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Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients

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Summary

Background Airway hyperresponsiveness (AHR) to stimuli that cause bronchial smooth muscle (BSM) contraction indirectly through the release of endogenous mediators is thought to reflect airway inflammation more closely compared with AHR measured by stimuli that act directly on BSM.

Methods Fifty-three adult non-smoking asthmatics (28 females, 18–56 years) who were not taking inhaled steroids were challenged with mannitol (up to 635 mg) and methacholine (up to 8 µmol). Induced sputum eosinophils, exhaled nitric oxide (eNO), peak flow variation and clinical severity of asthma according to the Global Initiative for Asthma guidelines were measured in addition to the health-related quality-of-life score using the Juniper asthma quality-of-life questionnaire.

Findings Both AHR to mannitol as well as to methacholine was associated with elevated markers of airway inflammation: in 83% of asthma patients with AHR to mannitol, and in 88% of asthma patients with AHR to methacholine, the eNO level was >20 p.p.b. Sputum% eosinophils >1% was measured in 70% of asthma patients with AHR to mannitol and in 77% of asthma patients with AHR to methacholine. In asthma patients without AHR, 15% had an eNO level >20 p.p.b., but none had sputum% eosinophils >1%. AHR to mannitol was more closely associated with the percentage of sputum eosinophils (PD₁₅ to mannitol vs. sputum% eosinophils: $r = -0.52$, $P < 0.05$), compared with AHR to methacholine (PD₂₀ to methacholine vs. sputum% eosinophils: $r = -0.28$, NS). Furthermore, there was a stronger correlation between AHR to mannitol and the level of eNO [PD₁₅ to mannitol vs. eNO (p.p.b.): $r = -0.63$, $P < 0.001$], compared with AHR to methacholine [PD₂₀ to methacholine vs. eNO (p.p.b.): $r = -0.43$, $P < 0.05$].

Interpretation In asthma patients not being treated with steroids, AHR to mannitol and to methacholine indicated the presence of airway inflammation. AHR to mannitol reflected the degree of airway inflammation more closely when compared with methacholine.

Keywords asthma, airway hyperresponsiveness, inflammation, mannitol, methacholine

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Introduction

Asthma is characterized by airway hyperresponsiveness (AHR) to a wide variety of stimuli. AHR is commonly measured using aerosols of pharmacological agonists, such as histamine and methacholine [1]. These agents act directly on bronchial smooth muscle (BSM), causing it to contract and the airways to narrow. AHR can also be

measured using stimuli that act indirectly through the release of mediators from inflammatory and neuronal cells in the airways. These stimuli include hyperpnoea of dry air as with exercise or eucapnic hyperventilation, aerosols of hypertonic saline or dry powdered mannitol or the pharmacological agent adenosine monophosphate (AMP) [2].

Because AHR to indirect stimuli is dependent on the presence of inflammatory cells and release of their

mediators in addition to a responsive muscle, it is thought that it should be more reflective of airway inflammation than airway geometry [3]. This concept is supported by the stronger association found between sputum eosinophils and AHR to the indirect stimulus AMP, compared with the association between eosinophils and AHR to the direct stimulus methacholine [4]. Similar findings might be expected using a dry powder of mannitol for inhalation, an indirect stimulus with many features in common with AMP [5, 6]. Mannitol acts as an osmotic stimulus to the cells in the airway mucosa and is thought to simulate the dehydration of the airway surface liquid that occurs as a result of evaporative water loss in conditioning the inspired air during exercise [5]. There is a significant relationship between the sensitivity to mannitol and other tests that act indirectly such as hypertonic saline, exercise, eucapnic hyperpnoea and AMP [6–8]. AHR to mannitol has been reported to be associated with improvement in symptom score and forced expiratory volume in 1 s (FEV₁) during treatment with inhaled steroids. Furthermore, AHR to mannitol is associated with deterioration of symptoms and peak flow as well as an increase in sputum eosinophils during dose reduction of steroids [9, 10]. However, there are no studies reporting the relationship between AHR to mannitol and to methacholine in relation to indices of airway inflammation in asthmatics not taking steroids at the time of the study.

