Bronchial hyperresponsiveness decreases through childhood

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Summary
Limited knowledge exists about development of bronchial hyperresponsiveness (BHR) through adolescence. We aimed to assess changes in and risk factors for BHR in adolescence.

From a Norwegian birth cohort of 517 subjects underwent clinical examinations, structured interviews and methacholine challenges at age 10 and 16. BHR was divided into four categories: no BHR (cumulative methacholine dose required to reduce FEV1 by 20% (PD20) >16 μmol), borderline BHR (PD20 ≤16 and >8 μmol), mild to moderate BHR (PD20 >16 and >1 μmol), and severe BHR (PD20 ≤1 μmol). Logistic regression analysis was used to assess risk factors and possible confounders.

The number of children with PD20 ≤8 decreased from 172 (33%) to 79 (15%) from age 10 to 16 (p < 0.001). Most children (n = 295, 57%) remained in the same BHR category from age 10 to 16 (50% with no BHR), whereas the majority 182 (82%) of the 222 children who changed BHR category, had decreased severity at age 16. PD20 ≤8 at age 10 was the major risk factor for PD20 ≤8 6 years later (odds ratio 6.3), without significant confounding effect (>25% change) of gender, active rhinitis, active asthma, height, FEV1/FVC, or allergic sensitization.

BHR decreased overall in severity through adolescence, was stable for the majority of children and only a minority (8%) had increased BHR from age 10 to 16. Mild to moderate and severe BHR at age 10 were major risk factors for PD20 ≤8 at 16 years and not modified by asthma or body size.

Introduction
Bronchial hyperresponsiveness (BHR) is associated with underlying chronic inflammation of the airways and characterized by episodes of excessive airway narrowing in response to provoking stimuli. Methacholine is a direct measure of BHR through stimulation of the bronchial smooth muscle. Most individuals may react with bronchial

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constriction to inhaled methacholine provided a sufficiently high dose, and a test is considered positive if a reduction in forced expiratory volume in the first second (FEV1) ≥ 20% occurs at any provocation dose (PD20) up to a pre-defined level. However, the PD20 cut-off levels for defining BHR varies. In addition to aid the diagnosis of asthma, BHR assessed by direct provocation methods can be a prognostic marker of asthma persistence and severity, and is used to monitor long term treatment effects. Asymptomatic BHR has been associated with development of asthma in children while others find no such association. BHR is associated with age, reported to decrease through childhood and to be a risk factor for BHR later in life. Its been proposed that gender, lung function, height and allergic sensitization may influence the development of BHR.

The natural development of BHR through puberty must be viewed in the context of its association with airways disease and poorly understood underlying mechanisms.

The aims of the present study were therefore to determine how BHR assessed by methacholine challenge at 10 years of age was related to the BHR at age 16.

Subjects and methods

Design

The present study reports results from the 10- and 16-year follow-up of children in the Environment and Childhood Asthma study (ECA) in Oslo. This prospective birth cohort established in Oslo, Norway in 1992 included 3754 healthy newborn children of whom 803 had lung function measured at birth and 306 cases (children with recurrent bronchial obstruction by two years) and 306 controls (children born closest in time to the cases but without bronchial obstruction) were included for a two-year investigation.

The 10-year and 16-year follow-up studies (completed in 2005 and 2009, respectively) recruited the 1215 children who had lung function measured at birth and/or a clinical examination at two years of age (Figs. 1 and 2), and thus represent an asthma-enriched general population.

The 10- and 16-year follow-up studies included structured parental (10 years) and study subject (16 years) interviews, skin prick tests (SPT), measures of lung function and fractional exhaled nitric oxide (FeNO), as well as methacholine provocation tests. All investigations required at least 4 weeks free from symptoms of a respiratory tract infection prior to the follow-up visits and no use of leukotriene antagonists for at least 72 h and short/long acting b2 agonists for at least 12/48 h respectively prior to the methacholine tests.

The study was approved by the Regional Medical Ethics Comity and the Norwegian Data Inspectorate, and registered in the Norwegian Bio-Bank. Written informed consent was obtained from all subjects’ parents at both investigations.

Subjects

At the 10-year follow-up study 1019 (84%) children attended the investigation, whereas 550 (45%) attended the 16-year study. However, 53% of the 10-year attendees were examined also at 16 years of age. The present study comprised those 517 children (52% boys) who successfully completed the methacholine challenges at 10 years of age (mean 10.7, range 9.0–12.5) and 16 years of age (16.7, 15.7–17.5). Demographic data are given in Table 1.

