

Comparison of bronchial challenge with ultrasonic nebulized distilled water and hypertonic saline in children with mild-to-moderate asthma

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Comparison of bronchial challenge with ultrasonic nebulized distilled water and hypertonic saline in children with mild-to-moderate asthma. C. Wojnarowski, K. Storm Van's Gravesande, J. Riedler, I. Eichler, C. Gartner, T. Frischer. ©ERS Journals Ltd 1996.

ABSTRACT: There is still controversy about the most suitable method to measure bronchial hyperresponsiveness in children. In epidemiological surveys, nonisotonic aerosols are being used increasingly for bronchial provocation testing. Our aim was to study the acceptability, safety and correlation between two published bronchial challenge tests.

Two standardized protocols - the inhalation of hypertonic saline (HS) and ultrasonically-nebulized distilled water (UNDW) - were performed in 36 children: 19 patients with the clinical diagnosis of mild-to-moderate asthma (7–12 yrs of age), and 17 control subjects (8–18 yrs of age). HS challenge involved stepwise inhalation of 4.5% saline (for 0.5, 1, 2, 4 and 8 min), whereas challenge with UNDW was performed as a single step protocol with 10 min inhalation of cold UNDW. Asthma medication was withheld prior to challenge testing.

Thirty five subjects completed both challenge tests (one asthmatic patient did not return after UNDW challenge) in random order within a 7 day time interval. For HS a $\geq 15\%$ reduction in forced expiratory volume in one second (FEV₁) from baseline was considered a positive response, and for UNDW a $\geq 10\%$ decrease. In 13 of the 19 asthmatic patients, but in none of the controls, a positive response was observed for UNDW. Fifteen out of 18 patients and one control subject had a positive response to HS. Twelve out of 18 asthmatic children responded to both challenges, three responded only to HS and three had no response to either challenge. There was a negative correlation between log provocative dose causing a 15% reduction in FEV₁ (PD₁₅) after HS and the maximum fall in FEV₁ after UNDW ($r_s = -0.63$; $p < 0.005$). The HS challenge had a lower acceptability than challenge with UNDW due to the unpleasant salty taste of HS. However, this did not inhibit the completion of the tests in any subject.

The results of this study suggest a good correlation between response to hypertonic saline and ultrasonically-nebulized distilled water in children with mild-to-moderate asthma. A multiple step protocol might be safer when applied in field studies involving children.

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Evidence has accumulated that the incidence of asthma has increased over the past decades [1], mainly among children. In order to study time trends in asthma epidemiology, one has to rely on standardized tests to characterize the disease. Since there is no gold standard of diagnosis and since asthma in children is a heterogeneous disease, information from questionnaire data, allergy testing and assessment of nonspecific bronchial hyperresponsiveness (BHR) is compiled to characterize the individual. Whilst there is growing consensus in standardization of questionnaires [2], there is still controversy about the most suitable method to assess BHR in children in epidemiological studies [3]. Both pharmacological and non-pharmacological tests have been used [4–16] but, since controversial data concerning the relationship between the two and among nonpharmacological tests have been

reported, it is difficult to compare results between studies [4, 6, 8–12]. Regarding time trends, only when an identical protocol has been used at different points in time can valid conclusions be drawn [13].

Pharmacological tests, such as methacholine or histamine challenges, offer a relatively high sensitivity but there is also a high rate of false positive tests [3]. Non-pharmacological challenge tests involve provocation with cold air [14], exercise [15], and inhalation of nonisotonic aerosols [8]. In general, these methods provide a high specificity but somewhat less sensitivity. Whereas some tests require sophisticated equipment (cold air) or a high degree of compliance (exercise), which is often difficult to obtain in teenagers, inhalation of nonisotonic aerosols is easy to perform and requires minimal equipment and personnel. For both the inhalation of

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ultrasonically-nebulized distilled water (UNDW) [13, 16] and hypertonic saline (HS) [4], population-based studies in children have been published [7, 13, 16].

In a clinical setting, we wanted to study whether the results obtained by these two published protocols correlate, which is what we would expect, conceptually, since a similar stimulus is exerted on the bronchial mucosa. To our knowledge, no such comparison has been made in children so far. Given a high correlation between the two methods, the issues of safety, acceptability and duration of the test would then influence the choice of a specific test for use in further studies.

