Official Journal of the Asian Pacific Society of Respirology
Respirology

ORIGINAL ARTICLE

Diagnostic properties of the methacholine and mannitol bronchial challenge tests: A comparison study

MIN-HYE KIM,[1,2]* WOO-JUNG SONG,[1]* TAE-WAN KIM,[1] HYUN-JUNG JIN,[3] YOU-SEOB SIN,[3] YOUNG-MIN YE,[3] SANG-HEON KIM,[4] HEUNG-WOO PARK,[1] BYUNG-JAE LEE,[5] HAE-SIM PARK,[3] HO-JOO YOON,[4] DONG-CHULL CHOI,[5] KYUNG-UP MIN[1] AND SANG-HEON CHO[1]

[1]*Department of Internal Medicine, Seoul National University College of Medicine,* [2]*Department of Internal Medicine, Ewha Womans University School of Medicine,* [3]*Department of Internal Medicine, Sungkyunkwan University School of Medicine,* [5]*Department of Internal Medicine, Hanyang University College of Medicine, Seoul and* [4]*Department of Internal Medicine, Ajou University School of Medicine, Suwon, Korea*

ABSTRACT

Background and objective: **Airway hyperresponsiveness is a common feature of asthma. Methacholine and mannitol are two representative agonists for bronchial challenge. They have theoretically different mechanisms of action, and may have different diagnostic properties. However, their difference has not been directly evaluated among Korean adults. In this study, we compare the diagnostic properties of methacholine and mannitol bronchial provocation tests.**

Methods: **Asthmatic patients and non-asthmatic controls were recruited prospectively from four referral hospitals in Korea. Participants were challenged with each of methacholine and mannitol inhalation on different days. Their diagnostic utility was evaluated by calculating their sensitivity and specificity for asthma diagnosis. Response–dose ratio was also compared.**

Results: **A total of 50 asthmatic adults and 54 controls were enrolled (mean age 43.8 years). The sensitivity and specificity of mannitol challenge (defined by a PD15 of <635 mg) were 48.0% and 92.6%, respectively, whereas those of methacholine (defined by a PC20 of <16 mg/mL) were 42.0% and 98.1%, respectively. Twenty asthmatic participants (24%) showed positive response to a single agonist only. In the receiver operating curve analyses using response–dose ratio values, area under the curve was 0.77 (95% confidence interval (CI): 0.68–0.86) for mannitol, and 0.89 (95% CI: 0.83–0.95) for methacholine. The correlations between log- transformed mannitol and methacholine response–dose ratios were significant but moderate ($r = 0.683$, $P < 0.001$).**

Conclusions: **The present study demonstrated overall similar diagnostic properties of two diagnostic tests, but also suggested their intercomplementary roles for asthma.**

The clinical trial registration number at ClinicalTrial.gov is NCT02104284.

SUMMARY AT A GLANCE

Methacholine and mannitol bronchial challenge are two representative tests for asthma. However, their difference has not been directly evaluated among Korean adults. We compared the diagnostic properties of these two tests. Two diagnostic tests showed similar diagnostic properties, but also suggested their intercomplementary roles for asthma.

Key words: bronchial provocation test, mannitol, methacholine, sensitivity, specificity.

Abbreviations: AHR, airway hyperresponsiveness; AUC, area under the curve; BMI, body mass index; CI, confidence interval; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; RDR, response–dose ratio; ROC, receiver operating characteristic.

INTRODUCTION

Airway hyperresponsiveness (AHR) is a common feature of asthma. It is defined as the hypersensitivity of airway contraction to provoking stimuli. Bronchial challenge tests are utilized to measure AHR. The history of using a direct agonist acetylcholine for this purpose began in the 1940s.[1]

Bronchial challenge tests are classified into direct and indirect methods according to their mechanisms.[2,3] Direct challenges tests utilize muscarinic analogues, histamine, leukotrienes or prostaglandins that directly stimulate airway smooth muscle

Correspondence: Sang-Heon Cho, Department of Internal Medicine, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 110-744, Korea. Email: shcho@snu.ac.kr

Conflict of interest statement: This study was funded by BL&H Co., Ltd. Aridol™. Mannitol was provided by BL&H Co., Ltd., South Korea. BL&H had no influence on data interpretation or writing the manuscript.

