

Assessment of EIB

What You Need to Know to Optimize Test Results

Sandra D. Anderson, PhD, DSc, MD(Hon)^{a,*}, Pascale Kippelen, PhD^b

KEYWORDS

- Exercise hyperpnea • Dry air • Voluntary hyperpnea • Mannitol
- Bronchial provocation

KEY POINTS

- Respiratory symptoms are poor predictors of either the presence or the severity of exercise-induced bronchoconstriction (EIB).
- There are many factors affecting the response to exercise, such as workload, ventilation, humidity, temperature, and time since medication.
- There is a high rate of negative test results using exercise ergometers in a laboratory setting.
- Surrogates for exercise have been developed to identify the potential for EIB in the laboratory setting.
- Eucapnic voluntary hyperpnea is a very potent and sensitive test to identify EIB and is recommended to identify EIB in elite athletes or in regular exercisers with suspected mild EIB.
- Challenge with mannitol dry powder aerosol, which uses prepacked capsules of increasing doses, reduces the risk of large decreases in the forced expiratory volume in the first second of expiration, helps to identify the presence of currently active asthma, and detects the potential for EIB.

BACKGROUND TO THE NEED TO IDENTIFY EXERCISE-INDUCED BRONCHOCONSTRICTION

“Exercise-induced asthma and exercise-induced bronchoconstriction (EIB) are the terms used to describe the transient increase in airways resistance that follows vigorous exercise.”¹ EIB occurs most commonly in people with clinically recognized asthma. History and symptoms cannot be relied on to predict EIB either in adults (**Fig. 1**)² or children.³ In one study, 40% of children considered well controlled by a

Funding Sources: Nil.

Conflict of Interest: Yes (S.D. Anderson); Nil (P. Kippelen).

^a Department of Respiratory and Sleep Medicine, Royal Prince Alfred Hospital, Camperdown, New South Wales 2050, Missenden road, Australia; ^b Centre for Sports Medicine & Human Performance, Brunel University, Kingston Lane, Uxbridge, Middlesex UB8 3PH, UK

* Corresponding author.

E-mail address: sandra.anderson@sydney.edu.au

Immunol Allergy Clin N Am 33 (2013) 363–380

<http://dx.doi.org/10.1016/j.iac.2013.02.006>

immunology.theclinics.com

0889-8561/13/\$ – see front matter © 2013 Elsevier Inc. All rights reserved.

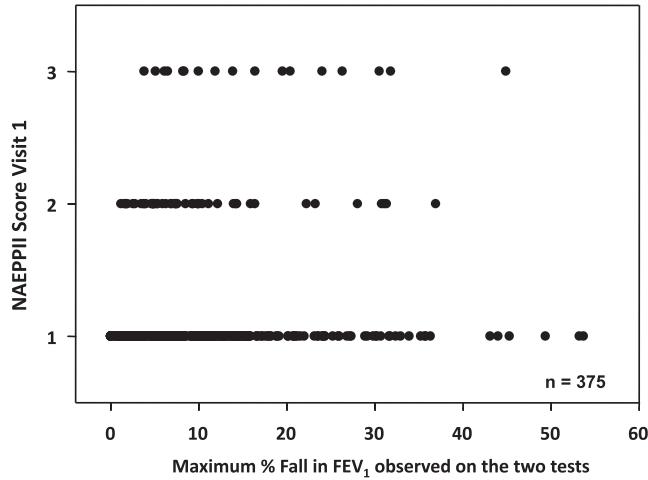


Fig. 1. Individual values for the highest percent decrease in FEV₁ documented after 2 exercise tests in relation to the National Asthma Education and Prevention Program (NAEPPII) asthma severity score at visit 1. Score 1 = symptoms 2 or less times per week; score 2 = symptoms 2 or more times a week but less than 1 time a day; score 3 = symptoms daily. (From Anderson SD, Pearlman DS, Rundell KW, et al. Reproducibility of the airway response to an exercise protocol standardized for intensity, duration, and inspired air conditions, in subjects with symptoms suggestive of asthma. *Respir Res* 2010;11:120; with permission.)

questionnaire had EIB⁴; in another study, 40% of children with EIB had never had a diagnosis of asthma.⁵ EIB also occurs in people without other symptoms of asthma, particularly elite athletes.^{2,6,7} In elite athletes, self-reported symptoms related to exercise are neither sensitive nor specific for identifying EIB (Table 1).^{2,8–11} An overdiagnosis and underdiagnosis of EIB from symptoms was realized when investigators started to measure the airway response to exercise in athletes with and without symptoms. The move away from symptoms to lung function measurement to diagnose EIB was facilitated by sporting bodies requesting objective evidence of asthma or EIB to justify the use of a beta₂ agonist aerosol before a sporting event.¹² Further, the recent large surveys on exercise-related symptoms reported in both adults¹³ and children¹⁴ in the United States suggest that objective measurement is required to identify EIB so that appropriate treatment can be prescribed.¹⁵

Table 1 The sensitivity, specificity, PPV, and NPV for respiratory symptoms to predict EIB				
	Sensitivity %	Specificity %	PPV %	NPV %
Cough	61	65	38	83
Wheeze	17	82	25	74
Chest tightness	20	80	26	74
Excess mucus	22	85	33	76

EIB is defined as a decrease in the FEV₁ of 10% or more following actual or simulated competition exercise in elite athletes.
Based on the findings of Rundell et al.²
Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

WHAT WE NEED TO KNOW TO IDENTIFY EIB

To develop successful protocols and reduce the possibility of false-negative tests, it is necessary to understand the stimulus and mechanism whereby exercise narrows the airways in susceptible people. This understanding also explains why surrogates for exercise, such as eucapnic voluntary hyperpnea (EVH) and inhaled mannitol, have been used successfully to identify the potential for EIB.

The stimulus for EIB is water loss, by evaporation, from the airway surface in humidifying large volumes of air in a short time (Fig. 2). This water loss cools the larger airways (>2 mm) and may increase the osmolarity of the airway surface liquid because of its small volume (<1 mL).¹⁶ Inspiring cool dry air enhances the possibility of EIB, and inspiring warm humid air reduces the possibility and may even prevent EIB.¹⁷ Exercise of high intensity is needed¹⁸ to identify EIB because it determines ventilation; the higher the ventilation, the greater the rate of water loss. The duration of exercise is also important. It cannot be too long because of the high intensity of exercise or too short because it will not result in sufficient water loss. In combination with the intensity of exercise and inspired air conditions, duration determines the number of generations of airways required to heat and to humidify the inspired air. The greater the number of generations, the greater the surface area of the airways involved in the exercise response. Exercising under conditions that require recruiting the small airways¹⁹ to heat and to humidify the air increases the chances of identifying EIB. The small airways have a high density of the mast cells²⁰ and are an important source of mediators of bronchoconstriction.

