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Increased airway closure is a determinant of airway hyperresponsiveness

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ABSTRACT: In order to investigate whether increased airway closure is a component of airway hyperresponsiveness (AHR), airway closure was compared during induced bronchoconstriction in 62 asthmatic, 41 nonasthmatic nonobese (control) and 20 nonasthmatic obese (obese) subjects.

Airway closure and airway narrowing were measured by spirometry as percentage change in forced vital capacity (%ΔFVC) and change in forced expiratory ratio (ΔFER), respectively. Multiple regression analyses were used to assess the determinants of AHR, assessed by the dose response slope (DRS).

The DRS was significantly increased in asthmatics compared with controls but did not differ between obese and controls. The spirometric predictors of logDRS were baseline FER, ΔFER, body mass index (BMI) and %ΔFVC. There was a negative relationship between BMI and logDRS in the regression, suggesting a protective effect.

The present findings suggest that the extent of airway closure during induced bronchoconstriction is a determinant of airway hyperresponsiveness, independent of the level of airway narrowing. However, after adjusting for airway closure, obesity appears to protect against airway hyperresponsiveness.

KEYWORDS: Airway hyperresponsiveness, asthma, small airways

Increased airway closure has been associated with a greater risk of severe asthma exacerbations [1, 2] and with a requirement for oral steroid treatment [3]. Radiological imaging studies have shown that the number of poorly ventilated or nonventilated lung regions correlates with the severity of asthma measured by clinical symptoms and spirometry [1, 4]. These studies suggest that increased airway closure in asthma is an important marker of disease severity. It has recently been proposed that airway hyperresponsiveness (AHR) in sensitised mice can be attributed to an increased susceptibility to small airway closure [2]. However, the role of increased airway closure as a contributing factor to AHR in asthma, defined as an exaggerated and unrestricted response to stimulation of airway smooth muscle, is not known.

It is not clear whether the extent of airway closure differs between asthmatic and nonasthmatic subjects. Using radionuclide imaging, KING *et al.* [5] found no difference in the volume of nonventilated lung measured at residual volume between asthmatic and nonasthmatic subjects. After methacholine-induced bronchoconstriction, airway closure increases in normal subjects [6, 7], but there have been few comparisons of the extent of airway closure during bronchoconstriction between asthmatic and nonasthmatic subjects. MILANESE *et al.* [8] found that airway closure, measured by the relative changes in forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), was similar in asthmatic and rhinitic subjects; however, both these groups had AHR. The mechanical effects of obesity [9–11] may predispose nonasthmatic subjects to increased airway closure during bronchial challenge [12]. If airway closure is an important mechanism for AHR then increased airway closure in obese subjects would be expected to increase responsiveness.

Airway closure is difficult to measure directly but has been estimated indirectly using a range of physiological techniques, such as spirometry [3, 8], nitrogen washout [1, 7] and radionuclide imaging [5, 6]. Using spirometry, airway closure has conventionally been represented by the change in FVC. Previous studies have reported changes in FVC at the provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) [3, 13] or concurrent changes in FVC and FEV_1 [8] to compare airway closure in subjects with

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varying magnitudes of bronchoconstriction. Since changes in FEV_1 are a cumulative effect of airway narrowing and airway closure [14], these indices reflect the proportion of the change in FEV_1 that is attributable to airway closure. GIBBONS *et al.* [3] have shown that the contribution of airway closure to the change in FEV_1 varies widely between asthmatic subjects, and does not correlate with the severity of AHR, measured by PC_{20}. This led them to speculate that airway closure and AHR are not due to the same mechanisms. However, to test this proposal it is necessary to evaluate the contribution of airway narrowing and airway closure separately. GIBBONS *et al.* [3] partitioned airway narrowing, measured by the change in the FEV_1/FVC ratio or forced expiratory ratio (FER), from airway closure, measured by the change in FVC. They identified a broad spectrum of responses in asthmatic subjects, ranging from those where changes in FER were predominant to those where changes in FVC were predominant. Similarly, using this approach, SORKNESS *et al.* [14] have examined the independent contribution of airway narrowing and airway closure in severe asthma at baseline. In the present study concurrent changes in FVC and FER were used to determine the independent contribution of airway narrowing and airway closure to AHR.

