Indirect bronchial provocation tests in childhood asthma
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INDIRECT BRONCHIAL PROVOCATION TESTS

In chapter 1 we describe the current approach to diagnosing, monitoring and treating childhood asthma. We discuss several clinical dilemmas of this approach that are debated in current literature. We postulate that indirect bronchial provocation tests (BPTs) can be used to monitor BHR and airway inflammation. In the following chapters of this thesis we used exercise challenge tests (ECTs) and mannitol tests for short term monitoring of asthmatic children.

The idea that indirect BPTs can be used to monitor airway inflammation is not new. In 2003 the European Respiratory Society task force on indirect airway challenges already concluded: ‘Indirect challenges may reflect acute changes in airway inflammation more closely and be clinically relevant markers to assess the clinical course of asthma.’ A review by Cockcroft and Davis compared the use of direct and indirect BPTs in the clinical assessment of asthma and concluded that indirect BPTs should be the challenges of choice for evaluating and monitoring asthma treatment.

BHR to an indirect stimulus has advantages over other parameters used to monitor the response to anti-inflammatory treatment. Symptoms and lung function are accessible parameters to monitor treatment, but poorly correlate to the underlying disease severity in asthmatic children. Questionnaires to assess asthma control, such as the Asthma Control Test (ACT), do not correlate with measures of airway inflammation in the follow-up of asthmatic children.

Invasive techniques, such as analysis of induced sputum, broncho-alveolar lavage and lung biopsies can be used to assess airway inflammation. In adults, tailoring treatment to sputum eosinophils reduces asthma exacerbations. In children, invasive tests are not suitable for routine use. Safety issues, technical demands and costs of sputum induction and analysis limit the use in clinical practice.

Analysis of Fraction of exhaled Nitric Oxide (FeNO) is relatively easy to perform in children. However, various other factors can influence FeNO levels, such as atopy, allergic rhinitis (AR), exercise, age, and viral respiratory tract infections. A 2012 pediatric (mean age 10-14y) meta-analysis concluded that tailoring the dose of inhaled corticosteroids (ICSs) to FeNO compared to symptoms and lung function cannot be recommended for clinical practice, as it leads to increased use of ICSs without meaningful changes in clinical outcomes.

BHR to direct stimuli reflects chronic inflammation and responds slowly to anti-inflammatory treatment. In adults, tailoring treatment to direct BHR for 2 years leads to fewer exacerbations, better lung function and a greater reduction in basement membrane thickness compared to tailoring treatment to symptoms and lung function alone. A similar study in asthmatic children (aged 6-16y) treated with ICSs over 2 years
showed no benefit in number of exacerbations, but a better pre-bronchodilator FEV1, in children whose treatment was tailored to direct BHR compared to symptoms alone. This could result from differences in the pathophysiology of asthma between adults and children. Direct BHR reflects chronic inflammation leading to functional and anatomic airway remodeling, which may play a larger role in adults.

BHR to indirect stimuli is closely associated with current airway inflammation. In adults, there is a significant relationship between sputum eosinophilia and the sensitivity to indirect, but not direct, BPTs. In adolescents and adults, the percentage of sputum eosinophils correlates with the severity of EIB and sensitivity to mannitol. In children (aged 8-14y), BHR to hypertonic saline is strongly associated with the presence of mast cells in sputum.

A response to an indirect BPT could identify patients who are likely to benefit from ICSs, as it reflects the presence of inflammatory cells that are targets for ICS treatment. The regular use of ICSs can attenuate airway sensitivity and reactivity to indirect stimuli. In adults, the increase in BHR to mannitol during down-titration of the dose of ICSs was predictive of an asthma exacerbation.

Airway sensitivity and reactivity to adenosine monophosphate (AMP; an osmotic indirect BPT) correlated significantly with guideline derived asthma control in adults. Abolishing the response to indirect stimuli, as a marker of asthma control, may be a treatment goal. Tailoring treatment with ICSs to BHR to mannitol was demonstrated to improve quality of life in asthmatic adults in a primary care setting. In a larger study in adults with mild-to-moderate asthma, tailoring ICS treatment to BHR to mannitol for a year led to reductions in number of mild exacerbations, FeNO, eosinophilic cationic protein, symptoms and rescue β2-agonist use, compared to tailoring treatment to symptoms and lung function.

In summary, indirect BPTs are clinically relevant parameters to assess and monitor airway inflammation and the short term response to anti-inflammatory treatment in asthmatic children.

Further research is necessary to assess the complex relationship between BHR and airway inflammation. Unraveling the exact pathophysiologic mechanisms of BHR to different stimuli would lead to a better understanding of childhood asthma and possible targets for therapy. Larger, randomized placebo controlled studies are necessary to evaluate how indirect BPTs can be used to monitor long-term changes in airway inflammation. The effect of tailoring treatment to indirect BHR should be studied for longer treatment periods and in different subgroups of patients.
MANNITOL PROVOCATION TEST

In chapter 2 we studied the clinical utility of the mannitol test to identify asthmatic children with exercise induced bronchoconstriction (EIB). In this study, 33 asthmatic children, aged 9-18 years, with a history of EIB, performed both mannitol and exercise tests. Data were composed of a cross tabulation comparing the reaction on an exercise test to a mannitol test. Correlations between post-exercise % fall in FEV₁ and RDR (response-dose ratio) and PD₁₅ (provoking dose to cause a 15% fall in FEV₁) of mannitol were calculated. Twenty-five children completed both tests. Pearson’s correlation between log-transformed RDR for mannitol and post-exercise % fall in FEV₁ was \( r_p = 0.666 \) (\( P < 0.001 \)). There was no significant relationship between the log PD₁₅ of mannitol and post-exercise % fall in FEV₁. Positive and negative predictive values of the mannitol test for EIB were respectively 69% and 92%. We concluded that a mannitol test is a suitable alternative for an exercise challenge test (ECT) to assess EIB in asthmatic children.

