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Original Article

Methacholine, cold air and exercise challenge tests in the diagnosis of bronchial responsiveness at school age: A follow-up study from birth to school age

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Abstract

Background: The aim of the present study was to compare three bronchial challenge tests for assessing bronchial hyperresponsiveness (BHR) in twin pairs followed up from birth to school age.

Methods: We studied three different bronchial challenge tests (methacholine inhalation challenge, cold air inhalation challenge and exercise–bronchodilation tests) at school age in 29 children born at or before 38 weeks gestation (median 35 weeks gestation) from multiple pregnancies. The children had been followed up from birth and were examined at the age of 7–15 years (median age 10 years).

Results: Bronchial hyperresponsiveness was found in 28–34% of children when these tests were analyzed separately. Eight children (28%) were exercise responders; two of them and three additional children were bronchodilator responders. Thus, 11 children (38%) had a pathologic result in the exercise–bronchodilation test. Ten children (34%) responded to cold air and nine children (31%) responded to methacholine inhalation. At least one test was pathologic in 18 children (62%), but only two children (7%) responded in all three challenges. A positive result in the exercise–bronchodilation test was associated with cold air reactivity, but not with methacholine reactivity. The exercise and cold air tests detected predominantly the same children. No differences were found in bronchial challenge test results between children who, at birth, were appropriately grown and those who had intrauterine growth retardation.

Conclusions: Bronchial hyperresponsiveness was common (up to 62%) at school age in children born as moderately preterm. The outdoor exercise–bronchodilation test found 61% of all BHR cases. Bronchial hyperresponsiveness was not associated with intrauterine growth status. The most sensitive test was the cold air inhalation challenge and a good agreement was seen between this test and the exercise challenge outdoors.

Key words: bronchial responsiveness, cold air, exercise test, intrauterine growth retardation, methacholine.

Introduction

Bronchial challenge tests offer an objective way to study airway reactivity allowing assessment of the presence and degree of bronchial hyperresponsiveness (BHR) and, finally, the diagnosis of reactive airway disease.

In bronchial challenge tests adaptable to children, the changes in air flow are usually demonstrated by peak expiratory flow (PEF) measurements,[1] although forced expiratory volume in 1 s (FEV_1) is the standard in adolescents and adults.[1] Methacholine or histamine,[2] cold air[3] and exercise[4] have been the most often used triggers in challenges. Free running outdoors mimics the everyday life of children and, in both clinical and epidemiologic settings, it has been sufficiently sensitive and specific for children over 5 years of age.[5] We have followed up from birth to school age a cohort of 67 non-asthmatic children

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from multiple pregnancies. Their basic lung function, cold air challenge and methacholine challenge results have been published recently.[6–8]

The aim of the present study was to evaluate exercise challenge by free running outdoors, for assessing bronchial responsiveness in twin pairs followed up from birth to school age. A bronchodilation test was performed after exercise challenge in all cases, irrespective of the test result. In order to test the hypothesis that intrauterine growth retardation (IUGR) is associated with 'intrauterine programming of pulmonary function abnormality in later life', as suggested previously by Barker *et al.*,[9] the responses in the exercise–bronchodilation tests were evaluated in relation to intrauterine growth. In addition, the study design allowed the comparison of these responses with responses in two other challenges, namely methacholine and cold air challenges.

METHODS

Subjects

Initially, 67 children from multiple pregnancies who were born at or before 38 weeks gestation over the period 1978–1986 were enrolled in the study.[6]

The methacholine inhalation challenge (MIC) test was performed in all 67 children[8] and the cold air challenge was performed in 63 children, as described in more detail elsewhere.[7] The provocative dose inducing a 20% or more fall in PEF (PD_{20}) in the MIC test was below 4900 μg in 23 children, including five twin pairs with a PD_{20} < 4900 μg in both children. This cumulative dose has been suggested for screening of increased bronchial reactivity.[10] These 23 reactive children and their 13 non-reactive counterparts were requested to perform the exercise test. Twenty-nine children (13 pairs of twins and one set of triplets) finally attended the test and they form the present study group.