The mannitol test has recently become commercially available in a kit form, making it easier and faster to administer than other challenge tests, taking approximately on average 15 min to perform, and only requiring a spirometer [5, 11].

We investigated the relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation [sputum eosinophils and exhaled nitric oxide (eNO)], and to variation in peak flow and quality of life (QOL) in a sample of adult asthma patients with a wide range of symptom frequencies who were not taking inhaled steroids.

Methods

Patients

Asthma patients, >18 years, were recruited through advertisements in local newspapers, and were included if they met the inclusion criteria of current asthma and the exclusion criteria of current smoking (past 6 months), current recent chest infection (past month) and lastly current or recent (past 3 months) use of oral or inhaled corticosteroids. A diagnosis of current asthma was made by a pulmonary physician who assessed all patients and on the basis of current asthma symptoms (past 4 weeks) in combination with current or previously detected peak

flow variation [$>20\%$ variation in peak expiratory flow rate (PEF)], reversibility to a β_2 -agonist (15% increase in FEV₁) or AHR to methacholine (PD₂₀ $<8 \mu\text{mol}$).

Study design

The study was performed at the Respiratory Research Unit, Bispebjerg Hospital, University Hospital of Copenhagen, Denmark. All participants visited the research unit twice [days between visits (median (range)): 7 (2–14)]: visit 1: interview, eNO, methacholine provocation, sputum induction. Visit 2: eNO, mannitol provocation, skin prick test (SPT), blood eosinophils. In order to avoid any effect of bronchial provocation with one agent on the outcome of provocation with another agent, there was an interval of at least 48 h between the two challenges. The study was approved by the local scientific ethics committee (protocol no. KF01-119/04); all subjects were informed and gave written consent.

Global Initiative for Asthma score

Asthma severity was further assessed and scored according to the 1999 Global Initiative for Asthma (GINA) guidelines on the basis of symptoms, the FEV₁% of predicted (FEV₁%pred) and the degree of peak flow variation [12].

Quality-of-life assessment

The Juniper asthma quality-of-life questionnaire [AQLQ(S)] on four domains of QOL – symptoms, activity limitation, emotional function and environmental exposures – was used [13]. The AQLQ score ranges from 1 (totally limited) to 7 (not at all limited); the minimal important difference for the AQLQ score is 0.5 [14].

Exhaled nitric oxide

At both visits, eNO was measured online, using the nitric oxide analyser (NIOX; Aerocrine AB, Solna, Sweden), a monitoring system that utilizes biofeedback to maintain a constant expiratory flow rate at the standard recommended rate of 0.05 L/s at each measurement [15]. The mean of three acceptable readings was taken, with at least 30 s of relaxed tidal breathing between manoeuvres, according to American Thoracic Society guidelines. The cut-off point for an increased level of NO was defined as 20 p.p.b. at a flow rate of 0.05 L/s [15].

Spirometry

Spirometry was performed according to the European Respiratory Society (ERS) recommendations [16]. The FEV₁ and forced vital capacity (FVC) were measured using

a 7 L dry wedge spirometer (Vitalograph, Buckingham, UK), which was calibrated weekly.

Bronchial provocation

Bronchial provocation with methacholine up to a cumulative dose of 8 µmol was performed with the Nebicheck nebulizer (#61650 DeVilbiss, Morgan, UK) using the dosimetric method described previously by Yan et al. [17]. A 20% decrease in FEV₁ to 8 µmol or less is regarded as a positive response for the methacholine test and indicates AHR. Bronchial provocation with mannitol powder (Aridol™; Pharmaxis, Frenchs Forest, Australia) contained in capsules and inhaled from an Osmohaler™ dry powder inhaler (Plastiap, Osnago, Italy) up to a cumulative dose of 635 mg was performed [5, 11]. Based on the findings in healthy non-asthmatics, a 15% decrease in FEV₁ to 635 mg or less is regarded as a positive response [5, 11] and indicates AHR. The cumulative dose of methacholine or mannitol required to provoke a 20% and a 15% fall in FEV₁, respectively (PD₂₀ and PD₁₅), was calculated by interpolation of the log-linear dose-response curve.

