

ORIGINAL ARTICLE

Effect of Aging on the Relationship between Asthma Severity and Bronchial Hyperresponsiveness in Children with Asthma

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An association between asthma and bronchial hyperresponsiveness (BHR) has been demonstrated. It is possible that the relationship between asthma severity and BHR in children with asthma is different in infants and in adolescents. The aim of this study is therefore to evaluate the effect of aging on the relationship between the severity of asthma and BHR in children with asthma.

We measured BHR in 386 subjects ranging from 2 to 20 years of age. The subjects consisted of 323 children with asthma (boys : girls = 193 : 130, mean age 9.7 years) and 63 age-matched controls (boys : girls = 25 : 38, mean age 8.2 years). BHR was measured using the methacholine inhalation challenge by measuring the transcutaneous oxygen pressure (tcPO₂) in children less than 6 years of age (Dmin-PO₂) and by measuring the respiratory resistance (Rrs) in children 6 years of age and older (Dmin-Rrs). Throughout the whole age range, both the Dmin-PO₂ and Dmin-Rrs in each asthma severity group were higher than those in the controls. In the asthmatics aged 2–5 years, the Dmin-PO₂ levels in the mild asthma group were higher than those in the moderate and severe asthma groups ($p < 0.001$, $p < 0.001$, respectively), and the Dmin-PO₂ levels in the moderate asthma group were also higher than those in the severe asthma group. This tendency was also found in the age ranges of 6–9 years and 10–13 years. In the asthmatics aged 14–20 years, the Dmin-Rrs levels were not significantly different among the three groups.

Taken together, these data show that aging has an effect on the relationship between the severity of asthma and BHR during childhood and that BHR may not be the sole determinant for the severity of asthma in adolescence.

Keywords bronchial hyperresponsiveness, childhood asthma, methacholine inhalation challenge, oscillation technique, transcutaneous oxygen pressure

INTRODUCTION

Bronchial asthma, one of the most common chronic respiratory diseases encountered in clinical practice (1), has shown an association with bronchial hyperresponsiveness (BHR)(2). BHR is a key feature of asthma, and some reports have suggested the degree of BHR to be more closely related to the severity of asthma than the pulmonary function parameters (3, 4). BHR is evident in infants with asthma and it persists after diagnosis (5). Although many children with asthma outgrow the disease after the onset of puberty (6), some of these adolescents without symptoms of asthma still continue to demonstrate persistent BHR (7).

The above observations suggest that the relationship between asthma severity and BHR in children with asthma thus differs between infants and adolescents. Previous reports have suggested the airway acetylcholinesterase activity to be lower in immature animals than in older ones, while the affinity of muscarinic receptors for their agonist ligands has been reported to decrease with age (8). This implies that airway smooth muscle may thus be more responsive to inhaled methacholine in younger animals than in older ones. However, some contrasting findings have also been reported (9); namely, disagreements concerning the

responses to specific agonists may involve different states of maturation as well as species differences. These various findings suggest a possible effect of aging on both severity of asthma and on BHR in pediatric patients with asthma.

We previously described a technique for evaluating BHR by monitoring the transcutaneous oxygen pressure (tcPO₂) (9), which can be used over a wide age range in children. This method demonstrated a close correlation between the clinical severity of bronchial asthma and bronchial reactivity to methacholine (10). To evaluate the effect of age on the relationship between asthma severity and BHR from infancy to late adolescence, we studied the responses to a methacholine inhalation challenge using the tcPO₂ monitoring method and an oscillation method in asthmatic patients ranging from 2 to 20 years of age, as well as age-matched controls (9), while paying special attention to the severity of asthma in the patients.

METHODS

Study Protocols

We studied the age-related changes in the bronchial reactivity in 323 asthmatic children and 63 age-matched control subjects ranging from 2 years to 20 years of age. The subjects from 2 to 6 years of age underwent a methacholine inhalation challenge using the tcPO₂-monitoring method, as described elsewhere (11, 12), while those 6 years of age and older were challenged measured simultaneously using

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TABLE 1.—Profiles of controls and asthmatic children within age, sex, and asthma-severity groupings.