The mechanism whereby the airways narrow is thought to relate to both the osmotic and thermal effects of evaporative water loss.²¹ Although water can rapidly return to the airway surface, a transient increase in osmolarity of the airway surface liquid will occur if the rate of water loss exceeds the rate of return.¹⁹ This transient hyperosmolar environment favors the release of mediators from cells near the airway surface.^{22,23} These mediators include prostaglandins (PGs), leukotrienes (LTs), and histamine, which can cause the bronchial smooth muscle to relax (PGE₂) during the first few

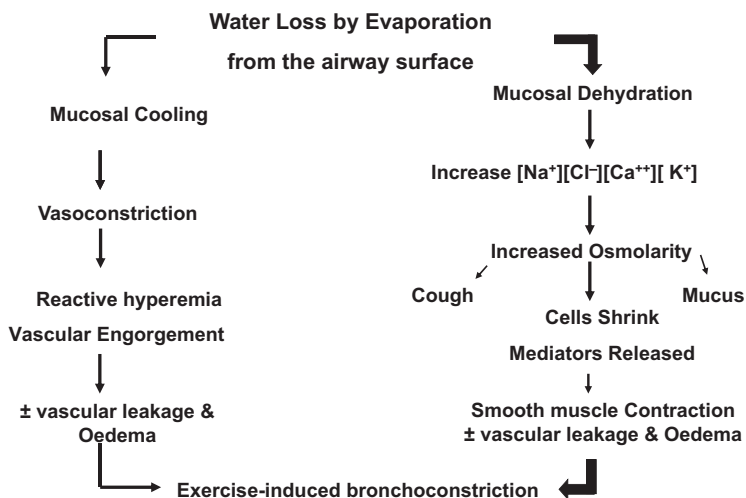


Fig. 2. The stimulus to exercise-induced bronchoconstriction is the water loss by evaporation from the airways surface in conditioning large volumes of air in a short time. The mechanism for airway narrowing relates to the thermal (cooling) and osmotic effects of this water loss.

minutes of exercise in all patients and then to contract (PGD₂, LTs, and histamine) in those with EIB.^{21,24} For this reason, medications that inhibit the production or release of these mediators or antagonize their effect need to be withheld for the appropriate time (Table 2). Further, patients should not have exercised within 2 hours²⁵ and preferably 4 hours of a test because a state of refractoriness during that period occurs in 50% of patients with EIB.²⁶ Even warm-up exercise can induce refractoriness.²⁷

During exercise under temperate or cold conditions, the airways cool because of the latent heat of vaporization in humidifying the inspired air. The colder the inspired air is during exercise, the greater the possibility for the thermal effects to enhance the severity of EIB. The thermal effects involve a reactive hyperemia of the bronchial vasculature, which is an event that follows the vasoconstriction in response to airway cooling.²⁸ This reactive hyperemia may also be accompanied by increased vascular permeability and mucosal edema. As previously discussed,²⁹ these events may not only serve to cause narrowing in their own right but may also amplify the effects of airway narrowing arising from bronchial smooth muscle contraction. The interplay of the thermal and osmotic effects is important in the assessment of cold-weather athletes who may need to exercise under conditions that reproduce their symptoms.³⁰

To optimize the chance of identifying EIB in untrained patients in a laboratory requires measuring the forced expiratory volume in the first second of expiration (FEV₁) before and at frequent intervals after exercising. The exercise should preferably

Table 2 Medications and their required withholding times before challenge by exercise, EVH, or mannitol to reduce the possibility of a false-negative test result and optimize the possibility of finding a positive test result	
Time to Withhold	Medication
8 h	Short-acting β_2 agonists (eg, <i>albuterol, salbutamol, levalbuterol terbutaline</i>) Inhaled nonsteroidal antiinflammatory agents Cromones (eg, <i>sodium cromoglycate, nedocromil sodium</i>)
12 h	Inhaled corticosteroids (eg, <i>beclomethasone dipropionate, budesonide, fluticasone propionate, ciclesonide</i>) Ipratropium bromide
24 h	Inhaled corticosteroids plus long-acting β_2 agonists (eg, <i>fluticasone and salmeterol, budesonide and formoterol</i>) Long-acting β_2 agonists (eg, <i>salmeterol, eformoterol</i>) Theophylline
72 h	Tiotropium bromide Antihistamines (eg, <i>cetirizine, fexofenadine, and loratadine</i>)
4 d	Leukotriene-receptor antagonists (eg, <i>montelukast sodium</i>) Nonsteroidal antiinflammatory (eg, <i>indomethacin, ibuprofen</i>)

Foods: Ingestion of caffeine-containing foods, such as coffee, tea, cola, and chocolate, may decrease bronchial hyperresponsiveness. These substances should be withheld on the day of the test.

Other factors that may confound results: Smoking and vigorous exercise should not be undertaken on the day of the test.

Abbreviations: HR, heart rate; HRmax, predicted maximum heart rate; VE, ventilation.

involve running (rather than cycling) and should last for 6 to 8 minutes. Patients should breathe dry air via the mouth with the nose clipped and exercise intensely enough to achieve a minute ventilation more than 21 times the baseline FEV_1 (in liters) by 4 minutes, but preferably within the first 2 minutes, and maintain this level until the end of exercise. In the absence of a measure of ventilation, heart rate can be used. The target heart rate should be between 85% and 95% of the predicted maximum for the duration of the test and preferably more than 95% predicted for the last 4 minutes.¹⁸ Protocols that have met these requirements for both running and cycling have been used successfully in laboratories or in the field.^{5,10,18,30–32}

There is a high frequency of negative exercise results in those tested in a laboratory for suspected EIB.^{31,33,34} This high frequency has resulted in fewer exercise tests being performed these days. Screening tests, in the field, for EIB have also had a low yield in some studies.³⁵ In part, this may be explained by the inadequacy of the exercise task, variable inspired air conditions, or the lack of attention to the conditions preceding the test, particularly recent exercise or the withholding of medication (see **Table 2; Table 3**).

Elite athletes are a special case in that sport-specific exercise in the field, under the appropriate conditions, may be required in the event of negative results of exercise tests or surrogates for exercise in the laboratory.^{2,6,10} The thermal and vascular effects are expected to be the greatest under conditions of inspiring air of subfreezing temperatures (such as in winter sports), less important when breathing warm humid air (such as during swimming),^{36,37} and absent under conditions of breathing hot dry air.^{38,39}

HOW IS THE DIAGNOSIS OF EIB MADE?

