Changes in maximum expiratory flow-volume curve configuration after treatment with inhaled corticosteroids

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ABSTRACT   The present study reports the changes in configuration of maximal flow-volume curves after eight weeks' treatment with inhaled corticosteroids in 14 asthmatic patients. The configuration was compared with that seen after bronchodilatation following inhalation of a single dose of ipratropium bromide. After inhaled corticosteroids the shape of the flow-volume curves was less bowed toward the volume axis, whereas the shape of the flow-volume curves after inhalation of ipratropium bromide showed no significant change. A significant correlation was observed between the decrease in blood eosinophil cell count and the straightening of the flow-volume curves, quantitatively expressed as shape factor and slope ratio. It is concluded that these changes in flow-volume curve configuration reflect a decrease in inhomogeneously distributed inflammatory airway narrowing.

Introduction

Bronchial obstruction in asthmatic patients is generally thought to be determined by bronchial smooth muscle contraction and by inflammatory processes in the bronchial wall.[1] Both factors can be treated separately. Inflammatory processes can be reduced by drugs such as corticosteroids, and smooth muscle contraction can be relieved by bronchodilating agents such as anticholinergic or sympathicomimetic drugs. Although corticosteroids and bronchodilators act in different ways, both drugs improve the forced expiratory volume in one second (FEV_1). Bronchodilatation is achieved in a short time after administration of the bronchodilator, but anti-inflammatory treatment takes several weeks to be effective, owing to the nature of inflammatory processes. In patients with mild asthma a rise in FEV_1 to their predicted values is often seen after administration of a bronchodilator, and such a change may occur after treatment with inhaled corticosteroids.[2] Other measures of pulmonary function could, however, show differences between the rapid bronchodilatation seen with bronchodilator drugs and the bronchodilatation occurring over several weeks with anti-inflammatory treatment.

Maximal expiratory flow-volume (MEFV) curves are generally thought to give additional information about the severity of bronchial obstruction.[3 4] The mechanical factors underlying airflow limitation during a forced expiration have received considerably more attention than the assessment of bronchial obstruction. On the basis of wave speed mechanics, Dawson and Elliott[5] concluded that the flow limiting segment moves towards the peripheral airways with decreasing remaining lung volume. Measuring flows at different lung volumes may therefore give an approximate indication of the site of airway obstruction.

In clinical practice flow-volume curves are usually analysed in terms of maximum flows at a given volume and often interpreted qualitatively with regard to the shape of the curve. Even in patients with mild bronchial obstruction[6 7] the flow-volume curves are more bowed towards the volume axis, and the question arises whether this increased convexity of the flow-volume curves reflects a specific pathological process that eventually may be influenced by treatment.

Several ways of quantifying the shape of an MEFV curve have been put forward. Mead[8] developed the slope ratio (SR), defined as tangent slope ($d\dot{V}/dV$) divided by the chord $\dot{V}/(FVC-V)$, as an index of curvilinearity of the MEFV curve. He introduced also the ratio $1/2(\dot{V}E\ max_{50}/\dot{V}E\ max_{25})$, here referred to as the shape factor at 50% remaining FVC (SF 50%) as

an index of non-linearity of the MEFV curve.[10] To extend the indices for curvilinearity of the MEFV curves over a larger lung volume, we calculated a similar index, making use of the flow at 75% remaining FVC: $1/3(\dot{V}E \ max_{75}/\dot{V}E \ max_{25})$, the shape factor at 75% (fig 1).

In the present study MEFV curves were measured before and after administration of a single dose of ipratropium bromide and before and after long term treatment with budesonide, as part of another study.[2] Ipratropium bromide is a potent bronchodilator that blocks the bronchoconstricting effects of acetylcholine, released by the vagal nerve, on muscarinic receptors on bronchial smooth muscle. Budesonide is a corticosteroid with a high local anti-inflammatory potency.[9]

The MEFV curves were analysed in 14 patients with extrinsic asthma with acute mild bronchial obstruction. The aim of the study was to compare the effects of bronchodilatation and bronchodilatation resulting from long term anti-inflammatory treatment in terms both of the maximal expiratory flow at various lung volumes and of change in the shape of the MEFV curve.

Methods

PATIENTS

Fourteen patients (12 male and two female) with allergic asthma (mean age 26, range 18–36 years) gave their informed consent to participate in the study. The clinical characteristics of the patients are shown in table 1.

