Prevalence of bronchial reactivity to inhaled methacholine in New Zealand children

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ABSTRACT   The prevalence of bronchial hyperreactivity to inhaled methacholine and of a clinical history of symptoms of asthma was determined in a birth cohort of 9 year old New Zealand children. A history of current or previous recurrent wheezing was obtained in 220 of 815 children. Of 800 who had spirometric tests, 27 (3.4%) had resting airflow obstruction (FEV_1/FVC < 75%). Methacholine challenge was undertaken without problem in 766 children, the abbreviated protocol being based on five breaths and four concentrations. A fall in FEV_1 of more than 20% was observed in 176 children (23% of challenges, 22% of the full cohort) after inhalation of methacholine in concentrations of up to 25 mg/ml. The prevalence of bronchial reactivity in children with symptoms was related to the frequency of wheezing episodes in the last year, and the degree of reactivity to the interval since the last episode. Sixty four children (8.0%) with no history of wheeze or recurrent dry cough were, however, also responsive to methacholine 25 mg/ml or less, while 35% of children with current or previous wheezing did not respond to any dose of methacholine. Bronchial challenge by methacholine inhalation was not sufficiently sensitive or specific to be useful as a major criterion for the diagnosis of asthma in epidemiological studies. The occurrence of airway reactivity in children without symptoms of asthma, however, raises the possibility that adult onset asthma and the development of airways obstruction in some subjects with chronic bronchitis could have origins in childhood.

Asthma, although one of the most common diseases of childhood, is underdiagnosed and under-treated.[1-3] The prevalence of childhood asthma is difficult to determine with certainty; estimates in New Zealand have ranged from 5% to 25%.[1 4-7] We have previously reported a high prevalence of a history of wheezing in 7 year old children.[1] Our results were in keeping with Australian findings that 11–12% of children have clinically important wheezing in the first 7–10 years of life, and that a further 20% may have mild or trivial wheezing not labelled as asthma.[8 9] Some of the variation in reported prevalence is attributable to differing interpretations of a history of wheezing, and the demonstration of bronchial reac-

tivity has been suggested as the ultimate standard for the diagnosis of asthma.[10 11] Non-specific bronchial reactivity to methacholine and histamine and to exercise has been well documented in subjects with symptomatic asthma,[12-17] methacholine challenge giving similar results to histamine challenge[15] and being more sensitive than exercise challenge.[16 17] Few epidemiological studies using these techniques for the detection of asthma have been published.[6 18] Lee *et al*[18] found that one third of 7 year old British children with recurrent wheezing did not respond to histamine inhalation challenge, while one third of a control group of 100 symptomless children did react in some instances to very low concentrations of histamine.

As part of a longitudinal multidisciplinary study of a birth cohort of New Zealand children now aged 9 years, we sought to determine the prevalence of bronchial hyperreactivity to inhaled methacholine and to assess the value of this challenge in determining the prevalence of asthma in childhood.

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Methods

Children aged 9 years, enrolled in the Dunedin Multidisciplinary Child Development Study, were the subjects of this study. A birth cohort of 1661 surviving infants born at Queen Mary Hospital, Dunedin, from 1 April 1972 to 31 March 1973 has been followed every two years from the age of 3, at which time 1037 children were located and were willing to participate; most were seen again at 5 and 7 years.[1] At 9 years 815 of these children attended the Dunedin study centre; children examined elsewhere in New Zealand and those whose details were obtained by correspondence have not been included in this report. The characteristics of the children in this longitudinal study, and their comparability at birth with those of the cohort not followed up, have been reported.[19]

The aim of the respiratory assessment was to determine the cumulative prevalence and characteristics of asthma between birth and age 9. A detailed questionnaire regarding past and current wheezing, coughing, and other respiratory symptoms was administered to the child's parent by a physician. Chest examination, spirometry, and methacholine inhalation bronchial challenge were performed after written informed parental consent had been obtained. All procedures were approved by the Dunedin Hospital ethics committee.