Methods

Normal controls

Baseline characteristics of the study population are presented in table 1. The control subjects were 17 children, 5 males and 12 females, aged 8–18 yrs (mean \pm SD age 16 \pm 3 yrs), who were recruited from the Paediatric and Orthopaedic out-patient clinic of the University Hospital of Vienna. None of these children had a history of wheeze or chronic cough, and none had ever required the use of a beta-receptor agonist. There was no history of respiratory tract infection during the 4 weeks prior to the study. None of the children had a personal or family history of atopy or asthma.

Patients with asthma

Nineteen patients (14 males and 5 females) were recruited from the Asthma out-patient clinic at the University Children's Hospital of Vienna. They were aged 7–12 yrs (mean \pm SD 11 \pm 3 yrs). All of them had a clinical diagnosis of mild-to-moderate asthma corresponding to the American Thoracic Society (ATS) criteria [17] for more than 1 year, and documented reversible airway obstruction (15% increase in forced expiratory volume in one second (FEV₁) after inhalation of salbutamol and/or a BHR documented as a positive methacholine challenge [18]. Sixteen patients used inhaled steroids (budesonide) on a regular basis, 13 patients were prescribed short-acting β_2 -agonist inhalations, six patients received long-acting inhaled bronchodilators and one patient required antihistamines. Asthma medication was withheld prior to challenge testing (cromoglycate and short-acting inhaled bronchodilators for at least 6 h, topical steroids and long-acting inhaled bronchodilators for at least 24 h, theophyllines for at least 12 h, and antihistamines for at least 48 h). At the time of the study, symptoms were well controlled and FEV₁ was greater than 75% of predicted value. There

was no history of respiratory tract infection during the 4 weeks prior to the study. Asthma severity was assessed by a standardized score [19], allowing a classification of six severity groups in which a score of 1 represented minor symptoms and 6 represented worst symptoms.

Study design

The study was a randomized, cross-over study. Patients were randomly assigned to start with either UNDW or HS. Children were asked to attend the lung function laboratory at the same time of day on two occasions, separated by 7 days, to perform both bronchial provocation tests. Asthmatics and healthy subjects followed the same protocol. The clinical status of the patients was initially evaluated by a paediatric pulmonologist, and the parents completed a questionnaire on current medication and the frequency and severity of their child's asthma. A visual analogue scale (VAS) was used to determine the acceptability of the two protocols. The VAS was mailed approximately 1–2 weeks after the completion of the study. For each of the two tests, children were asked whether it was experienced as being pleasant or unpleasant (scale 0–4). The study protocol was approved by the University Ethics Committee on human subjects in medical research. Parental written informed consent was obtained prior to enrolment after detailed study description.

Pulmonary function

Pulmonary function tests were performed at each visit. Forced vital capacity (FVC) and FEV₁ were recorded in the form of a maximum flow-volume curve (Masterlab; E. Jaeger, Germany) according to ATS standards [20]. Results were expressed as percentage predicted based on accepted reference standards [21]. Bronchodilatation *via* inhalation of salbutamol was performed when judged necessary by the physician attending the tests. In all patients, recovery from the challenges was followed by careful observation for 30 min.

Challenge with hypertonic (4.5%) saline (HS)

HS challenge tests were performed using a protocol developed by SMITH and ANDERSON [4] with some minor modifications as used in a recent population survey by RIEDLER *et al.* [7]: a solution of 4.5% HS was nebulized *via* a Devilbiss Ultraneb 2000 ultrasonic nebulizer, connected to 60 cm of corrugated aerosol tubing and a three-way nonrebreathing valve (Hans Rudolph No. 1410). The nebulizer canister was filled with 150 mL of HS and was refilled after the final challenge step. The output of the nebulizer was set to maximum position, guaranteeing an output ranging 1.4–4.6 mL \cdot min⁻¹ [22]. During the challenge, the nebulizer output was kept constant and the dose of saline increased successively by doubling the inhalation time, starting with 0.5 then 1, 2, 4 and 8 min with subjects inhaling at tidal volumes. FEV₁ measurements were made in duplicate 60 s after each challenge step. Where two successive FEV₁ values differed by more than 5%, a third measurement was taken and, according to ATS criteria, the best of two reproducible (and acceptable) FEV₁ was used for calculation. The challenge was

