Indirect bronchial provocation tests in childhood asthma
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Chapter 2

Mannitol and Exercise Challenge Tests in Asthmatic Children

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ABSTRACT

Background
Bronchial hyperresponsiveness (BHR), a characteristic feature of asthma, can be assessed through standardized bronchial provocation tests (BPTs). Exercise as a BPT is used in diagnosing and monitoring exercise induced bronchoconstriction (EIB). Recently a novel osmotic BPT has been developed, using dry powder mannitol. The aim of this study was to investigate the clinical utility of the mannitol challenge to identify asthmatic children with EIB.

Materials and Methods
Thirty-three clinically stable children, aged 9-18 years, with a history of EIB, performed both mannitol and exercise provocation challenges. Data were composed of a cross tabulation comparing the reaction on exercise provocation challenge to mannitol challenge. Correlations between post-exercise fall in FEV₁ and response-dose ratio (RDR) and PD₁₅ of mannitol were calculated.

Results
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. Children on long-acting β₂-agonists (LABAs) were significantly (\( P < 0.05 \)) more likely to have a positive response on the mannitol challenge. Positive and negative predictive values of the mannitol challenge for EIB were 69% and 92%.

Conclusion
Mannitol challenge appears to be a suitable alternative for an exercise provocation test to assess EIB in asthmatic children. Given the negative predictive value of 92%, it is especially useful to exclude EIB.
INTRODUCTION

Asthma is considered a chronic disease of the airways, characterized by inflammation, obstruction and hyperresponsiveness. Bronchial hyper-responsiveness (BHR) is the transient increase in airflow limitation in response to the exposure to a bronchoconstrictor stimulus. BHR leads to recurrent episodes of wheezing, chest tightness, breathlessness, and coughing. BHR can be assessed through the administration of bronchoconstrictor stimuli in standardized bronchial provocation tests (BPTs). BPTs are classified into two categories: ‘direct’ and ‘indirect’. Direct stimuli induce airflow limitation through a direct action on the effector cells, such as smooth muscle cells, bronchial vascular endothelial cells and mucus producing cells. Direct stimuli include pharmacological agents such as histamine and methacholine. Although these tests are sensitive for identifying BHR, they are not specific for asthma. Subjects with other pulmonary diseases and even healthy subjects demonstrated BHR to these agents. Indirect stimuli act on inflammatory cells that interact with the effector cells, such as mast cells and neuronal cells. Pro-inflammatory mediators and/or neurotransmitters released by these inflammatory cells act on the effector cells to cause airflow limitation. Indirect stimuli are more specific for identifying asthma, as they employ inflammatory cells resident in the asthmatic airway wall.

Exercise is used as a BPT to diagnose and monitor exercise induced bronchoconstriction (EIB) in children. EIB is defined as an acute, reversible bronchial obstruction occurring immediately after and occasionally during physical exercise. EIB is a highly prevalent symptom in adults and children with clinical asthma.

Drying of the airway wall during exercise is now considered an essential determinant to provoke EIB. The osmolarity of the airway surface liquid increases as water is lost by evaporation, which causes a shift of water from the epithelial cells to the airway surface. This osmotic gradient induces the release of mediators that cause bronchoconstriction from inflammatory cells resident in the airway wall.

The lack of sensitivity and specificity of self-reported symptoms necessitates the use of objective measures of lung function to confirm a diagnosis of EIB. Since exercise per se is not essential to induce the aforementioned osmotic gradient, other BPTs influencing the osmolarity of the airway surface liquid have been developed. These include eucapnic hyperpnoea of dry air and aerosols of hypertonic saline. An osmotic BPT using dry powder mannitol was developed and studied during the past decade. Mannitol is effective in identifying asthmatic adults who are responsive to hypertonic saline and exercise and children who are responsive to methacholine. Responses to mannitol are reproducible and can be used to monitor asthma therapy with corticosteroids. The aim of this study was to investigate the clinical utility of the mannitol challenge to identify asthmatic children responsive to an exercise challenge.
Inhaled corticosteroids (ICSs) can reduce responsiveness to mannitol and exercise. Regular use of long-acting β2-agonists (LABAs) can increase the airway response to BPTs, possibly due to a desensitization of β2-adrenoreceptors on the cell membrane of mast cells. Therefore, we compared the responses to mannitol and exercise provocation of children receiving treatment with LABAs or ICSs to those of children not using these medications.