Spirometry was performed with a water sealed Godart spirometer. At least three attempts were made to obtain the best forced expired volume in one second (FEV_1) and forced vital capacity (FVC). Values for FEV_1 and FVC were compared with established predicted values for children.[20] If FEV_1/FVC was less than 75% in a child with respiratory symptoms, or less than 70% in a symptomless child, methacholine challenge was not undertaken; responsiveness to an inhaled bronchodilator (salbutamol) was assessed instead.

METHACHOLINE CHALLENGE

Methacholine inhalation challenge was performed according to an abbreviated protocol modified from Chai et al.[21] Methacholine solutions (0.025, 0.25, 2.5, and 25.0 mg/ml) were prepared weekly from freeze dried methacholine chloride (Sigma, USA), stored at $-20°C$ and kept at $4°C$ between tests. After spirometry the child inhaled nebulised methacholine through a closely fitting facemask attached directly to a Hudson nebuliser delivering 0.2 ± 0.02 ml/min at an air flow rate of 6 l/min. The child was instructed to take five consecutive breaths from the mask, from functional residual capacity to total lung capacity. Spirometry was repeated 30 seconds and two minutes after challenge. Increasing concentrations of methacholine were inhaled at intervals of three to four minutes until FEV_1 fell to less than 80% of the best FEV_1 achieved before that test, or until the maximum concentration was reached. The provoking concentration of methacholine causing a 20% fall in FEV_1 ($PC_{20}FEV_1$) was calculated by interpolation.

Resting or induced bronchoconstriction was reversed by inhalation of salbutamol 0.5% administered undiluted through a Hudson nebuliser at an airflow rate of 6 l/min for 1.5 minutes. Improvement in FEV_1 was documented five minutes later; an increase of 10% or more was considered significant.

REPEATABILITY AND VALIDATION PROCEDURES

Within two months of the initial test a representative subsample of 79 subjects underwent a second study to assess the repeatability of the abbreviated methacholine challenge in children. To validate the abbreviated challenge procedure, a further 30 selected subjects, 22 of whom had shown reactivity with our protocol, were recalled for methacholine challenge according to the protocol of Hargreave et al,[22] in which twofold concentration increments (methacholine 0.03–32.0 mg/ml) were inhaled by tidal breathing for two minutes from a closely fitting facemask attached directly to a Wright's nebuliser delivering 0.13 ± 0.015 ml/min at an airflow rate of 6 l/min.

STATISTICAL ANALYSIS

The relationships between $PC_{20}FEV_1$ values and spirometric and clinical data were explored with χ^2 tests, t tests, and analyses of variance. Since differences in percentage fall in FEV_1 and in $PC_{20}FEV_1$ in the repeatability and validation studies were proportional to the size of the measurement, logarithmic transformations were carried out before analysis. Differences were analysed with the test of reliability described by Fleiss[23] and the analysis of comparison studies described by Altman and Bland.[24]

Results

Of the 1037 children entering the study at age 3, 815 (431 boys, 384 girls) were seen at the study centre at the age of 9. Although these represented only half of the birth cohort of 1661 children, they appear to have been a fair reflection of the original sample. The children not entered into the study at the age of 3 did not differ significantly in their birth and perinatal characteristics from those enrolled in the study,[19] and the gradual loss of 222 children over the subsequent six years has not altered significantly the socioeconomic or other epidemiologically important characteristics of the cohort.

PHYSIOLOGICAL STUDIES

Eight children did not perform adequately in the spirometric tests because of limited mental ability (2), lack of cooperation (3), or persistent variability of effort (3). Two were excluded because of a physical handicap that affected their ability to use a spirometer, three because they had used a bronchodilator drug within the preceding six hours, and two because they did not complete the methacholine challenge according to the protocol. Data from these 15 children, six of whom had a history of asthma, were excluded from the physiological analysis. Technically satisfactory spirometric tracings were obtained in the remaining 800 children. Methacholine challenge was declined by eight of these children.

