

# Adenosine, methacholine, and exercise challenges in children with asthma or paediatric chronic obstructive pulmonary disease

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## Abstract

**Background** – Bronchial hyperreactivity to methacholine is present in children with asthma and other types of paediatric chronic obstructive pulmonary disease (COPD), while hyperreactivity to exercise is more specific for asthma. Adenosine 5'-monophosphate (AMP) is a potent bronchoconstrictor and, like exercise, may provoke asthma by activating mast cells. This study investigated the suitability of AMP as a specific challenge for asthma in children.

**Methods** – Bronchial provocation challenges with methacholine and AMP were performed in a double blind fashion using tidal breathing in 51 children with asthma, 21 with paediatric COPD of various types, and in 19 control children. Each subject also underwent a standardised exercise challenge after inhalation challenges were completed. Sensitivity and specificity curves were constructed and the intersection point of sensitivity and specificity for each type of challenge was determined.

**Results** – When the asthmatic patients were compared with the children with COPD, the intersection points for AMP, exercise and methacholine were 90%, 85%, and 50%, respectively. When compared with the controls the same intersection points were 98%, 84%, and 92%, and when children with paediatric COPD were compared with controls they were 55%, 50%, and 82%.

**Conclusions** – Methacholine distinguishes both asthma and paediatric COPD from controls with a sensitivity of 82–92%, but does not distinguish between asthma and paediatric COPD; exercise and AMP distinguish asthma from controls with a sensitivity and specificity of 84–98% but they also distinguish asthma from paediatric COPD with a sensitivity and specificity of 85–90%. AMP inhalation is a practical aid for diagnosing asthma and distinguishing it from COPD in children of all ages.

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may also be associated with increased methacholine reactivity.<sup>4–8</sup> However, in adult patients with chronic infections, bronchial hyperreactivity by cold air or isocapnic hyperventilation has been found in a minority of the patients.<sup>9,10</sup> Moreover, we have recently shown<sup>11</sup> that children with asthma and those with chronic lung disease have hyperreactivity to methacholine, but only those with asthma are hyperreactive to physical exercise.

The pathophysiology of exercise-induced asthma is still disputed but there is increasing evidence of mediator release being involved. Exercise is associated with significant increases in plasma histamine and neutrophil chemotactic activity<sup>12–15</sup> which have been considered markers for the release of mediators by mast cells.<sup>16,17</sup> Adenosine is a potent bronchoconstrictor in asthmatic patients, possibly by stimulating or enhancing the release of mediators from mast cells.<sup>18,19</sup> Adenosine has been shown to potentiate the release of preformed but not newly generated mediators from mouse bone marrow-derived mast cells in tissue culture<sup>20</sup> and has also been used for bronchial challenge in asthmatic patients.<sup>21–23</sup> Thus, both exercise and adenosine may induce bronchoconstriction by releasing mediators from airway mast cells. Oosterhoff *et al*<sup>24</sup> compared adults with chronic obstructive pulmonary disease (COPD) with adult asthmatic patients and found that non-smoking adults with COPD were less responsive to adenosine than to methacholine, while the asthmatic patients were similarly responsive to both. They did not study the response to exercise or hyperventilation.

The differentiation of asthma from other types of COPD can be difficult in children since the clinical picture can be very similar. In the present study we have compared bronchial challenges by exercise, methacholine, and adenosine 5'-monophosphate (AMP) in children and young adults with bronchial asthma, in young patients with chronic obstructive lung diseases, and in healthy subjects of similar age. The object of the study was to determine whether AMP, like exercise, was a more specific stimulus than methacholine in differentiating asthma from chronic obstructive lung diseases in such patients.

