

Exercise and other indirect challenges to demonstrate asthma or exercise-induced bronchoconstriction in athletes

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Activity Objectives

1. To understand the role of indirect challenges in the diagnosis and monitoring of exercise-induced bronchoconstriction.
2. To describe optimal conditions for exercise, eucapnic voluntary hyperpnea, hypertonic saline, and mannitol challenges.
3. To improve interpretation of exercise, eucapnic voluntary hyperpnea, hypertonic saline, and mannitol challenges.

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The prevalence of exercise-induced bronchoconstriction is reported to be high among recreational and elite athletes, yet diagnosis is often symptom-based. Indirect challenges such as the laboratory exercise challenge provide objective criteria for proper diagnosis and treatment. However, a standardized protocol using appropriate exercise intensity, duration, and dry air inhalation is often not implemented, and thus a false-negative test may result. This article reviews and describes the symptom-based diagnosis, the exercise challenge, and other indirect challenges such as eucapnic voluntary hyperpnea, hypertonic saline inhalation, and inhaled powdered mannitol as methods to diagnose and evaluate exercise-induced

bronchoconstriction. Advantages and disadvantages of each diagnostic procedure are presented. (*J Allergy Clin Immunol* 2008;122:238-46.)

Key words: *Athlete, bronchoconstriction, diagnosis, EVH, exercise-induced asthma, hypertonic saline, mannitol*

Exercise-induced bronchoconstriction (EIB) is the transient airway narrowing after^{1,2} or during³⁻⁵ exercise. An estimated 90% of individuals with asthma^{6,7} and 10% to 50% of a given elite athlete population experience EIB.⁶⁻¹³ In this review, *EIB* is considered synonymous to *exercise-induced asthma*, although some reserve the term *exercise-induced asthma* for individuals with known asthma who bronchoconstrict from exercise. The wide range in reported prevalence in the athlete population is in part a result of the variability of specific environmental demands.¹³ This high prevalence of EIB reported in specific elite sports brings into question whether all individuals with EIB have asthma to some degree, or whether EIB is an entity exclusive from asthma and a natural occurrence related to lung injury. The 20% to 35% prevalence of EIB in the ice rink athlete has been attributed to the inhalation of cold dry air and *high-emission pollutants* from fossil-fueled ice resurfacing machines.¹⁴⁻¹⁶ The 30% to 50% prevalence among Nordic skiers is attributed to chronic inhalation of cold, dry air during training and competition,^{5,17,18} and the approximate 15% prevalence among distance runners is strongly associated with atopy, allergy, and asthma.^{19,20} Likewise,

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Terms in boldface and italics are defined in the glossary on page 239.

Abbreviations used

ATS:	American Thoracic Society
EIB:	Exercise-induced bronchoconstriction
EVH:	Eucapnic voluntary hyperpnea
FVC:	Forced vital capacity
HR _{peak} :	Peak heart rate
ICS:	Inhaled corticosteroid
IOC:	International Olympic Committee
MVV:	Maximal voluntary ventilation
RH:	Relative humidity
V _E :	Minute ventilation

the high prevalence of asthma and EIB reported in competitive swimmers (11% to 29%)²⁰ has been related to inhalation of *chloramines* in the air immediately above the water in indoor pools. Current literature suggests that the prevalence of EIB is higher in the elite athlete population than in the nonathlete population. Because of high β_2 -agonist use among elite athletes, the International Olympic Committee (IOC) requires objective evidence to demonstrate asthma or EIB as an indication for therapeutic use of β_2 -agonists during competition²¹; documented falls in FEV₁ from a specified indirect or direct challenge have been used since the 2002 Salt Lake City Olympic Games. This article reviews indirect challenges used to obtain an objective diagnosis of EIB.

SELF-REPORTED SYMPTOMS

Physician diagnosis of EIB is often based on self-reported symptoms without lung function tests.^{12,22-24} EIB in the athlete is most often accompanied by symptoms of cough, wheeze, chest tightness, dyspnea, or excess mucus production.¹² Although a clinical diagnosis of EIB based on self-reported symptoms may be marginally useful,^{12,25} recent studies have shown a lack of *sensitivity* and *specificity* of a symptom-based diagnosis in athletes.¹² Rundell et al¹² found that approximately half of elite athletes reporting EIB symptoms demonstrate normal airway function, whereas half of the athletes who reported having no symptoms tested positive for EIB. This study established the necessity of objective spirometry using a standardized challenge for appropriate diagnosis of EIB. Most recently, Parsons et al²⁴ confirmed that symptoms were not predictive of EIB in a study of 107 college athletes in whom *eucapnic voluntary hyperpnea (EVH)* challenge was performed. They defined a fall in FEV₁ of 10% or greater as consistent with EIB and found the prevalence of EIB was 36% in athletes with no symptoms and 35% for those with symptoms of EIB. Moreover, the athletes in *high-ventilation sports* were significantly more symptomatic (48%) than athletes in low-ventilation sports (25%; $P = .02$), with no difference in EIB prevalence between the groups. It is therefore necessary to confirm a diagnosis of EIB through objective measures of lung function by using standardized testing procedures.

