TITLE: Bronchial provocation testing for the identification of exercise-induced bronchoconstriction

AUTHORS: John D. Brannan PhD[1] & Pascale Kippelen PhD[2,3]

AFFILIATION

[1] Department of Respiratory and Sleep Medicine,

John Hunter Hospital

New Lambton NSW 2305 AUSTRALIA

john.brannan@health.nsw.gov.au

[2] Centre for Human Performance, Exercise and Rehabilitation, Brunel University London, Uxbridge, UK

[3] Division of Sport, Health and Exercise Sciences, College of Health and Life Sciences, Brunel University London, Uxbridge, UK.

pascale.kippelen@brunel.ac.uk

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ABBREVIATIONS:

EIB; exercise induced bronchoconstriction

FEV_1; forced expiratory volume in one second

ASL; airway surface lining

PDG_2; prostaglandin D_2

cystLT; cystenyl leukotrienes

AHR; airway hyperresponsiveness

ICS; inhaled corticosteroids

EVH; eucapnic voluntary hyperpnea

ATS; American Thoracic Society

ERS; European Respiratory Society

MVV; maximum voluntary ventilation

PD15; provoking dose of mannitol in milligrams to cause a 15% fall in FEV_1

PD10; provoking dose of mannitol in milligrams to cause a 10% fall in FEV_1

mg; milligrams

ABSTRACT

Exercise-induced bronchoconstriction (EIB) is a common occurrence in asthmatics, children and otherwise healthy athletes. Poor diagnostic accuracy of respiratory symptoms during exercise requires objective assessment of EIB. The standardised tests currently available for EIB diagnosis are based on the assumption that the provoking stimulus to EIB is dehydration of the airway surface fluid due to conditioning large volumes of inhaled air during exercise. 'Indirect' bronchial provocation tests that use stimuli to cause endogenous release of bronchoconstricting mediators from airway inflammatory cells include dry air hyperpnoea (e.g., exercise, eucapnic voluntary hyperpnoea) and osmotic aerosols (e.g., inhaled mannitol). The airway response to different indirect tests are generally similar in patients with asthma and healthy athletes with EIB. Further the airway sensitivity to these tests is modified by the same pharmacotherapy used to treat asthma. By contrast pharmacological agents, such as methacholine given by inhalation, act directly on smooth muscle to cause contraction. These 'direct' tests have been used traditionally to identify airway hyperresponsiveness in clinical asthma but are less useful to diagnose EIB. The mechanistic differences between 'indirect' and 'direct' tests have helped to elucidate the events leading to airway narrowing in asthmatics and elite athletes, while improving clinical utility of these tests to diagnose and manage EIB.

INTRODUCTION

Exercise-induced bronchoconstriction (EIB) describes the transient narrowing of the airways that occurs during or, most commonly following vigorous exercise.(1) EIB is common in patients with asthma who experience frequent respiratory symptoms (such as cough, wheeze, chest tightness, mucus hypersecretion) and is often an indicator of persistent asthma warranting treatment.(2) EIB can occur in otherwise healthy people, particularly in children and adolescents (de Aquiar KB Pediatr Pulmonol 2018) and in those performing regular exercise (e.g., army recruits, elite athletes).(2)

EIB is characterized by a transient fall in forced expiratory volume in the first second (FEV_1). Bronchial provocation tests that induce changes in FEV_1 in response to exercise, or surrogates of exercise (e.g., dry air hyperpnoea, hyperosmotic stimuli) are recommended for EIB diagnosis.(2, 5) This approach is strengthened by observations that exercise symptoms are poor predictors of EIB.(6)

Understanding the mechanisms of EIB is important in order to select the most appropriate test to assess EIB, as well as to justify and guide therapy.(7) This review is a summary of the pathophysiology of EIB, and describes the advantages and disadvantages of various diagnostic tests available for EIB assessment and management. In addition this review demonstrates how discrepancies between 'indirect' (e.g., exercise and its surrogates) and 'direct' (e.g., methacholine) tests advanced our understanding of the pathophysiology of EIB, and how the development of surrogates for exercise helped to improve clinical practice. According to current guidelines, 'direct' tests are not recommended for the assessment of EIB, due to discordance in the airway response in individuals with EIB alone and in those with mild clinical asthma with EIB.

Mechanisms of EIB: what have mechanistic studies taught us?