The objective of the present study was to determine whether increased airway closure contributes to the pathophysiology of AHR in human asthma. The hypothesis was that increased airway closure during bronchoconstriction, after standardising for airway narrowing, would be associated with increased responsiveness to methacholine in both asthmatic and obese nonasthmatic subjects. In order to test this multiple regression models were used to determine the factors that contributed to airway closure and then to determine whether the extent of airway closure contributed to AHR, after adjustment for airway narrowing and other significant factors.

METHODS

Subjects

Data were compiled from four studies in which protocols varied slightly but which employed similar techniques. Inclusion criteria and patient characterisation among studies were identical. Asthmatics were recruited from the asthma clinics at the Royal Prince Alfred Hospital (Camperdown, NSW, Australia), through the research volunteer database at the Woolcock Institute of Medical Research (Glebe, NSW, Australia) and from the University of Sydney (Sydney, NSW, Australia). Nonobese nonasthmatic (control) and obese nonasthmatic (obese) subjects were recruited from the staff at the Woolcock Institute of Medical Research, the University of Sydney and the Metabolism and Obesity Service and Sleep Disordered Breathing Clinics at Royal Prince Alfred Hospital. Asthmatic subjects had physician-diagnosed asthma, symptoms consistent with asthma in the preceding 12 months, and were taking inhaled corticosteroids and/or β_2-agonist medication. Nonasthmatic subjects had no history of respiratory disease. Obese subjects had a body mass index (BMI) >30 kg·m^{-2} [15]. All subjects were nonsmokers and had no other concomitant cardiac or respiratory disease. The studies were approved by the Central Sydney Area Health Service Ethics Review Committee (protocol No. X02-0217, X02-0057, X04-0014 and X05-0285) and all subjects gave written informed consent.

Study design

Subjects were studied during a single visit. Asthmatic subjects had a low-dose methacholine challenge (maximum dose of 12.2 μmol) and nonasthmatic subjects a high dose challenge (maximum dose of 200 μmol). Asthmatic subjects withheld the use of short acting β_2-agonists for 6 h and long acting β_2-agonists for 24 h prior to testing.

Data were obtained from a total of 62 asthmatic, 41 control and 20 obese subjects. Spirometry measurements were obtained throughout the methacholine challenge in 62 asthmatic and 16 control subjects, and only at baseline and after completion of the challenge in 25 control and 20 obese subjects

Exhaled nitric oxide

The fraction of exhaled nitric oxide (F_{ENO}) was measured using an offline method according to the specifications of the American Thoracic Society [16]. An expiratory flow rate was maintained at 200 mL·s^{-1} and the exhaled air was analysed offline by a chemiluminescent analyser (Model 42C; Thermo Environmental Instruments, Franklin, MA, USA). Two separate breaths were analysed from each subject and the average recorded. F_{ENO} values above 13 ppb were considered above the normal range [17].

Methacholine challenge

Methacholine challenges (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) were performed in asthmatic subjects, with cumulative doses ranging 0.1–12.2 μmol and, in nonasthmatic subjects, with cumulative doses 0.15–200 μmol, using a KoKo dosimeter (PDS Instrumentation Inc. Louisville, KY, USA) attached to oxygen at 30 psi. Spirometric measurements were made in accordance with American Thoracic Society recommendations [18]; FVC manoeuvres were held for a minimum of 6 s and until a plateau in the expiratory volume trace was observed. The predicted values of QUANJER *et al.* [19] were used.

Airway response to methacholine was measured by the percentage change in FEV_1 (%ΔFEV_1).Throughout challenge, airway narrowing was assessed by the change in FER (ΔFER) and airway closure by the percentage change in FVC (%ΔFVC). The proportion of the change in FEV_1 which was due to airway closure was measured as the %ΔFVC/%ΔFEV_1, termed the closing index. The dose–response slope (DRS) was calculated as the two point slope from the change in FEV_1 at the end of challenge divided by the dose in μmol [20, 21] to provide a continuous measure of airway responsiveness. AHR was defined as a DRS >6.8 %ΔFEV_1·μmol^{-1}.

Outcome variables and data analyses

Comparisons of baseline characteristics and response to methacholine between asthmatic and control, and obese and control subjects were examined using two-sample equal variance unpaired t-tests. The closing index was calculated after the last dose of challenge and compared between asthmatic, control and obese subjects using ANOVA. Multiple regression analyses were used to assess the determinants of airway closure and AHR, assessed by logDRS. All correlations were assessed using Pearson coefficients. A p-value <0.05 was considered to be statistically significant.