Airflow obstruction

Spirometric evidence of airflow obstruction at rest ($FEV_1/FVC < 75\%$) was found in 27 children (3.4% of 800 tested), 17 of them boys; only five of the 27 underwent methacholine challenge. Eight children with an FEV_1/FVC of less than 70%, 14 with an FEV_1/FVC of 70–74.9% who had had recent wheezing, and four whose FEV_1/FVC was 75% or more but who clinically had wheezing or a low absolute FEV_1 were given salbutamol only. Spirometric values improved after inhalation of salbutamol (> 10% increase in FEV_1) in all but three of these 26 children; in 22 the postbronchodilator FEV_1/FVC exceeded 75% and in 12 it exceeded 80%. Three children with an FEV_1/FVC of 70–74.9% with a trivial history of wheeze or cough and two with no such history were included in the group undergoing methacholine challenge.

Methacholine challenge

Of 766 children who underwent methacholine bronchial challenge, five (0.7%) had a more than 20% fall in FEV_1 after five breaths of methacholine 0.25 mg/ml, 31 (4.0%) after methacholine 2.5 mg/ml, and 140 (18.3%) after methacholine 25.0 mg/ml. In all, 176 children (23.0% of those without airway obstruction who were tested, or 22.0% of all children tested; 111 boys, 65 girls) showed a $PC_{20}FEV_1$ in response to methacholine of less that 25 mg/ml. All of the 176 children showed a greater than 10% increase in FEV_1 after a salbutamol inhalation given to terminate the methacholine response.

There was a weak positive correlation between $PC_{20}FEV_1$ and initial FEV_1 when this was expressed as percentage of predicted FEV_1 ($r = 0.21$, $p < 0.005$), but this accounted for only 4.6% of the variation in $PC_{20}FEV_1$. No correlation was found between $PC_{20}FEV_1$ and initial FVC. The prevalence of reactivity was related to the FEV_1/FVC ratio; 81.5% of children with an FEV_1/FVC below 75% and 62.5% of those with an FEV_1/FVC below 80% showed a response to salbutamol or methacholine.

No severe or protracted wheezing was induced despite a fall in FEV_1 by more than half in four children. Discomforting cough after methacholine in four children was reversed by inhalation of salbutamol. Oxygen and emergency drugs were always available but never required. Bronchial challenge was completed within 15 minutes, or 20 minutes if salbutamol was given to terminate bronchoconstriction.

RELATIONSHIP OF BRONCHIAL REACTIVITY TO CLINICAL HISTORY

A history of recurrent wheezing was obtained in 220 children. In 73 (9.0% of all children) wheeze was infrequent (one or two episodes a year) and asthma was classed as trivial; 113 (13.9%) had three or more episodes per year and were regarded as having mild asthma, and a further 34 (4.2%) had sufficiently frequent wheezing (more than one episode a month) to warrant regular treatment and these were classified as having moderate or severe asthma. Nineteen children had a single episode of wheezing only, while recurrent dry cough without wheeze was noted in 94 children; these children could not be considered symptom free and their data are given separately in table 1. Data

Table 1 *Relationship between symptoms before the age of 9 and response to bronchial challenge in 813 9 year old children*

| Symptoms	Total number	Number tested	No with reversible resting airflow obstruction	Number reacting to cumulative totals methacholine $PC_{20}FEV_1$			Total with reactive airways (% tested)
				<0.25	<2.5	≤25.0 (mg/ml)	
None ever	480	466	1	0	6	64	14
Recurrent dry cough	94	94	0	0	3	23	24
Single episode of wheezing	19	19	0	0	1	6	32
Trivial asthma (1–2 episodes/y)	73	73	1	0	2	18	26
Mild asthma (> 2 episodes/y)	113	108	10	2	10	46	52
Moderate or severe asthma (requiring regular treatment)	34	31	11	3	14	19	97
Total	813	791	23	5	36	176	245
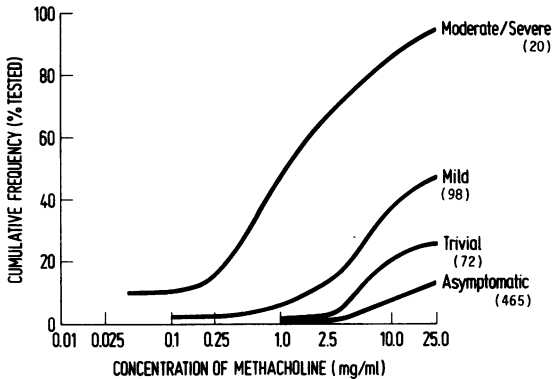
Fig 1 *Cumulative frequency of bronchial reactivity in response to increasing concentrations of methacholine in children with and without symptoms who did not have resting airflow obstruction. Numbers in brackets indicate numbers of children tested in each group.*