Water loss from the airway surface in response to conditioning large volumes of air to body conditions (i.e., 37°C, 100% relative humidity) during exercise is regarded as the primary stimulus to EIB.(1, 8) Severity of EIB varies with the water content of inhaled air(8) and inhalation of fully conditioned air during exercise completely blocks EIB.(9, 10) As cold air is always dry, EIB is usually more severe during winter(11) and is common in winter athletes.(12, 13) In addition to the amplifying effect on respiratory water loss, cold air breathing is thought to create intra-airway thermal gradients that trigger engorgement of the bronchial vasculature and mucosal oedema as soon as exercise ceases (14), thereby exaggerating airway narrowing.(Figure 1)

Mechanistically, water loss from the airways is likely to cause transient dehydration and hyperosmolarity of the airway surface liquid (ASL) in the first 10-12 generations where the volume of the periciliary fluid is estimated at less than 1 ml.(15, 16) Compensatory water movement across the airway epithelium restores the ASL osmolarity. It has been proposed that this event causes inflammatory cells (e.g., mast cells and eosinophils) to release histamine, prostaglanding-D_2 (PGD_2), cysteinyl leukotrienes (cystLT) and, in susceptible individuals, this leads to airway smooth muscle contraction and airway narrowing.(7) Reasons why patients with asthma are susceptible to EIB compared with healthy non-asthmatic subjects include; *i)* that asthmatics are likely to be allergic and have activated mast cells and eosinophils in greater numbers in their airways(17, 18)(Figure 2), as evidenced by mast cell and eosinophilic-derived mediators release (19, 20), and *ii)* their smooth muscle is hyper-responsive (consistent with observations of bronchial hyperresponsiveness to

113 methacholine in asthmatics with EIB).(21) In athletes (particularly endurance-trained
114 athletes), recruitment of the small airways in order to condition the large volumes of
115 inhaled air in a short time (up to 200L/min) likely amplifies the dehydration of the
116 small airways and osmotic stress.(22)

117 Evidence to support the osmotic theory of EIB arise from studies showing : *i)*
118 a good relationship between the severity of EIB and the airway sensitivity to surrogate
119 tests in known asthmatics(23), *ii)* consistent reports of an increase in urinary
120 metabolites of the potent bronchoconstrictors PGD_2 and cysLT after bronchial
121 provocation with dry air hyperpnoea and mannitol challenge(24-27); *iii)* reduced
122 severity and/or duration of induced bronchoconstriction, or enhanced airway recovery
123 in individuals with EIB pre-medicated with either an histamine antagonist (i.e.,
124 fexofenadine hydrochloride), or a mast cell stabilising agent (i.e., sodium
125 cromoglycate, nedocromil sodium) or leukotriene antagonist (e.g., montelukast)(25,
126 28-30); *iv)* attenuation of EIB using inhaled corticosteroids (ICS) at high dose acutely,
127 or in recommended doses regularly.(31, 32) Regular ICS in doses recommended for
128 the daily treatment of asthma can attenuate, or even completely abolish airway
129 sensitivity to exercise and to surrogate tests for EIB. A negative airway response
130 following ICS is suggestive of successful attenuation of airway inflammation (which
131 is the source of bronchoconstricting mediators). The abolition of EIB with
132 pharmacotherapy is considered a successful therapeutic end point.

133 *Clinical implication*: EIB is osmotically-driven and can be identified using
134 surrogate challenge tests that mimic exercise challenge, such as dry air hyperpnoea
135 and hyperosmotic stimuli.

136

137 **Challenge testing for the diagnosis of EIB: an historical perspective**

The development of tests for the diagnosis of EIB was derived from the understanding that exercise was a common stimulus for bronchoconstriction in patients with asthma. Assessing EIB is also useful and important in occupational settings where EIB could put individuals at risk of an attack of asthma (e.g., army recruits, scuba divers) and/or impair exercise performance (e.g., professional athletes). Prevalence of EIB in all these groups can differ significantly, as does the diagnostic sensitivity of bronchial challenge tests to assess EIB.(33) However, regardless of the diagnostic sensitivity and specificity of an individual test for EIB, the documentation of a positive response to exercise, or its surrogates, identifies the need for clinical intervention.(2) Little mechanistic differences exist in the airway responses to exercise (or its surrogates) between asthmatics and athletes. However, it is more likely to observe severe airway response to 'indirect' challenges in those with active asthma and EIB, compared to those with EIB alone. Some asthmatics may have significant airflow limitation during exercise, which can be observed in falls in minute ventilation. Occurrence of EIB during exercise (also referred to as breakthrough EIB) seems particularly common in children. Whilst not comprehensively analysed, treatment responses between individuals with EIB alone and those with asthma and EIB does not seem to differ.(Kippelen 2010, Kippelen 2010)