Methods

Except for the measures of BHR results from all tests are reported from the 10-year follow-up investigation only.

Structured interviews were paediatrician-guided towards the parents and included translated relevant ISAAC questions of airway symptoms in addition to detailed questions regarding lifestyle, disease and environmental exposures (for details see supplement).

Definitions based upon the structured interview;

Active asthma: at least two out of three criteria fulfilled: (1) doctor’s diagnosis of asthma, (2) the presence of asthma symptoms during the last 12 months, (3) use of asthma medication during the last 12 months.

Figure 1 Flow chart of the children included in the “Environment and Childhood asthma study in Oslo”. The overall attendance rate at the 10 year follow-up was 84% while it was 45% at the 16 year follow-up.

Figure 2 Box plot of natural logarithm of methacholine dose–response slope (LnDRS) at the age of 10 and 16 years. n = 517. The boxes show median values with 25 and 75% percentiles, Whiskers show minimum to maximum values.
Active rhinitis: at least two out of three criteria fulfilled: (1) doctor’s diagnosis of rhinitis, (2) present symptoms of rhinitis during the last 12 months, (3) treatment for eye nose or allergy symptoms during the last 12 months.

Lung function was measured by forced expiratory volume loops according to European guidelines at 10 and 16 years of age using Sensormedics V-max (Sensormedics Diagnostics, Yorba Linda, CA, USA) spirometer. Forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) are reported as raw values and % predicted and FEV1/FVC ratio (FEV1/FVC) as raw values only.

Fractional exhaled nitric oxide was measured with an Eco Medics CLD 88 Exhalyzer® (Eco Medics, AG 8635 Duernent, Switzerland) according to international recommendations.

Allergic sensitization was assessed by skin prick tests, Soluprick® (ALK Albello, Denmark), to 15 common inhalant and food allergens according to European guidelines. Allergic sensitization was considered positive with a wheal diameter of at least 3 mm (larger than the negative control) to at least one common inhalant or food allergen (see supplement for further details).

The exercise test was performed on a treadmill, 2–7 days after the methacholine challenge. The children ran for 6–8 min aiming at 95% of estimated maximum heart rate during the last 4 min. A positive exercise test was used as a fall in FEV1 ≥10% compared to baseline within 3–20 min after exercise.

A Methacholine challenge was used to assess BHR according to ATS guidelines using a SPIRA® dosimeter (Spira Respiratory Care Center Ltd., Hemeenlinna, Finland). Dose response slope (DRS) was calculated as maximal % reduction in FEV1cumulative dose methacholine administered and PD20 as the estimated cumulative methacholine dose required to reduce FEV1 by 20% compared to baseline. Based upon previously used cut-off values, we used the main classification of no BHR or positive BHR as a PD20 >16 or ≤16 μmol, respectively.

Severity of BHR was classified into borderline BHR as PD20 ≤16 and >8 μmol, mild to moderate BHR as PD20 ≤8 and >1 μmol and severe BHR was defined as PD20 ≤1 μmol. (see supplement for further details).

Outcomes, possible risk factors and explanatory variables

The main outcome was a PD20 ≤ 8 μmol methacholine, classified as mild to moderate or severe BHR.

The main risk factors were; no BHR or positive BHR, as well as the severity categories (no, borderline, mild to moderate and severe BHR, respectively).

The main explanatory variables were active asthma, active rhinitis, gender, allergic sensitization (as well to a positive SPT to D. farinae and/or D. pteronnynsinus), height, exercise test, use of inhaled corticosteroids (ICS) and lung function.

Statistical analyses

Continuous variables are presented as mean (SD or range when applicable), and categorical variables as count and percentages. Student’s two-sample t-test was applied for normally distributed variables and Wilcoxon–Mann–Whitney for non-normally distributed variables. For changes within groups paired student’s t-test was applied. For categorical variables Pearson’s Chi-Square for independent samples and McNemar’s test for paired samples. In the multivariate analysis we applied logistic regression for binary outcomes. The procedure used for the multivariate regression models was Hosmer’s manually backward elimination technique. Interactions and model assumptions were inspected in the final model and possible confounding effects were assessed Nagelkerke R² is given to describe the variation for the outcomes explained by the risk factors.

Statistical Package for Social Sciences (SPSS) 15.0 for Windows was used for statistical calculations. A p-value ≤ 0.05 was considered statistically significant.
Results

Demographics and data of personal and family history of allergic diseases, skin prick test and exercise test are given in Table 1 and results of lung function, DRS and FeNO are given in Table 1 supplement. The included 517 children (52% boys) were largely representative for the entire 10-year study group (Table 1), except for slightly, but significantly younger age and lower weight as well as being less often sensitized to allergens or reporting active rhinitis compared to the 501 non-included children.