Sputum induction and processing

Sputum induction was conducted according to ERS recommendations [18]. After inhalation of 1 mg terbutaline, sputum was induced by inhalation of hypertonic saline in increasing concentrations (3%, 4% and 5%) for three time periods each of 6 min (total duration 18 min). Sputum plugs were selected and processed, and cytopspins were prepared using standard methods, and a differential cell count was performed [18]. Two cut-off points were used for the level of sputum eosinophils: one of >1%, based on the distribution in healthy subjects compared with asthmatics [19] and one of ≥3% based on the level of eosinophils that has been shown to best predict a response to treatment with steroids [20].

Skin prick test

A SPT to 10 aeroallergens [birch (betula), grass (phl. Praetense) mugwort, horse, dog, cat (fel d), house dust mite (Der p 1 and Der f 2) and fungi (alternaria and cladosporium) (ALK-Abello, Hoersholm, Denmark)] was performed in duplicate with an ALK-Abello lancet with a 1 mm tip according to the European Academy of Allergology and Clinical Immunology (EAACI) recommendations. A positive result was defined as a weal at least 3 mm in diameter to one or more allergens [21].

Peak flow variation

Peak flow monitoring was performed for two weeks (Mini Wright; Clement Clarke International, Harlow, UK). The

variation in PEF was expressed as the min%max, i.e. the variation from the highest peak flow value recorded to the lowest morning pre-bronchodilator peak flow recorded during the 2-week period [i.e. ((highest peak flow – lowest peak flow)/highest peak flow) × 100] [22].

Statistical methods

SPSS (version 14) was used for data analysis. Summary statistics include median (min–max values), mean ± standard deviation values and geometric mean (GM) (min–max values).

The characteristics of asthma patients with and without AHR to methacholine and to mannitol were compared separately: (1) asthma patients with AHR to methacholine vs. asthma patients with no AHR and (2) asthma patients with AHR to mannitol vs. asthma patients with no AHR. χ^2 analysis was used for comparisons of the distribution of categorical variables [frequency of atopy, rhinitis and previous smoking, distribution of asthma severity (GINA score), frequency of significant peak flow variation (>20%), elevated eNO level (>20 p.p.b.), sputum eosinophilia (>1%/ >3%) and sputum neutrophilia (>61%)]. For continuous variables, the two-sample *t*-test was used for normally distributed variables [FEV₁%pred, FEV₁/FVC ratio, AQLQ score and PEF variation (%)], and the Mann-Whitney test for non-normally distributed data (eNO and sputum% eosinophils, sputum% neutrophils, sputum% lymphocytes, sputum% macrophages and sputum% epithelial cells).

The relationship between the sensitivity to methacholine (PD₂₀) and to mannitol (PD₁₅) and markers of airway inflammation, spirometric values, PEF variation and AQLQ scores was analysed using Pearson's correlation coefficient (*r_p*) for continuous variables.

Non-normally distributed data were log-transformed for further analysis (eNO, sputum% eosinophils, sputum% neutrophils, sputum% lymphocytes, sputum% macrophages and sputum% epithelial cells).

The reproducibility of the eNO measurement was assessed using the intraclass correlation coefficient [ICC (Cronbach's α)].

Results

Fifty-three asthma patients (28 females) were examined. Most (87%) had FEV₁ values within the normal range i.e. FEV₁ ≥ 80% of predicted [14], but a wide range of asthma severity according to the GINA guidelines classification based on symptom frequency, peak flow variation and FEV₁%pred, with an almost equal distribution of subjects within all four GINA groups of asthma severity (Table 1).