The children in the final study had typical prematurity associated diseases with no chronic lung problems either during the neonatal period or later. During the neonatal period, four children (14%) had respiratory distress syndrome, seven (24%) needed ventilator therapy and 16 (55%) needed supplemental oxygen (median 3.5 days; range 1–138 days). None of the children had used bronchodilators or any anti-inflammatory medication during the 3 months preceding the study. The children had to have been free of respiratory infection for over 1 month before the pulmonary function tests (PFT) and bronchial challenges were scheduled.

Definition of intrauterine growth

Based on growth status at birth, the study children were classified as appropriate for gestational age (AGA) or IUGR. By using Finnish gender- and gestational age-specific fetal growth charts,[11] the AGA was defined as the presence of the standardized birth weight between – 2SD and + 2SD. As described earlier, IUGR was defined as the standardized birth weight being below – 2SD or the twin-pair birth weight difference being more than 1.3SD.[6] Triplets consisted of two IUGR children and one AGA child; thus, they formed two IUGR/AGA pairs and one IUGR/IUGR pair. In total, the 29 children formed 12 discordant (IUGR/AGA) pairs and, in addition, two pairs had AGA/AGA and two pairs had IUGR/IUGR constellations.

Pulmonary function testing

Baseline lung function was studied by the 2200 Computerized Pulmonary Function Laboratory (Sensor Medics, Yorba Linda, CA, USA). Forced expiratory flow rates, including PEF, FEV_1 and forced mid-expiratory flow ($FEF_{25–75}$) were measured by the flow volume spirometer (FVS). In addition, PEF was measured by Wright's peak flow meter (WPEF; Airmed, Harlow, England). Lung functions were measured according to the recommendations of the American Thoracic Society.[12]

Baseline lung functions were expressed as percentages from the height-related reference values (% predicted).[13] The lower limit of normality was defined as 75% for WPEF, as well as for PEF and FEV_1 in the FVS. The lower limit of normality for $FEF_{25–75}$ was 65%.[14]

Bronchial challenge tests

Each of the three following challenge tests was performed on a different day to avoid refractoriness of the airways.[15]

Methacholine inhalation challenge test

The MIC test was performed by a method allowing the estimation of the degree of BHR. Drug delivery took place via a Spira electro 2 dosimeter (Respiratory Care Center, Hämeenlinna, Finland). In this dosimeter, the total, cumulative amount of methacholine inhaled by the patient can be calculated.[10] Before each MIC test, three separate WPEF values were measured; the best value was recorded for further analysis. The cumulative dose was increased until WPEF fell by 20% or more

(PD_{20}; provocative dose), the highest cumulative dose was 4900 μg ($PD_{20} = 4900$ μg). An increased responsiveness was considered to be present if PD_{20} by WPEF was less than 1000 μg, being found in nine of 29 children (31%), and these children were classified as MIC responders in the present study.

Cold air inhalation challenge test

The isocapnic hyperventilation of cold air challenge was performed at room temperature, as described elsewhere.[7] Before the challenge, three technically acceptable baseline flow–volume curves were obtained using a pneumotachograph spirometer (Medikro 909; Medikro, Kuopio, Finland). Thereafter, air containing water less than 1.75 mg/L cooled in the heat exchanger (Jaeger RHES; Erich Jaeger GmbH & CoKG, Wurzberg, Germany) was hyperventilated for 4 min through a mouthpiece. By using the highest FEV_1, the target minute ventilation was set to $FEV_1 \times 25$. The temperature of the inspired air was monitored continuously (GTH 1200 Digital Thermometer; Greisninger Electronic, Regenstauf, Germany). The mean temperature was –15.0°C (range –11.9 to –16.4°C) during hyperventilation. At least three technically satisfactory maximal flow–volume curves were obtained at 3, 5 and 10 min after cold air hyperventilation. The best of the three FEV_1 values at each time was included in further analyses. The maximal percentage fall in FEV_1, calculated from the highest prechallenge and the lowest technically acceptable post-challenge FEV_1, was used to express the response and a fall of 9% or more was considered as pathologic.[3,16] According to this limit, 10 of 29 children (34%) were classified as cold responders.

