

DIAGNOSIS

Methacholine challenge is insufficient to exclude bronchial hyper-responsiveness in a symptomatic military populationJustin Stocks, MD¹, Michael Tripp, MD¹, and Thuy Lin, MD²¹Naval Medical Center San Diego, San Diego, CA, USA and ²Pulmonary Medicine Department, Naval Medical Center Portsmouth, Portsmouth, VA, USA**Abstract**

Background: Bronchial hyper-responsiveness in a military population has been evaluated by direct and indirect challenge methods. We hypothesized that negative methacholine challenge testing (MCT) was not sufficient to exclude significant bronchial hyper-responsiveness in a symptomatic military population with exertional dyspnea. The purpose of our study was to identify bronchial hyper-responsiveness in symptomatic military recruits and active duty personnel with normal baseline spirometry and negative pharmacologic bronchoprovocation testing. **Methods:** We performed a retrospective single center electronic chart review of symptomatic service members with a negative MCT who completed a subsequent exercise challenge test (ECT). **Results:** ECT was positive in 45 (26.4%) of 171 subjects (98 recruits). Subjects with a positive ECT had lower baseline forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and FEV1/FVC than those with a negative ECT, and these differences were statistically significant. The mean drop in FEV1 with exercise challenge positive patients was $17.9 \pm 9.2\%$, and the mean drop in FEV1 with MCT was significantly greater in exercise challenge positive patients (-9.5 ± 5.5 vs. -7.6 ± 5.5 , $p = 0.042$). Exercise-induced bronchoconstriction (EIB) was observed in 41% of all recruits who subsequently did not complete training. Only 1 recruit subject of 28 with EIB completed training. **Conclusions:** Methacholine challenge is an insufficient screening test to detect bronchial hyper-responsiveness in a symptomatic military population. In military recruits, EIB is associated with training failure.

Keywords

Exercise challenge testing, exercise-induced bronchoconstriction, methacholine challenge testing, military recruit

History

Received 15 January 2014

Revised 15 April 2014

Accepted 24 April 2014

Published online 28 May 2014

Introduction

Exercise-induced bronchoconstriction (EIB) is defined by the American Thoracic Society (ATS) as acute airway narrowing that occurs with exercise [1]. EIB commonly occurs in association with asthma but is prevalent in up to 19% of asymptomatic adults without a clinical history of asthma [2]. In elite athletes, the prevalence ranges from 2% to 50% depending on the sporting activity and objective testing modality [2,3]. Screening methods to diagnose bronchial hyper-responsiveness include pharmaceutical challenge tests (methacholine or histamine), eucapnic voluntary hyperventilation (EVH), osmotic challenge tests (hypertonic saline or mannitol) or exercise challenge testing (ECT) [4]. Methacholine challenge testing (MCT) is most often considered when spirometry is normal and asthma is likely. The highest combination of positive and negative predictive power occurs when the pretest probability of asthma is 30–70%. MCT has excellent sensitivity and negative predictive value but mediocre specificity for asthma [5].

Airway hyper-responsiveness is a characteristic feature of asthma, but it can be associated with other conditions (e.g. allergic rhinitis, cystic fibrosis, bronchiectasis and COPD) or occur with exercise as in EIB. A positive methacholine bronchoprovocation test is diagnostic for the presence of airway hyper-responsiveness (a necessary component of the asthma syndrome), but a negative bronchoprovocation may be more helpful to rule out asthma. The documented sensitivity of MCT for EIB has been highly variable depending on the population studied [6–13].

Military recruit and active duty populations routinely engage in intense endurance training. Physical fitness is essential to mission preparation. EIB can cause significant exertional impairment which may adversely affect member deployability, force preparedness and mission success. Prompt and accurate diagnosis is crucial. Previous literature has supported the use of MCT and demonstrated variable sensitivity of ECT for asthma and EIB [6,7,9,13,14]. Historically MCT has shown more sensitivity than ECT for bronchial hyper-responsiveness [13–15]. Brown et al. performed an observational study of 210 military recruits who were referred for evaluation of exertional dyspnea after normal baseline spirometry. In the study, the subjects performed an ECT. If the ECT was negative (defined by

