Prevalence of bronchial reactivity to inhaled methacholine in New Zealand children

MALCOLM R SEARS, DAVID T JONES, M DAVID HOLDAWAY, CHRIS J HEWITT, ERIN M FLANNERY, G PETER HERBISON, PHIL A SILVA

From the Departments of Medicine, Paediatrics and Child Health, and Preventive and Social Medicine and the Dunedin Multidisciplinary Health and Development Research Unit, University of Otago Medical School, Dunedin, New Zealand

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 (FEV₁/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 FEV₁ 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 undertreated.1-5 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 reactivity 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 challenge15 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 al18 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.
Methods

Children aged 9 years, enrolled in the Dunedin Multi-disciplinary 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. 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 (FEV₁) and forced vital capacity (FVC). Values for FEV₁ and FVC were compared with established predicted values for children.20 If FEV₁/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 airflow 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 FEV₁ fell to less than 80% of the best FEV₁ achieved before that test, or until the maximum concentration was reached. The provoking concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀FEV₁) 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 FEV₁ was documented five minutes later; an increase of 10% or more was considered significant.

REPEATABILITY AND VALIDATION PROCEDURE

Within two months of the initial test a representative subsample of 79 subjects underwent a second test 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 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 PC₂₀FEV₁ values and spirometric and clinical data were explored with \(\chi^2\) tests, \(t\) tests, and analyses of variance. Since differences in percentage fall in FEV₁ and in PC₂₀FEV₁ 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 Fleiss23 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 socio-economic or other epidemiologically important characteristics of the cohort.
Prevalence of bronchial reactivity to inhaled methacholine in New Zealand children

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 from eight of these 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.

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

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Total number</th>
<th>Number tested</th>
<th>No with reversible resting airflow obstruction</th>
<th>Number reacting to cumulative totals methacholine PC$_{20}$FEV$_1$ &lt;0.25</th>
<th>&lt;2.5</th>
<th>≤25.0 (mg/ml)</th>
<th>Total with reactive airways (% tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None ever</td>
<td>480</td>
<td>466</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>64</td>
<td>14</td>
</tr>
<tr>
<td>Recurrent dry cough</td>
<td>94</td>
<td>94</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Single episode of wheezing</td>
<td>19</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>Trivial asthma (1–2 episodes/y)</td>
<td>73</td>
<td>73</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>Mild asthma</td>
<td>113</td>
<td>108</td>
<td>10</td>
<td>2</td>
<td>10</td>
<td>46</td>
<td>52</td>
</tr>
<tr>
<td>(&gt;2 episodes/y)</td>
<td>34</td>
<td>31</td>
<td>1</td>
<td>3</td>
<td>14</td>
<td>19</td>
<td>97</td>
</tr>
<tr>
<td>Moderate or severe asthma (requiring regular treatment)</td>
<td>34</td>
<td>31</td>
<td>1</td>
<td>3</td>
<td>14</td>
<td>19</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>813</td>
<td>791</td>
<td>23</td>
<td>5</td>
<td>36</td>
<td>176</td>
<td>245</td>
</tr>
</tbody>
</table>
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 PC20 FEV1 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, invariably 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 wheezing, a shorter interval being associated with a lower PC20 FEV1 (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 PC20 FEV1 less than 25 mg/ml, and the remaining 35% failed to achieve a 20% fall in FEV1 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 FEV1 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 PC20 FEV1 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 PC20 FEV1 < 10 mg/ml, 18 < 5.0 mg/ml, and six < 2.5 mg/ml.

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

<table>
<thead>
<tr>
<th>Wheezing episodes in last year</th>
<th>Total number</th>
<th>Number tested</th>
<th>No with reversible resting airflow obstruction</th>
<th>Number reactive to cumulative totals methacholine PC20FEV1</th>
<th>Total with reversible airflow (% tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>60</td>
<td>59</td>
<td>2</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>1-2</td>
<td>68</td>
<td>65</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>&lt; Monthly</td>
<td>47</td>
<td>45</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>&gt; Monthly</td>
<td>24</td>
<td>22</td>
<td>7</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>&gt; Weekly</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Daily</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

**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 FEV1 in the first test had a fall of under 20% on repeat.
testing, and eight vice versa; of these 16 whose classification changed, six had a 19.0–19.9% fall in FEV₁ in response to the concentration which in the other test had induced a greater than 20% fall in FEV₁. When the analysis of Fleiss²³ 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 PC₂₀FEV₁ in test 1 and test 2 was 0.94, not significantly different from 1.00 (95% confidence interval 0.63–1.41²⁴—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 Hargrave et al²² in 30 selected children. In seven children neither method produced a significant fall in FEV₁. In the remaining 23 children comparison of the methacholine concentrations required to provoke a 15% or 20% fall in FEV₁ showed good agreement between the two techniques (fig 2). In only four children was the difference in PC₂₀FEV₁ 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 PC₂₀FEV₁ determined by our study method and the Hargrave method was 1.02 (95% confidence interval 0.60–1.73²⁴—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 Hargrave and colleagues¹⁴,²² 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 al²⁵ 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 PC₂₀ values obtained by the short and long protocols. In only four children, three of whom had current asthma, was the difference in PC₂₀FEV₁ 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 airway obstruction reversed by a β sympathomimetic aerosol, or had a PC₂₀FEV₁ 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 wheeze did not show a 20% fall in FEV₁ 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 FEV₁ 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 al¹⁸; 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 Kava28 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 repeatedly29-32 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—that bronchial hyperreactivity may be present in children in the absence of respiratory symptoms.

Simonsson33 tabulated 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.

The assistance of many professional and volunteer staff and the cooperation of parents and children enrolled in the Dunedin Multidisciplinary Child Development Study is gratefully acknowledged. This work was supported by grants from the Otago Medical Research Foundation, the Medical Research Council of New Zealand, the National Children's Health Research Foundation, the Medical Research Distribution Committee, the University of Otago bequest funds, the Department of Health, and the Department of Education.

References

6 McQueen F, Holdaway MD, Sears MR. A study of asthma in a Dunedin suburban area. NZ Med J 1979;89:335-8.
Prevalence of bronchial reactivity to inhaled methacholine in New Zealand children


