

American Thoracic Society

Guidelines for Methacholine and Exercise Challenge Testing-1999

THIS OFFICIAL STATEMENT OF THE AMERICAN THORACIC SOCIETY WAS ADOPTED BY THE ATS BOARD OF DIRECTORS, JULY 1999

- I. Purpose and Scope
- II. Methacholine Challenge Testing
 - A. Indications
 - B. Contraindications
 - C. Technician Training/Qualifications
 - D. Safety
 - E. Patient Preparation
 - F. Choice and Preparation of Methacholine
 - G. Dosing Protocols
 - 1. Two-Minute Tidal Breathing Dosing Protocol
 - 2. Five-Breath Dosimeter Protocol
 - H. Nebulizers and Dosimeters
 - I. Spirometry and Other End-point Measures
 - J. Data Presentation
 - K. Interpretation
- III. Exercise Challenge
 - A. Indications
 - B. Contraindications and Patient Preparation
 - C. Exercise Challenge Testing
 - D. Assessing the Response

References

- Appendix A: Sample Methacholine Challenge Test Consent Form
- Appendix B: Sample Methacholine Challenge Pretest Questionnaire
- Appendix C: Sample Report Format
- Appendix D: Equipment Sources

I. PURPOSE AND SCOPE

This statement provides practical guidelines and suggestions for methacholine and exercise challenging testing. Specifically, it reviews indications for these challenges, details factors that influence the results, presents brief step-by-step protocols, outlines safety measures, describes proper patient preparation and procedures, provides an algorithm for calculating results, and offers guidelines for clinical interpretation of results. The details are important because methacholine and exercise challenge tests are, in effect, dose-response tests and delivery of the dose and measurement of the response must be accurate if a valid test is to be obtained. These guidelines are geared to patients who can perform good-quality spirometry tests; they are not appropriate for infants or preschool children. They are not intended to limit the use of alternative protocols or procedures that have been established as acceptable methods. We do not discuss the general topic of bronchial hyperresponsiveness (BHR).

The bronchial challenge tests chosen for review are the two most widely used, with enough information in the literature to evaluate their utility. Of the two, methacholine challenge testing is better established; a number of aspects in the exercise challenge protocol will benefit from further evaluation. We do not cover specific challenges with allergens, drugs, or occupational sensitizers, and recommend that such tests be performed only in laboratories with considerable experience in their techniques. For more extensive details or other challenge procedures the reader is referred to previously published guidelines for bronchial challenge testing (1-5) and reviews on the general topic of BHR (6-9).

As with other American Thoracic Society (ATS) statements on pulmonary function testing, these guidelines come out of a consensus conference. The basis of discussion at the committee's September 1997 meeting was a draft prepared by three members (P.E., C.I., and R.C.). The draft was based on a comprehensive Medline literature search from 1970 through 1997, augmented by suggestions from other committee members. The final recommendations represent a consensus of the committee. For issues on which unanimous agreement could not be reached, the guidelines reflect both majority and minority opinions.

The committee recommends that the guidelines be reviewed in 5 years and, in the meantime, encourages further research in the areas of controversy.

II. METHACHOLINE CHALLENGE TESTING

A. Indications

Methacholine challenge testing is one method of assessing airway responsiveness. Airway hyperresponsiveness is one of the features that may contribute to a diagnosis of asthma. It may vary over time, often increasing during exacerbations and decreasing during treatment with antiinflammatory medications. Methacholine challenge testing (MCT) is most often considered when asthma is a serious possibility and traditional methods, most notably spirometry performed before and after administration of a bronchodilator, have not established or eliminated the diagnosis. Symptoms that suggest asthma include wheezing, dyspnea, chest tightness, or cough in the following circumstances: (1) with exposure to cold air, (2) after exercise, (3) during respiratory infections, (4) following inhalant exposures in the workplace, and (5) after exposure to allergens and other asthma triggers. A history of such symptoms increases the pretest probability of asthma. The optimal diagnostic value of MCT (the highest combination of positive and negative predictive power) occurs when the pretest probability of asthma is 30-70% (10). Methacholine challenge testing is more useful in excluding a diagnosis of asthma than in establishing one because its negative predictive power is greater than its positive predictive power.

Methacholine challenge testing is also a valuable tool in the evaluation of occupational asthma. Methacholine challenge

testing is sometimes used to determine the relative risk of developing asthma, assess the severity of asthma, and assess response to asthma therapy although its clinical use in these areas has not been well established.

Rationale. Even asthma specialists cannot accurately predict MCT results in patients with an intermediate probability of asthma (I 1). The MCT has excellent sensitivity but mediocre positive predictive value for asthma (8). Most subjects with current asthma symptoms will have BHR. However, bronchial hyperresponsiveness is also seen in a wide variety of other diseases, including smoking-induced chronic airway obstruction (COPD), congestive heart failure (CHF), cystic fibrosis, bronchitis, and allergic rhinitis (12-14).

Because improvement in the clinical severity of asthma is associated with improvement in airway responsiveness (1.5, 16) clinical studies of asthma therapies often use change in airway responsiveness as an objective outcome measure (9, 17-25). Sont and colleagues have demonstrated the efficacy of a treatment program that included measures of airway hyperactivity in the management approach (26). However, we believe the routine use of MCT to examine patients with asthma in a clinical setting should await further exploration of the utility of such testing.

B. Contraindications

The contraindications to methacholine challenge testing, summarized in Table 1, are all conditions that may compromise the quality of the test or that may subject the patient to increased risk or discomfort. They are identified in the pretest interview or questionnaire. If contraindications are identified, they should be discussed with the physician who ordered the test or the medical director of the laboratory before proceeding.

Rationale. Low **FEV₁**. Occasional dramatic falls in FEV₁ may occur during MCT and the risk of such events may be increased in individuals with low baseline lung function. Reduced lung function is a relative contraindication because the overall risk of serious adverse events is small, even in patients with asthma who have severe airway obstruction (27). The level of lung function at which MCT is contraindicated is controversial. A baseline FEV₁ of < 1.5 L or < 60% predicted in adults is proposed as a relative contraindication by Sterk and coworkers (1) and Tashkin and coworkers (28). Half of 40 investigators polled in one study considered an FEV₁ of < 70% predicted to be a contraindication (29); 20% used cutoff points of 60% of predicted and 20% used 80% of predicted. The Second National Asthma Expert Panel Report used an FEV₁ < 65% predicted (30).

Airway obstruction. It is difficult to interpret a "positive" methacholine challenge result when baseline spirometry shows airway obstruction (a low FEV₁/FVC and low FEV₁), because

airway responsiveness correlates strongly with the degree of baseline airway obstruction in COPD. In the presence of a good clinical picture for asthma, if baseline spirometry shows airflow obstruction and there is a significant bronchodilator response (> 12% and > 0.2-L increases in either FEV₁ or FVC) the diagnosis of asthma is often confirmed and MCT is usually unnecessary.

Spirometry quality. An acceptable-quality methacholine challenge test depends on the ability of the patients to perform acceptable spirometric maneuvers. Patients who cannot perform acceptable spirometry tests in the baseline session should perhaps be rescheduled or be tested using an end-point measure that is less dependent on patient effort.

Cardiovascular problems. A history of cardiovascular problems may also be a contraindication, depending on the problem. The additional cardiovascular stress of induced bronchospasm may precipitate cardiovascular events in patients with uncontrolled hypertension or recent heart attack or stroke. Induced bronchospasm causes ventilation-perfusion mismatching (31, 32), which can result in arterial hypoxemia and compensatory changes in blood pressure, cardiac output, and heart rate (33, 34). On the other hand, cardiac arrhythmia rates actually fall during the performance of FVC maneuvers (35).

Pregnancy and nursing mothers. Methacholine is a pregnancy category C drug, meaning that animal reproductive studies have not been performed and it is not known whether it is associated with fetal abnormalities. It is not known whether methacholine is excreted in breast milk.

C. Technician Training/Qualifications

There is no recognized certification program for persons who perform methacholine challenge testing. The pulmonary laboratory director is responsible for evaluating and/or verifying the training and qualification of the person(s) who perform the test. At a minimum, the technician should:

1. Be familiar with this guideline and knowledgeable about specific test procedures
2. Be capable of managing the equipment including set-up, verification of proper function, maintenance, and cleaning
3. Be proficient at spirometry
4. Know the contraindications to MCT
5. Be familiar with safety and emergency procedures
6. Know when to stop further testing
7. Be proficient in the administration of inhaled bronchodilators and evaluation of the response to them

Rationale. These requirements are standard testing elements designed to ensure good-quality results and patient safety. It is estimated that about 4 d of hands-on training and at least 20 supervised tests are required for a new technician to become proficient in methacholine challenge testing (36).

D. Safety

Inhaled methacholine causes bronchoconstriction. The safety of both patients and technicians should be considered in the design of the test room and the testing procedures.

Precautions for patient safety. The medical director of the laboratory, another physician, or another person appropriately trained to treat acute bronchospasm, including appropriate use of resuscitation equipment, must be close enough to respond quickly to an emergency. Patients should not be left unattended during the procedure once the administration of methacholine has begun.

Medications to treat severe bronchospasm (30) must be present in the testing area. They include epinephrine and atropine for subcutaneous injection, and albuterol and ipratro-

TABLE 1
CONTRAINDICATIONS FOR
METHACHOLINE CHALLENGE TESTING

Absolute:

- Severe airflow limitation (FEV₁ < 50% predicted or < 1.0 L)
- Heart attack or stroke in last 3 mo
- Uncontrolled hypertension, systolic BP > 200, or diastolic BP > 100
- Known aortic aneurysm

Relative:

- Moderate airflow limitation (FEV₁ < 60% predicted or < 1.5 L)
- Inability to perform acceptable-quality spirometry
- Pregnancy
- Nursing mothers
- Current use of cholinesterase inhibitor medication (for myasthenia gravis)

pium in metered-dose inhalers or premixed solutions for inhalation. Oxygen must be available. A small-volume nebulizer should be readily available for the administration of bronchodilators. A stethoscope, sphygmomanometer, and pulse oximeter should be available.

Rationale. Thousands of methacholine challenge tests have been performed by laboratories with no serious side effects (3, 27–29, 37–40). Transient symptoms including wheezing, cough, mild dyspnea, and chest tightness are common in patients with BHR, although many experience no symptoms. The technician should be alert to patient symptoms and make a record of any that occur. When 1,000 participants with COPD in a multicenter trial were asked to report all symptoms after MCT, 25% had cough, 21% had dyspnea, 10% had wheezing, 6% had dizziness, and 2% had headache. Two-thirds had no symptoms. They were asymptomatic when leaving the clinic, and only 3 of 1,000 reported symptoms, such as chest soreness, in the days after the test (28). Fewer than 20% of 700 subjects undergoing histamine challenge testing in an occupational setting noted cough, chest tightness, or flushing (41).

Delayed or prolonged responses to methacholine are rare. One study reported prolonged responses to methacholine in four of six adult subjects with asthma who received high doses of methacholine after pretreatment with atropine (42). We are unaware of delayed or prolonged responses to methacholine with usual clinical testing doses. Although we are not aware of any deaths associated with methacholine challenge testing, the potential for severe bronchospasm is present and prudent measures to minimize risk should be in place. There have been reports of a fatality after a specific antigen challenge (8) and in association with a distilled water challenge (43).

Precautions for technician safety. Measures should be taken to minimize technician exposure to methacholine aerosol. The testing room must have adequate ventilation (with at least two complete exchanges of air per hour). Other, optional methods to reduce methacholine exposures include using low-resistance exhalation filters, a laboratory fume hood, supplemental local exhaust ventilation, and/or a high-efficiency particulate air (HEPA) cleaner. The dosimeter technique will reduce technician exposure to methacholine because there is only a 0.6-s actuation of the nebulizer with each inhalation. Technicians may also want to stand well away from the patient when methacholine is being nebulized. See Figure 1 for an illustration of configurations of exhalation filters to minimize exposure to methacholine.

Technicians with asthma are at increased risk of bronchospasm during testing and should take extra precautions to minimize their exposure to aerosolized methacholine. Performing methacholine challenge tests on technicians who will be testing patients may be a useful precaution. Knowing that a technician reacts to methacholine could lead a supervisor to reassign technicians or take additional precautions to minimize their exposure to methacholine.

Rationale. In a survey of 600 allergy specialists, about 20% reported symptoms among staff who performed methacholine challenge tests (3). Two cases of asthma have been reported in nurses who frequently administered methacholine challenge tests over a period of more than 2 yr (44). Technicians with known active asthma should not perform methacholine challenges unless appropriate methods are used to avoid exposure to methacholine. The use of exhalation filters and good ventilation of the testing room should reduce exposure.

E. Patient Preparation

1. Preparation when scheduling. When tests are scheduled, patients should be given a list of items/medications to avoid before the test. Table 2 (45–62) lists medications that can decrease airway responsiveness and the time period for which each should be withheld before the test. The goal is to withhold the medication for its biological duration of action (for 90% of patients taking the usual dose). Table 3 lists factors that may increase airway responsiveness.

2. Preparation at testing.

- Explain the test to the patient. Patients should be told they may experience some minor symptoms, such as cough or chest tightness, but that most patients have no symptoms. They should be warned that occasional severe symptoms may occur. Care should be taken to ensure that the test description does not bias the result. For example, avoid stating that the test induces an asthma attack.
- Ask the patient if they would like to urinate before the test (stress incontinence could be precipitated, especially in older women).
- Some hospitals require informed consent for the test (an example of an informed consent document is presented in APPENDIX A).
- Evaluate the patient for contraindications and review medication use. A pretest questionnaire is useful for this purpose (APPENDIX B).

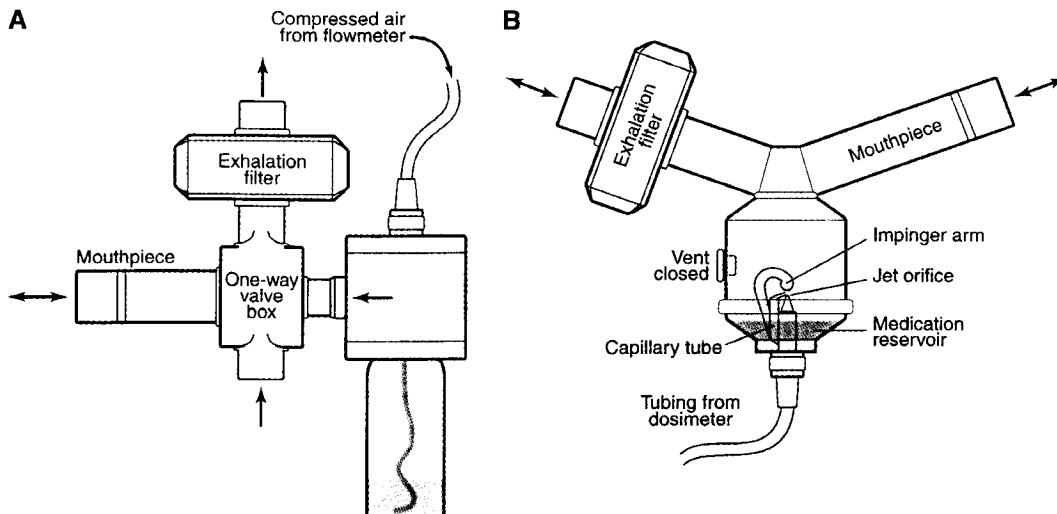


Figure 1. Schematic diagram illustrating typical nebulizer configurations for both the 2-min tidal breathing protocol (A, an English Wright nebulizer) and the five-breath dosimeter protocol (B, a DeVilbiss model 646 nebulizer). Both include an exhalation filter. Other models of nebulizers may be substituted (see Section II, H).

TABLE 2
FACTORS THAT DECREASE BRONCHIAL RESPONSIVENESS

Factor	Minimum Time Interval from Last Dose to Study	Ref. No.
Medications		
Short-acting inhaled bronchodilators, such as isoproterenol, isoetharine, metaproterenol, albuterol, or terbutaline	8 h	45, 46
Medium-acting bronchodilators such as ipratropium	24 h	20, 47
Long-acting inhaled bronchodilators, such as salmeterol, formoterol, tiotropium	48 h (perhaps 1 wk for tiotropium)	48, 49
Oral bronchodilators		50, 51
Liquid theophylline	12 h	
Intermediate-acting theophyllines	24 h	
Long-acting theophyllines	48 h	
Standard β_2 -agonist tablets	12h	
Long-acting β_2 -agonist tablets	24 h	
Cromolyn sodium	8 h	
Nedocromil	48 h	
Hydroxazine, cetirizine	3 d	
Leukotriene modifiers	24 h	
Foods		
Coffee, tea, cola drinks, chocolate	Day of study	52

Note: The authors do not recommend routinely withholding oral or inhaled corticosteroids, but their antiinflammatory effect may decrease bronchial responsiveness (53, 54). Inhaled corticosteroids may need to be withheld depending on the question being asked.

3. Testing.

- Subjects must be able to understand the procedure and perform reliable spirometric maneuvers.
- Subjects should be seated comfortably throughout the test.
- A brief physical examination of the chest and lungs may be useful but is not required.

Rationale. The pretest evaluation will alert the technician to important issues, including (1) the presence of contraindications to proceeding with the test; (2) conditions or exposures, such as a recent viral infection, that could temporarily increase airway responsiveness (58, 62-64) and cause a false-positive response; (3) the presence of medications that may alter airway responsiveness. Influenza vaccination, the menstrual cycle, antihistamines, and oral contraceptives do not significantly affect airway responsiveness (65-67). A number of medications, most notably anticholinergic and P-agonist inhalers, can temporarily reduce airway responsiveness, potentially causing a false-negative response (20, 23-25, 46, 52).