Table 1. Patient characteristics I

	n = 53
Gender (females)	47.2%
Age [years, median (range)]	33 (18–56)
Atopy*	66.0%
Rhinitis†	70.2%
Ex-smokers [pack-years (median (min–max))]	41.5% [8 (1–40)]
Severity of asthma (GINA‡)	
Mild intermittent	22.6%
Mild persistent	20.8%
Moderate persistent	22.6%
Severe persistent	34.0%
Lung function	
FEV ₁ [L, mean (SD)]	3.60 (0.82)
FVC [L, mean (SD)]	4.70 (1.1)
FEV ₁ [% pred; mean (SD)]	90.9 (11.5)
FVC [% pred; mean (SD)]	96.9 (9.2)
FEV ₁ /FVC ratio [mean (SD)]	0.77 (0.07)
PEF variation >20%§	60.8%

*Positive skin prick test.

†Symptoms of rhinitis in the past 4 weeks.

‡Classified according to the GINA guidelines on symptoms, PEF variation and lung function.

§Min–max values, 14 days monitoring.

GINA, Global Initiative for Asthma; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow rate; SD, standard deviation; min, minimum; max, maximum.

Exhaled nitric oxide, sputum inflammatory cell counts

The median eNO level was 31 p.p.b. (range 7–231), and eNO was increased above 20 p.p.b. in 27 subjects (64%) (Table 2). The values for eNO measured on the two study days were comparable [ICC (Cronbach's α): 0.97].

Thirty-eight subjects (68%) were able to produce a satisfactory sputum sample, among whom 19 (50%) had significant eosinophilia (>1%) and 14 (37%) had an eosinophil percentage >3% (Table 2).

Airway hyperresponsiveness

Of the 53 asthma patients, 34 (64%) had AHR to mannitol and 34 (64%) had AHR to methacholine (Table 2). Twenty-eight asthma patients (53%) had AHR to both tests, whereas six only had AHR to mannitol and six only had AHR to methacholine. In total, 40 asthma patients (76%) had AHR to at least one of the two tests, whereas 13 did not have AHR to either mannitol or methacholine.

Characteristics according to airway hyperresponsiveness

In Tables 3 and 4, patient characteristics such as allergic sensitization, rhinitis, smoking history, lung function, asthma severity, health-related QOL and markers of inflammation are presented according to AHR status.

Table 2. Patient characteristics II – airway hyperresponsiveness (AHR) and airway inflammation

	n = 53
Airway responsiveness	
AHR to mannitol*	64.2%
AHR to methacholine†	64.2%
AHR to mannitol and methacholine	52.8%
PD ₁₅ to mannitol (mg)[GM (min–max)]	119 (6–617)
PD ₂₀ to methacholine (μ mol)(GM(min–max))	1.36 (0.08–7.76)
Exhaled NO	
Exhaled NO >20 p.p.b.	66.0%
Exhaled NO (p.p.b.) [median (min–max)]	31 (7–231)
Sputum (n = 38)	
Eosinophils >3%	36.8%
Eosinophils (%) (median, min–max)	1.1 (0.0–51.0)
Neutrophils (%) (median, min–max)	34.0 (1.1–90.5)
Lymphocytes (%) (median, min–max)	2.2 (0.0–12.3)
Macrophages (%) (median, min–max)	34.1 (5.8–85.5)
Epithelial cells (%) (median, min–max)	14.3 (0.0–76.3)
Total cell count ($\times 10^6$ /mL) (median, min–max)	2.65 (0.15–19.72)
Viability (%) (median, min–max)	71 (53–97)
Blood eosinophils ($\times 10^{-9}$ /L) [median (min–max)]	0.23 (0.03–0.52)

*Positive reaction to methacholine: PD₂₀<8 μ mol.

†Positive reaction to mannitol: PD₁₅< 635 mg.