Age group (yr)	Control		Asthma		Severity		
	Total	Sex (boy:girl)	Total	Sex (boy:girl)	Mild	Moderate	Severe
2–5	24	8:21	50	28:22	28	18	4
6–9	15	10:5	105	63:42	56	39	10
10–13	15	4:6	115	68:47	55	35	25
14–20	9	3:6	53	34:19	29	18	6
Total	63	25:38	323	193:130	168	110	45

The average age of the asthmatic children and the controls is similar ($p > 0.1$, Kruskal-Wallis method). The distribution of the three groups was similar in each of the 4 age group ($p > 0.1$, Kruskal-Wallis method).

the tcPO_2 -monitoring method and the oscillation method (11–13).

Subjects

We studied 323 asthmatic patients (male : female, 193 : 130; mean age, 9.7 years) and 63 age-matched control subjects (male : female, 25 : 38; mean age, 8.2 years). The age distributions for the asthmatic subjects and the controls were similar (Table 1, $p > 0.1$; Kruskal-Wallis method). All asthmatic subjects satisfied the criteria for bronchial asthma (14), a diagnosis that we further confirmed during a clinical follow-up of longer than 1 year. No subject has undergone long-term inhaled steroid therapy before the methacholine inhalation challenge. All subjects were diagnosed to have atopic asthma based on their reaction to common environmental allergens administered via the skin and/or based on the results of radioallergosorbent tests.

The patients were assigned to groups according to the following age ranges: 2 to 5 years ($n = 50$), 6 to 9 years ($n = 105$), 10 to 13 years ($n = 115$), and 14 to 20 years ($n = 53$) (Table 1). Based on each patient's clinical history, the severity of asthma was graded in accordance with the Global Initiative for Asthma (GINA) classification (15). Among our patients, 168 (52.0%) had mild intermittent or mild persistent asthma (mild group); 110 (34.1%) had moderate persistent asthma (moderate group); and 45 (13.9%) had severe persistent asthma (severe group). The distributions of severity were similar between the four different age groups (Table 1, $p > 0.1$, Kruskal-Wallis method). In asthmatic patients more than 6 years of age, the inhalation challenge was carried out when baseline forced expiratory volume in 1 second (FEV_1) exceeded 70% of the predicted values; baseline FEV_1 was $85.4 \pm 1.5\%$ in the mild group, $80.2 \pm 1.2\%$ in the moderate group, and $75.5 \pm 1.5\%$ in the severe group.

All age-matched control subjects were free of chronic respiratory diseases and atopic diseases. All subjects had been free of respiratory tract infections for more than 4 weeks before enrollment and had received no medication for at least 12 hours before testing. Approval was obtained from the hospital Ethics Committee, and informed parental consent for each child was obtained before the study.

Methacholine Inhalation Challenge

The methacholine inhalation challenge was carried out in all subjects as previously described (11, 12). Subjects 6 years of age and older underwent the challenge in a seated posi-

tion wearing a nose clip. An Astograph (TCK 6100H; Chest Company, Tokyo, Japan) was used as the inhalation device and as the device measuring the real-time respiratory resistance (Rrs). The subjects from 2 to 5 years of age underwent the challenge during sleep in a supine position after the administration of trichlorethyl phosphate monosodium syrup (70 mg/kg), as they wore an oxygen mask with a constant bias flow. For these children the Astograph was used only as a delivery system. No difference in the bronchial responsiveness to methacholine has been found between wakefulness in a sitting position and sleep in a supine position (12).