GLOSSARY

AMP: AMP is used as an indirect provocation agent by stimulating the degranulation of bronchial mast cells with subsequent release of histamine, leukotrienes, and other inflammatory mediators. As such, it measures the level of airway inflammation.

CHLORAMINES: The interaction of chlorine with human pollutants (eg, sweat, urine) forms chloramines, which are respirable above swimming pools and include chloramide, chlorimide, and nitrogen trichloride. Inhalation of chloramines has been associated with asthma exacerbations, occupational asthma, and increased levels of tryptase, eosinophil cationic protein, and basophils in nasal lavage fluid.

EUCAPNIC VOLUNTARY HYPERPNEA (EVH): An indirect provocation test that was developed as a surrogate marker for exercise-induced bronchospasm. Other indirect tests were developed to mimic the various airway insults that occur during exercise. Hypertonic saline and dry powders mimic airway dehydration associated with water loss with exercise. Indirect tests have the advantage of assessing the presence of inflammatory cells and their mediators compared with direct tests that directly provoke smooth muscle contraction.

HIGH-EMISSION POLLUTANTS: Pollutants termed *criteria pollutants* are known to cause health effects at ambient air concentrations and consist of carbon monoxide, lead, nitrogen dioxide, ozone, particulate matter, and sulfur dioxide. Sulfur oxides, carbon monoxide, nitrogen oxides, and particulates are made by burning fossil fuels (coal, oil, and natural gas) and can contribute to the formation of tropospheric ozone. Chlorofluorocarbons cause depletion of stratospheric ozone. Particle pollution includes acids, organic chemicals, and aeroallergens.

HIGH-VENTILATION SPORTS: High-ventilation sports are defined as endurance sports in which ventilation is increased for prolonged periods—for example, during cross-country skiing or long distance running. Although the prevalence of exercise induced bronchospasm

was thought to be higher in these endurance sports, this might not be the case.

MAXIMAL VOLUNTARY VENTILATION (MVV): The maximum amount of air that can be breathed in a given period, often 1 minute. The maximal voluntary ventilation is effort-dependent and subject to variability.

MINUTE VENTILATION (V_E): Minute ventilation maintains the correct amounts of oxygen and carbon dioxide available to the alveoli and is defined as frequency of breathing multiplied by the volume of the breath—that is, respiratory rate per minute $\times$ tidal volume. In contrast, the alveolar ventilation takes into account only the gas that is available for alveolar gas exchange by subtracting the dead space from the tidal volume.

PERICILIARY FLUID/AIRWAY SURFACE LIQUID: The airway surface fluid is a bilayer composed of a superficial mucous layer and a periciliary fluid layer that bathes the airway epithelium up to the cilia. The mucous layer is composed of mucin glycoproteins made up mainly of Mucin (MUC) 5AC and MUC5B. The periciliary and mucous layers are separated from one another by a layer of surfactant. Water evaporation creates a hypertonic environment, and hyperosmolar fluid causes mast cell and basophil degranulation with histamine and leukotriene release. Eosinophils can also be stimulated by mannitol *in vitro* to cause the release of leukotriene C₄.

SENSITIVITY/SPECIFICITY: Sensitivity is the presence of a positive test among all patients with a disease and gauges the ability of finding true-positives among diseased states. Specificity is the presence of a negative test among all patients without a disease and gauges the finding of true-negatives among all healthy patients. By contrast, the positive and negative predictive values are parameters that assess the ability of a test to find true-positives and true-negatives among all patients with positive and negative tests, respectively.

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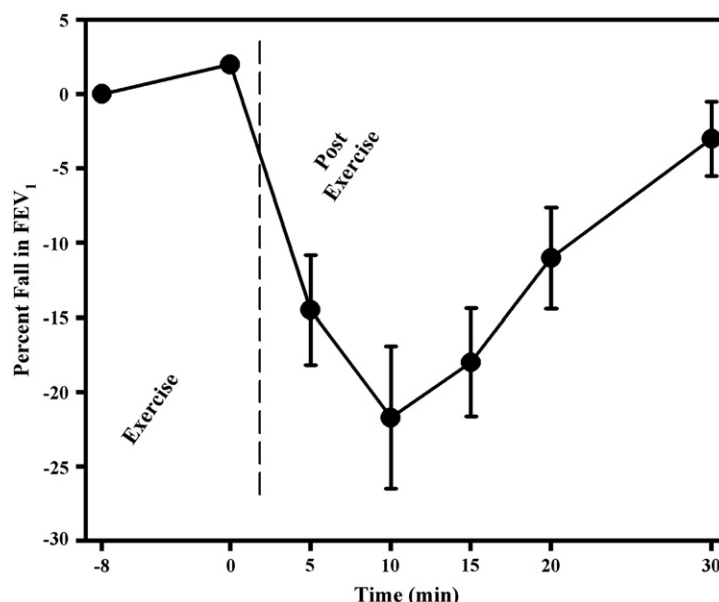


FIG 1. Typical change in FEV₁ in response to an 8-minute exercise challenge in EIB-positive individuals. Note the small improvement in FEV₁ immediately after exercise followed by significant falls in FEV₁ at 5 minutes after the cessation of exercise. Spontaneous recovery is most often nearly complete by 30 minutes postchallenge. Recovery can be accelerated by administration of an inhaled β_2 -agonist.