were inadequate in two children. The remaining 480 children (58.9%) did not admit to respiratory symptoms up to the age of 9 (table 1) and were classified as asymptomatic.

The cumulative frequency of $PC_{20}FEV_1$ values in wheezing and symptomless children without resting airflow obstruction is shown in figure 1. Reactivity to methacholine occurred more frequently at lower concentrations in those with more severe asthma. Reactivity was not, however, invariable among children with symptoms (table 1). The prevalence of reactivity to salbutamol and methacholine was directly related to the frequency of episodes during the year before the methacholine challenge (table 2); all with daily wheeze were reactive to methacholine, as were 88% of those whose episodes occurred at least monthly. But 35% of children with three or more wheezing episodes in the year before challenge and without resting airflow obstruction did not respond to methacholine 25 mg/ml. The degree of reactivity was related to the interval since the last episode of wheez-

ing, a shorter interval being associated with a lower $PC_{20}FEV_1$ (p < 0.001).

Despite these relationships, 50.5% of the 220 children with a history of recurrent wheezing did not respond to the highest dose of methacholine. Of the 147 children with mild or moderate or severe asthma at some time during their nine years, 16% had resting airflow obstruction, 5% declined methacholine challenge, 44% had a $PC_{20}FEV_1$ less than 25 mg/ml, and the remaining 35% failed to achieve a 20% fall in FEV_1 after inhaling methacholine 25 mg/ml. This was not simply related to the frequency or severity of episodes, since in a selected group of 38 children with three to 12 episodes of wheezing in the last year 11% had resting airflow obstruction, 42% responded to methacholine, and 47% did not achieve a 20% fall in FEV_1 after methacholine 25 mg/ml. In this group, those with reactivity and those without did not differ in mean age of onset of wheezing or in the interval since their last episode.

On the other hand, not all children with bronchial reactivity had had respiratory symptoms. Twenty six of the 27 children with resting airflow obstruction had a previous or current history of wheeze or recurrent cough, but only 64% of the 176 children with a methacholine $PC_{20}FEV_1$ value less than 25 mg/ml had had symptoms. Bronchial reactivity without any previous symptoms was found in 65 children. One had resting reversible airflow obstruction and 64 (41 boys and 23 girls; 13.3% of all symptomless children) showed a response to the doses of methacholine given; of these, 40 had $PC_{20}FEV_1$ <10 mg/ml, 18 <5.0 mg/ml, and six <2.5 mg/ml.

REPEATABILITY AND VALIDATION STUDIES
The abbreviated challenge was repeated in 79 children, 49 of whom did not respond to methacholine 25 mg/ml in either test while 14 responded in both tests. Thus 80% of children retained the same classification of "reactive" or "non-reactive" on repeat testing. Eight with a greater than 20% fall in FEV_1 in the first test had a fall of under 20% on repeat

Table 2 *Relationship between frequency of wheezing episodes in the last year and response to bronchial challenge in 220 children with recurrent wheezing*