Table 1. – Baseline characteristics of the study population prior to first challenge

	Patients (n=19)	Controls (n=17)
Age yrs	11 \pm 3	16 \pm 3
Sex M/F	14/5	5/12
FEV ₁ % pred	89 \pm 11	114 \pm 11
FVC % pred	105 \pm 8	110 \pm 9

Values are presented as mean \pm SD. FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; % pred: percentage of predicted value; M: male; F: female.

terminated when the FEV₁ had fallen by at least 15% of baseline, or a cumulative inhalation time of 15.5 min had been achieved. The nebulizer canister plus tubing was weighed on an electronic balance (Mettler, SM-6000) before challenge and after the final step to assess the total amount of aerosol nebulized. The mean output during the provocation was 2.5 mL·min⁻¹ with a SD of 0.84 mL·min⁻¹.

Challenge with UNDW

The challenge tests were performed according to a published protocol [5, 13, 16]. Aerosols of distilled water were generated by an ultrasonic nebulizer (Habel M608; Vienna, Austria), connected to 20 cm of corrugated aerosol tubing and a mouthpiece with a three-way valve to permit the expired air to be exhaled into the atmosphere. The water canister was filled with 150 mL cold (4°C) distilled water. A constant water temperature was maintained by adding ice cubes to the canister after each challenge test. The patients, with noseclips attached, had to breathe tidally through the mouthpiece inhaling the aerosol continuously over 10 min. Immediately and 5 min after the inhalation, maximum expiratory flow-volume curves were recorded. A positive response was defined as a ≥10% decrease in FEV₁ occurring after UNDW inhalation. The water volume output during the provocation was 1.0 mL·min⁻¹ (SD 0.37), delivering an aerosol with a mass median aerodynamic diameter of 8 μm (as described by the manufacturer).

Data analysis

A 15% reduction in FEV₁ from baseline was considered a positive response to HS. Furthermore, the provocation dose causing a 15% reduction in FEV₁ (PD₁₅) was calculated by linear interpolation of individual dose-response curves, plotting the % fall in FEV₁ from baseline against the cumulative dose of aerosol delivered (mL). PD₁₅ values were log-transformed to achieve a normal distribution. A positive UNDW challenge was defined as a ≥10% decrease in FEV₁. Spearman's rank correlation test was used to assess the association between variables. Analyses were performed using the Statistical Analysis System (SAS) software package (version 6.04).

Results

Thirty six children were enrolled: 17 control subjects without any personal or family history of atopy or asthma and 19 patients with mild-to-moderate asthma. By means of allergy tests, as available from medical records (skin tests, specific immunoglobulin E (IgE)), three asthmatic patients were nonatopic, two patients were sensitized to seasonal, 9 to perennial and 5 to combined allergens. Regarding the asthma severity score, seven patients were considered to have a symptom severity of first grade, five patients of second, four patients of third, two patients of fourth, and one patient of fifth grade. All asthmatic patients were able to withhold their medication as required.

Children were randomly allocated to one of the two tests: 18 children started with the HS- and 18 with the UNDW-challenge protocol. Seventeen percent of the challenge tests were performed in the morning and 83% in

the afternoon. One asthmatic child withdrew from the study after the first challenge test (UNDW) because of unwillingness to return for a second provocation. Thirty five subjects completed both challenge tests. Another asthmatic patient failed to repeat a lung function test 5 min after the UNDW-challenge because of dyspnoea, and one control subject did not complete the flow-volume curve 5 min after the UNDW-challenge because of lack of compliance.

The VAS was returned by 24 children. The average score for HS was 2.9 (SD 0.85) and for UNDW 1.8 (SD 1.09), suggesting lower acceptability for the HS-challenge (Wilcoxon sign rank test: $p < 0.001$). The inhalation of HS provoked a transient coughing and irritation of the throat in 29 of the 35 subjects, excessive saliva production in 21, and most of the children complained about an unpleasant salty taste. However, this did not inhibit the completion of the tests in any subject. The inhalation of UNDW provoked coughing in 28 subjects in the first minute of the bronchial challenge.

There were no significant differences in baseline lung function assessed before the two challenges in asthmatic patients and controls (table 1). For asthmatic children average FEV₁ was 2.03 (SD 0.5) L at the first challenge and 1.99 (SD 0.4) L at the second. Corresponding values for controls were 3.42 (SD 0.8) L at the first challenge and 3.48 (SD 0.8) L at the second, respectively.

