Benefits of high altitude allergen avoidance in atopic adolescents with moderate to severe asthma, over and above treatment with high dose inhaled steroids

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Summary

Background   Some patients with severe asthma cannot be controlled with high doses of inhaled steroids (ICS), which may be related to ongoing environmental allergen exposure.
Objective   We investigated whether 10 weeks of high altitude allergen avoidance leads to sustained benefits regarding clinical and inflammatory markers of disease control in adolescents with persistent asthma despite treatment with high dose ICS.
Methods   Eighteen atopic asthmatic adolescents (12–18 yr, 500–2000 µg ICS daily) with established house dust mite allergy, participated in a parallel-group study. Quality of life (PAQL), lung function, bronchial hyperresponsiveness (BHR) to adenosine and histamine, induced sputum and urine samples were collected repeatedly from 10 patients during a 10-week admission period to the Swiss Alps (alt. 1560 m) and at 6 weeks after return to sea level. Results were compared with those in eight patients, studied in their home environment at sea level for a similar time period. Throughout the study, asthma medication remained unchanged in both groups.
Results   During admission to high altitude, PAQL, lung function, BHR to adenosine and histamine, and urinary levels of eosinophilic protein X (U-EPX), leukotriene E4 (U-LTE4) and 9α11β prostaglandin F2 (U-9α11β PGF2) improved significantly (P < 0.05), with a similar tendency for sputum eosinophils (P < 0.07). Furthermore, the changes in PAQL and BHR to adenosine and histamine were greater in the altitude than in the control group (P < 0.05). At 6 weeks after renewed allergen exposure at sea level, the improvements in PAQL (P < 0.05), BHR to adenosine (P < 0.07) and histamine (P < 0.05), as well as U-EPX (P < 0.05) and U-LTE4 (P < 0.05) were maintained.
Conclusion   A short period of high altitude allergen avoidance, on top of regular treatment with ICS and long-acting B2-agonists, results in improvement of asthma, as assessed by clinical and inflammatory markers of disease severity. These findings indicate that short-term, rigorous allergen avoidance can improve the long-term control of severe asthma over and above what can be achieved even by high doses of inhaled steroids.

Keywords: Allergen avoidance, house dust mite, severe asthma, induced sputum, high altitude, urine, EPX, LTE4, 9α11β-PGF2
Introduction

Asthma is a chronic inflammatory disease, characterized by recurrent episodes of wheeze, variable airways obstruction and bronchial hyperresponsiveness (BHR) [1]. In patients with asthma, the inflammatory infiltrate in the airway wall mucosa consists mainly of eosinophils, mast cells and lymphocytes [2]. One of the major causes of asthma is sensitization to house dust mite (HDM) allergen [3]. In patients with asthma who are sensitized to HDM, clinical markers of asthma severity, such as peak expiratory flow (PEF) variability, FEV1 and BHR, are correlated to the number of mite allergens in their bedding [4]. Therefore, avoidance of mite allergen exposure is often recommended in asthma management [1]. However, creating a low allergenic environment in patient homes is often difficult to achieve and, hence, conflicting data on its effectiveness have been reported [5,6].

Highly effective allergen avoidance can be obtained by sending patients with severe allergic asthma to high altitude clinics. At high altitude, the number of HDM is extremely low, which is mainly due to a lower relative humidity in these regions [7]. When atopic asthmatic children are admitted to a high altitude clinic, FEV1, PEF rate and PEF variability improve rapidly [8–10], together with reductions in BHR and eosinophilic granulocytes in sputum [10–12]. However, there appears to be a relapse in these variables when patients are re-exposed to allergen in their home situation [9–11], but this may have been due to concurrent discontinuation of anti-inflammatory treatment in previous studies. As controlled studies on the effect of allergen avoidance are lacking, it was considered of interest to use the model of high altitude allergen avoidance to examine whether control of asthma, as established by quality of life and lung function, improves with high altitude allergen avoidance in atopic adolescents with moderate to severe persistent asthma treated with regular ICS, using a controlled, parallel-group design. Secondly, we questioned whether improvement in control of asthma is paralleled by a decrease in allergic inflammation, as indirectly determined by BHR and markers of eosinophilic inflammation in blood and induced sputum. Thirdly, eosinophil protein X (EPX), leukotriene E4 (LTE4) and 9α11β-prostaglandin F2 (9α11β-PGF2), each markers of activation of inflammatory cells, have been implicated in the pathophysiology of asthma and can be measured in the urine [15–17]. Since urine samples can easily be collected frequently, we examined whether urinary EPX, LTE4 and 9α11β-PGF2 provided non-invasive markers which paralleled the changes in control of asthma, BHR and the inflammatory markers in blood and induced sputum.

