

Relationship of Airway Wall Thickness to Airway Sensitivity and Airway Reactivity in Asthma

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Airway wall thickening has been assumed to cause airway hyperresponsiveness, but a protective effect against airway narrowing has also been suggested. We investigated the relationship between airway wall thickness as assessed by helical computed tomography and two components of airway responsiveness, airway sensitivity and reactivity, in patients with stable asthma with ($n = 23$) and without ($n = 22$) inhaled steroid treatment. A cross-section of the apical bronchus of the right upper lobe was obtained. Airway wall area corrected by body surface area was measured as an index of wall thickness. Airway sensitivity and reactivity were measured by continuous inhalation of methacholine, on the basis of the methacholine respiratory resistance dose-response curve. The eosinophil count in sputum was determined in 16 patients [steroid (+) group] and 14 patients [steroid (–) group]. In both groups of patients, airway sensitivity was not related to airway reactivity. Airway sensitivity was related to eosinophil count [$r = 0.57$ in the steroid (+) group and $r = 0.49$ in the steroid (–) group], but not to airway wall thickness. In contrast, airway reactivity negatively correlated with airway wall thickness [$r = -0.56$ in the steroid (+) group and $r = -0.55$ in the steroid (–) group] but not with eosinophil count. Our results suggest that airway wall thickening attenuates airway reactivity in patients with asthma. These findings may have important implications in pathophysiology and in the treatment of airway remodeling.

Keywords: airway hyperresponsiveness; airway remodeling; airway wall thickening; excessive airway narrowing

Postmortem studies have shown thickened airway walls in patients dying of acute exacerbations of asthma (1–4). Wall thickening results from inflammatory changes, such as edema and inflammatory cell infiltration, and from structural changes, such as mucous gland hyperplasia, reticular basement membrane thickening, vascular proliferation, and airway smooth muscle (ASM) hypertrophy and hyperplasia. These structural changes are features of airway remodeling associated with chronic inflammation (5–8). Mathematical models indicate that modest airway wall thickening results in disproportionately severe airway narrowing due to ASM shortening (1, 9–11). Airway wall thickening may thus lead to airway hyperresponsiveness (AHR), an essential feature of asthma (12, 13).

Several studies provide evidence against this widely accepted theory. Okazawa and coworkers (14) have investigated the relationship between ASM shortening induced by carbachol administered through the main bronchus and airway dimensions, assessed by examining all measurable airways in excised canine lungs. They have found that ASM of airways with thicker walls

was less likely to shorten. Physiological studies indicate that airways of patients with asthma are less distensible during forced inspiration (15, 16) or less collapsible during forced expiration (17) than those of normal subjects. The authors of these studies attributed such phenomena to increased stiffness of the asthmatic airway wall, possibly resulting from increased wall thickness. However, airway wall thickness was not assessed in these studies.

Computed tomography (CT) has been used to measure airway wall dimensions in patients with asthma (18–23). Patients have thicker airways on CT scans than do healthy control subjects (19–22), and the degree of thickening is related to the severity of disease (20, 21, 23) and airflow obstruction (21, 22). However, the relationship between airway wall thickness and inducible airway narrowing or AHR has rarely been investigated. Boulet and coworkers (18) and Little and coworkers (23) compared airway wall thickness, as assessed by CT, with AHR in patients with asthma. AHR was measured as the provocative concentration of methacholine (18) or histamine (23) that produced a 20% fall in FEV_1 (PC_{20} - FEV_1). One study (18) demonstrated a positive correlation of AHR with airway wall thickness in a subgroup of patients, whereas the other (23) found no significant relationship.

We addressed these inconsistent findings by studying the relationship between airway wall thickness, assessed by our validated method for helical CT (21), and AHR, evaluated by continuous inhalation of methacholine (24), in patients with stable asthma. As compared with using PC_{20} - FEV_1 as the sole index of AHR, the latter method can separately evaluate the two distinct and possibly independent components of AHR: airway sensitivity, and airway reactivity or exaggerated airway narrowing (12, 13). Airway inflammation as assessed by induced sputum was also analyzed. Some of the results of these studies have been previously reported in the form of an abstract (25).