Correspondence: Justin Stocks, MD, Naval Medical Center San Diego, Pulmonary Medicine Bldg 3-3, 34730 Bob Wilson Drive, San Diego, CA 92134, USA. Tel: (909)-222-7447. Fax: (619)-532-7625. E-mail: justinstocks77@gmail.com; justin.stocks@med.navy.mil

a <15% decrease from baseline FEV1 post-exercise), the patient underwent a MCT, and 59% of patients with negative ECT results had positive MCT results. For the 128 patients that completed both the ECT and MCT, the sensitivity for ECT was calculated at 32.5% and false-negative rate for ECT was 40.6%. The negative predictive value for ECT as the initial diagnostic test for airway hyper-responsiveness was calculated at 49.4%. Brown concluded that ECT may not be the best initial diagnostic test for exercise-induced bronchospasm [6]. Moreover, a low prevalence of EIB has been observed in asymptomatic military populations. Bronchial hyper-responsiveness was identified in 6% of Air Force personnel by ECT [16]. Sonna and colleagues demonstrated an EIB prevalence of 7% by ECT in an asymptomatic army recruit population [17]. However, a recent study at a military medical center suggests that MCT may miss EIB in a significant proportion of subjects [12]. In the study of 131 symptomatic military personnel EIB was found in 24.4% by EVH, and 19.8% of patients with a positive EVH had a negative MCT; only 3.8% of subjects had a positive MCT and negative EVH [12].

The Pulmonary and Critical Care department of the Naval Medical Center San Diego routinely evaluates military populations with exertional dyspnea. We perform MCT as a standard initial diagnostic test and 458 MCT were conducted in 2012 and over 4000 MCT were performed in the last decade. At our institution, a negative MCT (defined by the ATS as the provocative concentration of methacholine causing a 20% drop in FEV1 (PC20) > 16 mg/ml) is used to exclude a diagnosis of asthma, but subsequent ECT to evaluate for EIB is only rarely done for subjects with suggestive history. Such a practice may miss a substantial number of patients with EIB.

The purpose of our study was to identify the proportion, characteristics and outcomes of symptomatic recruits and active duty personnel with EIB that had normal baseline spirometry, negative bronchoprovocation testing and a subsequent positive ECT. We hypothesized that a negative MCT was not sufficient to exclude bronchial hyper-responsiveness and that ECT would identify a significant number of subjects with EIB.

Methods

Data collection

We conducted a single center retrospective chart review of military recruits and personnel between the ages of 17 and 25 evaluated in the pulmonary clinic for exertional dyspnea, cough and/or wheezing who underwent basic spirometry, MCT and subsequent ECT from 1 January 2002 to 30 June 2009. Patients were excluded if they had a PC20 < 16 mg/ml, a history of asthma, active respiratory infection or abnormal chest radiograph consistent with pneumonia. Subjects were identified with a searchable electronic pulmonary function database (Medgraphics Breeze Cardiorespiratory Diagnostics, Version 6.4.23). Data collected included age, height, weight, sex, ethnicity for each patient and FVC, FVC (percentage of predicted), FEV1, FEV1 (percentage of predicted), ratio of FEV1/FVC, PC20, percentage decrease from baseline FEV1 and positive or negative outcome for ECT. The study

was approved by the Naval Medical Center San Diego institutional review board (CIP #: NMCS.D.2010.0116) and was compliant with the Health Insurance Portability and Accountability Act.

Methacholine challenge testing

All subjects adhered to pretesting pulmonary function ATS recommendations and suggestions for abstinence from inhaled corticosteroids, leukotriene modifiers, anticholinergics and long acting bronchodilators for both MCT and ECT. MCT was conducted in a pulmonary function laboratory utilizing a five-breath dosimeter technique in accordance with ATS guidelines [5]. While wearing a nose clip subjects performed five timed breaths of nebulized methacholine (with serial concentrations of 0.0625, 0.25, 1, 4 and 16 mg/ml) and subsequent measurements of FEV1. The PC20 was calculated per ATS guidelines and considered negative when the PC20 was >16 mg/ml. All MCT was completed prior to ECT (minimum one day between tests).