INDIRECT VERSUS DIRECT CHALLENGES

The methods used to test for EIB are critical in making the correct diagnosis and developing a treatment strategy. Indirect challenges such as exercise, EVH, inhaled powdered mannitol, nebulized hypertonic saline, or *AMP* appear to be more effective in identifying EIB in the elite athlete population than direct challenges such as methacholine or histamine.^{26,27} Indirect challenges may be the preferred strategy for monitoring the effectiveness of asthma therapy, because airway hyperresponsiveness is associated with inflammation,²⁸ and inflammation is reduced by inhaled corticosteroid (ICS) treatment.²⁹⁻³¹ The indirect challenge is thought to cause inflammatory cells to release mediators such as leukotrienes, prostaglandins, and histamine that provoke airway smooth muscle constriction.¹ Thus, the indirect challenge reflects the level of inflammation in the airways,²⁸ whereas a direct challenge acts directly with airway smooth muscle receptors to cause constriction independent of airway inflammation.

EXERCISE CHALLENGE

The lack of using a standardized exercise challenge for EIB may explain the wide range in reported prevalence within specific sports, whereas the variety of exercise challenges at different intensities with no control over challenge *minute ventilation* (V_E) or water content of inhaled air may result in poor test-retest reliability. Therefore, diagnosis should be based on objective measurement of variable or partially reversible airflow obstruction using a standardized test with prespirometry and postspirometry.³²⁻³⁴

The use of spirometry to identify EIB includes baseline lung function measures followed by an EIB-provoking challenge and a series of lung function measurements after the provocation. Typical postprovocation spirometry times are at 5, 10, 15, and 30 minutes after the completion of the challenge with 2

reproducible maneuvers within 3% of each other performed at each time point, although variations with maneuvers at 1-minute, 3-minute, or 20-minute time points have been performed (Fig 1). However, IOC-Medical Commission recommendations dictate that FEV₁ should first be recorded at least 3 minutes after the challenge and should be sustained over the period of the next 5 minutes to be consistent with EIB. Postprovocation spirometry values are compared with preprovocation spirometry values to calculate a percent change from baseline. If there is a 10% or greater decrease in the FEV₁ of a FEV₁ or a forced vital capacity (FVC) maneuver at any time point, then the patient is diagnosed as being positive for EIB. If vocal cord dysfunction is suspected, a FVC maneuver should be performed immediately after completion of the challenge and may reveal a flattened inspiratory loop and a decreased FEV₁ with no change in the FEV₁/FVC ratio.^{35,36}

Guidelines that permit the diagnosis of EIB on the basis of pulmonary function change after an exercise challenge have been clearly defined³³ and statistically justified. However, post-exercise declines in FEV₁ greater than 10% to 25% have been used.^{7,12} A 15% fall in FEV₁ for field exercise challenges and a 10% fall in FEV₁ for laboratory challenges have been suggested.⁶ The American Thoracic Society (ATS)³³ and the European Respiratory Society³⁴ recommend a 10% decrease in FEV₁, based on 2 SDs from the mean baseline FEV₁ in healthy people, as a criterion for EIB.

It is necessary to confirm a diagnosis of EIB through quantitative objective measures of lung function by using standardized procedures because of the lack of sensitivity and specificity of self-reported symptoms and history questionnaire. The use of an exercise test for identifying EIB can be quite sensitive and specific if the V_E and water content of the inhaled air are standardized.³⁷⁻³⁹ Some⁴⁰⁻⁴⁴ have performed laboratory-based EIB testing by using an exercise challenge of 6 to 8 minutes in ambient

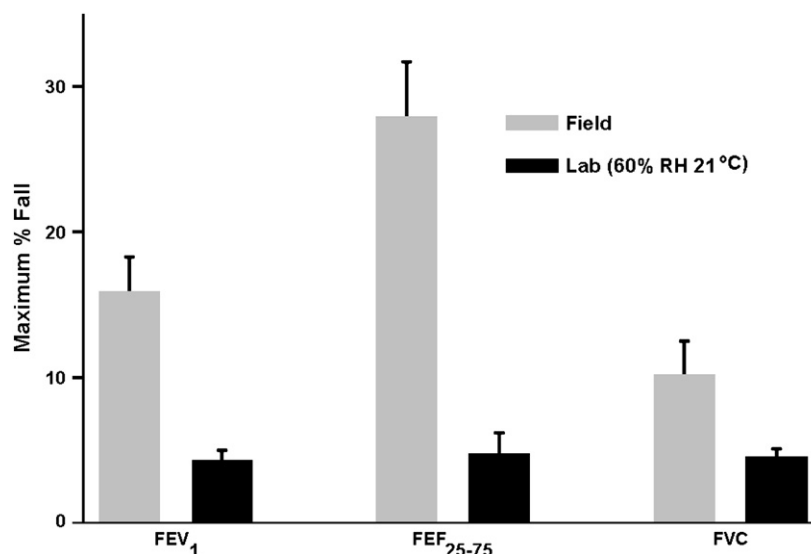


FIG 2. Eighteen of 23 elite winter athletes who tested positive by a field-based sport-specific exercise challenge but tested negative by laboratory treadmill run in ambient conditions of 21°C, 60% RH. Redrawn from Rundell KW, Wilber RL, Szmedra L, Jenkinson DM, Mayers LB, Im J. Exercise-induced asthma screening of elite athletes: field versus laboratory exercise challenge. *Med Sci Sports Exerc* 2000;32:309-16.¹⁰ FEF₂₅₋₇₅, Forced expiratory flow at 25% to 75% of FVC.

