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EARLY RISE IN EXHALED NO AND MAST CELL ACTIVATION IN REPEATED LOW DOSE ALLERGEN CHALLENGE

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ABSTRACT

Aims: Repeated low dose allergen inhalation challenge mimics natural allergen exposure, providing a model for early mechanisms in the triggering of asthma. We performed a controlled study to evaluate the time course of changes in exhaled nitric oxide (F_{ENO}) and urinary biomarkers of airway inflammation.

Methods: Eight subjects with mild allergic asthma completed two seven-day repeated low-dose challenge periods, with diluent and allergen, respectively. Subjects were symptom free at inclusion and investigated when not exposed to specific allergen. Pulmonary function and symptoms were followed, and F_{ENO} and urinary mediators were correlated to changes in airway responsiveness to histamine and adenosine.

Results: Despite no change in pulmonary function (FEV_1 mean fall ($\pm$ SE): $0.3(\pm 0.7)$ vs $0.6(\pm 1.0)$ %, for diluent and allergen, respectively) and no asthma symptoms, repeated allergen exposure, in contrast to diluent, caused significant increases in histamine responsiveness (2.3 doubling doses), an early and gradual increase in F_{ENO} (up to a doubling from baseline) and a small increase in the mast cell marker $9\alpha11\beta$ -PGF₂ after adenosine challenge.

Conclusion: Serial measurements of F_{ENO} have the potential to provide a very sensitive strategy for early detection of emerging airway inflammation and subsequent changes in airway hyperresponsiveness to histamine.

INTRODUCTION

Repeated low-dose allergen inhalation challenge has been introduced as a method to mimic and standardise natural exposure to environmental allergens [1]. In this challenge setting, patients with atopic asthma are subjected to inhalations of fixed doses of allergen, titrated to cause minimal bronchoconstriction and administered once daily on four to ten consecutive weekdays [1,2,3,4,5,6]. The procedure generates a distinct increase in airway hyperresponsiveness (AHR) to direct bronchoconstrictors [1- 6]. The increase in AHR occurs despite only few symptoms of asthma are reported by the subjects. The challenge model is therefore particularly suitable to investigate early events in the development of AHR, a central feature of the asthmatic phenotype. The relevance of the model is supported by the established effects of inhaled corticosteroids on AHR and sputum eosinophilia induced by the low-dose challenge procedure [7,8].

Investigations of mechanisms of airway inflammation in this particular model have, however, so far been rather limited, and findings in peripheral blood are largely negative [5,7,9]. In a diluent controlled evaluation of the challenge, the percentage of eosinophils, interleukin (IL)-5 and ECP in induced sputum were shown to increase [2], and early effects on eosinophils and macrophages in bronchoalveolar lavage fluid have also been reported [9,10]. The aim of this study was to further characterise the influence of repeated low-dose allergen challenge on the development of airway inflammation. The primary endpoint was to establish if there was an association between exhaled nitric oxide ($F_{E}NO$) and changes in airway responsiveness during repeated low-dose allergen exposure. This would provide evidence on whether or not $F_{E}NO$ is an appropriate early marker of airway inflammation that may precede symptomatic exacerbations of asthma. Previous data suggest increased $F_{E}NO$ during low dose allergen exposure [8], but in that particular study, the increase in $F_{E}NO$ was

not associated with an increase in airway responsiveness to methacholine, nor was diluent control used.

As secondary surrogate marker of inflammation, urinary levels of leukotriene E₄ (LTE₄) were measured along with urinary levels of the prostaglandin D₂ metabolite, 9α11β-prostaglandin F₂ (PGF₂), which serves as a specific marker of mast cell activation [11]. For example, the allergen-induced early and late asthmatic response in the conventional high dose allergen challenge is associated with an increase in urinary levels of 9α11β-PGF₂ [12]. While the activation of eosinophils has been shown to occur in the low-dose challenge [2,7,8], the activation of mast cells has been less explored. It is becoming increasingly evident that the pathogenetic role of the eosinophil in asthmatic airway inflammation is uncertain [eg.13]. We hypothesised that priming of mast cells takes place during the development of AHR and that measurements of urinary excretion of 9α11β-PGF₂ could test this hypothesis. As another attempt to test whether mast cells were activated, challenges with adenosine 5'-monophosphate (AMP) were included in the protocol. Adenosine 5'-monophosphate is an indirect bronchoconstrictor which, by acting primarily on mast cells, causes release of bronchoconstricting mediators [14,15,16]. Responsiveness to AMP has been suggested to be more closely associated with airway inflammation than challenges with direct bronchoconstrictors such as methacholine [17], and population studies have shown a relation between sensitization to common aeroallergens and AMP responsiveness [18]. The effects of the low dose allergen challenge procedure on responsiveness to AMP have not been assessed previously.