## Methods

### SUBJECTS

Ninety one children and young adults aged 6–25 years participated in the study; 51 met the

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Bronchial hyperreactivity to methacholine or histamine is a characteristic feature of asthma.<sup>1–3</sup> Other chronic lung diseases, especially those associated with chronic infection,

Table 1 Anthropometric and spirometric data of the subjects

Group	n	Mean (range) age (years)	Sex (M/F)	Mean (SE) baseline FEV <sub>1</sub> (%)
Asthma	51	13.0 (6–25)	32/19	86.4 (1.6)
Paediatric COPD				
Cystic fibrosis	7	11.1 (9–12)	4/3	78.9 (6.2)
Bronchiolitis obliterans	7	11.4 (7–15)	3/4	69.6 (3.8)
Primary ciliary dyskinesia	4	13.7 (7–19)	2/2	80.8 (7.1)
Bronchiectasis	3	11.3 (9–13)	2/1	80.3 (9.9)
Control	19	14.0 (8–23)	10/9	95.0 (2.1)

American Thoracic Society’s diagnostic criteria for asthma<sup>25</sup> and 21 had chronic obstructive lung disease (seven with cystic fibrosis, seven with bronchiolitis obliterans, four with primary ciliary dyskinesia, and three with bronchiectasis). We have termed this latter group paediatric chronic obstructive pulmonary disease (COPD). A further 19 patients were originally referred to the department for lung function and bronchial provocation tests because of subjective complaints of mild cough or breathlessness. Five children had anxiety-related sighs, five mild post-viral cough, five mild intermittent cough, three were normal children with shortness of breath during exercise without asthma, and one had heartburn. In every case full investigation and follow up failed to reveal any objective evidence of lung disease and were used as a control group for the analyses. The exclusion of asthma or COPD was established before performing the challenges. All subjects were recruited from the paediatric clinic of the Institute of Pulmonology, Hadassah University Hospital, Jerusalem. Anthropometric and spirometric data of the subjects are presented in table 1. Of the asthmatic patients, 30 were using inhaled bronchodilators as needed, four used sodium cromoglycate, and 17 used inhaled beclomethasone dipropionate. There was no history of upper or lower respiratory tract infection

during the four weeks before the studies in any of the subjects. At study entry all the control subjects and all but 10 patients (two with asthma, four with bronchiolitis obliterans, two with cystic fibrosis, one with bronchiectasis, and one with primary ciliary dyskinesia) had a forced expiratory volume in one second (FEV<sub>1</sub>) above 70% of the predicted value. All patients avoided bronchodilator therapy for at least 12 hours and sodium cromoglycate for at least 20 hours before the study. Inhaled corticosteroid therapy was continued unchanged. None of the 72 subjects was treated with theophylline-related compounds. The study was approved by the local ethics committee and written informed consent was obtained from the older subjects or from one parent.

STUDY DESIGN

The three challenges were performed on the same day in 55 patients (86%), within seven days in 78 patients (86%), and within 15 days in 86 patients (95%). In the five remaining subjects the challenges were performed within 30 days. The inhalation challenges were always performed in random order on the same day. When the exercise challenge was also performed on the same day as the inhalation challenge it was always the last challenge of the day. The exercise test was performed on a separate day in 16 subjects and on the same day as one of the inhalation challenges in 20 subjects. The interval between challenges on any one day was 2.5–3 hours which allowed the FEV<sub>1</sub> to return to within 10% of baseline value of the first challenge in every case before the second and third challenges were performed. Salbutamol inhalation was administered after the last challenge if the fall in FEV<sub>1</sub> was greater than 20%, if the patient was dyspnoeic, or if the FEV<sub>1</sub> did not return to within 10% of the baseline value.

BRONCHIAL PROVOCATION AND MEASUREMENT OF PULMONARY FUNCTION

Fresh solutions of methacholine and AMP were made up in phosphate buffer solution in a range of concentrations from 0.03 to 32 mg/ml (0.15–163.5 mmol/l) for methacholine and from 0.39 to 400 mg/ml (1.12–1152 mmol/l) for AMP. Two sets of 11 syringes (2.0 ml) with doubling concentrations of methacholine and AMP were prepared and coded so that neither the patient nor the person performing the investigation knew whether methacholine or AMP was being used. Inhalation challenges were performed by the method described by