Compared to the girls, the included boys were more often sensitized to allergens, had more asthma (active and ever), used more ICS, and had lower FEV₁/FVC ratio and higher DRS to methacholine at 10 years of age and taller at the age of 16 years.

BHR at 10 and 16 years

Fifty per cent of all subjects (n = 256) had positive BHR on at least one occasion (Fig. 3a) and the number of children with positive BHR decreased from 224 (43%) to 118 (23%) (p < 0.001) from 10 to 16 years of age, respectively. The corresponding number of children with a PD₂₀ ≤ 8 µmol methacholine decreased from 172 (33%) to 79 (15%) (p < 0.001).

The majority of subjects (n = 295, 57%) remained in the same BHR category from the 10–16 years investigation (Fig. 3b), whereas most (82%) of the 222 subjects who changed BHR category had decreased severity of BHR at the 16-years investigation; 138 lost their BHR altogether (Fig. 3b).

Of the 39 children with increased bronchial responsiveness, 22 became hyperresponsive whilst 17 became increasingly hyperresponsive. Significantly more girls than boys (10.5 vs 5.6%) (p < 0.05) changed towards more severe BHR, while more boys than girls (39.0 vs 30.6%) (p < 0.05) changed to a less severe BHR category.

At 10 years of age boys had significantly more often severe BHR than girls (p = 0.002).

Risk of BHR at 16 years of age

In bivariate analysis PD₂₀ ≤ 8 µmol at the age of 10 years was a significant risk factor for PD₂₀ ≤ 8 µmol six years later (odds ratio (OR) 6.3 (3.7, 10.6)) for all children, explaining 17% of the variation of in BHR at 16 years of age, with ORs 9.9 (4.3, 22.6) and 4.5 (2.2, 9.1) for boys and girls respectively. The corresponding values for PD₂₀ ≤ 16 µmol at 10 years are given in the supplement.

In the multivariate analysis including all BHR severity categories and explanatory variables only mild to moderate BHR, severe BHR, gender and FEV₁/FVC remained significant risk factors at 10 years of age for PD₂₀ ≤ 8 µmol at 16 years of age (Fig. 4). The confounding effect of the covariates was negligible (<25%) in bivariate and multivariate analysis (Table 3).

Stratified by gender, mild to moderate or severe BHR at the age of 10 years were significant risk factors for mild to moderate or severe BHR at the age of 16 years, explaining 23% of the variation of BHR in boys and 12% in girls. The highest risk for mild to moderate or severe BHR at 16 years was found in boys with severe BHR at 10 years (Table 2).

Table 4 demonstrates cross sectional and longitudinal bivariate associations between age, height or lung function and PD₂₀ ≤ 8 µmol.

The current use of ICS was not associated with rate of improvement in BHR severity category from 10 to 16 years (81% vs 86% among children not using ICS (p = 0.54)).

Discussion

The present study demonstrated that the severity of BHR overall decreased from 10 to 16 years of age. Although 57% of the children were classified in the same BHR category at both investigations, 82% of those who changed category, had decreased severity. Positive BHR, mild to moderate BHR and severe BHR at the age of 10 years increased the risk of mild to
moderate or severe BHR six years later while borderline BHR did not. Active asthma, active rhinitis, gender, height, lung function and allergic sensitization at the age of 10 years did not confound these associations. Reduced forced expiratory flow values, were associated with current and future BHR, whereas height or FVC were not.

**Bronchial responsiveness at the age of 10 and 16 years**

The present study confirms that BHR decreases from childhood to adolescents, as has been shown previously in patient populations\(^{11,14}\) and general populations.\(^5,9,12\)

Our findings of individual changes in BHR over a time period of six years demonstrated that BHR was stable in most subjects, but was reduced in more than 80% of those changing BHR category. This is supported by one other study reporting that 70% of those changing BHR status lost their BHR.\(^9\) This supports the view that although BHR decreases through childhood, it is a dynamic process that can vary over time\(^9\) and may change with various environmental exposures such as tobacco smoke, pollens, temperature and airway infection.