A diagnosis of EIB is commonly made on the measurement of an abnormal reduction in the FEV_1 after exercise. The FEV_1 does not need to be accompanied by a full forced vital capacity maneuver. Measurements of the FEV_1 are usually made in duplicate or triplicate 5, 10, 15, 20, and 30 minutes after exercise, with the best FEV_1 value at each

Table 3
Key determinants of the airway response to exercise challenge

Factors that Enhance the Possibility of EIB	Factors that Reduce the Possibility of EIB
Inspiring cold dry air	Inspiring warm humid air
Exercise of high intensity ($VE > 21 \times FEV_1$ or $HR > 85\% - 95\%$ pred HR_{max} within 2–4 min)	Exercise of low intensity ($VE < 22 \times FEV_1$ or $HR < 85\%$ pred HR_{max})
Duration of 6–8 min	Duration of less than 6 min or more than 8 min
Constant load exercise with rapid (in the first 2 min) workload increment	Incremental exercise protocol
Recent exposure to allergens	Strenuous exercise within 2–4 h before testing
Recent exposure to irritants or high densities of particulate matters	Warm-up exercise
Recent URTI or chest infection	Ingestion of caffeine-containing foods (eg, coffee, tea, cola, and chocolate) on the day of testing
	Inappropriate withholding time of drugs (cf Table 2)
	Out-of-season testing (for athletes only)

Abbreviations: HR, heart rate; HR_{max} , predicted maximum heart rate; URTI, upper respiratory tract infection; VE, ventilation.

time point being recorded. Earlier and more frequent measurements may be required to identify and avoid severe bronchoconstriction. The lowest value for the FEV₁ is usually observed within 15 minutes after exercise and recovers spontaneously within 30 to 60 minutes. Inhalation of a beta₂ agonist bronchodilator is recommended if there is a need to enhance recovery to the baseline FEV₁. A decrease in the FEV₁ after exercise of 10% or more from the pre-exercise value “is generally accepted as abnormal,” but a cutoff of 15% or more is often used when exercise has been performed in the field.⁴⁰ The severity of EIB is expressed as the maximum percent decrease in the FEV₁ after exercise, and suggested grades of severity are given in **Table 4**. For elite athletes to identify EIB, a decrease of 10% or more in the FEV₁ is preferable at 2 consecutive time points (eg, 5 and 10 minutes after exercise). This practice reduces the possibility of a false-positive result from recording a low FEV₁ immediately at the end of exercise because of respiratory muscle fatigue.⁴¹

A decrease in the FEV₁ of 10% and 15% from the baseline is close to the 95% confidence interval observed in healthy subjects in the laboratory^{42,43} and the field.^{5,44} A value of 13% has been reported to provide the optimal sensitivity (63%) and specificity (94%) for identifying children with asthma and young adults.⁴⁵ The choice of cutoff will be determined by the investigator’s need to be either more sensitive or more specific. Although a 10% decrease in the FEV₁ is acceptable for sporting bodies seeking clearance to use an inhaled beta₂ agonist, a value of 20% has been suggested to be included in a clinical trial to assess new drugs for EIB.⁴⁶

VARIABILITY IN THE RESPONSE TO EXERCISE

Since the early studies in EIB 50 years ago, variability of the airway response to exercise has been recognized.⁴⁷ There is a recent report in 373 subjects with signs and symptoms of asthma but without a definite diagnosis who performed running exercises on 2 occasions within 2 to 4 days under the optimal conditions described earlier.³² In 19% of the subjects, a decrease in the FEV₁ of 10% or more was documented after both tests. The mean of the highest percent decrease in the FEV₁ was 24.7% ± 9.7%, leaving the diagnosis of EIB in no doubt. For 24% of the subjects, only one test was positive, with the mean highest decrease being 14.3% ± 4.8%. For 57% of the subjects, the 2 tests were 10% or less with the mean of the highest percent decrease being 4.9% ± 2.9%. A value of 0% was assumed in 55 of the 210 subjects who had only an increase in the FEV₁ in response to exercise. The 5-times difference in the mean percent decrease in those with 2 positive tests and those with 2 negative tests suggests that 2 concordant test results may be useful to include or exclude a definitive diagnosis of EIB.

The variability in the percent decrease in the FEV₁ on the 2 test days was ± 9.7% in the 278 adults, ± 13.4% in the 95 children, and ± 14.6% for the 72 subjects with 2 positive exercise tests (with an FEV₁ decrease of 10% or more) (**Fig. 3**). This variability

Table 4 Classification of severity of EIB	
Degree of Severity of EIB	Maximum Percent Decrease in FEV ₁ After Exercise
Mild	≥10% but <25%
Moderate	≥25% but <50%
Severe	≥50% for steroid-naïve patients ≥30% for steroid-treated patients

A suggested classification for severity of EIB based on the percent decrease in FEV₁.

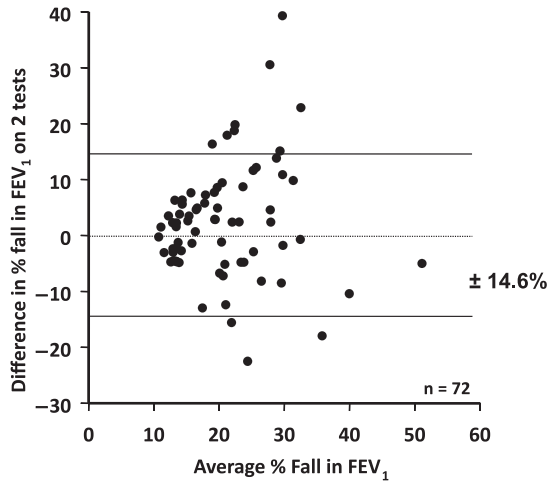


Fig. 3. Reproducibility of the percent decrease in FEV₁ measured after 8 minutes of running exercise of high intensity while breathing dry air by mouth. The difference between values for the percent decrease in FEV₁ on the 2 exercise tests performed within 2.6 ± 3.2 days in relation to the average value on the 2 tests for those 72 subjects who had a 10% or more decrease following both exercise tests. The interval defines the 95% probability that the difference between a single measurement and the true value for the subject is within that range. (From Anderson SD, Pearlman DS, Rundell KW, et al. Reproducibility of the airway response to an exercise protocol standardized for intensity, duration, and inspired air conditions, in subjects with symptoms suggestive of asthma. *Respir Res* 2010;11:120; with permission.)

in the percent decrease in the FEV₁ needs to be considered when selecting a subject for inclusion in a drug trial.