A positive response for UNDW was observed in 13 of the 19 asthmatic patients compared to none of the controls. Six patients did not respond to UNDW challenge. In 12 asthmatics, the positive response was observed immediately after the provocation, the largest fall in FEV₁ being 42% in one patient (No. 14) and 38% in another (No. 6) (table 2). Their bronchial obstruction was

Table 2. – Individual PD₁₅ (HS), maximum percentage fall in FEV₁ after UNDW and current medication in children with asthma

Pt No.	Current medication	PD ₁₅ (HS) mL	Maximum fall in FEV ₁ after UNDW %
1	B, sβ ₂ -A	*	8
2	B, lβ ₂ -A	13.3	9
3	B, sβ ₂ -A	*	9.8
4	B, sβ ₂ -A	*	9
5	B	0.8	10
6	lβ ₂ -A	1.7	38
7	B, sβ ₂ -A	2.2	19
8	B, sβ ₂ -A, lβ ₂ -A	2.5	24
9	B, sβ ₂ -A, AH	6.0	6
10	B, sβ ₂ -A	2.6	21
11	B, sβ ₂ -A	1.4	24
12	B, sβ ₂ -A	1.0	33
13	B, lβ ₂ -A	1.3	22
14	B, lβ ₂ -A	3.0	42
15	B, sβ ₂ -A, lβ ₂ -A	10.5	11
16	B	4.4	2
17	B, sβ ₂ -A	2.2	17
18	sβ ₂ -A	3.5	13
19	sβ ₂ -A	**	14

*: Patients Nos. 1, 3 and 4 did not respond after a cumulative HS-inhalation time of 15.5 min; **: patient No. 19 did not return for the HS-challenge. Pt: patient; HS: hypertonic saline; UNDW: ultrasonically-nebulized distilled water; FEV₁: forced expiratory volume in one second; PD₁₅ (HS): provocation dose of HS causing a 15% fall in FEV₁; B: inhalation of budesonide; lβ₂-A: inhalation of long-acting β₂-agonist; sβ₂-A: inhalation of short-acting β₂-agonist; AH: antihistamines.

rapidly reversible: at the third measurement, 15 min after the provocation, they had a mean increase in FEV₁ of 6%. Only two patients had a further decrease in FEV₁ after 5 min, (2.7 and 5%). One patient having an 8% fall after the UNDW-inhalation, had a 15% fall in FEV₁ at the third measurement. In the control group the minimum fall in FEV₁ was 0% and the maximum was 5.2% (figs. 1 and 2).

A positive response to HS was noted in 15 out of 18 patients. Three asthmatic patients did not respond after the cumulative 15.5 min inhalation time. After the first 0.5 min of inhalation, a 24–26% fall FEV₁ was observed in four asthmatics. The maximum fall (34%) was observed in one patient after the following 1 min inhalation interval (fig. 3).

In the control group, all children completed the whole 15.5 min inhalation time (fig. 4). One subject responded with a 23% fall in FEV₁ after the last inhalation step. For this child, a continuous decrease was observed (FEV₁: 2.72→2.64→2.56→2.52→2.48→2.08 L·s⁻¹) and FEV₁ returned to baseline value after salbutamol inhalation.

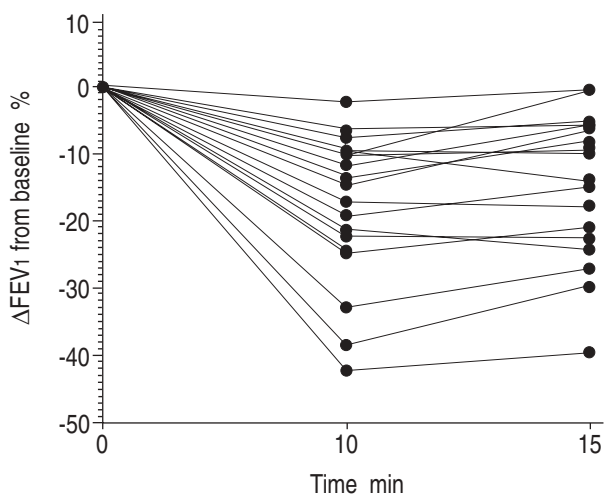


Fig. 1. — Change in forced expiratory volume in one second (Δ FEV₁) from baseline in asthmatic children (n=19) immediately after and 5 min after challenge with ultrasonically-nebulized distilled water (UNDW). Time 0 = start of inhalation.