RESULTS

Subject characteristics

Table 1 contains baseline characteristics for asthmatic, control and obese subjects. The asthmatic group did not differ from the control group in age, height or BMI, but had impaired baseline lung function. F_{ENO} levels were higher in the asthmatic subjects than in control subjects. Compared with controls, asthmatics had an increased response to methacholine, measured by FEV_1 and FVC, but not by FER. These changes were induced by lower doses of methacholine in asthmatics than in controls ($p<0.001$), resulting in a higher mean DRS in asthmatic subjects ($p<0.001$). A total of 54 out of 62 asthmatic subjects had AHR.

The obese subjects were older than the control subjects and had increased F_{ENO} but did not differ in baseline lung function. Response to methacholine, measured by FEV_1, FER and FVC was similar in obese and control subjects. DRS values were similar in both groups.

Airway closure measured by spirometry

In order to estimate the contribution of airway closure to the change in FEV_1, %ΔFVC was plotted against %ΔFEV_1 in the 62 asthmatic and 16 control subjects who had FVC and FEV_1 measured throughout challenge (fig. 1). In both asthmatic and control subjects there were significant linear relationships within individuals (median R^2 (inter-quartile range) of 0.95 (0.89–0.98) in asthmatics and 0.77 (0.68–0.94) in controls). Obese subjects had spirometric data measured at baseline and after the final dose step of challenge only, necessitating the calculation of the closing index (%ΔFVC/%ΔFEV_1) as the change from baseline at the final dose step of challenge. In order to compare all three groups, the closing index was calculated in asthmatic, control and obese subjects. The closing index in asthmatic and control subjects did not differ from the individual regressions measured throughout challenge ($p=0.43$ for asthmatic and 0.69 for controls). The mean±SEM closing index in 41 controls (0.54±0.03) did not differ significantly from that in 62 asthmatic subjects (0.60±0.02; $p=0.18$) but was significantly lower than in the 20 obese subjects (0.72±0.04; $p=0.001$), indicating greater closure occurring in the obese subjects. There was no correlation between the closing index and logDRS ($p=0.37$).

In order to estimate the independent effects of airway narrowing and airway closure during challenge, changes in FEV_1, FVC and FER were compared throughout challenge in asthmatic and control subjects. Figure 2 illustrates representative changes in two asthmatic and two control subjects. In figure 2a and b, the changes in FEV_1 are predominantly due to changes in FER; whereas in figure 2c and d, the changes in FEV_1 are predominantly due to changes in FVC. These examples represented extremes of a continuum of responses, with the majority of subjects falling in between. In order to determine the association between airway closure and airway narrowing, %ΔFVC was plotted against FER throughout challenge in asthmatic and control subjects (fig. 3). Absolute FER was plotted, rather than %ΔFER, to illustrate the role of baseline FER in the extent of airway closure during challenge.

Univariate analyses were undertaken using combined data for asthmatic, control and obese subjects to determine the spirometric predictors of airway closure, showing that %ΔFVC correlated with baseline FER ($r= -0.31$; $p=0.0004$), but not with ΔFER ($r=0.11$; $p=0.21$) or with BMI ($r=0.14$; $p=0.12$). Furthermore, logF_{ENO} did not correlate with %ΔFVC ($p=0.43$) but did correlate with ΔFER ($r=0.25$; $p=0.009$). Using multiple regression analysis, decreased baseline FER, increased ΔFER and increased BMI were significant independent predictors of increased %ΔFVC ($R^2_{adj}=0.17$, $F=9.53$; $p<0.0001$).

In order to determine the independent contribution to AHR of airway narrowing and airway closure, univariate and multivariate analyses were undertaken with logDRS as the outcome. LogDRS correlated with baseline FER ($r= -0.48$; $p<0.0001$),

TABLE 1 Anthropometric and lung function data of the asthmatic, control and obese populations