Seasonal differences can contribute to the variability of EIB.^{48,49} Although variability in the percent decrease is recognized when conditions of the inspired air alone are altered, it may also be important if the environmental conditions are altered.⁵⁰ Thus, exercising in subfreezing conditions may enhance responses via a facial reflex.^{51,52} These subfreezing conditions, although uncommon, have been used in a laboratory environment.^{30,53} Other factors that can affect the exercise outcome are recent exposure to allergens or irritants or to high densities of particulate matter.^{54–56}

During exercise testing, other conditions than EIB may cause a turbulent sound in the tracheal area. Inspiratory stridor,⁵⁷ vocal cord dysfunction,⁵⁸ or laryngeal obstruction⁵⁹ have all been reported to masquerade as EIB. It is important to consider these as a cause of symptoms (especially wheeze) if an exercise or surrogate challenge proves negative and there is no significant bronchodilator response to inhaling a beta₂ agonist.⁶⁰

SURROGATE TESTS TO IDENTIFY POTENTIAL FOR EIB

EVH

The many factors that determine the outcome of an exercise test and the inherent variability of EIB prompted the development of surrogates that could be more easily standardized for use in a hospital or office laboratory. That exercise itself was not necessary to elicit an airway response to hyperpnea with dry air was recognized in the 1970s. One test protocol, standardized for evaluating defense force recruits and

involving 6 minutes of voluntary hyperpnea of dry air at room temperature, has become the most widely used (Table 5).⁶¹ As with exercise, the FEV₁ is measured and the maximum percent decrease in the FEV₁ usually occurs within 10 minutes of finishing the test. A value of 10% or more has been taken as abnormal, based on the finding of a specificity of 90% and a sensitivity of 63% for identifying patients with asthma. A decrease in the FEV₁ of more than 14% was 100% specific for identifying patients with asthma.⁶² The variability for the percent decrease to EVH between 2 tests is reported within $\pm$ 6%.⁶³ The fixed concentration of 4.9% to 5.0% carbon dioxide (CO₂) allows eucapnia at ventilations between 40 and 105 L/min.⁶⁴

This EVH test has been increasingly used to test elite athletes⁶⁵ in addition to defense force recruits.⁶⁶ For elite athletes, the EVH test needs to be performed during the training season to enhance the chance of a positive test result,⁶⁷ and a sustained 10% or more decrease in the FEV₁ is used as criteria for a positive test.⁶⁸ For athletes and recruits, 6 minutes of breathing at a high level of ventilation (30 times the FEV₁) is used as a target to reduce the possibility of false-negative results. Because this high ventilation can lead to a severe percent decrease in the FEV₁ (Fig. 4),⁶⁹ it is recommended for use only in those who exercise regularly and intensely at high ventilation.⁷⁰ Because of the high potency of the test, EVH is often used to exclude EIB in elite athletes, especially in those with suspected upper respiratory tract dysfunction or dysfunctional breathing.

For those who do not exercise regularly, the potency of the stimulus using EVH can be reduced by decreasing the target ventilation to 17 to 21 times the FEV₁. Provided the ventilation is 21 or more times the baseline FEV₁ (in liters) for 6 to 8 minutes, it should be a valid test to exclude EIB in most untrained patients whose ventilation during exercise is unlikely to exceed this level.³¹ Using a standard 6-minute protocol, 81% of 178 patients achieved a diagnostic test.⁷¹ Women were less likely to achieve the target ventilation of 21 times the FEV₁ compared with men. For many untrained women, however, a ventilation of 17.5 or more times the FEV₁ would be the maximum level achieved during exercise.³¹

There are many advantages in using EVH to identify EIB in the laboratory, particularly for athletes. Providing the resistance of the equipment is low and permits high flow, voluntary hyperpnea of dry air is less stressful than exercise-induced hyperpnea, particularly at high ventilation. The duration of the ventilation and the temperature of the air inspired can be varied if required to evaluate the conditions under which exercise is performed (eg, cold air and so forth). Importantly, the target ventilation is based on the FEV₁ of the individual patient and is not dependent on muscle power of the limbs. Although it is easy to set up a circuit to perform an EVH test, attention should

Table 5 Protocol for EVH for identifying EIB	
Measurement	FEV ₁ before and 5, 10, and 15 min after EVH and as required
Target ventilation	30 times ^a or 21 times ^b FEV ₁
Duration	6 min
Inspired air	Dry air containing 4.9% to 5.0% CO ₂ , 21% O ₂ , and balance N ₂ inhaled at room temperature
Positive response	FEV ₁ percent decrease 10% or more preferably at 2 consecutive time points

^a For those who exercise intensely, Anderson et al, 2001.⁷⁰

^b For untrained subjects, Brummel et al, 2009.⁷¹

Equipment for Eucapnic Hyperventilation Test

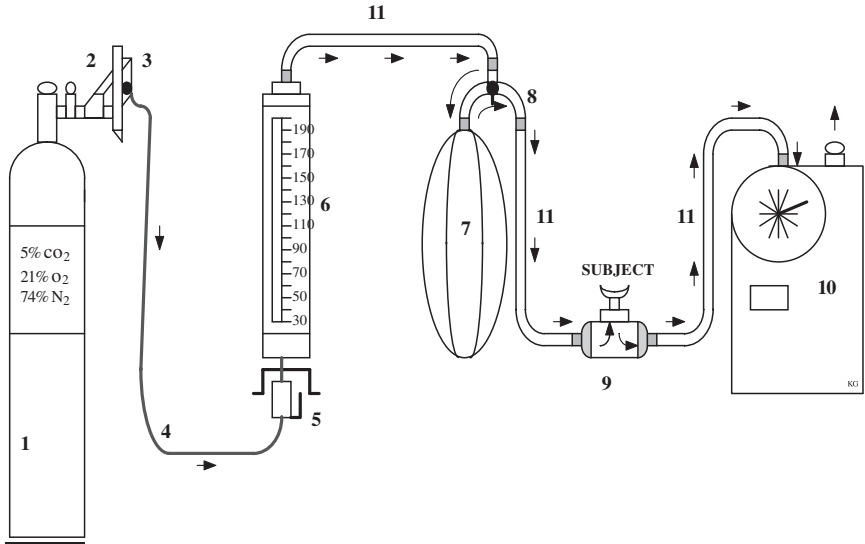


Fig. 5. Some equipment used to perform the eucapnic voluntary hyperpnea test. The gas cylinder contains 4.9-5% carbon dioxide, 21% oxygen, balance nitrogen (1). A regulator (2) a demand resuscitator 30-150 L/min (3) with high pressure tubing (4) and a demand valve in the fully open position, (5) allows the required flow of gas, to meet the target ventilation, to pass through a rotameter (6) via a three way tap (8) that allows gas to simultaneously enter and leave the large meteorological balloon (7) that acts as a reservoir. The subject inhales the dry gas via wide bore tubing (11) and a large low resistance low dead space two way valve (9) as close to the target ventilation as possible and exhales through a meter (10) that measures ventilation. (From Anderson SD, Argyros GJ, Magnussen H, et al. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med* 2001;35:344-7; with permission.)

causing bronchoconstriction.⁸⁰ Therefore, small concentrations of PGs and LTs released during exercise or EVH may provoke a 10% or more decrease in the FEV₁ in some patients negative to methacholine.

