

Is bronchodilator response in 2-yr-old children associated with asthma risk factors?

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Factors that might influence lung function bronchodilator response by 2 yr of age is largely unknown, thus we aimed to assess this in the 'Environment and Childhood asthma' (ECA) study in Oslo. A clinical investigation at mean age 26 months was attended by 516 (84%) children included in a nested case-control study [children with recurrent bronchial obstruction (rBO)] ($n = 265$) and controls without a history of lower respiratory disease ($n = 251$). Tidal lung function measures before and after inhaled nebulized salbutamol (bronchodilator response) (when clinically without BO) were obtained in 46%. Clinical characteristics and personal and family history of allergic/respiratory diseases (asthma risk factors) were ascertained by structured interview and clinical examination. Allergic sensitization was assessed by skin prick test/specific IgE antibody analyses to common allergens. Mean (95% CI) per cent change in time to reach peak flow/total expiratory time (t_{PTEF}/t_E) from before to after salbutamol was significantly larger in children with rBO [17.3 (9.4–25.3) than controls (–2.2 (–7.7 to 3.0)]. The bronchodilator response increased significantly ($p = 0.001$) with increasing number of asthma risk factors, but was not significantly associated with allergic sensitization, parental 'atopy', or maternal smoking alone. Children treated with inhaled corticosteroids had greater bronchodilator response than those treated with bronchodilators alone. Bronchodilator response in asymptomatic 2-yr-old children was most closely associated with the presence of rBO, but increasing number of asthma risk factors and treatment with inhaled corticosteroids were associated with increased bronchodilator response.

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Measurements of lung function in very young children have made it possible to perform epidemiological studies of natural growth and development of the lungs and airway, as well as to evaluate objectively development of airways and lung pathology.

Reduced lung function assessed by tidal breathing parameters has been implicated in wheezy illnesses in young children (1–3). Furthermore, measures of bronchial hyper-reactivity (BHR) and bronchodilator response have been assessed as the response to metacholine or histamine in infants, young children as well as adults by tidal flow volume (TFV) loops (4–8) or by the response to inhaled, nebulized bronchodilators (9, 10). However, no consensus has been reached

on standardization of a response test using TFV loops.

The characteristic relief of bronchoconstriction after inhalation of a β -2 agonist in asthma is well established also in young children, although obstructive airways disease (OAD) may have different aetiologies especially in children younger than school age (11). Improved lung function parameters have previously been demonstrated in young, asymptomatic children with recurrent wheeze (10) or asthma (4) after administration of salbutamol, even in the absence of detectable clinical symptoms or signs of bronchial obstruction (BO). Although the clinical implications of this are less well understood, we previously found a close correlation between response to

salbutamol (measured by tidal breathing parameters) and serum eosinophilic cationic protein in very young children. This led to the speculation that airway response to β -2 agonist (or bronchodilator response) in some way may be associated with eosinophil activation in infants (10). This has still not been clarified. To our knowledge, there are no published longitudinal studies assessing factors that may be associated with bronchodilator response in asymptomatic children under 3 yr.

Thus, the main objective of the present study was to determine whether allergic sensitization or identifiable asthma risk factors influenced the lung function response to an inhaled β -2 agonist in very young children (without present symptoms of BO) as measured by TFV loops.

Methods

Study design

The present study is part of the 'Environment and Childhood Asthma' (ECA) study in Oslo. Briefly, a cohort of 3754 newborns was established in Oslo during 15 months (from 1 January 1992) at the two main birth clinics, Ullevål and Aker Hospitals, and followed for 2 yr. The Regional Medical Ethics Committee had no objections to the study, and children were enrolled after written, informed consent was obtained from the parents. Inclusion criteria were: permanent Oslo address, minimum birth-weight 2000 g, absence of illness likely to impair respiration (severe respiratory, cardiovascular, neuromuscular or metabolic disease), no requirement for assisted ventilation or oxygen therapy after 6 h of life, at least one Norwegian speaking family member and the child living with at least one biological parent.

Questionnaires including the child's health and diseases, family history of disease, socio-economic factors and environmental exposure were completed by the parents every 6 months from birth to their second birthday. Several measures were instituted to identify all possible children with recurrent/persistent BO (with no attempt to define asthma) from the cohort and invite the children for examination at the clinic: the questionnaires were assessed for symptoms suggestive of OAD, the parents were encouraged to liberally report symptoms or signs suggestive of recurrent or persistent wheeze, all local family doctors and well baby clinics were asked to refer children with possible or established OAD to the project doctor, and special cards (returned and replaced every 6 months) with boxes for each of five signs

related to OAD were distributed to the parents to be completed by any doctor who examined the child during a respiratory tract illness.