Tests for EIB have evolved since the early investigations into the stimulus and mechanisms of EIB and the establishment of exercise protocols.(34) Historically, the work began using treadmill exercise to diagnose asthma in children(35) on the understanding that EIB was one of the first clinical features of asthma. Subsequently EIB in children was also shown to be one of the last features to resolve with regular ICS.(36) This was soon followed by the investigation of surrogate tests to identify EIB, most notably the development of the Eucapnic Voluntary Hyperpnea (EVH) test

with dry air for occupational screening of US Army recruits.(37) This development was associated with the emerging understanding that airway drying associated with exercise hyperpnea was the primary stimulus to EIB. This led to the development of osmotic challenges (using nebulised aerosols of hypertonic saline and dry powder mannitol) to identify potential for EIB.(38) Collectively, exercise, EVH and osmotic challenges are classified as 'indirect' tests, as they cause the release of mediators of bronchoconstriction from resident airway inflammatory cells. These mediators act on smooth muscle receptors to cause contraction and airways narrowing.(39, 40)

Throughout this period, and before the development of 'indirect' tests, it was common to use bronchial provocation tests using nebulised methacholine or histamine to identify AHR for assessing the potential for EIB. (41, 42) The rationale was that EIB is in fact a type of AHR and it can be associated with clinical asthma. Known as 'direct' tests for AHR, these pharmacological agents act directly on airway smooth muscle receptors to cause airway narrowing.(40) However, tests using these pharmacological agents are neither sensitive nor specific for identifying EIB (particularly in those with EIB alone or with an early diagnosis of asthma).(21, 43) Thus, there is dissociation between airway responses to exercise, or its surrogates (e.g., dry air hyperpnoea and osmotic challenges), and AHR to methacholine or histamine.(33, 43-45) Several reasons may serve to explain these findings: *i)* pharmacological agents act directly on the airway smooth muscle, thus a positive response is not dependent on the endogenous release of inflammatory mediators; *ii*) cysLT and PGD_2 are far more potent that methacholine- or histamine for provoking bronchoconstriction(46); *iii)* positive responses to 'direct' challenges (in the absence of a negative 'indirect' challenge test result) may result from airway injury from smoking, cold air hyperpnea or airway remodelling.(47) For example, elite skiers can

be positive to methacholine, with signs of airway epithelial injury and remodelling, yet many of these athletes are negative to exercise, EVH and mannitol challenges and do not respond to regular ICS.(48-50)

Clinical implication: Major clinical guidelines on EIB moved away from recommending methacholine or histamine for the assessment of EIB. However, these tests may remain important in identifying airway injury in elite athletes.(2, 5)

Measurement of change in airway calibre

For all bronchial provocation tests it is essential that quality baseline spirometry is performed (i.e., strictly employing ATS/ERS recommendations).(51) Baseline FEV_1 should be ≥70-75% of predicted normal value, and not <1.2L).(2) For both safety and efficacy reasons, the baseline FEV_1 must be stable. FEV_1 should be measured in duplicate at each time-point during or following the challenge with a difference of no more than 150ml or 5%. As the primary outcome is a change in FEV_1 from baseline, full forced expiratory manoeuvres to vital capacity are not essential.

Medications that can protect against EIB need to be withheld before a diagnostic challenge test.(2)(Table 1) Post-challenge, bronchoconstriction is usually reversed with a standard dose of inhaled $beta_2$-agonists. Recovery following inhaled $beta_2$-agonist may be slower in individuals with more severe falls in FEV_1 and also in those who are taking inhaled $beta_2$-agonists daily.(2, 52)