In 2011, Stickland et al. performed a systematic meta-analysis to determine the sensitivity and specificity of the mannitol test to diagnose EIB.\(^{38}\) They concluded that the sensitivity and specificity ranged from 58–96% and 65–78% respectively.\(^{38}\) Since then, several other studies have been performed to assess the concordance between BHR to mannitol and exercise and/or eucapnic voluntary hyperpnoea (which is considered a sensitive test for EIB, especially in athletes).\(^{39}\) The results of these and previous studies are summarized in Table 1.

These studies demonstrate a variable sensitivity and specificity of mannitol, depending on the research population. Especially in elite athletes, sensitivity of mannitol appeared low.\(^{34-37}\) However, the pathophysiology of EIB differs between asthmatic children and adult athletes. In childhood asthma, EIB is a consequence of the inflammatory substrate present in the airway wall. In adult athletes, EIB results from repetitive thermal, osmotic and mechanical stress due to extensive training, causing injury to the airway epithelium.\(^{40,41}\) Only one other study was performed in asthmatic children (aged 6-16y) and found similar positive and negative predictive values of the mannitol test for EIB compared to our study.\(^{32}\)

Although a mannitol test can be used as an alternative for an ECT, there are some differences between the physical responses to exercise and mannitol (Table 2.)

Firstly, exercise induces a variety of other physiologic changes, such as the release of steroids and catecholamines and an increase in minute ventilation, cardiac output and oxygen uptake. Some of these mechanisms protect the airway from narrowing and compensate for the ventilation-perfusion (\( V_{A}/Q \)) imbalance caused by bronchoconstriction.\(^{42}\) These compensatory mechanisms do not occur during a mannitol test.\(^{42}\)
### Table 1. Studies using inhaled mannitol to assess EIB

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Subjects</th>
<th>Compared to</th>
<th>N</th>
<th>ICS use (%)</th>
<th>Sens. (%)</th>
<th>Spec. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson 28 (2009)</td>
<td>Suspected asthma, age 6-50y</td>
<td>Standardized ECT</td>
<td>372</td>
<td>0</td>
<td>58 - 79*</td>
<td>61 - 65*</td>
</tr>
<tr>
<td>Aronsson 29 (2011)</td>
<td>Adults with mild asthma</td>
<td>EVH</td>
<td>34</td>
<td>79</td>
<td>55</td>
<td>92</td>
</tr>
<tr>
<td>Brannan 30 (1998)</td>
<td>Adult asthmatics with diagnosis of EIB</td>
<td>Standardized ECT</td>
<td>36</td>
<td>44</td>
<td>96</td>
<td>n/a</td>
</tr>
<tr>
<td>Kersten 31 (2009)</td>
<td>Children with asthma and EIB, age 9-18y</td>
<td>Standardized ECT</td>
<td>25</td>
<td>60</td>
<td>90</td>
<td>73</td>
</tr>
<tr>
<td>Barben 32 (2011)</td>
<td>Suspected asthma, age 6-16y</td>
<td>Standardized ECT</td>
<td>99</td>
<td>11</td>
<td>81</td>
<td>85</td>
</tr>
<tr>
<td>Holzer 33 (2003)</td>
<td>Adult elite athletes (some with EIB)</td>
<td>EVH</td>
<td>50</td>
<td>18</td>
<td>96</td>
<td>92</td>
</tr>
<tr>
<td>Clearie 34 (2010)</td>
<td>Adult elite swimmers (some with asthma)</td>
<td>Sport specific exercise test</td>
<td>61</td>
<td>5</td>
<td>11</td>
<td>85</td>
</tr>
<tr>
<td>Osthoff 35 (2013)</td>
<td>Paralympic adult athletes (some with asthma)</td>
<td>EVH</td>
<td>44</td>
<td>n/r</td>
<td>33</td>
<td>86</td>
</tr>
<tr>
<td>Sue-Chu 36 (2010)</td>
<td>Adult elite athletes (some with symptoms of EIB)</td>
<td>Sport specific exercise test</td>
<td>33</td>
<td>10</td>
<td>13</td>
<td>96</td>
</tr>
<tr>
<td>Stenfors 37 (2010)</td>
<td>Adult elite athletes (some with asthma)</td>
<td>EVH</td>
<td>46</td>
<td>n/r</td>
<td>17</td>
<td>95</td>
</tr>
</tbody>
</table>

*When sensitivity and/or specificity were not presented, values were calculated based on absolute numbers in the article. *depending on cut-off value for positive exercise test (10 vs. 20%) ECT = exercise challenge test, EVH = eucapnic voluntary hyperpnoe, n/r = not reported, sens = sensitivity, spec = specificity.
Secondly, mannitol is primarily deposited in the large central airways, because of its particle size. The amount of mast cells is greater in the peripheral airways, where only a small part of the inhaled mannitol powder will penetrate. When exercise is more vigorous and/or longer, producing a higher ventilation rate, or when the inspired air is colder and/or dryer, the smaller airways beyond the 10th generation are recruited in the humidifying process. Therefore, a change in inflammation of the small airways is more likely to be detected with an ECT.

Thirdly, the osmotic stimulus provoked by mannitol is different because it is caused by active withdrawal of water from the airway epithelium instead of evaporative water loss. The rate of change in osmolarity, which is suggested to be a determinant of BHR, is faster and greater with an ECT.