Cases ($n = 306$) for the nested case-control study were defined as soon as they had experienced at least two episodes of BO or persistent BO for at least 4 wk [hereafter referred to as recurrent BO (rBO)]. At least one episode of BO had to be verified by a doctor, with a history highly suggestive of a further BO. A control ($n = 306$) was chosen as soon as possible after definition of a case, as the child born closest in time to the case, but without lower respiratory illness. However, as lack of resources prevented enrolling extra controls if any of the investigations were unsuccessful, the cases and controls were not matched for the present study, as this would decrease the number of subjects available for analysis.

Verification of eligibility of case and control status was performed at completion of the study by a group of senior paediatricians at Ullevål Hospital to ensure that inclusion and exclusion criteria were met appropriately.

All children were asked to attend two investigations. The first occurred as soon as possible after the second episode of BO. The second and major investigation was scheduled for their second birthday. (Children attending for the first time at an age 20 months or more underwent a visit at 2 yr only.) However, limited resources demanded after some time that controls be postponed until the major investigation of all cases and controls at 2 yr of age, which had an 84% attendance rate (265 with rBO and 251 controls). The present paper reports lung function and bronchodilator response results only from the 2-yr investigation.

At the first visit a structured interview (detailed medical and allergic family history, pet keeping and tobacco smoke exposure), clinical examination, lung function assessed by TFV loops before, and after inhalation of nebulized salbutamol (whenever possible) as well as serum sampling for total IgE were performed. The main visit (at age 2) performed in the absence of any clinical signs of bronchial obstruction or upper respiratory infection (no further specified time limit since last wheeze or respiratory tract infection was defined) was the same as the first visit, with additional skin prick test (SPT) to food and local common inhalant allergens performed after lung function measurements had been obtained.

The present study comprises all children with rBO and controls in whom measurements of bronchodilator response were obtained at 2 yr of age.

Subjects

Measurements of airways response to salbutamol were obtained in 235/516 (46%) of the subjects attending the clinic at 2 yr. Characteristics of the children are given in Table 1. There were no significant differences in age, atopic dermatitis (AD), allergic sensitization or parental history of asthma and/or rhinoconjunctivitis among children with bronchodilator response measurements compared to those without.

Methods

Information on symptoms of allergic or other diseases in the child as well as parental allergic diseases (positive response to question of ever having had parental asthma and/or rhinoconjunctivitis) and smoking was obtained from a structured interview at the last clinical investigation.

The clinical investigations were performed when the child had no signs or symptoms of lower respiratory disease.

Measurements of TFV loops (SensorMedics 2600; Sensor Medics, Anaheim, CA, USA) were performed in all subjects awake, by investigators blinded to parental smoking habits. Measurements were performed as described elsewhere, with a face mask (Vital Signs Inc., Totowa, NJ, USA) connected to a pneumotachograph (4500 series, Hans Rudolph, MO, USA) with a flow range of 0–30 l/min. Dead-space of the system was 2.4 ml, and of the face mask 8.4–11 ml (4).

Table 1. Demographic data of children in the Environment and Childhood Asthma (ECA) study with measures of lung function response to inhaled nebulized salbutamol at 2 yr of age

	Response measures (n = 235)	No response measures (n = 279)
Age (±s.d.)	26.9 ± 3.7	27.0 ± 3.3
Percentage of boys	49.6	57.2
Percentage with rBO†	56.8	46.6*
IgE (geometric mean, 95% CI)	10.9 (9.2–12.8)	13.6 (11.4–16.3)
Percentage with allergic sensitization	6.0	6.8
Percentage with AD‡	33.3	32.9
Percentage with parental 'atopy'§	52.1	52.0
Percentage maternal smoking at 2 yr		
Never	70	63
Occasionally	3	5
Daily	27	32

*p = 0.02, related to with or without response measures at 2 yr of age.

†Percentage with recurrent bronchial obstruction (BO) or BO lasting for minimum 4 wk.

‡Presence ever of atopic dermatitis.

§Reported parental asthma and/or rhinoconjunctivitis.

Four TFV loops were stored for the final analysis, selected from eight temporarily stored curves according to specified selection criteria. Details of ratio calculations are described elsewhere (4, 12).