Thus, with a two-period, cross-over design, subjects allergic to pollen or animal dander and with no current asthma symptoms, were exposed to low doses of inhaled allergen and its diluent, respectively, for seven consecutive weekdays. Bronchial responsiveness to histamine and AMP was assessed before and after the two challenge periods, urinary mediator

metabolites were measured and determinations of $F_E\text{NO}$ were performed throughout the respective periods. In addition, a control group of eight healthy individuals were subjected to diluent challenge, using the same study protocol, to establish if baseline $F_E\text{NO}$ values and urinary markers differ over time between subjects with mild asthma and healthy individuals.

METHODS

Subjects

Eight non-smoking subjects with a history of asthma symptoms on exposure to pollen or animal dander were recruited to the study (Table 1). They were required to have a positive screening histamine challenge with a PD₂₀ FEV₁ (provocative dose causing a 20% fall in FEV₁) in the range of 110 to 2090µg, and a positive skin prick test to birch pollen, timothy grass pollen and/or animal dander but not to house dust mite. Pollen-sensitive subjects were investigated outside the pollen season and subjects sensitive to animal dander did not have pets of their own and were asked to strictly avoid animal contact. Thus on entering the study, subjects were asymptomatic, their asthma had been stable and they had had no respiratory tract infection for the last four weeks. Their sole medication was occasional use of β₂-agonists. A screening allergen challenge was performed with the allergen considered of most clinical importance in each individual, and their current sensitivity expressed as PD₂₀ FEV₁ was calculated (Table 1). Four of the eight subjects displayed a dual (early and late) response.

A group of eight non-smoking, age and sex matched healthy control subjects without a history of allergy or asthma were included (seven females and one male; age 24-43 years; mean baseline FEV₁ 106 % of predicted [range; 93-125%]). All control subjects were skin prick test negative and displayed a negative screening histamine challenge (PD₂₀ FEV₁ >2,090µg).

The study protocol was approved by the Local Ethics Committee of Karolinska Hospital (Dnr: 98:248), Stockholm and all subjects gave their informed consent to participate. All eight asthmatic subjects and eight control subjects completed the study.

Study design

Four weeks after the screening allergen challenge, the asthmatic subjects entered a two-period, cross-over repeated low-dose inhalation challenge with allergen and diluent, respectively. A summary of the study protocol is presented in Table 2.

Since our original description of the low-dose challenge [1] suggested that the increase in airway responsiveness might take some weeks to resolve, we chose to perform the diluent challenge single-blindly in the first period followed by the allergen challenge period after a four week wash-out. The subjects, the study technician measuring F_{ENO} , and the investigators analysing urinary mediators were, however, not aware of the predefined sequence and the study procedure was identical during the respective period.

First, a histamine challenge was performed followed by an AMP challenge on the next day. After the weekend, always starting on a Monday, the repeated low-dose allergen/diluent inhalation period started. On the day after the last allergen/diluent inhalation, a second histamine challenge was done and on the following day a second AMP inhalation challenge. The control subjects were subjected to the same procedure, but received the diluent challenge only.

Inhalation challenges

Subjects reported to the clinic at the same time in the morning (7:30-8:00 am) on all challenge days. Beta₂-agonists were not allowed for 8 h prior to challenge. Lungfunction was measured as FEV₁ on a spirometer (Vitalograph MDI Compact; Förbandsmaterial, Stockholm, Sweden), and the baseline, defined as the best of three recordings, had to be $\geq 70\%$ of predicted. Allergen, histamine and AMP provocation tests were performed by the use of a dosimeter controlled jet nebulizer (Spira Elektro 2, Respiratory Care Center, Hameenlinna, Finland) as described [19]. Challenges started by the inhalation of diluent. Provided FEV₁ did not change by more than 10%, the rising dose bronchoprovocation with the respective

active substance was started, and the postdiluent FEV₁ value used as baseline. At the screening allergen challenge, half-log increments in the cumulated dose of allergen (range 7-7,100 SQ Aquagen™, ALK, Copenhagen, Denmark) were inhaled every 15 minutes until a 20% fall in FEV₁ was obtained. Bronchial responsiveness to histamine (histamine diphosphate prepared by The Karolinska Hospital Pharmacy) was assessed with a similar protocol but with dose increments every third minute. Two concentrations (1.6 mg/mL and 16 mg/mL) and a variable number of breaths were used to create increasing cumulative doses (range 11-2,090 µg) of the agonist [19]. The AMP challenges employed a protocol using doubling concentrations every 5 minutes (1.56-400 mg/mL, Sigma chemical Co, St Louis, US) [20]. The PD₂₀-values for allergen, histamine and AMP, respectively, were calculated by linear interpolation from the log dose-response curves.