F. Choice and Preparation of Methacholine

Methacholine (acetyl- β -methylcholine chloride), available as a dry crystalline powder, is the agent of choice for nonspecific bronchoprovocation challenge testing. Food and Drug Administration (FDA)-approved methacholine (Provocholine) is

available in prepackaged, sealed 100-mg vials. Industrial sources of methacholine appear to work as well as Provocholine (68). The advantages of FDA-approved methacholine are that it is approved for human use and is required to meet good manufacturing practices for quality, purity, and consistency. The bromide salt of methacholine may be substituted for the chloride salt, although it is not currently available in an FDA-approved form. Methacholine powder is very hygroscopic. Bulk powder should be stored with a desiccator in a freezer. Sealed prepackaged vials do not require desiccation or freezing. Sterile normal saline (0.9% sodium chloride) with or without 0.4% phenol may be used as the diluent. The committee prefers the use of normal saline without phenol. Phenol-containing saline is specified for the dilution of Provocholine. There is no evidence that adding a preservative such as phenol to sterile saline diluent is necessary (69), nor is there evidence that use of phenol adversely affects MCT. Both diluents are widely used. The potential benefit of adding phenol is reducing the potential for bacterial contamination. The pH of methacholine in normal saline solution is weakly to moderately acidic depending on the concentration of methacholine. Buffered solutions are less stable and should not be used as the diluent (69-71).

Methacholine solutions should be mixed by a pharmacist or other well-trained individual using sterile technique. The vials should be labeled as methacholine with the concentration and an expiration date and stored in a refrigerator at about 4° C. When prepared with saline diluent and stored at 4° C, methacholine solutions of 0.125 mg/ml and greater should be stable for 3 mo (69, 71, 72). The package insert for Provocholine specifies the use of normal saline containing 0.4% phenol as the diluent and recommends the solution not be stored longer than 2 wk and that the 0.025-mg/ml solution be mixed on the day of testing. We are not aware of published information on the stability of methacholine in normal saline with phenol solution; such studies are needed. In the absence of this information, we recommend that the package insert recommendations for Provocholine storage be followed when methacholine is mixed with saline containing phenol. Solutions of methacholine should be warmed to room temperature before testing begins. Any unused methacholine solution remaining in a nebulizer should be discarded.

TABLE 3
FACTORS THAT INCREASE BRONCHIAL RESPONSIVENESS

Factor	Duration of Effect	Ref. No.
Exposure to environmental antigens	1-3 wk	25
Occupational sensitizers	Months	55, 56
Respiratory infection	3-6 wk	57, 58
Air pollutants	1 wk	59
Cigarette smoke	Uncertain*	60
Chemical irritants	Days to months	61

*Studies of the acute effects of smoking on airway hyperreactivity and methacholine challenge testing are not consistent (60). There is some evidence of a brief acute effect that can be avoided by asking subjects to refrain from smoking for a few hours before testing.

Rationale. Choice of agent. Methacholine and histamine produce bronchoconstriction at nearly equivalent concentrations (73, 74). Methacholine is currently more commonly used (29) and is preferred to histamine because histamine is associated with more systemic side effects, including headache, flushing, and hoarseness. In addition, BHR measurements may be less reproducible when using histamine (75-77).

Methacholine is a synthetic derivative of the neurotransmitter acetylcholine, a substance that occurs naturally in the body. Methacholine is metabolized more slowly by cholinesterase; its effects can be blocked or lessened by atropine or similar anticholinergic agents.

Conditions. Acidic methacholine solutions with concentrations greater than 0.3 mg/ml (pH < 6) remain stable for at least 3 mo when stored at 4° C (69, 71, 72, 78). One committee member's experience is that concentrations of 0.125 mg/ml are clinically stable for 3 mo, but this observation has not been documented in the literature and a conservative approach should be taken until more information is available on the stability of lower concentrations. Methacholine is rapidly decomposed by hydrolysis as the solution pH increases above six (71). Lower concentrations of methacholine lose potency faster when stored at room temperature because they are less acidic than higher concentrations (71, 72). The concentration of methacholine will change during nebulization if cold solutions are not allowed time to warm to room temperature before use (79). Solution remaining in a nebulizer after it has been used will concentrate by evaporation and should not be reused.

Preparing solutions. Accurate sterile mixing is very important for the accuracy of the test results and for the safety of patients. Only trained individuals should mix and label methacholine solutions.

C. Dosing Protocols

Many different dosing protocols have been used. Each has advantages and disadvantages and the committee was unable to come to a single recommendation. We were able to narrow the choices to two: (1) the 2-min tidal breathing method and (2) the five-breath dosimeter method. The FDA approval for the Methapharm (Brantford, ON, Canada) methacholine (Provocholine) is based on the five-breath technique and a dosing schedule using methacholine concentrations of 0.025, 0.25, 2.5, 10, and 25 mg/ml. A dilution scheme provided in their product information is designed to accurately produce these concentrations. The concentrations in the Provocholine product information can be used in the five-breath dosimeter method, although the committee prefers the dosing schedule described below because the dosing steps are even and because of concerns about the safety of the 10-fold changes in dilution strength in the Provocholine protocol. Dilution schemes for the two recommended dosing schedules, based on a 100-mg vial of Provocholine, are presented in Table 4.

Rationale. Four common methods of aerosol generation and inhalation are used (1): (1) dosing during a deep inhalation, using a Y tube occluded by the thumb; (2) five breaths of a fixed duration, using a dosimeter at the beginning of a deep inhalation (5, 80, 81); (3) a hand bulb nebulizer activated during inhalation (82); and (4) continuous nebulization while tidal breathing for 2 min (4, 83). All of these methods give similar results (74, 81, 84-88). The two techniques recommended in this statement are those most widely used in North America and Europe. The hand bulb nebulizer (Yan protocol) is infrequently used in the United States (3) and has poorer reproducibility when used with patients who have not previously performed methacholine challenge tests (87). It is widely used for epidemiological surveys.

1. Two-minute tidal breathing dosing protocol. The 2-min tidal breathing method is, with some variation, based on the protocol recommended by the Canadian Thoracic Society (4, 89). Refer to the flow chart in Figure 2. Details on spirometry technique are found in the section, Spirometry and Other Endpoint Measures.

- Prepare the following 10 doubling concentrations of methacholine in sterile vials, place them in a holder, and store them in a refrigerator:

Diluent: 0.03 0.06 0.125 0.25 0.50 1 2 4 8 16 mg/ml (see Table 4)

The use of the diluent step is optional. An optional shortened dosing regimen can be used under certain circumstances (see Rationale).

- Remove the vials from the refrigerator 30 min before testing, so that the mixture warms to room temperature before use. Insert 3 ml of the first (diluent or lowest) concentration into the nebulizer, using a sterile syringe.
- Perform baseline spirometry and calculate a target FEV₁ that indicates a 20% fall in FEV₁ (baseline [or diluent] FEV₁ × 0.8).
- Use a nebulizer setup such as shown in Figure 1. Use dry compressed air to power the nebulizer, and set the pressure regulator to 50 lb/in². Flow meter accuracy should be checked with a rotameter (Matheson Tri-Gas, Montgomeryville, PA). Adjust the flow meter to deliver the output established during the calibration procedure (0.13 ml/min, ± 10%). Attach a new exhalation filter (optional).
- Instruct the patient to relax and breathe quietly (tidal breathing) for 2 min. Apply a noseclip. Set the timer for 2 min.
- Ask the patient to hold the nebulizer upright, with the mouthpiece in his/her mouth. A face mask with a noseclip is a valid alternative and may be easier for some subjects (90). Start the timer and begin nebulization.
- Watch the patient to ensure that he/she is breathing comfortably and quietly, and not tipping the nebulizer. After

TABLE 4
DILUTION SCHEMES FOR THE TWO RECOMMENDED
METHACHOLINE DOSING SCHEDULES

Label	Strength	Take	Add NaCl (0.9%)	Obtain Dilution
A. Dilution schedule* using 100-mg vial of methacholine chloride and the 2-min tidal breathing protocol				
100 mg	100 mg		6.25 ml	A: 16 mg/ml
	3 ml of dilution A		3 ml	B: 8 mg/ml
	3 ml of dilution B		3 ml	C: 4 mg/ml
	3 ml of dilution C		3 ml	D: 2 mg/ml
	3 ml of dilution D		3 ml	E: 1 mg/ml
	3 ml of dilution E		3 ml	F: 0.5 mg/ml
	3 ml of dilution F		3 ml	G: 0.25 mg/ml
	3 ml of dilution G		3 ml	H: 0.125 mg/ml
	3 ml of dilution H		3 ml	I: 0.0625 mg/ml
	3 ml of dilution I		3 ml	J: 0.031 mg/ml
B. Optional dilution schedule using 100-mg vial of methacholine chloride and five-breath dosimeter protocol				
100 mg	100 mg		6.25 ml	A: 16 mg/ml
	3 ml of dilution A		9 ml	B: 4 mg/ml
	3 ml of dilution B		9 ml	C: 1 mg/ml
	3 ml of dilution C		9 ml	D: 0.25 mg/ml
	3 ml of dilution D		9 ml	E: 0.0625 mg/ml

* Schedule obtained from Methapharm (Brantford, ON, Canada).

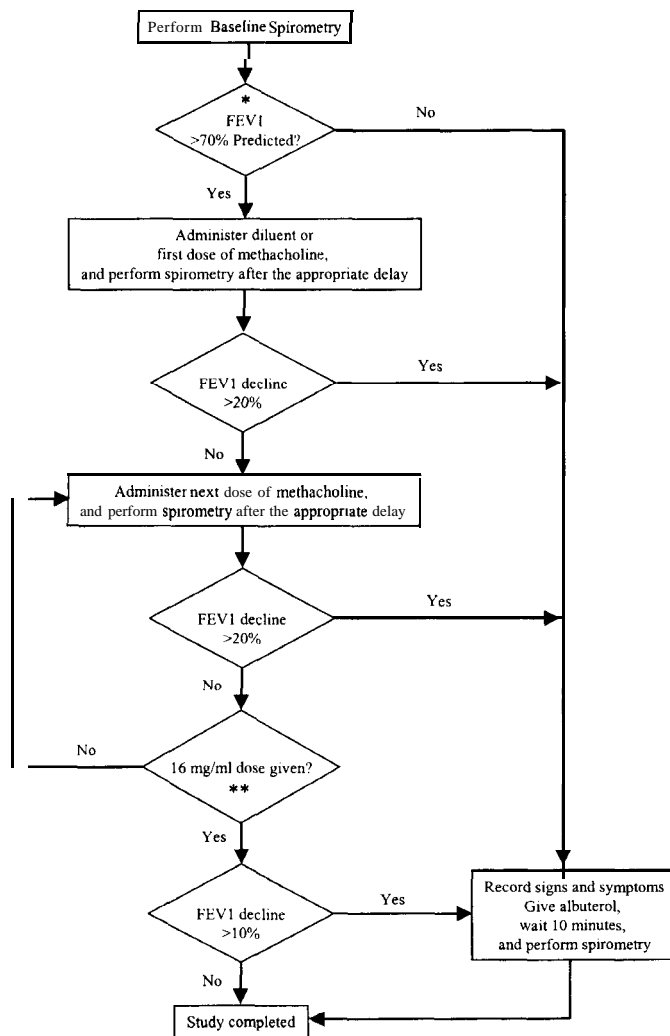


figure 2. Methacholine challenge testing sequence (flow chart).
*The choice of the FEV₁ value considered a contraindication may vary from 60 to 70% of predicted. **The final dose may vary depending on the dosing schedule used. Final doses discussed in this statement are 16, 25, and 32 mg/ml.

- exactly 2 min, turn off the flow meter and take the nebulizer from the patient.
- Measure the FEV₁, about 30 and 90 s after the nebulization is completed. Obtain an acceptable-quality FEV₁ at each time point. This may require repeated attempts. Perform no more than three or four maneuvers after each dose. It should take no more than 3 min to perform these maneuvers. To keep the cumulative effect of methacholine relatively constant, the time interval between the commencement of two subsequent concentrations should be kept to 5 min.
 - At each dose, report the highest FEV₁ from the acceptable maneuvers.
 - If the FEV₁ falls less than 20%, empty the nebulizer, add 3 ml of the next highest concentration, and repeat steps e-h above.
 - If the FEV₁ falls more than 20% from baseline (or the highest concentration has been given), give no further methacholine, note signs and symptoms, administer inhaled albuterol, wait 10 min, and repeat the spirometry. If vocal cord dysfunction is suspected and the patient's symptoms allow it, full inspiratory and expiratory flow volume loops may be performed before giving the bronchodilator.

2. *Five-breath dosimeter protocol.* The five-breath dosimeter protocol was first standardized by the National Institutes of Health (NIH) Institute of Allergic and Infectious Diseases in 1975 (5) and is presented as an alternative method by the European Respiratory Society (1). It is widely used in research studies. We have modified it by recommending quadrupling doses rather than doubling doses. The Provocholine dosing schedule is also acceptable. Refer to the flow chart in Figure 2.

- Set up and check the dosimeter.
- Prepare the following five concentrations of methacholine in sterile vials; place them in a holder; and store them in a refrigerator. (Note: in contrast to the 2 min tidal breathing method, only every other concentration is used, resulting in increments of quadrupling doses.) Use of a 32 mg/mL concentration is optional; it would primarily be used for research and epidemiological studies.

Diluent: 0.0625 0.25 1 4 16 mg/ml (see Table 4)

Use of the diluent step is optional.

- Remove the vials from the refrigerator 30 min before testing, so that the contents warm to room temperature before use. Insert 2.0 ml of the first concentration into the nebulizer, using a sterile syringe. (Some nebulizer models may require more than 2.0 ml of solution for reliable aerosolization.)
- The patient is seated throughout the test.
- Perform baseline spirometry.
- Briefly open the dosimeter solenoid to make sure the nebulizer is nebulizing.
- Ask the patient to hold the nebulizer upright with the mouthpiece in his/her mouth. Watch the patient during the breathing maneuvers to ensure that the inhalation and breathhold are correct and that the nebulizer is not tipped. The patient should wear a noseclip while inhaling from the nebulizer.
- At end exhalation during tidal breathing (functional residual capacity), instruct the patient to inhale slowly and deeply from the nebulizer. Trigger the dosimeter soon after the inhalation begins; dosimeters may do this automatically. Encourage the patient to continue inhaling slowly (about 5 s to complete the inhalation) and to hold the breath (at total lung capacity, TLC) for another 5 s.
- Repeat step h for a total of five inspiratory capacity inhalations. Take no more than a total of 2 min to perform these five inhalations.
- Measure the FEV₁ at about 30 and 90 s after the fifth inhalation from the nebulizer. Obtain an acceptable-quality FEV₁ at each time point. This may require repeated attempts. Perform no more than three or four maneuvers after each dose. It should take no more than 3 min to perform these maneuvers. To keep the cumulative effect of methacholine relatively constant, the time interval between the commencement of two subsequent concentrations should be kept to 5 min.
- At each dose, report the highest FEV₁ from acceptable maneuvers.
 - If the FEV₁ falls less than 20%, empty the nebulizer, shake it dry, and trigger the dosimeter once to dry the nebulizer nozzle. Add 2.0 ml of the next higher concentration, and repeat steps g-j.
 - If the FEV₁ falls more than 20% from baseline (or the highest concentration has been given), give no further methacholine, note signs and symptoms, administer inhaled albuterol, wait 10 min, and repeat the spirometry. If vocal cord dysfunction is suspected and the patient's symptoms allow it, full inspiratory and expiratory flow volume loops may be performed before giving the bronchodilator.

3. Rationale. Dosing schedules. Many different dosing protocols have been used by investigators and laboratories. Doubling concentrations are widely recommended for research protocols and are mathematically attractive but the smaller steps increase the time needed for a test. As a compromise, for clinical testing, we recommended quadrupling increments for clinical testing with the five-breath dosimeter method. Fewer concentrations have been used by many investigators in order to save time (41, 91–95) without any apparent increase in risk of severe bronchospasm. If MCT is used to determine changes in airway reactivity after therapy in patients known to have asthma, using doubling doses will give more precise PC_{20} (provocative concentration causing a 20% fall in FEV₁) values.

Optional shortening of the tidal breathing protocol. The 2-min tidal breathing protocol may be shortened (depending on the clinical situation) by adjusting the starting concentration (4). For example, in a diagnostic test for a subject not known to have asthma, taking no asthma medications, with normal lung function, and no response to diluent, a starting dose of 1 mg/ml is quite safe. In addition, when the FEV₁ has fallen less than 5% in response to a dose of methacholine, the next concentration may be omitted; a fourfold increase in dosage is quite safe under these circumstances. **Caution:** Small children with asthma symptoms are more likely than adults to have severe airway hyperresponsiveness and more caution in increasing the concentration at each step is warranted. This apparent increased sensitivity in children may reflect an increased dose per unit weight (96).

Test techniques. The speed at which methacholine is inhaled affects how it is deposited and, consequently, affects test results. Rapid inhalation flow (>1 L/s), instead of the recommended slow inhalation over 5 s, will reduce measured PC_{20} in many patients (97, 98). Nose clips are used to prevent dilution of the inhaled solution with air through the nose during the slow inhalations. The nebulizer is held upright because tipping may reduce or stop aerosol generation as the fluid intake nozzle moves above the fluid line. In addition, the output of some nebulizer models may depend on a vertical position. Inhalation of the aerosol via face mask (with the nose occluded) gave equivalent results compared with using a mouthpiece (90).

FEV₁: Timing and selection of values for interpretation. The timing of FEV₁ measurements at 30 and 90 s after the inhalation is based on considerable experience with measurements at these times. Recommendations for interpretation are largely based on measurements performed according to this timing schedule and use of the largest acceptable FEV₁ as the outcome variable is widely accepted. Some investigators prefer to report the lowest FEV₁, reasoning this will avoid the effect of an increase in FEV₁ as the methacholine wears off (99). However, this approach assumes the technician will be able to ensure high-quality tests and discard all unacceptable maneuvers before selecting the lowest FEV₁. If all FEV₁ maneuvers are completed in the recommended time (< 3 min) it is highly unlikely that the effect of methacholine will wear off.