NO, nitric oxide; min, minimum; max, maximum.

To illustrate the differences in these characteristics, asthma patients with AHR to methacholine and to mannitol were compared separately with asthma patients without AHR.

Asthma patients with AHR to mannitol or to methacholine had more severe asthma as defined by the GINA classification and a poorer AQLQ(S) score compared with asthma patients without AHR to either test (Table 3).

Furthermore, an elevated level of eNO (>20 p.p.b.) was more frequently observed in asthma patients with AHR to methacholine (88%) or to mannitol (82%) than in those without AHR (15%) (Table 4).

Significant eosinophilia ($\geq 1\%$) was observed in 69.6% of asthma patients with AHR to mannitol and 79.2% of subjects with AHR to methacholine, whereas none of the asthma patients without AHR had sputum eosinophils $\geq 1\%$ (Table 4).

Correlation between the degree of airway hyperresponsiveness to mannitol and to methacholine and the degree of peak flow variation, percent eosinophils in sputum, exhaled nitric oxide and the quality-of-life score

There was a significant positive correlation between the degree of AHR to mannitol (PD₁₅) and the eNO level, the % eosinophils in sputum, the variation in peak flow and the AQLQ score, whereas the degree of AHR to methacholine (PD₂₀) correlated significantly only with the eNO level and

Table 3. Patient characteristics according to airway hyperresponsiveness (AHR) status

	Mannitol positive [†] (n = 34)	Methacholine positive [‡] (n = 34)	Non-responders [§] (n = 13)
Atopy [*]	73.5%*	73.5%*	38.5%
Rhinitis	88.2%	94.1%	69.2%
Ex-smokers	51.5%*	47.1%	15.4%
Lung function			
FEV ₁ [% pred; mean (SD)]	89.7 (9.9)	89.1 (10.1)	95.5 (14.8)
FEV ₁ /FVC ratio [mean (SD)]	0.76 (0.08)	0.75 (0.07)	0.80 (0.07)
Severity of asthma (GINA ^{††})			
Mild intermittent	8.8%	11.7%	46.2%
Mild persistent	20.6%	20.6%	30.8%
Moderate persistent	32.4%	23.5%	0
Severe persistent	38.2%*	44.7%*	23.0%
Asthma quality-of-life score [mean (SD)] ^{‡‡}	5.54 (0.85)*	5.48 (0.83)*	6.1 (0.93)
Peak flow variation (PEF)			
PEF variation (>20%)	76.5%*	73.5%*	27.3%
PEF variation % [mean (SD)]	24.1 (9.1)*	23.7 (8.9)*	14.7 (7.0)

P-values: comparison of responders with non-responders.

* $P < 0.05$.

^{*}Positive skin prick test.

^{||}Symptoms of rhinitis.

^{††}Classified according to the GINA guidelines on symptoms, PEF variation and lung function.

^{‡‡}Asthma quality-of-life questionnaire by Juniper.

[†]Positive response to methacholine: $PD_{20} < 8 \mu\text{mol}$.

[‡]Positive response to mannitol: $PD_{15} < 635 \text{ mg}$.

[§]Negative responses to both tests.

GINA, Global Initiative for Asthma; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow rate; SD, standard deviation.