All patients had a history of episodic wheezing, and showed strongly positive skin test responses to at least two common allergens, including house dust mite (Allergens Diephuis Laboratories, Groningen, The Netherlands). All had increased serum concentrations of IgE specific for house dust mite. All patients had airway hyperresponsiveness to inhaled methacholine (provocation concentration of methacholine causing a 20% decrease in FEV_1 PC_{20} <8 mg/ml) according to the method of Juniper et al,[11] and had mild symptoms controlled by low doses of inhaled bronchodilators or prophylactic drugs (sodium cromoglycate or inhaled corticosteroids). None of the patients used oral corticosteroids. The initial FEV_1, measured before the treatment period, was $>70\%$ of the predicted value.[12]

STUDY DESIGN

Before entering the study the patients discontinued their usual maintenance treatment (including sodium cromoglycate or inhaled corticosteroids) for at least three weeks. After this period the patients were treated for two weeks with placebo inhalations (single blind) followed by an active treatment period of eight weeks, in which the patient inhaled budesonide 800 µg/day by
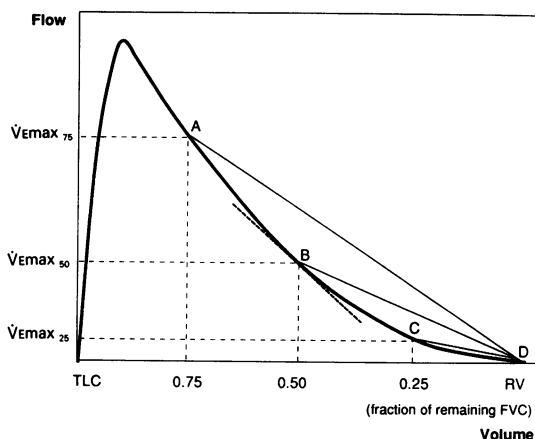

Fig 1   Schematic drawing of a curvilinear maximum expiratory flow-volume (MEFV) curve, showing flows at 75%, 50%, and 25% of forced vital capacity (FVC) and the tangent at 50% FVC. Slope ratio at 50% FVC is defined as the slope of the tangent at point B divided by the slope of the chord BD. Both shape factors and slope ratios are greater than 1 for a convex MEFV curve. TLC—total lung capacity; RV—residual volume.

metered dose aerosol (two puffs of 200 µg a puff twice daily).

MEFV curves were recorded before and after the active treatment period and blood was collected for an eosinophil cell count. Drug canisters were weighed every two weeks to assess treatment compliance. Patients were allowed to use inhaled ipratropium bromide on an "if necessary" basis, to control symptoms throughout the study. No ipratropium bromide or budesonide was allowed for 12 hours preceding the visit to the clinic. In general, the patients had only mild symptoms and ipratropium bromide was taken infrequently.

Table 1   Lung function values (% predicted[12]) at entry

Patient No.	Age (y)	FVC	FEV_1	PEF	$\dot{V}E\ max_{75}$	$\dot{V}E\ max_{50}$	$\dot{V}E\ max_{25}$
1	22	99	97	85	100	91	56
2	31	97	96	93	73	60	35
3	25	95	81	92	56	57	52
4	24	91	88	99	77	58	45
5	26	106	82	76	69	51	34
6	32	74	98	90	55	42	28
7	21	101	91	102	83	82	87
8	32	87	73	100	49	41	27
9	26	89	97	117	111	102	107
10	36	103	88	84	39	38	25
11	20	100	89	83	45	46	48
12	18	95	79	74	60	52	62
13	28	114	92	103	60	51	48
14	23	97	70	84	43	35	24
Mean	26	98	86	92	66	58	48
SEM	1	2	2	3	6	5	6

FVC—forced vital capacity; FEV_1—forced expiratory volume in one second; PEF—peak expiratory flow; $\dot{V}E\ max_{75}$, $\dot{V}E\ max_{50}$, $\dot{V}E\ max_{25}$—maximal expiratory flow at 75%, 50%, and 25% FVC.