Methods

Patients

Eighteen non-smoking adolescents with moderate to severe persistent asthma participated in this study (Table 1). All patients were seen on a regular basis by their paediatric pulmonologist and were treated with regular ICS, in moderate to very high doses daily [18] according to recent guidelines for children and adolescents [19]. Fifteen patients used fluticasone propionate (range 500–2000 mg daily), two patients used budesonide (range 800–1600 mg daily) and one patient used beclomethasone dipropionate (1000 mg daily). Furthermore, 15 patients used regular long-acting ICS, using a controlled, parallel-group design. Secondly, we questioned whether improvement in control of asthma is paralleled by a decrease in allergic inflammation, as indirectly determined by BHR and markers of eosinophilic inflammation in blood and induced sputum. Thirdly, eosinophil protein X (EPX), leukotriene E4 (LTE4) and 9α11β-prostaglandin F2 (9α11β-PGF2), each markers of activation of inflammatory cells, have been implicated in the pathophysiology of asthma and can be measured in the urine [15–17]. Since urine samples can easily be collected frequently, we examined whether urinary EPX, LTE4 and 9α11β-PGF2 provided non-invasive markers which paralleled the changes in control of asthma, BHR and the inflammatory markers in blood and induced sputum.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control patients</th>
<th>Admitted patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (m/f)</td>
<td>0/5</td>
<td>8/2</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>14.0 (1.0)</td>
<td>15.5 (0.6)</td>
</tr>
<tr>
<td>ICS (µg/day)*</td>
<td>1060 (500–2000)</td>
<td>1260 (1000–2000)</td>
</tr>
<tr>
<td>FP/Bud/BDP</td>
<td>4/1/0</td>
<td>8/1/1</td>
</tr>
<tr>
<td>Long-acting β-agonists (n)</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>FEV1 (%pred)</td>
<td>87.3 (5.8)</td>
<td>85.4 (4.3)</td>
</tr>
<tr>
<td>FEV1 reversibility (%pred)</td>
<td>13.0 (5.1)</td>
<td>13.7 (3.5)</td>
</tr>
<tr>
<td>PC20HIS (mg/mL)†</td>
<td>0.10 (1.1)</td>
<td>0.24 (0.7)</td>
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</table>

Data are mean (SEM), *mean (range), †geometric mean (SEM in doubling doses). ICS = inhaled corticosteroids, FP = fluticasone propionate, Bud = budesonide, BDP = beclomethasone dipropionate.
β2-agonists twice daily, whereas three patients used regular short-acting β2-agonists because they did not tolerate therapy with long-acting β2-agonists. Moreover, one patient was additionally treated with 600 mg theophylline daily, and two patients additionally with 8 mg nedocromil daily. Only patients with good compliance to their treatment and good inhalation technique of their medication were selected for this study. Despite their treatment with ICS and β2-agonists, patients were not optimally controlled, as indicated by at least one of the following during the 6 months before inclusion in the study: more than 1 day of school absenteeism due to asthma, one or more extra, unplanned visits to the general practitioner or paediatric pulmonologist for asthma, short course(s) of prednisolone and/or hospital admission(s) for an exacerbation of asthma.

FEV1 was > 60% predicted and was reversible by > 12% predicted or by reaching the normal range after 200 μg inhaled salbutamol. All patients were hyperresponsive to histamine as shown by a provocative concentration causing a 20% fall in FEV1 (PC20) < 4 mg/mL, and atopic as indicated by a positive RAST to at least one common aeroallergen including HDM (RAST class > 2). All patients were free from symptoms of a respiratory tract infection during the 4 weeks before entry of the study. The study was approved by the ethic committees of the participating hospitals. Informed consent was obtained from the adolescents and their caretakers.