MATERIALS AND METHODS

Subjects
Thirty-three children with a history of allergic asthma and EIB, aged 9-18 years were recruited from the outpatient clinic of the pediatric department of the Medisch Spectrum Twente. Clinically stable, otherwise healthy children, with a forced expiratory volume in one second (FEV₁) of at least 70% of predicted normal value, were included. Children had to be able to run on a treadmill and perform reproducible spirometry (i.e., coefficient of the predicted value variation in three of five consecutive measurements < 5%). Children were required to withhold the use of intranasal or systemic corticosteroids for 4 weeks; antihistamines, cromoglycates and anticholinergics for 2 weeks; LABAs and ICSs for 24h and short-acting β2-agonists for 8h before the tests. No vigorous exercise was permitted for 4h before the exercise challenge. All children and their parents or legal guardian signed an informed consent.

Study design
The study is of an observational prospective cohort design. All children first performed an exercise challenge in cold air, with pulmonary function tests before and after the challenge, at the local ice rink, Euregio Kunstijsbaan, Enschede. Standard pulmonary function test in this study consisted of a duplicated full flow-volume loop. The second visit was scheduled in the local pulmonary function laboratory within 4 weeks after the exercise challenge. During the second visit children performed a mannitol challenge with pulmonary function tests before the challenge and after each subsequent dose of mannitol. The protocol was approved by the Medical Ethics Committee of the Medisch Spectrum Twente.

Pulmonary function test
A Masterscope® Jaeger®, (IBM PS 235X) was used to measure pulmonary volumes and flow-volume loops. This spirometer was calibrated before testing. The expiratory flow-volume loop was recorded by instructing the children to perform a maximal expiratory effort from inspiratory vital capacity to residual volume. All measurements were per-
formed in duplicate. Pulmonary function was calculated from the best curve. FEV$_1$ was used as an index of BHR.

**Exercise challenge**

Exercise challenge testing was performed by running with nose clipped on a treadmill (Reebok®, TR1 premium run) using a standardized protocol.$^{24}$ Baseline spirometry was performed and the pre-challenge FEV$_1$ documented as the best FEV$_1$ of two measurements. During the test, heart rate was continuously monitored by a radiographic device (Polar Sport Tester). Cold, dry air was obtained by testing in the local ice rink with a constant temperature of 1°C. The test started with running at low speed on the treadmill with an incline of 10%. The running speed of the treadmill was increased, raising the heart rate to approximately 90% of the predicted maximum ((220-age) x 0.9). This speed was maintained for a maximum of 6 min. After the exercise challenge, flow-volumes were measured at $t = 1, 3, 6, 9, 12, 15$ and 20 min. A fall of > 15% in FEV$_1$ from baseline was considered a positive response.

**Mannitol challenge**

Baseline spirometry was performed and the prechallenge FEV$_1$ documented as the best FEV$_1$ of two measurements. The dose protocol consisted of 0 (empty capsule acting as a placebo), 5, 10, 20, 40, 80, 160, 160, and 160 mg mannitol. Dry powdered mannitol was supplied in capsules of 0, 5, 10, 20, and 40 mg. Doses of 80 and 160 mg were obtained by inhaling two or four capsules of 40 mg. The dry powder device used for inhalation was an Osmohaler® inhaler device.