METHODS

Subjects

We studied 2 groups of adult patients with stable asthma diagnosed according to American Thoracic Society criteria (26): 23 patients receiving inhaled beclomethasone dipropionate [steroid (+) group], and 22 steroid-naïve patients treated with inhaled short-acting β_2 -agonists alone [steroid (–) group]. No subject had ever smoked cigarettes, or had respiratory infections or exacerbation of asthma during the 8 weeks before enrollment.

The study was approved by the Ethics Committee of Kyoto University (Kyoto, Japan). Written informed consent was obtained from all participants.

CT Scans

Helical CT scanning was performed at full inspiration as described previously (21, 27). Briefly, a cross-section of the apical bronchus of the right upper lobe at its origin was examined to determine absolute airway thickness (T), airway wall area (WA), and percentage wall area (WA%, [WA/outer area of the bronchus] $\times$ 100). WA and T were normalized on the basis of body surface area (BSA). The CT data were transferred to a Power PC personal computer and automatically analyzed (27).

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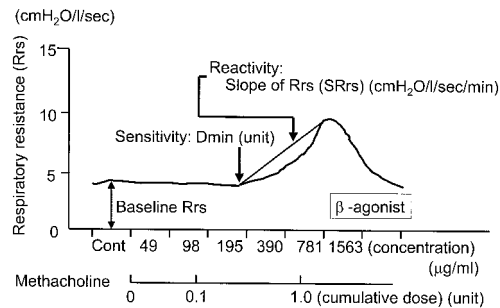


Figure 1. Scheme of the continuous methacholine inhalation method with simultaneous measurement of respiratory resistance (Rrs) (24). After recording the baseline Rrs during inhalation of physiologic saline, methacholine was inhaled sequentially, beginning with the lowest concentration, at 1-minute intervals. Dmin, the cumulative dose of inhaled methacholine at the inflection point at which Rrs begins to increase, was used as the index of airway sensitivity. Inhalation of methacholine was continued until Rrs reached twice the baseline value. The slope of the methacholine respiratory resistance dose–response curve (SRrs) was used as the measure of airway reactivity. (See online supplement for details.)

Methacholine Challenge

AHR was examined by continuous methacholine inhalation with simultaneous measurement of respiratory resistance (Rrs, cm H₂O/L/second) (Astograph; Chest, Tokyo, Japan) (Figure 1) (24). Briefly, twofold increasing concentrations of methacholine chloride diluted in physiologic saline in 10 dose steps (49 to 25,000 µg/ml) were prepared. They were inhaled during tidal breathing from nebulizers with an output of 0.15 ml/minute. After recording the baseline Rrs during inhalation of physiologic saline for 1 minute, methacholine was inhaled sequentially, starting with the lowest concentration, at 1-minute intervals. Dmin, the cumulative dose of inhaled methacholine at the inflection point at which Rrs begins to increase, was used as the index of airway sensitivity. This variable was measured in terms of a unit defined as 1-minute inhalation of methacholine (1 mg/ml). Inhalation of methacholine was continued until Rrs reached twice the baseline value (24). The plateau of the dose–response curve was not, therefore, examined. The slope of the methacholine respiratory resistance dose–response curve (SRrs) was used as the measure of airway reactivity. The total cumulative dose of methacholine at the end of inhaling the highest dose was 50 units.

As separate studies, we examined the reproducibility of the method in eight patients by measuring Dmin and SRrs repeatedly at an interval of 7 days, and compared the doubling of Rrs with the change in FEV₁ in seven patients.

Sputum Induction

After premedication with inhaled salbutamol, patients inhaled hypertonic (3%) saline solution for 15 minutes from an ultrasonic nebulizer, and sputum was induced (28). Adequate plugs of sputum were treated with 0.1% dithiothreitol (Sputasol; Oxoid, Hampshire, UK) followed by Dulbecco's phosphate-buffered saline. Eosinophil count (number per gram of sputum) was determined on centrifuged preparations stained with May–Grünwald–Giemsa.