Dry air hyperpnea challenges

Exercise for bronchial provocation

Laboratory exercise tests (usually performed on treadmills or cycle ergometers) require participants to perform a 6-8 min high intensity effort.(2, 5) The warm-up

period prior to reaching the target workload should be short (2-3 min maximum) and the remaining exercise (5-6 min) should be performed at 80-90% of predicted maximum heart rate (calculated as 220 minus age) or 17.5-21 times FEV_1 (when ventilation is recorded). The rationale for such protocols is to permit high ventilatory rates to be reached rapidly and to be sustained, in order to maximise the dehydrating stimulus to the airways. Recommended protocols outlined in guidelines(2, 5) are useful to assist in optimising the dehydrating stimulus and, thereby, potentiating the airway response and avoiding false negative tests. Of note, absolute humidity should be maintained below 10 mg H_2O/L (<50% relative humidity at 20°C) and a nose clip should used to avoid humidification of inhaled air from the nasal passage. Post-challenge, serial measurements of FEV_1 are taken (usually at 5, 10, 15 and 20 min), with a fall in FEV_1 of 10% or more over two consecutive time points considered as diagnostic for EIB.(Figure 2,3)

It is well known that laboratory exercise tests may not be sensitive enough to identify EIB in some individuals. For example, it is common for elite athletes to have EIB in their chosen sporting activity, yet have a negative running or cycling exercise test in the laboratory.(53) Negative tests more commonly occur in those with mild disease (i.e., when the FEV_1 fall may be close to the 10% cut-off for a positive test).(54) Possible reasons are that; *i)* the exercise test in the laboratory may not be sufficiently vigorous to require a ventilation rate to cause adequate airway dehydration(55); *ii)* it is not always possible to control water content of inspired air(55); and *iii)* airway irritants (e.g., airborne allergens, traffic-related pollutants, chlorination by-products in swimming pools) can enhance EIB in the field.(56) In addition, in individuals with an FEV_1 fall around the 10% threshold, there can be a variation in the airway response when multiple tests are performed.(54) While this is a

problem diagnostically, it also suggests that, in these individuals, EIB is likely to be mild.

Clinical implication: After a negative exercise test, if EIB is still highly suspected, the test should be repeated.(2)

Eucapnic Voluntary Hyperpnoea

The disadvantages of exercise in the laboratory motivated the development of alternative methods to improve diagnostic sensitivity. EVH testing(58) requires individuals to breathe for 6 min a dry gas mixture containing 21% O_2, 5% CO_2, balance N_2, at a ventilation level equating 60% of maximum voluntary ventilation (calculated as 21 times baseline FEV_1).(2) In order for athletes, to reproduce the ventilatory demand of their field exercise, the target ventilation should be increased to 85% of maximum voluntary ventilation (i.e., 30 times baseline FEV_1). Post challenge, FEV_1 should be measured soon after completion of the test and should be monitored for at least 15 min, with recordings taken at 5-min intervals. The cut-off for a positive EVH test is a fall in FEV_1 of 10% or greater. In athletes, it is recommended the fall be sustained over at least two consecutive time points.(2, 39)

EVH challenge is more sensitive for identifying AHR compared to laboratory exercise. Further, EVH has been demonstrated to be useful in elite athletes for confirming EIB documented during field exercise.(59) However, some individuals (especially young athletes) may not reach the minimum required ventilation of 21 times FEV_1, reducing the sensitivity of the test.(60) Further, in elite athletes, the use of a 10% cut-off may make the test too sensitive, and a 15% fall in FEV_1 may be recommended, as more specific (61). The variability in the airway response, particularly when the response is mild (i.e. around the 10% cut off), has also led some

263 authors to suggest that more than one EVH test should be performed to confirm
264 diagnosis (57). Finally, in some athletes – particularly those engaging in winter and
265 aquatic sports – a negative EVH test does not always exclude EIB.(62, 63)(Figure 2,3)
266 The apparatus for performing an EVH challenge can be sourced by pulmonary
267 function laboratories and 'home-made' set-ups can be easy to assemble.(39) However
268 they necessitate use of pre-made gas mixtures that can be expensive. There are now
269 commercially available devices for gas mixing. These usually require a higher initial
270 cost but potentially are less expensive due to lower ongoing costs.(64)
271 EVH has both practical and mechanistic advantages over laboratory-based
272 exercise tests. EVH permits the subject to reach a high rate of ventilation faster than
273 exercise, with an ability to sustain this high level of ventilation more easily, leading to
274 a more reliable dehydrating stimulus to the airway surface. Through the use of
275 compressed air, the inspired water content can be maintained close to zero and airway
276 dehydration potentiated. It is important to understand that with a more potent stimulus
277 comes the potential for severe falls in FEV_1 (>30%). This is more likely as the EVH
278 protocol is a single bolus dose of hyperpnea. This is in contrast to dose-response
279 challenge tests (such as mannitol and hypertonic saline) that reduce the possibility of
280 severe falls in FEV_1.
281 *Clinical implication*: It is recommended for EVH to be used only in
282 individuals; *i)* with EIB alone (i.e. not in those individuals with established clinical
283 asthma), *ii)* with normal, to near normal lung function (i.e., baseline FEV_1>75%
284 predicted), and *iii)* who are not taking inhaled medications regularly.(2) During EVH,
285 ventilation should also be closely monitored throughout the 6-min period. If falls in
286 ventilation are observed during the test, this may be an early sign of

bronchoconstriction and may lead to a severe airway response. It is best in this case to consider ceasing the EVH challenge before the end of the 6-min period.