The challenge started with a capsule of 0 mg mannitol. Children were asked to inhale from the device from near to functional residual capacity to near to total lung capacity and to subsequently hold their breath for 5 sec. Children had a nose clip on during inhalation and were asked to exhale through their mouth to minimize deposition in the nasopharynx. Sixty seconds after inhalation flow-volumes were measured in duplicate and the best FEV$_1$ values were retained for analysis. After flow-volume measurement children were given the subsequent dose within 60 sec. The procedure was repeated for each dose step. At each dose step the presence of cough was recorded.

Baseline FEV$_1$ was calculated from pre-test flow-volume loops. A fall of >15% in FEV$_1$ from baseline was considered a positive response. The test ended when a >15% fall in FEV$_1$ occurred or the cumulative dose of 635 mg mannitol had been administered. The lowest dose of mannitol that provoked a cough response was documented.
Statistical analysis

Best values of spirometric measurements of FEV₁ were used for statistical calculations. Baseline % predicted FEV₁ before both challenges were expressed as mean ± SD and compared using Student’s paired t-test.

For children positive on the mannitol challenge, the provoking dose required to cause a 15% fall in FEV₁ (PD₁₅, measuring airway sensitivity) was calculated by linear interpolation. The response-dose ratio (RDR; measuring airway reactivity) was calculated by taking the final percent fall in FEV₁ recorded and dividing it by the cumulative dose of mannitol administered to induce that percent fall.

Geometric means (Gmean) ± 95% confidence intervals (CI) were calculated using the log-transformed values of PD₁₅ and RDR. Pearson’s correlation (r_p) and significance values were used to investigate the relationship between log RDR and log PD₁₅ and post-exercise fall in FEV₁.

Positive and negative predictive values for mannitol challenge to predict EIB were calculated. Responses of children receiving treatment with LABAs or ICSs were compared to those of children not using these medications using a χ²-test.

The relationship between the lowest dose of mannitol that provoked a cough response and log RDR and log PD₁₅ was investigated using Pearson’s correlation and significance values.

RESULTS

Research population consisted of 33 children. Two children did not perform both tests: one child had a baseline FEV₁ of < 70% predicted value and one was not able to perform reproducible spirometry. During mannitol challenge, three children (9.7%) experienced a cough persistent enough to terminate the challenge. Of the 28 children who completed both challenges, three children had a change in medication between the challenges and were excluded. Data from 25 children were used for comparison of the tests (Table 1). Among them were 17 boys and 8 girls. Mean age (±SD) was 12.4 ± 2.0 years. Baseline FEV₁ before mannitol had a normal distribution with a mean (±SD) of 97.4 ± 16.6% predicted value, which was not significantly different from baseline FEV₁ before exercise (99.3 ± 20.1% predicted value).

Thirteen children (52%) had a positive response on the mannitol challenge. Their dose-response curves are shown in Fig. 1. Mean (±SD) difference between FEV₁ at baseline and FEV₁ after 0 mg mannitol was 0.024 (±0.096) L/sec, which was not significant using Students’ paired t-test.

Geometric mean [95% CI] for the RDR for mannitol was 0.0086 [CI: 0.0031-0.0248]. Pearson’s correlation between log-transformed RDR for mannitol and fall in FEV₁ after
exercise was $r_p = 0.666 (P < 0.001)$ (Fig. 2). Geometric mean [95% CI] for the PD$_{15}$ for children positive on the mannitol challenge was 84 mg [CI: 26-266]. There was no significant relationship between the log PD$_{15}$ of mannitol and the percent fall in FEV$_1$ after exercise ($r_p = -0.29, P = 0.34$) (Fig. 3).

No significant correlation was found between the lowest dose of mannitol that provoked a cough response and log PD$_{15}$ and log RDR to mannitol. Of the three children that terminated the mannitol challenge due to coughing, two were unresponsive to exercise, suggesting that the severity of the cough was not related to the presence of EIB.