*M-HK and W-JS equally contributed to this article.

Received 30 December 2013; invited to revise 28 January 2014; revised 19 February 2014; accepted 10 March 2014 (Associate Editor: Giorgio Piacentini).

Article first published online: 26 June 2014

receptors.[2] Indirect tests induce airway contraction through intermediate pathways to induce mediator release from local inflammatory cells.[3] Mannitol, hypertonic saline, adenosine monophosphate or exercise are indirect stimulators.[3]

At present, methacholine and mannitol are the most commonly used agonists in direct and indirect challenge tests, respectively. Because these two tests theoretically have different mechanisms of action, they are considered to have different diagnostic properties. The most important difference is that the indirect mannitol test is dependent on the asthmatic airway inflammation status, whereas the direct methacholine test is less dependent. In addition, methacholine AHR may be seen in other conditions, such as chronic obstructive pulmonary disease, congestive heart failure or rhinitis.[4] Therefore, the methacholine test has been suggested for asthma screening for its high sensitivity, whereas the mannitol test may be useful in confirming asthma for high specificity. To date, several studies have compared the utility of methacholine and mannitol tests in various populations;[1,5–10] however, to our knowledge, there has been no prospective study among Korean adults. The present study compared the diagnostic properties of methacholine and mannitol bronchial challenge tests for asthma in a Korean adult population.

METHODS

Study design

This study was a prospective, multicentre study performed at four referral hospitals in Korea: Seoul National University Hospital, Samsung Medical Center, Ajou University Hospital and Hanyang University Medical Center. Hospital-based recruitment was performed for asthmatic patients and non-asthmatic controls. Asthmatic patients were defined as those who had been diagnosed with asthma by specialist physicians; they had recurrent symptoms of asthma (wheezing and dyspnea) and used anti-asthmatic medication for 6 months before the enrollment. Non-asthmatic controls were voluntarily recruited from hospital visitors; they were included if they had never had wheeze or physician-diagnosed asthma.

Korean adults between the age of 18 and 70 years who could understand and perform lung function tests and bronchial challenges were included. Exclusion criteria included history of recent respiratory infection (within the last 4 weeks), history of a recent surgery, history of heart disease that could impose risks during bronchial challenges, history of uncontrolled hypertension, current smokers or ex-smokers with more than 10 pack-years, history of known pulmonary diseases with the exception of asthma, pregnancy or lactation, severe obesity (body mass index (BMI) of >35 kg/m^2), history of any health condition considered inappropriate for participation in this study and a pre-bronchodilator predictive value of forced expiratory volume in 1 s (FEV_1) of <70%.

All participants underwent methacholine bronchial challenge test and mannitol challenge test on different days, separated by least 24 h, as in previous reports.[7,10] All asthmatic patients stopped their anti-asthma medications before the tests according to the predetermined protocols (Table S1). The protocol was approved by the Institutional Review Board of Seoul National University Hospital. All subjects gave written informed consent.

Methacholine challenge test

The methacholine challenge test was performed as previously described.[11,12] Pulmonary function testing was carried out using a spirometry system (SensorMedics 2130; SensorMedics, Yorba Linda, CA, USA). The methacholine challenge test was performed using the Chai method[13] with minor modifications. Briefly, methacholine was prepared at the following concentrations, diluted with saline: 0.25, 0.625, 1, 4, 16, and 25 mg/mL. Methacholine was delivered as an aerosol by a Rosenthal-French dosimeter (Laboratory for Applied Immunology, Inc., Baltimore, MD, USA) and a nebulizer. Subjects were instructed to inhale five inspiratory capacity breaths while increasing the methacholine concentration from 0.25 to 25 mg/mL. The methacholine concentration that caused a 20% decrease in the FEV_1 from baseline was defined as PC20. AHR was defined as positive at PC20 < 16 mg/mL.