conditions (20-25°C; relative humidity [RH] <50%) at 80% to 90% of peak heart rate (HR_{peak}). Others⁴⁵ suggested that the exercise intensity be less than 85% of predicted HR_{peak}. This recommendation is based on the assumption that catecholamine release at high exercise intensity levels may cause bronchodilation, and thus affect test results, although evidence suggests that vagal tone withdrawal and not circulating catecholamines is responsible for bronchodilation during exercise. In addition, this exercise intensity may not be sufficient to trigger a response in all subjects. Although ATS guidelines³³ and studies by others⁴⁰⁻⁴⁴ recommend that laboratory-based EIB testing include an exercise challenge of 6 to 8 minutes in ambient conditions (20-25°C; RH <50%) at 80% to 90% of predicted HR_{peak} with prespirometry and postspirometry, the elite athlete population may require a greater exercise intensity under dry air conditions. Carlsen et al⁴⁶ examined the role of exercise load in relationship to EIB by comparing 20 children with asthma (9-17 years old) with EIB with 2 treadmill tests at 85% and 95% of calculated maximal heart rate. The peak fall in FEV₁ was significantly greater at 95% than at 85% peak heart rate (25.11% vs 8.84% fall); only 9 of 20 subjects had falls >10% at the 85% load, whereas all 20 subjects had falls >10% at the 95% work load. This study supports the necessity to standardize the exercise protocol by using high exercise intensity; the differences in peak fall in FEV₁ were most likely related to differences in V_E during the challenge.

Field-based challenges

Free running^{10,47} and sport-specific exercise challenges^{5,8,9,11,48} have been proven to be valid for the assessment of EIB and have been shown to be more sensitive than ambient temperature and RH laboratory challenges in elite winter athletes.¹⁰ Rundell et al¹⁰ examined sport/environment-specific field-based exercise challenges of varied duration to a standardized 8-minute-duration laboratory exercise challenge; laboratory conditions were 21°C, 60% RH, and exercise intensity for the last

6 minutes was ~95% HR_{peak}. Five athletes tested positive by both challenges, whereas 18 of the 23 athletes who tested positive for EIB by field-based challenge tested normal by laboratory challenge at 21°C, 60% RH (Fig 2). The remarkable differences in postexercise lung function between the field-based and ambient condition laboratory-based challenges provided strong evidence that water content of inhaled air is the primary stimulus of EIB. For some sports, sports-specific tests have been used with success; Wilber et al¹¹ used sports-specific tests (Nordic skiing, speed skating, ice hockey, ice skating) to identify EIB in Winter Olympic athletes, and Ogston and Butcher⁴⁸ used a 15-minute ski exercise to identify EIB in Nordic skiers. Free running or a 6-minute run has often been used in screening large groups for EIB because of the ease in performing such challenges. However, because of a general lack of control in terms of stimulus and the varied environmental conditions, this type of testing may not be reliable and is not appropriate to use as a means of monitoring treatment. To monitor the efficacy of EIB treatment effectively, it is imperative to use a challenge that can reproduce the stimulus and physiologic response (eg, exercise or EVH V_E).

Laboratory challenges

The laboratory exercise challenge for EIB may have high specificities,⁴⁹ but the sensitivity can be low because of high water content of inspired ambient air and the level of minute ventilation achieved and maintained.¹⁰ Anderson and Schoeffel⁵⁰ proposed that the increase in osmolarity of the *periciliary fluid* from water loss caused by conditioning of the inspired air during exercise or hyperventilation is the primary trigger of EIB. Mathematical modeling by Daviskas et al^{51,52} suggested that the loss of water during exercise was sufficient to increase osmolarity in the periciliary fluid to greater than 900 mOsm, a value that can cause airway narrowing. Airway water loss because of low RH inhaled air is the critical trigger that initiates the release of mediators leading to airway narrowing in susceptible individuals, and is thus a key

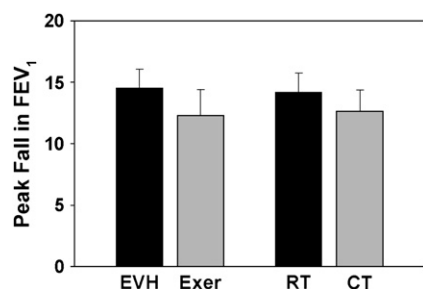


FIG 3. EVH and exercise challenge (*Exer*) were performed by EIB-positive athletes at room temperature (22°C) inhaling dry medical-grade air at either room temperature (*RT*) or chilled (−1°C; *CT*). Falls in FEV₁ were not different between challenge modes or between inhaled air temperature conditions. This provides further evidence supporting the notion that inhaled air water content and not air temperature per se is the major trigger for the EIB response. Redrawn from Evans TM, Rundell KW, Beck KC, Levine AM, Baumann JM. Cold air inhalation does not affect the severity of EIB after exercise or eucapnic voluntary hyperventilation. *Med Sci Sports Exerc* 2005;37:544-9.³⁹

component to the EIB challenge. The model developed calculated cumulative water loss of periciliary fluid to the 12th generation after 4 minutes of exercise or hyperventilation to be >2 mL, which exceeds the ~1.4 mL of airway surface liquid available.