**Table 1.** Research population: Baseline characteristics and responses to challenges

<table>
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<tr>
<th>Gender</th>
<th>Use of ICS</th>
<th>Use of LABA</th>
<th>Baseline FEV$_1$ % pred.</th>
<th>Mannitol PD$_{15}$ (mg)</th>
<th>Mannitol % fall in FEV$_1$</th>
<th>Exercise % fall in FEV$_1$</th>
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<td>10.3</td>
<td>+</td>
<td>85</td>
<td>151</td>
<td>15.3</td>
</tr>
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</table>

*As measured before the mannitol challenge. FEV$_1$ = forced expiratory volume in 1 s, ICS = inhaled corticosteroid, LABA = long-acting β2-agonist, PD$_{15}$ = provoking dose to cause a 15% fall in FEV$_1$. 
Fig. 1. Individual dose-response curves relating % fall in FEV\textsubscript{1} after mannitol to the cumulative dose of mannitol inhaled in children responsive to mannitol.

\textit{FEV\textsubscript{1} = forced expiratory volume in 1s.}

Fig. 2. Relationship between log-transformed response-dose ratio to mannitol and % fall in FEV\textsubscript{1} after exercise. Pearson's correlation coefficient, \( r_p = 0.666, P < 0.001 \).

\textit{FEV\textsubscript{1} = forced expiratory volume in 1s, RDR = response dose ratio, % fall in FEV\textsubscript{1} / cumulative dose.}
Ten children (40%) had a positive response on the exercise challenge. Nine (36%) were positive on both challenges and 11 (44%) negative on both challenges (Table 2). Positive predictive value of the mannitol challenge for EIB was 69%. Negative predictive value was 92%.

Using $\chi^2$-test children on LABAs were significantly ($P < 0.05$) more likely to have a positive response on the mannitol challenge. Using Students’ independent samples t-test, there was no significant difference ($P = 0.258$) in mean fall in FEV$_1$ after mannitol for children on LABAs. There was no difference in response to mannitol or exercise for children on ICSs.

Median time to perform a mannitol challenge was 24 min (range 6-30). Median time for children who were responsive to mannitol was 16 min (range 6-24). Mean maximum percent fall in FEV$_1$ after mannitol was 13.3 ± 7.3%. None of the children had a fall of >30% in FEV$_1$. FEV$_1$ recovered spontaneously in eight children (62%) and after the administration of a bronchodilator in all children within 10 min.

**Table 2.** Responses to mannitol and exercise provocation challenges

<table>
<thead>
<tr>
<th></th>
<th>Exercise positive</th>
<th>Exercise negative</th>
<th>Total</th>
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<tbody>
<tr>
<td>Mannitol positive</td>
<td>9</td>
<td>4</td>
<td>13</td>
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<tr>
<td>Mannitol negative</td>
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</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>15</td>
<td>25</td>
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</table>
Median time for an exercise challenge was 40 min (range 30-70). In children positive on the exercise challenge, maximum percent fall in FEV$_1$ occurred 2.9 ± 2.0 min post-exercise. Mean maximum fall in FEV$_1$ was 18.9 ± 15.3%, and six children had a fall of >30% in FEV$_1$.

DISCUSSION

The mannitol challenge can be used in children as a screening tool to assess EIB. There was a significant relationship between reactivity to inhaled mannitol, measured by log RDR, and fall in FEV$_1$ after exercise. Sensitivity to inhaled mannitol, measured by log PD$_{15}$, was not related to fall in FEV$_1$ after exercise. There were three children (9.7%) who experienced a cough severe enough to terminate the challenge. Positive and negative predictive values of the mannitol challenge for EIB were 69% and 92%. All subjects positive on the exercise challenge had a positive response to mannitol, except for one subject, who had only a borderline (17.5% fall in FEV$_1$) response to exercise. This indicates that the mannitol challenge can be useful as a diagnostic test to exclude EIB.

This study was the first to demonstrate that the mannitol challenge is valuable in identifying EIB in children. Similar results have been observed in adults. In a study by Brannan et al. among 23 asthmatic adults responsive to exercise, all but one were responsive to mannitol (sensitivity 95.7%).