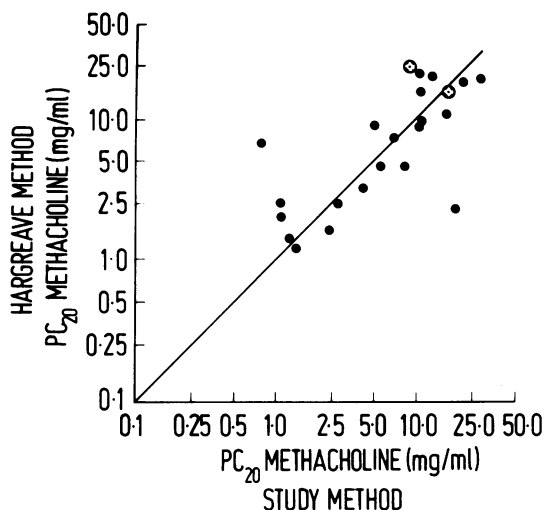| Wheezing episodes in last year	Total number	Number tested	No with reversible resting airflow obstruction	Number reacting to cumulative totals methacholine $PC_{20}FEV_1$			Total with reactive airways (% tested)
				<0.25	<2.5	≤25.0 (mg/ml)	
None	60	59	2	0	0	13	25
1–2	68	65	3	0	6	25	43
<Monthly	47	45	5	0	5	20	56
≥Monthly	24	22	7	2	6	12	86
>Weekly	13	12	1	2	5	9	83
Daily	8	8	4	1	4	4	100

Fig 2 *Comparison of $PC_{20}FEV_1$ obtained by the abbreviated method (abcissa) and by the Hargreave protocol (ordinate) in 23 children. Results from seven non-responders are not shown. The open circles indicate PC_{15} data for both methods when PC_{20} was not achieved by one protocol.*

testing, and eight vice versa; of these 16 whose classification changed, six had a 19.0–19.9% fall in FEV_1 in response to the concentration which in the other test had induced a greater than 20% fall in FEV_1. When the analysis of Fleiss[23] was applied to these data, the test gave a moderate coefficient of reliability (kappa = 0.50 (SE 0.01; p < 0.001)). In addition, the paired t test applied to the logarithmically transformed data from the 14 children responding on both occasions showed no bias from one test to the other ($t = 0.08$, df = 13; p = 0.94). The mean ratio of $PC_{20}FEV_1$ in test 1 and test 2 was 0.94, not significantly different from 1.00 (95% confidence interval 0.63–1.41[24]—that is, less than one doubling concentration of methacholine).

Methacholine reactivity measured with the abbreviated protocol was compared with that determined with the longer protocol of Hargreave *et al*[22] in 30 selected children. In seven children neither method produced a significant fall in FEV_1. In the remaining 23 children comparison of the methacholine concentrations required to provoke a 15% or 20% fall in FEV_1 showed good agreement between the two techniques (fig 2). In only four children was the difference in $PC_{20}FEV_1$ between the two techniques greater than a twofold concentration step; three of these four had current asthma. A paired t test on the logarithmically transformed data showed no bias from one method to the other ($t = 0.38$, df = 22; p = 0.71). The mean

ratio of $PC_{20}FEV_1$ determined by our study method and the Hargreave method was 1.02 (95% confidence interval 0.60–1.73[24]—that is, less than one doubling concentration of methacholine).

Discussion

In this study we added measurement of bronchial reactivity to a clinical and questionnaire survey of asthma prevalence in a birth cohort of over 800 9 year old children. An abbreviated methacholine challenge protocol proved practicable in an epidemiological setting and was accomplished without incident. The longer methacholine challenge protocol of Hargreave and colleagues[14 22] was not used because of time limitations and the likelihood that children subjected to a lengthy protocol would become fatigued and bored and so perform less well in the spirometric tests. Chatham *et al*[25] compared a similar abbreviated methacholine inhalation challenge with the longer protocol and found the abbreviated protocol provided a comparable measure of bronchial reactivity. Our validation study showed close agreement between the PC_{20} values obtained by the short and long protocols. In only four children, three of whom had current asthma, was the difference in $PC_{20}FEV_1$ between the two methods greater than a twofold concentration increment. The interval of up to two months between tests in the repeatability study was longer than ideal, and may have allowed bronchial reactivity to change in some children as a result of intercurrent infection or allergen exposure.