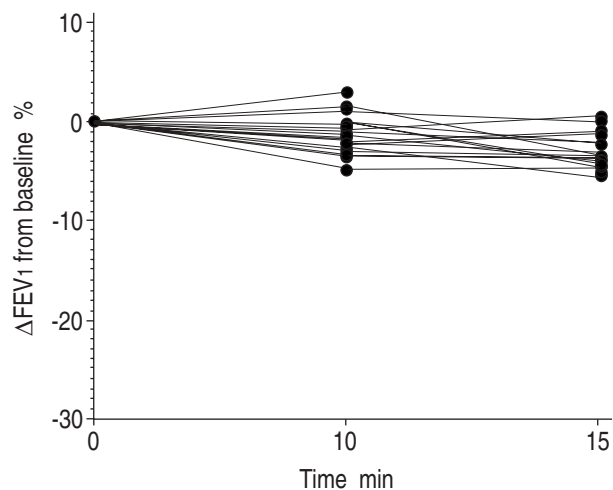


Fig. 2. — Change in forced expiratory volume in one second (Δ FEV₁) from baseline in controls (n=17) immediately after and 5 min after challenge with ultrasonically-nebulized distilled water (UNDW). Time 0 = start of inhalation.

His baseline FEV₁ was 106% of predicted. He had no personal or family history of atopy, asthma or smoking, and no respiratory tract infection was apparent during the last 4 weeks. In another subject, having a 15% fall in FEV₁ after the 1 min challenge step, without any clinical symptoms of bronchial obstruction, this 15% fall was interpreted as a measurement error and the challenge was continued. Subsequent FEV₁ values improved and this subject was not considered to be a responder.

Bronchial provocation with HS appeared to be more sensitive, with 15 out of 18 (83%) asthmatic patients responding, and slightly less specific (94%) than UNDW, because one control subject reacted. There was a trend for UNDW to be less sensitive (68%), because six asthmatics failed to respond (three of them also being missed by the HS provocation) but more specific (100%), with no false positive result; however, the differences were not statistically significant. The odds ratio linking a positive response to asthma was 5.6 (95% confidence interval (95% CI) 2.7–11.9) for challenge with HS and 3.2 (95% CI 1.9–5.4) for UNDW.

All patients having a PD₁₅ of 0.8–3.5 mL (HS) also responded to the UNDW provocation (table 2). The three

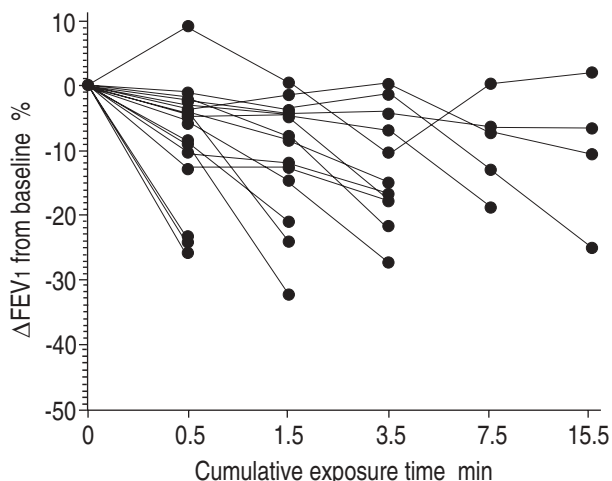


Fig. 3. — Change in forced expiratory volume in one second (Δ FEV₁) from baseline in asthmatic children (n=18) after successive inhalations of hypertonic saline (HS) for 30 s, 1, 2, 4 and 8 min.