Exercise challenge testing

ECT was performed by using a treadmill protocol adhering to ATS guidelines [5]. Baseline spirometry was obtained in the seated position at rest. While wearing nose clips and breathing room air subjects exercised on a treadmill with progressive speed and grade advancement. Specifically subjects started at 1.8 mph every 2 min. If a subject was capable of advancing to 6 mph the grade was then increased to a 15% grade, and if tolerated speed was advanced up to 8 mph. Heart rate and oxygen saturations were monitored with a pulse oximeter. Exercise was terminated once the subject had achieved 80% of predicted maximum heart rate (calculated as 220-age) for at least 4 min of exercise, developed symptoms or terminated the test due to fatigue. Subsequently, FEV1 was measured twice at testing intervals of 5, 10, 15, 20 and 30 min after cessation of exercise per ATS guidelines [5]. Testing was considered positive for EIB if post exercise FEV1 declined by $\geq 10\%$ of baseline consistent with current guidelines [1]. EIB severity was graded as mild, moderate or severe if the percentage decrease in FEV1 from the pre-exercise level was $\geq 10\%$ but <25%, $\geq 25\%$ but <50% and $\geq 50\%$, respectively.

Data analysis

Descriptive statistics were calculated as rates of occurrence with confidence intervals for categorical variables and means with standard deviations for continuous variables. The rate of negative MCT associated with positive ECT was tested against 6% (reported in the medical literature) by a test of proportions [14]. Based on the test of two proportions, the prevalence of bronchial hyper-responsiveness as measured by ECT would have to exceed 9.5% to be significantly different from the reported 6%. Tests of significance were calculated using the two sample *t* test for continuous variables and Fisher's exact test for categorical variables. A *p* value < 0.05 was considered statistically significant. Multivariate modeling was performed using logistic regression to test for demographic and spirometric variables that predicted a positive ECT.

Table 1. Comparison of ECT positive and negative subjects.

Parameter	All subjects <i>N</i> = 171	ECT positive <i>N</i> = 45	ECT negative <i>N</i> = 126	<i>p</i> Value
Age (±SD)	20.5 (±2.2)	20.2 (±2.2)	20.6 (±2.2)	0.32
Sex				0.84
Male, number (%)	131 (76)	34 (76)	97 (77)	
Female, number (%)	40 (24)	11 (24)	29 (23)	
Ethnicity				0.89
Caucasian, number (%)	113 (66)	31 (69)	82 (64)	
African American, number (%)	21 (12)	4 (9)	17 (14)	
Asian, number (%)	9 (5)	2 (4)	7 (6)	
Hispanic, number (%)	28 (17)	8 (18)	20 (16)	
Baseline FEV1, L (±SD) ^a	–	3.39 (±0.71)	3.97 (±0.66)	<0.001
Baseline FVC, L (±SD) ^a	–	4.15 (±0.94)	4.66 (±0.084)	<0.001
Baseline FEV1/FVC ^a	–	82.8 (±8.9)	85.7 (±6.8)	0.027
ΔFEV1 with MCT, % (±SD)	–	–9.5 (±5.5)	–7.6 (±5.5)	0.042
ΔFEV1 with ECT, % (±SD) ^a	–	–17.9 (±9.2)	–3.3 (±3.8)	<0.001

^aMean values for ECT ± standard deviation.

Table 2. Baseline spirometry prior to challenge test and response to challenge testing for all subjects.

Test	Liters (±SD) ^a	% Predicted (±SD)
MCT, baseline spirometry		
FEV1	3.78 (±0.74)	92.7 (±11.9)
FVC	4.72 (±0.91)	96.8 (±12.9)
FEV1/FVC (absolute value)	81.1 (±7.6)	
Mean decrease in FEV1, %	–8.1 (±5.56)	
ECT, baseline spirometry		
FEV1	3.82 (±0.72)	94.8 (±12.8)
FVC	4.53 (±0.90)	95.3 (±13.1)
FEV1/FVC (absolute value)	84.9 (±7.5)	
Mean decrease in FEV1, %	–7.1 (±8.7)	

^aAll numbers are mean values ± SD.