SPT was performed in all children at the age of 2 yr, according to Nordic standard (13). The following standardized extracts from ALK (Greer Laboratories INC, Lenoir, NC, USA) were used: egg white, cows milk, dermatophagoides pteronyssinus, timothy grass, cat dander, dog dander, silver birch, mugwort and *Cladosporium herbarum*, as well as saline negative control and histamine control (10 mg/ml). Sensitization was regarded as positive with a wheal for the allergen in question of at least half the size of wheal for the histamine positive control.

Serum was analysed for total IgE as well as specific IgE to the allergens as described above using the UniCAP fluoroenzyme immunoassay (FEIA) according to the manufacturer's instructions (Pharmacia Upjohn, Uppsala, Sweden). Specific IgE antibodies were analysed if the SPT was possibly or clearly positive. Allergic sensitization was regarded positive with a positive SPT and/or radioallergosorbant test (RAST) class of minimum two to at least one allergen.

Outcome measures. The main tidal breathing parameter was ratio t_{PTEF}/t_E which has been shown to differ according to OAD (5, 11, 12, 14–17) as well as predict wheeze in children under 3 yr of age (t_{PTEF}/t_E) (18–20).

The subjects were classified as responders (to salbutamol), non-responders or paradoxical responders based upon intra-individual variation, defined as:

Responder: t_{PTEF}/t_E improved > 2 s.d. of the intra-subject variation in t_{PTEF}/t_E .

Non-responder: t_{PTEF}/t_E changed < 2 s.d. of the intra subject variation in t_{PTEF}/t_E .

Paradox responder: t_{PTEF}/t_E declined > 2 s.d. of the intra-subject variation in t_{PTEF}/t_E .

The degree of change in t_{PTEF}/t_E was calculated as $[(t_{PTEF}/t_E \text{ after salbutamol} - t_{PTEF}/t_E \text{ before salbutamol})/t_{PTEF}/t_E \text{ before salbutamol}] \times 100$ [as has earlier been reported in ref. (10)] and is reported as per cent change from baseline.

Statistics. Statistical analyses were performed using the statistical package for social sciences (SPSS) version 11. Demographic results are given as mean values with standard deviation (s.d.) unless otherwise stated. Intra-individual variation is given as coefficient of variation and

main results are given as mean with 95% confidence interval (CI) given in parenthesis unless otherwise stated. Statistical significance for possible differences in categorical results was compared using the chi-square test, and between groups of continuous variables non-parametric tests (Wilcoxon's signed rank test or Kruskal–Wallis) were used. Correlation analyses were performed with Pearson's two-tailed test for significance.

For analyses of possible associations between bronchodilator response and the various 'asthma risk factors' [parental asthma and/or rhinoconjunctivitis, AD ever (by 2 yr of age) and/or rBO] a classification according to increasing load of supposed unfavourable factors was performed (Fig. 2), from the extreme of all factors negative to all positive. Possible statistical significance of differences between groups was assessed by non-parametric (Wilcoxon's or Mann–Whitney) tests, whereas possible differences between groups within analyses for the influence of allergic factors were performed by a one-way ANOVA test. To ascertain possible modifying effects upon bronchodilator response of inhaled steroids, a linear regression model was used, correcting for asthma risk factors, sex and allergic sensitization.

Results

Measures of TFV loops before and after inhalation of salbutamol were obtained in 235 children

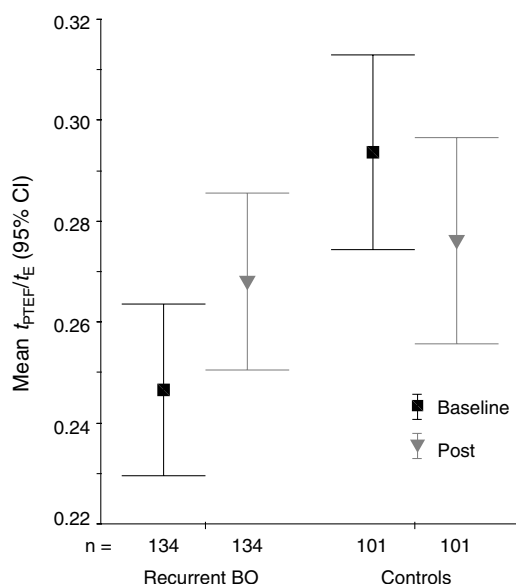


Fig. 1. Baseline and post-bronchodilator t_{PTEF}/t_E (ratio of time to reach peak flow to total expiratory time) at mean 26 months in 235 children with and without recurrent bronchial obstruction (BO).