Especially in children, it is important to identify and treat EIB. Physical exercise not only has physical benefits for asthmatic children, but also plays a key role in their social and neuromotor development. The mannitol challenge was already recognized as a useful test in identifying children with currently active asthma and those who are responsive to methacholine. It showed good repeatability in asthmatic children and its safety was demonstrated in 592 asthmatic and non-asthmatic subjects, including 126 children.

This study demonstrated a significant relationship between the log RDR to mannitol and fall in FEV$_1$ after exercise. The RDR data and calculations were previously described in a phase 3 study by Brannan et al. and Koskela et al. found that log RDR to mannitol correlated with log RDR to histamine provocation challenge and that it was reduced by treatment with budesonide. The log RDR to mannitol could therefore be used to assess the effect of treatment.

The mannitol challenge is a progressive dose-response test that is terminated when FEV$_1$ falls >15% from baseline. This prevents a vigorous fall in FEV$_1$ (>30%), which is an important safety feature. In this study, six children experienced a fall in FEV$_1$ of >30% after the exercise challenge, while none had such a fall in FEV$_1$ after the mannitol challenge.

The mannitol challenge was faster to perform than the exercise challenge. Median time for a mannitol challenge was 24 min (range 6-30). Median time for children re-
responsive to mannitol was 16 min, which is similar to challenge time reported in other studies.\textsuperscript{15,16} Median time for an exercise challenge was 40 min (range 30-70). This was mostly attributable to a prolonged recovery time (> 10 min after the administration of a β2-agonist) in nine children. Most of them (eight) were on long-term treatment with a LABA, which could have caused the prolonged recovery. Storms et al.\textsuperscript{25} found that after 4 weeks of treatment with salmeterol approximately 20% of patients failed to return to their baseline status 30 min after rescue β2-agonist administration.

The protocol used for the exercise challenge slightly deviates from that suggested by the ATS.\textsuperscript{24} A different measurement of reproducibility was used, that appears more suitable for children: a <5% difference between the two highest FEV\textsubscript{1} measurements.\textsuperscript{26,27} The criterion for a positive response to exercise is controversial. A fall of 10% or more 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.\textsuperscript{28} when testing 8-11 year old healthy children in the field, found that the fall in FEV\textsubscript{1} for 1.96 SD above the mean in this ‘normal’ group was 15.3%. Godfrey et al.\textsuperscript{5} suggested an optimal cut-off point of 13% for children and adolescents. Because the environmental circumstances in which the test was performed were more similar to outdoor circumstances than to laboratory settings, a cut-off value of 15% was used. A cut-off value of 10% would change the results. Six children that were considered negative on the exercise challenge are then considered positive. This would lead to a reduction in false positive subjects on the mannitol challenge from 4 to 2 children, raising the positive predictive value to 85%. The number of false negative subjects would increase, reducing the negative predictive value to 58%.

In this study we used the FEV\textsubscript{1} value at baseline prior to the mannitol challenge to calculate the percent fall in FEV\textsubscript{1} and PD\textsubscript{15} values. The standardized procedure is to use the FEV\textsubscript{1} value measured after the 0 mg capsule. There was no significant difference between FEV\textsubscript{1} measured at baseline and after 0 mg mannitol. Therefore, using FEV\textsubscript{1} values measured after the 0 mg capsules would not have changed the results of this study.

Mannitol challenge was performed within 4 weeks after the exercise provocation challenge. The length of this interval could have influenced the results of this study. During these 4 weeks, two children had a change in medications and were excluded from the study. None of the other children had an asthma exacerbation or a respiratory tract infection during this period. Baseline FEV\textsubscript{1} measured before both challenges was not significantly different.

Mannitol was generally well tolerated and there were no serious adverse events reported. However, all children experienced a cough. This was usually a mild cough, but in three children it was a reason to terminate the challenge. These three children were excluded because their coughing was persistent enough to interfere with the fixed
time schedule of the mannitol challenge protocol. Coughing can be a limitation of the challenge when it prolongs the time between inhalation and spirometry. The mannitol challenge should be performed quickly, as the rate of change in osmolarity is suggested to be the determinant of BHR.\textsuperscript{15} Prolonging the time could therefore lead to a milder osmotic stimulus and give falsely negative results.