Mannitol challenge test

A commercial mannitol (Aridol™; BL&H Co., Ltd., Seoul, South Korea) kit was used, and the challenge test was performed according to the manufacturer's protocols.[14] The mannitol capsule dose started at 0 mg and increased to a total cumulative dose of 635 mg. Each capsule was placed in the inhalation device, and a hole was made by pressing the device button before inhalation. After a deep breath of mannitol, the FEV_1 was measured after 60 s. The test was considered positive if the FEV_1 value decreased by more than 15% compared with the baseline FEV_1. The cumulative mannitol dose that caused a 15% decrease in the FEV_1 from baseline was defined as PD15. If the FEV_1 did not decline by more than 15%, the dose was increased until a cumulative dose of 635 mg was reached. If the FEV_1 did not fall by more than 15% until the last dose, the test was considered negative.

Statistical analysis

Descriptive data are expressed as means ± standard deviation or as percentages. Comparisons between groups were performed by Student's *t*-test, the Mann–Whitney test, the chi-square test or Fisher's exact test. The diagnostic properties of the mannitol challenge test and methacholine challenge test (sensitivity, specificity, positive predictive value and negative predictive value) were calculated against the diagnosis of asthma. In addition, response–dose ratio (RDR) was calculated by percentage of reduced FEV_1 at the last dose divided by cumulative dose. Pearson correlation tests were performed for the relationships between log-transformed RDRs of both results. Receiver operating characteristic (ROC) curve was configured and

Table 1 Characteristics of the study participants

	Asthma ($n = 50$)	Control ($n = 54$)	*P*-value*
Age (years)	46.4 ± 14.1	41.4 ± 16.0	0.090
Sex (%, female)	66.0	75.9	0.264
BMI (kg/m^2)	22.1 ± 3.0	21.9 ± 3.0	0.783
FEV_1 (% pred)	91.2 ± 12.2	95.0 ± 19.4	0.255
FVC (% pred)	95.6 ± 12.8	93.9 ± 9.3	0.457
FEV_1/FVC ratio (%)	81.5 ± 9.4	89.8 ± 8.7	<0.001
Underlying diseases, *n* (%)			
Hypertension	7 (14.0)	6 (11.1)	0.770
Diabetes	2 (4.0)	1 (1.9)	0.607
Liver disease	1 (2.0)	0 (0.0)	0.481
Other allergic diseases, *n* (%)			
Allergic rhinitis	33 (66.0)	8 (14.8)	<0.001
Atopic dermatitis	9 (18.0)	0 (0.0)	0.001
Allergic conjunctivitis	11 (22.0)	1 (1.9)	0.001

* *P*-values were determined by Student's *t*-test or the chi-square test.

BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; pred, predicted value.

Table 2 Outcomes of methacholine and mannitol challenge tests in asthmatics and controls

		Asthma ($n = 50$)		Control ($n = 54$)	
		Methacholine		Methacholine	
		+	−	+	−
Mannitol	+	17	7	1	3
	−	5	21	0	50
P-value*		<0.001		0.074	

* *P*-values were determined by the chi-square test or Fisher's exact test to identify differences between the methacholine and mannitol challenge tests.

Methacholine test was defined as positive if PC20 < 16 mg/mL. Mannitol test was defined as positive if PD15 < 635 mg/mL.

Table 3 Diagnostic properties of the methacholine and mannitol challenge tests

	Methacholine	Mannitol
Sensitivity (%)	44.0	48.0
Specificity (%)	98.1	92.6
Positive predictive value (%)	95.7	85.7
Negative predictive value (%)	65.4	65.8
Youden's index†	0.42	0.41

† Youden's index = sensitivity + specificity − 1.

area under the curve (AUC) value was compared. The *P*-value was considered to indicate statistical significance at <0.05. Statistical analysis was performed using Stata package (release 12.0; StataCorp, College Station, TX, USA).