(134 children with rBO) at 2 yr of age. The groups of children with and without bronchodilator response measurements were comparable for age, sex, AD (ever), allergic sensitization, total IgE, maternally reported smoking and parental atopy, whereas a small, but significant over-representation of children with rBO were found among those with such measurements (Table 1).

Children with rBO had significantly greater bronchodilator response than did controls (17.2 (9.4–25.3) vs. –2.2 (–8.3 to 2.5), respectively ($p = 0.001$)) (Fig. 1). Significantly more children with rBO compared to without rBO were defined as responders (27.3% vs. 20.0%, respectively) whereas the opposite was found for paradoxical response (11.4% vs. 22.0%, respectively) ($p = 0.04$). Intra-individual variation of t_{PTEF}/t_E did not differ between children with or without rBO neither at baseline nor after salbutamol, although there was a small, but significant ($p = 0.01$) increase in intra-individual variation after [15.8 (14.2–17.3) compared to before (13.5 (11.9–15.0)] salbutamol among controls. This was not found for cases [15.3 (13.7–16.9) vs. 15.3 (13.6–16.9)] or all children as one group [15.5 (14.4–16.6) vs. 14.5 (13.3–15.6), respectively] ($p = 0.08$).

The response to salbutamol tended to be larger among the 29 children with persistent (4 wk or more) BO symptoms compared to those with reported episodic wheeze only (29.4, 11.8–46.9 vs. 13.0, 4.1–21.9) ($p = 0.058$). There was no significant association between the reported number of episodes of BO and bronchodilator response (data not shown).

Although bronchodilator response did not differ significantly between subjects with or without atopic parents [8.2 (1.5–14.8) vs. 9.0 (1.0–17.0) ($p = 0.71$) or AD (13.6 (3.4–23.7) vs. 5.7 (0.1–11.5), respectively ($p = 0.22$)], an increase in bronchodilator response was seen with increasing number of 'asthma risk factors' ($p = 0.001$) (Fig. 2). Children with the maximum load of asthma risk factors (rBO, AD at some time as well as parental asthma and/or rhinoconjunctivitis) had significantly higher bronchodilator response to salbutamol than did children with none of the factors present ($p = 0.001$). The bronchodilator response was not independently associated with parental atopy when correcting for rBO (Fig. 2), but was significantly higher among children with AD compared to those without in the rBO children who had atopic parents ($p = 0.044$).

Neither the bronchodilator response (% change: mean (95% CI)) nor baseline or post-bronchodilator t_{PTEF}/t_E (data not shown) differed significantly between children with

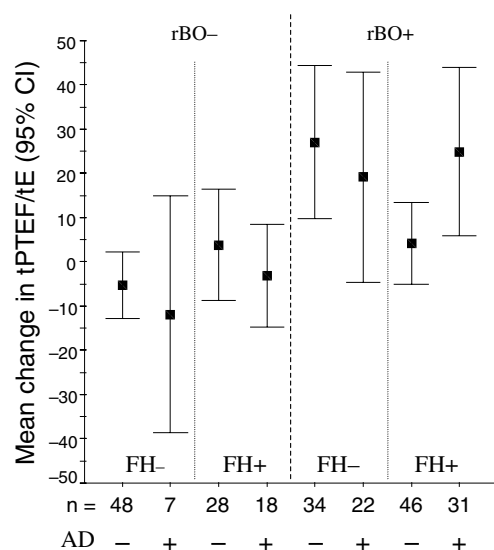


Fig. 2. The response to inhaled bronchodilator, assessed as the change in t_{PTEF}/t_E according to the formula: $(t_{PTEF}/t_E \text{ after} - t_{PTEF}/t_E \text{ before}) \times (t_{PTEF}/t_E \text{ before salbutamol})^{-1}$, increased significantly ($p = 0.001$) at mean age 26 months in 235 children with increasing number of asthma risk factors according to the absence (-) or presence (+) of parental asthma and/or allergic rhinitis (F), atopic dermatitis (AD) or recurrent/persistent bronchial obstruction (BO).

($n = 14$) (8.2 (-12.2–28.5)) compared to without [8.6 (3.3–14.0)] allergic sensitization, respectively. The bronchodilator response was not associated with exposure to maternal smoking reported at 2 yr of age [7.0 (0.7–13.3) for non-smokers, -0.2 (-27.7 to 27.2) for occasional smokers and 13.9 (3.9–23.9) for daily smoking, respectively ($p = 0.4$).