Mannitol provoked coughing was described previously and can be divided in an immediate cough and one occurring after a time lag. In the three children excluded from the study coughing occurred immediately after inhalation, suggesting it was due to the impaction of powder on the oropharynx.\textsuperscript{14,15} The Osmohaler\textsuperscript{a} is a low-resistance inhaler, permitting inhalation at high flow rates, which increases the rate of impaction on the oropharynx. Coughing occurring after a time lag can be attributed to the deposition of powder in the lower respiratory tract. Nerve fibers can be stimulated by hyperosmolality\textsuperscript{29,30}, which may lead to coughing. Koskela et al.\textsuperscript{31} found coughing to be related to the increased sensitivity of the asthmatic airways, making it useful in the diagnosis of asthma.

Out of the three children that were excluded, two were unresponsive to exercise, suggesting that the severity of the cough was not related to the extent of EIB. Furthermore, we recorded the lowest dose that provoked a cough response in all children and found no correlation between the dose that first provoked a cough response and log PD\textsubscript{15} and log RDR to mannitol.

The development of the mannitol provocation challenge was based on the theory that EIB is provoked by an increase in airway osmolarity. Water is lost by the humidifying of large quantities of dry air during exercise, causing a shift of water from the epithelial cells to the airway surface. Loss in cell volume leads to intracellular events that cause the release of inflammatory mediators from mast cells and eosinophils.\textsuperscript{10} The intracellular events that occur are the same whether the hyperosmolarity is caused by evaporation or by adding a hyperosmolar stimulus like mannitol. An essential difference however is the rate of change in osmolarity. Mannitol might be a more potent dehydrating stimulus to the airway than exercise. This could explain why some children with a positive response on the mannitol challenge were negative on the exercise challenge. Another possible explanation for this is that exercise induces a variety of other physiologic changes, such as the release of adrenal hormones (steroids and catecholamines), protecting the airway from narrowing.

Children on LABAs were significantly (\(P < 0.05\)) more likely to have a positive response on the mannitol challenge. These children did not have a more sensitive response to exercise. An increased airway response to BPTs after regular use of \(\beta\)\textsubscript{2}-agonists has been reported in other studies.\textsuperscript{20-22} Although single doses of LABAs can inhibit mediator release from mast cells, it was proposed that regular use desensitizes \(\beta\)\textsubscript{2}-adrenoreceptors on
Mannitol test in children

Mannitol test in children

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mast cells and thereby decreases cell membrane stability. This hypothesis is supported by the finding of a significant increase in mast cell mediator tryptase following an allergen challenge after treatment with salbutamol compared to placebo. A decreased stability of mast cells could explain the more sensitive response to mannitol.

In this study, half of the children using ICSs were still positive on either one or both of the challenges, although ICSs have been shown to reduce responsiveness to mannitol and exercise. These children might need an adjustment in their medication or dosage.

Mannitol challenge appears to be a suitable alternative for an exercise provocation test to assess EIB in asthmatic children. Given the negative predictive value of 92%, it is especially useful to exclude EIB. It is a practical option for use as an office-based test and has the potential to appeal to the wider health-care community, because of the simplicity and inexpensive nature of the equipment used. It is faster and easier to perform than an exercise provocation test, and does not require specifically trained personnel or specialized equipment such as treadmills and/or a dry air source. It does not require strenuous exercise or specific motor skills from the patient, and is therefore useful to assess EIB in disabled children. Furthermore, it has a built-in safety feature of a progressive dose-response challenge; the test can be stopped before severe falls in FEV₁ occur. Further research is required to assess the clinical utility of the mannitol challenge in monitoring asthma therapy in children and to define the sensitivity and specificity of the mannitol challenge in a larger study population of asthmatic children.
REFERENCES