Statistical Analysis

Data were expressed as means $\pm$ SD or median (range) and analyzed with the StatView 4.5 program (SAS Institute, Cary, NC). An unpaired *t* test, the Mann–Whitney U test, or the χ^2 test was used to compare groups. Spearman's rank correlation test or Pearson's correlation test was used to analyze relations between variables. *p* Values less than 0.05 were considered significant.

RESULTS

Validation Study of Methacholine Challenge Test

Of the eight patients with asthma examined, reproducibility of Dmin and Rrs measurements was good, as shown in Figure E1 (see the online supplement).

In seven patients, FEV₁ was measured before challenge and immediately after the Rrs had doubled postchallenge. The change in FEV₁ was $-19.3 \pm 6.9\%$ (median, -19.7% ; range, -8.0 to -31.4%), and the decrease in FEV₁ exceeded 16% in six of seven patients. The doubling of baseline Rrs thus caused a relevant degree of airway narrowing in most of the patients.

Comparison of Two Asthmatic Groups

The characteristics of the two groups of patients with asthma are summarized in Table 1. The methacholine inhalation test and CT scanning were successfully performed in all subjects. Sputum specimens suitable for cell counting were obtained from 16 patients of the steroid (+) group and from 14 patients of the steroid (–) group. Compared with patients without inhaled steroid treatment, patients receiving inhaled steroid showed a longer duration of disease, lower FEV₁/FVC, and greater reversibility of FEV₁ with inhaled β_2 -agonist. The two groups were similar with regard to airway sensitivity (log Dmin) and reactivity (SRrs) and the indices of airway wall thickness as assessed by CT.

Relationship between Variables

In both groups of patients with asthma, log Dmin did not relate to SRrs (Figure 2) but was negatively related to the number of eosinophils in sputum (Figure 3). The latter relationship was statistically significant in the steroid (+) group but was marginal in the steroid (–) group (Figure 3). However, in both groups, log Dmin did not correlate with any index of airway wall thickness, such as WA/BSA (Figure 4), WA% [$r = -0.10$, $p = 0.66$ for the steroid (+) group and $r = -0.21$, $p = 0.34$ for the steroid (–) group], and $T/(BSA)^{1/2}$ [$r = -0.00$, $p = 0.98$ for the steroid (+) group and $r = -0.06$, $p = 0.79$ for the steroid (–) group].

In contrast, irrespective of inhaled steroid treatment, SRrs was unrelated to the number of eosinophils in sputum (Figure 5), but negatively correlated with WA/BSA (Figure 6). SRrs also showed a negative relationship with $T/(BSA)^{1/2}$, which was statistically significant in steroid (+) group ($r = -0.60$, $p = 0.0019$) but was marginal in the steroid (–) group ($r = -0.38$, $p = 0.081$). There was no correlation between SRrs and WA% [$r = -0.23$, $p = 0.29$ in steroid (+) group and $r = 0.29$, $p = 0.19$ in steroid (–) group].

In the steroid (+) group, the duration of inhaled steroid treatment was 3.1 years (0.7–5.0 years), and the cumulative dose of treatment (sum of [dose $\times$ duration]) was 1,950 (267–5,400) ([µg/day] $\times$ years). These indices, as well as the final dose of treatment as shown in Table 1, did not correlate with FEV₁, log Dmin, SRrs, sputum eosinophil count, WA/BSA, WA%, or $T/(BSA)^{1/2}$ in these patients (data not shown). Also, in both groups of patients with asthma, the sputum eosinophil count did not correlate with WA/BSA [$r = 0.09$, $p = 0.74$ in the steroid (+) group and $r = -0.21$, $p = 0.45$ in the steroid (–) group], WA% [$r = 0.15$, $p = 0.55$ in the steroid (+) group and $r = 0.12$, $p = 0.66$ in the steroid (–) group], or $T/(BSA)^{1/2}$ [$r = 0.08$, $p = 0.76$ in the steroid (+) group and $r = -0.14$, $p = 0.61$ in the steroid (–) group].