Low-dose allergen inhalation challenge

The dose of allergen causing an early fall in FEV₁ of approximately 5% (PD₅) was determined from the screening allergen challenge in each individual (Table 1). The allergen PD₅ was then administered as a single challenge every day for 7 successive weekdays, with a break for the weekend (Table 2). The asthmatic patients inhaled the diluent by the same number of breaths as the allergen dose during the respective challenge periods, whereas the control subjects inhaled the diluent by 3 breaths. Spirometry recordings were taken before and 15 min following inhalation. Patients recorded morning and evening peak expiratory flow rate (PEFR) values during the study (Jaegher electronic peak flow meter), and they were requested to make further recordings in the event of asthma symptoms. Day and night time asthma symptom scores (0= no symptoms, 1=mild symptoms, 2=moderate symptoms and 3= severe symptoms) and short-acting β₂-agonist usage were also recorded on each study day.

NO measurements in exhaled air

F_ENO was measured according to the recommendations for online NO measurements published by the American Thoracic Society (ATS) [21], using an Aerocrine prototype NO system (Aerocrine AB, Stockholm, Sweden), including a CLD 77 AM chemiluminescence analyser [Eco Physics AG, Dürnten, Switzerland; sensitivity, 0.1 parts per billion (ppb) NO; rise time 0–90%, <0.1 s; sample flow rate, 110 ml/min; lag time from mouthpiece, 0.7 s] for online NO measurements, and a pneumotachygraph for monitoring of flow and pressure. Exhalation rate (250 ml/s) was kept constant by visual feedback during exhalation at 5 cm H₂O through a linear flow resistor (Hans Rudolph Inc, Kansas City, Kansas, USA). A two-point calibration was performed before each study session using mass-flow controlled dilutions of certified calibration gas (stock concentration 2 parts per million NO in N₂; AGA AB, Älvsjö, Sweden).

Urine analyses

On the AMP challenge days, urine was collected upon arrival just before the start of the challenge and one hour after the last dose of AMP. Likewise, a pre dose urine sample was obtained on challenge days 1 and 7 during each repeated low-dose period, whereas the post dose sample was taken at 30 minutes after the inhalation. The samples were frozen and the concentrations of 9 α 11 β -PGF₂ and LTE₄ were determined according to a validated semi-automated enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA) methodology [22,23].

Statistical analyses

For the primary outcome the sample size of eight subjects was based on our own measurements allowing for the detection of a 50% increase in F_ENO with 80% power and alpha=0.05. This is in agreement with the publication by Kharitonov et al [24] on the repeatability and sample size estimates for F_ENO measurements.

Calculations of geometric mean PD₂₀, PD₁₀ and PD₅ values were performed on log-transformed raw data. The data for pulmonary function, NO-values and urinary mediators were found to be normally distributed, and analysis of variance and paired t-tests were used to compare different periods and different treatment groups. Data are generally presented as mean $\pm$ SE. Differences were considered to be significant when p was less than 0.05. Statistical calculations were performed by the use of a validated statistical software package for personal computers (SigmaStat, Jandel Scientific, Sausalito, CA).

RESULTS

Lung function

The repeated low-dose allergen challenge did not affect baseline pulmonary function in the subjects with asthma. Thus, FEV₁-values obtained each day before low-dose allergen inhalation were no different from values during the diluent period (Figure 1a). Baseline values on the respective histamine and adenosine challenge days were also stable throughout the whole study and showed no significant variability between visits ($p = 0.16$).

None of the subjects had an early asthmatic response following the low dose allergen challenge. The group mean changes in FEV₁ during each challenge day are displayed in Figure 1b with the mean ($\pm$ SE) immediate fall in FEV₁ during the allergen challenge and diluent period being 0.6 ($\pm$ 1.0) % and 0.3 ($\pm$ 0.7) %, respectively. Neither did any subject exhibit a clinically significant late fall in lung function as assessed by PEF_R measurements and symptom reporting during the respective challenge period (data not shown).

The group of healthy controls had normal lung function that did not change during the observation period (mean ($\pm$ SE) FEV₁; 104 $\pm$ (13.6) vs 105 ($\pm$ 13.9) per cent predicted on challenge day 1 and 7, respectively, $p = 0.39$).

Symptoms

None of the subjects reported any night-time symptoms of asthma or use of β_2 -agonists, during either challenge period. During the diluent period no subject reported any symptoms of asthma (symptom score =0) whereas during the low-dose allergen period four subjects reported mild symptoms (symptom score =1) on occasional days (group mean ($\pm$ SE) symptom score: 0.16 [$\pm$ 0.07]).

Airway responsiveness to histamine

Repeated low-dose allergen inhalation produced an increase in the airway responsiveness to histamine in all eight subjects (Figure 2). There was a significant reduction in geometric

mean histamine PD₂₀ following the allergen period (724; range (324-1,622) µg before, vs 316 (166-603) µg after, $p < 0.01$), corresponding to 2.3 doubling doses. In contrast, histamine PD₂₀ was unaffected by repeated doses of the diluent 457(178-1,175) µg before vs 562(302-1,047) µg after, $p=0.48$) (Figure 2). While there was no variability in pre challenge histamine responsiveness between the two periods ($p>0.05$), the shift in histamine PD₂₀ was significantly different between the challenge periods (Log shift histamine PD₂₀: -0.36 vs 0.09 for allergen and diluent, respectively, $p<0.01$).