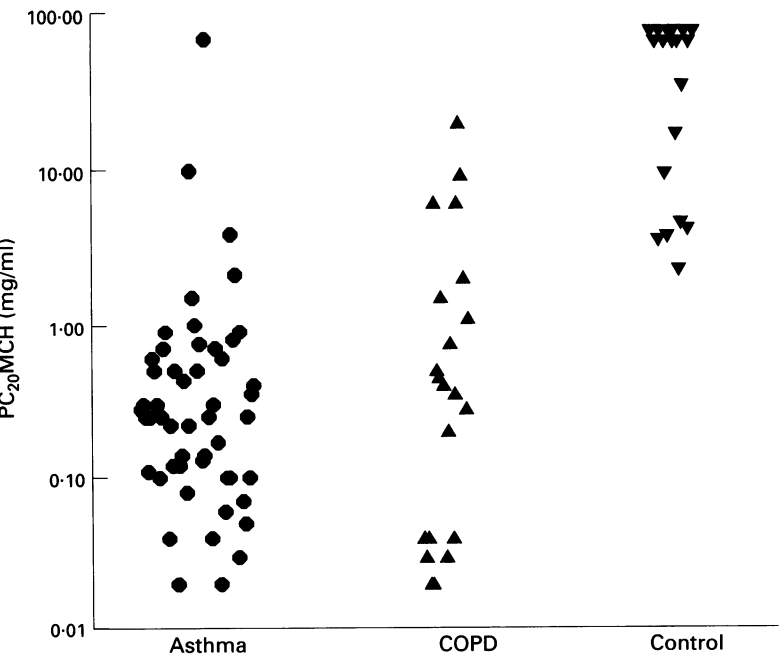


Figure 1 PC<sub>20</sub> for methacholine (MCH) in children with asthma, paediatric COPD, and control subjects.

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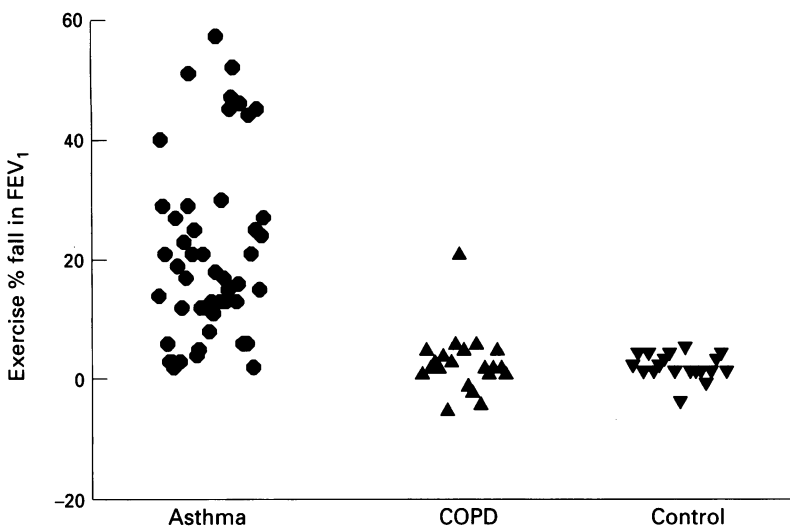


Figure 2 Percentage fall in FEV<sub>1</sub> after exercise in children with asthma, paediatric COPD, and control subjects.

Cockcroft *et al.*<sup>1</sup> The nebuliser chamber (Accorn II, Marquest, Englewood, USA) was filled with 2.0 ml of test solution and an airflow of 5 l/min resulted in a mean rate of nebulisation of 0.39 ml/min. The nebuliser was connected through a one-way valve system to a mouth-piece through which the child breathed normally while wearing a nose clip. The first inhalation comprised phosphate buffer solution, and then methacholine or AMP in increasing concentrations. Each solution was inhaled for two minutes during tidal breathing with measurement of lung function at 30, 90, and 180 seconds after each inhalation until the FEV<sub>1</sub> had fallen by 20% or more from the post-buffer value, or until the maximum concentration was reached. Lung function was measured in duplicate with a pneumotachograph based system (Vitalograph Compact, Buckingham, UK) and the highest value