Table 2   *Mean (SEM*) maximal expiratory flow volume values (% predicted[12]) before and after treatment*

	Budesonide				Ipratropium bromide			
	Baseline		After treatment	p†		Baseline	After treatment	p†
FVC	98	(2)	103 (2)	< 0·005	101	(2)	104 (2)	< 0·001
FEV$_1$	86	(2)	96 (2)	< 0·001	86	(3)	95 (2)	< 0·001
PEF	91	(3)	108 (4)	< 0·01	95	(4)	108 (4)	< 0·001
V̇E max$_{75}$	66	(6)	80 (4)	< 0·01	67	(7)	82 (6)	< 0·001
V̇E max$_{50}$	58	(5)	70 (4)	< 0·005	59	(5)	72 (6)	< 0·001
V̇E max$_{25}$	48	(6)	69 (5)	< 0·001	51	(6)	63 (6)	< 0·001

*The sample SEM.
†Significance of differences assessed by paired *t* tests.
Abbreviations as in table 1.

At least six weeks after the active treatment period the patients came to the clinic to produce flow-volume curves before and 30 minutes after the inhalation of a single dosage of 80 µg ipratropium bromide (two puffs containing 40 µg). The patients used only inhaled bronchodilator during the three weeks preceding this test and no inhaled drugs during the 12 hours preceding the test.

The protocol was approved by the hospital's ethics committee and the written informed consent of each patient was obtained.

LUNG FUNCTION
MEFV curves were recorded with a dry rolling seal type spirometer (Mijnhardt, Vica test 5). After inspiring slowly to total lung capacity (TLC) the patient performed a forced expiration followed by a forced inspiration. From three technically adequate curves where the forced vital capacity (FVC) did not differ by more than 5% the curve with the largest V̇E max$_{50}$ was selected. Values of FVC, FEV$_1$, peak flow (PEF), V̇E max$_{75}$, V̇E max$_{50}$, and V̇E max$_{25}$ were expressed as percentages of the predicted values.[12] The rate constant was calculated as V̇E max$_{50}$/FVC.

Curvature parameters were determined in three ways (fig 1). The index of shape as introduced by Mead *et al*[10] was defined as $1/2$(V̇E max$_{50}$/V̇E max$_{25}$) (SF 50%). We extended the volume range by also calculating a similar index $1/3$(V̇E max$_{25}$/V̇E max$_{25}$) (SF 75%). Mead's slope ratio (SR) is defined as the tangent slope to the curve (dV̇/dV) divided by the chord V̇E/ (FVC-V).[9]

As the microcomputer incorporated in the spirometer does not provide values for the slope of the MEFV curves, we estimated the slope as follows. A

quadratic curve was fitted to flows at 75%, 50%, 25%, and 0% of FVC to derive the slope ratio at these points. These values were compared with those obtained graphically, estimated directly from the tracings. The values from the quadratic fit matched the values drawn by hand well. We estimated the error in the slope values to be less than 3%. From values of V̇E max$_{75}$ and V̇E max$_{50}$, computed by spirometer, we calculated the chords and hence the slope ratios at 75% and 50% of the remaining FVC.

ANALYSIS
We calculated intraindividual standard deviations from two consecutive measurements on the same day using one way analysis of variance. The intraindividual SD for the shape factor was 0·08 in absolute units (5%) at 75% FVC and 0·08 (6%) at 50% FVC. The intraindividual SD for the slope ratios at FVC was 0·17 in absolute units (12%) at 75% and 0·21 (16%) at 50% FVC.

STATISTICAL ANALYSIS
Student's *t* test for paired samples was used to compare post-treatment with baseline values of MEFV curve values and curvature parameters. Correlations between variables were calculated by the Rank-Spearman test.

Results

Changes in lung function with budesonide and ipratropium are summarised in table 2. There were no significant differences between the baseline values before the two treatments, indicating that six weeks after cessation of budesonide the MEFV curve had

Table 3   *Measures (mean (SEM)) of the shape of the maximal expiratory flow-volume curve*

	Budesonide					Ipratropium bromide			
	Baseline		After treatment		p†		Baseline	After treatment	p†
RC	0·64	(0·06)	0·74	(0·05)	< 0·001	0·64 (0·06)	0·77 (0·07)	< 0·001
SF$_{50}$	1·36	(0·09)	1·12	(0·07)	< 0·01	1·29 (0·07)	1·27 (0·06)	NS
SF$_{75}$	1·59	(0·13)	1·30	(0·11)	< 0·01	1·49 (0·09)	1·46 (0·09)	NS
SR$_{50}$	1·33	(0·06)	1·26	(0·06)	NS	1·32 (0·06)	1·31 (0·05)	NS
SR$_{75}$	1·43	(0·07)	1·30	(0·08)	< 0·025	1·41 (0·07)	1·40 (0·06)	NS

