet of response and may suggest a possible practicable clinical test of the presence of the capacity for unlimited narrowing.

By chance the subjects in this study who demonstrated a plateau all had maximal falls in FEV1 of less than 20% thus in no subject from this group could a PD_{20}(FEV1) be established. There is no absolute reason why this should be so, maximal responses could lie between 20% and 40%. The fact that in this group no subjects were in that range implies a clear separation between the two groups in the study. The 'predictive power' of the indices under investigation therefore may be somewhat overestimated and some caution should be exercised when interpreting the results.

The results in this study show that baseline lung function in the form of FEV1/FVC has no predictive power for ultimate outcome to challenge.

In this study PD_{20}(FEV1) has 100% sensitivity and specificity for predicting the ultimate outcome of challenge. That is to say subjects in whom a PD_{20}(FEV1) could be established i.e. demonstrated a 20% fall in FEV1 all failed to demonstrate a plateau in response. However in the study doses of up to 51,200µg were delivered before the failure to establish a PD_{20}(FEV1) was confirmed in all subjects. Thus in this study at least the presence or absence of a 20% fall in FEV1 did not provide an 'early warning' of the presence or absence of a plateau. PD_{20}(Vm20) and PD_{20}(Vm35) were also 100% sensitive and specific. Interestingly though these indices, PD_{20}(Vm20) in particular, were established at a much earlier stage of challenge than PD_{20}(FEV1). In fact the percent change in FEV1 at the dose after which PD_{20}(Vm20) was established (ie a 20% fall in Vm20 had occurred) was small and not different between the two groups. Albeit in a post hoc analysis we see that after just 5 doses of methacholine (cumulative dose 50mcg) the ultimate outcome to challenge was entirely predictable in all subjects from the change in Vm20 at that point. To use FEV1 in this way would have required a minimum of 9 doses (cumulative dose 800mcg).

One important caveat needs to be added at this point. In order to properly assess the response to challenge in terms of 'partial' flows – without the distorting effect of deep inspiration, the measurement of the 'partial' flow was performed first at each stage of challenge and the total number of deep breaths at each stage was limited to one (to allow measurement of FEV1 and 'maximal' flows). One limitation of this protocol therefore is the absence of averaging and the associated increase in 'noise' in the measurement of each index at each stage of challenge. This did not appear to have any significant bearing on the determination of PD_{20}(FEV1) in that there was no obvious random distortion of the response curve. However in the assessment of PD_{20}(Vm20) in 3 subjects (1 asthmatic, 2 controls) once the entire dose response curve was observed it was apparent the 'outlying' results could have led to a falsely low assessment of this index had it been used exclusively to determine end point of challenge. For example in subject 1a (fig 6.7) observation of the whole dose response curve suggests a PD_{20}(Vm20) between 25µg and 50µg. However had the challenge been performed with the sole purpose of determining PD_{20}(Vm20) and the occurrence of the first value for Vm20 below 80% of the baseline been taken as the end point of the challenge then a very different value for PD_{20}(Vm20) would have been determined.

By definition PD_{10}(Vm20) is established even earlier in challenge than PD_{20}(Vm20). However what is gained in speed may be lost in precision. As can be seen in fig 6.2, the ranges of values for the two groups showed considerable overlap implying a weaker predictive power.

Interestingly PD_{20}(Vp35), like PD_{10}(Vm20) although highly significantly different between the two groups, shows overlap in its value ranges and thus demonstrates a weaker predictive power. This may be due to the variability in response to deep

inspiration. The 'ultimate outcome' in challenge is usually determined by the change in a DI dependant value, FEV1, perhaps it is therefore best predicted by another DI dependent index, Vm35 say. Vp35 is one step further removed from FEV1 by the response to DI, which has the effect of adding 'noise' (a variable factor) to the index. In summary all the 'within challenge' indices have considerable predictive power for the ultimate outcome of challenge. Indices relying on very small changes in a value such as PD_{10}(Vm20) or those based on a flow independent of a DI may suffer from excess 'noise' which in turn weakens their predictive power. PD_{20}(FEV1), PD_{20}(Vm35) and PD_{20}(Vm20) in this study all predict outcome with 100% sensitivity and specificity. The indices of small airway function are however predictive at a much earlier stage of challenge. These data are supportive of the argument that the presence or absence of unlimited airway narrowing lies in the function of the small airways. It clearly suggests that the behaviour of these airways early in challenge reflect behaviour of FEV1 late in challenge. To establish a practicable test for 'early detection' of the ultimate outcome of challenge however requires further work. What the indices of small airway function gain in sensitivity they may lose in specificity, they are generally more volatile, less reproducible than FEV1. The presence of 'outlying' results in the case of PD_{20}(Vm20) as discussed above is perhaps a manifestation of this, though it should be remembered that because of the limitations of this protocol only one measurement of each value was performed at each stage of challenge. In a clinical context at least 3 measurements even of FEV1 would be performed at each stage of challenge with reproducibility being monitored. It seems likely that under such conditions PD_{20}(Vm20) for example would be more reproducible. Also in a practical sense the sensitivity of the index may compensate for a degree of volatility. For example PD_{20}(Vm20) varied by a factor of 44 within the

group that demonstrated apparent unlimited narrowing yet the mean values of the 2 groups differed by a factor of 397. Thus, in this group at least, the volatility of the index did not significantly hinder its predictive power. Of course this study did not provide us with any data on within subject reproducibility. Further studies may conclude that an 'optimum' index such as PD_{20}(Vm35) or even PD_{20}(Vm50) offers the best solution, being less sensitive than PD_{20}(Vm20) but probably more reproducible.

Chapter 7

The relationships between small airway mechanical function, the concentration of exhaled nitric oxide, the response to bronchial challenge and the response to deep inspiration.

INTRODUCTION

Exhaled nitric oxide (NO) is an index of airway inflammation in asthma. In various studies it has been shown to correlate with indices of asthma severity such as eosinophils in induced sputum and airway responsiveness. Most studies however report no correlation between NO and FEV1. The reason for this is not clear. It may be that the indices reflect different aspects of the disease process (one cellular function, one mechanical function), which are not necessarily closely correlated. Alternatively the two indices may be reflecting changes in different parts of the bronchial tree. FEV1 is an index largely dependent on the function of larger airways, whereas the principal site of production of the NO measured in exhaled breath is the terminal airways (74, 81). In chapter 6 I discussed the importance of the function of the small airways in determining the response to challenge and the relatively poor correlation between that response and baseline FEV1. This may go some way towards explaining the said disparity. I.e. exhaled NO may be reflecting inflammation (and thus function) principally in the small airways, this in turn would be expected to correlate with the response to bronchial challenge yet may be more loosely related to indices such as FEV1 which in the absence of significant airway obstruction is probably determined principally by the function of the more central airways.

In chapter 6 I discussed the importance of another facet of responsiveness, the presence or absence of a maximal (plateau) response. The function of the small airways is particularly important in determining this. In the study reported in chapter 6 changes in the function of small airways, very early in bronchial challenge, as measured by PD_{20}(Vm20) were 100% sensitive and specific for the presence of a plateau response. I therefore examined the relationship between exhaled NO and PC_{20}(Vm20), as a surrogate index for the ultimate response to challenge (plateau or unlimited narrowing). In chapter 6 the relationship examined was between changes in small airway function within challenge and the ultimate outcome. Baseline indices such as FEV (% predicted) were found not to correlate with the ultimate outcome. Focusing more specifically on smaller airway function at baseline and using Vm50 and Vm20 as indices of this, in this study I also looked directly at the relationship of Pre challenge Vm50 & Vm20 with PC_{20}(Vm20). I wished to know whether the function of the smaller airways at baseline (prior to the delivery of the first dose of methacholine) correlated with the ultimate outcome of challenge even though FEV1 (% predicted) may not.

My aim was to examine the relationships between: Pre challenge exhaled NO, baseline indices of smaller airway function (Vm50 & Vm20) and methacholine responsiveness (in terms of the standard index PC_{20}(FEV1) and PC_{20}(Vm20) as a surrogate marker of 'ultimate' outcome). The relationship of each of these indices to baseline FEV1 would also be considered.

The study reported in chapter 5 and the paper by Skloot et al (9) together establish the fact that although an abnormal response to DI in asthmatic subjects cannot in isolation explain asthmatic hyper-responsiveness to bronchial challenge it is a contributory factor. Similarly the study reported in chapter 6 together with previously published

studies (10, 41, 49)demonstrates that although an absolute limitation to narrowing in response to bronchial challenge is not entirely determined by the response to DI it does have a protective effect. The bronchodilating effect of DI will reduce the extent of (maximal) narrowing. In some subjects this may limit airway narrowing to a safer, non-fatal degree. Thus the relationship of the response to DI to both these facets of the response to bronchial challenge is complex. I therefore examined the relationship of that response, as measured by both M/P and SGaw ratio (which appear to be determined by different mechanisms (chapter 4) to both indices of the response to bronchial challenge PC_{20}(FEV1) and PC_{20}(Vm20), exhaled NO, baseline FEV1, Vm50 and Vm20.

METHOD

I studied 15 steroid naïve asthmatic and 10 normal subjects (tables 3.1, 3.2 and 7.1). Normal subjects were all hospital employees. They reported no symptoms of asthma, had never received a diagnosis of asthma from a physician and had normal spirometric volumes. All subjects were non-smokers and had had no recent upper respiratory tract infection. Approval was obtained from the local Ethics Committee and written informed consent was obtained. The various indices of respiratory function were derived according to the following protocols.

Methacholine Challenge (tidal breathing method), exhaled NO, spirometric values, SGaw, SGaw ratio (pre and post DI) and maximal / partial (M/P) ratio were all derived by the methods described in chapter 3. The tidal breathing method for methacholine challenge was used as this method allowed a greater DI free period prior to the measurement of partial flows than the dosimeter method. The maximum expiratory flow at 50% and 20% vital capacity (Vm50 & Vm20) were derived from the same manoeuvres as FEV1. Because of the inter-individual variability in EILV the greatest lung volume at which an M/P ratio could be derived varied between subjects. The highest volume common to all subjects was 30% VC (70% VC expired). Three technically good manoeuvres were obtained in each subject. The mean M/P ratio at 30% VC was calculated.

Protocol

After an initial screening visit subjects returned at a later date and performed the respiratory function tests in the following order:

1. M/P ratio followed by a 15 minute rest period. **2.** SGaw ratio. **3.** Exhaled NO. **4.** MCh challenge, preceded by baseline spirometry from which FEV1, Vm50 and Vm20 were derived.

After methacholine challenge subjects were given an inhaled short acting beta-2 agonist if needed. They were monitored until lung function had returned to 95% of pre challenge values and they were feeling well.

RESULTS

There was no significant difference in the mean values of FEV1 (%predicted), NO, M/P or SGaw ratio between the two groups.

Pre challenge Vm20 displayed no significant correlation with any index of airway function or exhaled NO. The correlation between pre challenge Vm50 and exhaled NO did not reach significance in a two tailed t test (Table 7.2). Across all subjects pre challenge Vm50 demonstrated a significant correlation with PC_{20}(Vm20) (Fig 7.1), though failed to reach significance in either subgroup alone (Table 7.3). In asthmatic subjects (Table 7.4) there was a statically significant correlation between the concentration of exhaled NO and each index of the response to challenge, the same correlations were also significant across all subjects (control and asthmatic). The strength of the relationship and its statistical significance was greater in the case of $LogPC_{20}$(Vm20) (Fig 7.2) than $LogPC_{20}$ (FEV1). There were no significant relationship between NO and FEV1(% predicted) either in the asthmatic (Fig 7.3) or control subjects nor across all subjects (Table 7.5). In both asthmatic and control subjects the response to DI, as measured by either M/P or SGaw ratio, showed no statistically significant correlation with any index of airway function, or with the concentration of exhaled nitric oxide (Table 7.6).

Table 7.1

Baseline Values

	Control Subjects	N	**Asthmatic Subjects**	N	p
FEV1 (% pred)	98.7 (10.7)	10	91.9(13.41)	15	0.19
FEV1/FVC	0.84(0.05)	10	0.77(0.08)	15	0.02
SGaw	0.146(0.039)	10	0.110(0.042)	15	0.04
Log PC_{20} (FEV1)			-0.26(0.94)	12	
Log PC_{20} (Vm20)	0.209(0.453)	7	- 0.95(0.81)	15	0.002
Vm50	273.5(88.1)	10	209.7(45.0)	15	0.025
NO	6.48(4.62)	10	15.7 (18.7)	15	0.14
M/P	0.98(0.23)	10	1.05(0.26)	15	0.55
SGaw ratio	1.06(0.17)	10	1.00(0.13)	15	0.36

All values stated as mean(SD)

p value from 2 tailed Student's T test

N: number of subjects in the respective group.

Table 7.2

Correlations: Exhaled Nitric Oxide vs Pre Challenge Vm50 and Vm20

		Vm50			Vm20		
		Asthma	Control	All subjects	Asthma	Control	All subjects
NO	r	-0.44	-0.07	-0.34	-0.38	-0.06	-0.30
	p	0.10	0.84	0.93	0.17	0.88	0.15
	N	15	10	25	15	10	25

'p' = 2 tailed Significance, 'r' = Pearson Correlation's co-efficient.