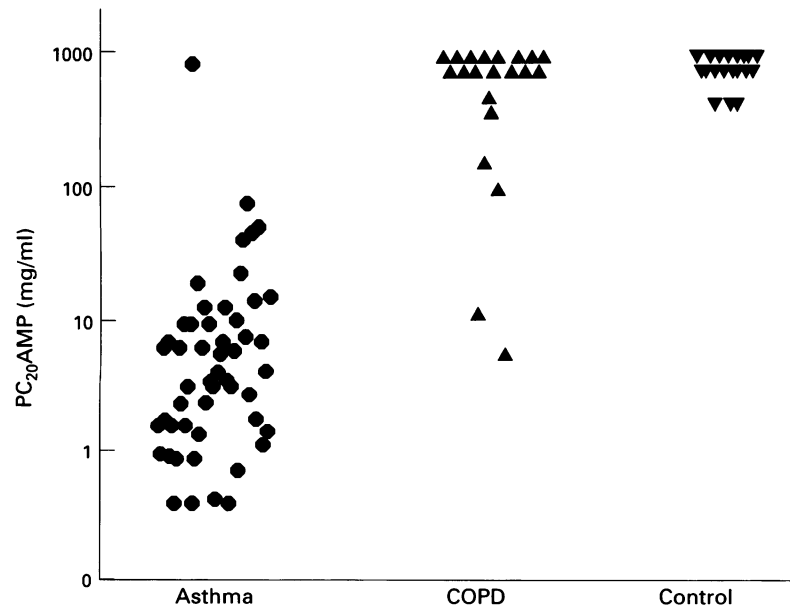


Figure 3 PC<sub>20</sub> for AMP in children with asthma, paediatric COPD, and control subjects.

of FEV<sub>1</sub> at each interval was recorded. The concentrations of methacholine or AMP causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>) from the post-phosphate buffer value were derived from a plot of percentage fall in FEV<sub>1</sub> against log methacholine or log AMP concentration. If the fall in FEV<sub>1</sub> was less than 10% at the maximal concentration, the next doubling values – that is, 64 mg/ml for methacholine and 800 mg/ml for AMP – were arbitrarily taken as PC<sub>20</sub>.

The exercise challenge was carried out using six minutes of treadmill running as described previously.<sup>26</sup> The treadmill was set at a slope of 10° and the subjects ran continuously at a speed of 5 km/h. This produced a heart rate of 160–180/min and represented approximately two thirds of the maximal working capacity. FEV<sub>1</sub> was measured before and 3, 5, 10, and 15 minutes after exercise. The challenge was performed in an air conditioned laboratory and the subjects breathed room air with a temperature of 22–26°C and a relative humidity of 48–56%. The result of the exercise challenge was calculated as the greatest fall in FEV<sub>1</sub> after exercise expressed as a percentage of the pre-exercise value.

STATISTICAL ANALYSIS

PC<sub>20</sub> values were log transformed before statistical analysis. Data are expressed as means (SE). Statistical comparisons between groups and within groups were made by analysis of variance (ANOVA) and paired Student *t* tests as appropriate. All tests of significance were two tailed. Differences were taken as significant when *p* was <0.05.

Results

There was no significant difference between the mean age of the subjects in all three groups. The mean baseline FEV<sub>1</sub> (table 1) was significantly (*p*<0.01) lower in the paediatric COPD (77.9 (3.4)%) and the asthmatic groups (86.4 (1.5)%) than in the control group (95.0 (2.1)%), and the difference between the asthmatic and paediatric COPD patients was also significant. There was no significant difference between the mean baseline FEV<sub>1</sub> before the three types of challenge within any of the three groups.