RC—rate constant: V̇E max$_{50}$/FVC (unit: s^{-1}); SF—shape factor, at 50% FVC and 75% FVC; SR—slope ratio, at 50% FVC and 75% FVC.
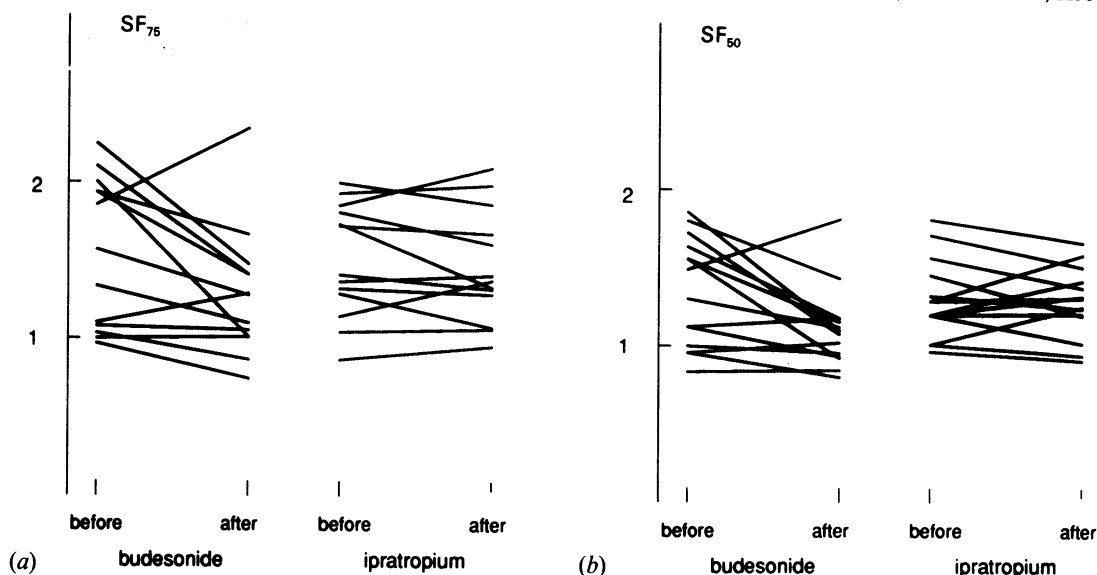
Fig 2 *Individual changes in the shape factor at 75% forced vital capacity (FVC) (SF$_{75}$) (a), and 50% FVC (SF$_{50}$) (b) before and after the treatment with budesonide and ipratropium bromide.*

returned to its pretreatment shape.

Budesonide treatment for eight weeks caused a significant improvement in all lung function measurements. The percentage change in end expiratory flows, especially in $\dot{V}E$ max$_{25}$ (59%), was much greater than the change in FEV$_1$ (12%). Ipratropium bromide also caused a significant improvement in expiratory volumes and flows. Change in $\dot{V}E$ max$_{25}$ (28%) was less pronounced in relation to change in FEV$_1$ (11%) than after treatment with budesonide.