Table 4. Airway hyperresponsiveness (AHR) status and airway inflammation

	Mannitol positive [†] (n = 34)	Methacholine positive [‡] (n = 34)	Non-responders [§] (n = 13)
Exhaled NO			
Exhaled NO > 20 p.p.b.	82.4%*	88.2 (30)*	15.4%
Exhaled NO (p.p.b.) [median (min-max)]	52 (15-231)**	48 (10-231)**	14 (7-50)
Induced sputum (n = 38)			
Eosinophils > 1%	69.6%**	79.2%**	0
Eosinophils > 3%	56.5%*	56.0%*	0
Neutrophils > 61%	37.5%*	39.1%*	10.0%*
Eosinophils [% (median, min-max)]	4.6 (0.0-51.0)*	4.2 (0.0-51.0)*	0.0 (0.0-0.9)
Neutrophils [% (median, min-max)]	29.3 (1.1-80.5)	30.5 (1.1-90.5)	30.1 (4.0-77.5)
Lymphocytes [% (median, min-max)]	3.4* (0.0-12.3)	2.5* (0.0-12.3)	1.8 (0.3-4.5)
Macrophages [% (median, min-max)]	32.3 (5.8-85.8)	34.0 (7.8-85.5)	40.5 (12.0-66.1)
Epithelial cells [% (median, min-max)]	11.9 (2.0-76.3)	9.5 (0.0-76.3)	16.9 (0.0-54.6)
Blood eosinophils [median (min-max)]	0.26 (0.07-0.56)	0.26 (0.10-0.52)	0.13 (0.03-0.24)

P-values: comparison of responders with non-responders.

* $P < 0.05$.

** $P < 0.001$.

[†]Positive response to methacholine: $PD_{20} < 8 \mu\text{mol}$.

[‡]Positive response to mannitol: $PD_{15} < 635 \text{ mg}$.

[§]Negative responses to both tests.

NO, nitric oxide; min, minimum; max, maximum.

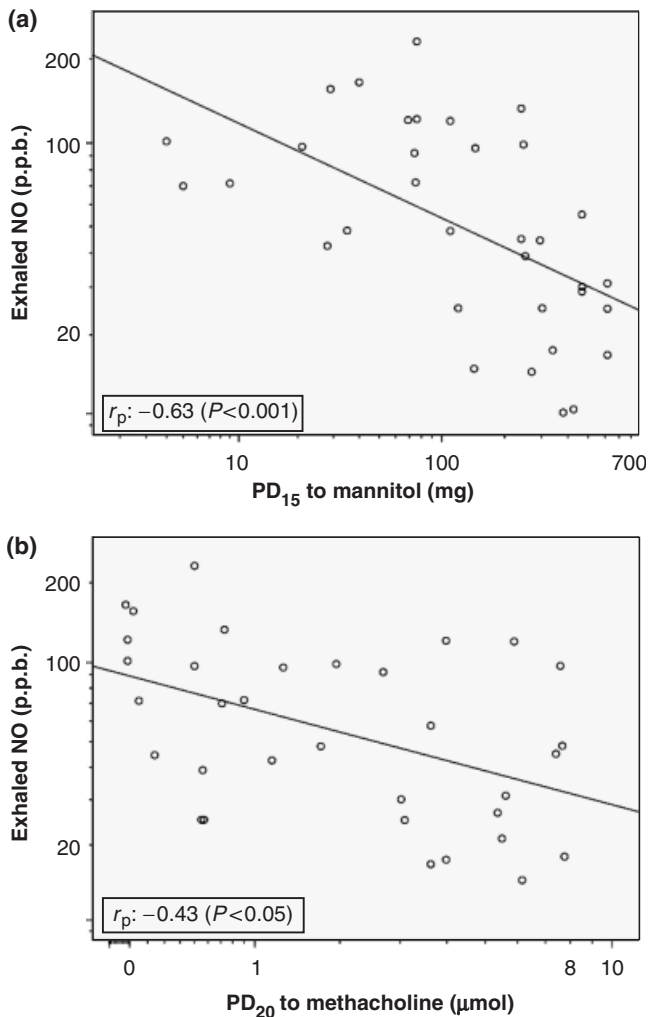


Fig. 1. (a) Scatterplot of PD₁₅ to mannitol vs. exhaled nitric oxide (eNO) (p.p.b.) and (b) PD₂₀ to methacholine vs. eNO (p.p.b.).

the variation in peak flow, but not with the % eosinophils in sputum, the FEV₁%pred, the FEV₁/FVC ratio or the QOL score (Fig. 1, Table 5). There was no correlation between the degree of AHR to mannitol or to methacholine and the percentage of neutrophils, lymphocytes, macrophages or epithelial cells in sputum (Table 5).