Individual results for the three challenges in the three groups are shown in figs 1–3. It can be seen that in the control group 11 subjects (58%) failed to respond to the highest concentration of methacholine used and 16 subjects (84%) failed to respond to the highest concentration of AMP. In the paediatric COPD group all responded to methacholine but 15 subjects (71%) failed to respond to AMP. In the asthmatics all but one subject responded to methacholine and all but one (not the same subject) to AMP. By its nature the exercise challenge provided a result in every case even if there was a small rise (negative fall) in FEV<sub>1</sub> after exercise and the values were normally distributed in each group. The mean percentage fall in FEV<sub>1</sub> after exercise in the asthmatic group (20.5 (14.8)%) was significantly

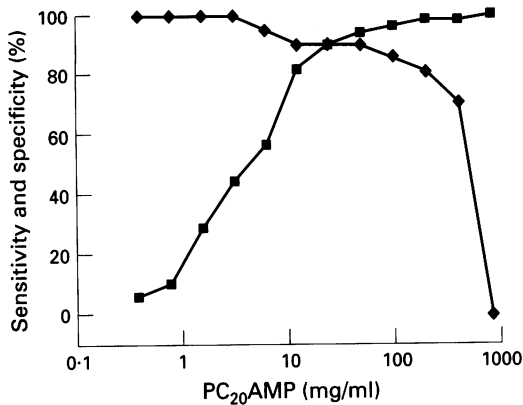


Figure 4 Sensitivity (■) and specificity (◆) of PC<sub>20</sub> AMP in differentiating asthma from paediatric COPD at various AMP concentrations.

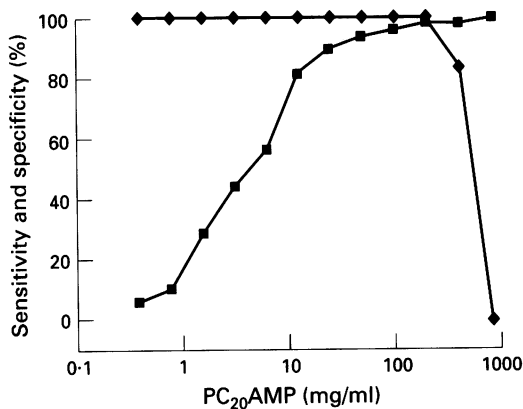


Figure 5 Sensitivity (■) and specificity (◆) of PC<sub>20</sub> AMP in differentiating asthma from controls at various AMP concentrations.

different ( $p<0.0001$ ) from that of the paediatric COPD (2.8 (5.1)%) and control groups (1.8 (2.1)%). There was no significant difference in the response to exercise between the paediatric COPD and control groups. One of the patients with paediatric COPD (primary ciliary dyskinesia) had a 21% fall in FEV<sub>1</sub> after exercise even though he had no personal or family history of atopic disease or asthma. Since all but one asthmatic and all the patients with paediatric COPD responded to methacholine it was possible to compare the responses in these groups. The geometric mean PC<sub>20</sub> for methacholine of the asthmatic group (0.28 mg/ml, range 0.22–0.34 mg/ml) was not sig-

nificantly different from that of the paediatric COPD group (0.36 mg/ml, range 0.22–0.58 mg/ml).

With the reservation that a nominal (minimum) value for PC<sub>20</sub> was arbitrarily assigned to non-responders to methacholine and AMP, the geometric mean PC<sub>20</sub> for AMP of the asthmatic group (4.43 mg/ml, range 3.57–5.50 mg/ml) was significantly different ( $p<0.0001$ ) from that of the paediatric COPD group (320.38 mg/ml, range 294.3–550.7 mg/ml) which was not significantly different from that of the control group (800 mg/ml).

Sensitivity and specificity in differentiating between asthma and paediatric COPD (which are not affected by arbitrary values assigned to non-responders) were calculated for every concentration of methacholine and AMP used and for every 5% fall in FEV<sub>1</sub> after exercise. Sensitivity of a challenge for asthma compared with paediatric COPD was defined as the ratio of the number of asthmatics with a positive test at the chosen concentration (or fall in FEV<sub>1</sub>) to the total number of asthmatics. Specificity for asthma was defined as the ratio of the number of patients with paediatric COPD with a negative test at the chosen concentration (or fall in FEV<sub>1</sub>) to the total number of patients with paediatric COPD. Curves of sensitivity and specificity for every step of the challenges were constructed as shown for the AMP challenge in fig 4. As sensitivity increases with increasingly strong stimuli, so specificity falls, and the best combination of sensitivity and specificity that can be obtained is at the point where they cross. These intersection points are given in table 2 from which it can be seen that the value for AMP was higher than that for exercise, and that both AMP and exercise yielded considerably greater sensitivity and specificity than methacholine in separating asthma from paediatric COPD.