Use of a diluent step. The opinions of the committee members regarding the desirability of starting with a diluent (control) were divided; most current protocols start with a diluent step. An advantage of starting with a diluent is that it gives patients an opportunity to learn the technique of inhaling from the nebulizer and practice in performing spirometry. In addition, most reference data used for interpretation are based on studies that used a diluent step and use of a diluent step provides a better link to interpretative data by ensuring technical comparability. Other committee members felt the rationale for using a diluent was weak. They argued: the lowest concentration of methacholine was chosen so that only the most hy-

perresponsive patient with asthma will respond and the use of a diluent control does not improve the safety of the test. The PC_{20} is not affected by starting with a diluent (100). The addition of a diluent control adds 5 min to each test. Only 1% of patients tested using a diluent (control) respond to the diluent with a 20% or greater fall in FEV₁ (101) and the clinical meaning of a positive response to the diluent is unknown. Such patients may be experiencing FVC maneuver-induced bronchospasm, but this should have been detected before the methacholine study was scheduled.

When a diluent step is used, the postdiluent FEV₁ is the reference point for comparison. We recommend a 20% fall in FEV₁ following diluent as the threshold of significance for consistency with the thresholds used in the rest of the test. Although some investigators have used a 10% fall in FEV₁ as the threshold to determine a positive response to the diluent, this threshold is close to the 95th percentile confidence interval of FEV₁, repeatability in patients with BHR and increases the chance of a test failure.

H. Nebulizers and Dosimeters

Nebulizers for the tidal breathing method. The nebulizer must deliver an aerosol with a particle mass median diameter (MMD) between 1.0 and 3.6 μm [e.g., the English Wright nebulizer (Roxon Medi-Tech, Montreal, PQ, Canada) (4) generates particles between 1.0 and 1.5 MMD and is generally used for this method]. It is acceptable to perform this technique with other brands of nebulizers with similar characteristics. For other nebulizer brands, reviews of nebulizer performance may be useful in making initial selections (102, 103); further validation of nebulizer performance is recommended. Avoid the use of nebulizers with MMD less than 1.0 μm . Flow must be adjusted for each nebulizer to obtain an output within 10% of 0.13 ml/min. Variation of MMD between 1.3 and 3.6 μm does not influence measurement of airway responsiveness when using the 2-min tidal breathing method (104). It is not, therefore, necessary to check the particle size generated by each individual nebulizer once the MMD range for a nebulizer model has been found to be between 1.0 and 3.0 μm .

To measure nebulizer output for the tidal breathing method, perform the following steps:

- Put 3 ml of room temperature saline into the nebulizer.
- Weight the nebulizer, using a balance accurate to 1.0 mg (preweight).
- Adjust the flow meter to 7.0 L/min and nebulize for exactly 2 min.
- Reweigh the nebulizer (postweight). Empty the nebulizer.
- Repeat steps 1–4 three times for each of the following air flows: 7.0, 8.0, and 9.0 L/min (or 4.0, 5.0, and 6.0 L/min for some nebulizer models).
- Calculate and plot the average nebulizer output at each air-flow.
 - The nebulizer output in milliliters per minute, assuming 1 ml of saline equals 1,000 mg, is calculated as

$$\text{Output (ml/min)} = [(\text{preweight (mg)} - \text{postweight (mg)}) / \text{time (min)}] / 1,000. \quad (1)$$
 - By interpolation, determine the airflow that will generate an output of 0.26 ml over 2 min (0.13 ml/min). Record the airflow for the nebulizer and the date of the calibration check.
- Subsequent checks of nebulizer output need only test the nebulizer output at the flow that generates the correct output. If the output is within specification (0.13 ml/min, $\pm 10\%$) testing at other flows is not necessary. Alternative

techniques for nebulizer calibration checks have been described that may be more accurate and that are applicable to respiratory function laboratories (105).

Nebulizers for the five-breath dosimeter method. Nebulizers for the five-breath method should deliver $9 \mu\text{l}$ (0.009 ml) \pm 10% of solution per **0.6-s** actuation during inhalation (104). The DeVilbiss (Somerset, PA) model 646 nebulizer is commonly used for this technique (Figure 1). Other nebulizers with equivalent characteristics and output are acceptable. Because the DeVilbiss 646 model has high between-unit output variability (106, 107), each nebulizer must be adjusted and its output checked before it is put into service. The extra vent on the DeVilbiss 646 nebulizer must be closed during methacholine challenge testing, as the nebulizer output increases and is variable when air can be entrained into the nebulizer (108). A flow regulator may be added to the nebulizer to control the rate of inspiratory flow. If used, the flow regulator must be included in the system during calibration. Nebulizer output is influenced by the gap between the jet orifice and the tip of the capillary tube (Figure 1). The position of the impinger arm also influences the output of the nebulizer but the effect varies from nebulizer to nebulizer, so no single position can be recommended (107). In the Lung Health Study, the impinger arm was placed so that its large curve pointed toward the mouthpiece when the nebulizer was assembled. A jet orifice-capillary tube gap of 0.01 in. (0.254 mm), measured with a spark plug tool, was used (P. Enright, personal communication). These are reasonable starting places. Once the nebulizer output is satisfactory, the impinger arm should be carefully glued in the position used during calibration. An exhalation filter may be added to the nebulizer cap opposite the mouthpiece (Figure 1). A single nebulizer may be used for all concentrations, provided it is emptied and the nozzle dried between doses. Alternatively, six or seven separate calibrated nebulizers may be filled before the test. If separate nebulizers are used, they must be carefully labeled to avoid dosing errors.

For the five-breath dosimeter technique, outputs are checked by weighing the nebulizer containing 2 ml (or the volume used for testing) of room temperature, sterile normal saline before and after 10 actuations with a technician simulating the test by inhaling slowly from the nebulizer. The scale used must be accurate to at least 0.5 mg. For the DeVilbiss 646 nebulizer, the target output is $90 \mu\text{l} \pm 10\%$ (0.09 ml [90 mg] $\pm 10\%$) for the 10 actuations.

Monitoring nebulizer performance. Nebulizer output varies from model to model and from unit to unit and may vary with time depending on how the nebulizer is maintained and cleaned (109). The actual output of each nebulizer used must be measured initially and at regular intervals. Because nebulizer performance over time may vary depending on individual laboratory use, maintenance, and cleaning practices, each laboratory should establish its own nebulizer monitoring schedule. We recommend that each laboratory check output after every 20 uses until an appropriate testing schedule is established for the laboratory.

The dosimeter. The dosimeter is an electrically valved system that enables the technician to administer aerosol for 0.6 s during inhalation from the nebulizer. The dose may be triggered manually by pressing a button or by an automatic system that delivers a single dose soon after the onset of a deep breath. Useful dosimeter options include a dose counter display (to remind the technician how many doses have been given), a feedback tone slowly increasing in frequency for 5 s after the dose is delivered, followed by a steady tone lasting for 5 s (to encourage a slow inhalation and breath holding at TLC), and a 5-min timer.

Dosimeter systems should be set up and checked according to the manufacturer recommendations. Manufacturers should give explicit instructions for (1) dosimeter and nebulizer setup and use to deliver the recommended methacholine concentrations and (2) operational checks to ensure proper ongoing function of the system.

The committee divided on the **need** for a dosimeter. Dosimeters may improve the accuracy and repeatability of the dose delivered to the airways but add additional expense. They are widely used in both clinical and research challenge testing. Automatic triggering of the dosimeter during the onset of inhalation is an attractive feature but manual triggering is acceptable. At least one researcher (110) has reported that exact timing of the dose did not affect the methacholine response, an argument against the need for automatic triggering. Further studies are needed to resolve this issue. It is important to simulate an actual test when nebulizers are calibrated because nebulizer output may be less if the dosimeter is activated without the inhalation.

Rationale. Nebulizers. Commercially available nebulizers used to deliver bronchodilator drugs have a wide range of characteristics. Early investigators used DeVilbiss 40 glass nebulizers. DeVilbiss 646 nebulizers have also been used by many investigators performing inhalation challenges. Their output characteristics have been well described (111, 112) and they are relatively inexpensive. Inexpensive plastic nebulizers are generally not manufactured with tight output tolerances and their volume output should be checked before use (107, 113, 114).

The output of a DeVilbiss 646 nebulizer varies 2:1 with the extra vent open or closed. It should be permanently closed (115) for methacholine challenge use, as the output is increased by inhaling through the nebulizer and also varies over time and after disassembly and reassembly of the baffle (109, and personal communications from Robert Wise and Paul Enright). At least 1 ml of solution should remain at the end of nebulization, because output decreases below this level. A $\pm 10\%$ range in nebulizer output is considered acceptable with doubling or quadrupling concentration increments. Nebulizer output is also directly proportional to the airflow driving the nebulizer (115).

Effect of nebulizer and technique differences on test outcomes. The two proposed techniques appear to give similar results in both children (116) and adults despite the substantial differences in administration technique and equipment. The Wright nebulizer used for the 2-minute tidal breathing has a very small particle size distribution with close to 80% of the output in the respirable fraction (RF). The RF is defined as the fraction of aerosol contained in droplets of $< 5 \mu\text{m}$, which, if inhaled via a mouthpiece, would have a high probability of deposition below the vocal cords (117, 118). For a calibrated output of 0.13 ml/min and a respiratory duty cycle (inspiratory time divided by total respiratory cycle time, T_i/T_{tot}) of 0.43, for 2 min of tidal breathing, approximately 0.089 ml of methacholine solution would be delivered below the cords (119). While much of this would be deposited, many smaller particles will be exhaled.

For the five-breath method, a DeVilbiss 646 nebulizer is used with an output of $9 \mu\text{l}$ (0.009 ml) per 0.6-s activation for a total delivery of 0.045 ml (104). Approximately 70% of the total delivery would be within the RF (107) for an estimated total dose of 0.032 ml delivered below the cords. It would appear, therefore, that the tidal breathing method should cause bronchoconstriction at a lower concentration of methacholine. The reasons such differences are not apparent include the following: (1) methacholine is rapidly metabolized. The tidal

breathing method ensures that there will be at least 2.5 min between the start of the inhalation and the first FEV₁, while the five-breath technique moves more quickly with less time for the effect of methacholine to wear off; (2) many of the small particles from the Wright nebulizer may either be exhaled or deposited in the alveoli, where they cannot cause bronchoconstriction; (3) the logarithmic increase in dose would make small differences in airway deposition very difficult to detect; and (4) the repeated deep inhalations in the five-breath technique may affect airway caliber (see Section I, Rationale).

By chance rather than by design, the two methods appear to yield very similar results. Because the inspiratory flow of the subject will greatly exceed the driving flow to the nebulizer, the parameters that will influence deposition will be T_i/T_{tot} for the tidal breathing method and the total duration of inspiration for the five-breath technique, neither of which is size dependent. This means that the expected deposition will be the same but the deposition per unit weight (or lung size) will be greater in smaller subjects than larger ones. This increased dose per unit size may be one of the factors that explains the apparent increased sensitivity in small children (96).

I. Spirometry and Other End-point Measures

Spirometry. Change in FEV₁ is the primary outcome measure for MCT. Spirometry should meet ATS guidelines (120). Special care should be taken to obtain high-quality baseline FEV₁ measurements because unacceptable maneuvers may result in false-positive or false-negative results. The quality of the flow-volume curves should be examined after each maneuver. Full FVC efforts lasting at least 6 s should be performed at baseline (and after diluent, if applicable). If FEV₁ is the only outcome being measured, the expiratory maneuver can be shortened to about 2 s. If a shortened expiratory time is used, technicians should take care that the inspiration is complete because incomplete inhalations will result in a false reduction in FEV₁, and abbreviated flow-volume tracings may not show an inadequate inhalation. If other spirometric outcome variables are used or if vocal cord dysfunction is suspected, full FVC maneuvers should be performed throughout the test. The highest FEV₁ value from acceptable tests is selected for the outcome variable after each dose.

The quality of the maneuvers contributes to the confidence with which an interpretation can be made. The following scheme of quality control (QC) grades reported (printed) after each level of methacholine may be used to assist the interpreter.

- A = two acceptable FEV₁ values that match within 0.10 L
- B = two acceptable FEV₁ values that match within 0.20 L
- C = two acceptable FEV₁ values that do not match within 0.20 L
- D = only one acceptable FEV₁ maneuver
- F = no acceptable FEV₁ maneuvers

All acceptable quality tests performed at 30 and 90 s after each dose of methacholine are used to calculate repeatability. Bear in mind that the repeatability criteria are based on traditional spirometric measurements and may not apply directly to tests performed after the administration of methacholine. Failure to meet repeatability standards should be used only to assist interpretation and not to exclude data from analysis. Studies are needed to better define FEV₁ repeatability criteria for methacholine challenge tests.

Forced inspiratory maneuvers. If vocal cord dysfunction is suspected, or if inspiratory stridor is noted during the baseline examination or after the final dose of methacholine, perform at least three full spirometry tests that include forced inspiratory

vital capacity (FVC) maneuvers. Vocal cord dysfunction (VCD) may be revealed as spontaneous or MCT-induced limitation of forced inspiratory flow resulting in a plateau in flow on the FVC curves (121-124). Patients with vocal cord dysfunction or central airway obstruction are not common, but often have a history suggesting asthma and may be referred for bronchoprovocation testing when the diagnosis of asthma is either considered or questioned.

Body plethysmography. Measures of airway resistance (Raw), usually expressed as specific conductance (sGaw), are alternative end points for MCT but should be used primarily in patients who cannot perform acceptable spirometry maneuvers (125,126). In patients with asthma and COPD, changes in Raw usually parallel changes in FEV₁, with MCT (127-129), but both Raw and sGaw are more variable than FEV₁. A larger percent change (e.g., 45%) is therefore required for a positive test. It is not necessary to measure total lung capacity (TLC) during MCT, because TLC usually does not change (130-132). A decrease in vital capacity reflects an increase in residual volume.

Transcutaneous oxygen. Measurements of transcutaneous oxygen tension (PtcO₂) correlated well with measurements of lung function in children (133-137). PtcO₂ may be a useful end point in infants, young children, and adults (138) whose cooperation cannot be obtained for spirometry. Use of PtcO₂ should be restricted to laboratories with experience in its use as an MCT end point.

Forced oscillation. Forced oscillation or impulse techniques and occlusion or interrupter techniques have recently been assessed (136,137). These approaches do not require patient effort and may be useful in testing patients who cannot perform acceptable spirometry maneuvers. At this time, their use should be reserved for patients who cannot perform acceptable spirometry maneuvers and should be restricted to laboratories with expertise in their application and interpretation.

Rationale. The bronchial smooth muscle stimulation induced by methacholine inhalation results in airway narrowing and airway closure. In healthy persons and patients with mild asthma, the deep inhalation that precedes an FVC maneuver causes transient bronchodilation that may last for up to 6 min (139-141). In patients with more severe asthma, this response is blunted or absent and the maneuver may result in bronchoconstriction (132, 141-143). In patients being clinically tested for a diagnosis of asthma, this difference in the effect of deep inhalations on airways may contribute to the better ability of FEV₁, in comparison with measurements of Raw or sGaw, to separate patients without asthma from patients with asthma (127, 144). However, because most diagnostic challenge tests are performed on individuals who are either nonasthmatic or mildly asthmatic, both are likely to have the bronchodilator response. Changes in peak expiratory flow (PEF) often parallel changes in FEV₁ during bronchoconstriction but have the disadvantages of being (1) more effort dependent and less reproducible and (2) less sensitive in detecting bronchoconstriction (145-148). Although not commonly used as an end point, change in FVC is reported to correlate with disease severity (149).

Changes in airway resistance may be more sensitive than changes in FEV₁ for detecting bronchoconstriction, but FEV₁ is superior to other parameters for discriminating relatively healthy persons from those with asthma (127,144). Other end points such as transcutaneous oxygen and forced oscillation have promise but are still experimental and are not recommended for routine clinical testing at this time.

J. Data Presentation

The results are reported as a percent decrease in FEV₁ from baseline (or postdiluent if a diluent step is used). Data should

be presented for each step in the protocol, including the post-bronchodilator test. At a minimum, all of the elements in the sample bronchoconstriction report in APPENDIX C should be included, including volume-time or flow-volume curves. A single number, PC₂₀ (with one decimal place), may be used to summarize the results for clinical purposes. Unless stated otherwise, it may be assumed that the PC₂₀ is calculated from the change in FEV₁.

If the FEV₁ does not fall by at least 20% after the highest concentration (e.g., 16 mg/ml) then the PC₂₀ should be reported as “> 16 mg/ml.” Do not extrapolate beyond the final concentration. If the FEV₁ falls by more than 20% after inhalation of the diluent, a PC₂₀ is not reported. Instead, state “there was a significant decrease in lung function after inhalation of the diluent and methacholine was not given.”

For manual graphic calculation of PC₂₀, the change in FEV₁ as a percentage of the reference value may be plotted on the ordinate against the log concentration on the abscissa. The following equation can be used to calculate the interpolated PC₂₀ (1, 4, 150):

$$PC_{20} = \text{antilog} \left[\log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{R_2 - R_1} \right] \quad (2)$$

where

- C₁ = second-to-last methacholine concentration (concentration preceding C₂).
- C₂ = final concentration of methacholine (concentration resulting in a 20% or greater fall in FEV₁)
- R₁ = percent fall in FEV₁ after C₁
- R₂ = percent fall in FEV₁ after C₂

Rationale. Exponential models are better than linear models for interpolating between concentrations or doses (150, 151). The provocative concentration that results in a 20% fall in FEV₁ (PC₂₀) was selected as the outcome variable because it is simple to calculate and avoids the complicated and controversial aspects of estimating a provocative dose (PD₂₀).