DISCUSSION

This is the first study, to our knowledge, to investigate the relationship between airway wall thickness, as assessed by CT, and

TABLE 1. CHARACTERISTICS OF PATIENTS WITH ASTHMA

	Inhaled Steroid (+)	Inhaled Steroid (–)	p Value
n	23	22	
Age, yr	59 ± 9	52 ± 18	0.11
Sex, male/female	12/11	6/16	0.088
BSA, m ²	1.6 ± 0.1	1.6 ± 0.2	0.58
Disease duration, yr	10 ± 14	4 ± 6	0.043
Atopy*/nonatopy	12/11	11/11	0.88
Dose of BDP, µg/d	800 (400–1,600)	0 (0–0)	
FEV ₁ , %pred	92 ± 4	101 ± 16	0.095
FEV ₁ /FVC, %	71 ± 10	78 ± 9	0.024
FEV ₁ reversibility, %†	8.0 ± 6.8	3.9 ± 4.3	0.041
Sputum eosinophils, number/g‡	1.6 (0.0–35.2)	0.2 (0.03–2.6)	0.057
Baseline Rrs, cm H ₂ O/L/sec	4.4 ± 1.8	3.9 ± 1.2	0.34
Log Dmin, units	–0.17 ± 0.19	0.16 ± 0.13	0.16
SRrs, cm H ₂ O/LI/sec/min)	1.4 ± 0.9	1.8 ± 1.1	0.29
WA/BSA, mm ² /m ²	21.2 ± 7.2	21.1 ± 7.6	0.98
WA%, %	64.5 ± 6.3	66.1 ± 7.0	0.45
T/(BSA) ^{1/2} , mm/m	1.3 ± 0.2	1.3 ± 0.3	0.97

Definition of abbreviations: BDP = beclomethasone dipropionate; BSA = body surface area; Dmin = cumulative dose of inhaled methacholine at the inflection point at which respiratory resistance begins to increase (marker of airway sensitivity); SRrs = slope of methacholine respiratory resistance dose–response curve (marker of airway reactivity); T = absolute airway thickness; WA = airway wall area; WA% = WA corrected by outer airway area.

* Determined by the presence of specific serum IgE antibody against at least one common inhalant allergen.

† Measured with inhalation of 200 µg of salbutamol.

‡ Examined in 16 steroid (+) subjects and in 14 steroid (–) subjects.

airway sensitivity and airway reactivity, measured separately in patients with asthma. We found consistent results in patients treated with inhaled steroid and those treated with β_2 -agonists alone. Airway reactivity negatively correlated with airway wall thickness. Airway sensitivity was related to the intensity of airway inflammation as assessed by sputum eosinophil count, but not to any index of airway wall thickness. It is suggested that airway wall thickening protects against excessive airway narrowing in asthma.

Our results may be against a widely accepted theory that airway wall thickening leads to AHR or excessive airway narrowing in patients with asthma (1, 9–11). Computational analyses of postmortem lungs by Pare, Hogg, and colleagues have indicated that a modest degree of airway wall thickening, associated with only a mild increase in baseline airway resistance, can exaggerate the airway closure induced by ASM shortening (1, 9–11). These elegant studies have provided the theoretical basis for the common notion mentioned above. However, when the elastic property of airway wall constituents is considered, this may not always be the case, as pointed out by Pare and coworkers (11). Evidence has been emerging to support this concept, as discussed below.