**Design**

This was set up as a controlled, parallel group study. Ten teenage patients were admitted to the Dutch Asthma Centre, which is situated in the Swiss Alps at 1560 m above sea level, for 10 weeks. Eight patients, frequency matched for age, need of asthma medication, level of lung function and BHR to histamine, participated in the control group.

During the first 2 days after admission, urine and blood samples were collected, and paediatric asthma quality of life (PAQL) was determined. At day 2 after admission, BHR to adenosine 5′-monophosphate (AMP) was established in the morning, and hyperresponsiveness to histamine was measured 4 h after spontaneous recovery from the AMP challenge. Furthermore, sputum was induced at day 4 after admission. This cluster of measurements was repeated at week 4 and week 8 after admission, and at 6 weeks after return to sea level (week 16). Furthermore, in order to evaluate the onset of altitude effects, BHR to AMP and urine samples were additionally obtained at week 2 after admission. In the control group, the cluster of measurements was performed at inclusion (week 0), week 8 and week 16.

Throughout the 16 weeks study, dosages of asthma medication were kept constant in all patients. If patients experienced an increase in asthma symptoms lasting several days, and/or if the treating pulmonologist judged it clinically necessary, such exacerbations were treated with a short course of prednisolone (30 mg/day for 7 days). Challenge tests were not performed if the patient had used prednisolone in the preceding 4 weeks. During the study period, patients followed their regular school programme. No additional education programmes were given to either of the two study groups. Prior to challenge tests, all patients withheld theophylline, cromoglycates and long-acting β2-agonists for 48 h and short-acting β2-agonists for 8 h, whilst they continued their regular ICS. In addition, patients were asked to refrain from caffeine-containing beverages in the 4 h preceding each challenge.

**Paediatric asthma quality of life (PAQL)**

Quality of life was assessed using the PAQL questionnaire [20]. At each of the 23 items, patients were asked to recall which impairment they had experienced during the previous week. Response options were recorded on a 7-point scale where 1 indicates maximum impairment and 7 no impairment. Results were expressed as mean score of all items. At follow-up, patients filled in the questionnaire while seeing their previous responses [21].

**Spirometry and challenge tests**

FEV1 was recorded from maximal expiratory flow-volume curves using a calibrated Masterlab pneumotachograph (Jaeger, Würzburg, Germany) or water-sealed spirometer (Sensormedics, Bilthoven, The Netherlands) [22]. Values of FEV1 were compared to reference values for adolescents [23]. Challenge tests with doubling concentrations of AMP (0.15–320 mg/mL; Merck, St Louis, MO, USA) or histamine diphosphate (0.03–8 mg/mL) were performed using the 2-min tidal breathing method [24]. PC20 to AMP (PC20AMP) and to histamine (PC20HIS) were calculated by linear interpolation on log concentration-response curves. If FEV1 had not fallen by 20% after administration of the highest concentration, PC20 was censored to the highest concentration of AMP (320 mg/mL) or histamine (8 mg/mL).

**Inflammatory markers in blood, induced sputum and urine**

Blood eosinophils were counted by automated counter and expressed as total x10⁹/L.

Prior to sputum induction, patients inhaled 200 μg inhaled salbutamol administered per metered dose inhaler connected to an aerosol chamber, for safety reasons [25]. Furthermore, FEV1 was measured before and 15 min after administration of salbutamol and reversibility in FEV1 was
calculated as difference between pre- and post-salbutamol values of \( FEV_1 \), expressed in \% predicted [25]. Subsequently, sputum was induced by inhalation of 4.5\% saline aerosols. Patients inhaled hypertonic saline aerosols during 5-min periods, for a maximum duration of 15 min [25]. Every 5 min, patients were asked to rinse their mouth and throat thoroughly with water, and encouraged to expectorate sputum into a plastic container. Whole sputum samples were processed using a validated protocol [25,26]. Differential cell counts were made by a qualified cytologist, on coded May–Grünwald–Giemsa stained cytopsins and eosinophils were expressed as percentage of 500 nucleated cells, excluding squamous cells. Sputum samples containing >80\% squamous cells were excluded from analysis. At inclusion, the median\% (range) squamous cells in our samples was 12.6\% (0.4–59.5).