K. Interpretation

The following factors should be taken into consideration when interpreting PC₂₀ results for an individual patient:

- Pretest probability of asthma, including current asthma symptoms
- Presence or degree of baseline airway obstruction
- Quality of the patient’s spirometry maneuvers
- Pretest questionnaire results (effects modifiers; see Tables 2 and 3)
- Symptoms reported by the patient at the end-of-test
- Degree of recovery after bronchodilator administration

TABLE 5
CATEGORIZATION OF BRONCHIAL RESPONSIVENESS

PC ₂₀ (mg/ml)	Interpretation*
> 16	Normal bronchial responsiveness
4.0-16	Borderline BHR
1.0-4.0	Mild BHR (positive test)
< 1.0	Moderate to severe BHR

* Before applying this interpretation scheme, the following must be true: (1) baseline airway obstruction is absent; (2) spirometry quality is good; (3) there is substantial postchallenge FEV₁ recovery.

Sensitivity and specificity of methacholine challenge tests
Repeatability of methacholine challenge test

A general scheme for categorizing airway responsiveness using PC₂₀, for use when the patient has no baseline airway obstruction, is shown in Table 5. The relationship between levels of airway responsiveness and asthma are discussed below.

Relating the degree of airway responsiveness to questions about individual patients. Using the degree of airway responsiveness to answer questions about individual patients assumes the test is properly performed, that no modifiers that would artificially alter airway responsiveness were present, and that the patient’s prior probability of having current asthma can be reasonably estimated. If the prior probability of asthma is 30–70% and the PC₂₀ is > 16 mg/ml it may be stated with a high degree of confidence that the patient does not currently have asthma (Figure 3) (152, 153). If the same patient has a PC₂₀ < 1.0 mg/ml, the test provides strong confirmation of the clinical diagnosis of asthma. When the PC₂₀ is between 1 and 16 mg/ml, one must be more cautious about stating whether or not the patient has asthma. It has been suggested that when the PC₂₀ is low and the “asthma-like” symptoms induced by MCT are similar to those previously reported by the patient, confidence is a diagnosis of asthma increases. This is intuitively attractive but we know of no published evidence supporting it.

In people with a PC₂₀ between 1 and 16 mg/ml and who have no asthma symptoms (an unusual circumstance because MCT would not be clinically indicated in such a setting) several possibilities exist: (1) there is mild intermittent asthma but the patient is a “poor perceiver” of asthma symptoms; (2) after exercise or inhalation challenges, the patient experiences chest tightness that is perceived but not recognized as abnormal (154); (3) the patient never exercises or experiences environmental triggers of bronchospasm; (4) the mild BHR is due to a cause other than asthma (postviral upper respiratory in-

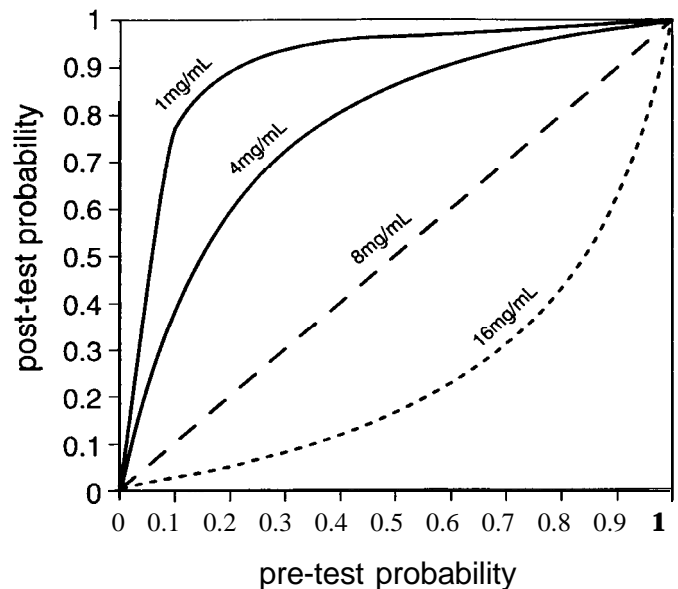


Figure 3. Curves illustrating pretest and posttest probability of asthma after a methacholine challenge test with four PC₂₀ values. The curves represent a compilation of information from several sources (10, 152, 153). They are approximations presented to illustrate the relationships and principles of decision analysis. They are not intended to calculate precise posttest probabilities in patients.

fection [URI, cigarette smoking, etc.); or (5) there is subclinical (asymptomatic) asthma that will become clinical asthma in the future (155, 156). Between 1.5 and 45% of asymptomatic persons with BHR may develop asthma during 2-3 yr of follow-up (157, 158).

In patients with a diagnosis of asthma, the correlation between degree of airway responsiveness and clinical severity of asthma is significant, but not strong enough by itself to categorize the severity of asthma in individual patients (83, 159-162). Recent exposures causing airway inflammation or residual effects of antiinflammatory therapy (systemic or topical to the airways) can easily change the degree of airway responsiveness so that the PC_{20} does not reflect the "usual" untreated severity of the patient's asthma.

It is difficult to interpret the meaning of a low PC_{20} in a patient with baseline airway obstruction (163). For instance, most patients with smoking-related COPD and mild to moderate baseline airway obstruction have BHR, but most have no acute or chronic bronchodilator response or symptoms of asthma (28, 128). It is even more difficult to interpret the significance of a change in PC_{20} when there has also been a change in baseline FEV_1 (which often occurs after successful therapy).

Decision analysis. The most common clinical indication for MCT is to evaluate the likelihood of asthma in patients in whom the diagnosis is suggested by current symptoms but is not obvious. The continuous nature of airway responsiveness and the overlap in PC_{20} between the response of persons with healthy lungs and patients who have unequivocal asthma requires decision analysis whether this is done formally or intuitively (1.52, 164-166).

The pretest probability (prior probability) is the likelihood that the patient has asthma before MCT results are considered. The posttest probability is the likelihood of asthma considering both the pretest probability and MCT results (posterior probability). The difference between the pre- and posttest probabilities represents the contribution of MCT. The MCT results can be helpful or misleading (1.52).

From an epidemiologic standpoint, the prior probability of asthma for a given individual is equal to the prevalence of asthma when a randomly selected population sample is being tested and the subject's medical history is not considered (e.g., when methacholine testing is used to screen hundreds or thousands of military recruits or job applicants for asthma). The prevalence of asthma is relatively low in the general population, usually about 5% (1.59, 167, 168), and the pretest probability in the example is also likely to be about 5%. In this example, an MCT test that is positive with a PC_{20} of 1 mg/ml (using the curves in Figure 3) gives an estimated posttest likelihood of asthma of approximately 45%. Note that with pretest likelihoods in the 5-15% range, the curves are steep and the pretest likelihood is a very strong determinant of the posttest likelihood of asthma.

When a patient presents with symptoms suggestive of asthma, the pretest probability is much higher than in the general population and is more difficult to define precisely. However, when the prior probability is between 30 and 70% MCT can be quite useful. For example, with a PC_{20} of 1 mg/ml, the posttest likelihood of asthma is roughly 90-98% with pretest likelihood estimates ranging from 20 to 80% (152; see Figure 3). If the prior probability is 30% and the PC_{20} is 4 mg/ml, the posttest likelihood is about 70% (Figure 3). Optimal test characteristics (the highest combination of positive and negative predictive power) **occurs** when the pretest probability of asthma is about 50% (10).

Categorical method of interpreting a methacholine challenge test. The alternative "categorical" method for the clinical interpretation of methacholine challenge tests makes three assumptions: (1) MCT results are either positive or negative for BHR; (2) asthma is either present or absent; and (3) there is a "gold standard" for diagnosing asthma. This popular method ignores the continuous spectrum of airway responsiveness, the continuous nature of the degree of uncertainty in the diagnosis of asthma, and the lack of a gold standard for the diagnosis.

Using the categorical method, sensitivity is defined as the fraction (or percentage) of patients with the disease (asthma) who have a positive test. Specificity is defined as the fraction of patients without asthma who have a negative test. A negative methacholine challenge result is commonly defined as non-response to the highest concentration (a $PC_{20} > 8-25$ mg/ml). A positive test is often defined as a $PC_{20} < 8$ or < 16 mg/ml. The optimal cutoff point (threshold) for separating a positive from a negative test is best accomplished using receiver-operator characteristic (ROC) curves. When using ROC analysis, the best PC_{20} cut point to separate patients with asthma from those without asthma is in the range of 8-16 mg/ml (10, 169).

The false-positive rate for asthma is of concern when interpreting MCT results; in such cases the PC_{20} is < 8 mg/ml but the patient does not actually have asthma. In testing general population samples, patients with allergic rhinitis, and smokers with COPD, MCT has relatively high false-positive rates and, therefore, poor positive predictive power (8, 169). About 30% of patients without asthma but with allergic rhinitis have a PC_{20} in the borderline BHR range (144, 170-172). Our recommendation to use an intermediate area of "borderline BHR" when the PC_{20} is between 4 and 16 mg/ml will improve the specificity of MCT in comparison with previous studies. A lower false-positive rate (with better test specificity) can be obtained by considering the pretest probability of asthma (using decision analysis).

A false-negative methacholine challenge result occurs when the PC_{20} is greater than 8-25 mg/ml (no response to the highest concentration) in a patient who has asthma. This occurs much less frequently than false-positive results. The negative predictive power of MCT is more than 90% when the pretest probability of asthma is in the range of 30-70% (153, 172) and most authors conclude that a negative MCT rules out asthma with reasonable certainty in patients who have had asthma symptoms during the previous 2 wk. Three factors should be considered before accepting a negative test as ruling out asthma: (1) airway responsiveness may have been suppressed if the patient was taking intensive antiinflammatory medications prior to the MCT. This issue may not be relevant if the patient has current symptoms; (2) in patients without current symptoms, the season for aeroallergen exposure may have passed (173, 174); and (3) a small fraction of workers with occupational asthma due to a single antigen or chemical sensitizer may respond only when challenged with the specific agent (175, 176).

Effect of test repeatability on interpretation. Optimal repeatability of MCT results is most important when change in airway responsiveness is used in clinical research studies to measure outcome of asthma therapy in clinical research studies. However, knowledge of short-term repeatability of the PC_{20} is also useful when interpreting the results from the first MCT performed by a single patient for clinical purposes.

Short-term within-subject repeatability studies (1-8 wk) when patients are in a stable clinical state show that the 95% confidence intervals for repeat determinations of methacholine PC_{20} lie within ± 1.5 doubling doses (7.5, 81, 82, 87, 88,

177-183). In other words, if the PC_{20} is measured as 4 mg/ml during a baseline clinic visit, repeat MCT 2 wk later will give a PC_{20} between 1.5 and 12 mg/ml in 95% of cases.

In addition to technical issues in standardization there are a number of nontechnical or subject-related factors (which will worsen or improve airway responsiveness) to be considered. Such factors should either be controlled in order to maximize repeatability, or recorded if they might explain changes in airway responsiveness. Factors that must be considered here include recent antigen exposure (which may have a considerable influence on airway responsiveness), exposure to chemical sensitizers (which may also have a large effect), recent respiratory tract infections (likely to have a relatively small effect), variations in airway caliber (probably a small effect), and alteration in asthma medications (may have a large effect depending on the circumstances).

Finally, partial tolerance of methacholine may occur in non-asthmatic subjects but not in asthmatic subjects when tests are repeated at less than 24-h intervals (184, 185). The observed tolerance may have been related to the higher cumulative doses of methacholine given to the nonasthmatic subjects (185).

III. EXERCISE CHALLENGE

Exercise induces airway narrowing in the majority of patients with asthma. Exercise-induced airway narrowing is called exercise-induced asthma (EIA) and exercise-induced bronchoconstriction (EIB); the latter term is used here. The major factors that determine severity of EIB are the pulmonary ventilation reached and sustained during exercise and the water content and temperature of the inspired air. The stimulus by which exercise causes the airways to narrow is the loss of water in bringing large volumes of air to body conditions in a short time (1 86189). The mechanisms whereby water loss causes the airways to narrow is thought to involve the thermal (cooling and rewarming) and/or osmotic effects (190-195) of dehydration. While airway cooling during exercise and airway rewarming after exercise are important determinants of the magnitude of response in adults breathing air of subfreezing temperatures (190), they are not prerequisites for EIB (194). Thus, EIB can occur when the inspired air temperature is greater than 37° C and in the absence of airway cooling (192, 196-198). Rapid rewarming does not enhance EIB in children (199).

Airway cooling and drying are thought to stimulate the release of inflammatory mediators, such as histamine and the cysteinyl leukotrienes (200-203). Thus, exercise has become an important challenge method for assessing the effects of antiinflammatory and other asthma medications. Given the observations of EIB occurring both in cold and very hot inspired air conditions, it can be concluded that, for the purposes of exercise challenges, the most important factor to control is the rate of water loss from the airways by monitoring ventilation and controlling inspired water content.

A. Indications

Exercise is used as a challenge test to make a diagnosis of EIB in asthmatic patients with a history of breathlessness during or after exertion. Such a diagnosis cannot be made with a methacholine test and EIB cannot be excluded by a negative response to methacholine. When the presence of EIB would impair the ability of a person with a history suggesting asthma to perform demanding or lifesaving work (e.g., military, police, or firefighting work), a test for EIB may be indicated (204, 205). Exercise testing is used to determine the effectiveness and optimal dosages of medications prescribed to prevent EIB. Exercise is also used to evaluate the effects of antiinflammatory therapy given

acutely (e.g., cromolyn sodium and nedocromil sodium) and chronically (e.g., steroids and leukotriene antagonists).

B. Contraindications and Patient Preparation

Contraindications. The contraindications for this test are the same as for methacholine challenge testing (Table 1). In addition, the patient with unstable cardiac ischemia or malignant arrhythmias should not be tested. Those with orthopedic limitation to exercise are unlikely to achieve exercise ventilation high enough to elicit airway narrowing. For patients over 60 yr old, a 12-lead electrocardiogram (ECG) obtained within the past year should be available.

Patient preparation. The patient should report to the laboratory in comfortable clothes and running or gym shoes, having consumed no more than a light meal and having had pulmonary medications withdrawn as suggested (Table 2). In addition, antihistamines should have been withheld for 48 h. Vigorous exercise should be avoided for at least 4 h before testing, as prior exercise has been found to exert a protective effect. The interval between repeat testing must also be at least 4 h.

Rationale. Fifty percent of individuals with exercise-induced bronchoconstriction are refractory to a second challenge within 60 min (206). Most lose this refractory state within 2 h, but it occasionally takes as long as 4 h (207-210).

C. Exercise Challenge Testing

Modes of exercise. The preferred modes of exercise are the motor-driven treadmill with adjustable speed and grade or the electromagnetically braked cycle ergometer. Heart rate should be monitored from a three-lead electrocardiographic configuration as a minimum. Alternatively, a pulse oximeter or other device able to reliably determine heart rate may be used. For those at higher risk for coronary artery disease, a 12-lead ECG configuration is advisable.

Inhalate. The patient inspires dry air less than 25° C with a noseclip in place, as nasal breathing decreases the water loss from the airways (211). This can be accomplished by conducting the study in an air-conditioned room (with ambient temperature of 20-25° C) with low relative humidity (50% or less). Inspired air temperature and humidity should be measured and recorded. Optimally, the water content of the inspired air should be less than 10 mg/L. Alternatively, the subject can inspire dry air through a mouthpiece and a two-way breathing valve. A dry inhalate is obtained by filling talc-free meteorological balloons with gas from a medical-grade compressed air source (195, 212, 213). Dry air can also be inspired through a demand valve attached to the inspired port of the two-way valve, although this provides some extra inspiratory resistance at high flow rates (214).

Treadmill protocol. Treadmill speed and grade are chosen to produce 4-6 min of exercise at near-maximum targets with a total duration of exercise of 6-8 min. For children less than 12 yr of age, the time is usually 6 min; for older children and adults the time is usually 8 min. Starting at a low speed and grade, both are progressively advanced during the first 2-3 min of exercise until the heart rate is 80-90% of the predicted maximum (calculated as $220 - \text{age in years}$) (203, 210, 215-217). Ventilation rather than heart rate can be used to monitor exercise intensity. Ventilation should reach 40-60% of the predicted maximum voluntary ventilation (MVV, estimated as $FEV_1 \times 35$) (214, 218, 219). The degree of physical fitness and body weight will strongly influence the grade and speed necessary to obtain the desired heart rate. A reasonable procedure is to quickly advance to a rapid, but comfortable, speed and then raise the treadmill slope until the desired heart rate or ventilation is obtained. Treadmill speed and slope are chosen

to achieve a **target ventilation (or heart rate) that is maintained** for at least 4 min. Children are usually able to reach the target more quickly than adults and for them the exercise duration may be only 6 or 7 min. For older children and adults 8 min of exercise is usually required to elicit EIB when dry air temperature is inhaled. A treadmill speed greater than 3 mph (about 4.5 km/h) and a gradient greater than 15% or an oxygen consumption of 35 ml/min/kg or greater will usually achieve the target ventilation or heart rate in young healthy subjects. Nomograms have been proposed to predict speed and grade that will elicit the desired heart rate (210), but they have not been extensively validated. It may be preferable to use nomograms relating oxygen consumption per kilogram to speed and slope of the treadmill (220–222).

The test ends when the patient has exercised at the target ventilation or heart rate for at least 4 min. This usually requires a total of 6–8 min of exercise. The test may be terminated by the patient at any time.