Stensrud et al⁵³ demonstrated a 50% reduction in severity of EIB when comparing exercise challenge conditions of 40% to 95% RH at ambient temperature (24% and 12% falls in FEV₁, respectively). Further studies by Evans et al³⁷⁻³⁹ examined inhaled air temperature and the EIB response and noted that the severity of EIB was related to water content and not the coldness of inhaled air during the exercise challenge. In those studies, subjects exercised while breathing room temperature (22°C) dry air or cold (−1°C), dry air where inhaled air water content was less than 5 mg·L^{−1} air; exercising minute ventilations were equal between conditions. No significant difference in postexercise FEV₁ was noted between inhaled room temperature and cold air conditions; likewise, no difference was noted between exercise and EVH with matched V_E (Fig 3). In another study, Beuther and Martin⁵⁴ demonstrated that a heat exchange mask was as effective as pre-exercise treatment with albuterol, supporting the role of inhaled air water content/temperature in the EIB response.

Recommended exercise protocol

Current recommendations suggest that the optimal exercise challenge for the identification of EIB is an 8-minute exercise bout, either cycling or running, that allows the patient to achieve >90% of HR_{peak} by 2 minutes into the challenge and maintain it for the remaining 6 minutes of the challenge. This will, except for the very fit elite athlete, result in achieving and maintaining V_E at approximately 85% of *maximal voluntary ventilation* (MVV)⁵⁵; for the elite or highly fit athlete, an intensity of approximately 95% HR_{peak} is recommended. Inhaled air during the challenge should be dry, medical grade air (<5 mg H₂O·L^{−1} air) administered via gas cylinder with a reservoir bag (Douglas bag apparatus), and 1-way mouth valve and nose clips should be worn during the test. Because airway drying is the primary trigger, it is important that the challenge is not performed in ambient conditions (eg, 22°C, 50% RH), because this increases the risk for a false-negative test.¹⁰ Heart rate, arterial oxygen saturation (via pulse oximetry), and V_E should be measured throughout the test, and

heart rate and arterial oxygen saturation should be monitored through postchallenge spirometry to recovery. This will provide strict monitoring of the patient and enable tight control of test conditions that will allow high test-retest reliability and patient safety. The utility of being able to reproduce test conditions can be a valuable tool for monitoring patient treatment on subsequent visits.²⁸ Lung function tests should follow ATS standards for FVC or FEV₁ maneuvers.³³ Two reproducible maneuvers within 3% of each other should be performed before the challenge, with the best maneuver used to calculate postexercise falls in FEV₁. Two reproducible postexercise spirometry maneuvers should be performed at each time point of 5, 10, 15, and 30 minutes after the completion of the challenge. Again, if vocal cord dysfunction is suspected, a FVC procedure should be performed immediately postexercise. Otherwise, the FEV₁ procedure is preferred to decrease the likelihood of poor quality maneuvers caused by respiratory muscle fatigue. Before performing the exercise challenge, the 10% fall, 50% alert value, and 95% return value FEV₁ should be calculated from the pre-exercise baseline spirometry. Spirometry should continue through each time point unless the patient has a fall in FEV₁ greater than 50%, at which point a short-acting β₂-agonist should be administered to reverse the response. The patient should remain at the clinic or laboratory until the FEV₁ returns to 95% of baseline values.

EUCAPNIC VOLUNTARY HYPERPNEA

Eucapnic voluntary hyperpnea is a challenge based on the premise that increased ventilation rate causes bronchoconstriction in susceptible individuals. The mechanism is thought to occur through drying the airway surface liquid and increased osmolarity, resulting in degranulation of inflammatory cells and release of inflammatory mediators, similar to what occurs during exercise. Because EVH has demonstrated high sensitivity to identify those with EIB,⁵⁶⁻⁵⁸ EVH is currently the IOC Medical Commission–recommended challenge to identify EIB among Olympic athletes.²¹