RESULTS

Baseline characteristics

A total of 104 subjects (50 patients and 54 controls) were enrolled. The mean age was 43.8 years, and 71.2% were female. No significant difference was found in age, sex or BMI between the asthma and control groups (Table 1). The FEV_1/FVC ratio was significantly lower in patients than in controls (81.5% vs 89.8%, respectively; $P < 0.001$), whereas the FEV_1 and FVC were not. Comorbid allergic conditions such as allergic rhinitis, conjunctivitis or atopic dermatitis were more frequent in patients than in controls.

Diagnostic properties of methacholine and mannitol challenge tests

Diagnostic properties of both tests were summarized in Table 2. Methacholine challenge tests showed positivity in 44% of patients (22/50) and 1.9% of controls (1/54), whereas mannitol tests were positive in 48% of patients (24/50) and 7.4% of controls (4/54). Twelve asthmatics showed positive reactions to a single agonist (five to methacholine only and seven to mannitol only); however, no statistically significant difference was found in several assessed factors between the two 'single-responder' subgroups (Table S2).

Overall diagnostic properties of both challenge tests were comparable (Youden's index, methacholine 0.42 vs mannitol 0.41; Table 3). However, the mannitol challenge tests had slightly higher sensitivity and lower specificity than the methacholine challenge tests. The ROC curve analyses on the RDRs of mannitol and methacholine challenge test are presented in Figure 1. The areas under the curve were 0.77 (95% confidence interval (CI): 0.68–0.86) for mannitol, and 0.89 (95% CI: 0.83–0.95) for methacholine. In the bivariate correlation tests, log-transformed methacholine- and mannitol-RDR showed a moderate relationship ($r = 0.683$, $P < 0.001$) (Fig. 2).

DISCUSSION

The present study compared the diagnostic properties of two bronchial challenge tests for asthma among Korean adults. We found overall similar diagnostic utility, but also found some discrepant results between them, suggesting their intercomplementary roles.

As both bronchial stimulants have different theoretical basis, they are suggested to have different diagnostic properties.[1] Here we confirmed the previous findings in Korean adults, by showing that 24% of asthmatic subjects had different responses to each of agonist inhalation. However, rather unexpectedly, we found similar or slightly higher sensitivity of mannitol tests compared with methacholine. With regard to this, several explanations may be proposed. First, the

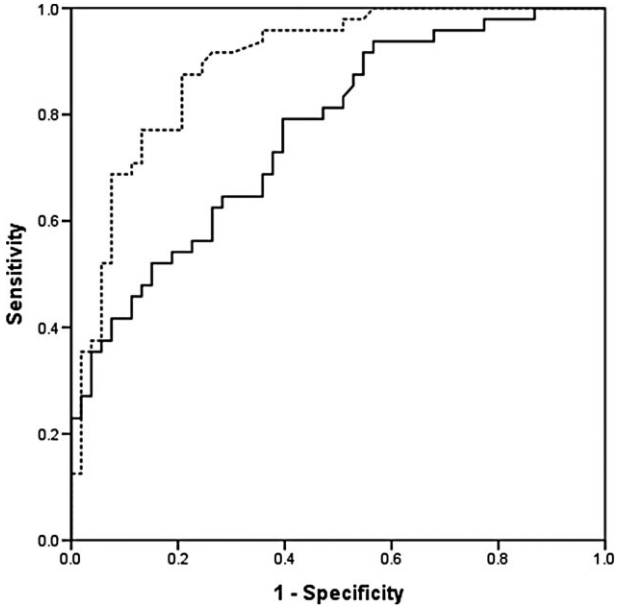

Figure 1 Receiver operating characteristic (ROC) curve of the response–dose ratio (RDR) value of mannitol and methacholine tests against the diagnosis of asthma. (—) mannitol, RDR, (- - -) methacholine, RDR.