Morning urine samples were stored in three portions at \(-20^\circ C\) until further analysis. EPX (Pharmacia, Uppsala, Sweden), LTE\(_3\) (Cayman Chemical, Ann Arbor, MI, USA) and 9\(\alpha\)11\(\beta\)-PGF\(_2\) (Cayman Chemical) in urine were measured by previously developed and validated immunoassays [15–17]. The limit of detection is <3 ng/mL for the EPX and <8 pg/mL for the LTE\(_4\) and 9\(\alpha\)11\(\beta\)-PGF\(_2\) immunoassays. Furthermore, creatinine was measured colourimetrically by alkaline picrate method (Sigma Chemical Company, St Louis, MO, USA). Results for EPX, LTE\(_4\) and 9\(\alpha\)11\(\beta\)-PGF\(_2\) were expressed as mean of replicate measurements in amount excreted/mmol creatinine.

**Statistical analysis**

During each cluster of measurements, baseline \( FEV_1 \) was calculated as the mean morning \( FEV_1 \) on the consecutive study days. Prior to analysis, PC\(_{20,AMP}\), PC\(_{20, HIS}\) and eosinophils in induced sputum and blood were (natural) log-transformed because of their skewed distribution. Data were presented as mean (SEM) or, in case of non-normal distribution, as geometric mean (range). PC\(_{20}\) values are presented as mean (SEM) or, in case of non-normal distribution, as geometric mean (range). PC\(_{20}\) values were expressed as geometric mean with SEM in doubling doses (DD) while quality of life data are presented as median (DD) since quality of life is not measured on a continuous scale. Baseline differences between groups for all variables were analysed by unpaired Student’s \( t \)-test except for PAQL life data which were analysed by the non-parametric Mann–Whitney \( U \)-rank-sum test. Within-group differences for all parameters were first tested using repeated measures analysis of variance, and further explored by the paired Student’s \( t \)-test. Wilcoxon’s signed-rank test was used to further test within-group differences in PAQL. The measurements of urinary mediators were conducted in one run for each subject, thereby allowing within-group comparisons, but excluding the possibility of between-group comparisons for these particular analyses. Between-group differences with respect to changes from baseline were analysed by covariance analysis with baseline values as covariate. Analyses were performed using the Statistical Package for the Social Sciences (SPSS-PC+). A \( P \)-value < 0.05 was accepted as the level of significance.

**Results**

In the control group, three patients could not be followed-up due to illness or exacerbations of asthma at all the time-points of measurements, and were thus not included in the analysis. These three patients did not differ from the rest of the patients in medication usage and level of \( FEV_1 \). In addition, one patient in the control group needed a 7-day course of prednisolone between inclusion and the fourth week of follow-up. In the altitude group four patients were lost to follow-up after week 10 for reasons that were not disease related.

At inclusion, the two groups did not differ with respect to age, medication usage, \( FEV_1 \), reversibility, or PC\(_{20,HIS}\) (Table 1).

**Paediatric asthma quality of life (PAQL)**

PAQL improved significantly during the study in both groups (\( P < 0.05 \), Table 2). However, the median (range) change in PAQL at week 8 was significantly greater in the altitude (1.3 (0.1–2.6)) than in the control group (0.3 (0.2–0.5), \( P < 0.01 \)).

**Baseline lung function and bronchial hyperresponsiveness**

During admission to high altitude, mean (SEM) \( FEV_1 \) improved significantly by 9.8 (2.4)\% predicted (\( P < 0.01 \), Table 2). Six weeks after discharge from the clinic \( FEV_1 \) decreased again, being neither significantly different from admission, nor from discharge values (\( P > 0.1 \)). In the control group, \( FEV_1 \) was stable and did not change significantly (\( P > 0.1 \)). Changes in \( FEV_1 \) at week 8 were not different between the two groups (\( P > 0.5 \)).