Airway sensitivity and airway reactivity are the two major components of AHR (12, 13). Several groups of investigators have examined these variables in patients with asthma and normal subjects. Orehek and coworkers (29) reported that the difference in airway reactivity between patients with asthma and normal subjects was greater than the difference in airway sensitivity. Airway sensitivity was measured as the dose of carbachol causing a 25% decrease in specific airway conductance, and airway reactivity was derived from the slope of the dose–response curve. There was no correlation between airway sensitivity and airway reactivity. They concluded that different mechanisms underlie the sensitivity and reactivity of the bronchial tree and considered hyperreactivity the main feature of the asthmatic response (29). A number of studies of the complete dose–response curves have shown a direct relationship between airway sensitivity, reactivity, and maximal airway narrowing (30, 31), but we found no correlation between airway sensitivity and airway reactivity, consistent with the results of Orehek and coworkers (29). A 1-year follow-up study of patients with asthma by Beaupre and Malo (32) showed that airway sensitivity measured as PC₂₀-FEV₁ to histamine paralleled clinical status, whereas airway reactivity assessed as the slope of the dose–response curve showed little change.

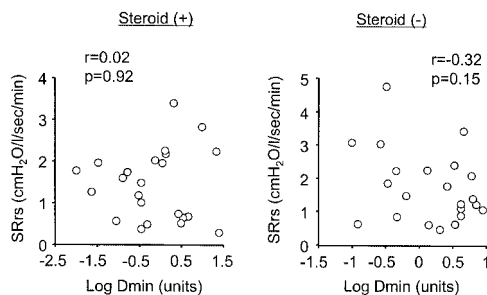


Figure 2. Relationship between airway sensitivity and airway reactivity. Log Dmin was not significantly related to SRrs in either the steroid (+) group or the steroid (–) asthmatic group.

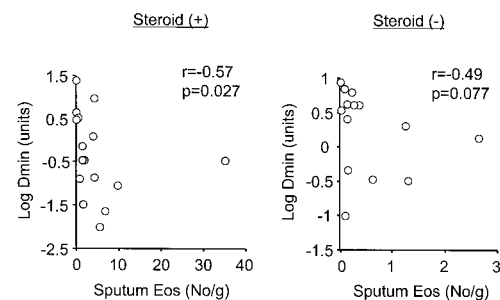


Figure 3. Relationship between airway sensitivity and airway inflammation. Log Dmin was negatively related to sputum eosinophil count in both the steroid (+) and steroid (–) asthmatic groups.

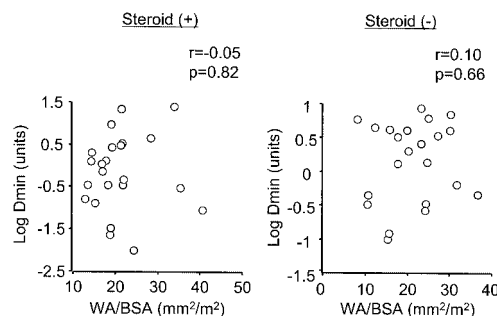


Figure 4. Relationship between airway sensitivity and airway wall thickness. Log Dmin was not significantly related to WA/BSA in both the steroid (+) and steroid (-) asthmatic groups.

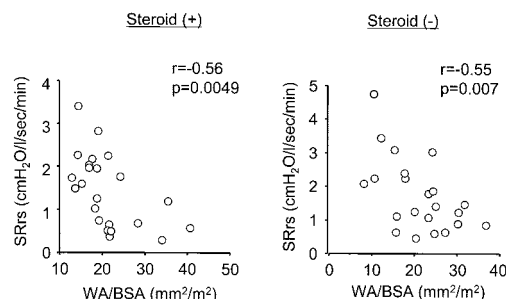


Figure 6. Relationship between airway reactivity and airway wall thickness. SRs was negatively correlated with WA/BSA in both steroid (+) and steroid (-) asthmatic groups.

They concluded that airway sensitivity ($PC_{20}\text{-FEV}_1$) is a more accurate index of the clinical status of patients with asthma than is airway reactivity (32). These results provide additional evidence that the origin(s) of airway sensitivity and airway reactivity may differ and suggest that airway reactivity may have more fixed or chronically determined components than airway sensitivity.