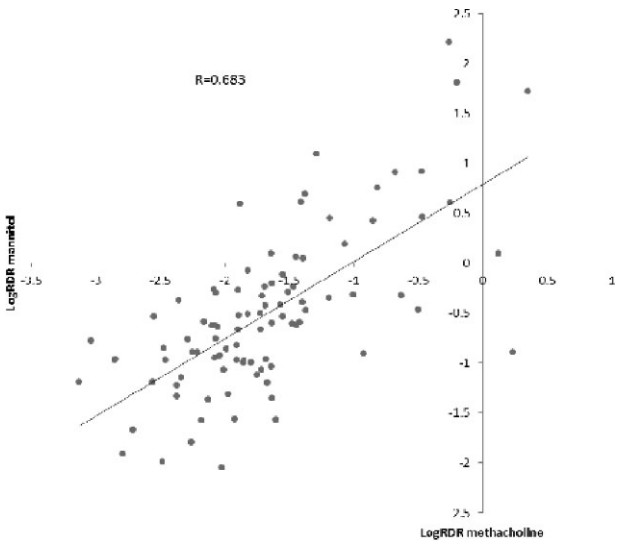

Figure 2 Scatterplot showing the correlation between log-transformed response–dose ratios (RDR) of methacholine and mannitol among asthmatics.

five-breath dosimeter method may possibly have lowered the sensitivity of methacholine inhalation test, as suggested by recent studies.[15–17] The dosimeter inhalation method may deliver less methacholine or may have bronchoprotective effects.[18] Indirect challenge tests are considered to be less influenced by deep inhalations than the direct tests.[2] In this regard, a few recent studies suggested that negative methacholine test by dosimeter methods is not sufficient to exclude asthma.[19,20] It is also notable that four of six comparative studies[5,7,8,21–23] have reported slightly higher sensitivity of mannitol tests, similar to our findings. Anderson *et al*. reported a sensitivity of 55% for the mannitol test and 51% for the methacholine test in the clinical diagnosis of asthma.[8] Miedinger *et al*. reported the sensitivity of mannitol test to be 43%, which is comparable with 41% for the methacholine test for asthma in the military conscripts.[7]

Another explanation for low sensitivity of methacholine tests could be the characteristics of our asthma participants having normal or near-normal lung function, which could be related to false-negative methacholine responses. We excluded asthma patients whose baseline predictive value of FEV_1 was <70%. Methacholine responses are known to be partly dependent on airway caliber, resulting to false responses. In addition, among physician-diagnosed asthma patients, negative methacholine response was associated with better lung functions.[4]

In the RDR-based ROC analyses, we found slightly better diagnostic utility of methacholine compared with mannitol tests (AUC: 0.89 vs 0.77, respectively). However, our findings do not claim the diagnostic superiority of methacholine over mannitol, as contradictory findings have also been reported in previous studies (AUC: 0.85 vs 0.89, respectively).[5] We suppose that the difference could have been related to our lower specificity of mannitol tests. We recruited control groups on the basis of their prior asthma history and symptoms; however, asthmatic symptoms may not preclude asymptomatic airway inflammation. The control groups recruited from hospital visitors could have potential limitations in specificity, as they could have higher probability of having airway inflammation than community population samples. One previous study reporting similar AUC values had utilized the community-based samples randomly selected from the civil registration list.[5]

Our generally low sensitivity (<50%) for both challenge tests might have been influenced by recent asthma medication status, particularly by inhaled corticosteroids (ICS). In fact, current American Thoracic Society (ATS) guidelines provide recommendations on how to withhold medication before methacholine testing, but not for ICS.[4] We made efforts to reduce ICS effects by instructing to withhold ICS at least for 12 h prior to the test, but the potential effects of ICS may not have been fully excluded.[20,24,25] Recent evidence suggests that ICS influences the outcomes of bronchial provocation tests.[2,3,5,14,17–30]

In addition, low sensitivity could have been related to our defining criteria for asthma groups. We included the asthmatics by the combination of their physician diagnosis, recent symptoms and asthma medication, but not by the use of objective challenge tests. However, we later confirmed their asthma diagnosis by reviewing medical records and lung function variability.