The methacholine inhalation challenge was performed according to the procedure described by Takishima (16). Briefly, methacholine (Daiichi Kagaku Yakuhin, Tokyo, Japan), was serially diluted twofold on the day of the test with saline to provide a series of 10 strengths, ranging from 25 mg/mL to approximately $49 \mu\text{g/mL}$. The drug was then administered via an Astograph, which included 12 identical nebulizers connected to a mainline tube and an air compressor shifted from one nebulizer to the next at 1-minute intervals. Saline was set up in the first nebulizer as a control. The twelfth nebulizer ordinarily contained salbutamol hemisulfate (1.7 mg/mL in saline, to be administered for 2 minutes).

tcPO_2 was measured as previously described using a tcPO_2 monitoring system (Cutaneous PO_2 Monitor 820; Roche, Basel, Switzerland) (11, 12). Successive doses of methacholine began with the second nebulizer, and thereafter being doubled until tcPO_2 had decreased 10% from the baseline. The cumulative dose of methacholine at the inflection point where tcPO_2 decreased in a linear fashion was taken as the BHR (Dmin-PO_2). Dmin-PO_2 was significantly related to the change in Rrs (Dmin-Rrs) obtained by an oscillation method (Figure 1) (11, 12). One Dmin unit was considered to be equal to 1 minute of inhaling an aerosolized methacholine solution (1.0 mg/mL) during tidal breathing. Routine auscultation was performed before and during the methacholine challenge to document wheezing.

In subjects older than 6 years of age the oscillation technique was used during the methacholine inhalation challenge, and the threshold point of Rrs (Dmin-Rrs) was calculated. A constant-amplitude pressure generator connected to the mouthpiece produced pressure conforming to the sine waves of a constant amplitude at the mouth, using a "loud-speaker box" system. A constant-bias flow was introduced between the nebulizer and the flow meter to minimize any dead space. The airflow at the mouth was measured with a pneumotachometer and a differential pressure transducer, which also was used to measure the mouth pressure relative to the ambient pressure. To record the dose-response curves, Rrs was calculated by an analog computer and then was continuously displayed on the time scale of an X-Y recorder.

Data Analysis

A parametric analysis of variance (ANOVA) was performed to assess any differences in Dmin-PO_2 and Dmin-Rrs between the groups. The data are expressed as the means $\pm$ SEM. The logarithmic values ($\log_{10}$ [milli-units]) of Dmin-PO_2 or Dmin-Rrs were used for the statistical analysis and plots in the figures.

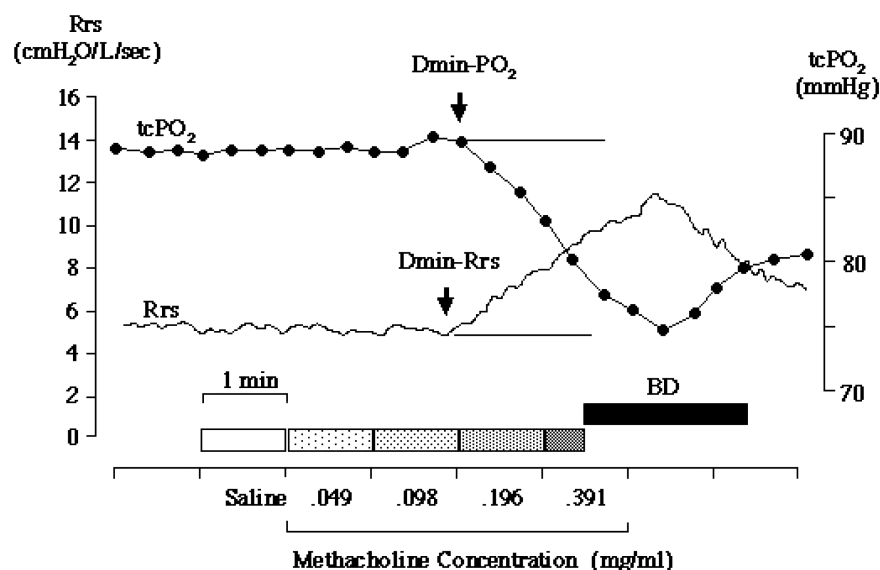


FIGURE 1.—Analysis of typical dose-response curves. With inhalation of an incremental challenge of methacholine, Rrs increased and tcPO₂ decreased curvilinearly. The cumulative dose of methacholine at the inflection point where Rrs increased and tcPO₂ decreased linearly (Dmin-Rrs, Dmin-PO₂) was taken as the bronchial responsiveness of methacholine.