The general procedure involves voluntary breathing of hypercapnic air (4.5% to 5% CO₂, 21% O₂, balance N₂) at a predetermined rate of 60% to 85% MVV; with the elite athlete, the breathing rate should be at a minimum of 85% MVV.⁵⁵ Pre-EVH and post-EVH spirometry is performed as it is with exercise. The respiration rate for the challenge is estimated by assuming MVV to be 35 × FEV₁,⁵⁵ although this assumption has been challenged. Spiering et al⁵⁹ found high variability in using FEV₁ to standardize EVH target ventilation (range, 64% to 109% maximum minute ventilation) and suggested that underdiagnosis of low-end outliers could occur. The recommended ventilation rate is 30 × FEV₁ (estimated 85% MVV) for 6 minutes.⁵⁶ Argyros et al⁶⁰ demonstrated that EVH at 30 × FEV₁ for 6 minutes resulted in a greater fall in FEV₁ than (1) EVH at 20 × FEV₁, (2) EVH at 15 × FEV₁ for 12 minutes, or (3) EVH at 30 × FEV₁ for 2 minutes repeated 3 times (26.7% ± 11.3% vs 16.6% ± 10.9% vs 11.0% ± 8.1% vs 19.6% ± 9.9% falls in FEV₁).

The dry EVH air mixture contains 4.5% to 5% CO₂^{55,56} to ensure eucapnia because hyperventilation-induced hypocapnia can cause bronchoconstriction in people with and without asthma.^{4,61} As with the exercise challenge, it is important to measure V_E to assure adequate and consistent ventilation throughout the 6-minute test. A variation of the EVH challenge is to chill the inspired air, potentially causing a greater change in airway

osmolarity or vascular response. However, the temperature of the inspired air has been subsequently shown to be unimportant to the degree of bronchoconstriction from this challenge.^{38,39} Using dry ambient temperature air (22°C) and dry cold air (−1°C), Evans et al³⁹ found no difference in post-EVH falls in FEV₁ between inhaled air temperature conditions (15.2 vs 13.8 for room temperature and cold air inhalation, respectively). Because the cold temperature inhaled air did not have an additive effect to the bronchoconstrictive response in this study, Evans et al³⁸ suggested that the water content and not temperature of the inhaled air is essential to test conditions. This conclusion is in agreement with others.⁵⁰

HYPERTONIC SALINE CHALLENGE

Nebulized hypertonic saline is considered an indirect challenge because it acts by increasing airway surface liquid osmolarity, triggering sensitized cells (in particular, mast cells) to release inflammatory mediators.⁶² An osmotic challenge such as nebulized hypertonic saline demonstrates effectiveness similar to exercise and EVH, but is more economical and easier to administer.⁶³⁻⁶⁶ An additional advantage of using this challenge is the ability to collect sputum concurrently with the measure of airway responsiveness for mediator and cellular analysis.⁶⁵⁻⁶⁷

The hypertonic saline challenge consists of baseline spirometry measurements followed by nebulized hypertonic saline inhalation. Most researchers have concluded that a 4.5% hypertonic saline concentration is most appropriate to deliver quicker (usually 15-20 minute) results without severe falls in FEV₁.⁶⁵⁻⁶⁷ The initial exposure to the hypertonic saline is 30 seconds.⁶⁶ If the percent fall from baseline FEV₁ is less than 10%, the exposure time is doubled (eg, 30 seconds, 60 seconds, 2 minutes, 4 minutes, 8 minutes).⁶⁶ Standard protocol indicates that FEV₁ is measured 60 seconds after every exposure. If FEV₁ fall is greater than 10%, the same exposure is administered a second time. The test is terminated after a 15% or greater fall in FEV₁ is observed or when a total minimum dose of 23 g has been administered in 15.5 minutes.⁶⁶ As with the mannitol challenge (see “Inhaled powdered mannitol challenge”), the hypertonic saline challenge produces a dose-dependent response, shown in Fig 4. This enables a classification to be made concerning the severity of the patient's asthma and level of inflammation.

A disadvantage of using the hypertonic saline challenge is that it may not be useful for evaluating treatment with low doses of ICSs. Jones et al⁶⁷ treated subjects with uncontrolled asthma for 8 weeks with inhaled beclomethasone of 50, 100, 200, and 500 µg/d. A dose-dependent change in responsiveness to hypertonic saline could be attained, but only the largest dose appeared to be effective.⁶⁷ Thus, hypertonic saline may function best observing dose-dependent changes of high-dose ICS therapy, which resembles the direct challenge measures. Although hypertonic saline has some pitfalls, it can still be used to identify those with exercise-induced asthma, current active asthma, or a history of asthma but no current symptoms.⁶⁵

INHALED POWDER MANNITOL CHALLENGE

Mannitol was developed as a bronchial provocation test in 1994 but has not yet received final US Food and Drug Administration approval for use as a bronchoprovocation test in the United States. Mannitol is a naturally occurring sugar alcohol found in most