Table 7.3

Correlations: Pre challenge Vm50 and Vm20 vs Responses to Challenge

		$LogPC_{20}$ (FEV)		$LogPC_{20}$ (Vm20)		
		Asthma	Control	Asthma	Control	all subjects
Vm50	r	0.32	n/a	0.43	0.65	0.54
	p	0.31		0.11	0.12	0.009
	N	12		15	7	22
Vm20	r	0.28		0.19	0.70	0.29
	p	0.38		0.49	0.08	0.19
	N	12		15	7	22

'p' = 2 tailed Significance, 'r' = Pearson Correlation's co-efficient.

Table 7.4

Correlations: Responses to Challenge vs Exhaled Nitric Oxide

		NO	
		Asthma	Control
$LogPC_{20}(FEV)$	r	-.586	
	p	.045	
	N	12	
$LogPC_{20}(Vm20)$	r	-.694	-.686
	p	.004	.089
	N	15	7

'p' = 2 tailed Significance,

'r' = Pearson's Correlation co-efficient.

Table 7.5

Correlations: FEV1 (% predicted) vs Pre challenge Vm50, Responses to Challenge and Exhaled Nitric Oxide

		Vm50	$LogPC_{20}$(FEV)	$LogPC_{20}$(Vm20)	NO
Asthma	r	0.78	.159	.200	-.183
FEV1 (% pred)	p	0.001	.621	.474	.514
	N	15	12	15	15
Control	r	0.83		.498	-.376
FEV1 (% pred)	p	0.003		.256	.284
	N	10		7	10

'p' = 2 tailed Significance, 'r' = Pearson's Correlation co-efficient.

Table 7.6

Correlations: Response to DI vs FEV1(% predicted), Pre challenge Vm50, Responses to Challenge and Exhaled Nitric Oxide

		FEV1 (% pred)	LogPC$_{20}$(FEV) Vm50		LogPC$_{20}$(Vm20)	NO
Asthma						
M/P	r	-.125	-0.33	.038	.129	.113
	p	.656	0.24	.905	.648	.687
	N	15	15	12	15	15
SGaw ratio	r	-.056	-0.38	.313	.171	-.043
	p	.844	0.16	.322	.541	.878
	N	15	15	12	15	15
Control						
M/P	r	.107	-0.18		.118	-.489
	p	.768	0.62		.801	.152
	N	10	10		7	10
SGaw ratio	r	-.426	-0.40		-.211	-.020
	p	.220	0.25		.650	.956
	N	10	10		7	10

'p' = 2 tailed Significance, 'r' = Pearson's Correlation co-efficient.

Figure 7.1

$LogPC_{20}$ (Vm20) vs. Pre challenge Vm50

in all subjects

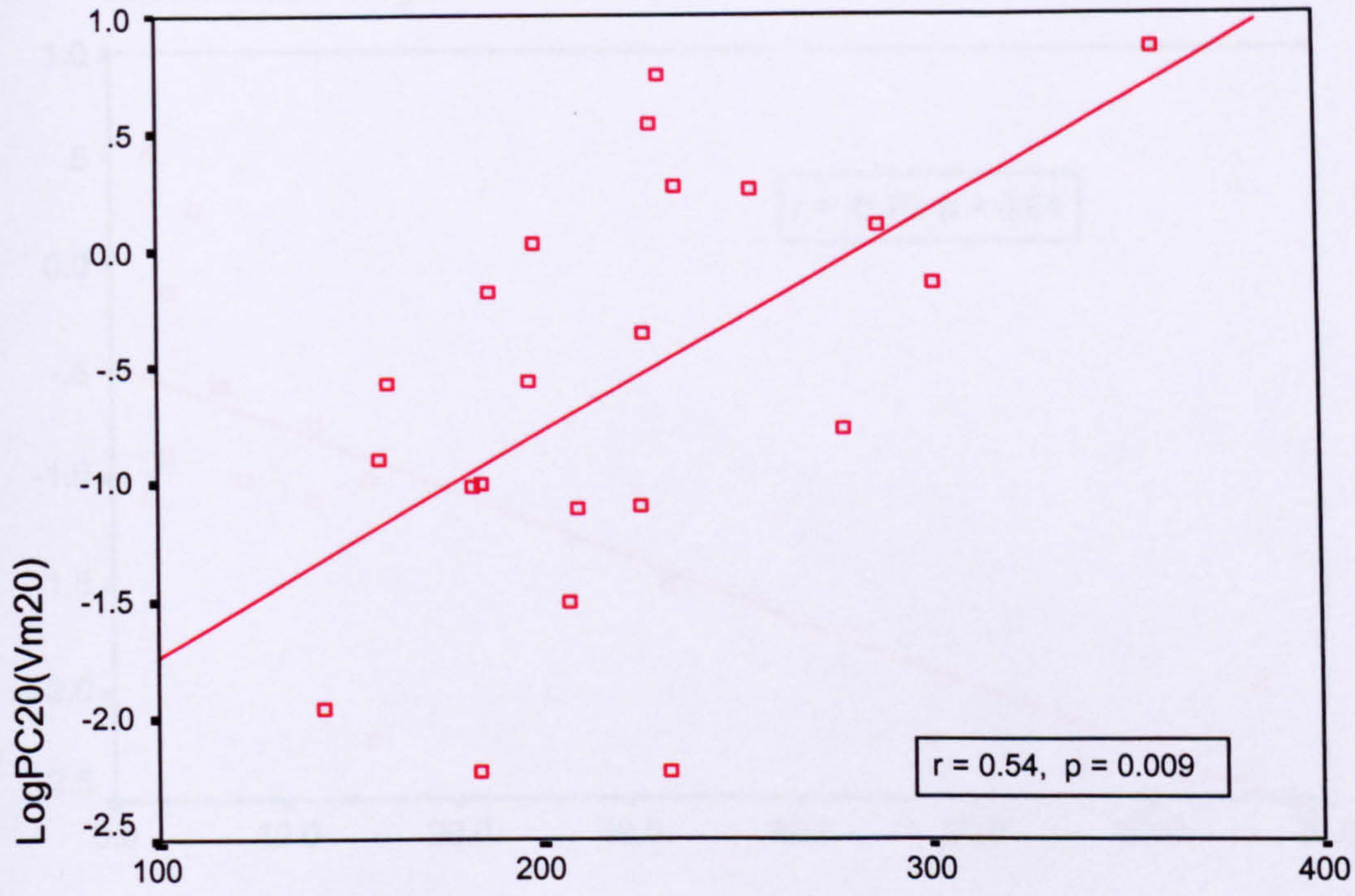
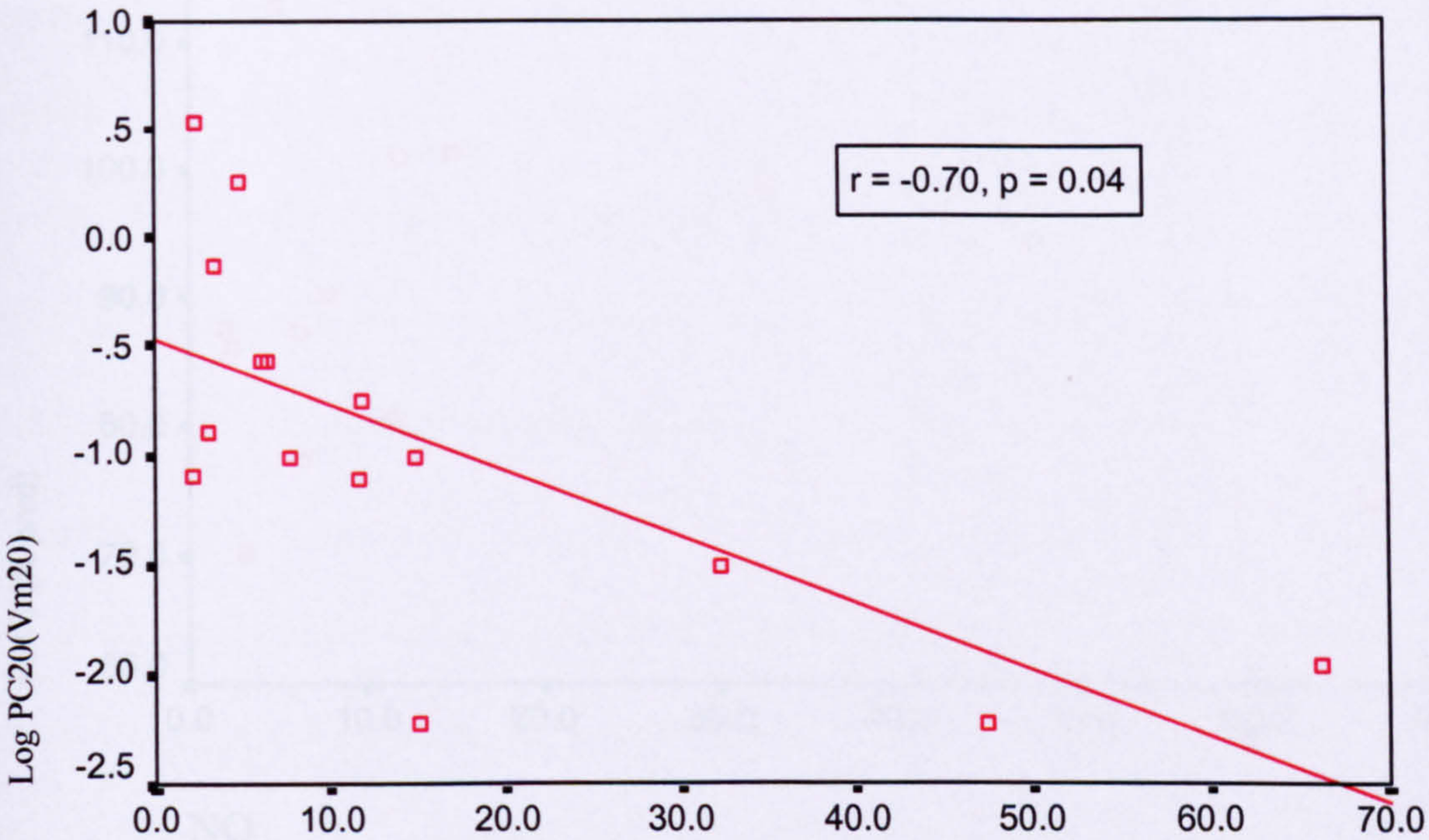


Figure 7.2

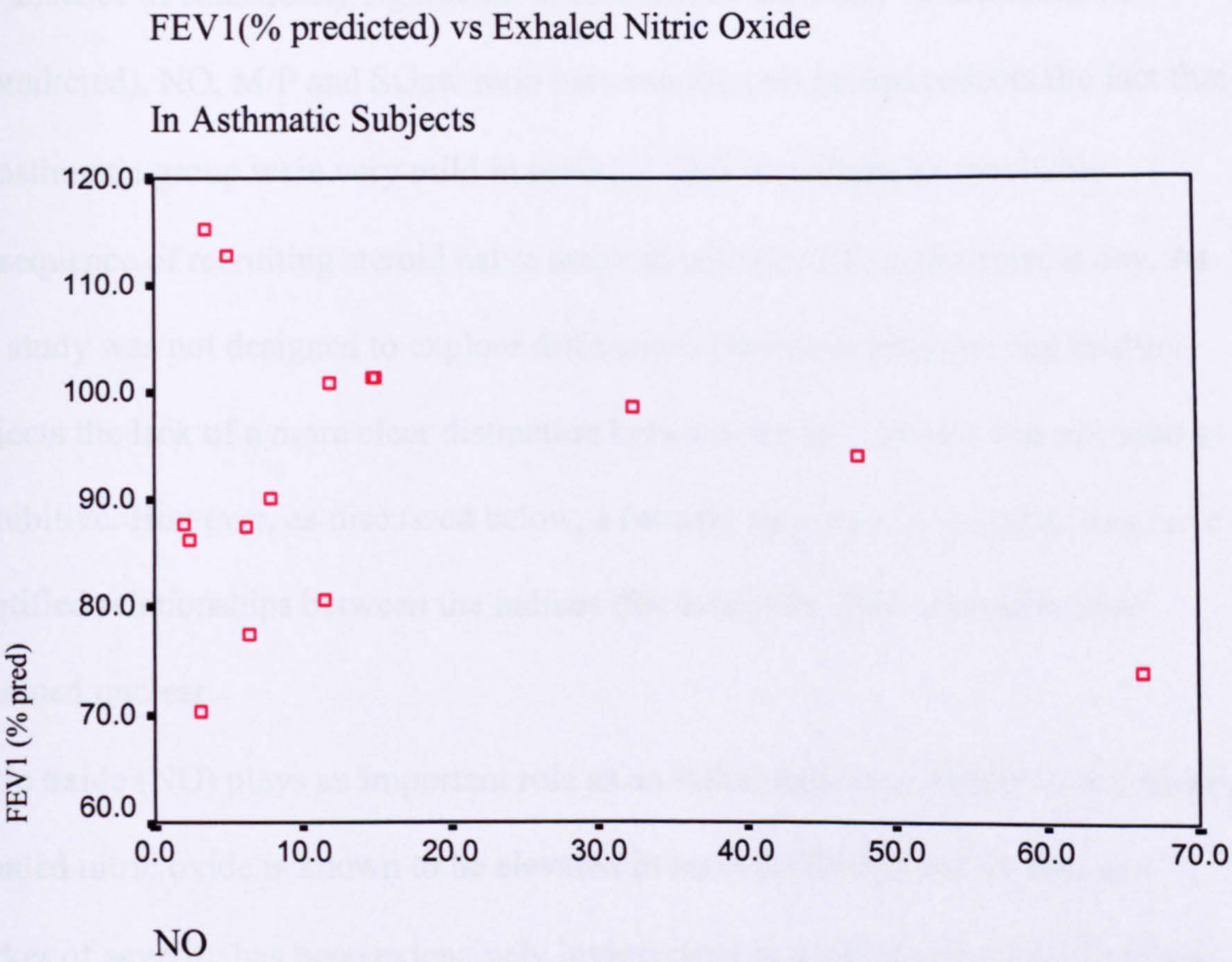
LogPC20(Vm20) vs Exhaled Nitric Oxide

In Asthmatic Subjects



NO

Figure 7.3


FEV1(% predicted) vs Exhaled Nitric Oxide
In Asthmatic Subjects
120.0
110.0
100.0
90.0
80.0
70.0
60.0
FEV1 (% pred)
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
NO