A mannitol test is faster and easier to perform than an ECT, and does not require specifically trained personnel or specialized equipment. Mannitol tests showed good

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**Table 2. Characteristics and differences of exercise challenge and mannitol tests**

<table>
<thead>
<tr>
<th></th>
<th>Exercise challenge test</th>
<th>Mannitol test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Safety</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burden for patient</td>
<td>-vigorous exercise</td>
<td>-side effect: coughing⁴⁸</td>
</tr>
<tr>
<td></td>
<td>-time consuming</td>
<td></td>
</tr>
<tr>
<td>Safety during test</td>
<td>-risks of running at high speed</td>
<td>-approved for safety &amp; efficacy⁴⁸</td>
</tr>
<tr>
<td></td>
<td>-(uncontrolled) large fall in FEV₁</td>
<td>-dose-response protocol avoids large fall in FEV₁⁴⁶</td>
</tr>
<tr>
<td>Recovery</td>
<td>rapid (&lt; 10 min after SABA)</td>
<td>rapid (&lt; 10 min after SABA)⁴⁹</td>
</tr>
<tr>
<td><strong>Technical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment</td>
<td>-space occupying ergometer</td>
<td>-office based test kit</td>
</tr>
<tr>
<td></td>
<td>-dry air source, cold air environment</td>
<td></td>
</tr>
<tr>
<td>Personnel</td>
<td>trained personnel</td>
<td>easy standard procedure</td>
</tr>
<tr>
<td>Duration</td>
<td>45 - 50 min</td>
<td>20 - 30 min⁴₆</td>
</tr>
<tr>
<td>Costs</td>
<td>high</td>
<td>moderate</td>
</tr>
<tr>
<td>Repeatability</td>
<td>variation ± 12 % in children⁵⁰</td>
<td>variation ± 1.1 doubling dose in children⁵¹</td>
</tr>
<tr>
<td><strong>Diagnostic value for asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulus</td>
<td>natural stimulus; real life test</td>
<td>osmotic stimulus; surrogate test</td>
</tr>
<tr>
<td>Specificity</td>
<td>high (90%)</td>
<td>high (95%)⁵²</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>moderate (65%); depends on study population and air conditions</td>
<td>moderate (40 - 75%); depends on study population</td>
</tr>
<tr>
<td><strong>Monitoring value for asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment effect</td>
<td>improves with ICS treatment²⁰</td>
<td>improves with ICS treatment²¹,²²</td>
</tr>
<tr>
<td>Asthma control</td>
<td>can be used to monitor asthma control</td>
<td>can be used to monitor asthma control²³,²⁷</td>
</tr>
</tbody>
</table>

FEV₁ = forced expiratory volume in 1s, ICS = inhaled corticosteroid, SABA = short acting β₂-agonist
repeatability in asthmatic children (aged 6-16y). Its safety was demonstrated in 592 asthmatic and non-asthmatic subjects, including 126 children (aged 6-18y). In this study, only 14/592 subjects experienced > 30% fall in FEV₁, as the test can be stopped before major falls in FEV₁ occur. The mannitol test is also feasible in younger children, aged 3-7 years.

A limitation of the mannitol test is the occurrence of a dry cough, which almost all patients experience. This cough can interfere with the fixed time schedule. The test should be performed quickly (next dose within 60s after spirometry). Prolonging the time between the inhalation of subsequent doses of mannitol could lead to a milder osmotic stimulus and give falsely negative results. Severe coughing, leading to disruption of the test, occurs in 1.3% of tested subjects. Mannitol provoked coughing can occur immediately after inhalation due to the impaction of powder on the oropharynx or later due to deposition of powder in the lower respiratory tract, stimulating nerve fibers by hyperosmolarity. Mannitol induced coughing is related to the increased sensitivity of the asthmatic airways, making it useful in the diagnosis of asthma.

In summary, mannitol has a high specificity and moderate sensitivity to diagnose EIB in asthmatic children; but there are some differences in the physiological responses to exercise and mannitol. As mannitol is a practical, fast and safe test, it has the potential to become an office based test to monitor treatment alterations in asthmatic children with EIB.

Future research studying the differences in physical responses to mannitol and exercise could present new information on the pathophysiology of EIB. The effects of tailoring treatment to BHR to mannitol in children with asthma and EIB should be further explored.

**β2-AGONISTS**

In chapter 3 and 4 we investigated the effect of the development of tolerance to long-acting β2-agonists (LABAs) on BHR to indirect BPTs. We analyzed clinically stable asthmatic children who were stepped down from LABA/ICS combination therapy to monotherapy with the same dose of the ICS. As regular use of β2-agonists can increase BHR to indirect stimuli, we hypothesized that this step down could do the opposite.

In chapter 3 we analyzed the effect of stepping down combination therapy to monotherapy with an ICS on EIB in clinically stable asthmatic children with a history of mild to moderate EIB. Nineteen children, aged 8-16 years, were analyzed in this open-label pilot study. Children performed a baseline ECT after a 4 week run-in period on combination
therapy and 3 weeks after the medication was stepped down. Maximum % fall in FEV₁ was significantly lower after 3 weeks of ICS monotherapy ($P = 0.03$).

Eight children had a ≥ 15% fall in FEV₁ after exercise at the initial ECT. The cutoff value to diagnose EIB on an ECT is controversial. A fall of ≥ 10% is considered abnormal; a fall of ≥ 15% appears to be more diagnostic of EIB, particularly if exercise has been performed in the field. Haby et al. found that 1.96 SD above the mean fall in FEV₁, when testing 8-11 year old healthy children in the field was 15.3%. Because the environmental circumstances in which the test was performed in our study were more similar to outdoor circumstances than to laboratory settings, a cut-off value of 15% was used.