Similarly, sensitivity and specificity curves for differentiating between asthma and controls and between paediatric COPD and controls were calculated from the data of the asthmatic versus control and paediatric COPD versus control groups respectively. The intersection points of these analyses are also given in table 2 and the plot for AMP in distinguishing asthma from controls is shown in fig 5. This analysis shows that, to distinguish between asthma and controls, all these types of challenge yielded a similarly high level of sensitivity and specificity. Neither exercise nor AMP were particularly helpful in distinguishing paediatric COPD from controls (because the patients with paediatric COPD mostly failed to respond to these challenges), but methacholine did yield a reasonable specificity and sensitivity.

Table 2 Intersection points of sensitivity and specificity curves of the three challenges

	Sensitivity and specificity at intersection (%)	Value of test at intersection
Methacholine:		
Asthma v paediatric COPD	50	0.28 mg/ml
Asthma v controls	92	2.20 mg/ml
Paediatric COPD v controls	82	3.80 mg/ml
Exercise:		
Asthma v paediatric COPD	85	5% fall in FEV <sub>1</sub>
Asthma v controls	84	4% fall in FEV <sub>1</sub>
Paediatric COPD v controls	50	3% fall in FEV <sub>1</sub>
Adenosine:		
Asthma v paediatric COPD	90	25 mg/ml
Asthma v controls	98	210 mg/ml
Paediatric COPD v controls	55	>400 mg/ml

Discussion

Methacholine challenge has been widely used for the detection and quantitation of bronchial hyperreactivity in asthmatic patients.<sup>1-3</sup> Hargreave and coworkers<sup>27</sup> suggested a cut-off limit of 8 mg/ml for the PC<sub>20</sub> to methacholine since all their non-asthmatic subjects had a PC<sub>20</sub> above this value while all their asthmatic sub-

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jects with recent symptoms had a lower  $PC_{20}$  value. In two previous studies<sup>11,28</sup> performed in our laboratory on 52 and 182 asthmatic children the upper limits (least reactivity) of methacholine responsiveness (using log mean + 2 log SD) were 4.4 and 7.7 mg/ml, respectively. In the present study the upper limit of  $PC_{20}$  for methacholine (log mean + 2 log SD) in the asthmatic group was 5.6 mg/ml. Although a few of the control group had  $PC_{20}$  values below this range, the analysis of sensitivity and specificity (table 2) showed that, in concurrence with our previous study,<sup>11</sup> methacholine was useful in distinguishing the asthmatic subjects from the controls but not in differentiating between asthma and other types of chronic obstructive lung disease in children.

Exercise-induced bronchoconstriction has also been regarded as an important hallmark of asthma, but the percentage fall in  $FEV_1$  taken as the cut-off limit for asthma in the literature ranges from about 10% to 20%.<sup>29-31</sup> Burr and coauthors<sup>32</sup> studied a group of 812 children aged 12 years who were healthy and unrelated to asthmatic subjects and found that 92% had a fall in peak expiratory flow rate after exercise of less than 10%. In our previous study<sup>11</sup> the upper limit of percentage fall in  $FEV_1$  in control children (taken as mean + 2SD) was 8.2% and in the present study it was 6.0%. In the present study 40 of the 51 asthmatic subjects (78%) had a fall in  $FEV_1$  of more than 6.0% while only one of the patients with paediatric COPD showed significant bronchoconstriction after exercise. This is very similar to the results of our previous study where none of the 22 children with paediatric COPD responded to exercise by bronchoconstriction.<sup>11</sup> The inhalation challenges were performed randomly but the exercise test was performed at the end of the day in most cases. There is a slight possibility that the inhalation challenges might have influenced the exercise test. However, the protocol was identical for the different groups of patients and therefore, if there was any effect of one challenge on the response of the other, it would be the same in the three groups. From table 2 it can be seen that the intersection of sensitivity and specificity for exercise was a little less than for methacholine in distinguishing the asthmatic from the control subjects, but it was much greater in distinguishing asthma from paediatric COPD.