DISCUSSION

The absence of statistically significant differences in the mean values of FEV1 (%predicted), NO, M/P and SGaw ratio between the two groups reflects the fact that the asthmatic group were very mild in severity. This is perhaps an inevitable consequence of recruiting steroid naïve asthmatics in the UK in the current day. As this study was not designed to explore differences between asthmatic and healthy subjects the lack of a more clear distinction between the two groups was not seen as prohibitive. However, as discussed below, a broader spectrum of severity may have identified relationships between the indices discussed here that have otherwise remained unclear.

Nitric oxide (NO) plays an important role as an inflammatory mediator in the airways. Exhaled nitric oxide is known to be elevated in asthma (73-78) and its role as a marker of severity has been extensively investigated in a variety of clinical settings. The relationships reported to various clinico-pathological indices of asthma however have been somewhat variable. Many studies, in a variety of clinical contexts, have identified a correlation between exhaled NO and bronchial hyper-responsiveness, including: steroid naïve asthma (76, 78) and asthma associated with seasonal allergic rhinitis (74) as well as chronic stable asthma (79). In the context of steroid naïve asthma it has also been shown to correlate with diurnal variability in peak expiratory flow (PEF) (76). Although NO was found to correlate with FEV1(% predicted) in a large group across a broad spectrum of severity ranging from subjects attending the emergency department with acute severe asthma through chronic stable asthma to non-asthmatic controls (75), it is usually reported as not correlating significantly with FEV1(% predicted) (76-78, 80) other functional markers of severity such as symptom

scores (76) or beta-agonist use (76). The reason for this disparity between the correlation of NO with bronchial responsiveness (PD_{20}(FEV1)) and the absence of correlation with FEV1 is not clear.

In chapter 6 I discussed the importance of the function of the small airways in determining the 'ultimate' outcome of bronchial challenge (plateau or unlimited narrowing) and the relatively poor correlation between this facet of response and baseline FEV1. Although the PD_{20}(FEV1) and the ultimate outcome are different facets of the response to challenge, in chapter 6 I established that a close correlation exists between the two. A relationship may therefore exist between the baseline function of the small airways and PD_{20}(FEV1). If so and if as reported exhaled NO is principally derived from the terminal airways (74, 81) then this may go some way towards explaining the said disparity. I.e. exhaled NO may be reflecting inflammation (and thus function) principally of the small airways, this in turn would be expected to correlate with the response to bronchial challenge (chapter 6) yet may be only loosely related to indices such as FEV1 which in the absence of significant airway obstruction is probably determined principally by the function of the more central airways.

The findings in this study were broadly consistent with this hypothesis. In asthmatic subjects and across all subjects although the correlation between pre challenge Vm50 (an index more dependent on small airway function than FEV1) and exhaled NO did not reach significance in a two-tailed t test. A one tailed test is significant and given published data on the relationship between exhaled NO and airway function may have been more appropriate. Notwithstanding this, the current study has to be read as inconclusive on the issue of a relationship between pre challenge Vm50 and exhaled NO. This may well be due to a flaw in the design of the study. Vm50 pre challenge, as with Vm20, was derived from a single expiration. Random variability could have

been reduced had a mean of three manoeuvres been used. This same error may also have been responsible for the lack of correlation seen between baseline Vm20, an index even more prone to random variability than Vm50, and either the response to challenge or exhaled NO.

Across all subjects although the correlation between baseline Vm50 and log PC_{20}(FEV1) did not reach statistical significance, the correlation between baseline Vm50 and log PC_{20}(Vm20) (which in chapter 6 was shown to predict the ultimate outcome of challenge with a very high degree of sensitivity and specificity) was statistically significant. Further and to close the loop, the correlation between exhaled NO and response to challenge was statistically significant as has been previously reported. This was the case using either index of response: log PC_{20}(FEV1) or logPC_{20}(Vm20). Interestingly the strength of the correlation and its statistical significance was greater with logPC_{20}(Vm20) than logPC_{20}(FEV1).

Consistent with the findings in chapter 6, previously published data and the proposed linking mechanism in this chapter baseline FEV1(% predicted) demonstrated no correlation with exhaled NO, logPC_{20}(Vm20) or logPC_{20}(FEV1). Log transformation of NO did not qualitatively alter the statistical significance of any of these correlations. The correlation between baseline Vm50 and FEV1(% predicted) is not surprising. It may have been more appropriate to have tested the hypothesis using an index more dependent on small airway function and less dependent on central airway function than Vm50, such as Vm20 (appropriately averaged) or Closing Volume, a measurement reported to be an index of terminal airway function.

The response to DI

We know too from the study in chapter 6 and other published data (10, 41, 49) that within challenge the response to DI is an important determinant of the level at which a maximal response occurs. In some cases as discussed above that could make the difference to an individual subject demonstrating a plateau or the apparent capacity for unlimited narrowing. Would the response to DI at baseline therefore have any predictive power as to the outcome of challenge? Lim et al (11) reported on a positive relationship between the magnitude of the increase in residual volume following deep breaths (Δ RV) at baseline and the degree of fall in FVC following histamine inhalation (Δ FVC) though this was at a dose of histamine inducing only a 20% fall in FEV1, not the maximal, plateau response.

In chapter 4 I introduced a hypothesis (to be tested in chapter 8) that the abnormal asthmatic response to DI is due to fluid flux. This would be more specifically dependent on the degree of acute inflammation / oedema than the overall increase in thickness of the airway wall. The overall thickness is due to the combined effects of oedema and chronic re-modelling. Although these two factors are distinct one might expect some correlation between the two. The more 'severe' the asthma the greater the inflammation, the inflammatory process over time being ultimately responsible for the re-modelling. As explained in the model by James et al (64) the increased thickness of the airway wall can explain most, if not all, of the observed reduction in FEV1 and PC_{20}(FEV1) etc. in asthma. Thus one might, in a large cross-sectional study, find a correlation between the response to DI (related to the degree of oedema) and the FEV1 (related to total wall thickness). Such a correlation is reported by Lim et al (5). However, the relationship between the increase in airway wall thickness due to re-modelling and that due to oedema will not always be the same. The severity of the

inflammatory process at a particular point in time may determine the degree of airway wall oedema but factors such as the duration of the inflammatory process and the previous use of steroids (113) for example will also influence the degree of re-modelling. Certainly when the greater part of the airway wall thickening is due to acute inflammation and oedema, during an acute exacerbation for example, one might expect to find a good correlation between the response to DI and FEV1. Such a correlation was observed in a longitudinal study by Lim et al (16) when M/P and FEV1 were monitored in subjects recovering from an acute exacerbation of asthma. However in circumstances when the greater part of the airway wall thickness is not due to acute inflammation one might expect the correlation between the response to DI and FEV1, to be less strong.

The asthmatic subjects in this study were steroid naïve and therefore, for the reasons described above, may have had a disproportionate amount of re-modelling for the severity of asthma observed at the time of the study. They were also a fairly homogeneous group. The absence of a statistically significant correlation between the response to DI (by either index, M/P or SGaw ratio) and the indices of airway function: PC_{20}(FEV1), PC_{20}(Vm20), baseline FEV1 and Vm50 or even NO may therefore be explained by the likely weakness of the correlation between the degree of acute inflammation and the degree of re-modelling.

In summary the findings in this study (in conjunction with those in chapter 6) may explain the disparity in previously reported studies of the presence of a correlation between exhaled NO and the response to bronchial challenge and the general absence of a correlation between exhaled NO and the standard indices of baseline airway function such as FEV1. Exhaled NO would appear to be reflecting function of the small airways which are crucial in determining the response to bronchial challenge,

particularly the 'ultimate' outcome. On the other hand, in the absence of significant airway obstruction, FEV1 is, more dependent on the function of the larger airways. The absence of a correlation between the response to DI and any other index of airway function in this steroid naïve, relatively homogeneous asthmatic group is consistent with the hypothesis that the bronchoconstrictor response to DI is dependent on the degree of acute inflammation/oedema only, whilst many of the other indices of airway function depend on the overall thickness of the airway wall.

Chapter 8

A Novel Hypothesis to Explain the Bronchoconstrictor Effect of Deep Inspiration in Asthma

INTRODUCTION

The bronchodilatation post DI in healthy subjects is generally attributed to a reduction in airway smooth muscle tone but the mechanism underlying the bronchoconstriction observed post DI in many asthmatics is not fully established.

In the inflamed asthmatic airway wall, fluid flux across leaky capillaries between the intra and extra vascular compartments in response to changes in hydrostatic pressure is likely to be greater than in a healthy, non-inflamed airway. I therefore hypothesised that the large, negative intra thoracic pressure generated during a rapid deep inspiration would cause extravasation of fluid into the asthmatic airway wall, increasing its thickness and reducing the lumen, while in the healthy airway without inflammatory changes such an effect would be absent or insignificant.

I have tested this hypothesis by comparing the bronchoconstricting effect of DI in asthmatic and healthy subjects when inspiration was performed both with and without added resistance. Inspiration against added resistance was used to enhance the negative intra thoracic pressure during DI. My hypothesis predicted that asthmatic subjects would show increased airway narrowing following DI against resistance as compared with resistance free inspiration and that this difference would be greater than that seen in healthy subjects.

METHOD

I studied 10 mildly asthmatic and 11 normal subjects (tables 3.1, 3.2 and 8.1). SGaw was measured by the method described in chapter 3. Additional resistance for inspiration was provided by the attachment, in series, to a standard mouthpiece of an orifice 3mm wide and 3.4cm long.

Preliminary Studies

A pilot study was conducted in 10 subjects to confirm the practicability of this degree of resistance and the time to inspire a full breath through it. In each subject I measured the time (t) to perform a forced maximal inspiration from residual volume (RV) to total lung capacity (TLC) through resistance and the inspiratory vital capacity (IVC) during unencumbered breathing. This study demonstrated that 't' was, to a very good approximation, a linear function of IVC: $t = 2.12 \times IVC - 0.05$ ($R^2 = 97\%$, $P < 0.0005$). Inspiratory times varied between 5 and 14 seconds.

In a second pilot study the magnitude of the intra thoracic pressure generated during the two manoeuvres was assessed in four subjects after swallowing an oesophageal balloon. The subjects performed the two inspiratory manoeuvres that were to be performed in the main study. I found that maximum (negative) pleural pressure during forced inspiration against resistance was achieved early in inspiration and was sustained for most of the duration of the inspiration. During the controlled, resistance free, inspiration the negative pressure generated gradually increased throughout inspiration, achieving its maximal (negative) value at the end of inspiration. The peak (negative) pressure generated during forced inspiration against resistance was

significantly greater than the peak (negative) pressure during controlled, resistance free, inspiration (mean, - 62.9cmH_2O vs. - 23.1cmH_2O, p=0.019).

Protocol

The full study was performed at a later date. A series of respiratory manoeuvres was performed in the following sequence:

1. Inspiratory Vital Capacity (IVC).

Following 2 or 3 tidal breaths subjects exhaled to RV and then performed a non forced inspiration to TLC. The best of three technically good manoeuvres was recorded and used to predict the inspiratory time 't' through resistance using the previously derived equation: t = 2.12 x IVC - 0.05.

Subjects then rested for five minutes.

2. Specific Airway Conductance (SGaw) 'pre DI'.

The 'pre DI' manoeuvre began with tidal breathing for one minute. The volume time trace was monitored on screen to ensure that no deep breaths occurred. After a one-minute DI free period, SGaw, TLC, FRC and RV were measured. The sequence was repeated at least twice more after at least 1 minute of tidal breathing on each occasion. The mean of three technically good measurements was calculated and recorded as the 'pre DI' SGaw.