The change in curvilinearity of the MEFV curve (shape factor and slope ratio at 75% and 50% FVC) is summarised in table 3, and individual changes are given in figures 2 and 3. At 75% FVC both slope ratio and shape factor had fallen significantly after treatment with budesonide; ipratropium bromide caused no change in either measure. At 50% FVC only the shape factor showed a significant decrease with budesonide. Changes in shape factor were larger when the initial slope factor was higher (see figs 2 and 3), indicating that the more convex to the volume axis the
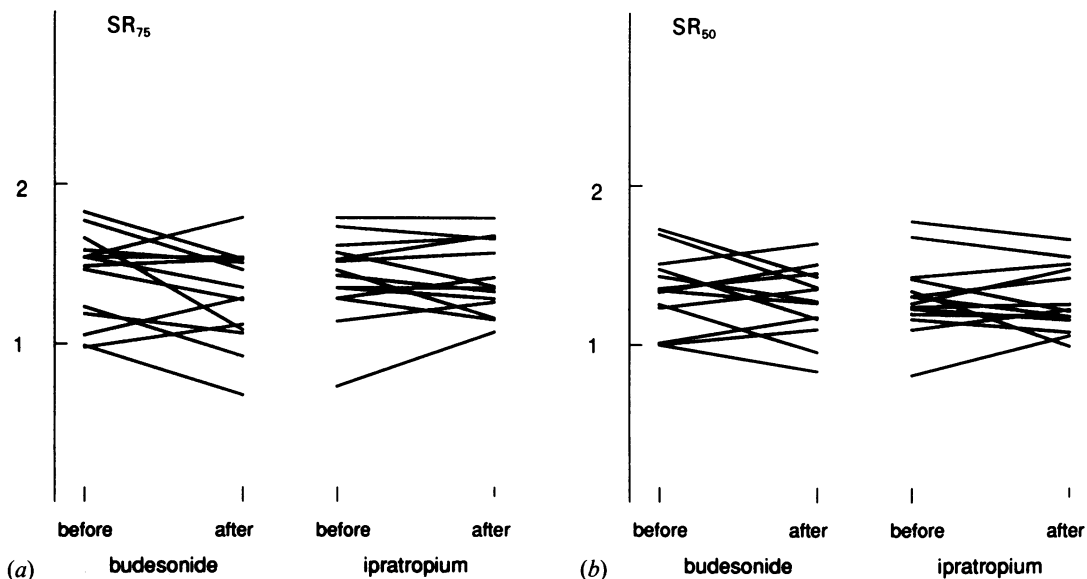

Fig 3 *Individual changes in the slope ratio at 75% forced vital capacity (FVC) SR$_{75}$ (a), and 50% FVC (SR$_{50}$) (b) before and after treatment with budesonide and ipratropium bromide.*
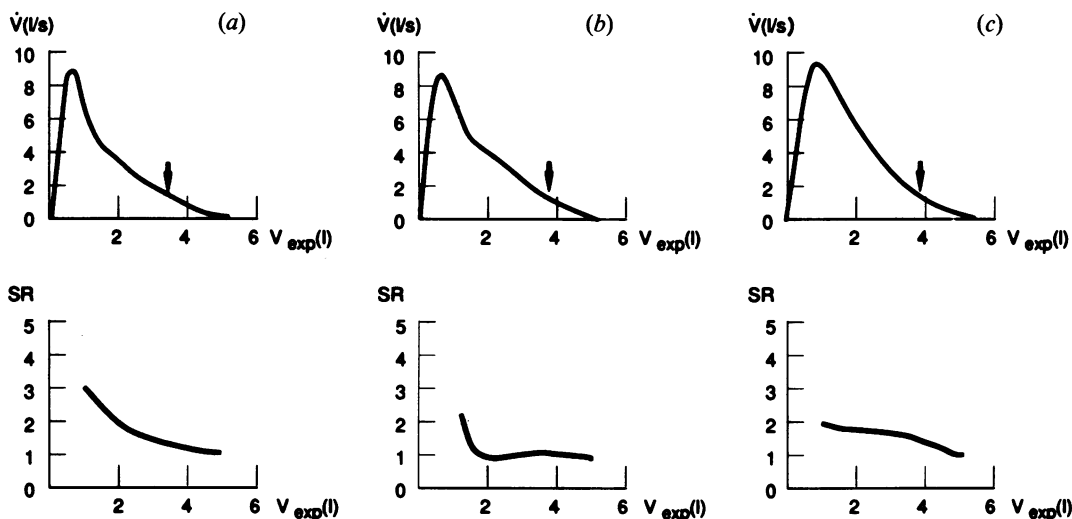
Fig 4 *Example of maximum expiratory flow-volume (MEFV) curves of a single patient: (a) baseline; (b) after eight weeks' treatment with budesonide; (c) after six weeks' withdrawal of budesonide but after inhalation of ipratropium bromide. Arrows indicate FEV$_1$. In the lower panel the course of the slope ratio versus volume (V) is shown.*
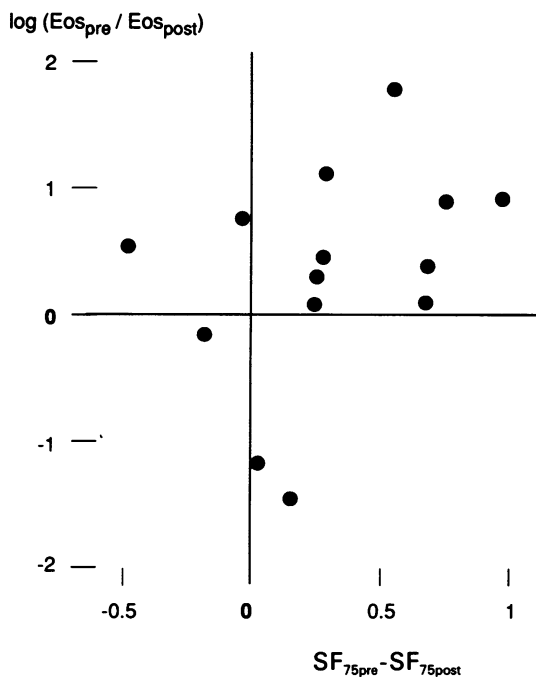