During admission to high altitude, PC\(_{20,AMP}\) improved significantly by 3.3 (1.0) doubling doses (DD) (\( P < 0.02 \)), and PC\(_{20,HIS}\) by 3.0 (0.7) DD (\( P < 0.001 \), Fig. 1). Six weeks after discharge PC\(_{20,HIS}\) was still significantly higher as compared to admission by 2.5 (0.8) DD (\( P < 0.05 \), with a similar tendency for PC\(_{20, AMP}\) (2.4 (1.5) DD, \( P = 0.07 \)). In the control group neither PC\(_{20,AMP}\) nor PC\(_{20,HIS}\) changed significantly (\( P > 0.2 \)). At week 8 of the study, the changes in both PC\(_{20,AMP}\) and PC\(_{20,HIS}\) were significantly different between the groups (\( P < 0.05 \) and \( P < 0.01 \), respectively).
Eosinophils in peripheral blood and induced sputum

Blood eosinophils decreased significantly during admission to high altitude \((P < 0.05)\), whereas these numbers increased again after discharge \((P > 0.6, \text{Table 3})\). Eosinophil numbers did not change in the control group \((P > 0.1)\). The change in eosinophil numbers at week 8 tended to be greater in the altitude than in the control group \((P < 0.06)\).

Adequate sputum samples were obtained on 80% of all the induction occasions. At week 0 the geometric mean (range) %eosinophils was 3.1% (0–5.6) in the altitude and 3.0% (0–10.8) in the control group. During admission to high altitude, sputum %eosinophils tended to decrease \((P < 0.07)\), but eosinophil numbers were not different from admission levels at 6 weeks after discharge \((P > 0.8, \text{Table 3})\). There were no significant changes in sputum %eosinophils in the control group \((P > 0.2)\). The changes in sputum %eosinophils at week 8 were not different between the altitude and control group \((P > 0.9)\).

**LTE\(_4\), EPX and 9\(\alpha\)11\(\beta\)-PGF\(_2\) in urine samples**

At baseline, the mean (SEM) levels of urinary LTE\(_4\), EPX and 9\(\alpha\)11\(\beta\)-PGF\(_2\) were 62.7 (8.5) ng/mmol creatinine, 110.7 (25.5) \(\mu\)g/mmol creatinine and 64.1 (6.8) ng/mmol creatinine, respectively, in the altitude group. Levels of LTE\(_4\), EPX and 9\(\alpha\)11\(\beta\)-PGF\(_2\) decreased significantly during admission to high altitude \((P < 0.05, \text{Fig. 2})\). After renewed allergen exposure (wk 16), urinary LTE\(_4\) and EPX were still lowered as compared to admission \((P < 0.05)\), while 9\(\alpha\)11\(\beta\)-PGF\(_2\) levels were not different from baseline \((P > 0.4)\). In the control group, the baseline levels of urinary LTE\(_4\), EPX and 9\(\alpha\)11\(\beta\)-PGF\(_2\) were 40.6 (2.5) ng/mmol creatinine, 89.6 (34.8) \(\mu\)g/mmol creatinine and 38.6 (2.2) ng/mmol creatinine, respectively. These levels did not change significantly during the study period \((P > 0.05)\).

**Discussion**

In patients with asthma who are not optimally controlled...
Table 3 Eosinophils in peripheral blood and induced sputum during the study

<table>
<thead>
<tr>
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<th>Blood eosinophils ($\times 10^6$/L)</th>
<th>% Sputum eosinophils</th>
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<tbody>
<tr>
<td></td>
<td>Control (range)</td>
<td>Altitude (range)</td>
</tr>
<tr>
<td>Wk 0</td>
<td>353.2 (200–1045)</td>
<td>326.4 (31–1316)</td>
</tr>
<tr>
<td>Wk 4</td>
<td>326.4 (20–1045)*</td>
<td>3.0 (0–10.8)</td>
</tr>
<tr>
<td>Wk 8</td>
<td>288.3 (200–374)†‡</td>
<td>173.6 (75–544)‡</td>
</tr>
<tr>
<td>Wk 16</td>
<td>246.7 (143–517)</td>
<td>306.7 (100–840)</td>
</tr>
</tbody>
</table>

Data are geometric mean (range). *$P < 0.01$ compared to week 0; †$P < 0.05$ compared to week 0; ‡$P < 0.05$ for changes compared to week 0 between the groups; §$P < 0.07$ compared to week 0.

Fig. 2. Mean (SEM) changes, expressed as percentage change from baseline, in urinary LTE$_4$ (open bars), EPX (crossed bars) and 9α,11β-PGF$_2$ (solid bars) during the 16-week study in the altitude group (upper panel) and control group (lower panel). *$P < 0.05$ compared to baseline values.