In the subgroup of patients with EIB, maximum % fall in FEV₁ was significantly lower ($P < 0.01$) after the medication was stepped down and in 6 children it decreased to < 15%. We concluded that in clinically stable asthmatic children with mild to moderate EIB despite LABA/ICS combination therapy, the cessation of the LABA can reduce and in most cases even abolish EIB.

In chapter 4 we performed a similar study design, analyzing the effect of stepping down combination therapy to ICS monotherapy on BHR to a mannitol test. Seventeen children, aged 12-17 years, with clinically stable asthma and a history of mild to moderate EIB were analyzed in this prospective open-label study. Children performed a mannitol test after a 4 week run-in period on combination therapy and 30 ± 4 days after their medication was stepped down. The changes in mannitol PD₁₅, RDR and recovery time following a short-acting β2-agonist (SABA) to ≥ 95% of baseline FEV₁ were calculated. Mannitol PD₁₅ and RDR did not change after stepping down. The recovery time following a SABA was significantly shorter ($P = 0.01$) after the cessation of the LABA. We concluded that in clinically stable asthmatic children with mild to moderate EIB despite LABA/ICS combination therapy, the cessation of the LABA does not change BHR to mannitol, but does shorten recovery time to baseline lung function following a rescue SABA.

Due to concerns about the safety of LABA use, the US Food and Drug Administration (FDA) advised that in asthmatic children on combination therapy, LABAs should be withdrawn once asthma control is achieved. A recent meta-analysis reviewed the effect of stepping down to ICS monotherapy compared to continuation of LABA/ICS combination therapy in asthmatic adults. They concluded that the LABA step-down regimen resulted in worse asthma control and quality of life. However, they did not assess the effect on BHR, and no pediatric studies were included.

The results from our studies are consistent with findings from studies that stepped up treatment with a LABA. Regular treatment with β2-agonists leads to downregulation and desensitization of the β2-adrenoreceptor (β2AR), resulting in a reduced protection
against BHR\textsuperscript{61-66} and a prolonged recovery time after rescue therapy with a SABA.\textsuperscript{61,64,65} Because this process occurs faster in mast cells than smooth muscle cells, the effects of tolerance are more pronounced when measured with an indirect BPT compared to a direct BPT.\textsuperscript{67-70} As BHR to exercise decreased after the cessation of the LABA, and BHR to mannitol did not, we speculate that EIB gives a better reflection of inflammation in the peripheral airways, where more mast cells reside\textsuperscript{45} and the amount of β2ARs on smooth muscle is highest.\textsuperscript{58}

Downregulation of β2ARs on the mast cell increases its susceptibility to degranulate, as was shown by increased levels of mast cell mediators following an allergen challenge in adults treated with a SABA.\textsuperscript{71} This could lead to more instable asthma and an increase in BHR.

The number of β2ARs on leukocytes in asthmatic children (aged 11-16y) has a negative correlation with BHR to exercise.\textsuperscript{72} Regular treatment of steroid-naïve asthmatic adults with a SABA increases BHR to exercise\textsuperscript{73,74} and hypertonic saline.\textsuperscript{75} This has not been studied after regular treatment with LABA monotherapy. However, in studies comparing a step-up to LABA/ICS combination therapy to the same dose of ICS monotherapy, asthmatic children (aged 6-18y) showed a significantly lower improvement in BHR to methacholine\textsuperscript{76} and a non-significantly lower improvement in BHR to exercise\textsuperscript{63} on LABA/ICS combination therapy. This suggests that LABAs may attenuate the beneficial effects of ICSs on BHR.

In daily life, the effect of LABA tolerance may be less apparent than in clinical trials due to poor compliance. The effects of tolerance have been shown to be reversed within three days after a LABA is discontinued.\textsuperscript{77} Therefore, patients who regularly take a ‘drug holiday’ might not experience the adverse effects of LABA tolerance.

The clinical relevance of β2AR downregulation and β2-agonist associated increased BHR to indirect stimuli is still debated. In chapter 5 we review current literature on the benefits and safety of β2-agonists in childhood asthma. We hypothesize that the combination of increased BHR and a reduced response to rescue bronchodilators could account for the association between serious asthma related adverse events and regular β2-agonist use.

Asthmatic patients with specific genotypes might be more susceptible to this. β2AR-gene polymorphisms result in changes in the amino acid sequence of the β2AR, leading to alterations of its properties, possibly representing a risk factor for adverse responses to β2-agonist therapy.

The rare Thr164Ile polymorphism results in a decreased β2AR ligand binding in vitro\textsuperscript{78} and was associated with severe exacerbations requiring hospitalizations and systemic corticosteroids in African American adults treated with a LABA.\textsuperscript{79}
The more extensively studied Arg16Gly polymorphism, which is prevalent in 12-15% of the Caucasian population, has also been shown to interfere with treatment responses to β2-agonists. In vitro, receptors with the homozygous Arg16 genotype show enhanced susceptibility for homologous desensitization and receptor downregulation.

Retrospective analyses of data from trials in adults on ICSs have demonstrated adverse effects of the Arg16 homozygous variant on BHR and exacerbations after receiving a SABA as regular therapy. In a genotype stratified prospective trial in steroid-naïve asthmatic adults the response to 16 weeks regular albuterol was compared to placebo plus ipratropium rescue treatment. In this study, Arg16 homozygotes did not demonstrated a deterioration of symptom control during albuterol treatment, in contrast to Gly16 homozygotes.

Studies searching for the effect of the Arg16 homozygous genotype on the response to treatment with LABAs have shown conflicting results, which appear to be dependent on the age of the study group.