Exercise challenge test

Eight minutes of outdoor free running at a heart rate > 170 b.p.m. (approximately 85% of predicted maximum) constituted the exercise challenge and was performed during the cold season, from August 1993 to March 1994. Heart rate was monitored by telemetry (Polar Sport tester; Polar Elektro, Kempele, Finland) at 15 s intervals. Both WPEF and FEV_1 values were monitored. The WPEF was measured before and immediately after the test and 5, 10, 15, and 20 min later; FEV_1 was measured by dynamic spirometry (Vitalograph dry spirometer; Vitalograph, Buckingham, UK) before and immediately after the test and 10 and 20 min later. The childrens' lungs were auscultated before and 5 and 15 min after the exercise. Symptoms and signs, if present, and auscultatory findings were recorded. The median outdoor temperature during the exercise was –1°C (range –12°C to +10°C). A positive exercise test result was defined as a 15% or greater fall in WPEF or FEV_1 at any time after the exercise calculated from the baseline pre-exercise values.[17]

Bronchodilation test

Twenty-five minutes after exercise, all children received salbutamol (0.15 mg/kg) via a Spira nebulizer (Spira Oy, Hämeenlinna, Finland). The WPEF and FEV_1 were measured 15 min after bronchodilator inhalation. A positive bronchodilation test result was defined as a 15% or greater rise in WPEF or FEV_1 after bronchodilator inhalation calculated from the baseline pre-exercise values.[18]

In the present study, children with positive responses in the exercise test were termed as exercise responders and children positive in the bronchodilation test were classified as bronchodilator responders. The children with positive responses either in the exercise test or in the bronchodilation test were called exercise–bronchodilation responders.

Ethics

The parents of the children provided informed consent for the participation of the children in the present study. The study was approved by the Research Ethics Committee of Kuopio University Hospital.

Statistical analysis

Data were analyzed using SPSS for Windows 6.1 software (SPSS, Chicago, IL, USA). The Mann–Whitney *U*-test was used for non-normally distributed continuous variables and Student's *t*-test for normally distributed continuous variables to examine the differences between IUGR and AGA children and between cold, methacholine, exercise or bronchodilation responders and non-responders. The non-continuous variables were tested with Fischer's exact test. The McNemar test was applied to evaluate the association between BHR and IUGR within discordant twin pairs.

The Pearson correlation coefficients between baseline FEV_1 and WPEF, as well as between exercise-induced falls of FEV_1 and WPEF, were calculated.

Cohen's κ values were calculated to evaluate the agreement between the results yielded by different

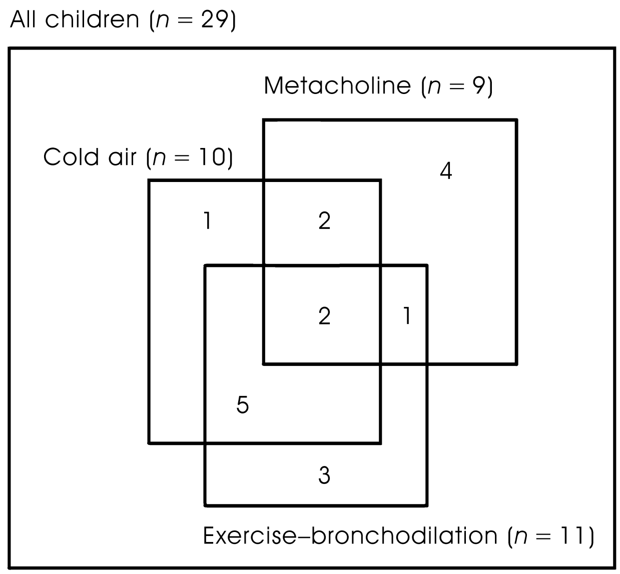

Fig. 1 Distributions of the nine metacholine, 10 cold air and 11 exercise–bronchodilation responders between the different bronchial challenge tests.

bronchial challenges. The closer the value is to 1, the better the agreement. A value below 0.4 signifies no agreement.