The only gender difference in BHR was a male predominance of severe BHR at the age of 10 years (11% vs. 4%, \(p = 0.004\)), but significantly more girls than boys increased their BHR from the age of 10 to the age of 16 years. This may reflect the start of a shift towards more severe BHR in post pubertal females as described by Tantisira et al.\(^{14}\) This gender shift is further strengthened by the more pronounced decrease in BHR in boys between the two investigations in the present study. In contrast to Tantisira et al.\(^{14}\) Paoletti et al.\(^{30}\) found more BHR in childhood girls and in childhood-adolescent boys while other studies have failed to discover gender difference in BHR.\(^{11}\) If the trend of girls becoming more and boys becoming less responsive continues and the follow-up was conducted at a later stage, we might have experienced a gender difference in the BHR categories similar to the findings in a recently published study in mice demonstrating a gender related age difference in BHR with a male preponderance for BHR early in life, and female preponderance later in life.\(^31\)

**Risk of BHR at the age of 16**

In the present study the different definitions of BHR (except borderline BHR) at 10 years of age significantly increased the risk of PD\(_{20} \leq 8 \mu mol\) 6 years later confirming the results of Ulrik and Backer.\(^{12}\) and Roorda et al.\(^{13}\) reporting associations between logarithmic dose–response to histamine in childhood and BHR in adolescence and early adulthood.

Although allergic sensitization in general and to HDM in particular significantly increased the risk of BHR six years later in bivariate analyses, this was no longer significant after relevant adjustments in the present study. This reflects findings from other studies, where a positive skin prick test has been associated with BHR in some\(^{32,33}\) but not all studies.\(^{34}\) Allergic sensitization to HDM has as been linked to BHR in cross sectional\(^{22,33}\) and longitudinal studies\(^{12}\) and has been reported to be a risk factor in childhood for BHR in adolescence even after adjusting for asthma.\(^{15}\)

**BHR, lung function and body size**

There was no association (in cross sectional data) between height and mild to moderate or severe BHR, even with a height range of 47 cm and 50 cm, respectively. These findings agree with those reported by Ownby et al.\(^{35}\) Crude associations between height and BHR have been reported,\(^{16,36}\) whereas after adjusting for FVC or FEV\(_1\)/FVC such associations appear to be non-significant.\(^{36}\) Our data showed no significant association between height and mild to moderate or severe BHR and FVC while Peat et al.\(^{36}\) reported a small but significant effect of FVC (in cross sectional data among 1613 children aged 7–12 years). Measures of airway obstruction as FEV\(_1\) (% predicted and l/s) and FEV\(_1\)/FVC were associated with BHR six years later, similar to the findings of Ulrik and Backer\(^{12}\) but FEV\(_1\)/FVC was the stronger predictor and was used for the multivariate prediction model in the present study.

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**Figure 4** Final prediction model for mild to moderate or severe BHR (PD\(_{20} \leq 8 \mu mol\)) at 16 years of age adjusted for at active asthma, active rhinitis, gender, allergic sensitization, height, exercise test, use of inhaled corticosteroids and FEV\(_1)/FVC\) at the age of 10 years. Borderline bronchial hyperresponsiveness (BHR): PD\(_{20} >8\) and \(\leq 16 \mu mol\), mild to moderate BHR: PD\(_{20} >1\) and \(\leq 8 \mu mol\) and severe BHR: PD\(_{20} \leq 1 \mu mol\).

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**Table 2** The risk of having mild to moderate or severe bronchial hyperresponsiveness at the age of 16 years with different severity of bronchial responsiveness at the age of 10 years in 269 males and 248 females. The odds ratios are based on bivariate relationships, and no bronchial responsiveness (PD\(_{20} > 16 \mu mol\)) is the reference. The odds ratios are based on bivariate relationships, and no bronchial responsiveness (PD\(_{20} > 16 \mu mol\)) is the reference.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Risk factor</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Borderline BHR</td>
<td>1.8</td>
<td>0.3, 9.2</td>
</tr>
<tr>
<td>Male</td>
<td>Mild to moderate BHR</td>
<td>4.8</td>
<td>1.7, 13.4</td>
</tr>
<tr>
<td>Male</td>
<td>Severe BHR</td>
<td>50.7</td>
<td>16.3, 157.8</td>
</tr>
<tr>
<td>Female</td>
<td>Borderline BHR</td>
<td>0.9</td>
<td>0.2, 4.2</td>
</tr>
<tr>
<td>Female</td>
<td>Mild to moderate BHR</td>
<td>4.1</td>
<td>1.9, 8.8</td>
</tr>
<tr>
<td>Female</td>
<td>Severe BHR</td>
<td>6.5</td>
<td>1.6, 25.7</td>
</tr>
</tbody>
</table>

BHR: Bronchial Hyperresponsiveness, severe BHR: PD\(_{20} \leq 1 \mu mol\), mild to moderate BHR: PD\(_{20} \leq 8 >1-\mu mol\), borderline BHR: PD\(_{20} \leq 16>8 \mu mol\), OR: Odds Ratio, CI: Confidence Interval.
Table 3 The risk of severe to moderate or severe bronchial hyperresponsiveness at the age of 16 years with different categories of bronchial hyperresponsiveness at the age of 10 years and the confounding effect of active asthma, active rhinitis, gender, allergic sensitization and FEV1/FVC.

