Supplementary text:

Methods of bronchial provocation tests

For all bronchial provocation tests, baseline FEV_1 was measured in triplicate using ATS criteria for paediatric lung function testing [1] and the best measurement was recorded. Children were excluded from the challenge if their baseline FEV_1 was ≤65% of predicted or if they were unwilling to cooperate. If 15 minutes after the bronchial provocation test, FEV1 had not returned within 5% of baseline or in case of dyspnoea, salbutamol 100 μg (2-4 puffs Ventolin® pMDI via spacer) was given to reverse the bronchoconstriction.

Exercise provocation test

The children performed the exercise challenge using a treadmill (T-2100, GE Healthcare, Freiburg, Germany) or a bicycle ergometer (ER Ergoselect 200, Ergoline GmbH, Bitz, Germany) for 8 min, inspiring room air according to published ATS and ERS guidelines [2, 3]. At one site, children chose between treadmill and bicycle, at the other only a treadmill was available. We performed exercise testing under controlled conditions (maintaining inspired air temperature at 20–25°C and humidity of <10 mg water/L) [4] and measured heart rate and oxygen saturation by pulse oximeter with a forehead sensor (Nellcor N595 OxiMax, Tyco Healthcare, Neustadt/Donau, Germany). After baseline spirometry we started exercise testing at 60% target workload (defined as Watt = measured FEV_1 x 53.76-11.07), rapidly increasing workload aiming at 75% of the target in the second minute, 90% in the third minute, and 100% in the fourth minute, sustaining the latter for ≥4 min. We increased workloads more rapidly if the heart rate was not expected to reach at least 85% of the predicted maximum (220-age in years).[2] Spirometry was performed 1, 3, 5, 7, 10, and 15 min after exercise, in duplicate [5].

We reported the results as the maximum fall of FEV_1 during the exercise provocation test.

Methacholine provocation test

The children performed the methacholine provocation test based on the Five-Breath Dosimeter Protocol [2, 4]. They first inhaled NaCL 0.9% to measure baseline values, then they inhaled stepwise 0.05mg, 0.05mg, 0.2mg, 0.3mg, 0.6mg and 1.2mg of methacholine (cumulative dose of 2.4 mg in children <14 years old) via a nebulizer. Children older than 14 years old had an additional inhalation step with a cumulative dose of 3.2mg methacholine. At end exhalation during tidal breathing, the children inhaled slowly and deeply from the nebulizer. The dosimeter was triggered after the inhalation begins, and the subject was encouraged to continue inhaling slowly and to hold the breath for another 5 seconds. This step was repeated for a total of five inspiratory capacity inhalations which should not take more than 2 minutes. The challenge was terminated when FEV_1 fell by 20% or more, or the highest dose was given. Lung function was measured in 5-min intervals until it had returned to within 5% of the baseline value. We reported the results of the methacholine provocation test as provocation dose causing a 20% decrease of FEV_1 from baseline (PD-20).

Mannitol dry powder provocation test

The mannitol provocation test was performed according to the protocol recommended by Anderson et al. [6], with slight modifications [7, 8]. Baseline FEV_1 was measured in triplicate and the highest of these measures was recorded. The mannitol dry powder (MDP) provocation test (AridolTM, Pharmaxis, French Forests, New South Wales, Australia) was conducted as described in our previous study [8]. The children were asked to inhale the

contents of an MDP capsule through the delivery device (OsmohalerTM). The following dosing steps were used: 0 mg (empty capsule acting as a placebo to measure baseline FEV_1), 5, 10, 20, 40, 80, 160, 160, and 160 mg. We administered multiples of 40 mg capsules to achieve doses of 80 mg and more. After each dose, children performed a 5-sec breath-hold, followed one minute later by spirometry in duplicate, and the higher FEV1 was recorded. If the children had a decrease in FEV_1 >10%, then the dose producing this was repeated for safety reasons. This process was repeated until either FEV_1 had fallen by 15% or the subject had reached the maximum dose (cumulative dose of 635 mg mannitol). We reported the results of the mannitol provocation test as provocation dose causing a 15% decrease of FEV_1 from baseline (PD-15).

References

1. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-1136.
2. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, et al. Guidelines for methacholine and exercise challenge testing-1999. Am J Respir Crit Care Med. 2000;161(1):309-329.
3. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J. 1993: 16: 53-83.
4. Barben J RJ. Measurement of bronchial responsiveness in children. In: Hammer J, Eber E, editors. Paediatric pulmonary function testing. Prog Respir Res. Basel: Karger;2005;33:125-136.
5. Riedler J. Nonpharmacological challenges in the assessment of bronchial responsiveness. Eur Respir Mon. 1997: 5: 115-135.
6. Anderson SD, Brannan J, Spring J, Spalding N, Rodwell LT, Chan K, Gonda I, Walsh A, Clark AR. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. Am J Respir Crit Care Med. 1997;156(3.1):758-765.
7. Barben J, Roberts M, Chew N, Carlin JB, Robertson CF. Repeatability of bronchial responsiveness to mannitol dry powder in children with asthma. Pediatr Pulmonol. 2003: 36(6): 490-494.
8. Barben J, Kuehni CE, Strippoli MP, Schiller B, Hammer J, Trachsel D, Swiss Paediatric Respiratory Research Group. Mannitol dry powder challenge in comparison with exercise testing in children. Pediatr Pulmonol. 2011;46:842–848.