3. SGaw 'post DI without resistance' ($SGaw_{DI}$)

Using the same apparatus, after 2 or 3 tidal breaths, subjects performed a non-forced expiration to RV. From RV they inspired slowly (at a steady rate) to TLC. The inspiratory rate was such that the entire inspiration took 't' seconds. This precise timing was achieved by practice and an audible second count '1,2,...,t' during inspiration. The actual time taken was measured to ensure parity with the time 't'

predicted for the inspiration performed against resistance. From TLC subjects performed a swift but non-forced expiration to RV. The manoeuvre was repeated twice more and following the third timed inspiration to TLC subjects returned to FRC. Panting at FRC as before allowed calculation of SGaw. The mean of three technically good measurements was calculated and recorded as the '$SGaw_{DI}$'.

4. SGaw 'post DI against resistance' ($SGaw_{DI\ RES}$)

Using the same apparatus, subjects began by performing 2 or 3 tidal breaths without added resistance. They then performed a non-forced expiration to RV, followed by inspiration with maximum effort through resistance to TLC. Subjects were required to achieve a full inspiration as quickly as possible. The time taken was noted to ensure the accuracy of the predicted time 't' and thus matching with the time of the earlier controlled, resistance free, inspiration. From TLC subjects performed a swift but non-forced expiration to RV against no resistance. The manoeuvre was repeated twice more. Following the third maximal inspiration to TLC subjects returned to FRC. Panting at FRC as before allowed calculation of SGaw. The mean of three technically good measurements was calculated and recorded as the '$SGaw_{DI\ RES}$'.

<u>Analysis</u>

In order to assess the effect of the negative intrathoracic pressure generated by forced inspiration against resistance I compared $SGaw_{DI\ RES}$ with $SGaw_{DI}$ in each subject group. The % change $SGaw_{DI}$ to $SGaw_{DI\ RES}$ in each subject was calculated. Comparison was made of this % change between the 2 groups. $FRC_{DI\ RES}$, the lung volume at which $SGaw_{DI\ RES}$ was measured, and FRC_{DI}, the lung volume at which $SGaw_{DI}$ was measured, were also compared within each group.

The time taken to inspire when inspiration was performed at a voluntarily controlled rate without additional resistance (t_{DI}) was compared with the calculated time to inspire with maximal force performed against added resistance ($t_{DI\,RES}$). Within group results were compared using a paired t test and between group comparisons were made using a non paired t test.

RESULTS

All results are expressed as mean (standard deviation). Subject groups were of similar mean age and sex distribution (Table 8.1). The asthmatic subjects had mild airways obstruction.

In asthmatic subjects, SGaw following forced inspiration against resistance ($SGaw_{DI\ RES}$) was on average 13.5% less than SGaw measured following the voluntarily controlled deep inspiration against no added resistance ($SGaw_{DI}$) (Table 8.2). This difference was statistically significant ($p = 0.003$). In healthy subjects there was no difference in the SGaw following the two types of inspiratory manoeuvre. The % change, $SGaw_{DI}$ to $SGaw_{DI\ RES}$ was significantly different between the two groups. In the asthmatic subjects there was no statistically significant difference between FRC_{DI} and $FRC_{DI\ RES}$ although in healthy subjects a difference of similar average magnitude was statistically significant.

There were no differences in the inspiratory time when measured at the voluntarily controlled rate without added resistance {mean(SD): asthma 9.48(2.26) and normals 10.49(3.15)} and the calculated time to inspire with maximal force performed against added resistance {asthma 9.46(3.02) and normals 10.15(2.92)}.

Table 8.1

Baseline Data

	Asthma	**Healthy**
Age	32.8 (5.1)	30.2 (5.0)
FEV1 (% predicted)	90.8 (27.8)	97.5 (10.5)
FEV1/FVC	0.72 (0.14)	0.80 (0.07)
SGaw (pre DI)	0.094 (0.037)	0.154 (0.048)
Sex	5M	6M

mean (standard deviation)

Table 8.2

	Asthma	Healthy
$SGaw_{DI}$	0.084 (0.036)	0.141 (0.041)
$SGaw_{DI\ RES}$	0.071 (0.031)	0.138 (0.039)
% change	-13.5 (11.0)*	- 0.5 (12.4)*
p	0.003	0.67
FRC_{DI}	4.29 (1.36)	3.69 (1.19)
$FRC_{DI\ RES}$	4.07 (0.93)	3.49 (1.12)
p	0.23	0.02

mean (standard deviation)

SGaw measured in $cmH_2O^{-1}.sec^{-1}$

* comparison of % change in the asthmatic vs. healthy group. p = 0.02

DISCUSSION

The different responses of asthmatic and healthy airways to deep inspiration probably reflect a fundamental feature of asthmatic pathophysiology. Studies in healthy subjects consistently show a bronchodilating response to DI (7-10, 14, 18, 22) but in asthma the response is more variable (4-6, 11, 15, 16). While milder asthmatics simply display a more limited bronchodilator response, subjects with more severe asthma show bronchoconstriction post DI. Indeed some studies have identified an inverse relationship between the bronchodilating effect of DI and severity (5, 16, 17, 20). The bronchodilatation post DI in healthy subjects is largely attributable to a reduction in airway smooth muscle tone. This explanation is consistent with the enhanced bronchodilating effect of DI seen in the context of methacholine induced bronchoconstriction in both asthmatic (4-6) and healthy subjects (1, 8) and with diminution of the bronchodilating effect of DI following administration of $ß_2$ sympathetic agonists (6, 16, 23).

The mechanism underlying the bronchoconstriction observed post DI in some asthmatics is not fully established. A diminution of the bronchodilating effect of DI can be explained by a reduction in the degree of stretch imposed on smooth muscle by DI in asthmatic subjects (24). An additional mechanism however is required to explain the observed bronchoconstrictor response. Hysteresis within the parenchyma resulting in lower lung recoil pressure post DI and a diminished retractile force on the airway wall has been proposed as such a mechanism with the net effect on airways post DI being a balance of the hysteresis within the airways and parenchyma - the 'relative hysteresis hypothesis' (4, 7, 14, 23, 29). This mechanism explains many reported findings but it does not account for the observations in a study by Burns (4)

and the study reported in chapter 4 in asthmatic subjects of an increase in isovolumic forced expiratory flow post DI occurring in conjunction with a reduction in SGaw. I therefore propose the following mechanism, which, together with the stretching effect of DI on airway smooth muscle, would account for all the published observations:

A large proportion of the increased thickness of the airway wall in asthma is due to inflammation, which includes: increased vascularity, increased mucosal blood flow (114), leaky capillaries, inflammatory exudate and oedema (115). Even in stable situations the equilibrium of intra /extra vascular fluid flux is dynamic and delicately balanced. This equilibrium is likely to be altered by the markedly negative intra thoracic pressure generated during a rapid deep inspiration. Such pressure applied to a leaky, low-pressure capillary bed across the capillary wall would cause extravasation of fluid into the airway wall, increasing its thickness and reducing its lumen (and thus reducing SGaw). The increased interstitial fluid would also render the airway wall more turgid, reducing its compliance as recently reported with airway wall inflammation in vitro (110, 111). Consequently the airway would be less susceptible to compressive forces during the subsequent forced expiration. Given the dependence of fluid flux on hydrostatic as well as oncotic pressure the argument that fluid flux would occur in response to a change in pressure is perhaps not controversial. A more debatable point is whether sufficient fluid would have moved from the intra- to extra-vascular space given the pressure changes and time frame involved to account for the changes in airway conductance observed. No direct measurements have been made however it is worthy of note that the intra-thoracic pressures generated by the two manoeuvres in this study were around $-63 cmH_2O$ in the case of the inspiration against resistance and only $-23 cmH_2O$ during resistance free inspiration. This compares with

a typical intra-capillary pressure in the bronchial circulation of around 28cmH_2O (116). The duration of the application of these pressures was between 5 and 14 seconds. It has also been shown that relatively small changes in airway wall oedema can potentiate the bronchoconstricting effect of smooth muscle constriction (64, 69) and lead to significant airway narrowing. In an animal study Uhlig et al (72) demonstrated a significantly enhanced response to methacholine, after airway wall vascular engorgement compared with before: 67.8% vs 10.8% increase in Raw. Yet the morphometric data suggested that the engorgement process had produced only small changes in airway wall cross sectional area: 3.2 mm^2 versus 2.8 mm^2 in airways with a mean size >3mm, for example.

Thus relatively small net movements of fluid could be responsible for the observed changes. In healthy subjects, without inflammatory changes, such a mechanism would be absent or insignificant.

The hypothesis is consistent with studies reporting an inverse relationship between the bronchodilating effect of DI and severity of asthma (and thus oedema) (5, 16, 17, 20). One animal study (117) appeared to contradict these earlier findings. CT imaging in anesthetized sheep demonstrated that lung inflation produced greater bronchodilatation following prior bronchoconstriction by increased airway wall oedema (bradykinin) than following bronchoconstriction induced by smooth muscle constriction (methacholine). The fundamental difference however is that in this study 'deep inspiration' was achieved by positive pressure ventilation. This is entirely consistent with the proposed hypothesis which would predict in this context that 'deep inspiration' would invoke not only bronchodilatation secondary to smooth muscle stretch but also a reduction in airway wall oedema secondary the intra-thoracic positive pressure (as opposed to negative intra-thoracic pressure normally associated

with a deep inspiration). In a second animal study by one of the same authors, in the same journal, in the same year (118) induced airway wall oedema was found not to be associated with enhanced responses to methacholine. The study, I believe, suffers from the same 'flaw'. The interaction between smooth muscle shortening, airway wall oedema and lumenal narrowing was again observed in the context of positive pressure ventilation.

In the current study I have tested the proposed hypothesis by comparing the bronchoconstricting effect of DI (reduction in SGaw) in asthmatic and healthy subjects inspiring both with and without added resistance. In the design of the study the order of the two manoeuvres was not randomised. This introduces a theoretical risk of carry over (order bias) however it was felt that the pause between manoeuvres as the equipment was prepared and the intrinsic duration of the second manoeuvre ($SGaw_{DI\ RES}$) with its three forced deep inspirations would minimise any residual effect from the previous slow DIs in the $SGaw_{DI}$ manoeuvre. The inspiratory manoeuvres were designed to be identical in their time-volume relationship in order to minimise differences in the behaviour of smooth muscle or any other element in which intrinsic tone varies in response to stretch, including factors contributing to both airway and parenchymal hysteresis. Inspiration against added resistance enhanced the negative intra thoracic pressure during DI. Thus the only difference between the two types of inspiratory manoeuvre was the change in intra thoracic pressure. The fluid flux hypothesis predicts enhanced airway narrowing in asthmatic subjects following DI against resistance as compared with resistance free inspiration. In healthy subjects without airway inflammation the hypothesis predicts substantially less sensitivity to changes in intra thoracic pressure and less difference in SGaw

following the two types of inspiratory manoeuvre. These results are entirely consistent with this hypothesis.

In conclusion this study suggests that the changes in airway function observed post DI in asthmatic subjects result not only from changes in airway and parenchymal components of the lung which are subject to the stretch/relaxation phenomenon (already extensively studied) but also from the effects of transient changes in intra-thoracic pressure on the inflamed asthmatic airway wall.

Further testing is required to investigate the full implications of this mechanism. The potential to influence airway wall oedema by manipulation of the intra-thoracic pressure could have clinical benefits. Positive pressure applied by non-invasive ventilation, for example, could have the effect of reducing airway wall oedema. If effective such interventions could have a role in clinical management in the acute setting.

Chapter 9

Discussion (General)

Defining the response to DI

Part of the purpose of this thesis has been to explore and hopefully better understand the physiological mechanisms that underpin the responses to a deep inspiration, both in healthy controls and asthmatics. Whilst there appeared to be some common consensus that asthmatic subjects gained less bronchodilatation than healthy subjects, with some even displaying frank bronchoconstriction after DI, the published studies actually reported a much more mixed and complex picture. To attempt to explore the mechanisms underpinning the responses to DI our first task therefore had to be to define precisely what those responses were, in both asthmatic and healthy subjects.

In the study reported in chapter 4 M/P ratio was found to vary in a systematic way with the volume at which it was measured, increasing as lung volume decreases both in asthmatic and healthy subjects. Several possible explanations for this finding are discussed in the chapter, the most plausible explanation however would appear to be that the dependence on lung volume reflects a differing effect of DI on different generations of airway, with the larger airways showing relatively less bronchodilatation, perhaps due to a lesser stretching affect on the smooth muscle during DI.

The study in chapter 4 also found that the response to DI as measured by forced expiration appeared to contradict the response as measured by SGaw. The finding is consistent with another study reported in the literature (4). In the relatively mild group of asthmatics studied SGaw ratio suggested bronchoconstriction post DI, yet mean

M/P ratio, at the same lung volume, suggested bronchodilatation. The healthy subjects showed the converse pattern, with SGaw ratio suggesting bronchodilatation and M/P ratio suggesting bronchoconstriction. On the basis of these findings four criteria were identified that must be satisfied by any mechanism (operational in conjunction with smooth muscle stretch/relaxation) explaining the abnormal asthmatic response to DI:

(i) It must account for bronchoconstriction post DI in asthmatics.