RESULTS

Three challenge tests (i.e. cold air inhalation, methacholine inhalation and exercise test) were performed in 29 children. Eighteen children (62%) had at least one positive response in these tests, 10 children (34%) had two or three positive responses, but only two children (7%) responded in all three challenges. The number of responders in the different challenge tests are given in Fig. 1 and the original results of the challenge tests are given in Table 1. The responses in the exercise and/or the exercise–bronchodilation test were associated with cold air responses, but not with methacholine-induced responses. The κ value was 0.68 between cold air and exercise tests ($P < 0.001$) and 0.48 between cold air and exercise–bronchodilation tests ($P = 0.01$). The κ values between all other challenge tests were less than 0.14.

Eleven responders (38%) were found in the exercise–bronchodilation test. Eight children (28%) were exercise responders; two of them and three additional children were bronchodilator responders (Table 2). In these children, WPEF was diagnostic in eight cases and FEV_1 was diagnostic in six cases, but both methods were diagnostic in only three cases. Thus, there was no agreement between WPEF and FEV_1 changes in the exercise–bronchodilation test (κ 0.25; $P > 0.05$).

There was a significant correlation between baseline FEV_1 and WPEF values ($r = 0.54$; $r^2 = 0.29$; $P = 0.003$), as well as between exercise-induced falls in FEV_1 and WPEF values ($r = 0.60$; $r^2 = 0.36$; $P = 0.001$). No correlation was found between FEV_1 and WPEF in post-bronchodilation measurements ($r = 0.12$; $r^2 = 0.01$; $P = 0.52$). There was no significant association between baseline lung function and exercise, bronchodilation and exercise–bronchodilation test results (Table 2).

When the exercise challenge test results were compared within the 12 discordant (IUGR/AGA) twin pairs, the median WPEF falls were 9.2% (IUGR) and 5.7% (AGA; NS) and the median FEV_1 falls were 7.8% (IUGR) and 1.2% (AGA; NS). Similarly, there were no significant differences in the number of responders between the IUGR and AGA groups (Table 3).

To sum up, BHR (i.e. is at least one pathologic result in the three challenges) was diagnosed in 11 of 14 IUGR children (79%) and in seven of 15 AGA children (47%; NS). The respective proportions for two pathologic results were 50 and 20% (NS). Similarly, no differences were seen between IUGR and AGA children if exercise responders and cold responders were analyzed as a combined group (data not shown).

DISCUSSION

We evaluated the frequency of BHR by three different challenge tests (i.e. MIC, cold air inhalation challenge and exercise–bronchodilation tests) at school age in children born prematurely from multiple pregnancies. None of the children had clinically defined asthma or was on continuous medication for asthma. By single challenges, BHR was found in 28–38% of the study children. The figures are high compared with population data (e.g. in our area 4–5% of children have asthma and an additional 4–5% have asthma-like symptoms).[5] Based on the same population data, BHR assessed by exercise challenge was present in 35% of children with asthma, but in only 3% of children with no asthma or asthma-like symptoms.[19] In contrast, the BHR figures of the present study are within the limits of the European Community Respiratory Health Survey (ECRHS) study, in which BHR was measured by the MIC test and varied from 3 to 27% in the child populations of different countries.[20] This result, like our present observations, stresses the large variation in BHR between different studies and between