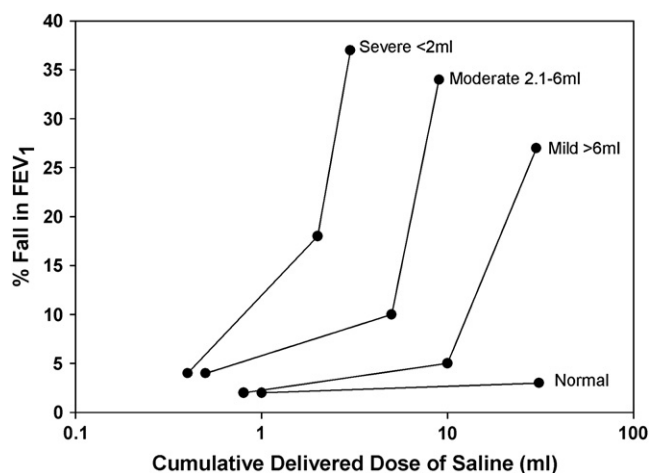


FIG 4. The hypertonic saline challenge provides a cumulative dose-response curve that can be used to define the severity of the EIB response. Redrawn from Anderson SD, Brannan JD. Methods for “indirect” challenge test including exercise, eucapnic voluntary hyperpnea, and hypertonic aerosols. *Clin Rev Allergy Immunol* 2003;24:27-54.⁶⁶

vegetables that resists absorption at high RH and is a stable substance. The inhalation of dry powder mannitol is thought to cause smooth muscle contraction by stimulating the release of inflammatory mediators from airway cells. Because mannitol does not act directly on the airway smooth muscle, it is thought to be a better representation of the neural and cellular contribution to airway hyperresponsiveness.^{28,65,68}

The preparation of dry powder mannitol has been previously described in depth.⁶⁵ Mannitol powder is encapsulated for administration through a dry powder inhaler device. Mannitol is delivered in progressive doubling doses of 5 mg, 10 mg, 20 mg, 40 mg, 80 mg, 160 mg, 160 mg, and 160 mg, with a maximal total cumulative dose of 635 mg, depending on the specific airway response.⁶⁵ One minute after each dose, FEV₁ is measured in duplicate. The initial test dose is a 0-mg capsule; the resultant FEV₁ is used to calculate the target FEV₁ ($0.85 \times$ measured FEV₁) for a 15% fall from subsequent doses. The challenge is stopped when a $\geq 15\%$ fall in FEV₁ from baseline is recorded, a between-dose fall of $\geq 10\%$ in FEV₁ occurs, or a cumulative dose of 635 mg has been administered.²⁸

The inhaled powdered mannitol challenge has many advantages over other indirect challenges as well as direct challenges. It is a practical option for use as an office-based test because of ease to administer and it does not require specialized equipment such as treadmills, a dry air source, and/or patient headgear. In addition, the inhaled powder mannitol challenge takes less time than other challenges and can be provided at the point of need.

A unique advantage to the mannitol challenge is the differentiation of responses to mannitol, as shown in Fig 5. A severe response to mannitol results in a decrease in FEV₁ greater than 15% from less than or equal to 35 mg, a moderate response results in a decrease in FEV₁ greater than 15% to 155 mg, a mild response results in a decrease in FEV₁ greater than 15% to greater than 155 mg, and a normal response does not express a significant decrease in FEV₁.

Mannitol may be superior to exercise and EVH because of the built-in safety feature of a progressive dose-response challenge; the test can be stopped before severe falls in lung function (FEV₁)

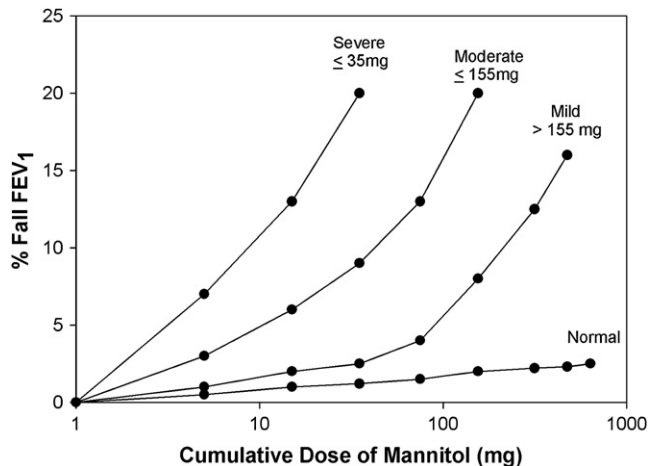


FIG 5. Similar to the hypertonic saline challenge, the cumulative dose-response curve obtained from the inhaled powder mannitol challenge can be used to define the severity of the response. Thus the efficacy of treatment can be monitored through serial challenges. Redrawn from Anderson SD, Brannan JD. Methods for "indirect" challenge test including exercise, eucapnic voluntary hyperpnea, and hypertonic aerosols. *Clin Rev Allergy Immunol* 2003;24:27-54.⁶⁶

occur. The fall in FEV₁ can also be seen much quicker with mannitol than with other procedures, such as hyperventilation, which may take as long as 10 minutes after cessation,⁶⁵ and exercise, which may take as long as 20 minutes after cessation.⁶⁶ In a phase III, multicenter, open-label, operator-blind, crossover design, randomized trial with follow-up, strong similarities between mannitol and hypertonic saline were observed.⁶⁹ The subjects who tested positive by both mannitol and hypertonic saline demonstrated equal mean percent falls in FEV₁ (mannitol, 21.0 ± 5.9; hypertonic saline, 21.3 ± 5.9).⁶⁹ This study also demonstrated similar time to completion for a positive test (mannitol, 17.3 ± 7.1 minutes; hypertonic saline, 15.0 ± 9.1 minutes).⁶⁹

Brannan et al⁶⁹ demonstrated the safety of inhaled mannitol and its ability to identify airway hyperresponsiveness; during the phase III trial, no serious adverse events were reported, and the overall safety profile was similar to that of hypertonic saline.