(ii) It must account for an increase in airway wall rigidity post DI in asthmatics.

(iii) It should be distinct from, and thus vary independently of, smooth muscle stretch/relaxation.

(iv) It should be absent or insignificant in healthy subjects.

Within the relative hysteresis hypothesis (4, 7, 14, 23, 29), greater hysteresis of the parenchyma than the airways in asthma could account for bronchoconstriction post DI (SGaw<1). However parenchymal hysteresis results in lower lung recoil pressure post DI and would lessen the retractile force on the airway wall. This would render the airway more, rather than less, susceptible to compressive forces during forced expiration, thus condition (ii) is not satisfied.

The following novel mechanism is proposed:

That the negative intra thoracic pressure generated during a rapid deep inspiration could cause a net extravasation of fluid into the airway wall, increasing its thickness and reducing its lumen (thus reducing SGaw and *tending* to reduce forced expiration). The increased interstitial fluid would also render the airway wall more turgid and less susceptible to compressive forces during the subsequent forced expiration, *tending* to enhance forced expiration. The net effect of this newly proposed mechanism on forced expiration being a balance of these two opposing effects. This novel hypothesis was subsequently tested, the results are reported in chapter 8. The overall effect of DI

however would remain a net balance between the effects of this newly proposed mechanism and effects on smooth muscle.

The previously well described DI associated reduction in smooth muscle tone due to stretching is likely to occur in both healthy and asthmatic subjects, although there is evidence that this effect may be diminished in asthma. The dilating effect of a reduction in muscle tone on airway calibre is clear. A reduction in smooth muscle tone however may also reduce airway wall rigidity. Thus, although SGaw would be increased, the effect on forced expiratory flow would be determined by the relative magnitude of these two opposing effects.

The response of maximal flow and SGaw to DI in any individual asthmatic is therefore the net result of the effects of the reduction of smooth muscle tone and fluid flux in the airway wall. In healthy subjects, in the absence of inflammation in the airway wall, the degree of fluid flux would be significantly diminished, perhaps negligible and the response to DI would be determined largely by the reduction in smooth muscle tone.

<u>Testing the Novel Hypothesis</u>

In chapter 8 the fluid flux hypothesis was tested by comparing the bronchoconstricting effect of DI (reduction in SGaw) in asthmatic and healthy subjects inspiring both with and without added resistance. Inspiration against added resistance enhanced the negative intra thoracic pressure during DI but the inspiratory manoeuvres were designed to be identical in their time-volume relationship in order to minimise differences in the behaviour of smooth muscle or any other element in which intrinsic tone varies in response to stretch, including factors contributing to

both airway and parenchymal hysteresis. Thus the only difference between the two types of inspiratory manoeuvre was the change in intra thoracic pressure. The fluid flux hypothesis predicts enhanced airway narrowing in asthmatic subjects following DI against resistance as compared with resistance free inspiration. In healthy subjects without airway inflammation the hypothesis predicts substantially less sensitivity to changes in intra thoracic pressure and less difference in SGaw following the two types of inspiratory manoeuvre. Our results are entirely consistent with this hypothesis.

In conclusion this study suggests that the changes in airway function observed post DI in asthmatic subjects result not only from changes in airway and parenchymal components of the lung which are subject to the stretch/relaxation phenomenon (already extensively studied) but also from the effects of transient changes in intra-thoracic pressure on the inflamed asthmatic airway wall.

<u>A word on Cellular biomechanics</u>

There has been some recent focus on the importance of periodic stretch and relaxation inherent in tidal breathing for normal functioning of airway smooth muscle (27, 54, 57, 119, 120). Shen et al (119) reported the response to methacholine in rabbits ventilated at varying frequency and with varying magnitudes of tidal volume, including 'zero volume' ("static conditions"). The increase in Raw with challenge was significantly greater under static conditions than during tidal ventilation at any frequency or volume. Increases in the volume or frequency of tidal ventilation resulted in significant decreases in Raw in response to methacholine. They concluded that the effect of lung volume changes on airway responsiveness in vivo is primarily related to the stretch of airway smooth muscle. Though interestingly, given the

positive pressure associated with ventilation, these findings could also be explained by the oedema hypothesis discussed in chapter 8.

An important in vitro study was reported by Fredberg et al (57) in which isolated, maximally contracted bovine tracheal smooth muscle was subjected to tidal stretches. When the amplitude of imposed tidal stretch was very small, the steady-state value of the active force approximated the isometric force, the muscle was stiff and displayed little hysteresis in the tidal cycle. When the amplitude of imposed tidal stretch was increased the active force and stiffness decreased and greater hysteresis was demonstrated. The muscle could be maintained in these steady, dynamically determined contractile states for as long as the tidal stretches were sustained.

It is thus argued that the reduced mechanical load on airway smooth muscle in asthma, due to the unlinking of airway and parenchyma (55, 56) would lead to diminished smooth muscle stretch in during normal tidal breathing and therefore a more 'latched' state (between actin and myosin) with increased active force and stiffness of the muscle. Whether this possible 'semi-permanent' contracted state has additional functional relevance to the diminution in asthma of the transient relaxation in tone following a deep inspiration or whether it is simply another manifestation of the same phenomenon is not clear. Also, the same group that first described the biomechanical effect on smooth muscle of periodic tidal stretches (57) in a later paper (120) quantified the stretch amplitude required to cause active force or muscle stiffness to fall by half, or hysteresivity to double, as slightly greater than 2%. By contrast, the authors report the stretch amplitude expected during quiet breathing at rest is 4%. The difference in stretch amplitude between smooth muscle in an asthmatic and healthy airway during tidal breathing would be difficult to quantify though intuitively would seem unlikely to differ by as much as a factor of 2. The

functional significance of this observed in vitro phenomenon in explaining the abnormal response of asthmatic airways therefore remains unclear. At most, the phenomenon may account for a diminution in the bronchodilating effect of DI in asthma. It would not appear to account for the bronchoconstriction observed in many subjects.

Whether functionally important or not, these apparent changes in smooth muscle behavior are not a primary facet of the asthmatic state but secondary to geometrical changes in the airway wall.

The Relationship between the response to DI and bronchial hyperresponsiveness

The possible relationship between the generally diminished bronchoprotective effect of DI in asthmatic subjects and the increased responsiveness to non-specific bronchial stimuli is intriguing. The response to bronchial stimuli is usually measured in terms of the change from baseline of FEV1, which by definition is preceded by a deep inspiration. It would seem obvious therefore that the abnormal response to DI must at least play a part in the observed hyperresponsiveness to bronchial challenge in asthma. This idea was first mooted by Fish in 1981 (13). In fact Fish's hypothesis was that: 'Asthmatic hyperresponsiveness is due to a problem of smooth muscle relaxation with deep inspiration'. The hypothesis was later tested by Skloot (9) who concluded that it was correct.

First a careful dissection of the hypothesis is required.

As discussed above and in chapter 4, whilst a failure of smooth muscle to relax post DI may account for a diminished bronchodilator effect in asthmatic subjects, it cannot account for frank bronchoconstriction. A ' problem of smooth muscle relaxation'

cannot therefore be the only difference between the asthmatic and healthy responses to DI. Thus the diminished bronchoprotective effect of DI in asthmatic subjects, even within the context of bronchial challenge is unlikely to be exclusively due to a problem of smooth muscle relaxation. At face value therefore, without further exploration, the hypothesis would appear to be false. However, although not stated as such, the more general hypothesis that: 'Asthmatic hyperresponsiveness is due to an abnormal response to DI' is actually what Skloot tested.

This, more limited, hypothesis would certainly seem worthy of testing. In doing so further careful cautious and rigorous interpretation of results is likely to be required. We know from numerous other studies (7, 8, 10, 17-20) including the study which lead to the development of the hypothesis (13) that there is a diminished bronchoprotective effect of DI in asthmatic subjects, the fact therefore that this abnormal response is <u>part</u> of the explanation for asthmatic hyperresponsiveness is not controversial, in fact it could be considered to be already established. Thus to prove the (more limited) hypothesis any study would have to demonstrate that without the benefit of DI the bronchial responsiveness of healthy subjects is not just closer to that of asthmatic subjects but is in fact the same.

Skloot (9) tested this hypothesis and declared it to be true. This was widely reported at the time, which included extensive coverage in the lay press, as a 'big breakthrough' in our understanding of asthma. The study is however fatally flawed. As discussed in chapter 5, the 'τ' index used the measure the response to challenge will systematically underestimate the difference in the responsiveness between the asthmatic and control subjects. The absence therefore of a statistically significant difference in the responsiveness between the two groups in this study cannot be used to conclude that the responses are in reality the same.

I retested the hypothesis using an index not prone to the same error. I found a highly significant difference between the asthmatic and control responses to methacholine challenge, implying that even in the absence of DI, asthmatics are more responsive to methacholine than controls. This leads us to the conclusion that hyperresponsiveness in asthma cannot be attributed entirely to an abnormal response to DI. A detailed comparison of my methodology and that used by Skloot is described in chapter 5.

The Response to DI and the Maximal Response to Bronchial Challenge

The presence of a maximal (plateau) response or the converse, the capacity for unlimited airway narrowing is a less familiar facet of the response to bronchial challenge than PD_{20}(FEV1) but potentially more important. The obvious clinical correlate would appear to be the susceptibility to very severe, potentially fatal, exacerbations of asthma. The phenomenon described by Woolcock (33) is now well established with most healthy subjects and some mild asthmatics demonstrating a plateau response. The proportion of subjects demonstrating the phenomenon has varied between studies. Much of this variability will be due to subject selection but of course the value to which FEV1 is allowed to fall before the presence or absence of a plateau response is determined will have a significant bearing on it. Intuitively it seems likely that the maximal fall in FEV1 at plateau will vary, with some being below a 40% or even 60% threshold. Those who do not plateau prior to the given 'safety' threshold in a particular study probably fall into two categories: Those in whom a plateau would be established at a lower value of FEV1 and those who have the capacity for true unlimited narrowing. In an absolute sense therefore it could be argued that any study may be mis-positioning the dividing line in this dichotomy,

however in a practical sense those who do plateau below such threshold (particularly 60%) are probably more appropriately categorised with those most susceptible to fatal exacerbations of asthma.

I considered whether the plateau of the dose-response to methacholine, could be explained by the airway dilation that follows deep inspiration (DI) in non-asthmatics. In common with a previous study by Sterk (49) in subjects in whom a plateau could be established the maximum fall in Vp35 was found to be greater than the maximum fall in Vm35. It is clear therefore that DI had offered some degree of bronchoprotection in these subjects. Indeed in this and similar studies it my well be that a large bronchodilating response to DI could cause a plateau to occur above rather than below whatever safety threshold is set (in this case 40%) thus re-classifying the subject. The response to DI is therefore clearly an important factor, at least in terms of limiting the level of maximal plateau response. However the very existence of a plateau in a partial flow response to challenge implies that a 'mechanism' limiting airway narrowing in response to challenge would appear to be operating independently of the effects of deep inspiration.

<u>Small Airways and the Maximal Response to Bronchial Challenge</u>

Given their cartilaginous support I reasoned that narrowing of the central airways during bronchial challenge will be limited and the presence or absence of apparent unlimited narrowing to challenge is more likely dependant on function of the smaller airways. Assuming too that, in the absence of significant bronchoconstriction, FEV1 is determined principally by the larger airways I further reasoned that early in challenge changes in FEV1 probably reflect changes in calibre of the larger airways

and may not therefore reflect the ultimate outcome to challenge. I reasoned that if the small airway were indeed the site where the capacity for unlimited narrowing resided then early changes of small airway function within challenge may predict the ultimate outcome.

In the study reported in chapter 6 although PD_{20}(FEV1) was 100% sensitive and specific for predicting the ultimate outcome of challenge, PD_{20}(Vm20) and PD_{20}(Vm35) were also 100% sensitive and specific and predicted outcome at a much earlier stage of challenge. In fact the percent change in FEV1 at the dose after which PD_{20}(Vm20) was established (ie a 20% fall in Vm20 had occurred) was small and not different between the two groups.

Indices relying on very small changes in a value such as PD_{10}(Vm20) or those based on a flow independent of a DI such as PD_{20}(Vp35) although highly significantly different between the two groups, showed overlap in their value ranges and thus demonstrated a weaker predictive power. PD_{20}(FEV1), PD_{20}(Vm35) and PD_{20}(Vm20) in this study all predicted outcome with 100% sensitivity and specificity. The indices of small airway function are however predictive at a much earlier stage of challenge. The findings in this study are consistent with the hypothesis that the presence or absence of a capacity for unlimited airway narrowing lies in the function of the small airways. It demonstrates that the behaviour of these airways early in challenge reflects behaviour of FEV1 late in challenge. To establish a practical test for 'early detection' of the ultimate outcome of challenge however requires further work. What the indices of small airway function gain in sensitivity they may loose in specificity, due to excess 'random variability', being generally more volatile and less reproducible than FEV1. Though it should be noted that in this group at least, the volatility of the index did not significantly hinder its predictive power. This study did not provide data on

within subject reproducibility. Further studies should look PD_{20}(Vm50) and PD_{30}(Vm20) as well as PD_{20}(Vm35) and PD_{20}(Vm20). The 'optimum' index should provide the appropriate balance between sensitivity and reproducibility.