Bicycle ergometer. For bicycle ergometer exercise, a target work rate to achieve the target ventilation can be determined from equations relating work rate to oxygen consumption and oxygen consumption to ventilation (193, 219, 222). One equation used to establish the target work rate is $\text{watts} = (53.76 \times \text{measured FEV}_1) - 11.07$. The work rate is set to 60% of the target in the first minute, 75% in the second minute, 90% in the third minute, and 100% in the fourth minute (214). Using this protocol the repeatability of the percent fall in FEV₁ is good. For example, the coefficient of variation for two tests performed within 1 mo is 21%. Thus, a patient who has a 30% fall in FEV₁ on one occasion would be expected to have a fall within 24–36% if tested within 1 mo. Ventilation and/or heart rate are checked to determine if the exercise targets are achieved. A valid test requires the target exercise intensity to be sustained for 4–6 min. To ensure that the target minute ventilation is sustained, the work rate may need to be reduced in the final minutes of exercise. It is important for the patient to reach the target heart rate or ventilation within 4 min because the rate of water loss is the determining factor for eliciting EIB and refractoriness can develop if exercise is prolonged at submaximal work. The test ends when the patient has exercised at the target work rate for 6 min. The patient may terminate the test at any time.

Pulmonary gas exchange. Measurement of pulmonary gas exchange during exercise is helpful, although not required. Measurement of minute ventilation allows an assessment of the magnitude of the stimulus to airway narrowing (223) and measurement of oxygen uptake makes it possible to quantify the intensity of exercise as a fraction of predicted peak oxygen uptake. These data can be used to confirm an adequate exercise level, which is especially important when a test is negative.

Safety. A licensed physician or an experienced technician should observe the patient during exercise and the recovery period and watch for undue stress (e.g., severe wheezing, chest pain, lack of coordination) or adverse signs (e.g., ECG abnormalities, falling blood pressure, severe decrease in O₂ saturation). The choice of who monitors the test and what parameters are monitored depends on the risk for adverse events. In patients felt to be low risk for adverse events, the test may be supervised by an experienced technician, provided that a licensed physician can be summoned quickly, if problems arise. The technician should be able to recognize the presence of indicators of respiratory distress and be able to recognize the presence of significant arrhythmias. In patients felt to be at higher risk for adverse events, a physician should directly monitor the test. In either case, a resuscitation cart should be immediately available.

Although not always accurate during exercise (224, 225), estimation of arterial O₂ saturation by pulse oximetry is recommended both during and after exercise. Measurement of blood pressure by sphygmomanometry is a useful adjunct but is not routinely required. ECG monitoring of all subjects is necessary to ensure accurate heart rate measurements but 12-lead monitoring is necessary only in high-risk cases.

Rationale. The choice of the recommended technique is based on physiologic considerations and on substantial experience reported in the literature (1, 201, 210, 213–215, 218, 219, 221, 223, 226–228).

Mode of exercise. Although early studies suggested that treadmill exercise was preferable to bicycle exercise (226, 229, 230), it appears this was most likely **due** to the more rapid increase in ventilation in response to treadmill running. Providing the work rate can raise the ventilation to the target within 4 min, cycling exercise can be used effectively. Although peak oxygen uptake averages approximately 10% less on the cycle ergometer than on the treadmill, peak ventilation is comparable (231). In those with established EIB, the cycle ergometer has been used successfully in assessing the effects of drugs (214). Its role in identifying those with EIB is less well defined. Theoretically, cycle ergometry should be a satisfactory alternative testing mode; well-designed comparative studies will be required to establish this. Free range running has been proposed as being useful for screening populations (232–235), although safety measures are difficult to provide in this testing mode.

Choice of the inhalate. The tendency to elicit airway narrowing is enhanced when the inhalate is cool and/or dry. However, it is not clear whether the ambient humidity in the typical air-conditioned laboratory (typically 30–50% water vapor saturation) is significantly less likely to induce airway narrowing than perfectly dry air (as can be obtained from a compressed air source). Anderson and coworkers (189) found in a group of 12 children a $35 \pm 13\%$ (SD) fall in FEV₁ with ambient air (23.3° C, 12 mg H₂O/L) and a $45 \pm 16.7\%$ (SD) fall in FEV₁ with compressed dry air (25.4° C). Although the difference was not significant, the greater response to compressed air may help to identify persons with mild EIB and diminish the chance of a negative test. Cold air generators, which produce dry air at below-freezing temperatures, are commercially available and are in use in some laboratories (236). In patients in whom symptoms are specifically associated with exercise in the cold, conducting an exercise challenge while breathing a cold dry inhalate may be useful.

Work rate profile. A range of testing configurations may produce a similar degree of bronchoconstriction is susceptible individuals. However, the target minute ventilation must be reached quickly and be sustained for 4 min to diminish the possibility that refractoriness will occur. The intensity of the exercise should be such that the person cannot exercise much beyond 6 or 8 min. If they can, it is unlikely that the workload was sufficiently hard to elicit EIB. The incremental work rate profile used in cardiopulmonary exercise testing, in which exercise intensity is progressively increased to tolerance over a 10-min period (231), is less likely to be effective in evaluating EIB (237), probably because high levels of ventilation are sustained for a relatively short time. Studies will be required to evaluate this possibility. The evidence suggests the most effective exercise is hard and relatively brief. Specifically, prolonging the warm-up period has the potential to induce refractoriness to EIB. Sustaining the heavy exercise period for a prolonged period may actually diminish the bronchoconstriction; a trend for a decreased response has been demonstrated for exercise periods of 12 min or longer (221).

Using a heart rate target is practical and generally effective. However, because pulmonary ventilation is more closely

related to the stimulus to bronchoconstriction than is heart rate, some authors prefer measuring ventilation to guide exercise intensity. Anderson and colleagues have suggested that ventilation be sustained for 4 min at between 40 and 60% of predicted maximum voluntary ventilation, calculated as $FEV_1 \times 35$ (214, 218, 219).

Safety. The most common problem encountered in exercising a patient with asthma is severe bronchoconstriction. This can usually be treated rapidly and successfully by administering nebulized bronchodilator with oxygen. The appropriate equipment should be immediately available and a pulse oximeter should be kept on the subject. As the patients most likely to have the most severe response are those with less than normal lung function a minimum FEV_1 for a patient to be allowed to proceed with the exercise test must be clearly defined. The European Respiratory Society (219) suggested an FEV_1 greater than 75% of the predicted normal value.

The approach to monitoring will vary depending on the setting in which exercise testing is conducted and an assessment of risk for the individual being tested. Risk of adverse events should be minimized and a rapid response should be available in the event of a serious adverse event. In a hospital setting, where a resuscitation team is readily available, a young, healthy subject who has symptoms of exercise-induced bronchoconstriction can be tested in a laboratory with minimum monitoring and only a technician present. In a setting with less support and a higher risk patient, more intense monitoring by individuals with the skills to appropriately diagnose and treat adverse events would be necessary. In their procedure manual, testing laboratories should define the appropriate levels of monitoring and the person who makes the decisions to test individual subjects.

D. Assessing the Response

Forced expiratory volume in 1 s (FEV_1) is the primary outcome variable. Spirometry should be performed in the seated position before exercise and then serially after exercise, utilizing the test method recommended by the American Thoracic Society (120). At least two and preferably three acceptable tests should be obtained at each testing interval. As a goal, the highest and second highest FEV_1 values should differ by no more than 0.2 L. The highest of the acceptable FEV_1 values is selected as the representative value at each interval. One exception to ATS-recommended techniques for spirometry is allowed. If the only outcome variable to be used is the FEV_1 , the duration of the expiration may be limited to 2-3 s. In all cases it is important to vigorously coach the patient to inhale fully even in the presence of chest tightness. Incomplete inhalations will result in false reductions in FEV_1 .

If vocal cord dysfunction or other possible causes of central airway obstruction are suspected, full inspiratory and expiratory flow-volume loops should be obtained.

An appropriate postexercise testing schedule is 5, 10, 15, 20, and 30 min after cessation of exercise. Some investigators include earlier measurements (1 and 3 min postexercise) because severe EIB can sometimes be present at the cessation of exercise (214, 219). Early recognition allows it to be dealt with promptly.

If the FEV_1 has returned from its nadir to the baseline level or greater, spirometry testing may be terminated at 20 min postexercise. A P-agonist bronchodilator may be administered at any time to reverse the bronchoconstrictive response if the patient experiences appreciable dyspnea, or if the FEV_1 has not recovered to within 10% of baseline when the patient is ready to leave the laboratory.

The presence of exercise-induced bronchoconstriction is defined by plotting FEV_1 as a percentage of the preexercise baseline FEV_1 at each postexercise interval. A decrease below

90% of the baseline FEV_1 (i.e., a 10% decrease) is a generally accepted abnormal response (1, 218, 221, 237-240). Some authors suggest a value of 15% is more diagnostic of EIB, particularly if exercise has been performed in the field (233).

Rationale. A large number of testing schedules for performing postexercise spirometry have been suggested. In most cases, the nadir in FEV_1 occurs within 5-10 min of cessation of exercise, although it is occasionally not reached until 30 min postexercise (239). Including a 30-minute postexercise observation is controversial, because such a delay is infrequently seen. Some laboratories terminate the test as soon as the FEV_1 falls below a certain threshold (e.g., 10%). Without confirming the FEV_1 has reached its nadir, it is not possible to assess the severity of exercise-induced bronchoconstriction. Other laboratories define the nadir as when the FEV_1 has increased from its lowest value at the two subsequent time intervals (e.g., the test is terminated at 15 min when the 10- and 15-min FEV_1 values are higher than that measured at 5 min). This reduces the time of challenge for most patients.

The criterion for a positive response is controversial. A fall of 10% or more is considered abnormal; a fall of 15% appears to be more diagnostic of EIB. A fall in FEV_1 of as little as 10% seems to be a reasonable criterion because healthy subjects generally demonstrate an increase in FEV_1 after exercise. Some authors have employed a more stringent criterion (e.g., a 15% fall) (223, 227). Three studies in presumably normal children have demonstrated an upper 95% confidence limit (defined as 1.96 SD) of the FEV_1 fall as 8.2% (241), 10% (242), and 15.3% (233). Further studies are needed to establish the validity of proposed thresholds for a positive test.

A positive response is seen in those with upper airway abnormalities such as abnormal posterior motion of the arytenoid region (243) or vocal cord dysfunction (244). These rare cases can be distinguished from exercise-induced bronchoconstriction by examining the flow-volume curve (245, 246).

ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories

Committee Members

ROBERT O. CRAPO, M.D., *Chairman*
 RICHARD CASABURI, Ph.D., M.D.
 ALLAN L. COATES, M.D.
 PAUL L. ENRIGHT, M.D.
 JOHN L. HANKINSON, Ph.D.
 CHARLES G. IRVIN, Ph.D.
 NEIL R. MACINTYRE, M.D.
 ROY T. MCKAY, Ph.D.
 JASK S. WANGER, M.S.

Ad Hoc Committee Members

SANDRA D. ANDERSON, Ph.D.
 DON W. COCKCROFT, M.D.
 JAMES E. FISH, M.D.
 PETER J. STERK, M.D.

Acknowledgment: The authors thank Janet Embry for editorial assistance.

References

1. Sterk, P. J., L. M. Fabbri, P. H. Quanjer, D. W. Cockcroft, P. M. O'Byrne, S. D. Anderson, E. F. Juniper, and J. L. Malo. 1993. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Statement of the European Respiratory Society. *Eur. Respir. J.* 6(Suppl. 16):53-X3.
2. Eiser, N. M., K. F. Kerrebijn, and P. H. Quanjer, editors. 1983. Guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstriction agents. *Bull. Eur. Physiopathol. Respir.* 19:495-514.
3. Shapiro, G. G., and R. A. Simon, for the American Academy of Allergy and Immunology Bronchoprovocation Committee. 1992. Bronchoprovocation committee report. *J. Allergy Clin. Immunol.* 89:775-778.
4. Juniper, E. F., D. W. Cockcroft, and F. E. Hargreave. 1994. Tidal breathing method. In *Histamine and Methacholine Inhalation Tests: Laboratory Procedure and Standardization*, 2nd ed. Astra Draco AB, Lund, Sweden.
5. Chai, H., R. S. Farr, L. A. Froehlich, D. A. Mathison, J. A. MacLean, R. R. Rosenthal, A. L. Sheffer, S. L. Spector, and R. G. Townley. 1975. Standardization of bronchial inhalation challenge procedures. *J. Allergy Clin. Immunol.* 56:323-327.

6. Spector, S. L. 1993. Bronchial provocation tests. In E. B. Weiss and M. Stein, editors. *Bronchial Asthma: Mechanisms and Therapeutics*, 3rd ed. Little, Brown, & Company, Boston.
7. Cockcroft, D. W., and F. E. Hargreave. 1991. Airways hyperresponsiveness: definition, measurement and clinical relevance. In M. A. Kaliner, P. J. Barnes, and C. G. A. Persson, editors. *Asthma: Its Pathology and Treatment*. Marcel Dekker, New York.
8. Fish, J. E. 1993. Bronchial challenge testing. In E. Middleton, editor. *Allergy: Principles and Practice*, 4th ed. Mosby-Year Book, St. Louis, MO.
9. Postma, D. S., and H. A. Kerstjens. 1998. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 158(Suppl.):S187-S192.
10. Perpiñá, M., C. Pellicer, A. deDiego, L. Compte, and V. Macian. 1993. Diagnostic value of the bronchial provocation test with methacholine in asthma: Bayesian analysis approach. *Chest* 104:149-154.
11. Dales, R. E., F. Nunes, D. Partyka, and P. Ernst. 1988. Clinical prediction of airways hyperresponsiveness. *Chest* 5:984-986.
12. Ramsdell, J. W., F. J. Nachtwey, and K. M. Moser. 1982. Bronchial hyperactivity in chronic obstructive bronchitis. *Am. Rev. Respir. Dis.* 126X29-832.
13. Du Toit, J. I., A. J. Woolcock, C. M. Salome, R. Sundrum, and J. L. Black. 1986. Characteristics of bronchial hyperresponsiveness in smokers with chronic air-flow limitation. *Am. Rev. Respir. Dis.* 134:498-501.
14. Yan, K., C. M. Salome, and A. J. Woolcock. 1985. Prevalence and nature of bronchial hyperresponsiveness in subjects with chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* 132:25-29.
15. Juniper, E. F., P. A. Frith, and F. E. Hargreave. 1981. Airways responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* 36:575-579.
16. Murry, A. B., A. C. Ferguson, and B. Morrison. 1981. Airway responsiveness to histamine as a test for overall severity of asthma in children. *J. Allergy Clin. Immunol.* 68:119-124.
17. Kraan, J., G. H. Koeter, T. W. van der Mark, H. J. Sluiter, and K. de Vries. 19X5. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J. Allergy Clin. Immunol.* 76:628-636.
18. Kerrebijn, K. F., E. E. van Essen-Zandvliet, and H. J. Neijens. 1987. Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. *J. Allergy Clin. Immunol.* 79:653-659.
19. Dinh Xuan, A. T., and A. Lockhart. 1991. Use of nonspecific bronchial challenges in the assessment of anti-asthmatic drugs. *Eur. Respir. Rev.* 1:19-24.
20. Crimi, N., F. Palermo, R. Oliveri, R. Polosa, I. Settineri, and A. Mistretta. 1992. Protective effects of inhaled ipratropium bromide on bronchoconstriction induced by adenosine and methacholine in asthma. *Eur. Respir. J.* 5:560-565.
21. Laitinen, L. A., A. Laitinen, and T. Haahtela. 1992. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta 2-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. *J. Allergy Clin. Immunol.* 90:32-42.
22. Van Essen-Zandvliet, E. E., M. D. Hughes, H. J. Waalkens, E. J. Duiverman, S. J. Pocock, and K. F. Kerrebijn. 1992. Effects of 22 months of treatment with inhaled corticosteroids and or beta-2-agonists on lung function, airway responsiveness, and symptoms in children with asthma. *Am. Rev. Respir. Dis.* 146:547-554.
23. Rabe, K. F., R. Jorres, D. Nowak, N. Behr, and H. Magnussen. 1993. Comparison of the effects of salmeterol and formoterol on airway tone and responsiveness over 24 hours in bronchial asthma. *Am. Rev. Respir. Dis.* 147:1436-1441.
24. Prieto, L., J. M. Berto, V. Gutierrez, and C. Tornero. 1994. Effect of inhaled budesonide on seasonal changes in sensitivity and maximal response to methacholine in pollen-sensitive asthmatic subjects. *Eur. Respir. J.* 7:1X45-1 X51.
25. Cockcroft, D. W., V. A. Swystun, and R. Bhagat. 1995. Interaction of inhaled β_2 agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. *Am. J. Respir. Crit. Care Med.* 152:1485-1489.
26. Sont, J. K., L. N. A. Willems, E. H. Bel, J. H. J. M. van Krieken, J. P. vandenBroucke, P. J. Sterk, and the AMPUL Study Group. 1999. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. *Am. J. Respir. Crit. Care Med.* 159:1043-1051.
27. Martin, R. J., J. S. Wanger, C. G. Irvin, B. B. Bartelson, R. M. Cherniak, and the Asthma Clinical Research Network (ACRN). 1997. Methacholine challenge testing: safety of low starting FEV₁. *Chest* 112:53-56.
28. Tashkin, D. P., M. D. Altose, E. R. Bleecker, J. E. Connett, R. E. Kanner, W. W. Lee, R. Wise, and the Lung Health Research Group. 1992. The Lung Health Study: airway responsiveness to inhaled methacholine in smokers with mild to moderate airflow limitation. *Am. Rev. Respir. Dis.* 145:301-310.
29. Scott, G. C., and S. R. Braun. 1991. A survey of the current use and methods of analysis of bronchoprovocational challenges. *Chest* 1(X1):322-328.
30. National Asthma Education and Prevention Program. 1997. Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma. U.S. Government Printing Office, Washington, DC. NHLBI Publication No. 97-405 1.
31. Rodriguez-Roisin, R., A. Ferrer, D. Navajas, A. G. Agusti, P. D. Wagner, and J. Roca. 1991. Ventilation-perfusion mismatch after methacholine challenge in patients with mild bronchial asthma. *Am. Rev. Respir. Dis.* 144:88-94.
32. Roca, J., and R. Rodriguez-Roisin. 1992. Editorial: asthma, allergen challenge and gas exchange. *Eur. Respir. J.* 5:1171-1172.
33. Goldman, M. D., M. Mathieu, J. M. Montely, R. Goldberg, J. M. Fry, J. L. Bernard, and R. Sartene. 1995. Inspiratory fall in systolic pressure in normal and asthmatic subjects. *Am. J. Respir. Crit. Care Med.* 151:743-750.
34. Stewart, I. C., A. Parker, J. R. Catterall, N. J. Douglas, and D. C. Flenley. 1989. Effect of bronchial challenge on breathing patterns and arterial oxygenation in stable asthma. *Chest* 95:65-70.
35. Fields, C. L., R. P. Byrd, M. A. Ossorio, T. M. Roy, M. J. Michaels, and R. L. Vogel. 1993. Cardiac arrhythmias during performance of the flow-volume loop. *Chest* 103:1006-1009.
36. Enright, P. L., M. D. Lebowitz, and D. W. Cockcroft. 1994. Physiologic measures: pulmonary function tests. Asthma outcome. *Am. J. Respir. Crit. Care Med.* 149:S9-S18.
37. Weiss, S. T., I. B. Tager, J. W. Weiss, A. Munoz, F. E. Speizer, and R. H. Ingram. 1984. Airways responsiveness in a population sample of adults and children. *Am. Rev. Respir. Dis.* 129X98-902.
38. Rijcken, B., J. P. Schouten, S. T. Weiss, F. E. Speizer, and R. van der Lende. 1988. The relationship between airway responsiveness to histamine and pulmonary function level in a random population sample. *Am. Rev. Respir. Dis.* 137:826-832.
39. Baake, P. S., V. Baste, and A. Gulsvik. 1991. Bronchial responsiveness in a Norwegian community. *Am. Rev. Respir. Dis.* 143:317-322.
40. Peat, J. K., C. M. Salome, and W. Xuan. 1996. On adjusting measurements of airway responsiveness for lung size and airway caliber. *Am. J. Respir. Crit. Care Med.* 154:870-875.
41. Kremer, A. M., T. M. Pal, M. Oldenziel, M. Kerkhof, J. G. R. de Monchy, and B. Rijcken. 1995. Use and safety of a shortened histamine challenge test in an occupational study. *Eur. Respir. J.* 5:737-741.
42. Thompson, N. C., P. O'Byrne, and F. E. Hargreave. 1983. Prolonged asthmatic responses to inhaled methacholine. *J. Allergy Clin. Immunol.* 71:357-362.
43. Seatta, M., A. Di Stefano, G. Turato, R. De Caro, D. Bordignon, S. T. Holgate, and L. M. Fabbri. 1995. Fatal asthma attack during an inhalation challenge with ultrasonically nebulized distilled water. *J. Allergy Clin. Immunol.* 95:1285-1287.
44. Lundgren, R., M. Söderberg, L. Rosenhall, and E. Norman. 1992. Case report: development of increased airway responsiveness in two nurses performing methacholine and histamine challenge tests. *Allergy* 47:188-189.
45. Ahrens, R. C., A. C. Bonham, G. A. Maxwell, and M. M. Weinberger. 1984. A method for comparing the peak intensity and duration of action of aerosolized bronchodilators using bronchoprovocation with methacholine. *Am. Rev. Respir. Dis.* 129:903-906.
46. Greenspon, L. W., and W. L. Morrissey. 1986. Factors that contribute to inhibition of methacholine-induced bronchoconstriction. *Am. Rev. Respir. Dis.* 133:735-739.
47. Wilson, N. M., S. Green, C. Coe, and P. J. Barnes. 1987. Duration of protection by oxitropium bromide against cholinergic challenge. *Eur. J. Respir. Dis.* 71:455-458.
48. Derom, E. Y., R. A. Pauwels, and M. E. F. Van Der Straeten. 1992. The effect of inhaled salmeterol on methacholine responsiveness in subjects with asthma up to 12 hours. *J. Allergy Clin. Immunol.* 89:811-815.
49. Cockcroft, D. W., and V. A. Swystun. 1997. Effect of single doses of S-salbutamol, R-salbutamol, racemic salbutamol, and placebo on the airway response to methacholine. *Thorax* 52:845-848.
50. McWilliams, B. C., R. Menendez, H. W. Kelley, and J. Howick. 1984.