	Asthmatic subjects	Control subjects	Obese subjects
Subjects n	62	41	20
Age yrs	29.8 (26.7–32.9)	35.2 (30.7–39.7)	44.7 (39.8–49.6)*
Sex % male	57%	45%	65%
Height m	1.71 (1.69–1.73)	1.72 (1.69–1.75)	1.70 (1.66–1.74)
BMI kg·m^{-2}	24.7 (23.59–25.81)	23.9 (23.14–24.66)	37.7 (34.4–41.0)***
FEV_1 % pred	87.3 (83.8–90.8)***	101.0 (97.8–104.2)	99.4 (94.0–104.8)
FVC % pred	93.7 (89.5–97.9)**	103.0 (99.5–106.5)	102.8 (97.4–108.2)
FER	0.75 (0.73–0.77)***	0.82 (0.80–0.84)	0.81 (0.79–0.83)
ΔFER at max$^{\#}$	0.083 (0.062–0.104)	0.073 (0.040–0.106)	0.053 (0.017–0.089)
%ΔFEV_1 at max	24.5 (22.8–26.2)***	17.9 (14.9–20.9)	17.8 (13.5–22.1)
%ΔFVC at max	15.0 (13.3–16.7)***	10.0 (7.7–12.3)	12.9 (9.2–16.6)
F_{ENO} ppb¶	18.3 (15.1–22.0)***	9.2 (7.8–10.7)	12.4 (10.3–15.0)*
Max dose μmol	6.10 (-0.1–12.3)***	165.3 (145.3–185.3)	193.3 (180.3–206.3)
DRS %fall·μmol^{-1}¶	16.2 (11.5–22.5)***	0.25 (0.11–0.39)	0.10 (0.06–0.14)

Data are presented as mean (95% confidence interval), except: $^{\#}$: median (interquartile range); and ¶: geometric range (95% confidence interval), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; FER: forced expiratory ratio; F_{ENO}: exhaled nitric oxide fraction; DRS: dose–response slope. *: $p<0.05$ *versus* controls; **: $p<0.01$ *versus* controls; ***: $p<0.001$ *versus* controls.