Hyperosmolar Aerosols

Hyperosmolar saline was developed as a bronchial provocation test during the 1980s. When it was observed that patients with asthma with EIB could have an attack of asthma provoked by inhaling hyperosmolar aerosols,⁸¹ the tests were standardized and became used as surrogates for exercise.^{82,83} However, there were limitations in that a large-volume ultrasonic nebulizer was needed, and the dose of the aerosol delivered could differ widely between patients and over time, depending on the inspiratory flow through the nebulizer and the age of the piezoelectric crystal.⁸⁴ The technical and hygienic limitations associated with the correct use of the 4.5% saline test were overcome by the development of a dry powder of mannitol for inhalation.

Mannitol as a dry powder was chosen as the osmotic stimulus because it is stable at high relative humidity, has many medical applications, and was generally regarded as a safe molecule.⁸⁵ Mannitol caused the release of mast cell histamine *in vitro*, and this release was enhanced in the presence of anti-immunoglobulin E.⁸⁶ Mannitol

inhalation in vivo has been shown to be associated with the same increase in urinary concentration of PGs and LTs as those observed in response to exercise or EVH.^{73,74,87,88}

The mannitol test kit (known as Aridol or Osmohale; Pharmaxis Inc, Exton, PA) contains prepacked capsules with increasing doses and an inhaler device, so there is no preparation or cleanup time required (**Table 6**). The dose response protocol reduces the risk of large decreases in the FEV₁ that can occur unexpectedly following exercise and EVH. A positive test is a 15% decrease in the FEV₁ at a cumulative dose of 635 mg or a 10% decrease between doses. The test result is expressed as the provoking dose to induce a 15% decrease in the FEV₁ (PD₁₅) and is a measure of sensitivity. This value is based on the 95% confidence interval documented in healthy subjects.⁸⁹ The repeatability of the PD₁₅ is within ± 1 doubling dose both in adults and children.^{90,91} The response dose ratio (RDR), a measure of reactivity, is calculated taking the final percent decrease in the FEV₁ after challenge divided by the cumulative dose of mannitol to provoke that percent decrease in the FEV₁. The 95% confidence interval for RDR in healthy subjects is a 0.004% decrease in the FEV₁ per milligram.⁸⁹ A 0% decrease after mannitol is taken as 0.1% in calculating the RDR.⁸⁹

Most patients will cough during the mannitol challenge, but in 71% this is occasional; in 13% it causes a delay in administering the capsule, and in 1.3% the challenge cannot be completed.⁸⁹ The mannitol test has regulatory approval and is available in 26 countries, including the United States, Europe, Australia, and South Korea, to identify bronchial hyperresponsiveness. As with hyperosmolar saline, sputum can be induced with mannitol inhalation and collected for analysis.⁹²

In groups of well-defined patients, hyperresponsiveness to mannitol has been shown to have high specificity for a clinical diagnosis of asthma (95%), with a sensitivity of approximately 60%.^{89,93,94} This sensitivity increases to 89% when the use of inhaled corticosteroids (ICS) is taken into account.⁸⁹ This finding means that like exercise, a positive mannitol test is consistent with the diagnosis of asthma, but a negative test does not exclude asthma. Most studies comparing responses to exercise and mannitol show concordant test results both for children at 84%⁹⁵ and 80%⁹⁶ and for adults at 74%.³⁴ Mannitol had a sensitivity of 59% and 69% to identify a 10% or 15% decrease in the FEV₁ in a group of patients with symptoms but without a definite diagnosis of asthma when 2 identical exercise tests were performed within a few days.³⁴ However, the frequency of a positive mannitol response was 1.41 times that of a 10% or more decrease on the first exercise test and 1.65 times greater than a 15% decrease.^{34,97}

Table 6
Mannitol provocation test kit^a protocol

Inhaled agent	Dry powder mannitol
Increasing doses	0, 5, 10, 20, 40, 80, 160, 160, 160 mg
Measurements	FEV ₁ before and 1 min after dose with highest value for FEV ₁ recorded
Positive response	Decrease FEV ₁ 15% or more or 10% between doses
Expression of result	
Sensitivity	PD ₁₅
Reactivity	Response dose ratio
Recovery	Bronchodilator or spontaneous

The time of testing should be kept to a minimum to progressively increase the osmotic gradient and less than 35 minutes.

^a Commercially available as Aridol or Osmohale.

There are individuals in whom a mannitol test is positive and EIB or EVH test is negative and vice versa.^{34,69} For those individuals with a positive mannitol test, the diagnosis is confirmed and the potential to get EIB is identified. The study in subjects with exercise wheeze is a good example of this, whereby 36 of 39 (92%) subjects tested positive to mannitol but only 14 (36%) had EIB to the exercise protocol.⁹⁸ When the exercise or EVH test is positive at a 10% or more decrease in the FEV₁ and the mannitol test is negative, the different cutoff points should be kept in mind and the percent decreases in the FEV₁ compared. Further, if exercise or EVH tests are performed with cold air inspire, borderline airway responses may be entirely mediated by reactive hyperemia, with ensuing vascular engorgement and edema in the mucosa and submucosa (but without inflammatory mediator release).²⁸ Some investigators have reported a 10% decrease or PD₁₀ to mannitol to compare responses with EVH (see **Fig. 4**),^{69,99} whereas others have compared mannitol and exercise in children using a 15% decrease for both tests.^{95,96} In addition, it is useful to calculate the reactivity to mannitol (ie, RDR) and relate it to the maximum percent decrease after exercise, which is also an index of reactivity.

A positive response to mannitol is consistent with other indices of inflammation and was observed in 82% of those with eosinophilic asthma.¹⁰⁰ The response to mannitol was greater in those with a higher exhaled nitric oxide.¹⁰¹ In 252 patients with asthma treated with ICS, the frequency of a positive mannitol test was 51%,¹⁰² a value similar to that reported for exercise.³¹ As with a negative exercise test, caution is needed when interpreting a negative mannitol test in those being treated with ICS. In these patients, the negative test is likely caused by a reduction in inflammatory cell number¹⁰³ or concentration of mediators rather than a loss of sensitivity of the bronchial smooth muscle, which remains hyperresponsive to methacholine even after months of treatment with high doses of ICS.¹⁰⁴

In elite athletes, one might commonly encounter a positive methacholine test result but a negative result to challenge with mannitol, exercise, or EVH. A possible reason for this is that epithelial injury can result in a positive methacholine test because the access of the methacholine to the smooth muscle is enhanced by the injury. The same athlete may have no significant airway inflammation and, as a result, have negative tests to mannitol, exercise, or EVH.¹⁰ For this reason, it is not appropriate to make a diagnosis of asthma or identify the potential for EIB in elite athletes from a positive methacholine test result alone.