NO, Baseline Function of the Small Airways and the Response to Bronchial Challenge

The findings in the study in chapter 6 are interesting but generate more questions than they answer. If it is the function of small airways that determines the ultimate response to challenge could an assessment of small airway function at baseline (pre challenge) be used to predict outcome?

The published data on the concentration of exhaled nitric oxide (NO) is interesting and perhaps of some relevance, it suggests an apparent parallel. NO is known to correlate well with the response to challenge, at least in terms of PD_{20}(FEV1) (74, 76, 78, 79) yet not with baseline FEV1(76-78, 80). The reason for this disparity is not clear. Exhaled NO is also thought to be derived from the peripheral airways (74, 81). The question arose as to whether exhaled NO would correlate with the mechanical function of the small airways and whether NO as well as the mechanical function of the small airways would correlate with the response to challenge.

On the issue of the relationship between Vm50 and NO the findings in the study reported in chapter 7 were inconclusive. The absence of a clear correlation between NO and an index of small airway function may have been contributed to by a number of design flaws in the study. An index more exclusively dependent on small airway function such as Closing volume or Vm20 should have been selected and an

appropriately averaged measurements taken. This aspect of the hypothesis requires further testing.

Across all subjects although the correlation between Vm50 and log PD_{20}(FEV1) did not reach statistical significance, the correlation between Vm50 and log PD_{20}(Vm20) (which in chapter 6 was shown to predict the ultimate outcome of challenge with a very high degree of sensitivity and specificity) was statistically significant.

Further, and to close the loop, the correlation between exhaled NO and response to challenge was statistically significant as has been previously reported (74, 76, 78, 79). This was the case using either index of response: log PD_{20}(FEV1) or logPD_{20}(Vm20). Interestingly the strength of the correlation and its statistical significance was greater with logPD_{20}(Vm20) than logPD_{20}(FEV1).

Consistent with the findings in chapter 6, previously published data and the proposed linking mechanism in this chapter baseline FEV1(% predicted) demonstrated no correlation with exhaled NO, logPD_{20}(Vm20) or logPD_{20}(FEV1).

In summary the findings in this study (in conjunction with those in chapter 6) go someway to explain the disparity in previously reported studies of the presence of a correlation between exhaled NO and the response to bronchial challenge and the general absence of a correlation between exhaled NO and the standard indices of baseline airway function such as FEV1. Exhaled NO may to be reflecting function of the small airways, which are crucial in determining the response to bronchial challenge, particularly the 'ultimate' response. On the other hand, in the absence of significant airway obstruction, FEV1 is, to a greater degree, determined by the function of the more central airways.

The response to DI and the response to Bronchial Challenge

We know too from the study in chapter 6 and other published data (10, 41, 49) that within challenge the response to DI is an important determinant of the level at which a maximal response occurs. However in chapter 7 no correlation was found between the response to DI at baseline and the response to challenge.

As explained using the model by James et al (64) the increased thickness of the airway wall may explain most, if not all, of the reduction in FEV1 and the enhanced responsiveness to bronchial challenge in asthma. The overall thickness of the wall would be due to the combined effects of oedema and chronic re-modeling. In chapter 4 I introduced a hypothesis (tested in chapter 8) that the abnormal asthmatic response to DI is due to fluid flux. This would be more specifically dependant on the degree of acute inflammation / oedema in the airway wall rather than its overall thickness. As discussed, although these two factors (overall thickness and degree of oedema) are likely to be closely linked, the relationship will vary. For the reasons discussed in chapter 7 one might expect the relationship to be weaker in this steroid naïve group, which may explain the weakness observed in the relationship between indices such as FEV1 & PC_{20}(FEV1) and the response to DI in this group.

In summary, the absence of a correlation between the response to DI and any other index of airway function in this steroid naïve relatively homogeneous asthmatic group is consistent with the hypothesis that the response to DI is dependent on the degree of acute inflammation/oedema only, whilst many of the other indices of airway function depend on the overall thickness of the airway wall.

Chapter 10

Conclusions and Possible Directions for Future Research

The Small Airways

Given their cartilaginous support narrowing of the central airways during bronchial challenge will be limited and the presence or absence of the capacity for unlimited narrowing to challenge is more likely to be dependant on the function of the smaller airways. The sensitivity and specificity of changes in small airway function very early in bronchial challenge in predicting the ultimate outcome (limited or unlimited airway narrowing) supports this hypothesis. Furthermore, it suggests a potential clinically practical and safe test of the capacity for unlimited airway narrowing. Further studies however are required to determine the optimum index.

M/P ratio is greater at lower lung volumes in both asthmatic and healthy subjects. This dependence of the response to DI on the lung volume at which it is measured suggests that the effect of DI on different generations of airway may vary – with a greater bronchodilating effect seen in the smaller airways. With progressive bronchoconstriction during challenge the small airways play an increasingly important role in determining the maximal expiratory flow. This in addition to the increased smooth muscle tone may account for the increase in M/P as bronchial challenge proceeds. If M/P ratio at lower lung volumes does indeed reflect the response to DI in smaller airways then, given the importance of the small airways in determining the response to challenge, this may be the more pertinent index of the DI response. This hypothesis requires further testing.

The study reported in chapter 5, in conjunction with the original paper by Skloot (9) established that in the context of bronchial challenge the diminished asthmatic response to DI (as compared to the healthy response to DI) plays a role in distinguishing the asthmatic and healthy responses to challenge. It is clear however from the study reported in chapter 5 that this mechanism alone is only part of the explanation, it cannot account fully for the abnormal asthmatic response to challenge. I.e. there must be a further factor or mechanism determining this fundamental feature of asthmatic pathophysiology.

The study reported in chapter 6 confirms previously reported findings that the maximal or plateau response to challenge, where it exists, occurs following a lesser percentage change from baseline in indices preceded by, and therefore dependent on the response to, DI such as Vm20 and FEV1 than indices independent of the effects of DI such as Vp20. It is clear therefore that the bronchodilating effect of DI does offer some protective effect in terms of the maximal response to bronchial challenge. However the existence of a maximal or plateau response in many subjects in DI independent indices such as Vp20 clearly demonstrates that whatever factor or mechanism ultimately limits narrowing, it operates independently of the effect of DI. Thus in terms of the maximal response to challenge, as with PD_{20}(FEV1), the response to DI is influential but does not explain all findings.

In the novel hypothesis proposed in chapter 4, the bronchoconstrictor response to DI in asthmatic subjects is explained by fluid flux within the airway wall in response to changes in intra-thoracic pressure during DI. The hypothesis is tested by exaggerating the intra-thoracic pressures generated by the inspiratory manoeuvre and measuring the

functional response. Care was taken in the design of the study to exclude changes in other parameters that could influence outcome. In could be argued that in the absence of any other explanation for the results then this study constitutes 'proof' of the hypothesis. However a study, which includes more direct evidence of the hypothesised fluid shift, is likely to be more convincing to the wider scientific community. The study by Uhlig et al (72) demonstrated that very small changes in airway wall cross sectional area had large functional effects. The magnitude of the morphometric changes reported are probably beyond the resolution of current (in vivo) imaging techniques. I suspect therefore that an animal study with direct measurement of pathological specimens would be required to demonstrate the small volumes of fluid flux likely to be involved.

On the premise that the hypothesis is correct, I argue that in the absence of acute inflammation/oedema, in non-asthmatic subjects say, the effect of this proposed mechanism would be diminished, perhaps negligible. It would seem probable that the magnitude of the effect of this mechanism, would be related to the severity and degree of acute inflammation within the airway wall. This in turn, although clearly related to, would be distinct from, the overall increase in thickness of the airway wall seen in asthma - comprising changes of chronic remodelling as well as the effects of acute inflammation/oedema. One might, nevertheless, expect some correlation between these two features. The more severe the asthma the greater the inflammation, and the greater the degree of re-modelling. As in the model by James (64) the overall thickness of the airway wall may explain many of the abnormal features of asthmatic airway function such as the reductions in FEV1 and PD_{20}(FEV1). Therefore one might, in a large cross-sectional study, find a correlation between the response to DI (related to the degree of acute inflammation) and FEV1 (related to total wall

thickness), such a correlation is reported by Lim (5). However the relationship between the thickening due to re-modeling and that due to oedema will not always be the same. Factors other than severity of the inflammatory process at the time of testing will determine the degree of re-modeling that is has occurred, the duration of inflammatory process and the previous use of steroids (113) for example. The steroid naïve asthmatic group studied in chapter 7 may therefore have had a disproportionate amount of re-modelling for the degree of acute inflammation present. In such circumstances one might expect the correlation between the response to DI and FEV1 say, to be less strong. Indeed in this group M/P and SGaw ratio did not correlate with FEV1 or the response to challenge.

Alternatively in circumstances when a greater part of the airway wall thickening is due to acute inflammation/oedema, during an acute exacerbation for example, one might again expect to find a good correlation between the response to DI and FEV1. Such a correlation was observed in a longitudinal study by Lim et al (16) when M/P and FEV1 were monitored in subjects recovering from an acute exacerbation of asthma.

It would seem therefore that the response to DI relates to, and therefore can give us information on, the degree of 'acute inflammation / oedema' only, not the totality of airway wall thickening. It is the overall increase in airway wall thickness rather than the changes of acute inflammation alone that would be expected to be most closely linked to airway lumenal narrowing and abnormalities of airway function such as the diminution of FEV1 and the response to bronchial challenge.

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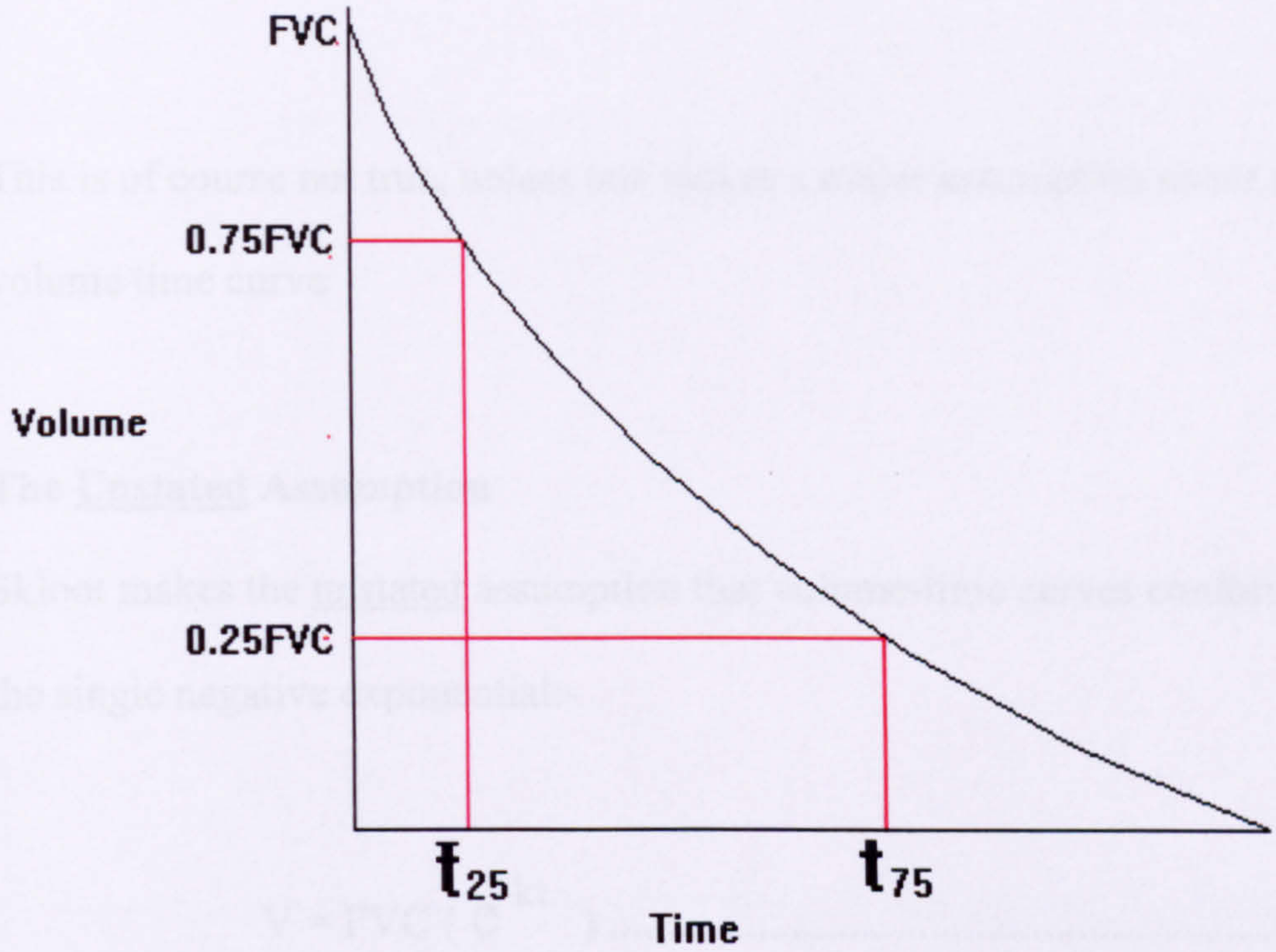
Appendix 1.
Abbreviations