In 1989, Sterk and Bel (12) reviewed the mechanisms of airway sensitivity and airway reactivity or excessive airway narrowing. They proposed that airway sensitivity is determined by the strength of the stimulus that triggers the airways to narrow ("prejunctional" mechanisms). Determinants of airway sensitivity include epithelial damage and malfunction, neural control, inflammatory cell number/activity, interactions among these factors, and altered metabolism or absorption of inflammatory mediators. Our results and those of previous studies (33) support the view of Sterk and Bel (12) that the degree of cellular inflammation, as measured here by the sputum eosinophil count, is related to airway sensitivity, but conflict with the results of others (34). Epithelial damage and increased levels of several inflammatory mediators have also been associated with airway hypersensitivity. As for airway reactivity, Sterk and Bel regarded this variable to represent the responsiveness of the target organ (i.e., airways) to the stimulus given ("postjunctional" mechanisms) (12). Determinants of airway reactivity include ASM contractility, viscous and elastic loads on ASM shortening, swelling of the airway wall, and intraluminal exudate and secretions. ASM contractility, airway wall swelling, and intraluminal exudate and secretions were considered to heighten airway reactivity. In con-

trast, Sterk and Bel proposed that viscous and elastic loads on ASM shortening are responsible for the plateau of the dose-response curve *in vivo*. Only scant evidence was available at that time to indicate that individual components of airway structure, or the net effect of alterations in such components, can protect against ASM shortening *in vivo*. However, the involvement of viscous and elastic loads, as well as ASM contractility, might be in accordance with the possible "fixed" nature of airway reactivity (32).

Several observations suggest a protective effect of airway wall thickening against ASM shortening or airway narrowing. In animal models, airway wall thickening (14), or deposition of extracellular matrix such as collagen or fibronectin in the airway wall after repeated allergen exposure (35), was associated with attenuated ASM shortening. Such shortening was ascribed to stiffening of the airways. Postmortem studies show that the ASM layer of patients with asthma is thickened (1–4). This may result in AHR, if the contractility of ASM cells remains constant (11, 36). However, thickening of the ASM layer is partly attributed to increased deposition of extracellular matrix around individual ASM cells (4), which may act against ASM shortening. In addition, ASM cells may differentiate to a less contractile phenotype during proliferation (11, 36, 37). Physiological studies also indicate that the airways of patients with asthma may be less collapsible or less distensible than those of normal subjects. Brackel and coworkers (17) have investigated the mechanical properties of the airways with the use of an esophageal balloon and a Pitot static probe, positioned at several locations in the central airways. They obtained area-versus-transmural pressure curves during forced expiration. Airway compliance and specific airway compliance were significantly lower in patients with long-standing asthma than in healthy subjects. Brackel and coworkers concluded that airway remodeling may have resulted in stiffer dynamic elastic properties of the airway wall (17), although airway remodeling was not assessed by bronchial biopsy or CT. The change in anatomic dead space (ΔV_D) with lung volume has been proposed as a noninvasive index of airway distensibility or stiffness (ΔV_D as corrected by the change of end-inspiratory volume [mL/L]). Wilson and coworkers (15) have shown that patients with mild asthma have less distensible airways, with a ΔV_D of 27.0 mL/L during inspiration as compared with 37.3 mL/L for control subjects ($p = 0.014$). Ward and coworkers (16) have attributed this phenomenon to reticular basement membrane thickening by showing an inverse correlation between ΔV_D and reticular basement membrane thickness ($r = -0.37$, $p = 0.03$) in patients with mild to moderate asthma. Moreover, Milanese and coworkers (38) have found a negative correlation between reticular basement membrane thickness and AHR, as measured as the provocative dose