Table 1 Original values in the three challenge tests

Case no.	Exercise–bronchodilation				Cold air	Methacholine
	Changes in exercise*		Changes after bronchodilation*		ΔFEV$_1$*	PD$_{20}$†
	PEF	FEV$_1$	PEF	FEV$_1$		(μmol/L)
1	–66.7‡	–65.2‡	–19.4	–17.4	–21.3‡	320‡
2	–32.3‡	–19.5‡	–1.4	12.2	–12.6‡	2500
3	–28.6‡	–2.9	19.1‡	11.8	–5.0	990‡
4	–20.8‡	–10.3	–3.8	13.8	–9.8‡	380‡
5	–18.8‡	–18.6‡	1.6	4.7	–11.5‡	4900
6	–17.0‡	3.6	19.2‡	10.7	–10.0‡	4900
7	–14.0	0	–2.0	9.7	–7.3	350‡
8	–12.1	–11.6	–6.1	2.3	–11.7‡	2300
9	–10.9	6.3	4.4	15.6‡	–6.1	4900
10	9.4	–16.0‡	–6.3	4.0	–9.9‡	3600
11	–9.2	5.9	0	5.9	–1.70	540‡
12	–8.7	–7.7	4.4	0	–6.10	620‡
13	–6.7	7.7	–2.2	11.5	–15.6‡	280‡
14	–6.5	–11.8	21.7‡	0	–5.3	2500
15	–6.3	6.7	12.6	13.3	–5.0	120‡
16	–3.8	–33.3‡	3.7	–3.3	–24.3‡	3800
17	–2.3	–5.7	9.3	11.4	–9.7‡	450‡
18	16.0	–10.0	20.0‡	–10.0	6.3	4600
19	–11.8	–4.7	–5.9	0	–5.3	4900
20	–10.9	–2.4	9.1	9.5	–1.0	2200
21	–9.1	–5.7	1.8	2.9	3.2	4900
22	–8.3	13.2	2.6	5.3	1.9	3400
23	–7.4	6.9	0	10.3	–1.9	3200
24	–6.4	5.6	4.3	5.6	–5.0	4900
25	–3.3	–4.8	11.7	2.4	–6.4	4900
26	–3.0	8.3	6.1	13.9	–8.8	4900
27	–1.6	–2.5	9.7	5.0	–8.4	4900
28	–1.2	0	0	4.1	–4.7	4900
29	10.2	–10.0	14.3	–10.0	–3.5	4900

*Decreases and increases are expressed as percentage changes from baseline values.
†The PD$_{20}$ is the cumulative provocative dose causing a 20% fall in Wright's peak flow meter.
Cases 1–18 are defined as bronchial hyperresponders.
‡Subnormal values.
PEF, peak expiratory flow; FEV$_1$, forced expiratory volume in 1 s.

Table 2 Association between baseline lung function and exercise and bronchodilation test results

Test/category	Subnormal results in baseline lung function			
	WPEF < 75% (*n* = 10)	FEV$_1$ < 75% (*n* = 4)	FEF$_{25–75}$ < 65% (*n* = 3)	At least one subnormal result (*n* = 12)
Exercise test				
Responders (*n* = 8)	3	0	0	3 (38%)
Non-responders (*n* = 21)	7	4	3	9 (43%)
Bronchodilation test				
Responders (*n* = 5)	2	1	0	2 (40%)
Non-responders (*n* = 24)	8	3	3	10 (42%)
Exercise–bronchodilation test				
Responders (*n* = 11)	5	2	2	5 (46%)
Non-responders (*n* = 18)	5	2	1	7 (39%)

Data show the number of subjects in each group.
WPEF, Wright's peak flow meter; FEV$_1$, forced expiratory volume in 1 s; FEF$_{25–75}$, forced mid-expiratory flow.