- Effects of theophylline on inhaled methacholine and histamine in asthmatic children. *Am. Rev. Respir. Dis.* 130:193-197.
51. Magnussen, H., G. Reuss, and R. Jorres. 1987. Theophylline has a dose-related effect on the airway response to inhaled histamine and methacholine in asthmatics. *Am. Rev. Respir. Dis.* 136:1163-1167.
 52. Henderson, J. C., F. O'Connell, and R. W. Fuller. 1993. Decrease of histamine induced bronchoconstriction by caffeine in mild asthma. *Thorax* 48:824-826.
 53. Juniper, E. F., P. A. Kline, M. A. Vanzieleghem, E. H. Ramsdale, P. M. O'Byrne, and F. E. Hargreave. 1990. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. *Am. Rev. Respir. Dis.* 142:832-836.
 54. Freezer, N. J., H. Croasdel, I. J. M. Doull, and S. T. Holgate. 1995. Effect of regular inhaled beclomethasone on exercise and methacholine airway responses in school children with recurrent wheeze. *Eur. Respir. J.* 8:1488-1493.
 55. Lemièrre, C., A. Cartier, J. Dolovich, M. Chan-Yeung, L. Grammar, H. Ghezzi, J. L'Archeveque, and J. L. Malo. 1996. Outcome of specific bronchial responsiveness to occupational agents after removal from exposure. *Am. J. Respir. Crit. Care Med.* 154:329-333.
 56. Merget, R., M. Reineke, A. Rueckmann, E. M. Bergmann, and G. Schultze-Werninghaus. 1994. Nonspecific and specific bronchial responsiveness in occupational asthma caused by platinum salts after allergen avoidance. *Am. J. Respir. Crit. Care Med.* 150:1146-1149.
 57. Empey, D. W., L. A. Laitinen, L. Jacobs, W. M. Gold, and J. A. Nadel. 1976. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am. Rev. Respir. Dis.* 113:131-139.
 58. Cheung, D., E. C. Dick, M. C. Timmers, E. P. A. deKlerk, W. J. M. Spaan, and P. J. Sterk. 1995. Rhinovirus inhalation causes long-lasting excessive airway narrowing in response to methacholine in asthmatic subjects in vivo. *Am. J. Respir. Crit. Care Med.* 152:1490-1496.
 59. Kreit, J. W., K. B. Gross, T. B. Moore, T. J. Lorenzen, J. D'Arcy, and W. L. Eschenbacher. 1989. Ozone-induced changes in pulmonary function and bronchial hyperresponsiveness in asthmatics. *J. Appl. Physiol.* 66:2 17-22.
 60. Jensen, E. J., R. Dahl, and F. Steffensen. 1998. Bronchial reactivity to cigarette smoke in smokers: repeatability, relationship to methacholine reactivity, smoking, and atopy. *Eur. Respir. J.* 11:670-676.
 61. Malo, J. L., A. Cartier, L. P. Boulet, J. L'Archeveque, F. Saint-Denis, L. Bherer, and J. P. Courteau. 1994. Bronchial hyperresponsiveness can improve while spirometry plateaus two to three years after repeated exposure to chlorine causing respiratory symptoms. *Am. J. Respir. Crit. Care Med.* 150:1142-1145.
 62. Little, J. W., W. J. Hall, R. G. Douglas, G. S. Mudholkar, D. M. Speers, and K. Patel. 1978. Airway hyperreactivity and peripheral airway dysfunction in influenza A infection. *Am. Rev. Respir. Dis.* 118:295-303.
 63. Annesi, I., M.-P. Oryszczyn, F. Neukirch, E. Orovén-Frija, M. Korobaeff, and F. Kauffmann. 1992. Relationship of upper airways disorders [rhinitis] to FEV₁ and bronchial hyperresponsiveness in an epidemiological study. *Eur. Respir. J.* 5:1104-1110.
 64. Prieto, J. L., V. Gutierrez, J. M. Berto, and B. Camps. 1996. Sensitivity and maximal response to methacholine in perennial and seasonal allergic rhinitis. *Clin. Exp. Allergy* 26:61-67.
 65. Woenne, R., M. Kattan, R. P. Orange, and H. Levison. 1978. Bronchial hyperreactivity to histamine and methacholine in asthmatic children after inhalation of SCH 1000 and chlorpheniramine maleate. *J. Allergy Clin. Immunol.* 62:119-124.
 66. Juniper, E. F., P. A. Kline, R. S. Roberts, F. E. Hargreave, and E. E. Daniel. 1987. Airway responsiveness to methacholine during the natural menstrual cycle and the effect of oral contraceptives. *Am. Rev. Respir. Dis.* 135:1039-1042.
 67. Duval, N. J., K. M. Lahren, and K. M. O'Neil. 1994. Effect of influenza vaccine on bronchoprovocation testing in normal subjects. *Chest* 106:750-752.
 68. Sherman, C. B., D. G. Kern, R. W. Corwin, and B. Andrus. 1994. A clinical and structural comparison of industrial methacholine and Provocholine. *Chest* 105:1095-1097.
 69. Hayes, R. D., J. R. Beach, D. M. Rutherford, and M. R. Sim. 1998. Stability of methacholine chloride solutions under different storage conditions over a 9 month period. *Eur. Respir. J.* 11:946-948.
 70. Rosenfeld, J., E. F. Juniper, and F. E. Hargreave. 1984. Gas chromatographic determination of methacholine chloride in pharmaceutical preparations. *J. Chromatogr.* 287:433-437.
 71. Watson, B. L., R. A. Cormier, and R. J. Harbeck. 1998. Effect of pH on the stability of methacholine chloride in solution. *Respir. Med.* 92:588-592.
 72. Pratter, M. R., T. F. Woodman, R. S. Irwin, and B. Johnson. 1982. Stability of stored methacholine chloride solutions: clinically useful information. *Am. Rev. Respir. Dis.* 126:717-719.
 73. Bhagat, R. G., and M. M. Grunstein. 1984. Comparison of responsiveness to methacholine, histamine, and exercise in subgroups of asthmatic children. *Am. Rev. Respir. Dis.* 129:221-224.
 74. Toelle, B. G., J. K. Peat, C. M. Salome, J. Crane, D. McMillan, J. Dermand, W. D'Souza, and A. J. Woolcock. 1994. Comparison of two epidemiological protocols for measuring airway responsiveness and allergic sensitivity in adults. *Eur. Respir. J.* 7:1798-1804.
 75. Juniper, E. F., P. A. Frith, C. Dunnett, D. W. Cockcroft, and F. E. Hargreave. 197X. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 33:705-710.
 76. Chatham, M., E. R. Bleecker, P. L. Smith, R. R. Rosenthal, P. Mason, and P. S. Norman. 1982. A comparison of histamine, methacholine, and exercise airway reactivity in normal and asthmatic subjects. *Am. Rev. Respir. Dis.* 126:235-240.
 77. Higgins, B. G., J. R. Britton, S. Chinn, T. D. Jones, A. S. Vathenen, P. G. J. Burney, and A. E. Tattersfield. 1988. Comparison of histamine and methacholine for use in bronchial challenge tests in community studies. *Thorax* 43:605-610.
 78. MacDonald, N. C., C. K. Whitmore, M. C. Makoid, and J. Cobby. 1981. Stability of methacholine chloride in bronchial provocation test solutions. *Am. J. Hosp. Pharmacy* 38:868-871.
 79. Kongerud, J., V. Soyseth, and B. Johansen. 1989. Room temperature influences output from the Wright jet nebulizer. *Eur. Respir. J.* 2:681-684.
 80. Rosenthal, R. R. 1989. Approved methodology for methacholine challenge. *Allergy Proc.* 10:301-310.
 81. Ryan, G., M. B. Dolovich, R. S. Roberts, P. A. Frith, E. F. Juniper, F. E. Hargreave, and M. T. Newhouse. 1981. Standardization of inhalation provocation tests: two techniques of aerosol generation and inhalation compared. *Am. Rev. Respir. Dis.* 123:195-199.
 82. Yan, K., C. Salome, and A. J. Woolcock. 1983. Rapid method for measurement of bronchial responsiveness. *Thorax* 38:760-765.
 83. Cockcroft, D. W., D. N. Killian, J. J. Mellon, and F. E. Hargreave. 1977. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin. Allergy* 7:235-243.
 84. Beaupre, A., and J. L. Malo. 1979. Comparison of histamine bronchial challenges with the Wright nebulizer and the dosimeter. *Clin. Allergy* 9:575-583.
 85. Britton, J., A. Mortagy, and A. E. Tattersfield. 1986. Histamine challenge testing: comparison of three methods. *Thorax* 41:128-132.
 86. Bennett, J. B., and R. J. Davies. 1987. A comparison of histamine and methacholine bronchial challenges using the DeVilbiss 646 nebulizer and the Rosenthal-French dosimeter. *Br. J. Dis. Chest* X1:252-259.
 87. Knox, A. J., A. Wisniewski, S. Cooper, and A. E. Tattersfield. 1991. A comparison of the Yan and a dosimeter method for methacholine challenge in experienced and inexperienced subjects. *Eur. Respir. J.* 4:497-502.
 88. Peat, J. K., C. M. Salome, A. Bauman, B. G. Toelle, S. L. Wachinger, and A. J. Woolcock. 1991. Repeatability of histamine bronchial challenge and comparability with methacholine bronchial challenge in a population of Australian schoolchildren. *Am. Rev. Respir. Dis.* 144:338-343.
 89. Hargreave, F. E., G. Ryan, N. C. Thomson, P. M. O'Byrne, K. Latimer, E. F. Juniper, and J. Dolovich. 1981. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance [Review]. *J. Allergy Clin. Immunol.* 68:347-355.
 90. Juniper, E. F., M. Syty-Golda, and F. E. Hargreave. 1984. Histamine inhalation tests: inhalation of aerosol via a facemask versus a valve box with mouthpiece. *Thorax* 39:565-571.
 91. Parker, C. D., R. E. Bilbo, and C. E. Reed. 1965. Methacholine aerosol as test for bronchial asthma. *Arch. Intern. Med.* 115:452-458.
 92. Chatham, M., E. R. Bleecker, P. Norman, P. L. Smith, and P. Mason. 1982. A screening test for airways reactivity. An abbreviated methacholine inhalation challenge. *Chest* 82:15-I 8.
 93. Schmidt, L. E., P. S. Thorne, J. L. Watt, and D. A. Schwartz. 1992. Is an abbreviated bronchial challenge with histamine valid? *Chest* 101:141-145.
 94. Sovijärvi, A. R. A., L. P. Malmberg, K. Reinkainen, P. Ryttilä, and H. Poppius. 1993. A rapid dosimetric method with controlled tidal breathing for histamine challenge: repeatability and distribution of bronchial reactivity in a clinical material. *Chest* 104:164-170.
 95. Jörres, R. A., D. Nowak, D. Kirsten, L. Gronke, and H. Magnussen. 1997. A short protocol for methacholine testing adapted to the Rosenthal-Chai dosimeter technique. *Chest* 111 I:866-869.
 96. LeSouëf, P. N., M. R. Sears, and D. Sherril. 1995. The effect of size and age of subject on airway responsiveness in children. *Am. J. Respir. Crit. Care Med.* 152:576-579.