Exhaled NO

The group mean ($\pm$ SE) F_ENO increased from 8.6 ± 1.4 ppb before the allergen challenge period to 14.7 ± 2.3 ppb 24 hours after the last allergen inhalation ($p<0.05$) (Figure 3). In contrast, there was no significant change in F_ENO during the diluent period (9.8 ± 1.7 ppb before vs 10.4 ± 1.6 after; $p>0.05$). When assessed for the individuals, there was however no significant mathematical correlation between the change in NO levels during the allergen period and the corresponding decrease in histamine PD₂₀ values ($r = 0.394$, $p = 0.33$).

There were gradually increasing levels of F_ENO in exhaled air during the low-dose allergen period, in contrast to the diluent period where the levels remained stable (Figure 4). The levels of F_ENO were also stable in the healthy control group (Figure 4). However, the mean levels of F_ENO during the observation period were significantly lower in the healthy control group in comparison with the asthmatic subjects ($p<0.05$) (Figure 4).

Interestingly, the coefficient of intra-individual variability of F_ENO in the asthmatic subjects was 20.7% (range 6.7-37) during the allergen challenge period and 12.8% (range 6.7-31) during the diluent period, similar to the coefficient of variability for F_ENO in the healthy subjects (14.2% [range 9.2-25]).

Airway responsiveness to adenosine

While all eight subjects with asthma by design were hyper-responsive to histamine, only four produced a PD₂₀ for AMP of less than 1,600 mg/ml at inclusion. In these subjects, there was no significant increase in responsiveness to AMP 48 hours after repeated allergen challenge in comparison to the diluent period (log shift PD₂₀ AMP being -0.37 and 0.17 after allergen and diluent, respectively, p=0.14). It was possible to calculate PD₁₀ AMP in seven subjects, but the shift in AMP responsiveness during the allergen period was not significantly different from the diluent period (log shift PD₁₀ AMP -0.18 vs 0.17 after allergen and diluent, respectively, p=0.06).

Urinary mediators

Baseline morning concentrations of urinary 9 α 11 β -PGF₂ and LTE₄ remained unaffected during either challenge period (Table 3). However, the asthmatic subjects had significantly higher baseline concentrations of urinary 9 α 11 β -PGF₂ (Figure 5a) and LTE₄ (Figure 5b) compared with the healthy control subjects.

There was no increase in the urinary excretion of either mediator after the allergen challenges on day 1 and 7 (Table 3). There was, however, an increase in urinary excretion of 9 α 11 β -PGF₂ after adenosine challenge at the end of the repeated allergen exposure period, but not at the end of the diluent period (Table 3). Urinary levels of LTE₄ were unchanged after adenosine challenge during both periods (Table 3).

DISCUSSION

The study documented increased airway responsiveness to histamine following seven repeated low dose allergen exposures during a nine day period in subjects with mild asthma. At the same time, the subjects had no symptoms of asthma and did not need to use rescue bronchodilator medications. The increased airway responsiveness was furthermore obtained despite the subjects had no change in baseline airway calibre. The study therefore adds to the indications that the low dose allergen challenge method is well suited to study both the early events in allergic airway inflammation and the mechanisms involved in AHR. In contrast to most previous studies using different protocols for low dose allergen challenge, we included a diluent period as it was felt that subjects with asthma according to the natural course of the disease may display increased spontaneous variability in airway responsiveness and inflammation. Furthermore, we also included a group of healthy individuals inhaling diluent for a similar challenge period. Another measure to enhance the resolution of the model was to minimize the accidental environmental exposure to the study allergens. We therefore studied subjects out of their season and specifically excluded house dust mite allergic subjects as elimination of that particular natural exposure is very difficult and presumably introduces a confounding background trigger of inflammation in many studies.

The main finding in the study was that of a progressive increase in NO-levels in exhaled air during the repeated low dose allergen exposures. The mean value of $F_E\text{NO}$ was thus almost doubled after the allergen challenges whereas the levels of $F_E\text{NO}$ were unchanged and stable after repeated challenge with the diluent in the same subjects. Our finding thus confirms and extends the reports from one group of investigators of increased exhaled NO levels after repeated low dose allergen challenge [8,25]. However, the increase in exhaled NO was only associated with increased AHR to methacholine in one of their studies [25]. As the subjects in our study had no symptoms of asthma nor changes in lung function, our

finding supports the hypothesis that increased $F_{E}NO$ may be an early and very sensitive sign of increased airway inflammation. It has been documented that measurements of $F_{E}NO$ at four to eight week intervals may improve the dosing of inhaled steroids required to achieve asthma control [26]. We found an early and progressive rise of $F_{E}NO$ within a few days after the start of the allergen challenge period. This suggests that monitoring of $F_{E}NO$ on a daily basis might represent a strategy for early detection of exacerbations, providing the opportunity for treatment before the exacerbation has reached the “point of no return” where no treatments so far have been able to abrogate an exacerbation [27].