Discussion

In this group of symptomatic asthma patients, AHR to methacholine and to mannitol was strongly associated with elevated markers of inflammation such as sputum eosinophils and/or eNO. Furthermore, the degree of AHR to mannitol (PD₁₅) reflected the degree of eosinophilic airway inflammation and the level of eNO more closely compared with AHR to methacholine (PD₂₀). These findings suggest that AHR to both an indirect and a direct stimulus are concomitant features with inflammation at

Table 5. Correlation of between the response to mannitol and methacholine and inflammatory and clinical parameters

	PD ₁₅ mannitol	PD ₂₀ methacholine
PD ₂₀ methacholine	0.50**	–
Log eNO (<i>r_p</i>)	– 0.63**	– 0.43*
Log sputum% eosinophils (<i>r_p</i>)	– 0.52*	– 0.28
Log sputum% neutrophils (<i>r_p</i>)	0.04	– 0.03
Log sputum% lymphocytes (<i>r_p</i>)	– 0.13	– 0.10
Log sputum% macrophages (<i>r_p</i>)	0.04	0.01
Log sputum% epithelial cells (<i>r_p</i>)	0.34	0.24
FEV ₁ % of predicted (<i>r_p</i>)	– 0.24	– 0.03
FEV ₁ /FVC (<i>r_p</i>)	– 0.15	0.12
PEF variation (%) (<i>r_p</i>)	– 0.55*	– 0.42*
Total AQLQ score (<i>r_p</i>)	0.35*	0.32

**P* < 0.05.

***P* < 0.001.

eNO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow rate; AQLQ, asthma quality-of-life questionnaire.

least in asthmatics with a good lung function not being treated with inhaled steroids.

In order to describe the relationship between AHR and measures of airway inflammation without the potential confounding benefit of steroids, the subjects included were without steroid treatment for at least 3 months and most (83%) for 12 months even though all had current asthma symptoms. The absence of inhaled steroids may explain why we observed a positive association between the level of eNO and AHR to both methacholine and mannitol. In comparison, Leuppi et al. [23] were not able to find an association between the level of eNO and the PD₁₅ to mannitol in a group of asthma patients treated with inhaled steroids. Recent treatment with steroids may also explain why, in the study by van den Berge et al. [4], AHR to AMP correlated with the percentage of sputum eosinophils, but not with the level of eNO. The asthma patients included in their study were examined only 3 weeks or less after discontinuing treatment with inhaled steroids. It is possible that the inflammatory response in the airways was still attenuated to some degree [4]. The results in the present study confirm that in asthma patients who have not been treated recently with steroids, there seems to be a closer relationship between the degree of airway inflammation and AHR to indirect bronchoprovocation tests such as mannitol compared with a direct test such as methacholine.

The mechanism behind the AHR to mannitol and methacholine may both be related to airway inflammation, albeit via different mechanisms: methacholine acts on specific receptors on the BSM to cause contraction and the airways to narrow. The response to methacholine has been shown to be partly dependent on airway calibre, and

AHR to methacholine has been reported in non-asthmatics with a low airway calibre including healthy children and chronic obstructive pulmonary disease patients [1]. In the present study, there was no relation between AHR to methacholine and airway calibre as measured by baseline FEV₁ or the FEV₁/FVC ratio, most likely because most subjects had values for lung function within the normal range. Thus, airway inflammation, rather than airway calibre, appeared to be a more important determinant of airway responsiveness in these subjects. An indirect association between the response to methacholine and airway inflammation may partly be explained by structural and functional abnormalities of airway smooth muscle cells, as well as thickening of the basement membrane related to the remodelling that may be observed in relation to airway inflammation [24]. Furthermore, eosinophils in the airway mucosa release cytotoxic chemokines such as eosinophil cationic protein, which may enhance access of methacholine through the basement membrane to the smooth muscle cells [24].