97. Laube, B. L., P. S. Norman, and G. K. Adams. 1992. The effect of aerosol distribution on airway responsiveness to inhaled methacholine in patients with asthma. *J. Allergy Clin. Immunol.* 89:510-518.
98. Ruffin, R. E., M. B. Dolovich, R. K. Wolffe, and M. T. Newhouse. 1978. The effects of preferential deposition of histamine in the human airway. *Am. Rev. Respir. Dis.* 117:485-492.
99. Cockcroft, D. W., B. A. Barseheid, and K. Y. Murdock. 1983. Measurement of responsiveness to inhaled histamine using FEV₁: comparison of PC-20 and threshold. *Thorax* 38:523-526.
100. Connolly, M. J., A. J. Avery, E. H. Walters, and D. J. Hendrick. 1988. The use of sequential doses of inhaled histamine in the measurement of bronchial responsiveness: cumulative effect and distortion produced by shortening the protocol. *J. Allergy Clin. Immunol.* 82:863-868.
101. Wanger, J., and C. G. Irvin. 1998. Responses to diluent inhalation in clinical methacholine challenges (abstract). *Am. J. Respir. Crit. Care Med.* 157(Suppl.):A672.
102. Loffert, D. T., D. Ikle, and H. S. Nelson. 1994. A comparison of commercial jet nebulizers. *Chest* 106:1788-1792.
103. Smith, L., and E. R. McFadden. 1995. Bronchial hyperreactivity revisited. *Ann. Allergy Asthma Immunol.* 74:454-469.
104. Ryan, G. J., M. B. Dolovich, G. Obminski, D. W. Cockcroft, E. Juniper, F. E. Hargreave, and M. T. Newhouse. 1981. Standardization of inhalation provocation tests: influence of nebulizer output, particle size, and method of inhalation. *J. Allergy Clin. Immunol.* 67:156-161.
105. Ward, R. J., D. W. Reid, R. F. Leonard, D. P. Johns, and E. H. Walters. 1998. Nebulizer calibration using lithium chloride: an accurate, reproducible and user-friendly method. *Eur. Respir. J.* 11:937-941.
106. Massey, D. G., D. Miyachi, and G. F. Massey. 1982. Nebulizer function. *Bull. Eur. Physiopathol. Respir.* 18:665-671.
107. Hollie, M. C., R. A. Malone, R. M. Skufca, and H. S. Nelson. 1991. Extreme variability in aerosol output of the DeVilbiss 646 jet nebulizer. *Chest* 100: 1339-1344.
108. Mercer, T. T., R. F. Goddard, and R. L. Flores. 1969. Effect of auxiliary air flow on the output characteristics of compressed-air nebulizers. *Ann. Allergy* 27:211-217.
109. Merkus, P. J. F. M., E. E. M. van Essen-Zandvliet, E. Parlevliet, G. Borsboom, P. J. Sterk, K. F. Kerrebijn, and P. H. Quanjer. 1992. Changes of nebulizer output over the years. *Eur. Respir. J.* 5:488-491.
110. Nieminen, M. M., H. Holli, A. Lahdensuo, A. Muittari, and J. Karvonen. 1987. Aerosol deposition in automatic dosimeter nebulizer. *Eur. J. Respir. Dis.* 71:145-152.
111. Ferron, G. A., K. F. Kerrebijn, and J. Weber. 1976. Properties of aerosols produced with three nebulizers. *Am. Rev. Respir. Dis.* 114:899-908.
112. Sterk, P. J., A. Plomp, J. F. van de Vate, and P. H. Quanjer. 1984. Physical properties of aerosols produced by several jet and ultrasonic nebulizers. *Bull. Eur. Physiopathol. Respir.* 20:65-72.
113. Arossa, W., F. Quagliotti, M. Sala, S. Spinaci, and G. DeCandussio. 1984. Different performance of two commercial nebulizers. *Respiration* 46: 12X-132.
114. Dennis, J. H., A. J. Avery, E. H. Walters, and D. J. Hendrick. 1992. Calibration of aerosol output from the Mefar dosimeter: implications for epidemiological studies. *Eur. Respir. J.* 5:1279-1282.
115. Dolovich, M. B. 1985. Technical factors influencing response to challenge aerosols. In F. E. Hargreave and A. J. Woolcock, editors. *Airway Responsiveness: Measurement and Interpretation*. Astra Pharmaceuticals, Mississauga, ON, Canada.
116. Asher, M. I., J. M. Bertrand, P. H. Beaudry, and A. L. Coates. 1983. Histamine inhalation challenge in children—a comparison of two methods. *Ann. Allergy* 50:389-392.
117. Clay, M. M., D. Pavia, S. P. Newman, T. Lennard-Jones, and S. W. Clarke. 1983. Assessment of jet nebulizers for lung aerosol therapy. *Lancet* 2:592-594.
118. Newman, S. P., P. G. D. Pellow, M. M. Clay, and S. W. Clarke. 1985. Evaluation of jet nebulizers for use with gentamicin solution. *Thorax* 40:671-676.
119. Brannan, J. D., P. Subbarao, B. Ho, S. D. Anderson, H.-K. Chan, and A. L. Coates. 1999. Inhaled mannitol identifies methacholine responsive children with current asthma (abstract). *Am. J. Respir. Crit. Care Med.* 159:A911.
120. American Thoracic Society. 1995. Standardization of spirometry: 1994 update. *Am. J. Respir. Crit. Care Med.* 152:1107-1136.
121. Christopher, K. L., R. P. Wood, R. C. Eckert, F. B. Blager, R. A. Raney, and J. F. Souhrada. 1983. Vocal-cord dysfunction presenting as asthma. *N. Engl. J. Med.* 308:1566-1570.
122. Goldman, J., and M. Muers. 1991. Vocal cord dysfunction and wheezing. *Thorax* 46:401-404.
123. Bucca, C., G. Rolla, I. Brussino, V. DeRose, and M. Bugiani. 1995. Are asthma-like symptoms due to bronchial or extrathoracic airway dysfunction? *Lancet* 346:791-795.
124. Newman, K. B., U. G. Mason, and K. B. Schmalting. 1995. Clinical features of vocal cord dysfunction. *Am. J. Respir. Crit. Care Med.* 152: 1382-1386.
125. Cockcroft, D. W., and B. A. Barseheid. 1983. Measurement of responsiveness to inhaled histamine: comparison of FEV₁ and sGaw. *Ann. Allergy* 51:374-377.
126. Habib, M. P., P. D. Pare, and L. A. Engel. 1979. Variability of airways response to inhaled histamine in normal subjects. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 47:51-58.
127. Michoud, M. C., H. Ghezzi, and R. Amyot. 1982. A comparison of pulmonary function tests used for bronchial challenges. *Bull. Eur. Physiopathol. Respir.* 18:609-621.
128. Greenspon, L. W., and E. Gracely. 1992. A discriminant analysis applied to methacholine bronchoprovocation testing improves classification of patients as normal, asthma or COPD. *Chest* 102:1419-1425.
129. Goldstein, M. F., S. M. Pacana, D. J. Dvorin, and E. H. Dunsky. 1994. Retrospective analyses of methacholine inhalation challenges. *Chest* 105:1082-1088.
130. Kirby, J. G., E. F. Juniper, F. E. Gargreave, and N. Zamel. 1986. Total lung capacity does not change during methacholine-stimulated airway narrowing. *J. Appl. Physiol.* 61:2144-2147.
131. Wasserman, K., G. Pothoff, J. Bahra, and H. H. Hilger. 1992. Reversible volume changes of trapped gas in nonspecific bronchoprovocation tests. *Chest* 101:970-975.
132. Moore, B. J., L. M. Vergurt, G. G. King, and P. D. Pare. 1997. The effect of deep aspiration on methacholine dose-response curves in normal subjects. *Am. J. Respir. Crit. Care Med.* 156:1278-1281.
133. van Broekhoven, P., W. C. Hop, E. Rasser, J. C. de Jongste, and K. F. Kerrebijn. 1991. Comparison of FEV₁ and transcutaneous oxygen tension in the measurement of airways responsiveness to methacholine. *Pediatr. Pulmonol.* 11:254-258.
134. Phagoo, S. B., N. M. Wilson, and M. Silverman. 1992. Repeatability of methacholine challenge in asthmatic children measured by changes in transcutaneous oxygen tension. *Thorax* 47:804-808.
135. Fontana, G., S. Cardelicchio, G. Camiciottoli, P. Panuccio, and V. Boddi. 1993. Changes in transcutaneous oxygen partial pressure as an index of response to inhaled methacholine in asthmatic patients. *Chest* 103:1375-1380.
136. Wilson, N. M., P. Bridge, S. B. Phagoo, and M. Silverman. 1995. The measurement of methacholine responsiveness in 5 year old children: three methods compared. *Eur. Respir. J.* 8:364-370.
137. Klug, B., and H. Bisgaard. 1996. Measurement of lung function in awake 2-4 year old asthmatic children during methacholine challenge and acute asthma: a comparison of the impulse oscillation technique, the interrupter technique and transcutaneous measurement versus whole-body plethysmograph. *Pediatr. Pulmonol.* 21:290-300.
138. Dal Negro, R., and L. Allegra. 1989. Blood gas changes during and after nonspecific airway challenge in asthmatic and normal patients. *J. Appl. Physiol.* 67:2627-2630.
139. Parham, W. M., R. H. Shepard, P. S. Norman, and J. E. Fish. 1983. Analysis of the time course and magnitude of lung inflation effects on airway zone: relation to airway reactivity. *Am. Rev. Respir. Dis.* 128:240-245.
140. Malmberg, P., K. Larsson, B. M. Sundblad, and W. Zhiping. 1993. Importance of the time interval between FEV₁ measurements in a methacholine provocation test. *Eur. Respir. J.* 6:680-686.
141. Pellegrino, R., P. J. Sterk, J. K. Sont, and V. Brusasco. 1998. Assessing the effect of deep inhalation on airway calibre: a novel approach to lung function in bronchial asthma and COPD. *Eur. Respir. J.* 12:1219-1227.
142. Wheatley, J. R., P. D. Pare, and L. A. Engel. 1989. Reversibility of induced bronchoconstriction by deep inspiration in asthmatics and normal subjects. *Eur. Respir. J.* 2:331-339.
143. Skloot, G., S. Permutt, and A. Toggias. 1995. Airway responsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J. Clin. Invest.* 96:2393-2403.
144. Fish, J. E., and J. F. Kelley. 1979. Measurements of responsiveness in bronchoprovocation testing. *J. Allergy Clin. Immunol.* 64:592-596.
145. Meltzer, A. A., M. H. Smolensky, G. E. D'Alonzo, R. B. Harnist, and P. H. Scott. 1989. An assessment of peak respiratory flow as a surrogate measurement of FEV₁ in stable asthmatic children. *Chest* 96:329-333.
146. Berube, D., A. Cartier, J. L'Archeveque, H. Ghezzi, and J. L. Malo. 1991. Comparison of peak expiratory flow rate and FEV₁ in assessing bronchomotor tone after challenges with occupational sensitizers. *Chest* 99:831-836.
147. Paggiaro, P. L., G. Moscato, and D. Giannini. 1993. The Italian working

- group on the use of peak expiratory flow in asthma. *Eur. Respir. Rev.* 3:438-443.
148. Gaurin, D., L. C. D'Aquino, G. Gagnon, J. L. Malo, and A. Cartier. 1994. Comparison between PEF and FEV₁ in the monitoring of asthmatic subjects at an outpatient clinic. *Chest* 106:1419-1426.
 149. Gibbons, W. J., A. Sharma, D. Lougheed, and P. T. Macklem. 1996. Detection of excessive bronchoconstriction in asthma. *Am. J. Respir. Crit. Care Med.* 153:582-589.
 150. Cockcroft, D. W., K. Y. Murdock, and J. J. Mink. 1983. Determination of histamine PC₂₀: comparison of linear and logarithmic interpolation [Letter]. *Chest* 84:505-506.
 151. Verlato, G., I. Cerveri, A. Villani, M. Pasquetto, M. Ferrari, F. Fanfulla, E. Zanolin, B. Rijcken, and R. de Marco. 1996. Evaluation of methacholine dose-response curves by linear and exponential mathematical models: goodness of fit and validity of extrapolation. *Eur. Respir. J.* 9:506-511.
 152. Gilbert, R., and J. H. Auchincloss. 1990. Post-test probability of asthma following methacholine challenge. *Chest* 97:562-565.
 153. Cockcroft, D. W., K. Y. Murdock, B. A. Berscheid, and B. P. Gore. 1992. Sensitivity and specificity of histamine PC-20 determination in a random selection of young college students. *J. Allergy Clin. Immunol.* 89:23-30.
 154. Salome, C. M., W. Xuan, E. J. Gray, E. Belousova, and J. K. Peat. 1997. Perception of airway narrowing in a general population sample. *Eur. Respir. J.* 10:1052-1058.
 155. Hopp, R. J., R. G. Townley, R. E. Biven, A. K. Bewtra, and N. M. Nair. 1990. The presence of airway reactivity before the development of asthma. *Am. Rev. Respir. Dis.* 141:2-8.
 156. Jansen, D. F., W. Timens, J. Kraan, B. Rijcken, and D. S. Postma. 1997. (A)symptomatic bronchial hyper-responsiveness and asthma. *Respir. Med.* 91:121-134.
 157. Zhong, N. S., R. C. Chen, M. O. Yang, Z. Y. Wu, J. P. Zheng, and Y. F. Li. 1992. Is asymptomatic bronchial hyperresponsiveness an indication of potential asthma? A two year follow-up of young students with bronchial hyperresponsiveness. *Chest* 102:1104-1109.
 158. Laprise, C., and L. P. Boulet. 1997. Asymptomatic airway hyperresponsiveness: a three year follow-up. *Am. J. Respir. Crit. Care Med.* 156:403-409.
 159. Woolcock, A. J., J. K. Peat, C. M. Salome, K. Yan, S. D. Anderson, R. E. Schoeffel, G. McCowage, and T. Killalea. 1987. Prevalence of bronchial hyperresponsiveness and asthma in a rural adult population. *Thorax* 42:361-368.
 160. Josephs, L. K., I. Gregg, M. A. Mullee, and S. T. Holgate. 1989. Nonspecific bronchial reactivity and its relationship to the clinical expression of asthma: a longitudinal study. *Am. Rev. Respir. Dis.* 140:350-357.
 161. Chhabra, S. K., S. N. Gaur, and A. K. Khanna. 1989. Clinical significance of nonspecific bronchial hyperresponsiveness in asthma. *Chest* 96:596-600.
 162. Avital, A., N. Noviski, E. Bar-Yishay, C. Springer, M. Levy, and S. Godfrey. 1991. Nonspecific bronchial reactivity in asthmatic children depends on severity but not on age. *Am. Rev. Respir. Dis.* 144:36-38.
 163. Ulrik, C. S. 1996. Factors associated with increased bronchial responsiveness in adolescents and young adults: the importance of adjustment for prechallenge FEV₁. *J. Allergy Clin. Immunol.* 97:761-767.
 164. Griner, P. F., R. J. Mayewsky, A. I. Mushlin, and P. Greenland. 1981. Selection and interpretation of diagnostic tests and procedures: principles and applications. *Ann. Intern. Med.* 94(Pt.2):557-592.
 165. Sox, H. C. 1986. Probability theory in the use of diagnostic tests: an introduction to critical study of the literature. *Ann. Intern. Med.* 104:60-66.
 166. Popa, V., and J. Singleton. 1988. Provocation dose and discriminant analysis in histamine bronchoprovocation: are the current predictive data satisfactory? *Chest* 94:466-475.
 167. Lebowitz, M. D., and S. Spinaci. 1993. The epidemiology of asthma. *Eur. Respir. Rev.* 3:41 S-423.
 168. Weiss, K. B., P. J. Gergen, and T. A. Hodgson. 1992. An economic evaluation of asthma in the United States. *N. Engl. J. Med.* 326:862-866.
 169. Rijcken, B., J. P. Schouten, S. T. Weiss, B. Rosner, K. De Vries, and R. Van der Lende. 1993. Long term-variability of bronchial responsiveness to histamine in a random population sample of adults. *Am. Rev. Respir. Dis.* 148:944-949.
 170. Townley, R. G., U. Y. Ryo, B. M. Kolotkin, and B. Kang. 1975. Bronchial sensitivity to methacholine in current and former asthmatics and allergic rhinitis patients and control subjects. *J. Allergy Clin. Immunol.* 56:429-442.
 171. Braman, S. S., A. A. Barrows, B. A. DeCottis, G. A. Settipane, and W. M. Corrao. 1987. Airway responsiveness in allergic rhinitis: a risk factor for asthma. *Chest* 91:671-674.
 172. Nieminen, M. M. 1992. Unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients. *Chest* 102:1537-1543.
 173. Boulet, L. P., A. Cartier, N. C. Thomson, R. S. Roberts, J. Dolovich, and F. E. Hargreave. 1983. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J. Allergy Clin. Immunol.* 71:399-406.
 174. Platts-Mills, T. A., E. R. Tovey, E. B. Mitchell, H. Moszoro, P. Nock, and S. R. Wilkins. 1982. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 2:675-678.
 175. Hargreave, F. E., E. H. Ramsale, and S. O. Pugsley. 1984. Occupational asthma without bronchial hypo-responsiveness. *Am. Rev. Respir. Dis.* 130:513-515.
 176. Banks, D. E., J. Sastre, B. T. Butcher, E. Ellis, R. J. Rando, H. W. Barkman, Y. Y. Hammad, H. W. Glindmeyer, and H. Weill. 1989. Role of inhalation challenge testing in the diagnosis of isocyanate-induced asthma. *Chest* 95:414-423.
 177. Dehaut, P., A. Rachiele, R. R. Martin, and J. L. Malo. 1983. Histamine dose-response curves in asthma: reproducibility and sensitivity of different indices to assess response. *Thorax* 38:516-522.
 178. Chinn, S., J. R. Britton, P. G. J. Burney, A. E. Tattersfield, and A. O. Papacosta. 1987. Estimation and repeatability of the response to inhaled histamine in a community survey. *Thorax* 42:45-52.
 179. Weiss, M. E., B. Wheeler, P. Eggleston, and N. F. Adkinson. 1989. A protocol for performing reproducible methacholine inhalation tests in children with moderate to severe asthma. *Am. Rev. Respir. Dis.* 139:67-72.
 180. Weeke, B., F. Madsen, and L. Frolund. 1987. Reproducibility of challenge tests at different times [Review]. *Chest* 91(Suppl. 6):83S-89S.
 181. Boulet, L. P., D. Morin, and H. Turcotte. 1990. Variations of airway responsiveness to methacholine and exercise in asthmatic and normal subjects over a 12 month period. *Clin. Invest. Med.* 13:60-66.
 182. Balzano, G., I. D. Carri, C. Gallo, G. Cocco, and G. Melillo. 1989. Intra-subject between-day variability of PD₂₀ methacholine assessed by the dosimeter inhalation test. *Chest* 95:1239-1243.
 183. Josephs, L. K., I. Gregg, M. A. Mullee, M. J. Campbell, and S. T. Holgate. 1992. A longitudinal study of baseline FEV₁ and bronchial responsiveness in patients with asthma. *Eur. Respir. J.* 5:32-39.
 184. Stevens, W. H., P. J. Manning, R. M. Watson, and P. M. O'Byrne. 1990. Tachyphylaxis to inhaled methacholine in normal but not asthmatic subjects. *J. Appl. Physiol.* 69:875-879.
 185. Beckett, W. S., M. E. Marenberg, and P. E. Pace. 1992. Repeated methacholine challenge produces tolerance in normal but not in asthmatic subjects. *Chest* 102:775-779.
 186. Chen, W. Y., and D. J. Horton. 1977. Heat and water loss from the airways and exercise-induced asthma. *Respiration* 34:305-313.
 187. Strauss, R. H., E. R. McFadden, R. H. Ingram, E. C. Deal, and J. J. Jaeger. 1978. Influence of heat and humidity on the airway obstruction induced by exercise in asthma. *J. Clin. Invest.* 61:433-440.
 188. McFadden, E. R., and R. H. Ingram. 1979. Exercise-induced asthma. Observations on the initiating stimulus. *N. Engl. J. Med.* 301:763-769.
 189. Anderson, S. D., R. E. Schoeffel, R. Follet, C. P. Perry, E. Daviskas, and M. Kendall. 1982. Sensitivity to heat and water loss at rest and during exercise in asthmatic patients. *Eur. J. Respir. Dis.* 63:459-471.
 190. McFadden, E. R. 1987. Exercise-induced asthma: assessment of current etiologic concepts. *Chest* 91:151S-157S.
 191. Gilbert, I. A., and E. R. McFadden. 1992. Airway cooling and rewarming: the second reaction sequence in exercise-induced asthma. *J. Clin. Invest.* 90:699-704.
 192. Anderson, S. D., R. E. Schoeffel, J. L. Black, and E. Daviskas. 1985. Airway cooling as the stimulus to exercise-induced asthma—a reevaluation. *Eur. J. Respir. Dis.* 67:20-30.
 193. Anderson, S. D. 1984. Is there a unifying hypothesis for exercise-induced asthma? *J. Allergy Clin. Immunol.* 73:660-665.
 194. Anderson, S. D., and E. Daviskas. 1992. The airway microvasculature and exercise induced asthma. *Thorax* 47:748-752.
 195. Brannan, J. D., H. Koskela, S. D. Anderson, and N. Chew. 1999. Responsiveness to mannitol in asthmatic subjects with exercise and hyperventilation-induced asthma. *Am. J. Respir. Crit. Care Med.* 158:1120-1126.
 196. Deal, E. C., E. R. McFadden, R. H. Ingram, R. H. Strauss, and J. J. Jaeger. 1979. Role of respiratory heat exchange in production of exercise-induced asthma. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* 46:467-475.
 197. Hahn, A., S. D. Anderson, A. R. Morton, J. L. Black, and K. D. Fitch. 1984. A reinterpretation of the effect of temperature and water content of the inspired air in exercise-induced asthma. *Am. Rev. Respir. Dis.* 130:575-579.
 198. Argyros, G. J., Y. Y. Phillips, D. B. Rayburn, R. R. Rosenthal, and J. J.