For comparison purposes with the age-matched control subjects the %Dmin in asthmatic subjects was calculated for each severity group as:

the Dmin in each asthmatic subject / the mean Dmin in all age-matched controls $\times 100(\%)$.

A *p* value of less than 0.05 was considered significant.

RESULTS

During the methacholine inhalation challenge, two patients briefly showed more than a 25% decrease in tcPO₂, but the maximum decrease of tcPO₂ ranged between 10 to 20 mmHg for most others. Both children showing more than a 25% decrease in tcPO₂ received orciprenaline sulfate via the final nebulizer, thereby increasing the tcPO₂ to normal levels within several minutes. As a result, all patients underwent the methacholine inhalation challenge safely. In all age ranges, both Dmin-PO₂ and Dmin-Rrs in asthmatic subjects were significantly lower than those in the control subjects.

In the 2- to 5-year-old subjects, Dmin-PO₂ differed significantly between the three asthma severity groups. Dmin-PO₂ in the mild group (3.79 ± 0.1 log [milli-units]) was higher than in either the moderate group (2.69 ± 0.1 log [milli-units]) or the severe group (2.47 ± 0.1 log [milli-units]); both *p* < 0.001, while Dmin-PO₂ in the moderate group was higher than that in the severe group (Figure 2). This tendency also was found in the 6- to 9-year olds: mild asthma, 3.09 ± 0.1 log (milli-units); moderate asthma, 2.97 ± 0.1 log (milli-units); and severe asthma, 2.51 ± 0.2 log (milli-units); (Figure 2). The same pattern prevailed in the 10- to 13-year-old subjects: mild asthma, 3.36 ± 0.1 log (milli-units); moderate asthma, 3.00 ± 0.1 log (milli-units); and severe asthma, 2.15 ± 0.1 log (milli-units); (Figure 2).

In the 14- to 20-year-old subjects, however, Dmin-Rrs did not differ significantly between the three groups: mild asthma, 3.14 ± 0.1 log (milli-units); moderate asthma,

2.96 ± 0.1 log (milli-units); and severe asthma, 3.05 ± 0.3 log (milli-units) (Figure 2).

In the mild asthma group, the %Dmin in the 14- to 20-year-old subjects was significantly smaller than in the other three age ranges (Figure 3). No significant differences were evident between the four age ranges for moderate asthma. However, in the severe asthma group, the %Dmin in the 14- to 20-year-old subjects was higher than that in the other three age ranges (Figure 3).

DISCUSSION

Most asthmatic children exhibit BHR, which describes an exaggerated constrictive response of the airways to a wide variety of specific and nonspecific stimuli that clearly plays a central role in the pathophysiology of asthma (17, 18). Although much has been learned about the clinical aspects of BHR in asthma, relatively little is known about the factors that affect BHR. It is therefore important to elucidate whether BHR correlates with asthma severity because an amelioration of BHR remains one of the goals of asthma treatment (19).

Methacholine-induced airway obstruction results in hypoxemia because a narrowing of the airways changes the local ventilation/perfusion ratio, thus resulting in a decrease in the arterial PO₂ (20, 21). We previously described a technique for evaluating BHR, involving the continuous monitoring of the respiratory resistance (Rrs) and transcutaneous oxygen pressure (tcPO₂), which can be used over a wide range of pediatric ages (10). This method, which is so simple and painless that it can be used in infants, yields effort-independent, highly reproducible results (11), although the tcPO₂ change is thought to reflect only ventilation/perfusion inequality (20).

One key finding of the present study is that in the three younger age groups of children with asthma, namely in those ranging from 2 to 5 years of age, 6 to 9 years of age, and 10 to 13 years of age, the degree of BHR was closely related to