We found that 25% of the 9 year old childhood population had resting airflow obstruction reversed by a β sympathomimetic aerosol, or had a $PC_{20}FEV_1$ of less than 25 mg/ml inhaled methacholine. Heightened bronchial responsiveness is a common phenomenon in childhood. Considerable overlap in bronchial responsiveness was, however, found between children with and without symptoms; 13.9% of symptomless children tested showed reversible bronchoconstriction or reactivity to methacholine 25 mg/ml, while 35% of those with clinically relevant wheezing did not show a 20% fall in FEV_1 with methacholine. The distribution of bronchial reactivity in the population is likely to be a continuum rather than bimodal. On the basis of a 20% fall in FEV_1 after five breaths of methacholine 25 mg/ml as evidence of heightened bronchial reactivity, some children with no recall of wheeze or recurrent dry cough showed bronchial reactivity, while some with symptoms suggestive of asthma did not. These findings are similar to those of Lee *et al*[18]; one third of their 7 year old subjects with recurrent wheeze failed to respond to inhalation of histamine 16 mg/ml, while one third of symptomless children did respond. Clearly, bronchial reactivity to methacholine is not

synonymous with asthma; a single negative response to challenge does not negate the clinical diagnosis. Bronchial reactivity to other potential provocations— exercise, temperature change, infection, exposure to allergens—could possibly be present in the absence of methacholine sensitivity, and the latter may vary with time. Aquilina *et al*[26] and Empey *et al*[27] showed that histamine reactivity was increased in normal subjects immediately after upper respiratory tract infections. Laitinen and Kava[28] found no increase in histamine induced change in specific airway conductance in healthy subjects given a nasal inoculation of influenza A virus, whereas asthmatic subjects given the same inoculation showed increased reactivity.

Children with a history of wheezing showed significant relationships between the degree of airway reactivity and both the frequency of symptoms over the previous year and the interval between the most recent episode and the methacholine challenge. A relationship between bronchial reactivity and the frequency and severity of clinical asthma has been found repeatedly[20 29–32] but bronchial reactivity without symptoms is less well documented. While some children reported as showing asymptomatic bronchial reactivity may have had symptoms not recalled by the mother, the interval from the episode to the methacholine challenge would be most likely to be long and the severity of the episode minimal for it not to be recalled on direct and searching questioning. The present study confirms the findings of Lee *et al*[18]—namely, that bronchial hyperreactivity may be present in children in the absence of respiratory symptoms.

Simonsson[33] tabled seven reports of bronchial reactivity to methacholine in "normal" adult subjects; the maximum number of subjects in any one study was 62.[13] The concentrations and doses of methacholine administered and the measurement of lung function varied but these studies suggest a degree of bronchial hyperreactivity in the normal adult population similar to that in children. Heightened bronchial responsiveness in adults may have its origins in childhood. Whether increased responsiveness continues to be asymptomatic, or perhaps predisposes to the later development of asthma or airflow obstruction in patients with chronic bronchitis,[34] is conjectural; and the question could be answered only by long term follow up of children such as these.

In summary, we have shown that 25% of 9 year old children had evidence of airway reactivity, revealed either by resting airflow obstruction relieved with salbutamol or by responsiveness to inhalation of methacholine. While the presence of airway reactivity was associated broadly with symptoms of asthma, a substantial number of children (8% of 800 tested) had mild or moderate increases in airway reactivity but denied any respiratory symptoms. On the other hand, 35% of children with recent or past histories of more than trivial wheezing did not show increased responsiveness to methacholine. We conclude that heightened bronchial responsiveness is a common phenomenon in childhood but that bronchial challenge is not sufficiently sensitive or specific to make its use in epidemiological studies of asthma mandatory. Rather, for both epidemiologist and clinician, a detailed history indicating recurrent episodes of wheezing should lead to a diagnosis of asthma.

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