In children, an increased risk for exacerbations in Arg16 homozygotes in a cohort of 1182 patients on ICSs (aged 3-22y) with daily use of salmeterol was reported. Another study described an increase in oral corticosteroid use and emergency department visits in the past year in 597 Arg16 homozygotes (aged 4-12y) on LABA/ICS combination therapy compared to Gly16 homozygotes. A prospective randomized controlled trial in asthmatic children (aged 5-18y) showed that in Arg16 homozygotes adding montelukast compared to salmeterol to inhaled fluticasone significantly improved asthma symptoms and quality of life. This study suggests that in children the Arg16Gly genotype could help to determine the appropriate step-up or step-down regimen.

In summary, regular treatment with β2-agonists leads to a loss of bronchoprotection and a reduced bronchodilator response to rescue SABAs. Downregulation of β2ARs on the mast cell could lead to more instable asthma, increased BHR and a higher risk of exacerbations. Therefore, if asthmatic children on LABA/ICS combination therapy experience EIB, a withdrawal of the LABA or a switch to another step 3 option should be considered. If asthmatic children with EIB need SABAs more than twice weekly, including pre-exercise use, a step-up in controller therapy should be considered.

Future trials should study the effects of different LABA treatment regimens, with concomitant ICSs, on BHR and other outcome measures of inflammation, asthma control and quality of life. More research in children < 12 years is necessary to provide evidence based recommendations on the safety and efficacy of LABA/ICS combination therapy in general, and in different genetic subgroups.
In chapter 6 we studied the effect of intranasal anti-inflammatory treatment on lower airway inflammation and BHR. In this study, children aged 12-17 years, with mild-to-moderate asthma, intermittent AR and ≥ 10% fall in FEV₁ at a screening ECT were randomized (double-blind) to 22 ± 3 days treatment with nasal fluticasone furoate or placebo. The primary outcome was change in exercise induced fall in FEV₁. Secondary outcomes were changes in scores on the asthma control questionnaire, pediatric asthma quality of life questionnaire and FeNO.

Twenty-five children completed the study. Exercise induced fall in FEV₁ decreased significantly in the fluticasone furoate group compared to the placebo group (P = 0.04). Although total quality of life score did not improve after nasal fluticasone furoate treatment, the activity limitation domain score improved significantly within the fluticasone furoate group (P = 0.03). No significant changes were observed in FeNO and asthma control questionnaire scores. We concluded that treatment with an intranasal corticosteroid reduces EIB in children with mild-to-moderate asthma.

Preceding studies on the effect of INCS on measures of BHR and lower airway inflammation have shown conflicting results. The 2010 Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines concluded there was no clear benefit from the use of INCS for asthma. However, since 2010 several new studies have been published and a meta-analysis of both adult and pediatric studies concluded that INCS significantly improve FEV₁, symptom scores, rescue medication use and BHR to methacholine and histamine compared to placebo in steroid-naïve patients. There was no significant change in asthma outcomes with the addition of INCS to ICSs. This may reflect the fact that most patients on ICSs already have good baseline asthma control with less room for improvement with additional INCS. In our study, we included children with a ≥ 10% fall in FEV₁ post-exercise, which is a sign of partially uncontrolled asthma. This may explain why we did demonstrate an improvement in EIB with INCS even in children on ICSs.

Although the effect of INCS on BHR to direct BPTs was statistically significant in the 2013 meta-analysis, it was only small (improvement in PC₂₀ of 0.43 doubling doses; 95% CI 0.16 – 0.69) and not clinically relevant. An indirect BPT might be more suitable to measure the short-term effects of INCS on the lower airways. The effect of INCS on BHR to indirect stimuli was only studied in one other study. Henriksen et al. investigated the effect of intranasal budesonide on EIB in room temperature in steroid-naïve allergic asthmatic children (mean age 11.6y) with chronic nasal obstruction and found a non-significant trend towards a reduction in EIB after 4 weeks treatment. The main differences compared to our study were the inclusion criteria and the condition of the inhaled air during the ECT. We included children with intermittent symptoms of AR
whereas Henriksen selected children with a persistently blocked nose. Furthermore, we used cold, dry air, which amplifies EIB, and may explain the greater difference between groups in our study.

Results of our study support the existence of an important physiologic relation between upper and lower airway inflammation.

It could be argued that treatment effects result from deposition of INCS into the lungs. However, studies assessing drug delivery of intranasal sprays in adults observed no significant deposition of medication into the lower airways.\textsuperscript{94,95} An effect of INCS through systemic absorption is also unlikely, since absorption of fluticasone furoate after intranasal administration is low.\textsuperscript{96}

It was suggested that post nasal drip may lead to aspiration of nasal secretions, spreading inflammatory and infectious cells and mediators to the lower airways. This concept was studied in adults by Bardin et al., who concluded that there was no seeding of the lower airways by nasal secretions.\textsuperscript{97}

Improved nasal breathing may reduce chronic irritation of the lower airways as the nose warms, humidifies and filters the inspired air. In contrast, nasal blockage leads to exposure of the bronchial mucosa to cold, dry air. This has an acute effect on EIB in daily life, as it amplifies evaporative water loss from the airway surface liquid, but it has no effect on EIB in laboratory testing, as patients wear a nose clip during an ECT. Chronic exposure of the bronchial mucosa to cold, dry and unfiltered air increases the deposition of allergen to the lower airways, leading to increased lower airway inflammation and BHR.