Results

A total of 171 subjects (98 recruits) completed both MCT and subsequent ECT between January of 2002 and June of 2009. All subjects had complete challenge testing data. Subject demographics are shown in Table 1. The majority of subjects were Caucasian (66%) and male (76%) with a mean age of 20.5 ± 2.2 years.

Baseline spirometry for each respective challenge test is displayed in Table 2. The mean time between challenge tests was 12 days. All subjects completed both challenge tests. Spirometric variables (FEV1, FVC and FEV1/FVC) matched a normal distribution and were not statistically different at baseline between tests.

ECT was positive in 45 (26.3%) subjects exceeding the expected 6% and threshold value of 9.5%, and this was statistically significant ($p < 0.001$). Recruits comprised 62% of ECT positive subjects. A comparison of ECT positive and negative subjects is summarized in Table 1. Subjects with a positive ECT had lower baseline FEV1, FVC and FEV1/FVC than those with a negative ECT, and these differences were statistically significant. The mean drop in FEV1 with MCT was slightly but significantly greater in ECT positive patients (-9.5 ± 5.5 vs. -7.6 ± 5.5 , $p = 0.042$). There were no significant differences in age, gender or ethnicity between ECT positive and negative subjects. Of those with positive ECT, 37 (82%) were classified with mild EIB and 8 (18%) with moderate EIB. No subjects met criteria for severe

EIB. On multivariate regression analysis no spirometric or demographic variables significantly correlated with a positive ECT (data not shown).

Training outcome data were available for all recruit subjects. In total, 98 of 171 subjects were recruits, and 66 recruit subjects did not complete training. ECT was positive in 41% (27/66) of all recruits who failed to complete training. Only 1 recruit subject with a positive ECT (1/28) completed training.

Discussion

We observed that a significant number of symptomatic recruits with a negative MCT had bronchial hyper-responsiveness on ECT. Moreover, almost all recruits with EIB (96%) did not complete training. To the best of our knowledge, no prior study has specifically focused on symptomatic MCT negative military recruits and their outcomes. Prior investigations have shown adequate utility for MCT in the military population. Historically MCT was used to screen Italian conscripts for asthma [18]. Morris and colleagues found that MCT yielded a diagnosis in 40% of 105 military patients with exertional dyspnea [19]. Roth et al. did a prospective, blinded cohort comparison study in 103 Reserve Officer Training Corps (ROTC) cadets to determine the prevalence of positive results for MCT. Ultimately, 21 of 23 cadets with a positive MCT result were diagnosed with asthma. In a recent Korean military study MCT confirmed bronchial hyper-responsiveness in 23 of 35 subjects that did not meet objective criteria for bronchodilator responsiveness, and all subjects with a positive MCT were eventually diagnosed with asthma [20]. MCT has also been observed to be more sensitive than ECT for bronchial hyper-responsiveness in certain non-military populations [13,21]. MCT has shown excellent utility in elite athletes as well. Stensrud and colleagues evaluated 24 elite cross-country skiers with exercise field testing vs. methacholine challenge and found methacholine challenge was more sensitive than a sport-specific exercise field test for identifying athletes with asthma and/or bronchial hyper-responsiveness [9].

However, recent investigations challenge the utility of MCT for detecting bronchial hyper-responsiveness.

Holley and colleagues observed a high false-negative rate for MCT in symptomatic military personnel with a positive EVH [12]. Anderson et al. conducted MCT, ECT and mannitol challenge testing in 509 adult and pediatric subjects with signs and symptoms suggestive of asthma to determine the sensitivity and specificity of challenge tests to detect bronchial hyper-responsiveness and EIB [22]. Physicians blinded to the challenge test results evaluated the participants at five visits and rendered a clinical diagnosis of asthma. The sensitivity and specificity of MCT were low (70% and 54.5%, respectively) compared with prior studies. MCT was negative in 118 subjects diagnosed with asthma clinically, and 73 of 163 (45%) subjects with a positive ECT had negative MCT. However, the study may have been limited by a high rate of misdiagnoses; 15% of all subjects diagnosed with asthma were negative to all three challenge tests, and 12% of subjects with two or more positive challenge tests were determined to not have asthma. Unblinding physicians to challenge test results may have caused a significant redistribution of asthma diagnoses.