AMP CHALLENGE

Although the results of a bronchoprovocation test using inhaled AMP will not be accepted by the IOC-MC as evidence of EIB, AMP challenge has been used to identify bronchial hyperresponsiveness in competitive cross-country skiers.⁷⁰ Unlike the previous indirect challenges mentioned, the mode of action for the inhaled AMP challenge is not osmotic. When inhaled, AMP quickly dephosphorylates into adenosine,⁷¹ causing mast cells to degranulate and release histamine and leukotrienes, which have a potent effect on bronchoconstriction.^{72,73} Adenosine is a protein signaling molecule that binds to specific G-protein-coupled receptors on cell surfaces throughout the body and are involved in multiple signaling pathways that elicit cellular responses.⁷¹

AMP is prepared for inhalation by mixing AMP with a phosphate-buffered solution.⁷⁴ Briefly, AMP is administered in progressively doubling concentration doses within the range of 0.09 to 800 mg/mL.⁷⁴ Avital et al⁷⁵ used doubling concentration doses within the range of 0.39 to 400 mg/mL, and Benckhuijsen et al⁷⁶ recommended using doubling concentrations of 0.15, 0.30,

to 320 mg/mL. Generally, the AMP protocol consists of baseline spirometry measurements, with a baseline FEV₁ ≥ 60% of predicted being acceptable to continue the procedure.⁷⁶ The doubling concentration doses of AMP are then delivered to the subject via a nebulizer for 2 minutes until a ≥ 20% fall in FEV₁ is recorded, at which point the protocol is terminated.^{74,76,77} Spirometry measurements are taken between nebulizer treatments in at least duplicate.^{74,76}

In a comparison study between PC₂₀ AMP and PC₂₀ methacholine, van den Berge et al⁷⁸ concluded that PC₂₀ AMP was more closely related to airway inflammation than PC₂₀ methacholine. This study also concluded that AMP provides a better representation of airway inflammation based on sputum eosinophils and exhaled nitric oxide.⁷⁸ van den Toorn et al⁷⁹ also observed a significant correlation between exhaled nitric oxide levels and AMP responsiveness but did not observe such a correlation between eNO and methacholine in adolescents 18 to 25 years of age.

Although AMP is noninvasive, has low cost, and is an excellent way to monitor inflammation, there is a pitfall associated with this challenge.⁷¹ van den Berge et al⁸⁰ demonstrated that the AMP challenge can result in the production of sputum eosinophilia within 1 hour of the challenge, which was not the case with the methacholine challenge. An inflammatory response after the AMP challenge could present a problem and needs to be considered before using the AMP challenge as a diagnostic tool.⁸⁰

Both AMP and mannitol are considered indirect challenges because they stimulate the release of inflammatory mediators from mast cells.^{72,77} It has been shown that mannitol produces results similar to those from the AMP challenge in terms of airway hyperresponsiveness and recovery time.⁷⁷ Studies involving the recovery time from AMP and mannitol challenges suggest that histamine is involved in initiating the response, whereas cysteinyl leukotrienes maintain the bronchoconstrictor response during recovery.^{72,77} Although not an osmotic challenge, these findings indicate that the AMP challenge is very similar in nature to the mannitol challenge.^{72,77}

CONCLUSION

In conclusion, a major determinant in making the correct diagnosis and in defining the severity of the EIB response is the methodology used in the challenge. For exercise and EVH challenge, the ventilation achieved and maintained and the water content of the inspired air modify the degree of reversible bronchoconstriction and therefore must be carefully regulated during challenges. This can be accomplished by measuring V_E during hyperpnea and using dry medical-grade air supplied via a gas cylinder and reservoir system. The effect of physical conditioning on serial exercise challenges and the EIB response should also be considered. For example, if the intensity of the exercise challenge is standardized and regulated by heart rate, the minute ventilation could change dramatically in an individual who was not in peak physical condition before an initial diagnostic challenge but subsequently began an exercise program before sequential challenges. This could result in lower minute ventilation at the predetermined heart rate and skew the bronchoconstriction toward normal, causing erroneous conclusions concerning treatment. Therefore, to monitor treatment effectively by exercise challenge results, it is important to standardize to measured minute ventilation based on an initial diagnostic challenge. Only when challenge conditions are standardized and tightly controlled

can the laboratory challenge be used to verify the efficacy of treatment. There is certainly an argument for the use of the standardized exercise challenge over surrogate challenges. After all, the evaluation is for EIB. However, the surrogate challenges described in this review show good sensitivity and specificity to exercise and should be considered. EVH, hypertonic saline, mannitol, and AMP are challenges that require less laboratory space and set-up equipment and can be performed in the typical clinical setting. Although not yet approved for use in the United States, the inhaled powdered mannitol challenge shows great promise as a diagnostic tool and as a tool to monitor the effectiveness of medical treatment for EIB.

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