Osmotic stimuli (e.g., mannitol challenge)

The methodology for the mannitol challenge arose from the need to make indirect tests more practical and accessible.(65) The test is standardised and simpler to perform than exercise or EVH, which both require complex equipment. The mannitol test comes as a kit consisting of increasing doses of mannitol powder (5, 10, 20 and 40mg in capsules) and a simple low resistance inhaler.(66) FEV_1 is measured at baseline and 60 sec following the inhalation of each dose. As the response to mannitol is dependent on progressively increasing the osmotic gradient at the airway surface, the test should be performed without significant delay between doses. Mannitol provokes cough in some patients(67, 68)., To minimise cough induced by upper airway impaction, individuals should be advised not to inhale the mannitol powder too rapidly.(69)

The fall in FEV_1 required for a positive mannitol test is 15%, which has been validated to aid in a clinical diagnosis of asthma. In individuals (especially athletes) who have a 10% fall in FEV_1 with the maximum dose of 635mg of mannitol, mild EIB may be present.(70) The mannitol challenge is the only regulatory approved indirect bronchial challenge test that has demonstrated adequate safety and efficacy in identifying asthma and EIB.(21, 66) (Figure 2,3)

The airway sensitivity to mannitol is reproducible(71, 72) and relates well to the severity of EIB in asthmatics and summer elite athletes.(23, 70, 73, 74) Further, in mild asthmatics with EIB, AHR to mannitol was 1.4 times more likely to identify AHR than a laboratory exercise test.(21) However, in swimmers, airway responses to

mannitol and field-based exercise are often discordant, particularly when the responses is a product of mild AHR.(75, 76)

Severity of the airway sensitivity is expressed by provoking dose of mannitol that causes a 15% fall in FEV_1 (PD_{15}) (with a PD_{15}<35mg classified as severe, 35-155mg, moderate, and 155-635mg, mild).(39) The airway response can also be expressed as response-dose ratio (i.e., the % fall in FEV_1/mg of mannitol), which is a measure of airway reactivity. The severity of the airway response can predict the severity of airway inflammation (e.g., mast cells, eosinophils)(77-80) (Figure 5) and regular ICS treatment has been shown to reduce the airway sensitivity and reactivity in patients with asthma.(32, 81) However, continued treatment with ICS can abolish the airway sensitivity to mannitol. Like the abolition of EIB with ICS, a negative mannitol test has been proposed as a signal for optimal ICS therapy(Brannan 2010) and a potential end-point to signal the down-titration of ICS.(Turton 2012)

Clinical implication: Mannitol may be used to identify and monitor ICS treatment in individuals with EIB; a goal for adequate therapy being non-responsive to the challenge.

Future Directions

Future directions in research in EIB have previously been discussed.(82) The role of the small airways in EIB is still unclear and few studies have used outcome measures other than FEV_1 to quantify the change in airway calibre, such as impulse or forced oscillometry.(83, 84) It is still not clear whether these outcome measures can provide complementary information to FEV_1. Future studies could investigate these methods on EIB, in particular those with mild EIB. The threshold for a positive EVH test, particularly in asymptomatic elite athletes, is still under debate, as is the minimum

ventilation to be reached by young athletes.(61) The lack of concordance in the response to various indirect bronchial challenges in some athletic groups (particularly swimmers and cold-weather athletes) warrants further investigation to establish which test (if any) can be considered as a 'Gold Standard'.

Conclusion

The development of surrogate tests for the diagnosis of EIB has assisted with the understanding of the mechanisms of EIB. EIB is an osmotically-driven and inflammatory-mediated condition that is primarily triggered by the loss of water from the airways during conditioning of inhaled air during exercise-hyperpnea. In spite of some limitations, surrogate 'indirect' bronchial tests (in particular, EVH and mannitol) reproduce, in a standardised manner, the osmotic changes that occur within the airways during exercise. 'Indirect' tests therefore constitute valuable tools for the assessment and management of EIB.