Our study also showed that even subjects with mild untreated asthma had higher baseline levels of $F_{E}NO$ than a group of healthy controls. During the diluent period, the asthmatic subjects nevertheless had stable levels of $F_{E}NO$ and the day-to-day variability was similar to that of the healthy controls. In contrast, during the allergen period, the variability in $F_{E}NO$ levels increased, suggesting that another early sign of increased airway inflammation may be increased day-to-day variability in $F_{E}NO$.

The secondary end-point in the study was to evaluate the usefulness of monitoring urinary levels of LTE_4 and the mast cell marker $9\alpha11\beta-PGF_2$. It was found that when assessed on four occasions during a ten day period, the asthmatic subjects had elevated baseline levels of both mediators in the urine compared with the healthy controls. Although the difference was small, it was significant and indicates that by performing repeated sampling one may detect differences even in relatively small groups of subjects. Most studies that have failed to find significant differences in urinary LTE_4 between healthy subjects and subjects with mild asthma [28] have compared cross-sectional data comprised of single measurements that clearly have insufficient power to detect small differences.

In the subjects with asthma, there was however no progressive change in baseline urinary excretion of either mediator during the allergen challenge period. The only significant

difference that was found on the group level, was an increase in $9\alpha11\beta$ -PGF₂ excretion after the AMP challenge 48 hours after the allergen period. Urinary $9\alpha11\beta$ -PGF₂ is established as the most sensitive method for detection of mast cell activation [11]. The finding of increased $9\alpha11\beta$ -PGF₂ therefore supports priming of the mast cells during the allergen period or increased infiltration of mast cells, in particular as the dose of AMP was similar before and after the period. Our observation suggesting increased mast cell activation may relate to the report of increased numbers of methachromatic cells in sputum in another low dose allergen challenge study [2], incidentally the only other study that has used a diluent control arm. Admittedly, the effect we observed of AMP on PGD₂ release was small, and further studies are required to establish this mechanism. As we did the AMP provocations 48 hours after the last allergen inhalation, it might also be that we missed the peak of increased mast cell activation. In addition, given that the predetermined PD₅ dose resulted in only small falls in FEV₁, it is conceivable that larger repeated allergen doses might have produced significant changes in urinary markers or in airway responsiveness to AMP.

One reason for the modest increase in the mast cell product PGD₂ in our study might relate to the relative insensitivity to AMP that was obtained with the protocol we used. Thus, only four out of eight subjects produced a PD₂₀ for AMP before the study period. As AMP challenges have been proposed to be very specific for asthma, it is a little surprising that only half of the subjects in a group with mild asthma displayed significant responsiveness to AMP. However, our protocol for AMP challenges may not have been optimal.

Obviously our data support the conclusion that increased F_ENO is a more sensitive and early marker of airway inflammation than urinary LTE₄. There may in fact be a mechanistic explanation of the unchanged levels of urinary LTE₄ in our study. Thus, it was recently observed in a model of the peripheral lung that NO specifically inhibits allergen-induced release of leukotrienes [29]. It might be that the increased levels of NO during early airway

inflammation in fact represents an important protective mechanism intended to limit the development of the inflammation.

While the debate on whether NO predominantly is pro- or anti-inflammatory continues, our study meanwhile undoubtedly supports that frequent serial measurements of F_ENO have the potential to provide a very sensitive strategy for early detection of emerging airway inflammation. Moreover, the low dose allergen challenge model [30] is here to stay as it provides opportunities for safe and controlled investigations of mechanisms in airway hyperresponsiveness and airway inflammation in the absence of baseline bronchoconstriction and ongoing asthmatic symptoms.

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Table 1: Characteristics of subjects with asthma

Subject no	Age	Sex	FEV ₁ % predicted	PD ₂₀ Histamine μ g	Allergen	SPT mm	PD ₂₀ Allergen SQ	Late reaction	PD ₅ Allergen SQ
1	32	F	85	620	Birch	5.5	500	No	100
2	25	F	96	788	Cat	9.5	350	No	14
3	40	F	106	1864	Cat	9.5	1525	Yes	213
4	29	F	81	420	Birch	6.5	500	Yes	21
5	29	F	89	128	Cat	12.0	170	Yes	7
6	41	F	103	197	Timothy	14.5	27	No	7
7	32	F	98	186	Cat	8.5	350	Yes	112
8	26	M	102	1069	Cat	14.5	270	No	42
Mean			95	457*			295*		33*
Range			81-106	128-1864			27-1525		7-213