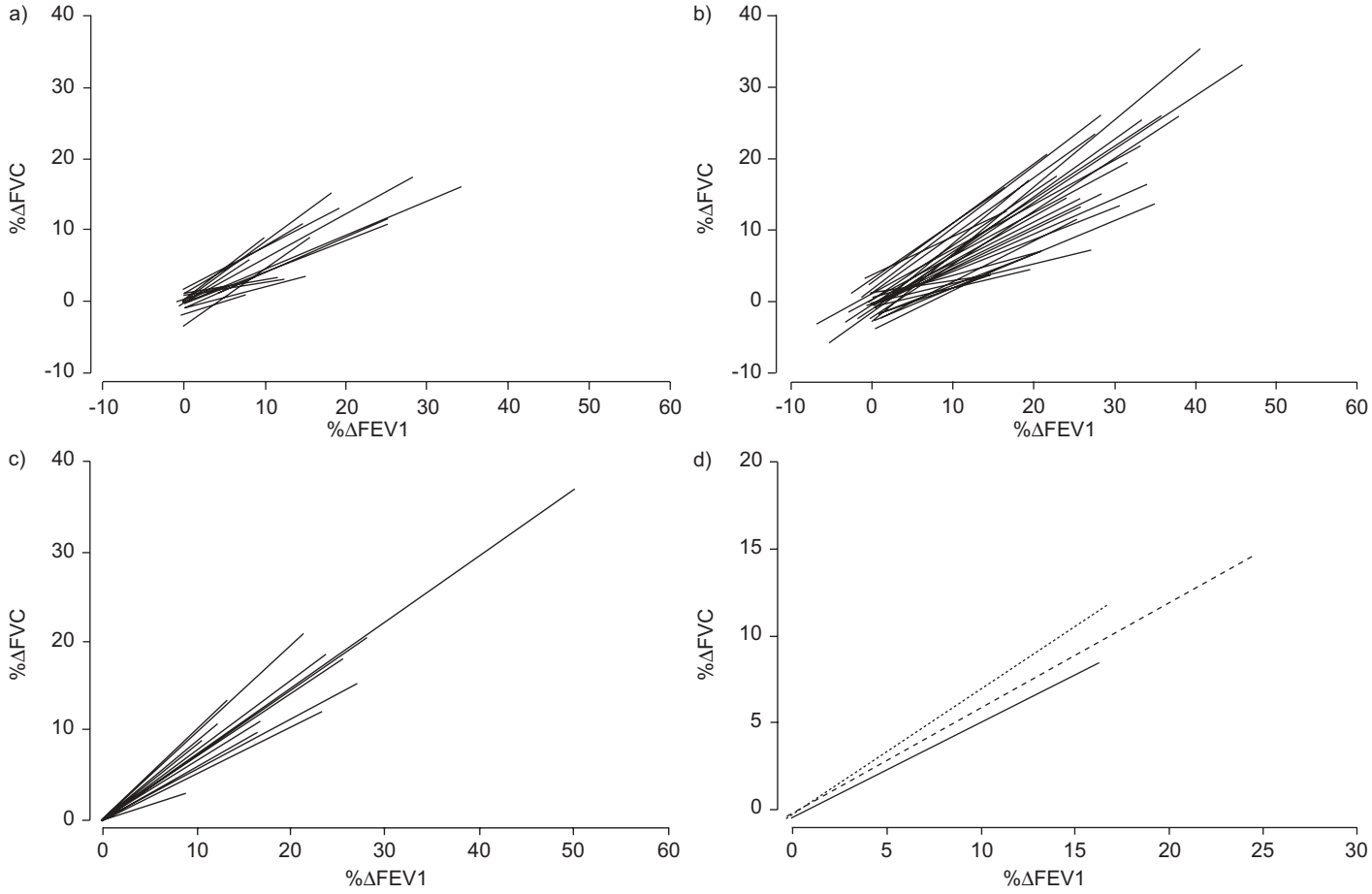

FIGURE 1. Relationship between percentage change in forced vital capacity (%ΔFVC) and percentage changed in forced expiratory volume in one second (%ΔFEV1) during challenge. Individual regression lines are shown for control (a) and asthmatic subjects (b). Obese subjects (c) did not have FVC measured throughout challenge and are shown as the two-point line between saline and last dose step of challenge. Mean regression lines have been plotted for each group (d) and are stated as y=m(±SE)×x+b; control (—; y=0.54(±0.03)×x-0.41), asthma (-----; y=0.60(±0.02)×x-0.26) and obese (·····; y=0.72(±0.03)×x-0.33).

%ΔFVC (r=0.41; p<0.0001), ΔFER (r=0.23; p=0.03) and BMI (r= -0.19; p=0.03). Multiple regression analysis showed that increased %ΔFVC, decreased baseline FER, increased ΔFER and BMI were all significant predictors of increased logDRS (R^2_{adj}=0.36, F=18.72; p<0.0001). In the latter regression, BMI had a negative β co-efficient, suggesting a protective effect of increased BMI.

DISCUSSION

The present study shows that the magnitude of airway closure induced by bronchial challenge is determined by baseline airway calibre, the extent of airway narrowing and BMI. After adjusting for these factors, the magnitude of airway closure makes a significant, independent contribution to the severity of AHR. However, after accounting for the effects of airway closure, increasing BMI has a protective effect against the severity of AHR.

The change in FVC is an indirect measure of airway closure, based on the assumption that any volume change is due to an increase in residual volume reflecting gas trapping [3]. It is possible that such changes represent near closure rather than complete closure of airways; however, both result in regions of nondetectable flow, representing severely under-ventilated lung units. Because FEV1 reflects both airway closure and airway narrowing, a strong correlation between the changes in FVC and FEV1 during challenge would be expected; however, it has not previously been demonstrated whether the slope of this relationship is increased in asthmatic subjects, compared with healthy controls, or if it changes with increasing bronchoconstriction.

The relationship between changes in FVC and FEV1 provides an estimate of the proportion of the change in FEV1 that is attributable to airway closure. There was a linear relationship between the changes in FVC and FEV1 throughout challenge in both asthmatic and control subjects in the present study, consistent with findings reported in young and old asthmatic subjects [22]. The strong linear relationship between changes in FVC and FEV1 implies that, within individual subjects, the contribution of airway closure to the change in FEV1 is consistent over the range of response usually observed in bronchial challenge tests. The wide variation in the slope of this relationship in both asthmatic and nonasthmatic subjects suggests that the contribution of airway closure to the change in FEV1 is much greater in some subjects than in others but the slopes do not differ between asthmatic and control subjects.