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593

TABLE 1: The recommended withdrawal times for medications, foods and physical activity prior to performing challenge testing with exercise, eucapnic voluntary hyperpnea or inhaled mannitol

Medication / Activity / Food	Recommended time to withhold prior to challenge testing
Short acting beta$_2$ agonist (albuterol, terbutaline)	8 hr
Long acting beta$_2$ agonist (salmeterol, eformoterol)	24 hr
Long acting beta$_2$ agonist in combination with an inhaled corticosteroid (salmeterol/fluticasone, formoterol/budesonide)	24 hr
Ultra long acting beta$_2$ agonists (indacaterol, olodaterol, vilanterol)	≥72 hr
Inhaled corticosteroid (budesonide, fluticasone propionate, beclomethasone)	6 hr
Long acting inhaled corticosteroid (fluticasone furoate)	24 hr
Leukotriene receptor antagonists (montelukast, zafirlukast)	4 days
Leukotriene synthesis inhibitors (zileuton /slow release zileuton)	12 hr / 16 hr
Anti-histamines (loratadine, cetirzine, fexofenadine)	72 hr
Short acting muscarinic acetylcholine antagonist (ipratropium bromide)	12 hr
Long acting muscarinic acetylcholine antagonist (tiotropium bromide, aclidinium bromide, glycopyrronium)	≥72 hr
Cromones (sodium cromoglycate, nedocromil sodium)	4 hr
Xanthines (theophylline)	24 hr
Caffeine	24 hr
Vigorous exercise	>4 hr

FIGURE LEGENDS

Figure 1

A schematic outlining the key events triggered by exercise-hyperpnea and eucapnic voluntary hyperpnea (EVH) of dry air, i.e. two 'indirect' bronchial provocation challenges for EIB. The mannitol test (i.e. an osmotic 'indirect' challenge) mimics the effects of dry air hyperpnea by increasing the osmolarity of the airway surface. For all these stimuli, an important feature is the presence of airway inflammation, in particular the mast cell, in association with a sensitive airway smooth muscle. When the airway response is more severe, eosinophils may also get involved. 'Direct' tests (e.g., methacholine) act directly on the airway smooth muscle to cause bronchoconstriction.

Figure 2

An example of the relationship between eosinophilic airway inflammation (obtained from sputum induction) and the severity of EIB (as measured by the % fall in FEV_1 after exercise) in asthmatic subjects. While the mast cell mediators play a key role in the airway response to mannitol, the presence of the eosinophil can augment the airway sensitivity to exercise. While the absence of eosinophilia (<2% eosinophils representing the cut off for normal) does not exclude the presence of EIB, the airway response is often milder.(18)

Figure 3

An algorithm for the decision to perform an 'indirect' bronchial provocation test in persons with symptoms suggestive of EIB. The figure includes: the test options, test

626 outcomes, cut-off values for a positive test, and classification of the severity of the
627 airway response. Adapted from Weiler et al.(2)
628
629 **Figure 4**
630 A summary of the fundamental similarities and differences in the protocols required
631 to perform indirect tests to identify exercise-induced bronchoconstriction (EIB);
632 laboratory exercise, eucapnic voluntary hyperpnea (EVH) and the mannitol bronchial
633 provocation challenge test. *denotes common to all tests. *Note*. The highest FEV_1 is
634 taken to calculate % fall in FEV_1 at each time point.
635
636 **Figure 5**
637 Data taken from two studies (n=36) where sputum eosinophils have been obtained in
638 steroid-naïve subjects performing a mannitol challenge test.(77, 78) There is a
639 significantly higher levels of eosinophils in patients with severe to moderate airway
640 hyperresponsiveness (AHR) to mannitol (n=22)(grey dots), compared to those who
641 have mild AHR (n=14)(black dots); the latter also have normal levels of eosinophils
642 in sputum (<2% eosinophils) (left). It is considered mast cells are playing the primary
643 role in AHR to mannitol, while eosinophils, if present, augment the airway response.
644 There was a significant difference in the provoking dose (in mg) of mannitol to cause
645 a 15% fall in FEV_1 (PD_{15}) between the severe to moderate group compared to the
646 mild group.(right). INSET: A summary of the dose response curves in those with
647 severe, moderate and mild AHR to mannitol. ***p<0.001
648
649