Nasal provocation with allergen leads to increased trafficking of eosinophils and progenitor cells from the bone marrow to peripheral blood\textsuperscript{98,99} and thereby increases the blood levels of eosinophils and inflammatory mediators.\textsuperscript{100} These could spread to the lower airways through the systemic circulation. It has been shown in adolescents and adults that INCS can reduce the number of circulating eosinophils in peripheral blood\textsuperscript{101} and surrogate markers of inflammation, such as pulmonary FeNO.\textsuperscript{102} In our study, we found no significant reduction in FeNO after treatment with intranasal fluticasone furoate, but steroid-naïve children tended to have a greater decrease in FeNO than ICS treated children. Previously, Pedroletti et al. described unchanged levels of FeNO in asthmatic children (mean age 13y) with mild to moderate AR treated with INCS added to ICSs.\textsuperscript{103} Conversely, Sandrini et al. found a significant decrease in FeNO in steroid-naïve asthmatic adults after treatment of AR with intranasal triamcinolone.\textsuperscript{102} This may reflect a difference in the severity of allergic inflammation due to ICS use.

In summary, in children with concomitant asthma and AR, treatment of the upper airways with an INCS can improve EIB and asthma outcomes, especially in steroid-naïve
children. This effect is most likely due to a combination of improved nasal breathing and a decreased number of circulating inflammatory cells and mediators.

Further research studying the cells and mediators involved in the pathophysiologic connection between the upper and lower airways could increase our understanding of both asthma and AR. Larger studies in adults and children, with different asthma severity and control status and intermittent vs. persistent AR are required to develop evidence-based integrated treatment strategies.

**PHARMACOLOGIC PHENOTYPING**

Asthma is a heterogeneous disease, which is also exemplified in the variability of individual patients’ responses to medications. Guideline recommendations for treatment are primarily based on evidence from trials comparing mean responses between medication regimens. However, there is a considerable inter-individual variability in response to medications. There is currently a lack of evidence to guide clinicians in selecting the medication most likely to achieve a favorable response in an individual patient.

In the past decade, the Childhood Asthma Research and Education (CARE) Network examined the variability in response to step 2 treatment options (i.e., initial controller therapy with an ICS or LTRA) to assess phenotypic factors indicative of a specific favorable response. They found that children (aged 6-17y) who responded better to ICSs had higher baseline FeNO, blood eosinophils, serum Eosinophil Cationic Protein and IgE and lower FEV₁ and methacholine PC₂₀. A parental history of asthma and prior ICS use also predicted a favorable response to ICSs. Those who responded better to MLK were significantly younger and had a shorter duration of asthma and a higher urinary leukotriene E4 (uLTE4) and uLTE4 / FeNO ratio. These results suggest that an eosinophilic inflammatory phenotype is associated with a better ICS response, and a high CysLT inflammatory phenotype is associated with a better LTRA response.

Subsequently, differential response to step 3 treatment (i.e., step-up therapy in children uncontrolled on low dose ICSs) was analyzed by the CARE network in the Best Add-On Giving Effective Response (BADGER) study. By using a composite outcome measure of exacerbations, asthma control days and FEV₁, 44.1% of asthmatic children (aged 6-17y) responded best to add-on LABA, 26.7% to medium dose ICS (250μg fluticasone twice daily) and 29.2% to add-on LTRA. In a multivariate analysis with data from this study, higher uLTE4 levels were associated with a favorable response to LTRA over LABA step-up therapy and higher impulse oscillometry reactance area with a favorable response of LABA over ICS step-up therapy. There were no apparent baseline predictors of differential responses comparing ICS with LTRA step-up therapy.
Phenotypic variables that predict a favorable response to treatment are dependent on the definition of response that is used, for example an improvement in lung function or asthma control, or the prevention of exacerbations.\textsuperscript{111} As a single dose of MLK and regular daily treatment with MLK both attenuate EIB, we hypothesized that we could pharmacologically phenotype children who would be protected against EIB by MLK by measuring the response to a single dose on EIB.

In chapter 7, we describe a prospective open-label study, in which 24 adolescents on low dose ICSs, aged 12-17 years, with a history of persistent asthma and $\geq 10\%$ post-exercise fall in FEV$_1$, were treated with MLK once daily for 30 ± 4 days. Children performed an ECT 3 times: at baseline, 20h after the first dose and 40-44h after the last dose of 4 weeks treatment. The relationship between the effect of a single MLK-dose and 4 weeks treatment on EIB was analyzed. The positive and negative predictive values of $\geq 25\%$ reduction in area under the curve for 20 min post-exercise (AUC$_{0-20\text{min}}$) after a single dose were calculated.

Twenty-one children completed the study. There was a moderate correlation between the effect of a single MLK-dose and the effect of 4 weeks treatment on AUC$_{0-20\text{min}}$, $r = 0.49$ ($P = 0.011$). The AUC$_{0-20\text{min}}$ decreased significantly after a single MLK-dose ($P = 0.001$), but not after 4 weeks treatment ($P = 0.080$). The positive and negative predictive values of $\geq 25\%$ reduction in AUC$_{0-20\text{min}}$ after a single dose were resp. 84.6\% and 50.0\% ($P = 0.146$). We concluded that the effect of a single MLK-dose on EIB only modestly predicts the effect of 4 weeks treatment on EIB in adolescent asthmatics on low dose ICSs. The single dose response to MLK was stronger than the response to 4 weeks regular treatment, implying that a high adherence is essential to profit from the full protective effect of MLK against EIB. If used on a daily base, MLK offered clinically significant protection against EIB in the large majority (80\%) of children uncontrolled by low dose ICS.