REFERENCES

1. Freed AN, Anderson SD. Exercise-induced bronchoconstriction. Human models. In: Kay AB, editor. Allergy & allergic diseases. Oxford (United Kingdom): Blackwell Scientific Publications; 2008. p. 806–20.
2. Rundell KW, Im J, Mayers LB, et al. Self-reported symptoms and exercise-induced asthma in the elite athlete. *Med Sci Sports Exerc* 2001;33:208–13.
3. De Baets F, Bodart E, Dramaix-Wilmet M, et al. Exercise-induced respiratory symptoms are poor predictors of bronchoconstriction. *Pediatr Pulmonol* 2005; 39:301–5.
4. Madhuban AA, Driessen JM, Brusse-Keizer MG, et al. Association of the asthma control questionnaire with exercise-induced bronchoconstriction. *J Asthma* 2011;3:275–8.
5. Haby MM, Peat JK, Mellis CM, et al. An exercise challenge for epidemiological studies of childhood asthma: validity and repeatability. *Eur Respir J* 1995;8: 729–36.

6. Mannix ET, Manfredi F, Farber MO. A comparison of two challenge tests for identifying exercise-induced bronchospasm in figure skaters. *Chest* 1999;115:649–53.
7. Parsons JP, Kaeding C, Phillips G, et al. Prevalence of exercise-induced bronchospasm in a cohort of varsity college athletes. *Med Sci Sports Exerc* 2007;39:1487–92.
8. Holzer K, Anderson SD, Douglass J. Exercise in elite summer athletes: challenges for diagnosis. *J Allergy Clin Immunol* 2002;110:374–80.
9. Dickinson JW, Whyte GP, McConnell AK, et al. Mid expiratory flow versus FEV1 measurements in the diagnosis of exercise-induced asthma in elite athletes. *Thorax* 2006;61:111–4.
10. Sue-Chu M, Brannan JD, Anderson SD, et al. Airway hyperresponsiveness to methacholine, adenosine5-monophosphate, mannitol, eucapnic voluntary hyperpnoea and field exercise challenge in elite cross country skiers. *Br J Sports Med* 2010;44:827–32.
11. Parsons JP. Exercise-induced bronchospasm: symptoms are not enough. *Expert Rev Clin Immunol* 2009;5:357–9.
12. Anderson SD, Fitch K, Perry CP, et al. Responses to bronchial challenge submitted for approval to use inhaled beta2 agonists prior to an event at the 2002 Winter Olympics. *J Allergy Clin Immunol* 2003;111:44–9.
13. Parsons JP, Craig TJ, Stoloff SW, et al. Impact of exercise-related respiratory symptoms in adults with asthma: Exercise-Induced Bronchospasm Landmark National Survey. *Allergy Asthma Proc* 2011;32:431–7.
14. Ostrom NK, Eid NS, Craig TJ, et al. Exercise-induced bronchospasm in children with asthma in the United States: results from the Exercise-Induced Bronchospasm Landmark Survey. *Allergy Asthma Proc* 2011;32:425–30.
15. Weiler JM, Anderson SD, Randolph C, et al. Pathogenesis, prevalence, diagnosis, and management of exercise-induced bronchoconstriction: a practice parameter. *Ann Allergy Asthma Immunol* 2010;105:S1–46.
16. Anderson SD, Daviskas E, Smith CM. Exercise-induced asthma: a difference in opinion regarding the stimulus. *Allergy Proc* 1989;10:215–26.
17. Anderson SD, Schoeffel RE, Follet R, et al. Sensitivity to heat and water loss at rest and during exercise in asthmatic patients. *Eur J Respir Dis* 1982;63:459–71.
18. Carlsen KH, Engh G, Mørk M. Exercise induced bronchoconstriction depends on exercise load. *Respir Med* 2000;94:750–5.
19. Daviskas E, Gonda I, Anderson SD. Local airway heat and water vapour losses. *Respir Physiol* 1991;84:115–32.
20. Carroll ML, Carroll NG, Gundersen HJ, et al. Mast cell densities in bronchial biopsies and small airways are related. *J Clin Pathol* 2011;64:394–8.
21. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is.... *J Allergy Clin Immunol* 2000;106:453–9.
22. Hjoberg J, Folkerts G, van Gessel SB, et al. Hyperosmolarity-induced relaxation and prostaglandin release in guinea pig trachea in vitro. *Eur J Pharmacol* 2000;398:303–7.
23. Gulliksson M, Palmberg L, Nilsson G, et al. Release of prostaglandin D2 and leukotriene C4 in response to hyperosmolar stimulation of mast cells. *Allergy* 2006;61:1473–9.
24. Anderson SD, Kippelen P. Exercise-induced bronchoconstriction: pathogenesis. *Curr Allergy Asthma Rep* 2005;5:116–22.
25. Edmunds A, Tooley M, Godfrey S. The refractory period after exercise-induced asthma: its duration and relation to the severity of exercise. *Am Rev Respir Dis* 1978;117:247–54.