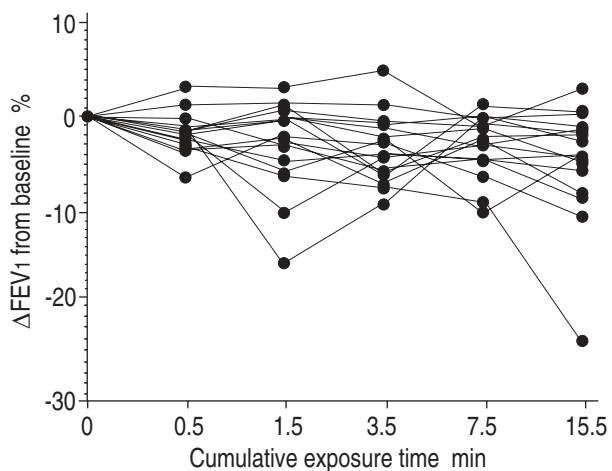


Fig. 4. — Change in forced expiratory volume in one second (Δ FEV₁) from baseline in controls (n=17) after successive inhalations of hypertonic saline (HS) for 30 s, 1, 2, 4 and 8 min.

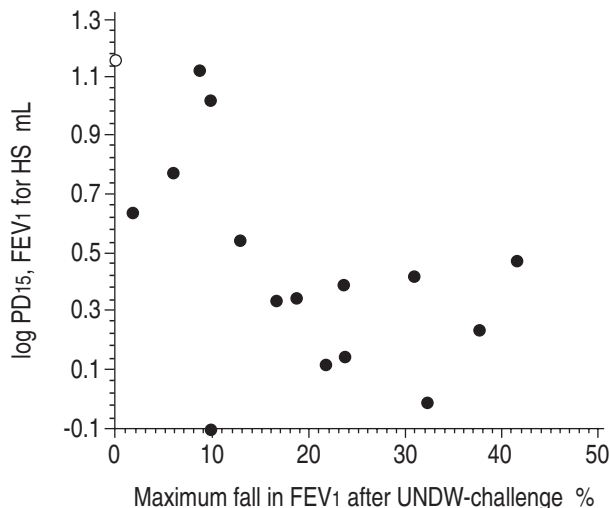


Fig. 5. — Correlation between dose of hypertonic saline (log-transformed) causing a fall in FEV₁ of 15% (logPD₁₅,FEV₁) and the maximum fall in FEV₁ from baseline after inhalation of UNDW for 15 asthmatic children (●), and one control subject (○), responding to HS. Three asthmatic children did not respond to HS, hence no PD₁₅ could be calculated. HS: hypertonic saline; FEV₁: forced expiratory volume in one second; PD₁₅: provocation dose of HS cause a 15% fall in FEV₁; UNDW: ultrasonically-nebulized distilled water.

patients and one control subject responding only to HS had PD₁₅ values of 4.2–14.4 mL. PD₁₅ could not be calculated for four patients: one did not return for the HS provocation after having the UNDW challenge and three patients did not respond to HS (these three also failed to respond to UNDW).

The correlation between log PD₁₅ in the HS challenge and the maximum fall in FEV₁ after UNDW is shown in figure 5 for the 15 asthmatic patients and one control subject who had a positive response to HS. A statistically significant negative correlation for log PD₁₅ after HS and the maximum fall in FEV₁ after UNDW was obtained with the Spearman's rank correlation ($r_s = -0.63$; $p < 0.005$). The correlation between maximum fall in FEV₁ after HS and UNDW was 0.69 ($p = 0.0001$).

In both groups, no significant correlation was obtained between response to the challenge and the asthma severity score (HS: $r_s = 0.24$; $p > 0.1$; UNDW: $r_s = 0.06$; $p > 0.1$).

Discussion

The results of the present study confirm the observations that both inhalation of UNDW and HS are effective bronchoconstrictor stimuli in children with mild-to-moderate asthma but not in control subjects. The aim of this study was not to compare two different nonisotonic aerosols but to test two well-established protocols [7, 13, 16] with regard to their utility in epidemiological studies, in order to make realistic recommendations as to which method should be used in further population-based studies. Criteria for an optimal test in such studies are: high safety; high sensitivity and specificity; high acceptability (particularly in children); and short duration.

Inhalation of nonisotonic solutions offers many advantages with respect to these demands as compared to inhalation of cold air, exercise or pharmacological challenges. Both protocols use minimal equipment, are relatively cheap, and require little patient co-operation. In

a recent study, RIEDLER *et al.* [7] used HS challenge in 382 children aged 13–15 yrs, and found a sensitivity of 47% for current wheeze. Distilled water challenge has been reported to be of somewhat lower sensitivity by FRISCHER *et al.* [16], who found a sensitivity of 36% in 446 children aged 7–10 yrs. These observations were confirmed by the present study, showing a somewhat higher sensitivity for HS as compared to UNDW challenge. However, one asymptomatic control subject had a positive response to HS challenge without a personal or family history of atopy, asthma or smoking, and without an apparent respiratory tract infection in the last 4 weeks, resulting in a lower specificity for HS.