Instead of phenotyping patients responsive to different treatment regimens, pharmacogenetic studies analyze how different genotypes predict individual responses to pharmacological therapies.\textsuperscript{112} Genetic variation can influence the drug response through pharmacodynamic mechanisms (for example variation in the receptor pathway) of pharmacokinetic mechanisms. As described in a previous paragraph, polymorphisms of the $\beta_2$AR can influence the response to LABA treatment. Similarly, polymorphisms of the glucocorticoid pathway and CysLT pathway can influence responses to ICSs and LTRAs.\textsuperscript{113}

In summary, our results suggest that pharmacologic phenotyping of children by measuring the single dose effect on an indirect BPT is not useful for MLK. Studies that assessed phenotypic factors indicative of a favorable response to different step 2 and step 3 treatment options showed no consistent pattern of baseline predictors. Phar-
macrogenetic studies may have the potential to identify genetic polymorphisms that predict a favorable response to treatments.

In future research, larger prospective genotype-stratified studies are required to further explore the impact of genetic variations on therapeutic responses. Furthermore, additional factors influencing variability in treatment response, such as gene-gene interactions and environmental effects (such as smoking) should be identified.

TREATMENT OF EXERCISE INDUCED BRONCHOCONSTRICTION

The presence of EIB in asthmatic children is a sign of partially uncontrolled asthma. Physical activity is the most important trigger for asthma symptoms and EIB has a great impact on a child’s quality of life. Therefore, protection against EIB should be a priority for pediatricians treating asthmatic children.

Non-pharmaceutical treatment options include warming up, maneuvers to pre-warm and humidify the air during exercise, improving cardiovascular condition and losing weight. Furthermore, co-morbid conditions should be treated. For example, allergic asthmatic children with concomitant AR can be treated with an INCS, as described in chapter 6.

Pharmaceutical management of EIB follows guidelines of asthma management in general. However, treatment directed at the prevention of EIB may lead to other step-up or step-down decisions than treatment directed at other outcome measures.

The first step in the treatment of EIB is the prophylactic use of SABAs shortly before exercise. A large body of evidence supports the use of SABAs and it was calculated that post-exercise maximum fall in FEV$_1$ was reduced with 18.99% (95% CI 16.60 – 21.38) by administration of a single dose of SABA. However, daily use of SABAs leads to tolerance and an increase in EIB. If a SABA is needed more than twice a week (even if only before exercise), controller therapy is recommended.

Alternative prophylactic treatment options include LABAs, LTRAs and ICSs. A single dose of a LABA reduces post-exercise maximum fall in FEV$_1$ with 15.60% (95% CI 12.92 – 18.29). A single dose of MLK was reported to reduce maximum fall in FEV$_1$ 4.65 to 17.0% within hours (Table 3). In our study presented in chapter 7, MLK provided 5.8% (95% CI 2.4 – 9.2) reduction in maximum fall in FEV$_1$ 20h after a single dose.

A single dose of an ICS has also been shown to attenuate maximum post-exercise fall in FEV$_1$. The 2012 ATS clinical practice guideline on EIB calculated that the single dose effect of ICSs is only small (mean difference in maximum fall in FEV$_1$ 4.68%, 95% CI -7.01 – 16.37) and pre-exercise ICS use is therefore not recommended. However, in two studies the effect of a high dose of 1 mg Fluticasone Propionate in asthmatic children
(aged 8-16y) was reported, and this dose attenuated the maximum fall in FEV₁ with resp. 9.5% (95% CI -1.8 – 20.8)¹¹⁹ and 9.9% (95% CI 0.9 – 18.8)¹²⁰

Controller treatment options include ICSs, LABAs and LTRAs. The first step is usually a low dose ICS. Daily treatment with ICSs for >4 weeks has been shown to reduce maximum post-exercise fall in FEV₁ with 10.98% (95% CI 7.11 - 14.86%).¹¹⁶ Daily treatment with MLK provides similar protection against EIB, as it reduces maximum post-exercise fall in FEV₁ with 10.70% (95% CI 7.15 - 14.25%).¹¹⁶

In our study presented in chapter 7, MLK as add-on therapy to ICSs provided only 4.8% (95% CI -1.9 – 10.9) reduction in maximum fall in FEV₁ after 4 weeks treatment. However, we performed the ECT 40-44h after the last dose of MLK, whereas previous pediatric studies performed an ECT 10-24h after the last dose.⁶¹,⁶³,¹²¹ As the acute antagonistic effect of MLK wanes within 36h, a high compliance is essential to profit from the full protective effect against EIB.

LABAs are less effective for long-term treatment of EIB, as their bronchoprotective effect decreases with daily use.¹¹⁷ As described in chapter 3 and 4, many children even profit from the discontinuation of LABA treatment. Studies directly comparing the protective effects of regular daily treatment with LABAs and LTRAs against EIB, with or without concomitant ICSs, consistently favor LTRAs (Table 4).