There is a problem in the evaluation of the mean  $PC_{20}$  of the paediatric COPD and control groups since we may have underestimated the true  $PC_{20}$  of the non-responders and the assumed values taken for statistical analysis were the least values for  $PC_{20}$ . Given these restrictions, the significance of the very large differences in the responses of the various groups is almost certainly underestimated.

The response of patients with paediatric COPD to AMP and exercise was poor or non-existent up to the highest concentration used in the challenge. These subjects had lower basal lung function but, since they responded very well to methacholine, this could not have accounted for their lack of response to AMP and exercise. As a further check on the possible

effect of baseline lung function we selected nine children with asthma and nine with paediatric COPD who were matched for age (11.16 (3.2) and 11.7 (3.2) years, respectively) and for baseline  $FEV_1$  (89.3 (10.0)% and 88.2 (8.9)%, respectively). There was no significant difference in the geometric mean  $PC_{20}$  to methacholine (0.29 mg/ml (range 0.14–0.6) for paediatric COPD and 0.47 mg/ml (range 0.31 to 0.72) for asthmatics), while there was a significant difference for mean percentage fall in  $FEV_1$  after exercise (1.7 (3.0)% in those with paediatric COPD and 20.6 (18.5)% in asthmatics,  $p < 0.01$ ) and for geometric mean  $PC_{20}$  to AMP (257.3 mg/ml (range 148.8–444.9) for patients with paediatric COPD and 6.35 mg/ml (range 5.06–7.97) for asthmatics,  $p < 0.0001$ ). These results closely reflect those of the whole groups so that baseline lung function was not the cause of the differences in response.

In all but two asthmatic patients, whenever the exercise challenge was positive ( $>6.0\%$  fall in  $FEV_1$ ), there was hyperreactivity to AMP at a concentration below the mean + 2SD of the asthmatic group (98.2 mg/ml). On the other hand, 12 of the 49 asthmatic patients with a positive AMP challenge failed to respond to exercise. The mean  $PC_{20}$  of these 12 asthmatic children compared with that of the 39 children positive to exercise was not significantly different either for methacholine or AMP. These observations are reflected in the greater intersection point of AMP (98%) compared with exercise (84%) in distinguishing asthma from controls (table 2). The reason for this is unclear but could be related to the multiple factors known to influence the response of asthmatic patients to exercise at any one time.<sup>33</sup>

Adenosine challenge has clear advantages over exercise in that it can be performed in patients of all ages including the very young and older subjects in whom an exercise challenge would be undesirable. Moreover, the present study shows adenosine to be as specific as exercise in differentiating asthma from paediatric COPD, but more sensitive than exercise in revealing bronchial hyperreactivity in asthmatic subjects. Adenosine challenge should therefore be a very useful tool in the differential diagnosis of asthma and COPD in patients of all ages in whom the diagnosis is clinically uncertain.

In conclusion, our control group was insensitive to AMP, exercise and low concentrations of methacholine. Asthmatic subjects were responsive to all three challenges while patients with paediatric COPD mainly responded to methacholine. These findings suggest a final common pathway of hyperreactivity to methacholine in all types of chronic lung disease in children, including asthma, while a more specific and possibly mast cell-related pathway may exist only in asthmatic subjects. Adenosine, like exercise, is of much more value than methacholine in the differentiation of asthma from COPD in children. These inhalation challenges, unlike exercise, are suitable for virtually all age groups and seem to be a useful tool in the differential

# diagnosis of asthma in children and young people.

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