Fig 5 *Scatterplot of the logarithm of the ratio of blood eosinophil cell counts (Eos) and change in the shape factor (SF$_{75}$) after treatment with budesonide.*

MEFV curve is the larger is the improvement with budesonide.

Shape ratios decreased significantly with decreasing lung volume, for both baseline measurements and after administration of ipratropium bromide. After budesonide slope ratios were generally smaller, and showed no relation to lung volume (fig 4).

The mean (SEM) number of eosinophil granulocytes in peripheral blood decreased significantly from 267 (51)/mm^3 to 198 (33)/mm^3 after treatment with budesonide (p < 0·05, non-parametric sign test). There was a correlation between the ratio of blood eosinophils before and after treatment and the change in slope factor with budesonide (Spearman correlation coefficient 0·47, p < 0·05: fig 5).

Discussion

In this study treatment with both budesonide for eight weeks and a single dose of ipratropium bromide increase FEV$_1$. Budesonide had a larger effect, however, on end expiratory maximal flows than ipratropium bromide. This may partly be due to the fact that end expiratory maximal flow values are numerically smaller and show wide variability. As the variability for these flows appears to be of the same order of magnitude as that for other maximal expiratory flow values in our patients (table 2), the differences we report are likely to reflect differences in the action of budesonide and ipratropium bromide.

End expiratory flow rates are frequently used to assess bronchodilator responses.[13][14] The present study shows that possible changes in curvilinearity of the

MEFV curve also need to be taken into account. In this study we quantified the curvilinearity of the MEFV curves and showed that treatment with budesonide caused a decrease in curvilinearity, whereas treatment with ipratropium bromide had no such effect. The straightening effect of budesonide on the MEFV curve was seen with all three methods of quantifying the curvilinearity, though the methods that used $\dot{V}E$ max$_{75}$ were more sensitive. The slope ratio at 50% FVC showed a non-significant difference whereas the change in slope ratio at 75% FVC was significant. Before treatment with budesonide there appears to be a decrease in slope ratio with decreasing lung volume. This confirms the findings of O'Donnell and coworkers,[15] who observed a similar decrease in subjects with mild asthma. They also found no such change in slope ratio in normal subjects. The fact that we found no significant difference in the slope ratios at 75% and 50% FVC after treatment with inhaled corticosteroids might indicate that change in slope ratio with decreasing lung volume has returned to normal. It may be argued that selection of MEFV curves may introduce bias with respect to the curvilinearity measures. Peslin *et al*[16] reviewed different methods of selection of MEFV curves, and found that the method of selection resulted in systematic changes in maximal expiratory flow values. The absolute values of the curvilinearity parameters may therefore depend on our selection criteria, but differences in these parameters before and after medication are unlikely to be influenced by selection; similarly, though measurements made by dry rolling seal spirometer may differ from those made by pneumotachograph,[17] the differences are systematic and should cancel out when values before and after medication are compared.

The shape factor seems to be a fairly repeatable parameter, as judged by intraindividual standard deviations. Slope ratio was more variable, perhaps because slope ratio was calculated from differentiation of volume. We made no corrections for change in absolute lung volume. Neither treatment would be expected to cause much change in absolute lung volume in these patients with mild asthma (table 2). As both shape factor and slope ratio are basically ratios of flows, which shift in the same direction with change in lung volume, the effect of a small change in lung volume on flow would be expected to cancel out in the ratio of flows, and probably therefore had only a minor effect on shape factor and slope ratio.