The bronchodilator response correlated significantly ($p < 0.001$), although not strongly ($r = -0.45$), with baseline t_{PTEF}/t_E (Fig. 3). The use of inhaled corticosteroids by 2 yr of age did not differ significantly whether the child had (ever) AD (17.2%), their parents were atopic (27.6), both (29.3), or neither (25.9) ($p = 0.35$). However, the bronchodilator response was significantly higher among the 31 children treated with inhaled corticosteroids compared to those steroid naive [25.9 (8.4–43.5) vs. 5.9 (0.68–11.3) ($p = 0.022$), but when correcting for asthma risk factors and sex this effect was only borderline ($p = 0.066$).

Discussion

Although lung function response to nebulized bronchodilator increased with increasing numbers of asthma risk factors (personal or parental allergic/respiratory diseases), this was mainly attributed to the presence of rBO. Allergic sensitization was not associated with the bronchodilator response to salbutamol at 2 yr.

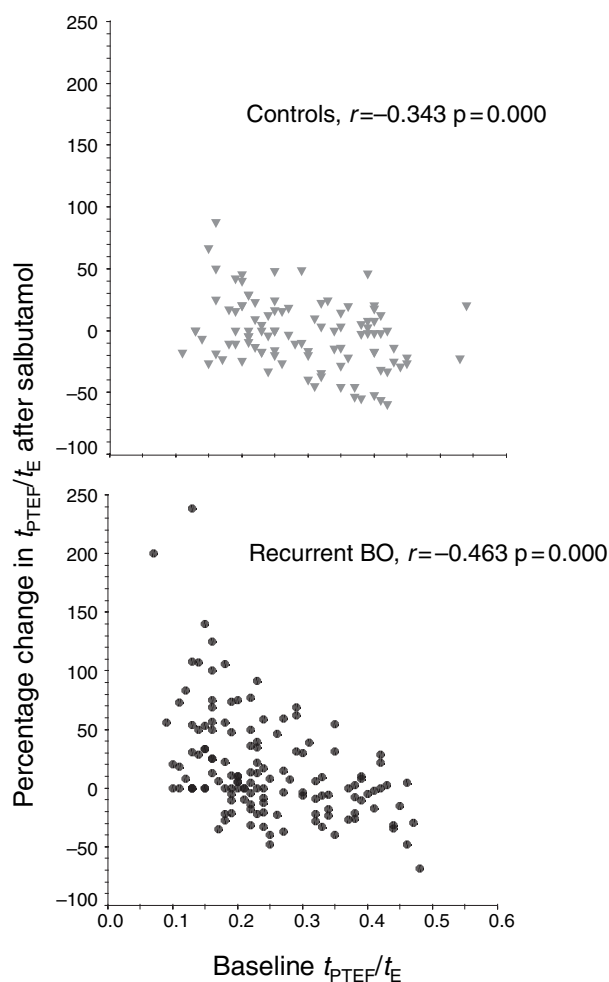


Fig. 3. Bronchodilator response $[(t_{PTEF}/t_E \text{ after} - t_{PTEF}/t_E \text{ before}) \times (t_{PTEF}/t_E \text{ before salbutamol})^{-1}]$ correlated ($r = -0.444$) significantly ($p = 0.000$) with baseline t_{PTEF}/t_E in 2-yr-old children with (black dots, below) and without (grey triangles above) recurrent bronchial obstruction (BO).

The lung function response to salbutamol at 2 yr found among children with rBO is in accordance with several studies among very young children with a history of BO, by TFV loops (4, 10), whole-body plethysmography (21), interrupter technique (Rint) (21) and oscillation techniques (21, 22), respectively. However, other studies could not demonstrate significant changes in lung function in asymptomatic children with OAD after inhaled salbutamol (aerosol with a spacer device) by forced expiratory flow volume loops (squeeze technique) (23) or the raised volume technique, respectively (11). These studies, however, cannot be compared directly. Firstly, the techniques of lung function measurements vary, and the physiological parameters assessed are different (9, 11, 24). Secondly, the administration form for salbutamol varies, with nebulized salbutamol in the Norwegian studies,

whereas pressurized aerosols with spacer devices were used in the others (21–23).