PD₂₀= Provocative dose causing a 20% fall in FEV₁

PD₅= Provocative dose causing a 5% fall in FEV₁

SPT – skin prick test mean diameter

* - geometric mean

Table 2: Schedule for each study period

Visit 1 Thursday	Visit 2 Friday	Visit 3 Monday	Visit 4 Tuesday	Visit 5 Wednesday	Visit 6 Thursday	Visit 7 Friday	Visit 8 Monday	Visit 9 Tuesday	Visit 10 Wednesday	Visit 11 Thursday
F _E NO FEV ₁ PD ₂₀ Hi	F _E NO FEV ₁ PD ₂₀ AMP urine	F _E NO FEV ₁ Ag/Dil #1 urine	F _E NO FEV ₁ Ag/Dil #2	F _E NO FEV ₁ Ag/Dil #3	F _E NO FEV ₁ Ag/Dil #4	F _E NO FEV ₁ Ag/Dil #5	F _E NO FEV ₁ Ag/Dil #6	F _E NO FEV ₁ Ag/Dil #7 urine	F _E NO FEV ₁ PD ₂₀ Hi	F _E NO FEV ₁ PD ₂₀ AMP urine

Abbreviations: F_ENO = Nitric oxide; FEV₁ = Forced expiratory volume in one second; PD₂₀= Provocative dose causing a 20% fall in FEV₁ ; Hi = histamine; AMP = Adenosine 5' -Monophosphate; Ag/Dil = Allergen or Diluent inhalation

Table 3: Excretion of mediators in urine (asthmatic subjects, n=8, mean $\pm$ SE, ng/mmol creatinine)

	Diluent period		Allergen period	
	9 α ,11 β -PGF ₂	LTE ₄	9 α ,11 β -PGF ₂	LTE ₄
Before AMP visit 2	77 $\pm$ 8	50 $\pm$ 8	101 $\pm$ 28	58 $\pm$ 9
After AMP visit 2	83 $\pm$ 6	47 $\pm$ 5	110 $\pm$ 30	51 $\pm$ 5
Before Ag/Dil visit 3	76 $\pm$ 12	58 $\pm$ 18	74 $\pm$ 8	46 $\pm$ 6
After Ag/Dil visit 3	82 $\pm$ 13	61 $\pm$ 14	94 $\pm$ 21	49 $\pm$ 3
Before Ag/Dil visit 9	83 $\pm$ 9	44 $\pm$ 5	84 $\pm$ 12	49 $\pm$ 4
After Ag/Dil visit 9	79 $\pm$ 7	53 $\pm$ 8	85 $\pm$ 13	53 $\pm$ 4
Before AMP visit 11	101 $\pm$ 10	71 $\pm$ 18	77 $\pm$ 10	46 $\pm$ 4
After AMP visit 11	119 $\pm$ 30	57 $\pm$ 3	101 $\pm$ 13*	46 $\pm$ 4

Abbreviations: AMP = Adenosine 5'-Monophosphate; Ag/Dil = Antigen or Diluent inhalation

* = p<0.05 vs value before challenge; p-value for all other pre- post comparisons > 0.05

Figure legends

1. Pulmonary function in asthmatic subjects. a) Baseline FEV₁ and b) per cent fall in FEV₁ 15 min after dosing during the diluent (○) and the repeated low dose-allergen (●) challenge period, respectively.
2. Individual and geometric mean histamine PD₂₀ values before and after the respective allergen and diluent challenge periods in subjects with asthma.

p<0.05 between changes during diluent vs allergen challenge, as well as before and after the allergen challenge period.
3. Individual and group mean concentrations of exhaled NO before and after the respective allergen and diluent challenge periods.

p<0.05 between changes during diluent vs allergen challenge, as well as before and after the allergen challenge period.
4. Time course of F_ENO measurements taken before allergen or diluent inhalation during the respective challenge periods (●: allergen in asthmatic subjects; ○: diluent in asthmatic subjects; open triangle: diluent in healthy control subjects).
5. Baseline urinary concentrations of a) 9α11β-PGF₂ and b) LTE₄ on visits 2, 3, 9 and 11 in subjects with asthma (○) and in healthy controls (open triangle) during the diluent period.

p<0.05 between mean values of the respective mediator level.