Table 3 Responses in exercise and bronchodilation tests and in the combined exercise–bronchodilation test in intrauterine growth-retarded and appropriate for gestational age children

Challenge test	Pathologic result	IUGR children ($n = 14$)	P	AGA children ($n = 15$)
Exercise test	8/29	6 (43%)	0.11	2 (13%)
Bronchodilation test	5/29	3 (21%)	0.65	2 (13%)
Exercise–bronchodilation test	11/29	7 (50%)	0.21	4 (27%)

IUGR, intrauterine growth retardation; AGA, appropriate for gestational age.

different populations, being highly dependent on study design and challenge tests used.

The design of the present study allowed us to evaluate the effects of intrauterine growth on subsequent lung function. Our present combined data revealed no association between BHR and IUGR. At the population level, BHR has been more common in low-birth weight than in normal birth weight children, the difference having continued throughout childhood.[21] Previously, we have observed that the parameters reflecting lung volumes had no correlation with the intrauterine growth pattern but, in agreement with the hypothesis of Barker *et al.*,[9] parameters reflecting airway flow had such a correlation.[6] However, in disagreement with the hypothesis, flow changes in cold air[7] or metacholine challenge[8] did not show any correlation with IUGR. In the present study, no association was found between IUGR and BHR measured by exercise challenge and/or bronchodilator responses. Thus, our combined results speak against the hypothesis of Barker *et al.*,[9] that low expiratory flow values are the result of small airway calibers as a consequence of restricted intrauterine growth.

All the children included in the present study performed three different challenge tests and, in order to avoid refractoriness of the airways, the tests were performed on different days. Our results suggest that different bronchial challenge tests reflect different types of bronchial responsiveness. The exercise challenge and cold air inhalation tests tended to identify the same children, as was demonstrated by the good agreement in the κ statistics. This agreement is not surprising because both tests are non-specific, acting in a similar way; both cold air inhalation and hyperventilation lead to cooling of the airways. When methacholine acts directly and predominantly on airway smooth muscle, cold and/or dry air exposure and hyperventilation activate cellular and neurogenic mechanisms, leading indirectly to contraction of the smooth muscle.[22] Thus, cold and dry air inhalation in the laboratory and exercise outdoors in cold weather as well probably measure the same BHR mechanism. In northern countries, exercise outdoors in cold weather is a part of normal life and, thus, free running tests outdoors simulate the everyday life of children. In addition, cold air and exercise challenges may be ethically more acceptable to children and their parents than bronchoprovocation tests using chemical stimuli.[23] Cold and dry air inhalation challenges in the laboratory are easier to quantify and standardize than are exercise challenges outdoors.

The correlations between FEV_1 and WPEF changes in the exercise–bronchodilation test were only weak to moderate; r^2 values were only 1–38%, in accordance with a recent study in primary health care settings.[24] It seems likely that FEV_1 and WPEF reflect different responses to bronchoconstrictors or bronchodilators; FEV_1 reflects changes in small peripheral airways, whereas WPEF reflects changes in large central airways.[25,26] Both WPEF and FEV_1 found reactive cases equally often, stressing the need to evaluate both central and peripheral airway changes in bronchial challenge tests. The bronchodilation test was positive only rarely, in 17% of cases. This was an expected result in the present cohort, because the children were non-symptomatic with no previous evidence of asthma.

A significant bronchial response to cold air or exercise is a very specific indicator of asthma.[3,16,27–29] The presence of inflammatory cells within the airways capable of releasing bronchoconstrictive mediators may be a prerequisite for responsiveness to cold air, dry air or exercise.[30] Thus, it is possible that the children responsive to cold air and/or to exercise in the present study were actually asthmatics with occasional, mild symptoms or a poor appreciation of the symptoms of asthma. By combining all challenge data, as many as 62% of children had some evidence on increased bronchial reactivity. This figure is relatively high, but not unexpected, because different challenge tests reflect different types of BHR and, so, the number of findings is related to the number of available tests.[22]

In conclusion, BHR was relatively common. We detected it at school age in up to 62% of children born prematurely, although none of the children was symptomatic for asthma. No significant association was seen between BHR and IUGR.

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