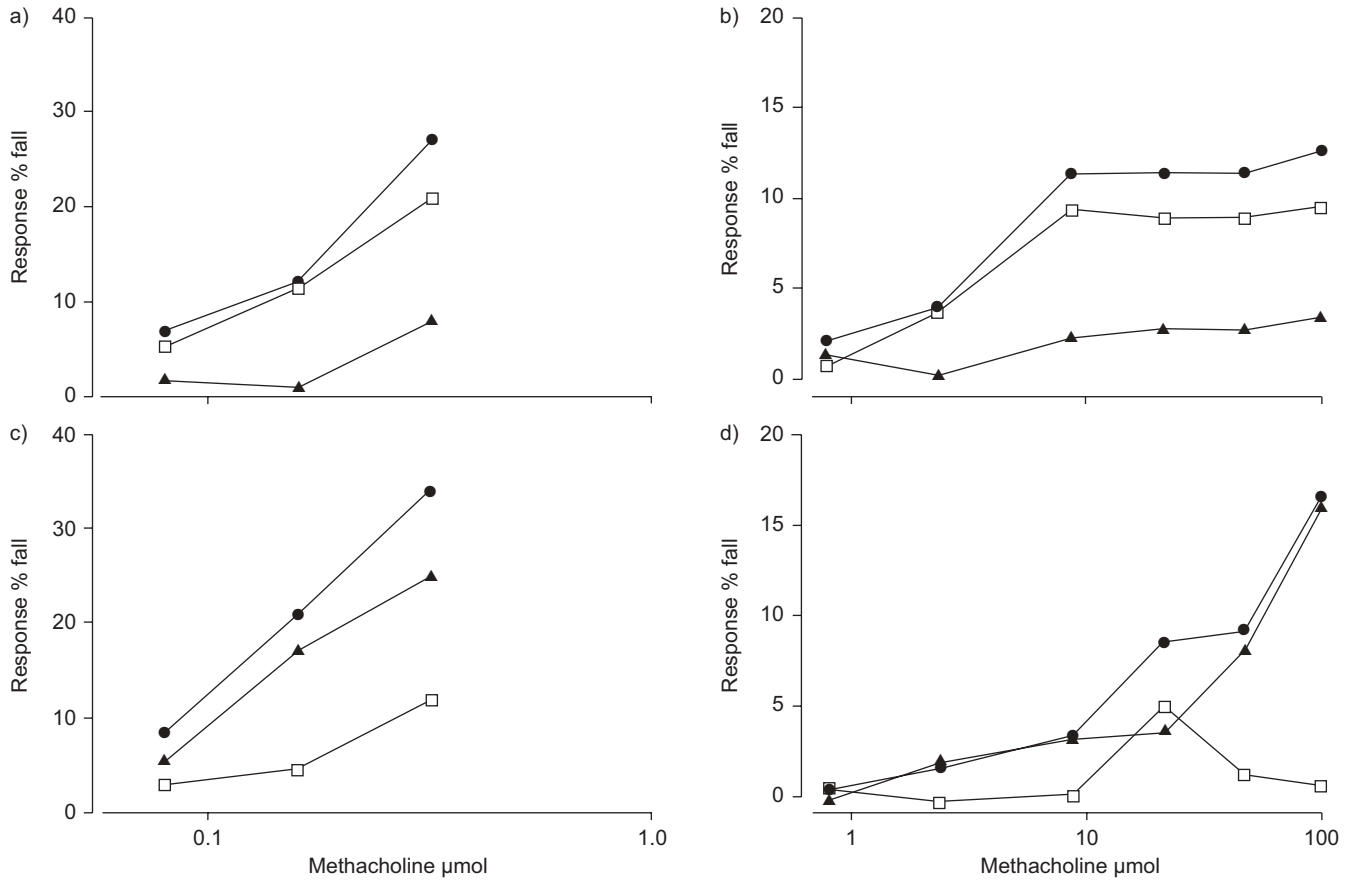

FIGURE 2. Per cent changes from baseline in forced expiratory volume in one second (FEV_1; ●), forced vital capacity (FVC; ▲) and forced expiratory ratio (FER; □) in two asthmatic subjects (a and c) and two control subjects (b and d). The change in FEV_1 in a and b is predominantly due to the change in FER whereas in c and d, it is predominantly due to changes in FVC.

Moreover, the consistency of the contribution of airway closure to the change in FEV_1 within subjects suggests that the wide variation in slopes between subjects may be attributed to baseline subject characteristics. MILANESE *et al.* [8] also reported that slopes were similar in asthmatic and rhinitic subjects with AHR. However, an increase in the slope has been found in older asthmatics, compared with younger asthmatics [22], in patients with COPD compared with asthmatics [8] and, in the present study, in the obese compared with controls. Changes in critical closing pressures, due to loss of elastic recoil in elderly asthmatics and in COPD and to increased intra-abdominal pressure on the diaphragm in the obese [11], are likely to result in airway closure occurring at higher lung volumes. Thus, airway closure would make an increased contribution to the change in FEV_1 during bronchial challenge.