26. Schoeffel RE, Anderson SD, Gillam I, et al. Multiple exercise and histamine challenge in asthmatic patients. *Thorax* 1980;35:164–70.
27. Reiff DB, Choudry NB, Pride NB, et al. The effect of prolonged submaximal warm-up exercise on exercise-induced asthma. *Am Rev Respir Dis* 1989;139:479–84.
28. McFadden ER, Lenner KA, Strohl KP. Postexertional airway rewarming and thermally induced asthma. *J Clin Invest* 1986;78:18–25.
29. Anderson SD, Holzer K. Exercise-induced asthma: is it the right diagnosis in elite athletes? *J Allergy Clin Immunol* 2000;106:419–28.
30. Stensrud T, Berntsen S, Carlsen KH. Exercise capacity and exercise-induced bronchoconstriction (EIB) in a cold environment. *Respir Med* 2007;101:1529–36.
31. Anderson SD, Lambert S, Brannan JD, et al. Laboratory protocol for exercise asthma to evaluate salbutamol given by two devices. *Med Sci Sports Exerc* 2001;33:893–900.
32. Anderson SD, Pearlman DS, Rundell KW, et al. Reproducibility of the airway response to an exercise protocol standardized for intensity, duration, and inspired air conditions, in subjects with symptoms suggestive of asthma. *Respir Res* 2010;11:120.
33. Rundell KW, Wilber RL, Szmedra L, et al. Exercise-induced asthma screening of elite athletes: field vs laboratory exercise challenge. *Med Sci Sports Exerc* 2000;32:309–16.
34. Anderson SD, Charlton B, Weiler JM, et al. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res* 2009;10:4.
35. Hallstrand TS, Curtis JR, Koepsell TD, et al. Effectiveness of screening examinations to detect unrecognised exercise-induced bronchoconstriction. *J Pediatr* 2002;141:343–9.
36. Clearie KL, Williamson PA, Vaidyanathan S, et al. Disconnect between standardized field-based testing and mannitol challenge in Scottish elite swimmers. *Clin Exp Allergy* 2010;40:731–7.
37. Romberg K, Bjermer L, Tufvesson E. Exercise but not mannitol increases Clara cell protein (CC16) in elite swimmers. *Respir Med* 2011;105:31–6.
38. Aitken ML, Marini JJ. Effect of heat delivery and extraction on airway conductance in normal and in asthmatic subjects. *Am Rev Respir Dis* 1985;131:357–61.
39. Anderson SD, Schoeffel RE, Black JL, et al. Airway cooling as the stimulus to exercise-induced asthma - a re-evaluation. *Eur J Respir Dis* 1985;67:20–30.
40. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing - 1999. *Am J Respir Crit Care Med* 2000;161:309–29.
41. Johnson BD, Babcock MA, Suman OE, et al. Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol* 1993;460:385–405.
42. Kattan M, Thomas CM, Keens TG, et al. The response to exercise in normal and asthmatic children. *J Pediatr* 1978;92:718–21.
43. Custovic A, Arifhodzic N, Robinson A, et al. Exercise testing revisited. The response to exercise in normal and atopic children. *Chest* 1994;105:1127–32.
44. Backer V, Dirksen A, Bach-Mortensen N, et al. The distribution of bronchial responsiveness to histamine and exercise in 527 children and adolescents. *J Allergy Clin Immunol* 1991;88:68–76.
45. Godfrey S, Springer C, Bar-Yishay E, et al. Cut-off points defining normal and asthmatic bronchial reactivity to exercise and inhalation challenges in children and young adults. *Eur Respir J* 1999;14:659–68.

46. Food and Drug Administration Guidance for Industry. Available at: <http://www.fda.gov/cder/guidance>. Accessed October 27, 2012.
47. Anderson S, Seale JP, Ferris L, et al. An evaluation of pharmacotherapy for exercise-induced asthma. *J Allergy Clin Immunol* 1979;64:612–24.
48. Helenius IJ, Tikkanen HO, Haahtela T. Occurrence of exercise induced bronchospasm in elite runners: dependence on atopy and exposure to cold air and pollen. *Br J Sports Med* 1998;32:125–9.
49. Choi IS, Ki WJ, Kim TO, et al. Seasonal factors influencing exercise-induced asthma. *Allergy Asthma Immunol Res* 2012;4:192–6.
50. Koskela H, Tukiainen H, Kononoff A, et al. Effect of whole-body exposure to cold and wind on lung function in asthmatic patients. *Chest* 1994;105:1728–31.
51. Koskela H, Tukiainen H. Facial cooling, but not nasal breathing of cold air, induces bronchoconstriction: a study in asthmatic and healthy subjects. *Eur Respir J* 1995;8:2088–93.
52. Zeitoun M, Wilk B, Matsuzaka A, et al. Facial cooling enhances exercise-induced bronchoconstriction in asthmatic children. *Med Sci Sports Exerc* 2004;36:767–71.
53. Evans TM, Rundell KW, Beck KC, et al. Cold air inhalation does not affect the severity of EIB after exercise or eucapnic voluntary hyperventilation. *Med Sci Sports Exerc* 2005;37:544–9.
54. Henriksen JM. Exercise-induced bronchoconstriction. Seasonal variation in children with asthma and in those with rhinitis. *Allergy* 1986;41:468–70.
55. Karjalainen J, Lindqvist A, Laitinen LA. Seasonal variability of exercise-induced asthma especially out-doors. Effect of birch pollen allergy. *Clin Exp Allergy* 1989;19:273–9.
56. Rundell KW, Spiering BA, Baumann JM, et al. Bronchoconstriction provoked by exercise in a high-particulate-matter environment is attenuated by montelukast. *Inhal Toxicol* 2005;17:99–105.
57. Rundell KW, Spiering BA. Inspiratory stridor in elite athletes. *Chest* 2003;123:468–74.
58. McFadden ER, Zawadski DK. Vocal cord dysfunction masquerading as exercise-induced asthma. A physiologic cause for “choking” during athletic activities. *Am J Respir Crit Care Med* 1996;153:942–7.
59. Christensen PM, Thomsen SF, Rasmussen N, et al. Exercise-induced laryngeal obstructions: prevalence and symptoms in the general public. *Eur Arch Otorhinolaryngol* 2011;268:1313–9.
60. Abu-Hasan M, Tannous B, Weinberger M. Exercise-induced dyspnea in children and adolescents: if not asthma then what? *Ann Allergy Asthma Immunol* 2005;94:366–71.
61. Argyros GJ, Roach JM, Hurwitz KM, et al. Eucapnic voluntary hyperventilation as a bronchoprovocation technique. Development of a standardized dosing schedule in asthmatics. *Chest* 1996;109:1520–4.
62. Hurwitz KM, Argyros GJ, Roach JM, et al. Interpretation of eucapnic voluntary hyperventilation in the diagnosis of asthma. *Chest* 1995;108:1240–5.
63. Stadelmann K, Stensrud T, Carlsen KH. Respiratory symptoms and bronchial responsiveness in competitive swimmers. *Med Sci Sports Exerc* 2011;43:375–81.
64. Phillips YY, Jaeger JJ, Laube BL, et al. Eucapnic voluntary hyperventilation of compressed gas mixture. A simple system for bronchial challenge by respiratory heat loss. *Am Rev Respir Dis* 1985;131:31–5.
65. Fitch KD, Sue-Chu M, Anderson SD, et al. Asthma and the elite athlete: summary of the International Olympic Committee’s Consensus Conference, Lausanne, Switzerland, January 22–24, 2008. *J Allergy Clin Immunol* 2008;122:254–60.