Mannitol is an osmotic stimulus that causes the airways to narrow by release of bronchoconstricting mediators such as leucotrienes, prostaglandins and histamine [25, 26]. The source of these mediators is likely to be mast cells and eosinophils in the airways and both these cells release mediators *in vitro* in response to mannitol [27, 28]. Airway eosinophilia and an increased release of cysteinyl leucotrienes are involved in the airway pathology of exercise-induced bronchoconstriction (EIB) [29], and AHR to mannitol has been shown to have a high sensitivity to identify EIB in elite athletes [30]. The positive association between airway sensitivity to mannitol (PD₁₅) and the level of eosinophils and eNO in the present study is in keeping with the concept that airway sensitivity to mannitol is a reflection of airway inflammation to a greater extent than is sensitivity to methacholine. Further airway reactivity to mannitol has been shown to increase in association with increasing percentage of eosinophils in sputum in asthmatic subjects who are undergoing a step-wise down-titration of inhaled steroids [10].

We did not set a limit on lifetime tobacco consumption in our exclusion criteria and a decrease in lung function associated with high total tobacco consumption could have contributed to AHR particularly to methacholine. Volunteers who had a recent history of smoking were excluded from participation because of the impact current smoking may have on airway inflammation and AHR [31]. However, in the present group of asthmatics, most had a normal lung function and there was a similar proportion of never smokers and ex-smokers with AHR to methacholine and to mannitol, suggesting that previous smoking did not influence the present results.

A correlation between the clinical severity of asthma and the airway responsiveness to an indirect stimulus has been described previously [9, 32]. To assess the association

between the clinical severity of asthma and AHR to mannitol and methacholine, we used the classification system suggested by the GINA guidelines, which categorizes asthma severity on the basis of symptom frequency, FEV₁ and PEF variability [12]. We chose the GINA classification because it is widely available to many clinicians, and we wanted to be able to relate the results on the response to methacholine and to mannitol to a clinical asthma severity score that was recognizable to most clinicians. One shortcoming of such a clinical evaluation is that it is partly based on symptom frequency, which may tend to overestimate asthma severity, and the limitations of the GINA classification system are reflected in the present findings. While we did find that asthma patients with AHR tended to have more severe asthma according to the GINA classification compared with asthma patients without AHR, it was remarkable that more than half of the group of asthma patients without AHR had persistent asthma according to this classification (GINA 2+), for which treatment with inhaled steroids is recommended. As most of the 13 non-responders had a normal lung function (11/13, FEV₁ >80% predicted) and normal PEF variation (11/13), their severity classification was primarily based on symptom frequency. All participants in the present study had a diagnosis of asthma based on a combination of asthma symptoms and at least one measurement of reversible airflow obstruction, peak flow variation or AHR, and the group of asthma patients without AHR is likely to represent a subgroup with currently very mild asthma: considering the low level of markers of airway inflammation in these asthma patients, and the absence of AHR, it seems unlikely that they would benefit by anti-inflammatory treatment, which exemplifies the need for objective assessments of asthma patients before commencing treatment regimens.

We found that asthma patients with AHR were characterized by having poor asthma control with a decreased QOL and a significant degree of peak flow variation as well as by having elevated levels of eNO and sputum eosinophils, whereas non-responders had few signs of active asthma or airway inflammation. This supports previous findings of asthma patients who have AHR to mannitol demonstrating a benefit with inhaled steroids [9] and the response to mannitol returns with the withdrawal of inhaled steroids [8]. However, in order to extrapolate the present results to a more general population of asthma patients encountered in the clinical setting, a study describing the response to mannitol in relation to markers of airway inflammation in a non-selected population of asthma patients consecutively referred to a pulmonary specialist is required.

In conclusion, the present study shows that, in subjects with current asthma who are not treated with steroids, the AHR to mannitol more closely reflected the degree of airway inflammation than did AHR to methacholine.

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