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despite the use of high doses of inhaled steroids, allergen avoidance was tested as an alternative intervention to understand mechanisms and probe if there was further room for improvement in asthma control. In practice, administration of oral steroids or other drugs with clear-cut unfavourable side-effects would have been the alternative to increase control of asthma. The results of this study show that in atopic adolescents with not optimally controlled, moderate to severe persistent asthma, 10 weeks of high altitude allergen avoidance, on top of regular treatment with high dose ICS, resulted in marked improvements of quality of life and BHR. These high altitude-induced improvements were accompanied by a decrease in blood eosinophils and the urinary markers of inflammation EPX, LTE4 and 9α11β-PGF2, with a similar tendency for sputum eosinophils. Interestingly, improvements in quality of life, in BHR to histamine and, to a lesser extent, AMP, and in urinary LTE4 and EPX were sustained after 6 weeks of re-exposure to allergen at sea level. In the control group studied at sea level, none of the studied parameters changed importantly during the study. These results suggest that short-term high altitude allergen avoidance is of clinical benefit in atopic adolescents with moderate to severe persistent asthma, and that attenuation of inflammation can be augmented by allergen avoidance, on top of what can be achieved by high dose ICS. In addition, at least for some of the outcome variables, the effects of high altitude allergen avoidance appeared to be persistent, suggesting a prolonged beneficial action on the underlying inflammation.

To our knowledge, this is the first study demonstrating prolonged benefits of high altitude treatment, added to regular treatment with high dose ICS, in moderate to severe asthmatic subjects using a controlled, parallel-group design. Contrary to previous studies, we included the patient’s quality of life and surrogate markers of airway inflammation, besides measuring lung function which disclosed the long-term benefits. Apparently, quality of life has improved already 8 weeks after admission to high altitude. The observed increase in quality of life at high altitude (1.3 PAQL) can be considered to be clinically significant, whereas its slight increase at sea level does not exceed the minimal important difference in quality of life [20]. More importantly, quality of life in the altitude group remained improved after re-exposure to allergen after discharge. The large reduction in urinary markers of inflammation during admission to altitude, but not in the control group, is a novel finding. Furthermore, the lowered levels of LTE4 and EPX were sustained after 6 weeks of renewed allergen exposure at sea level. This suggests that cellular activation, as measured by urinary markers, is relatively sensitive to allergen avoidance, despite adequate ICS treatment.

The improvements in FEV1 and BHR during the period at high altitude were in the same order of magnitude as has been demonstrated in other studies [9–12]. These observed benefits are most likely due to the very low levels of HDM at altitude [7]. However, although the housing conditions of most patients at sea level were optimal with respect to exposure to indoor allergens, we can not rule out that avoidance of other domestic factors such as pets, tobacco smoke and other environmental or climatic factors may have contributed to the present improvements in the altitude group [5]. Furthermore, in the present study, we may even have underestimated the altitude effect since we had several drop-outs in the control group due to exacerbations, which reduced the power of the study. Nonetheless, we were able to detect between-group differences for the changes in quality of life, and BHR to AMP and histamine. The current relapse in BHR after re-exposure to allergen in the home situation was less pronounced than in previous studies [9–11]. This difference in relapse may relate to the fact that anti-inflammatory treatment was kept constant in our study, suggesting that in previous studies the concomitant reduction in ICS may have masked the prolonged beneficial effect of high altitude.

The patients with asthma we selected for this study were all treated with high dose ICS and (long-acting) β2-agonists, according to step 4 of the treatment guidelines for schoolchildren with asthma [19]. In view of the high dosage of ICS the outcome of treatment in this group of patients is: to obtain the least possible symptoms, least possible need for relieving bronchodilators, least possible limitation of activity, best PEF, least possible PEF variability, and least adverse effects from medicine [19]. This is what we observed in our patients with asthma. Even though lung function was near normal under the current medication, they still had symptoms of asthma as described in the methodology section. The next pharmacological step to try to improve control of asthma would be the addition of oral steroids [19]. The paediatric pulmonologists, treating our patients, were very reluctant to do so because of their fear of side-effects. The addition of leukotriene modifying drugs was not a possibility, because these drugs were not available in The Netherlands at the time of the study. Therefore, we believe that the addition of short-term, radical allergen avoidance on top of the current treatment is very relevant in our patient group and apparently results in substantial improvements in the control of asthma.