66. Holley AB, Cohee B, Walter RJ, et al. Eucapnic voluntary hyperpnea is superior to methacholine challenge testing for detecting airway hyperreactivity in nonathletes. *J Asthma* 2012;49(6):614–9.
67. Bougault V, Turmel J, Boulet LP. Airway hyperresponsiveness in elite swimmers: is it a transient phenomenon? *J Allergy Clin Immunol* 2011;127:892–8.
68. Bougault V, Boulet LP, Turmel J. Bronchial challenges and respiratory symptoms in elite swimmers and winter sport athletes. *Chest* 2010;138:31S–7S.
69. Holzer K, Anderson SD, Chan HK, et al. Mannitol as a challenge test to identify exercise-induced bronchoconstriction in elite athletes. *Am J Respir Crit Care Med* 2003;167:534–47.
70. Anderson SD, Argyros GJ, Magnussen H, et al. Provocation by eucapnic voluntary hyperpnea to identify exercise induced bronchoconstriction. *Br J Sports Med* 2001;35:344–7.
71. Brummel NE, Mastronarde JG, Rittinger D, et al. The clinical utility of eucapnic voluntary hyperventilation testing for the diagnosis of exercise-induced bronchospasm. *J Asthma* 2009;46:683–6.
72. Rundell KW, Anderson SD, Spiering BA, et al. Field exercise vs laboratory eucapnic voluntary hyperventilation to identify airway hyperresponsiveness in elite cold weather athletes. *Chest* 2004;125:909–15.
73. Kippelen P, Larsson J, Anderson SD, et al. Effect of sodium cromoglycate on mast cell mediators during hyperpnea in athletes. *Med Sci Sports Exerc* 2010;42:1853–60.
74. Kippelen P, Larsson J, Anderson SD, et al. Acute effects of beclomethasone on hyperpnea-induced bronchoconstriction. *Med Sci Sports Exerc* 2010;42:273–80.
75. Stensrud T, Mykland KV, Gabrielsen K, et al. Bronchial hyperresponsiveness in skiers: field test versus methacholine provocation? *Med Sci Sports Exerc* 2007;39:1681–6.
76. Anderson SD, Kippelen P. Airway injury as a mechanism for exercise-induced bronchoconstriction in elite athletes. *J Allergy Clin Immunol* 2008;122:225–35.
77. Bougault V, Turmel J, St-Laurent J, et al. Asthma, airway inflammation and epithelial damage in swimmers and cold-air athletes. *Eur Respir J* 2009;33:734–9.
78. Pedersen L, Winther S, Backer V, et al. Airway responses to eucapnic hyperpnea, exercise and methacholine in elite swimmers. *Med Sci Sports Exerc* 2008;40:1567–72.
79. Norval J, Perry CP, Anderson SD. Responses to methacholine and eucapnic voluntary hyperpnea in relation to baseline lung function [abstract]. *Respirology* 2007;12:A1.
80. O'Byrne PM. Leukotrienes in the pathogenesis of asthma. *Chest* 1997;111:27S–34S.
81. Smith CM, Anderson SD. Hyperosmolarity as the stimulus to asthma induced by hyperventilation? *J Allergy Clin Immunol* 1986;77:729–36.
82. Smith CM, Anderson SD. Inhalation provocation tests using non-isotonic aerosols. *J Allergy Clin Immunol* 1989;84:781–90.
83. Riedler J, Reade T, Dalton M, et al. Hypertonic saline challenge in an epidemiological survey of asthma in children. *Am J Respir Crit Care Med* 1994;150:1632–9.
84. Anderson SD, Brannan JD. Methods for 'indirect' challenge tests including exercise, eucapnic voluntary hyperpnea and hypertonic aerosols. *Clin Rev Allergy Immunol* 2003;24:63–90.

85. Anderson SD, Brannan J, Spring J, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997;156:758–65.
86. Eggleston PA, Kagey-Sobotka A, Schleimer RP, et al. Interaction between hyperosmolar and IgE-mediated histamine release from basophils and mast cells. *Am Rev Respir Dis* 1984;130:86–91.
87. Brannan JD, Gulliksson M, Anderson SD, et al. Evidence of mast cell activation and leukotriene release after mannitol inhalation. *Eur Respir J* 2003;22:491–6.
88. Brannan JD, Gulliksson M, Anderson SD, et al. Inhibition of mast cell PGD2 release protects against mannitol-induced airway narrowing. *Eur Respir J* 2006;27:944–50.
89. Brannan JD, Anderson SD, Perry CP, et al. The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline. *Respir Res* 2005;6:144.
90. Brannan JD, Anderson SD, Gomes K, et al. Fexofenadine decreases sensitivity to and montelukast improves recovery from inhaled mannitol. *Am J Respir Crit Care Med* 2001;163:1420–5.
91. Barben J, Roberts M, Chew N, et al. Repeatability of bronchial responsiveness to mannitol dry powder in children with asthma. *Pediatr Pulmonol* 2003;36:490–4.
92. Wood LG, Powell H, Gibson PG. Mannitol challenge for assessment of airway responsiveness, airway inflammation and inflammatory phenotype in asthma. *Clin Exp Allergy* 2010;40:232–41.
93. Sverrild A, Porsbjerg C, Thomsen SF, et al. Diagnostic properties of inhaled mannitol in the diagnosis of asthma: a population study. *J Allergy Clin Immunol* 2009;124:928–932.e1.
94. Sverrild A, Porsbjerg C, Thomsen SF, et al. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study. *J Allergy Clin Immunol* 2010;126:952–8.
95. Barben J, Kuehni C, Strippoli M-P, et al. Mannitol dry powder challenge in comparison with exercise-testing in children. *Pediatr Pulmonol* 2011;46:842–8.
96. Kersten ET, Driessen JM, van der Berg JD, et al. Mannitol and exercise challenge tests in asthmatic children. *Pediatr Pulmonol* 2009;44:655–61.
97. Anderson SD, Brannan JD. Bronchial provocation testing: the future. *Curr Opin Allergy Clin Immunol* 2011;11:46–52.
98. Cowan SC, Hewitt RS, Cowan JO, et al. Exercise-induced wheeze: fraction of exhaled nitric oxide directed management. *Respirology* 2010;15:683–90.
99. Aronsson D, Tufvesson E, Bjerner L. Comparison of central and peripheral airway involvement before and during methacholine, mannitol and eucapnic hyperventilation challenges in mild asthmatics. *Clin Respir J* 2011;5:10–8.
100. Porsbjerg C, Lund TK, Pedersen L, et al. Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. *J Asthma* 2009;46:606–12.
101. Anderson WJ, Lipworth BJ. Relationship of mannitol challenge to methacholine challenge and inflammatory markers in persistent asthmatics receiving inhaled corticosteroids. *Lung* 2012;90(5):513–21.
102. Brannan JD, Perry CP, Anderson SD. Mannitol test results in asthmatic adults receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2012. [Epub ahead of print].

103. James A, Gyllfors P, Henriksson E, et al. Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi. *Allergy* 2012;67:958–61.
104. Reddel HK, Jenkins CR, Marks GB, et al. Optimal asthma control, starting with high doses of inhaled budesonide. *Eur Respir J* 2000;16:226–35.