- Jaeger. IYY3. Water loss without heat flux in exercise-induced bronchospasm. *Am. Rev. Respir. Dis.* 147:1419-1424.
199. Smith, C. M., S. D. Anderson, S. Walsh, and M. McElrea. 1989. An investigation of the effects of heat and water exchange in the recovery period after exercise in children with asthma. *Am. Rev. Respir. Dis.* 140:598-605.
200. Finnerty, J. P., and S. T. Holgate. 1990. Evidence for the roles of histamine and prostaglandins as mediators in exercise-induced asthma: the inhibitory effect of terfenadine and flurbiprofen alone and in combination. *Eur. Respir. J.* 3:540-547.
201. Randolph, C. 1997. Exercise-induced asthma: update on pathophysiology, clinical diagnosis, and treatment. *Curr. Probl. Pediatr.* 27:53-77.
202. Reiss, T. F., J. B. Hill, E. Harman, J. Zhang, W. K. Tanaka, E. Bronsky, D. Guerreiro, and L. Hendeles. 1997. Increased urinary excretion of LTE₄ after exercise and attenuation of exercise-induced bronchospasms by montelukast, a cysteinyl leukotriene receptor antagonist. *Thorax* 52:1030-1035.
203. Leff, J. A., W. W. Busse, D. Pearlman, E. A. Bronsky, J. Kemp, L. Hendeles, R. Dockhorn, S. Kundu, J. Zhang, B. C. Seidenberg, and T. F. Reiss. 1998. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N. Engl. J. Med.* 339:147-152.
204. Nish, W. A., and L. A. Schwietz. 1992. Underdiagnosis of asthma in young adults presenting for USAF basic training. *Ann. Allergy* 69:239-242.
205. Sinclair, D. G., M. M. Sims, N. A. Hoad, and C. R. Winfield. 1995. Exercise-induced airway narrowing in army recruits with a history of childhood asthma. *Eur. Respir. J.* 8:1314-1317.
206. Anderson, S. D. 1975. Exercise-induced asthma: the state of the art. *Chest* 87(Suppl. 5):191S-197S.
207. Godfrey, S., M. Silverman, and S. D. Anderson. 1973. Problems of interpreting exercise-induced asthma. *J. Allergy Clin. Immunol.* 52:199-209.
208. Edmunds, A. T., M. Tooley, and S. Godfrey. 1978. The refractory period after exercise-induced asthma: its duration and relation to the severity of exercise. *Am. Rev. Respir. Dis.* 117:247-254.
209. Stearns, D. R., E. R. McFadden, F. J. Breslin, and R. H. Ingram. 1981. Reanalysis of the refractory period in exertional asthma. *J. Appl. Physiol. Environ. Exercise Physiol.* 50:503-508.
210. Eggleston, P. A., R. R. Rosenthal, S. D. Anderson, R. Anderton, C. W. Bierman, E. R. Blecker, H. Chai, G. J. Cropp, J. D. Johnson, P. Konig, J. Morse, L. J. Smith, R. J. Summers, and J. J. Trautlein. 1979. Guidelines for the methodology of exercise challenge testing of asthmatics. *J. Allergy Clin. Immunol.* 64:642-645.
211. Shturman-Elistein, R., R. J. Zeballos, J. M. Buckley, and J. F. Souhrada. 1978. The beneficial effect of nasal breathing on exercise-induced bronchoconstriction. *Am. Rev. Respir. Dis.* 118:65-73.
212. Phillips, Y. Y., J. J. Jaeger, B. L. Laube, and R. R. Rosenthal. 1985. Eucapnic voluntary hyperventilation of compressed gas mixture: a simple system for bronchial challenge by respiratory heat loss. *Am. Rev. Respir. Dis.* 131:31-35.
213. König, P. 1989. Exercise challenge: indications and techniques. *Allergy Proc.* 10:345-348.
214. Anderson, S. D., L. T. Rodwell, J. Du Toit, and I. H. Young. 1991. Duration of protection by inhaled salmeterol in exercise-induced asthma. *Chest* 100:1254-1260.
215. Eggleston, P. A., and J. L. Guerrant. 1976. A standardized method of evaluating exercise-induced asthma. *J. Allergy Clin. Immunol.* 58:414-425.
216. Vathenen, A. S., A. J. Knox, A. Wisniewski, and A. E. Tattersfield. 1991. Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and eucapnic dry air hyperventilation in patients with asthma. *Thorax* 46:811-816.
217. Ahmed, T., J. Garrigo, and I. Danta. 1993. Preventing bronchoconstriction in exercise-induced asthma with inhaled heparin. *N. Engl. J. Med.* 329:90-95.
218. Woolley, M., S. D. Anderson, and B. M. Quigley. 1990. Duration of protective effect of terbutaline sulfate and cromolyn sodium alone and in combination on exercise-induced asthma. *Chest* 97:39-45.
219. ERS Task Force on Standardization of Clinical Exercise Testing. 1997. Clinical exercise testing with reference to lung diseases: indications, standardization and interpretation strategies. *Eur. Respir. J.* 10:2662-2689.
220. Givoni, B., and R. F. Goldman. 1971. Predicting metabolic energy cost. *J. Appl. Physiol.* 30:429-433.
221. Silverman, M., and S. D. Anderson. 1972. Standardization of exercise tests in asthmatic children. *Arch. Dis. Child.* 47:882-889.
222. Jones, N. L. 1997. Clinical Exercise Testing, 4th ed. W.B. Saunders, Philadelphia.
223. Eggleston, P. A. 1984. Methods of exercise challenge. *J. Allergy Clin. Immunol.* 73:666-669.
224. Ries, A. L., J. T. Farrow, and J. L. Clausen. 1985. Accuracy of two ear oximeters at rest and during exercise in pulmonary patients. *Am. Rev. Respir. Dis.* 132:685-689.
225. Hansen, J. E., and R. Casaburi. 1987. Validity of ear oximetry in clinical exercise testing. *Chest* 91:333-337.
226. Anderson, S. D., M. Silverman, P. König, and S. Godfrey. 1975. Exercise-induced asthma. *Br. J. Dis. Chest* 69:1-39.
227. Cropp, G. J. 1979. The exercise bronchoprovocation test: standardization of procedures and evaluation of response. *J. Allergy Clin. Immunol.* 64:627-633.
228. Anderson, S. D. 1993. Diagnosis and management of exercise-induced asthma. In M. E. Gershwin and G. M. Halpern, editors. *Bronchial Asthma: Principles of Diagnosis and Practice*, 3rd ed. Humana Press, Clifton, NJ. 513-547.
229. Anderson, S. D., N. M. Connolly, and S. Godfrey. 1971. Comparison of bronchoconstriction induced by cycling and running. *Thorax* 26:396-401.
230. Eggleston, P. A. 1975. The cycloergometer as a system for studying exercise-induced asthma. *Pediatrics* 56(Suppl.):899-903.
231. Wasserman, K., J. E. Hansen, D. Y. Sue, B. J. Whipp, and R. Casaburi. 1994. Principles of Exercise Testing and Interpretation. Lea & Febiger, Philadelphia.
232. Custovic, A., N. Arifhodzic, A. Robinson, and A. Woodcock. 1994. Exercise testing revisited: the response to exercise in normal and atopic children. *Chest* 105:1127-1132.
233. Haby, M. M., S. D. Anderson, J. K. Peat, C. M. Mellis, B. G. Toelle, and A. J. Woolcock. 1994. An exercise challenge protocol for epidemiological studies of asthma in children: comparison with histamine challenge. *Eur. Respir. J.* 7:43-49.
234. Heaman, D. J., and J. Estes. 1997. The free-running asthma screening test: an approach to screening for exercise-induced asthma in rural Alabama. *J. Sch. Health* 67:X3-88.
235. Helenius, I. J., H. O. Tikkanen, and T. Hahtela. 1998. Occurrence of exercise induced bronchospasm in elite runners: dependence on atopy and exposure to cold air and pollen. *Br. J. Sports Med.* 32:125-129.
236. Eliasson, A. H., Y. Y. Phillips, K. R. Rajagopal, and R. S. Howard. 1992. Sensitivity and specificity of bronchial provocation testing: an evaluation of four techniques in exercise-induced bronchospasm. *Chest* 102:347-355.
237. O' Cain, C. F., N. B. Dowling, A. S. Slutsky, M. J. Hensley, K. P. Strohl, E. R. McFadden, and R. H. Ingram. 1980. Airway effects of respiratory heat loss in normal subjects. *J. Appl. Physiol. Environ. Exercise Physiol.* 49:875-880.
238. Backer, V., and C. S. Ulrik. 1992. Bronchial responsiveness to exercise in a random sample of 494 children and adolescents from Copenhagen. *Clin. Exp. Allergy* 22:741-747.
239. Brudno, D. S., J. M. Wagner, and N. T. Rupp. 1994. Length of postexercise assessment in the determination of exercise-induced bronchospasm. *Ann. Allergy* 73:227-231.
240. Cropp, G. J. 1975. Relative sensitivity of different pulmonary function tests in the evaluation of exercise-induced asthma. *Pediatrics* 56(Suppl.):860-867.
241. Godfrey, S., C. Springer, N. Noviski, C. Maayan, and A. Avital. 1991. Exercise but not methacholine differentiates asthma from chronic lung disease in children. *Thorax* 46:488-492.
242. Kattan, M., T. G. Keens, C. M. Mellis, and H. Levison. 1978. The response to exercise in normal and asthmatic children. *J. Pediatr.* 92:718-721.
243. Bittleman, D. B., R. J. Smith, and J. M. Weiler. 1994. Abnormal movement of the arytenoid region during exercise presenting as exercise-induced asthma in an adolescent athlete. *Chest* 106:615-616.
244. McFadden, E. R., and D. K. Zawadski. 1996. Vocal cord dysfunction masquerading as exercise induced asthma: a physiologic cause for choking during athletic activities. *Am. J. Respir. Crit. Care Med.* 153:942-947.
245. Acres, J. C., and M. H. Kryger. 1981. Clinical significance of pulmonary function tests. Upper airway obstruction. *Chest* 80:207-211.
246. Tenholder, M. F., R. J. Moser, J. C. Koval, L. L. Bennett, and K. R. Rajagopal. 1987. The flow volume loop in upper airway obstruction masquerading as asthma. *Immunol. Allergy Pract.* 9:33-43.

APPENDIX A
 SAMPLE METHACHOLINE CHALLENGE TEST CONSENT FORM

PROCEDURE. The purpose of a methacholine challenge test is to determine the amount of airway irritability of a patient. You (or your child) will be asked to inhale a mist that contains different concentrations of methacholine. The mist is produced by a device called a nebulizer and inhaled through a mouthpiece or facemask. Before the test begins, and after each period of inhalation, you or your child will be asked to blow forcefully into a spirometer. The test usually takes about an hour.

DISCOMFORTS AND RISKS. This test does not cause an asthma attack but the inhalation of aerosols may be associated with mild shortness of breath, cough, chest tightness, wheezing, chest soreness, or headache. Many subjects do not have any symptoms at all. These symptoms (if they occur) are mild, last for only a few minutes, and disappear following the inhalation of a bronchodilator medication. There is a very small possibility of severe narrowing of your airways. This could cause severe shortness of breath. If this occurs, you will be immediately treated.

I have read the above information and understand the purpose of the test and the associated risks. With this knowledge I agree to having this test performed on me or my child.

Patient or Guardian	Date
Witness	Date

APPENDIX B
 SAMPLE METHACHOLINE CHALLENGE PRETEST QUESTIONNAIRE

Name: _____ Date of birth: _____

1. List all medications you have taken in the last 48 hours for asthma, hay fever, heart disease, blood pressure, allergies, or stomach problems, and the number of hours or days since your last dose for each medication.

Drug	Date and time of last treatment
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

- | | | |
|---|-----|----|
| 2. Has a physician told you that you have asthma? | Yes | No |
| 3. Have you ever been hospitalized for asthma? | Yes | No |
| 4. Did you have respiratory disease as a child? | Yes | No |
| 5. Have you ever experienced asthma symptoms such as wheezing, chest tightness, or shortness of breath within the last two weeks? | Yes | No |
| 6. If a smoker, when did you last smoke? _____ | | |
| 7. Have you had a respiratory infection in the last 6 weeks? | Yes | No |
| 8. Have you had a heart attack or stroke within the last three months? | Yes | No |
| 9. Do you have high blood pressure? | Yes | No |
| 10. Do you have an aortic aneurysm? | Yes | No |
| 11. Are you pregnant? | Yes | No |

APPENDIX C

SAMPLE **METHACHOLINE** CHALLENGE TEST REPORT FORM

Laboratory name, address, and phone number:
 Patient name, ID number, date of birth, sex, height:
 Test date, technician initials:
 Indication for the test (e.g., rule out asthma):
 Test method (e.g., five-breath dosimeter):
 Bronchodilator administered and dose:

Time	Test Phase	FEV ₁	QC Score	dFEV ₁ (% of baseline)
9:00	Baseline	3.10	A	
9:10	Diluent	3.00	B	100%
9:15	0.06 mg/ml	3.05	A	102%
9:20	0.25 mg/ml	2.94	C	98%
9:25	1.0 mg/ml	2.62	A	87%
9:30	4.0 mg/ml	2.16	A	72%
9:45	BD recovery	3.20	B	107%

(display flow-volume or volume-time curve)

Bronchial responsiveness: PC₂₀ 1.9 mg/ml (insert or attach a dose-response curve)
 Signs and symptoms after final dose: Chest tightness, wheezing.
 Symptoms completely resolved after bronchodilator (BD)
 Interpretation: Test quality is acceptable
 Mild bronchial hyperresponsiveness

Medical director's signature: _____

APPENDIX D
 EQUIPMENT SOURCES*

Company	Equipment
DeVilbiss Health Care, Inc. P.O. Box 635 Somerset, PA 15501-0635 (814) 443-4881	DeVilbiss 646 nebulizers
Mallinckrodt 675 McDonnell Blvd. Hazelwood, MO 63042 (800) 635-5267 http://mallinckrodt.com	Nebulizers Filters
Marquest Medical Products 11039 E. Lansing Circle Englewood, CO 80112 (303) 790-4835 http://www.marquestmedical.com	Filters
Matheson Tri-Gas 166 Keystone Drive Montgomeryville, PA 18936 http://www.mathesongas.com	Gas meters Rotameters
Methapharm Inc. 131 Clarence St. Brantford, Ontario N3T2V6, Canada (800) 287-7686 http://www.metapharm.com	Methocholine (Provocholine)
Pall Biomedical Products Corporation 2200 Northern Blvd. East Hills, NY 11548 http://www.pall.com	Filters
PDS Instrumentation 908 Main Street Louisville, CO 80027 (303) 666-8100 http://www.pulmonarydata.com	Dosimeters Methocholine (Provocholine) DeVilbiss 646 nebulizers, output characterized with a flow regulator Filters
Roxon Medi-Tech a500 Lafrenaie Montreal, Quebec H1P 284, Canada (514) 326-7780 http://www.biomed.nicolet.com	English Wright nebulizer

* This list is provided to simplify access to some sources of equipment. It is not a complete list of all possible sources of acceptable equipment.