How can the present results be interpreted? First, our data indicate that maximal clinical benefit and maximal suppression of airways inflammation can not be obtained by inhaled steroids in moderate to severe asthma, as long as pro-inflammatory stimulation continues. This is in keeping with current hypotheses of molecular interaction between...
transcription factors and steroids, in which elevated levels of the former, inhibit steroid efficacy during exposure to pro-inflammatory stimuli [27]. Avoidance of such exposure will then allow more powerful anti-inflammatory effects of steroids. Second, most likely, mast cells and T cells become less activated due to the sheer absence of HDM allergen at altitude, resulting in reduced secretion of cytokines and chemotactants to, for instance, eosinophils [2]. Indeed, eosinophils in blood and urinary markers of eosinophil and mast cell activation decreased markedly during admission to high altitude, whereas there was a small decrease in sputum eosinophils. In particular our finding that urinary LTE4 levels decreased during high altitude allergen avoidance supports reduced cell activation, since the biosynthesis of leukotrienes is not inhibited by glucocorticoids [28]. Together, our results in sputum and urine suggest either that urinary EPX is merely reflecting activation of circulating rather than sputum eosinophils, or that measurements in induced sputum are not suitable to monitor additional anti-inflammatory interventions in patients who are already treated with high dose ICS concomitant to current guidelines [1]. The relatively low sputum eosinophil counts and the absence of correlations between changes in urinary EPX and changes in eosinophil numbers in induced sputum or blood (results not shown) may favour the latter possibility.

Third, after re-exposure to allergen at sea level, urinary $9\alpha 11\beta$-PGF$_2$ levels were no longer different from admission, suggesting a renewed increase in basal stimulation of mast cells. In contrast, at this time-point BHR to AMP still was attenuated by more than two doubling concentrations. These findings suggest that trigger factor evoked release of mast cell mediators still was reduced 6 weeks after re-exposure to environmental allergens. Alternatively, there was reduced responsiveness of airway smooth muscle to secreted mast cell mediators, because in addition to BHR to AMP, BHR to histamine was also attenuated at 6 weeks after discharge from high altitude. The increase in (activated) mast cells after re-exposure to allergen at sea level may have contributed to renewed recruitment of eosinophils, as shown by increasing eosinophil numbers in induced sputum and blood after return to sea level. Presumably, eosinophils within the airways remained less activated after re-exposure to allergen since not only urinary levels of inflammatory cell activation (EPX and LTE$_4$), but also BHR to histamine were attenuated at this time-point. Together, these results confirm that once control of asthma is improved by the combination of inhaled steroid treatment and allergen avoidance, inhaled steroids alone can more adequately control the underlying (activity of) inflammation of the disease, as has been suggested before [29].

What are the clinical implications of the present study? First, high dose inhaled steroids are not optimally effective during on-going allergen exposure in moderate to severe asthma. It is remarkable that short-term, rigorous allergen avoidance can have long-term clinical benefits, despite subsequent re-exposure to allergens. Second, with the growing knowledge on the potential adverse effects of high dose ICS, such as reduced growth in children [30] and reduced bone density in women [31], it is becoming more important to assess which dose of ICS can be given safely to patients, and in particular to those with severe asthma. Short-term high altitude allergen avoidance seems to contribute to long-term better control of asthma, possibly with reduced need of oral and/or inhaled steroids. Third, it appears that urine can be used to monitor (changes in) allergic inflammation in patients using high dose inhaled steroids. In fact, the present study represents the first indication that urinary EPX, LTE$_4$ and $9\alpha 11\beta$-PGF$_2$ as new non-invasive markers of airway inflammation change in a fashion which is consistent with changes in clinical status. It now needs to be examined in long-term follow-up studies in larger groups of patients with severe asthma whether one or recurrent periods of allergen avoidance also results in improvements in airway wall remodelling, as has been observed when treatment is aimed at reducing airways hyperresponsiveness [32]. If so, high altitude treatment may improve the prognosis of severe asthma.

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