There was no significant correlation between the closing index (%ΔFVC/%ΔFEV_1) and AHR, measured by the dose response slope, in the present study. This finding is consistent with the study of GIBBONS *et al.* [3] which found no correlation between airway closure and the severity of AHR measured by the PC_{20}. GIBBONS *et al.* [3] used log–linear interpolation to calculate the change in FVC at the PC_{20} concentration, so the fall in FVC was standardised to a 20% change in FEV_1. The closing index in the present study also standardises the change in FVC relative to the change in FEV_1. Thus, in both studies, the measure of airway closure has been standardised relative to the change in FEV_1, rather than relative to the magnitude of airway narrowing. As has been seen, both in the present study and in the GIBBONS *et al.* [3] study, the relative contribution of airway narrowing and airway closure to the change in FEV_1 varies widely between subjects. Consequently, standardising the change in FVC for the change in FEV_1 is inappropriate for determining the independent contribution of airway closure to AHR.

Due to the fact that the change in FEV_1 reflects both airway narrowing and airway closure [14], any analysis of the independent contribution of airway narrowing and airway closure to AHR must use measures other than FEV_1. GIBBONS *et al.* [3] were the first to use FER to partition the contribution of airway narrowing from the overall airway response measured by FEV_1. In the present study, the magnitude of airway closure measured by %ΔFVC was determined by baseline airway calibre measured by baseline FER, the extent of airway narrowing measured by the change in FER and by BMI. The role of baseline calibre and narrowing as determinants of closure suggests that there is a critical airway size, below which airways completely close. Both reduced baseline calibre and increased narrowing would push airways towards this critical calibre. This effect is consistent with computational models of changes in lung impedance during induced bronchoconstriction [23]. Furthermore, decreased functional