<table>
<thead>
<tr>
<th>Possible confounders</th>
<th>Severe BHR</th>
<th>Moderate to mild BHR</th>
<th>Borderline BHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude, OR (95% CI)</td>
<td>21.8 (9.9, 48.1)</td>
<td>4.1 (2.2, 7.5)</td>
<td>1.1 (0.4, 3.5)</td>
</tr>
<tr>
<td>Active asthma, OR (change)</td>
<td>17.9 (–18%)</td>
<td>3.8 (–7%)</td>
<td>1.1 (0%)</td>
</tr>
<tr>
<td>Active rhinitis, OR (change)</td>
<td>18.5 (–15%)</td>
<td>3.9 (–5%)</td>
<td>1.1 (0%)</td>
</tr>
<tr>
<td>Gender, OR (change)</td>
<td>25.2 (+16%)</td>
<td>4.2 (+2%)</td>
<td>1.2 (+9%)</td>
</tr>
<tr>
<td>Allergic sensitization, OR (change)</td>
<td>20.6 (–5%)</td>
<td>4.0 (–2%)</td>
<td>1.2 (+9%)</td>
</tr>
<tr>
<td>FEV1/FVC, OR (change)</td>
<td>18.2 (–16%)</td>
<td>3.9 (–5%)</td>
<td>1.1 (0%)</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA and AR, OR (change)</td>
<td>16.9 (–23%)</td>
<td>3.8 (–7%)</td>
<td>1.1 (0%)</td>
</tr>
<tr>
<td>AA, AR and gender, OR (change)</td>
<td>19.3 (–12%)</td>
<td>3.8 (–7%)</td>
<td>1.1 (0%)</td>
</tr>
<tr>
<td>AA, AR, gender, AS and FEV1/FVC, OR (change)</td>
<td>16.6 (–24%)</td>
<td>3.5 (–15%)</td>
<td>1.2 (15%)</td>
</tr>
</tbody>
</table>


There was no significant association between height and mild to moderate or severe BHR and height or FVC as well as no gender difference in BHR at 16 years of age (when boys were taller than girls) in the present study. This questions that the observed decrease in BHR is a result of somatic and lung growth as was suggested by Le Souef et al. leading to reduced constrictor-agent-dose to body-size ratio.

Rather, a progressive loss of compliance or increased stiffness has been observed in other human tissues. A similar development in the airway wall could increase the mechanical load on airway smooth muscle and reduce bronchial responsiveness with age. This has to our knowledge not been confirmed, but Wang et al. found no age related change in airway mucosal membrane stiffness in rabbits.

### Strengths and limitations

The prospective design and the size of the study population in the present study strengthen the validity of the results. However, with only two measurement points, the possibility for determining natural development in longitudinal analyses is limited. With a follow-up rate of only approximately 50%, there is a risk that children with respiratory problems may be over-represented in the last investigation. However, such a case would lead to a possible underestimation of the decrease in BHR and an over-estimation of the risk estimates compared to a non-selected general population. However, controlling for clinical characteristics at 10 years revealed no significant differences between those who did and did not attend the follow-up investigation. The attendees were in fact less often sensitized to any allergen and had less often active rhinitis at 10 years of age than those who did not attend the 16-year investigation. Thus, it is likely that the results can be applicable to an age-appropriate general population of children.

The definition of active asthma and active rhinitis in the present study was based upon a structured clinical interview and considered to be stricter than doctors diagnose or questionnaire reported symptoms alone. The requirement of at last two out of three commonly used criteria reduce the risk of over or under diagnosing asthma and rhinitis.

### Conclusion

The majority of children had the same level of BHR at the age of 10 and 16 years, whereas 80% of those changing...
category had reduced BHR. Although most categories of BHR at 10 years of age were significant risk factors for mild to moderate and sever BHR 6 years later, this was most pronounced for severe BHR and not significant for borderline BHR. Asthma, rhinitis, allergic sensitization and gender did not modify these associations and the reduced BHR is unrelated to changes in body size.

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Conflict of interest

The authors have no conflict of interest.

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Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.rmed.2011.09.013.

References