CO_2	carbon dioxide
DI	Deep Inspiration
e	= 2.7182818...
EILV	End Inspiratory Lung Volume (the lung volume at the end of tidal inspiration)
FET_{25-75}	the time expire the middle 50% of vital capacity (25% to 75% expired) during forced expiration.
FEV1	Forced Expiratory Volume in one second
FRC	Functional Residual Capacity (the lung volume at the end of tidal expiration)
FVC	Forced Vital Capacity
ln	natural logarithm (to the base e)
IVC	Inspiratory Vital Capacity
M/P	the ratio of the maximal expiratory flow rate following a maximum inspiration to the maximal expiratory flow rate following a partial expiration (Vm / Vp) at a given isovolumic point (chapt. 3.3)
NO	Nitric Oxide (the concentration of)
PD_{20}(FEV1)	The provoking dose causing a 20% fall in FEV1 from baseline during bronchial challenge (dosimeter method, chpt 3.6)
PD_{20}(Vm20)	The provoking dose causing a 20% fall in Vm20 from baseline during bronchial challenge (dosimeter method, chpt 3.6)

PC_{20}(FEV1)	The provoking concentration causing a 20% fall in FEV1 from baseline during bronchial challenge (tidal breathing method, chpt 3.7)
Ptp-V	quasi-static transpulmonary pressure-volume
RV	Residual Volume
sd	Standard deviation
SGaw	Specific airway conductance
SGaw ratio	ratio of: SGaw ('post DI') performed immediately after a deep inspiration to SGaw ('pre DI') performed after a period of time in which deep inspiration was avoided.
$SGaw_{DI}$	Specific airway conductance performed after a slow, timed deep inspiration (chapter 8)
$SGaw_{DI\ RES}$	Specific airway conductance performed after a forced deep inspiration against added resistance (chapter 8)
τ	$= FET_{25-75} / \ln 3$
TGV	Thoracic Gas Volume, as measured by body plethysmography
TLC	Total Lung Capacity
VC	Vital Capacity
Vm35	expiratory flow rate at 35% vital capacity (remaining) during forced expiration preceded by a maximal inspiration
Vp50	expiratory flow rate at 50% vital capacity during forced expiration preceded by a partial inspiration

Appendix 2

Analysis of the 'τ' Index (9)



Firstly define...

$$\text{Mean Max Expiratory Flow (MMEF)} = \frac{0.5 \times FVC}{t_{75} - t_{25}}$$

$$\text{so } \frac{FVC}{MMEF} = 2 \times (t_{75} - t_{25}) = \text{twice time to expire middle 50\% of volume}$$

Define

$$\mathbf{t_{au}} = \frac{\mathbf{FVC}}{\mathbf{MMEF}} \times \frac{\mathbf{0.5}}{\mathbf{\ln 3}}$$

Skloot et al inform us that this index is the reciprocal of the mean slope of the flow volume curve between 25% and 75% of the forced expiration.

This is of course not true, unless one makes a major assumption about the shape of the volume time curve

The Unstated Assumption

Skloot makes the unstated assumption that volume-time curves conforms perfectly to the single negative exponential:-

$$V = FVC\,(e^{-kt}) \quad \text{.....1.}$$

or

$$e^{-kt} = \frac{V}{FVC} \quad \text{.....2.}$$

Given this assumption, let us show that Skloot's statement about the slope on the flow volume curve is true...

from 1.

$$f = \frac{dv}{dt} = -K\,(FVC)\,e^{-kt} \quad \text{.....3.}$$

from 2.

$$-Kt = \ln\left(\frac{V}{FVC}\right) \quad \text{.....4.}$$

rearranging

$$t = -\frac{1}{K} \ln\left(\frac{V}{FVC}\right) \quad \text{.....5.}$$

let t_{25} = the time to expire 25% of the FVC, by which time V = 0.75 FVC

so

$$t_{25} = -\frac{1}{K} \ln 0.75 \qquad t_{75} = -\frac{1}{K} \ln 0.25 \quad \text{.....6.}$$

recall

$$\text{MMEF} = \frac{0.5\ FVC}{t_{75} - t_{25}} = \frac{0.5\ FVC}{-1/K(\ln 0.25 - \ln 0.75)}.$$

$$= \frac{-K\ 0.5\ FVC}{\ln 1/3}$$

$$= \frac{K\ 0.5\ FVC}{\ln 3}$$

so

$$\mathbf{tau} = \frac{FVC}{MMEF} \times \frac{0.5}{\ln 3} = \frac{\ln 3}{0.5\ K} \times \frac{0.5}{\ln 3} = \frac{1}{K}$$

<u>Aside</u> **So what is K?**

recall

$$V = FVC\ (e^{-kt})$$

from 2. & 3.

$$f = -K(FVC)\frac{.\ V\ .}{(FVC)} = -KV$$

so slope on <u>flow volume</u> curve $= \frac{df}{dv} = -K$

So **tau** <u>is</u> the reciprocal of the slope of the flow volume curve.

<u>But what is tau</u> ?

Let us think of **tau** in a way which is easier to grapple with conceptually

Recall...

$$\mathbf{tau} = \frac{\mathbf{FVC}}{\mathbf{MMEF}} \times \frac{\mathbf{0.5}}{\mathbf{ln\ 3}} = 2\ (t_{75} - t_{25}) \times \frac{\mathbf{0.5}}{\mathbf{ln3}}$$

ie.

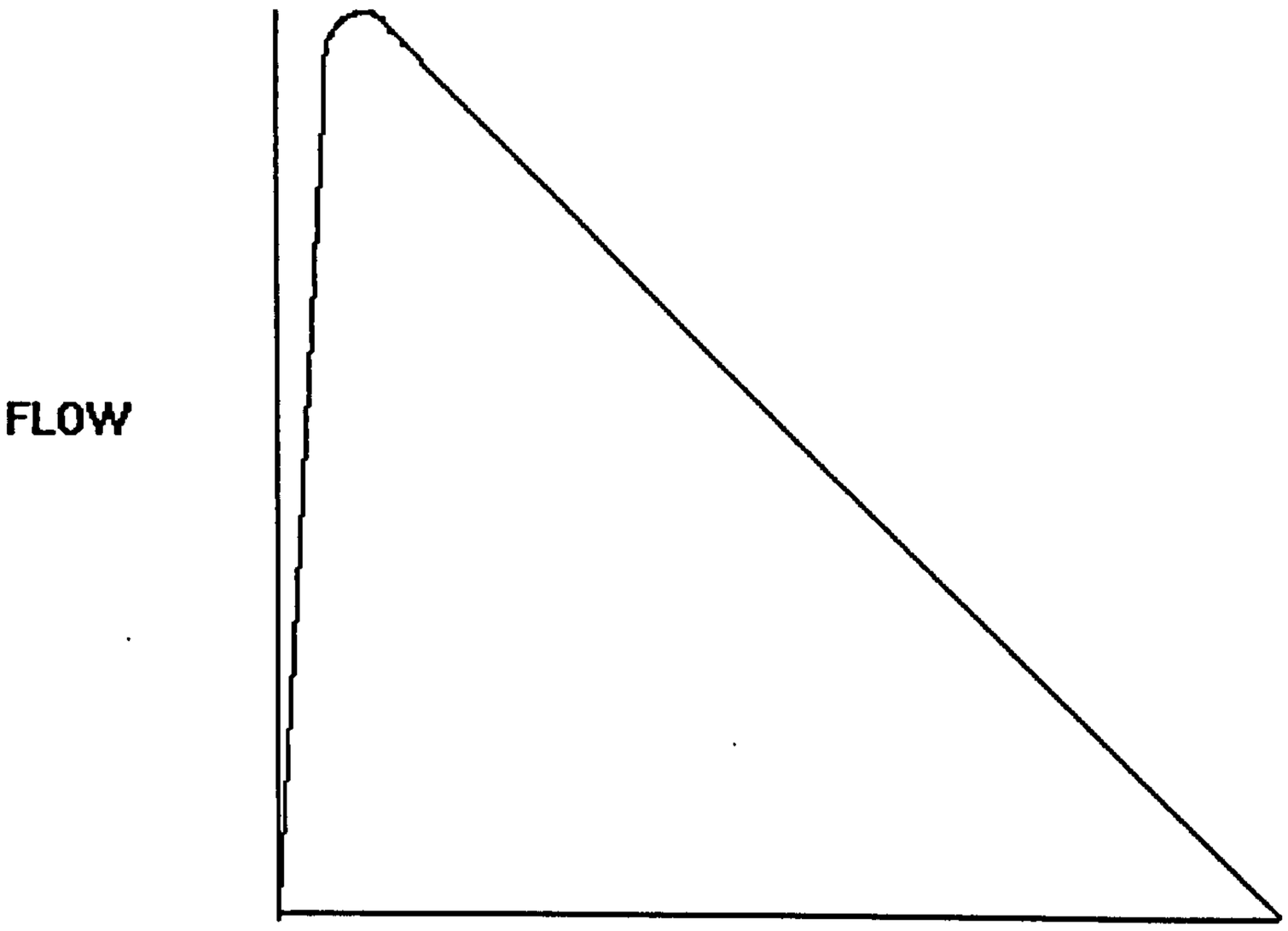
tau	*is proportional to*	the time taken to expire the middle 50% of the FVC

Question:

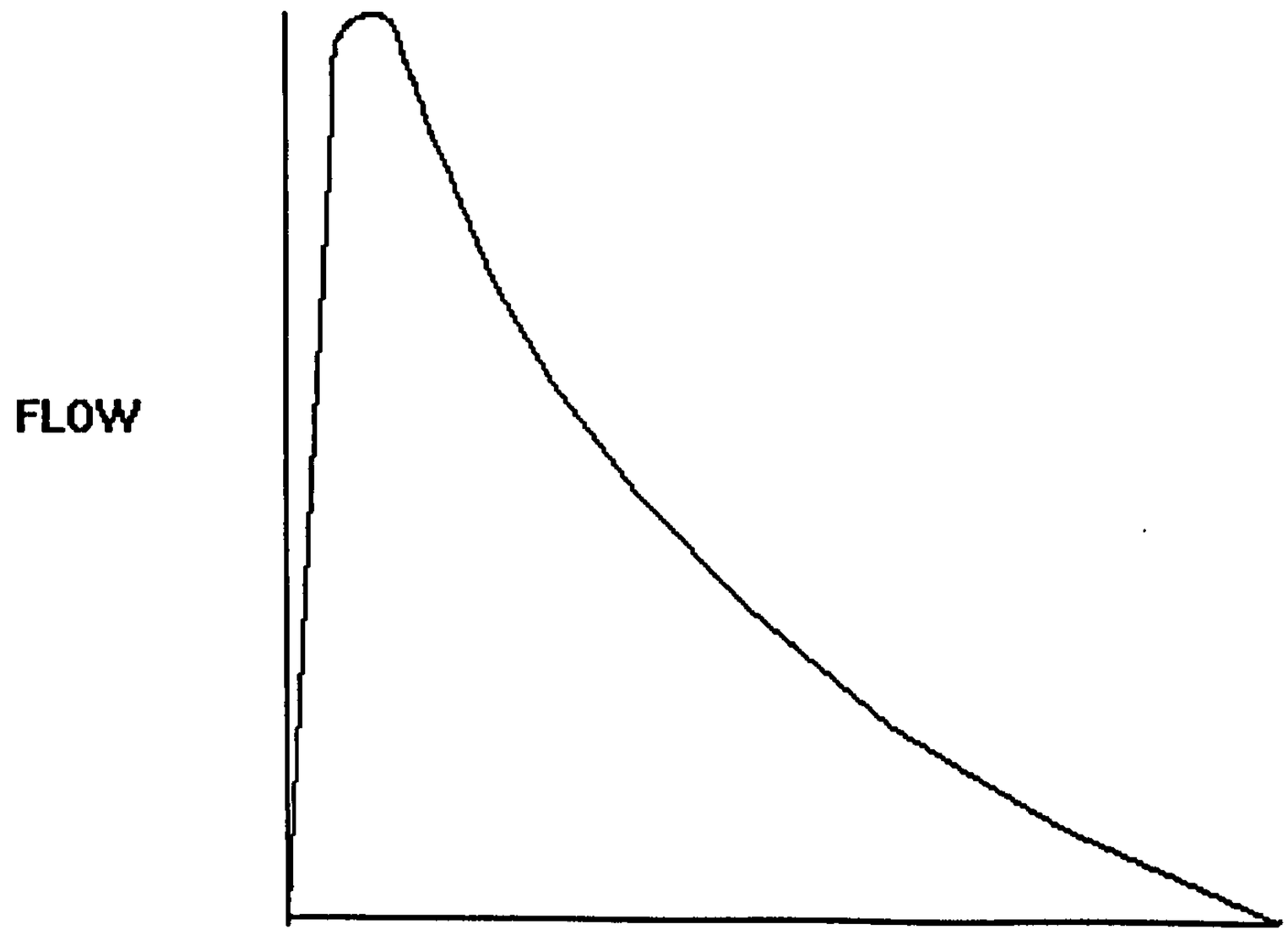
Is this volume independent?