Doubling the dose of ICSs does not result in significant differences in the airway response to exercise after 3 weeks treatment¹³²,¹³³, but quadrupling the dose of ICSs resulted in 26.1 - 34.8% protection in AUC¹³³. However, high dose ICSs may cause more side effects than LTRAs.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age</th>
<th>Intervention</th>
<th>Time interval after single dose</th>
<th>Absolute reduction % fall FEV₁</th>
<th>95% CI</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coreno 2000¹²²</td>
<td>10</td>
<td>29 ± 3</td>
<td>MLK 10 mg</td>
<td>1-12h</td>
<td>17</td>
<td>n/r</td>
<td>65.4</td>
</tr>
<tr>
<td>Peroni 2002¹²³</td>
<td>19</td>
<td>7-13</td>
<td>MLK 5 mg</td>
<td>2-24h</td>
<td>8.9</td>
<td>3.6-14.2</td>
<td>47.6</td>
</tr>
<tr>
<td>Pearlman 2006¹²⁴</td>
<td>51</td>
<td>15-45</td>
<td>MLK 10 mg</td>
<td>2-24h</td>
<td>11.5</td>
<td>0.3-14.7</td>
<td>51.6</td>
</tr>
<tr>
<td>Peroni 2011¹²⁵</td>
<td>69</td>
<td>6-14</td>
<td>MLK 5 mg</td>
<td>1-8h</td>
<td>9.9</td>
<td>n/r</td>
<td>34.7</td>
</tr>
<tr>
<td>Philip 2007¹²⁶</td>
<td>47</td>
<td>15-44</td>
<td>MLK 10 mg</td>
<td>2-24h</td>
<td>8.7</td>
<td>n/r</td>
<td>39.9</td>
</tr>
<tr>
<td>Philip 2007¹²⁷</td>
<td>53</td>
<td>15-41</td>
<td>MLK 10 mg</td>
<td>2h</td>
<td>5.8</td>
<td>2.7-8.9</td>
<td>33.1</td>
</tr>
<tr>
<td>Rundell 2005¹²⁸</td>
<td>9</td>
<td>19 ± 1</td>
<td>MLK 10 mg</td>
<td>6-8h</td>
<td>10.3</td>
<td>n/r</td>
<td>64.8</td>
</tr>
<tr>
<td>Rundell 2005¹²⁹</td>
<td>11</td>
<td>23 ± 7</td>
<td>MLK 10 mg</td>
<td>6h</td>
<td>11.8</td>
<td>n/r</td>
<td>52.7</td>
</tr>
<tr>
<td>Mastalerz 2002¹³⁰</td>
<td>19</td>
<td>19-54</td>
<td>MLK 10 mg</td>
<td>1h</td>
<td>12.3</td>
<td>n/r</td>
<td>54.7</td>
</tr>
<tr>
<td>Wasfi 2011¹³¹</td>
<td>66</td>
<td>4-14</td>
<td>MLK 4 / 5 mg</td>
<td>2-24h</td>
<td>4.65</td>
<td>0.75-8.55</td>
<td>23.3</td>
</tr>
</tbody>
</table>

CI = confidence interval, MLK = montelukast, n/r = not reported
In summary (Table 5), treatment of asthmatic children directed at the prevention of EIB may lead to other decisions than treatment directed at other outcome measures.

Based on the currently available evidence, we suggest the following steps for treatment of EIB in children:

All children: Non-pharmaceutical treatment (improve cardiovascular condition, lose weight). Consider treatment of co-morbid conditions, such as AR with INCS. Check inhalation technique and compliance.

1. Pre-exercise prophylactic SABA. Step up if SABA is needed more than twice per week.
2. Start daily low dose ICS.
   Alternative option: monotherapy with daily LTRA. This provides similar protection against EIB, but has less effect on other outcome parameters of asthma.
3. Add daily LTRA.
   Alternative option: double dose of ICSs. There are no studies comparing the effect of double dose ICS vs. LTRA on EIB, but a double dose of ICSs has a better effect on other outcome parameters of asthma.
4. Refer to expert.

### Table 4. Overview of studies comparing the effect of treatment with a LABA vs. a LTRA on post-exercise maximum % fall in FEV₁

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects Intervention ICS Duration</th>
<th>Absolute reduction % fall FEV₁</th>
<th>95% CI</th>
<th>% protection</th>
<th>P-value of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stelmach 2008&lt;sup&gt;53&lt;/sup&gt;</td>
<td>40 6-18 Bud+Form Bud+MLK</td>
<td>100% 4 wk</td>
<td>7.7</td>
<td>2.6-2.8</td>
<td>28.9</td>
</tr>
<tr>
<td>Fogel 2010&lt;sup&gt;51&lt;/sup&gt;</td>
<td>145 6-14 Flu+Salm Flu+MLK</td>
<td>100% 4 wk</td>
<td>11.3</td>
<td>9.3-13.3</td>
<td>45.0</td>
</tr>
<tr>
<td>Storms 2004&lt;sup&gt;54&lt;/sup&gt;</td>
<td>119 15-58 Flu+Salm Flu+MLK</td>
<td>100% 4 wk</td>
<td>3.1</td>
<td>n/r</td>
<td>22.8</td>
</tr>
<tr>
<td>Villaran 1999&lt;sup&gt;56&lt;/sup&gt;</td>
<td>197 15-45 Salm MLK</td>
<td>10% 4 wk</td>
<td>7.3</td>
<td>4.1-10.5</td>
<td>23.6</td>
</tr>
<tr>
<td>Edelman 2000&lt;sup&gt;55&lt;/sup&gt;</td>
<td>177 15-45 Salm MLK</td>
<td>No 8 wk</td>
<td>11.4</td>
<td>n/r</td>
<td>31</td>
</tr>
</tbody>
</table>

*Bud = budesonide, CI = confidence interval, Flu = fluticasone, Form = formoterol, ICS = inhaled corticosteroid, MLK = montelukast, NS = non-significant, Salm = salmeterol*
Table 5. Effects of different medications used in the treatment of EIB

<table>
<thead>
<tr>
<th></th>
<th>Acute effect single dose</th>
<th>Effect regular daily treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SABA</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>LABA</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>ICS</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>LTRA</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

ICS = inhaled corticosteroid, LABA = long acting β2-agonist, LTRA = leukotriene receptor antagonist, SABA = short acting β2-agonist
REFERENCES


19. Chauhan BF, Ducharme FM. Anti-leukotriene agents compared to inhaled corticosteroids in the management of recurrent and/or chronic asthma in adults and children. Cochrane Database Syst Rev 2012;5:CD002314.