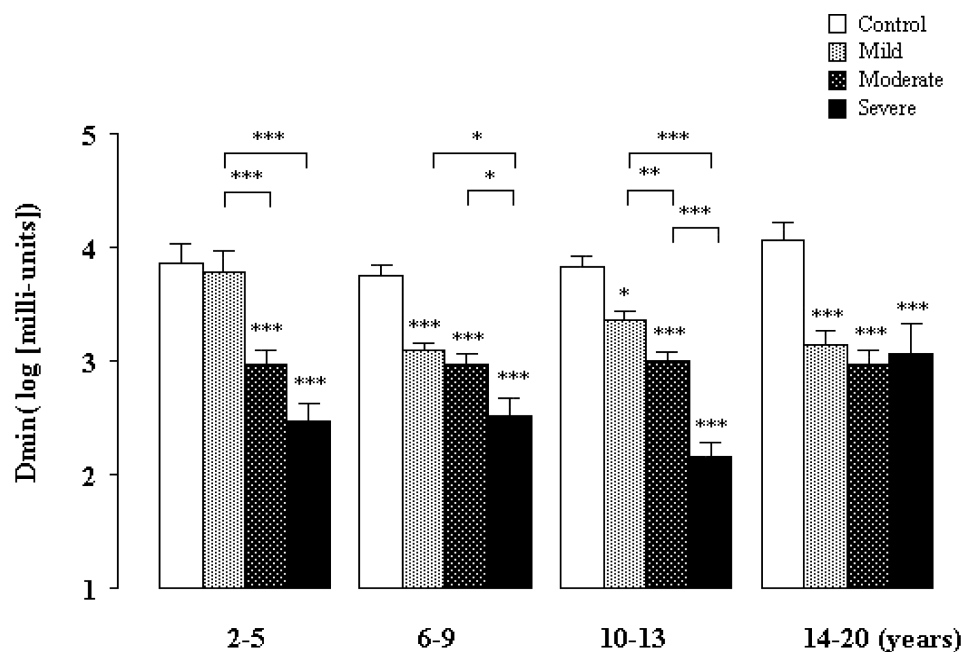


FIGURE 2.—Age-related Dmin-PO₂ changes in the three asthma-severity groups and control subjects. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the severity of asthma, while this was not true for adolescents from 14 to 20 years of age.

Furthermore, when we expressed Dmin in the subjects with asthma in the four age groups relative to the values in the age-matched controls (i.e., %Dmin), the adolescents with mild asthma showed a lower %Dmin, whereas those with severe asthma showed a higher %Dmin than that observed in other age groups. The values in the age-matched symptom-free control subjects were needed as a reference point for asthmatic subjects of different ages because the age-related changes in BHR have been reported to occur in asthmatic patients throughout childhood and adolescence (10).

BHR, which is considered to be closely related to the severity of asthma (3, 4), is influenced in turn by chronic airway disturbances and hereditary factors (22). There is a strong agreement that the mechanisms underlying persistent BHR usually involve chronic airway inflammation (23). Considering this association, children with severe asthma who show severe airway inflammation and/or airway damage may have a higher airway sensitivity to airway stimuli, thus resulting in severe symptoms (24, 25). The reported histologic changes in the airway walls and epithelium in severely asthmatic children resemble those seen in adults, namely, reticular basement membrane thickening and airway inflammation with

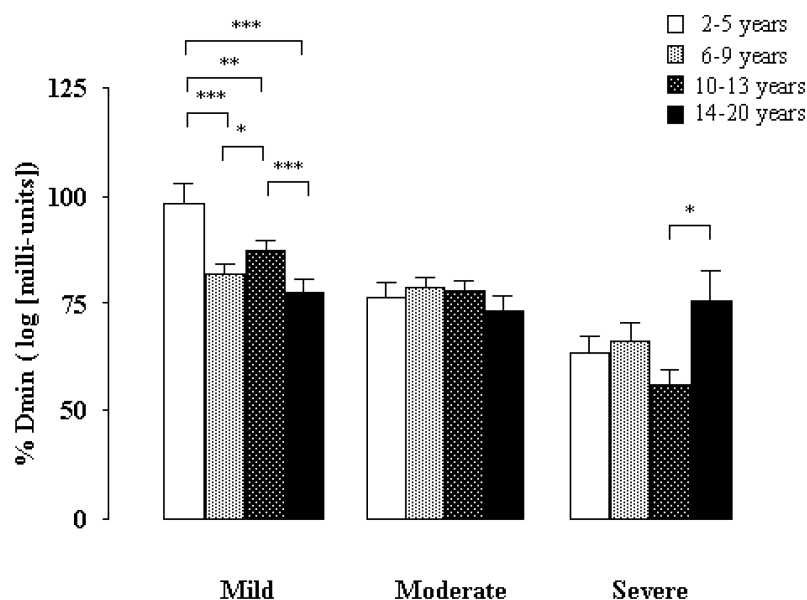


FIGURE 3.—Age-related %Dmin changes in the three asthma-severity groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