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Figure 1a

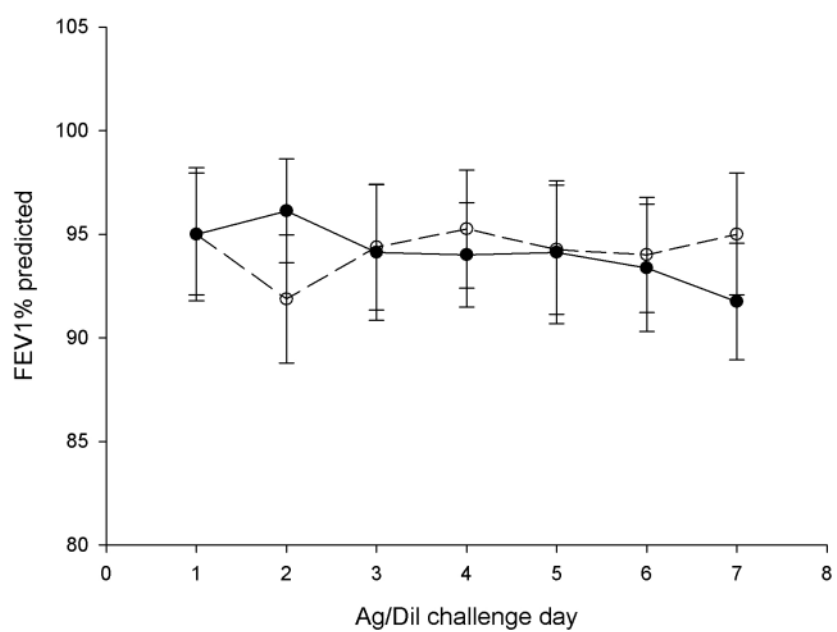


Figure 1b

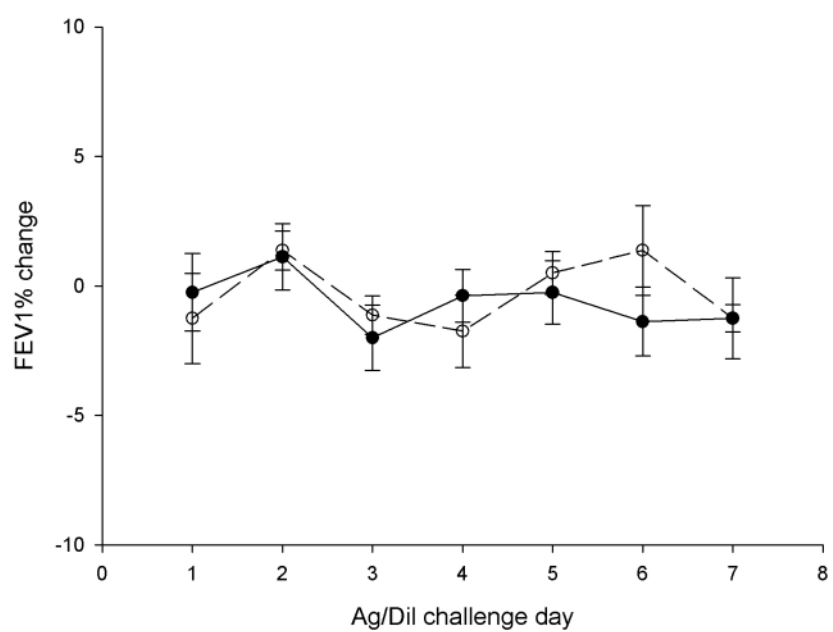


Figure 2