The present study aimed at revealing possible factors that may influence bronchodilator response in very young children in an observational study. We are not aware of other published studies where this has been explored. Not surprisingly, rBO was the asthma risk factor most strongly associated with bronchodilator response. Although the presence (ever) of AD was associated with increased bronchodilator response in wheezy children with atopic parents, there was no overall significant independent effect of AD or parental allergic diseases upon the bronchodilator response, and the between-subject variation was large. Allergic sensitization did not influence the lung function response to salbutamol in the present study. It is possible that the aetiology of rBO in very young children (demonstrated in the present study) may be more closely associated with lung function abnormalities than is the case for asthma in school age, which is more closely associated with allergen sensitization. However, only 14 of the children included in the present analyses were sensitized to at least one allergen by 2 yr of age, so that any definite interpretation of this finding should be made with caution.

The present study found that children treated with inhaled corticosteroids had significantly larger improvement in lung function after inhaled salbutamol, giving rise to several speculations. Firstly, the finding may indicate that the steroid-treated children had more severe obstructive airways, thus supporting the physician's choice of treatment. On the other hand, it may indicate that inhaled steroids do not influence bronchodilator response, that inhaled steroids may improve responsiveness to inhaled β -agonist (25, 26) or even, that a lack of inhaled steroids for treatment in children using bronchodilators alone may adversely affect the response to nebulized salbutamol (25, 26). However, it is important to stress that the present study was not designed to assess possible effects of corticosteroids on bronchodilator response. Thus, interpretation of our finding is limited to creating hypotheses.

The present study found no association between bronchodilator response and exposure to maternal smoking at the start of life. This may be supported by findings from other studies suggesting that tobacco smoke may adversely affect lung function (27, 28) but with a less clear effect on BHR (3). In the study by Young et al., increased BHR was found in infants of mothers who smoked during pregnancy and/or had a positive family history of atopic diseases (3).

However, as the present study assessed bronchodilator response and not BHR, no direct comparison between these studies and our study can be drawn.

There are several limitations to the present study. First, the design of the study did not identify asthma in children, but only rBO. Children with minimum entry rBO criteria (of two episodes of BO only) may not have received an asthma diagnosis. It may be argued that rBO, represents symptoms or signs of various aetiologies, in particular 'wheezy lower respiratory tract infections (LRTI)', which may not necessarily be associated with later asthma (30). Nevertheless, since rBO represents a risk factor for later asthma development, it is included as an 'asthma risk factor' in the present paper. Furthermore, measurements were performed when children were asymptomatic, rendering it less likely that acute wheezy LRTI was responsible for any effects.

Secondly, the methodology of TFV measurements is debated, and no agreement as to standardization of such measurements has been reached despite the many studies that employed TFV loops for assessing lung function in infants and very young children. Although it is not clear what physiological parameters TFV loops reflect, results from studies applying TFV loop measurements correspond to findings with conventional lung function measurements such as spirometry (14, 31). Furthermore, intra-individual variation (CV of the four selected curves for each measurement) was unlikely to significantly influence the results, as it was slightly different only among the children with less change after salbutamol, both measured as raw value as well as regarding responder status that was based upon intra-individual variation. Other studies have also found larger variability among healthy compared to subjects with airways disease, supporting our findings of slightly higher intra-individual variation among the controls (14). An intra-individual variation of approximately 15% is comparable to that found in previous studies of TFV measurements both in our own, (4, 32) and other groups (33, 34). Thus, even though only four curves were selected for each measurement, the authors believe the results are valid, since: (i) variability was similar in both measurements, (ii) results are similar to that found in other studies among different individuals, (iii) our results (27) with the same TFV selection method in previous studies have been supported by findings with different methods of measuring TFV loops (longer epochs of breath cycles) in other groups (exemplified by the effect

of *in utero* exposure to tobacco smoke products) (35).

The present study comprises children from a large prospective cohort, nested case-control design. From the total cohort of 3754 children, 306 cases were identified (we could identify all cases with documented rBO) and subsequently 306 controls were defined. The attendance rate for this case-control study at 2 yr was 84%. Lung function measurements in awake subjects of this age are challenging, and the success rate of repeated TFV measurements before and after salbutamol inhalation was 46%. The most commonly encountered problems were non-acceptance of the face mask or the fact that the child did not sit quietly sufficiently long enough to perform successful measurements. Apart from a slight over-representation of children with rBO among those in whom measurements were obtained, the groups with and without bronchodilator response measurements were otherwise similar. Thus, the study results would appear not to be influenced by selection bias.

Conclusion

Bronchodilator response measured by TFV loops in very young children was mainly associated with rBO, but also with AD in children with atopic parents, as well as in children receiving treatment with inhaled corticosteroids. Allergic sensitization appeared not to influence bronchodilator response at 2 yr.

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