Classically ECT has been observed to have poor sensitivity for bronchial hyper-responsiveness in military populations. Brown and colleagues observed a dismal sensitivity for ECT as the initial diagnostic modality for bronchial hyper-responsiveness in symptomatic recruits [6]. Our observations may differ due to the discrepancy in ECT positivity definition (10% drop in FEV1 vs. 15%) and different methodologies. Morris et al. performed spirometry and ECT on 222 asymptomatic Army personnel with less than one year of active military service. The study found 31 people (14%) had airway obstruction as defined by ATS standards, and ECT was positive in only 23% of personnel with obstruction.

Our study population had a high positive ECT frequency (26.4%) compared with prior studies of military populations. Subjects with EIB had significantly lower FEV1, FVC, FEV1/FVC and a greater drop in FEV1 to MCT (despite being a “negative” test) compared with ECT negative subjects (Table 1). Our findings reinforce and extrapolate on prior observations by Holley and colleagues. Furthermore, subjects in our study had more severe EIB compared with Holley et al. and prior military studies [12]. These results suggest that a substantial proportion of recruits with EIB may be undetected by primary MCT. This may be due to the proposed mechanism of EIB with evaporative water loss activating mast cells and subsequent BHR instead of allergens directly stimulating bronchoconstriction. The training environment and repetitive exercise may adversely condition this pathway of BHR. Moreover, moderate EIB may be more common in the symptomatic recruit population, and it may result in more significant adverse outcomes and loss of potential military personnel. Our data support the use of ECT in recruit subjects with exertional dyspnea and a negative MCT.

Our study has several limitations. It is retrospective with the associated shortcomings and biased toward a specific subpopulation of military members with exertional dyspnea in a physically demanding environment, and our findings may not be applicable to the civilian sector or elite athletes. Moreover, the five-breath dosimeter method may have resulted in a higher PC20 as observed in prior limited

studies [23]. Although we observed training failure in nearly all recruits with EIB, our observations do not necessarily establish the causality of EIB and training failure. Moreover, challenge test results are available for recruit commands, and these results may have influenced recruit disposition. However, EIB likely contributed to recruit training failures. Data are lacking on the medical management of recruits with EIB who failed training. Moreover, we did not report the outcomes of subjects with mild EIB that completed training and became full military personnel. It is possible that EIB may have shortened these subjects’ military careers or resulted in medical separation (discharge from military service due to a medical condition impairing the subject’s ability to complete one’s duty). Furthermore, a prospective trial in symptomatic recruits performing both MCT and ECT combined with direct physician evaluation may have yielded different results.

In conclusion, MCT cannot be relied upon as the diagnostic gold standard for bronchial hyper-responsiveness in a military population. EIB is associated with significant failure to complete military training. The ideal test or combination and sequence of tests to detect bronchial hyper-responsiveness in a symptomatic military population are still unclear. A direct prospective comparison between MCT, mannitol, histamine, ECT and EVH may improve the identification, management and outcomes of military service members with bronchial hyper-responsiveness.

Acknowledgements

Dr Stocks collected and analyzed data, wrote and edited the main body of the text and abstract, and submitted the manuscript for publication. Dr Lin was the PI of the study and submitted the IRB protocol, collected and analyzed data, wrote and edited the text and provided general oversight of the study. Dr Tripp analyzed data, edited the text and prepared the manuscript for publication. The authors wish to acknowledge Dr Robert Riffenburgh, PhD, for statistical analysis and Ms Juneva Julio (CRT, CPFT) for assistance with data acquisition.

Declaration of Interest

The authors have no conflicts of interest to report.