Apart from the provoking stimulus, different nebulizers were implemented. HS involved a multiple step protocol, whereas in UNDW challenge a single step protocol was used. Additionally, different cut-off points were used to assess the response. The Devilbiss nebulizer delivers approximately twice the amount of aerosol as compared to the Habel M608 nebulizer, which could lead to a higher dose of the provoking stimulus to the airways. However, for children responding to both stimuli, 0.8–3.5 mL HS resulted in an average fall in FEV₁ of 23%, whereas 10 mL of UNDW resulted in an average fall in FEV₁ of 22.8%, suggesting that HS constitutes a stronger bronchoconstrictor than UNDW in children. It is unlikely that a multiple step protocol for UNDW would have increased the sensitivity to a large extent. In a previous study, we compared the single step protocol to a multiple step protocol and observed the maximum reaction after 2–6 min of inhalation [5]. In this study, the sensitivity of 10 mL UNDW inhalation was 67% and increased to only 75% after a total of 30 mL UNDW was inhaled. Furthermore, it is possible that during the 10 min inhalation period refractoriness has occurred in some patients, with FEV₁ returning to baseline at the end of the inhalation. We cannot exclude the possibility that the smaller particle size delivered by the Devilbiss was responsible for the higher sensitivity of HS.

The disadvantages of the HS protocol are lower acceptability, longer duration and the need for a higher cut-off point. The probability of an "abnormal" result in a bronchial provocation test due to within-subject variability has been shown to increase with the number of measurements [23]. A higher cut-off point is needed in HS-challenge which involves up to six spirometric tests. Although in most children a dose response was observed for HS, four children reacted with a >20% fall in FEV₁ after 30 s of inhalation. Hence, the multiple step protocol does not exclude severe airway obstruction. However, after UNDW a considerable fall in FEV₁ (38 and 42%) was observed in two children. Hence, with regard to safety, the HS multiple step protocol offers an obvious advantage. For both challenge tests, resuscitation equipment should be at hand and only trained personnel should perform these tests.

There was a statistically significant correlation for PD₁₅ (HS) and the maximum fall in FEV₁ after UNDW tests, and both protocols identified the same children with asthma, which, conceptually, is what we would expect since a similar stimulus is exerted on the bronchial mucosa: a change in osmolarity of the airway surface liquid, causing the production and release of substances from cells in the airway epithelium and mucosa and resulting in airway narrowing [8].

A number of studies have investigated the relationship between nonisotonic aerosols and other challenge tests, particularly exercise and the inhalation of methacholine and histamine. In general, there is better agreement among indirect measures of BHR than between indirect and direct challenges. SMITH and ANDERSON [4] found a significant relationship between HS and methacholine and exercise but not between distilled water and HS. This difference in observation might relate to the older age range of subjects studied by SMITH and ANDERSON [4]. RIEDLER *et al.* [7] found only a moderate agreement of response to HS and exercise, whereas MAKKER and HOLGATE [11] found a close relationship between HS responsiveness and the exercise-induced asthma score, and BELCHER *et al.* [12] found the response to exercise and hypertonic aerosols to be closely related. Several investigators observed a significant relationship between water and exercise [9, 10, 24], whereas other investigators have not found a relationship between water and methacholine [6, 9, 10, 25]. MAKKER and HOLGATE [11] found no correlation between HS and methacholine or histamine.

These observations stress the need for standardized challenge tests in population-based surveys in order to compare results between centres.

In conclusion, the results of this study suggest that there is a good correlation between the response to hypertonic saline and to ultrasonically-nebulized distilled water in children with mild-to-moderate asthma. There was a trend for bronchial provocation with hypertonic saline to be more sensitive than ultrasonically-nebulized distilled water for identification of children with clinical asthma. The challenge with ultrasonically-nebulized distilled water appeared to be slightly more specific and had a higher acceptability. However, for safety reasons, a multiple step protocol, such as that used in the hypertonic saline challenge, is preferable, particularly in field studies, in order to minimize the risk of severe airways obstruction.

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