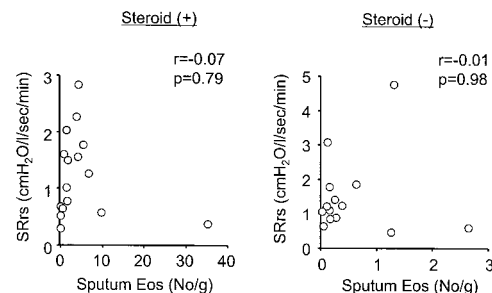


Figure 5. Relationship between airway reactivity and airway inflammation. SRs was unrelated to sputum eosinophil count in both steroid (+) and steroid (-) asthmatic groups.

of methacholine that produced a 20% fall in FEV₁ (PD₂₀-FEV₁), in 11 patients with asthma ($r = 0.77$, $p < 0.01$), suggesting an airway stiffening consequent to this pathologic change. Available evidence thus indicates that the airways of patients with asthma may be stiffer than those of healthy subjects. Increased stiffness may result from mechanisms such as deposition of extracellular matrix in the airway wall, which may lead to airway wall thickening (39).

Our study demonstrates, for the first time, an inverse association between airway wall thickening and airway reactivity in patients with asthma. This suggests a protective role of airway wall thickening against excessive airway narrowing in human subjects *in vivo*. The relative roles of different connective tissue proteins are unclear, with respect to their responses to compressive stress (11). Moreover, elastosis or fragmentation of elastic fibers (40) or degenerative changes of cartilage (41) in remodeled asthmatic airways may decrease airway elasticity and thus increase deformability. Because CT cannot discern histologic details of the airway wall and we did not pathologically examine the airways, we are unable to discuss the contribution of individual components of airway remodeling to airway elasticity. We can at least state, however, that the “net” effect of airway wall thickening may be protection against ASM shortening. This assumption has important implications regarding the pathophysiologic role, and treatment, of airway wall thickening or remodeling. Airway wall thickening is related to chronic airflow obstruction (21, 22) and may progress with increased duration of disease (4, 21). Thickening of the airway wall might be associated with persistent airflow obstruction but infrequent exacerbations of the disease (5), considered a possible characteristic of long-standing asthma (42). Long-term treatment with inhaled steroid has been widely recommended for patients with persistent asthma. Such treatment may reduce airway wall thickness, as it does in animal models (43), or reticular basement membrane thickness in patients with asthma (44), which might possibly lead to airway hyperreactivity (5). In our patients treated with inhaled steroid, the total duration, final dose, and cumulative dose of treatment did not correlate with the indices of airway sensitivity, airway reactivity, or airway wall thickness. In addition, these patients did not differ from steroid-naïve patients with regard to airway sensitivity, airway reactivity, or airway wall thickness. It may be difficult, however, to argue the effect of inhaled steroid on these indices by such cross-sectional analysis or comparison of two patient groups that differ in disease duration or pulmonary function.

Boulet and coworkers (18) showed a positive correlation between airway wall thickness, measured by CT, and AHR, based on PC₂₀-FEV₁. This correlation was observed in patients with asthma with fixed airflow obstruction ($n = 13$), but not in those without such obstruction ($n = 11$) or in healthy control subjects ($n = 10$). More recently, Little and coworkers (23) performed a similar study in 49 patients with optimally controlled asthma but failed to demonstrate a relationship between airway wall thickness and AHR. Both studies used PC₂₀-FEV₁ to estimate AHR, considered a marker of airway sensitivity, that may also reflect airway reactivity (13). In addition, the value of the study by Boulet and coworkers (18) may be limited by the small number of subjects. Unlike these previous studies, we separately evaluated airway sensitivity and airway reactivity.

We conclude that airway wall thickening may protect against excessive airway narrowing in patients with asthma. Airway sensitivity may be related to the degree of airway inflammation but not to airway wall thickness. These results have important implications regarding the pathophysiologic role, and therapeutic modulation, of airway wall thickening in asthma. Future studies should be conducted with large numbers of patients, to investigate

the relationship between CT findings and clinical characteristics such as the frequency of exacerbations and variability in peak expiratory flow, and the long-term effect of antiinflammatory treatment, such as inhaled steroid, on CT findings, functional parameters, and their interrelations.

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