The pathophysiological basis of the increased curvilinearity of the MEFV curve is unknown. Many authors are of the opinion that the decrease in expiratory flow near residual volume is due to preferential obstruction of the peripheral airways.[18 19] Mead[9] developed the concept of inhomogeneous emptying of the lung during forced expiration and has shown on theoretical grounds that when such inhomogeneity occurs the flow-volume curve should be convex towards the volume axis. This has also been seen by O'Donnell[15] in asthmatic subjects, by Landau[20] in patients with more advanced chronic obstructive lung disease, and recently by Kapp *et al*[21] in an epidemiological study. The increased curvilinearity of the MEFV curve may therefore be caused by regional inhomogeneity of forced expiratory flow—that is, the existence of regions with a different time constant (flow divided by volume) in the lung.[8] Our results might therefore suggest that inhaled corticosteroid drugs improve regional ventilatory inhomogeneity whereas rapid bronchodilatation with ipratropium does not exert such an effect, though the overall time constant was decreased.

Bronchial obstruction in asthma seems to be the consequence of smooth muscle contraction and increased thickness of the airway wall caused by inflammatory processes.[1] The inflammatory changes are seen in central and peripheral airways.[22] The eosinophil cell, which is predominant in bronchial biopsy material from allergic patients[22] and in bronchalveolar lavage fluid[23] from asthmatic subjects, is seen in samples taken from central airways.[22 23] The eosinophil may be a marker of disease activity as suggested by Durham and Kay,[24] who observed that the number of blood eosinophils correlated well with airway responsiveness.

Budesonide has a strong anti-inflammatory effect[9] and this is thought to account for the fall in peripheral blood eosinophils during treatment with budesonide. As budesonide has low oral bioavailability when inhaled,[9] we assume that the decrease in peripheral blood eosinophils is due to inhaled and not ingested drug. The fact that we found a correlation between change in peripheral blood eosinophils and change in curvilinearity of the MEFV curve suggests that inflammatory processes might be responsible for the abnormal shape of the MEFV curve in asthmatic patients. Limitation of expiratory flow is related not only to airway narrowing but also to the mechanical properties of the airway wall, in particular to airway compliance,[5] and airway wall thickening due to inflammation may be an important cause of flow limitation. Such changes in the thickness of the airway wall are unlikely to be spread uniformly throughout the airways, and this may lead to inhomogeneous emptying during forced expiration.

Ipratropium bromide is a powerful bronchodilator. Several studies have shown that regional ventilation inhomogeneity in asthma is not improved by administration of a bronchodilator.[25 26] This might be due to better penetration of the aerosol to relatively well ventilated areas than to relatively poor ventilated areas. The effect of long term treatment with an inhaled corticosteroid might differ from the effect of

an inhaled bronchodilator because the former causes a gradual decrease in airway narrowing in poorly ventilated areas, thereby decreasing ventilation inhomogeneity.

The question whether the anti-inflammatory effect occurs preferentially in central or in peripheral airways cannot be answered from this study. The detection of inhomogeneous emptying of the lung during a forced expiration is determined by the resistance that different lung regions have in common. If this common resistance is large in relation to the resistances peripheral to the common resistance, the total time constant would be primarily determined by the common resistance. From the results reported here we may conclude that, before anti-inflammatory treatment, the major inhomogeneity is located peripherally, whereas after treatment the flow limiting segment has moved to more central sites. This does not, however, rule out the possibility that central airways are also affected by inflammation. In that case the main effect of treatment with inhaled corticosteroids would be a change in central airway compliance.

We conclude that treatment with an inhaled corticosteroid causes an increase in maximal expiratory flows and a decrease in the abnormal curvilinearity of the MEFV curve in patients with allergic asthma. This effect is probably due to a decrease in inhomogeneously distributed inflammatory airway narrowing. This reduction in the curvilinearity of the MEFV curve was not seen after bronchodilatation by a single dose of ipratropium despite a similar increase in FEV_1.

References

1 Dunnill MS. The pathology of asthma with special reference to the changes in the bronchial mucosa. *J Clin Pathol* 1960;**13**:27–33.

2 Kraan J, Koeter GH, Van der Mark ThW, *et al.* Dosage and time effects of inhaled budesonide in bronchial hyperreactivity. *Am Rev Respir Dis* 1988;**137**:44–8.