Several limitations need to be considered in interpreting our findings. First, the present study had relatively small sample size, the statistical power may have been insufficient to draw firm conclusions. Second, we recruited asthmatics and controls from tertiary hospitals, which may limit the external

validity of our findings. Third, we could not include the assessment for airway inflammatory status in study design, which might have influenced the specificity of mannitol tests.

Nevertheless, the present study prospectively compared the diagnostic utility of methacholine and mannitol challenge tests for asthma in Korean adults. Non-inferior sensitivity of mannitol tests suggested several possibilities in practical interpretation of these two challenge tests. In addition, intra-individual difference in response to methacholine or mannitol suggested their complementary roles for asthma diagnosis.

REFERENCES

1 Cockcroft D, Davis B. Direct and indirect challenges in the clinical assessment of asthma. *Ann. Allergy Asthma Immunol.* 2009; **103**: 363–9, quiz 9-72, 400.
2 Cockcroft DW. Direct challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010; **138**: 18S–24S.
3 Anderson SD. Indirect challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010; **138**: 25S–30S.
4 Crapo R, Casaburi R, Coates A, Enright P, Hankinson J, Irvin C, MacIntyre N, McKay R, Wanger J, Anderson S. 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. *Am. J. Respir. Crit. Care Med.* 2000; **161**: 309.
5 Sverrild A, Porsbjerg C, Thomsen SF, Backer V. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study. *J. Allergy Clin. Immunol.* 2010; **126**: 952–8.
6 Anderson WJ, Lipworth BJ. Relationship of mannitol challenge to methacholine challenge and inflammatory markers in persistent asthmatics receiving inhaled corticosteroids. *Lung* 2012; **190**: 513–21.
7 Miedinger D, Mosimann N, Meier R, Karli C, Florek P, Frey F, Scherer K, Surber C, Villiger B, Michel F. Asthma tests in the assessment of military conscripts. *Clin. Exp. Allergy* 2010; **40**: 224–31.
8 Anderson SD, Charlton B, Weiler JM, Nichols S, Spector SL, Pearlman DS, A305 Study Group. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir. Res.* 2009; **10**: 4.
9 Porsbjerg C, Brannan J, Anderson S, Backer V. Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. *Clin. Exp. Allergy* 2008; **38**: 43–50.
10 Gade E, Thomsen S, Porsbjerg C, Backer V. The bronchial response to mannitol is attenuated by a previous methacholine test: but not vice versa. *Clin. Exp. Allergy* 2009; **39**: 966–71.
11 Cho SH, Oh SY, Bahn JW, Choi JY, Chang YS, Kim YK, Min KU, Kim YY. Association between bronchodilating response to short-acting beta-agonist and non-synonymous single-nucleotide polymorphisms of beta-adrenoceptor gene. *Clin. Exp. Allergy* 2005; **35**: 1162–7.
12 Kim MH, Jung JW, Kang HR. The usefulness of job relocation and serum eosinophil cationic protein in baker's asthma. *Int. Arch. Allergy Immunol.* 2013; **161**: 252–7.
13 Chai H. Antigen and methacholine challenge in children with asthma. *J. Allergy Clin. Immunol.* 1979; **64**: 575–9.
14 Brannan JD, Anderson SD, Perry CP, Freed-Martens R, Lassig AR, Charlton B. The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline. *Respir. Res.* 2005; **6**: 144.
15 Cockcroft DW, Davis BE, Todd DC, Smycniuk AJ. Methacholine challenge: comparison of two methods. *Chest* 2005; **127**: 839–44.