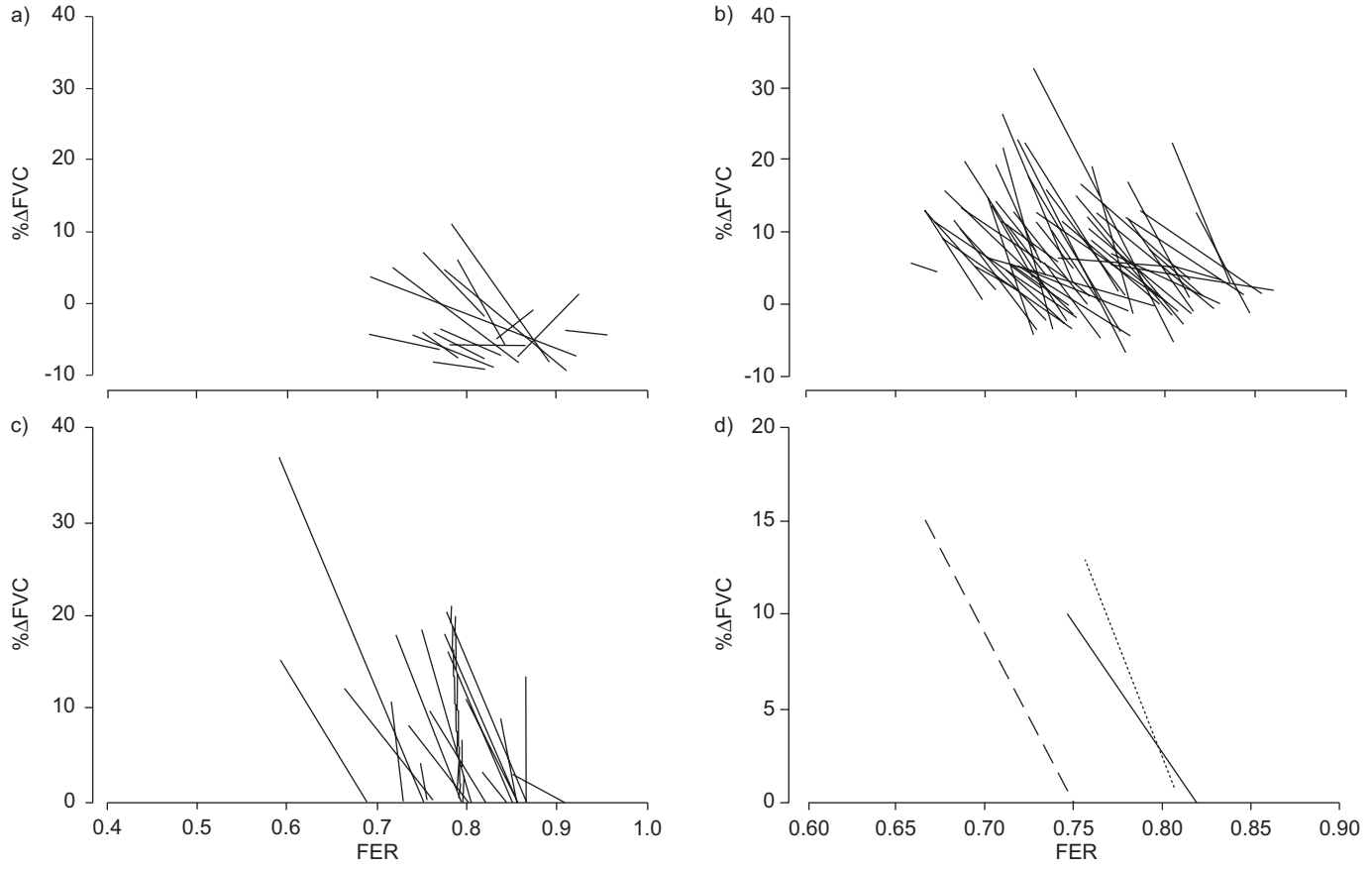

FIGURE 3. Relationship between the percentage change in forced vital capacity (%ΔFVC) and absolute forced expiratory ratio (FER) during challenge. Individual regression lines are shown for control (a) and asthmatic subjects (b). Obese subjects (c) did not have FVC measured throughout challenge and are shown as the two-point line between saline and last dose step of challenge. Representative regression lines have been plotted for each group (d) and have been calculated from the mean baseline FER, mean ΔFER and mean %ΔFVC; control (——), asthma (-----) and obese (·····).

residual capacity in obese subjects [9] and loss of elastic recoil reported in subpopulations of asthmatic subjects [24, 25] would also push airways towards this critical calibre. The role of BMI as a determinant of airway closure is consistent with recent research showing an exaggerated reduction in FVC with age in obese subjects [26].

After adjusting for the level of airway narrowing and baseline calibre, the magnitude of airway closure was still a significant determinant of the severity of AHR. This suggests that excessive changes in FEV_1 in asthma are an effect of both widespread narrowing and peripheral closure. This is consistent with previous studies reporting both uneven ventilation and complete loss of ventilation following bronchoconstriction in asthmatic subjects [5, 27].

In the present study, after accounting for other significant factors, BMI had a significant negative association with DRS. The explanation for this apparent protective effect of BMI against AHR is unknown but there are several possible mechanisms, including an increase in the elastic recoil of the lungs [28] or a redistribution of methacholine and/or airflow to other lung regions following closure of basal airways. The present authors speculate that the distribution of airway closure may be an important factor differentiating between increased closure that leads to AHR in asthmatics, and increased closure that protects against AHR in the obese. Using radionuclide imaging, KING *et al.* [5] reported that the distribution of airway closure differed between asthmatic and nonasthmatic subjects. Closure in nonasthmatics was predominantly in the lung bases, whereas in asthmatic subjects the distribution of closure was patchy, with peripheral wedge-shaped defects in both apical and basal lung regions [5]. Basal ventilation appears to be impaired in obese subjects [29], suggesting that closure may be confined to the basal lung zones *i.e.* topographically similar to that in nonasthmatics [5]. VENEGAS *et al.* [27] used computer modelling to show that airflow redistribution will occur in the event of extreme constriction or closure of airways, promoting dilatation and protection against large scale closure in the airways that remain open. Therefore, homogeneously increased basal airway closure in the obese could redirect airflow, resulting in widespread dilatation and thus protect against extreme bronchoconstriction.

The present study shows that baseline airway calibre, the extent of airway narrowing and body mass index are all factors that determine the magnitude of airway closure during bronchoconstriction. After adjusting for these factors, increased airway closure measured by spirometry remained an independent determinant of the severity of airway

hyperresponsiveness. However, although body mass index increases the magnitude of airway closure, it protects against airway hyperresponsiveness, suggesting that the topographical distribution rather than the magnitude of airway closure may be an important determinant of airway hyperresponsiveness.

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