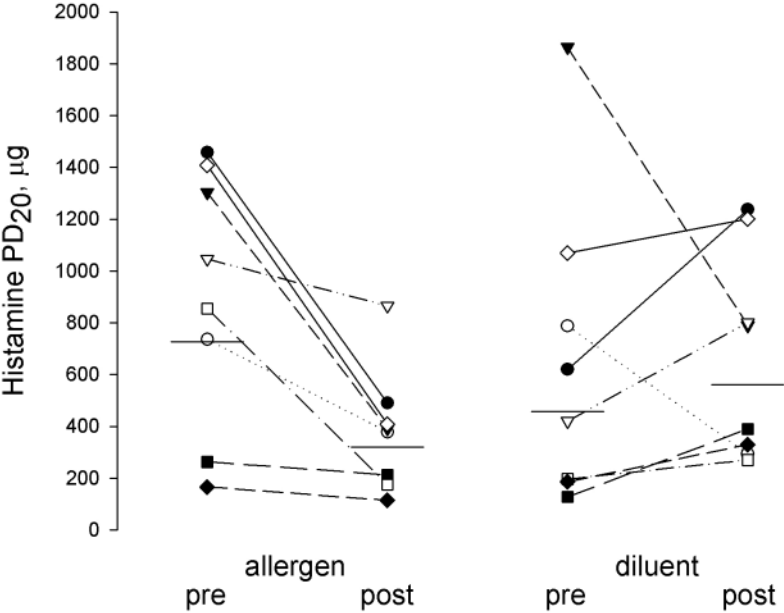


Figure 3

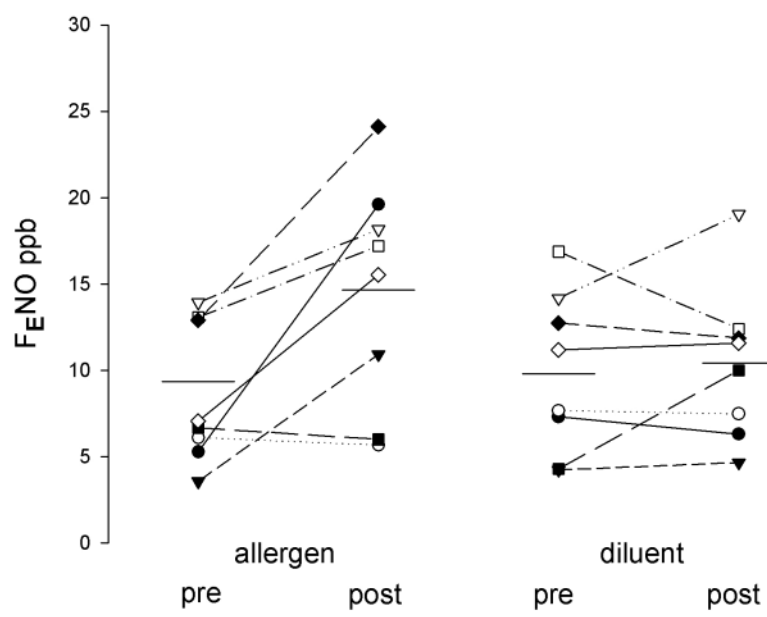


Figure 4

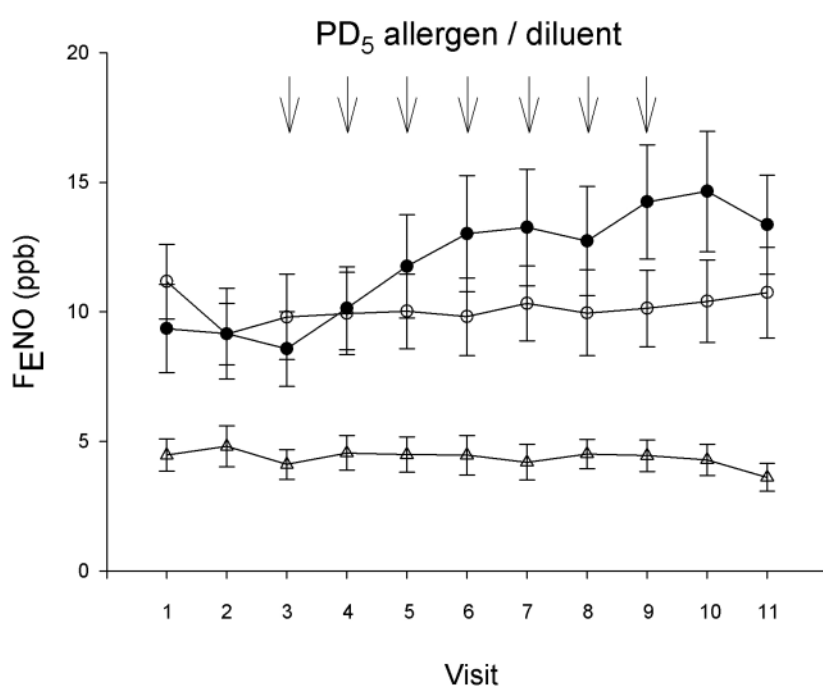


Figure 5a

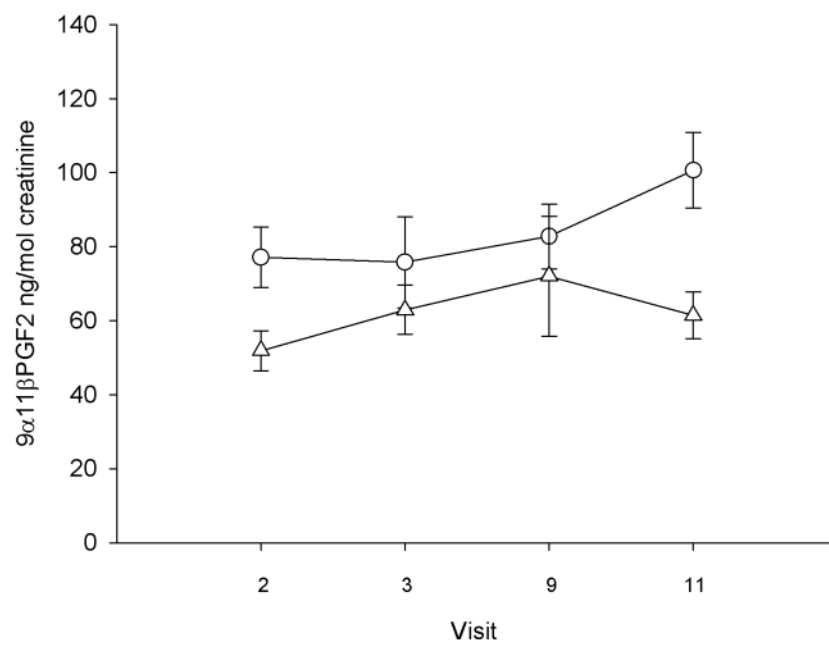


Figure 5b