eosinophilic infiltration have already been established (26–28). Reticular basement membrane thickening appears to be persistent in children with severe asthma, showing changes of a degree similar to that seen in adults with asthma (28). Conversely, the airway walls and epithelium in younger children with milder asthma have shown a different histologic appearance (29, 30); namely, reticular basement membrane thickening and eosinophilic inflammation, characteristic of asthma in older children and adults, are not present in symptomatic infants with reversible airflow obstruction (29). Studies of infants who wheeze have identified some features similar to the findings in adults with asthma, but also some differences (no increase in either the mast cell counts or prostaglandin D₂ concentrations) (30). Importantly, children with persistent asthma tend to show an increased number of epithelial cells in the bronchoalveolar lavage fluid (30). The increased number of epithelial cells in the bronchoalveolar lavage fluid suggests that a degree of structural damage with epithelial shedding thus occurs in asthma. We believe that BHR in infants and younger children is therefore a particularly accurate indicator of the severity of asthma because the airway wall structure in younger mildly asthmatic children may be simpler, thus reflecting immaturity and a relative absence of cumulative damage.

Our second finding was that Dmin in adolescents with mild asthma was significantly less than in the other age ranges. Adolescents have sometimes been observed to outgrow their asthma (6). We suspect that the clinical changes in asthma in adolescence involve the dramatic physiologic changes that occur during this period. Previous studies have shown that almost all asthmatic children develop symptoms before 5 years of age, while asthma remission increases from 10 to 19 years of age. Lombardi reported that only 37% of asthmatic adolescents had developed symptoms of asthma before the age of 6 years, while 29% developed symptoms during adolescence (31). These results suggest that the age-related changes of BHR in adolescent asthma are affected by changes in various clinical factors. However, previous reports have suggested that BHR is persistent (32, 33). In addition, the present study also confirmed the BHR in children with varying clinical severities of asthma to be higher than in the control subjects (Figure 2). We suspect that adolescents with mild asthma who had previously demonstrated more severe asthma who show the same degree of BHR as previously observed.

Our third finding was that Dmin in severe asthma in adolescence was higher than that in the younger age ranges. The airway smooth muscle activity and airway wall rigidity are both considered to be potent factors affecting bronchial reactivity in asthmatic patients. A relationship between the pathophysiologic changes in the airways as well as airway rigidity and both growth and development have been reported. For example, bronchial cartilage hardens during childhood development (34), and such a change is likely to induce airway rigidity.

Furthermore, previous reports have suggested that airway remodeling related to airway inflammation can affect BHR (28, 35). The thickening of the epithelial reticular basement membrane is a characteristic feature of the remodeling process in asthmatic adults, but when this process begins during the development of asthma is not exactly known (29). Biopsy specimens from children with asthma have demonstrated that

the reticular basement membrane has already thickened at 16 years of age (27). The duration of asthma is one of the important factors affecting airway remodeling (25, 36). Niimi et al. reported that airway wall thickening attenuates airway reactivity in patients with asthma (37). Although the precise relationship between airway rigidity and age in humans remains unclear, we speculate that the decrease in BHR in severe asthmatic adolescents results in part from the airway rigidity induced by remodeling. Consequently, BHR is an unreliable gauge of severity of asthma during adolescence, while BHR is an important factor underlying the clinical aspects of childhood asthma. Adolescence is a unique period in life, encompassing a wide spectrum of physical and psychosocial changes that lead an individual into adulthood. More detailed studies of asthma in adolescence are thus called for to identify clinically useful predictors of the outcome of this disease in adult life.

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