References

1. Parsons JP, Hallstrand TS, Mastrorade JG, Kaminsky DA, Rundell KW, Hull JH, Storms WW, et al. An official American Thoracic Society clinical practice guideline: exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2013;187:1016–1027.
2. Khan DA. Exercise-induced bronchoconstriction: burden and prevalence. *Allergy Asthma Proc* 2012;33:1–6.
3. Rundell KW, Slee JB. Exercise and other indirect challenges to demonstrate asthma or exercise-induced bronchoconstriction in athletes. *J Allergy Clin Immunol* 2008;122:238–246.
4. Anderson SD, Brannan JD. Bronchial provocation testing: the future. *Curr Op All Clin Immunol* 2011;11:46–52.
5. American Thoracic Society. Guidelines for methacholine and exercise testing. *Am J Respir Crit Care Med* 2000;161:309–329.
6. Brown L, Martin BL, Morris MJ. Airway hyperresponsiveness by methacholine challenge testing following negative exercise challenge. *J Asthma* 2004;10:553–558.

7. Morris MJ, Schwartz DS, Nohrenberg JL, Dooley SN. Airway hyperreactivity in asymptomatic military personnel. *Mil Med* 2007; 172:1194–1197.
8. Roth BJ, Hammers LM, Dillard TA. Methacholine challenge testing in Reserve Officer Training Corps cadet. *Chest* 2001;119:701–707.
9. Stensrud T, Mykland KV, Gabrielsen K, Carlsen KH. Bronchial hyperresponsiveness in skiers: field test versus methacholine provocation? *Am Col Sports Med* 2007;39:1681–1686.
10. Bougalt V, Turmel J, Boulet LP. Bronchial challenges and respiratory symptoms in elite swimmers and winter sport athletes. *Chest* 2010;138:31S–37S.
11. Pedersen L, Winther S, Backer V, Anderson SD, Larsen KR. Airway responses to eucapnic hyperpnea, exercise, and methacholine challenge in elite swimmers. *Med Sci Sports Exerc* 2008;40: 1567–1572.
12. Holley AB, Cohee B, Walter RJ, Shah AA, King CS, Roop S. Eucapnic voluntary ventilation is superior to methacholine challenge testing for detecting airway hyperreactivity in nonathletes. *J Asthma* 2012;49:614–619.
13. Hildebrand K, Przybylowski T, Maskey-Warzechowska M, Chazan R. Usefulness of selected tests in the diagnosis of exercise-induced bronchoconstriction. *Pneumonol Alergol Pol* 2011;79: 397–406.
14. Eggleston P. A comparison of the asthmatic response to methacholine and exercise. *J Allergy Clin Immunol* 1979;63: 104–110.
15. Chatham M, Bleecker ER, Smith PL, Rosenthal RR, Mason P, Norman PS. A comparison of histamine, methacholine and exercise airway reactivity in normal and asthmatic subjects. *Am Rev Respir Dis* 1982;126:235–240.
16. O'Donnell AE, Fling J. Exercise-induced airflow obstruction in a healthy military population. *Chest* 1993;103:742–744.
17. Sonna L, Angel KC, Sharp MA, Knapik JJ, Patton JF, Lilly CM. The prevalence of exercise-induced bronchospasm among US Army recruits and its effects on physical performance. *Chest* 2001;119: 1676–1684.
18. Ferrante E, Grasso S, Corbo GM, Ciappi G. [The measurement of bronchial hyperreactivity for military service fitness]. *Recenti Prog Med* 1991;82:520–522.
19. Morris MJ, Grbach VX, Deal LE, Boyd SY, Morgan JA, Johnson JE. Evaluation of exertional dyspnea in the active duty patient: the diagnostic approach and the utility of clinical testing. *Mil Med* 2002;167:281–288.
20. Lee SM, Ahn JS, Noh CS, Lee SW. Prevalence of allergic diseases and risk factors of wheezing in Korean military personnel. *J Korean Med Sci* 2011;26:201–206.
21. Sin BA, Yildiz OA, Dursun AB, Misirlilik Z, Demirel YS. Airway hyperresponsiveness: a comparative study of methacholine and exercise challenges in seasonal allergic rhinitis with or without asthma. *J Asthma* 2009;46:486–491.
22. 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.
23. Prieto L, Lopez V, Llusar R, Rojas R, Marin J. Differences in the response to methacholine between the tidal breathing and dosimeter methods: influence of the dose of bronchoconstrictor agent delivered at the mouth. *Chest* 2008;134:699–703.