Well if we allow Skloot's implicit assumption that the volume time curve conforms perfectly to a single negative exponential, then yes

But from the above we see the implication of this is that the slope on the Flow - Volume curve is a <u>constant</u> (= -K)



Which may be a reasonable assumption in normal subjects, but breaks down in asthmatic subjects



& recall as **tau** = 1/K,

So we see that if subjects start their expiration at a higher lung volume then the mean slope on the expiratory curve (K) of the F-V loop increases. As K increases, **tau** decreases. **tau** being the index of bronchoconstriction then using this index, subjects starting their expiration at a higher lung volume will appear to have less bronchconstriction.

I.e. this index is **not** volume independent

For a given degree of bronchocnstriction, the higher the volume at which the partial expiration begins, the lower the value of **tau**

Worked Example

In the following example the numbers and calculations are taken from actual (typical) asthmatic and healthy subjects in the study in chapter 5, the figures are for illustrative purposes only.

Fig.1 shows the partial expiratory flow on volume-time axes at the end point of challenge (after 50mcg MCh) in the asthmatic subject. FET_{25-75}, from which τ is calculated, is **1.23 seconds.**

The curviliniarity of the volume-time curve is determined by the degree of bronchoconstriction. FET_{25-75} (and τ) is an index of this curviliniarity. Skloot argues that the index is independent of the volume at which the expiration is begun (EILV). In fact such independence is a mathematical property of a single exponential. In healthy subjects, to a good approximation, the volume time curve does conform to a single exponential, however this is not so in asthmatics. To demonstrate this, In fig. 2

we have re-calculated FET_{25-75} (from the same curve) as if EILV had remained at its baseline value. The position of the 'baseline EILV' is calculated using the mean change in EILV in the asthmatic group (15% of baseline FVC) and the assumption of constant TLC throughout challenge. This re-calculated FET_{25-75} is **1.88 seconds.**

In this subject baseline FET_{25-75} was **0.68 seconds.** The change in FET_{25-75} (from baseline to end of challenge) recorded was therefore (1.23 minus 0.68) **0.55 seconds** or 81%. When we re-calculate FET_{25-75} as if EILV had remained at its baseline value we find the change in FET_{25-75} is (1.88 minus 0.68) **1.20 seconds** or 176%. Thus the rise in EILV that occurred during challenge had caused the measured rise in FET_{25-75} (and τ) to be only 46% of that which would have been recorded had EILV remained at the baseline level. Ie the rise in EILV has masked the true degree of bronchoconstriction in the asthmatic subject.

In the healthy control group the rise in EILV is less important for two reasons:

(i.) The, within challenge, rise in EILV is less than in the asthmatic group (2.5% vs 15%)

(ii.) Due to the shape of the volume-time curve the rise in EILV has less effect on FET_{25-75}.

In a typical healthy subject we found the mean 2.5% rise in EILV represented a change of 0.16 litres, this had no measurable effect on the FET_{25-75}.

In conclusion, the rise in EILV during challenge would appear to be masking a significant difference in response between asthmatic and healthy subjects.

Figure 1

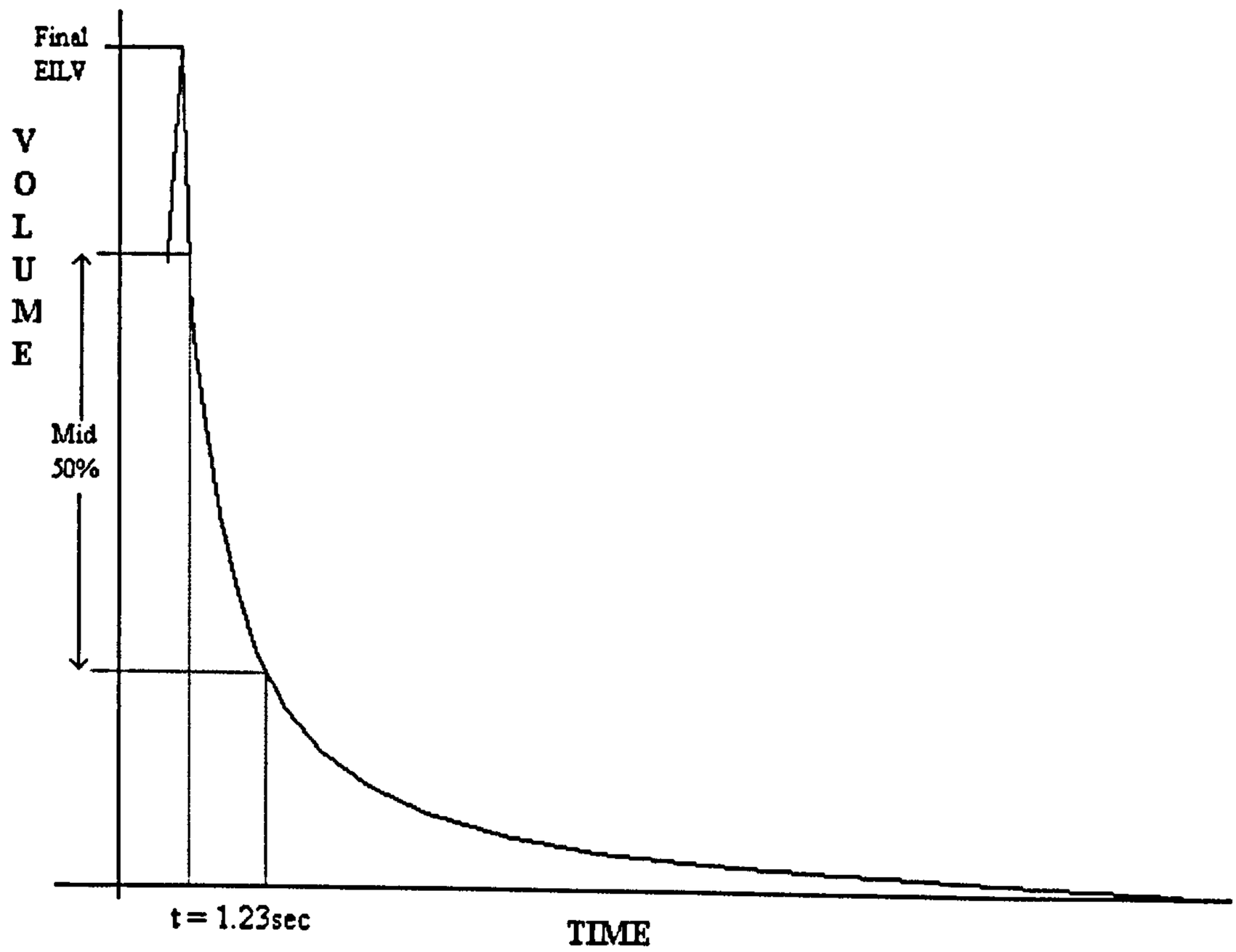
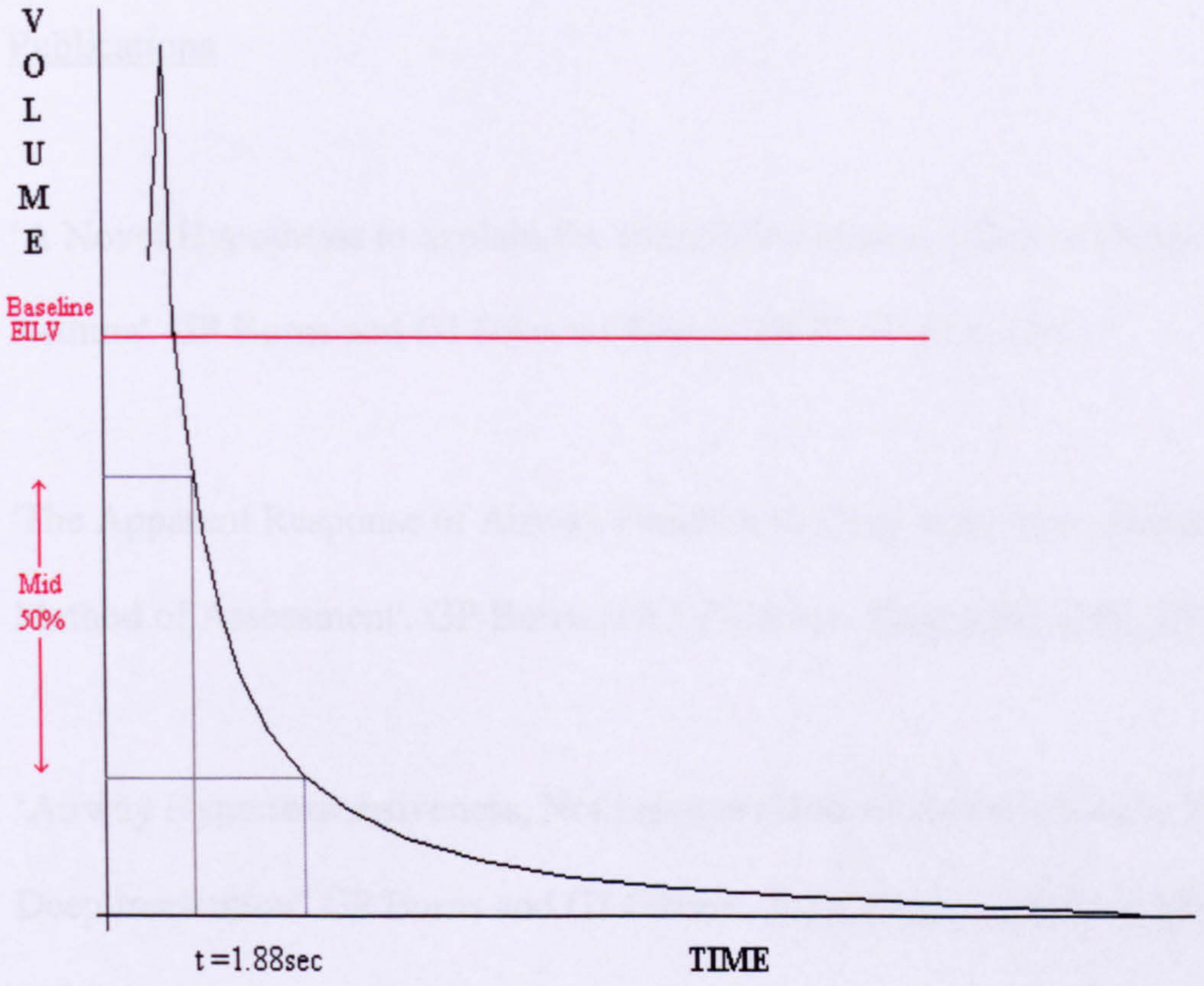


Figure 2



Appendix 3

PUBLICATIONS AND PRESENTATIONS

to date arising from this body of work:

Publications

'A Novel Hypothesis to explain the Bronchoconstrictor effect of Deep Inspiration in Asthma'. GP Burns and GJ Gibson (Thorax 2002; 57: 116-119)

'The Apparent Response of Airway Function to Deep Inspiration Depends on the Method of Assessment'. GP Burns and GJ Gibson. Resp Med 2001; 95: 251-7

'Airway Hyperresponsiveness, Not just a problem of Smooth Muscle Relaxation with Deep Inspiration' GP Burns and GJ Gibson. Am J Respir Crit Care Med 1998:158, p203-6.

Presentations

'Relation of Exhaled Nitric Oxide Concentration to Airway Function in Mild Asthma'. GP Burns et al. ERJ 2000:16 suppl 31, p443s

'A Novel Mechanism to Explain the Bronchoconstrictor Effect of Deep Inspiration in Asthma'. GP Burns and GJ Gibson. ERJ 1998: 12 suppl 29. P22s

'Mechanism of the Bronchoconstrictor Effect of Deep Inspiration in Asthma'.

GP Burns and GJ Gibson. Thorax 1998: 53 suppl 4, pA41

'Prediction of Plateau Response to Bronchial Challenge by early changes in Small Airway Function' GP Burns and GJ Gibson Am J Respir Crit Care Med 1998:157;3.pA672

'Dependence of the Observed Response to Deep Inspiration on the Method of Assessment' GP Burns and GJ Gibson ERJ 1997:10 s25. p196s

'Airway Hyperresponsiveness, Not just a problem of Smooth Muscle Relaxation with Deep Inspiration' GP Burns and GJ Gibson. Am J Respir Crit Care Med 1997:155;4. pA544

'Airway Narrowing in Asthma' presentation The Breathing Club, Cambridge FIRST PRIZE 1997

'Asthmatic Hyperresponsiveness: an abnormal response to DI?' presentation MRC Fellows conference, Edinburgh 1997