16 Prieto L, Ferrer A, Domenech J, Perez-Frances C. Effect of challenge method on sensitivity, reactivity, and maximal response to methacholine. *Ann. Allergy Asthma Immunol.* 2006; **97**: 175–81.
17 Cockcroft DW, Davis BE. The bronchoprotective effect of inhaling methacholine by using total lung capacity inspirations has a marked influence on the interpretation of the test result. *J. Allergy Clin. Immunol.* 2006; **117**: 1244–8.
18 Scichilone N, Pyrgos G, Kapsali T, Anderlind C, Brown R, Permutt S, Togias A. Airways hyperresponsiveness and the effects of lung inflation. *Int. Arch. Allergy Immunol.* 2001; **124**: 262–6.
19 Ban GY, Park HL, Hwang EK, Ye YM, Shin YS, Nahm DH, Park HS. Clinical characteristics of asthmatics having negative results to methacholine bronchial challenge test. *Korean J Asthma Allergy Clin Immunol* 2012; **32**: 152–8.
20 Sumino K, Sugar EA, Irvin CG, Kaminsky DA, Shade D, Wei CY, Holbrook JT, Wise RA, Castro M, American Lung Association Asthma Clinical Research Centers. Methacholine challenge test: diagnostic characteristics in asthmatic patients receiving controller medications. *J. Allergy Clin. Immunol.* 2012; **130**: 69–75, e6.
21 Miedinger D, Chhajed PN, Tamm M, Stolz D, Surber C, Leuppi JD. Diagnostic tests for asthma in firefighters. *Chest* 2007; **131**: 1760–7.
22 Sue-Chu M, Brannan JD, Anderson SD, Chew N, Bjermer L. Airway hyperresponsiveness to methacholine, adenosine 5-monophosphate, mannitol, eucapnic voluntary hyperpnoea and field exercise challenge in elite cross-country skiers. *Br. J. Sports Med.* 2010; **44**: 827–32.
23 Stenfors N. Self-reported symptoms and bronchial hyperresponsiveness in elite cross-country skiers. *Respir. Med.* 2010; **104**: 1760.
24 Brannan JD, Perry CP, Anderson SD. Mannitol test results in asthmatic adults receiving inhaled corticosteroids. *J. Allergy Clin. Immunol.* 2012; **131**: 906–7.
25 McGrath KW, Fahy JV. Negative methacholine challenge tests in subjects who report physician-diagnosed asthma. *Clin. Exp. Allergy* 2011; **41**: 46–51.
26 Prosperini G, Arcidiacono G, Ciamarra I, Crimi N, Polosa R. Beclomethasone dipropionate attenuates airways hyperresponsiveness to neurokinin a and histamine in asthma. *Respir. Med.* 2006; **100**: 1006–12.
27 Swystun VA, Bhagat R, Kalra S, Jennings B, Cockcroft DW. Comparison of 3 different doses of budesonide and placebo on the early asthmatic response to inhaled allergen. *J. Allergy Clin. Immunol.* 1998; **102**: 363–7.
28 Ward C, Reid DW, Orsida BE, Feltis B, Ryan VA, Johns DP, Walters EH. Inter-relationships between airway inflammation, reticular basement membrane thickening and bronchial hyper-reactivity to methacholine in asthma; a systematic bronchoalveolar lavage and airway biopsy analysis. *Clin. Exp. Allergy* 2005; **35**: 1565–71.
29 Nomura S, Kanazawa H, Asai K, Nomura N, Hirata K, Yoshikawa J. Potential mechanisms of improvement of airway hyperresponsiveness by inhaled corticosteroid therapy in asthmatic patients. *J. Asthma* 2006; **43**: 113–17.
30 Liu M, Liu K, Zhu N, Xia J, Chen X. Inflammatory mediators in induced sputum and airway hyperresponsiveness in cough variant asthma during long-term inhaled corticosteroid treatment. *Mediators Inflamm.* 2012; **2012**: 403868.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Guideline to stop the anti-asthma medications before provocation test

Table S2 Characteristics of mannitol test only-positive and methacholine test only-positive patients