3 Hyatt RE, Black LF. The flow-volume curve. A current perspective. *Am Rev Respir Dis* 1973;**107**:191–9.

4 Olive JT, Hyatt RE. Maximal expiratory flow and total respiratory resistance during induced bronchoconstriction in asthmatic subjects. *Am Rev Respir Dis* 1972;**106**:366–76.

5 Dawson SV, Elliott EA. Wave-speed limitation on expiratory flow—a unifying concept. *J Appl Physiol* 1977;**43**:498–515.

6 Black LF, Offord K, Hyatt RE. Variability in the maximum expiratory flow-volume curve in asymptomatic smokers and in non-smokers. *Am Rev Respir Dis* 1974;**110**:282–92.

7 Green M, Mead J, Turner JM. Variability of maximum expiratory flow-volume curves. *J Appl Physiol* 1974;**37**:67–74.

8 Mead J. Analysis of the configuration of maximum expiratory flow-volume curves. *J Appl Physiol* 1978;**44**:156–65.

9 Johansson SA, Andersson KE, Brattsand R, Gruvstad E, Hedner P. Topical and systemic glucocorticoid potencies of budesonide, beclomethasone diproprionate, and prednisolone in man. *Eur J Respir Dis* 1982;**63** (suppl 122):74–82.

10 Hyatt RE, Rodarte JR, Mead J, Wilson ThA. Changes in lung mechanics: flow-volume relation. In: Macklem PT, Permutt S, eds. *The lung in the transition between health and disease.* New York: Dekker, 1979.

11 Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978;**33**:705–10.

12 Quanjer Ph H, ed. Standardised lung function testing. *Bull Eur Physiopathol Respir* 1983;**19** (suppl 5):45–51.

13 Cohen AA, Hale FC. Comparative effects of isoproterenol aerosols on airway resistance in obstructive pulmonary diseases. *Am J Med Sci* 1965;**249**:309–15.

14 Clay MM, Pavia D, Clarke SW. Effect of aerosol particle size on bronchodilatation with nebulised terbutaline in asthmatic subjects. *Thorax* 1986;**41**:364–8.

15 O'Donnell CR, Castile RG, Mead J. Changes in flow-volume curve configuration with bronchoconstriction and bronchodilation. *J Appl Physiol* 1986;**61**:2243–51.

16 Peslin R, Bokadana A, Hannhart B, Jardin P. Comparison of various methods for reading maximal expiratory flow-volume curves. *Am Rev Respir Dis* 1979;**119**:271–8.

17 Pincock AC, Miller MR. The effect of temperature on recording spirograms. *Am Rev Respir Dis* 1983;**128**:894–8.

18 Knudson RJ, Burrows B, Lebowitz MD. The maximal expiratory flow volume curve: its use in the detection of ventilatory abnormalities in population study. *Am Rev Respir Dis* 1976;**114**:871–9.

19 Gelb AF, Zamel N. Simplified diagnosis of small airway obstruction. *N Engl J Med* 1973;**288**:395–8.

20 Landau LI, Taussig LM, Macklem PT, Beaudry PH. Contribution of inhomogeniety of lung units to the MEFVC in children with asthma and cystic fibrosis. *Am Rev Respir Dis* 1975;**111**:725–31.

21 Kapp MC, Schachter EN, Beck GJ, Maunder LR, Witek ThJ. The shape of the maximum expiratory flow-volume curve. *Chest* 1988;**94**:799–806.

22 Holgate ST, Roche W, Roberts A, Beasley RC. Inflammation as the basis of asthma. In: Sluiter HJ, van der Lende R, Gerritsen J, Postma DS, eds. *Bronchitis IV. Fourth international symposium.* Assen: van Gorcum, 1989.

23 De Monchy JGR, Kauffman HF, Venge P, *et al.* Broncho-alveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;**131**:373–6.

24 Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy* 1985;**15**:411–8.

25 McFadden ER, Lyons HA. Airway resistance and uneven ventilation in bronchial asthma. *J Appl Physiol* 1968;**25**:365–70.

26 Siegler D, Fukuch Y, Engel L. Influence of bronchomotor tone on ventilation distribution and airway closure in asymptomatic asthma. *Am Rev Respir Dis* 1976;**114**:123–30.