Effects of high altitude and cold air exposure on airway inflammation in patients with asthma

Sven F Seys,1,2 Marc Daenen,3 Ellen Dilissen,1 Ruud Van Thienen,1 Dominique M A Bullens,5 Peter Hespel,4 Lieven J Dupont2,6

ABSTRACT

Aims Eighteen patients with asthma were evaluated during preparation to climb to extreme altitude in order to study the effects of low fractional inspired oxygen (FiO₂), prolonged exposure to cold air and high altitude on lung function, asthma control and airway inflammation.

Methods Spirometry and airway inflammation (fractional exhaled nitric oxide (FeNO) and induced sputum) were studied under different test conditions: hypoxic (FiO₂=11%) exercise test, 24-hour cold exposure (−5°C) and before, during and after an expedition that involved climbing the Aconcagua mountain (6965 m).

Results Forced expiratory volume in 1 s (FEV₁) and FeNO values were slightly lower (p<0.04) after 1 h of normobaric hypoxia. FEV₁ decreased (p=0.009) after 24-hour cold exposure, accompanied by increased sputum neutrophilia (p<0.01). During the expedition FEV₁ and forced vital capacity decreased (maximum FEV₁ decrease of 12.3% at 4300 m) and asthma symptoms slightly increased. After the expedition the Asthma Control Test score and prebronchodilator FEV₁ were reduced (p<0.02), sputum neutrophil count was increased (p=0.04) and sputum myeloperoxidase levels, sputum interleukin 17 mRNA, serum and sputum vascular endothelial growth factor A levels were significantly higher compared with baseline. Patients with asthma with the lowest oxygen saturation during the hypoxic exercise test were more prone to develop acute mountain sickness.

Conclusions Exposure to environmental conditions at high altitude (hypoxia, exercise, cold) was associated with a moderate loss of asthma control, increased airway obstruction and neutrophilic airway inflammation. The cold temperature is probably the most important contributing factor as 24-hour cold exposure by itself induced similar effects.

INTRODUCTION

Asthma is a chronic inflammatory disease with reversible airway obstruction accompanied by chest tightness, dyspnoea and cough.1 However, most patients with asthma are able to deal with external physical triggers (eg, cold temperature or high-intensity physical efforts) when their asthma is adequately controlled.2 Travelling to high altitude is assumed to be safe in patients with stable asthma3–4; decreases in peak flow rates may occur5 but the incidence of acute mountain sickness (AMS) is not increased.6 Analysis of spirometry in a small group of subjects with asthma climbing to high altitude showed a decrease in forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) which were not significantly different from control subjects.7 Asthma symptom scores did not change, but 40% of the patients used more medication and 8% experienced a severe exacerbation.8

Asthma control at high altitude is affected by several environmental factors including allergen burden, cold air, hypoxia and air density.1 The decreased presence of allergens and lower air pollution at high altitude may explain the beneficial effect of high altitude training in subjects with asthma.6 Cold air is a known trigger for asthma, especially in combination with increased ventilation during exercise,7 which may explain the increased prevalence of asthma among cross-country skiers8,9 and emphasises the need for optimal treatment in these athletes.8 Exposure to cold air at high altitude may thus counteract the beneficial effects of improved air quality.

In this study, 18 patients with asthma who participated in an expedition to climb the Aconcagua mountain (6959 m) were evaluated both during the 1-year preparation phase as well as during the actual ascent. This allowed us to study the effects of low fractional inspired oxygen (FiO₂), prolonged exposure to cold air and high altitude on lung function, asthma control and airway inflammation.
METHODS
A more detailed description of the methodology is available in the online supplement.

Subjects
Eighteen patients with asthma (13 men) were recruited to take part in an expedition to climb the Aconcagua mountain (6965 m) in Aconcagua National Park, Argentina.

Study design
Patients were evaluated at different time points during the year prior to the expedition as well as during the actual ascent (figure 1 and online supplement).

Hypoxic exercise test
A hypoxic exercise test was performed with exposure to normobaric (760 mm Hg) hypoxia (FiO₂ 11%). Patients were continuously monitored at rest for 30 min and during exercise (oxygen saturation, heart rate, ECG). Fractional exhaled nitric oxide (FeNO) measurement and spirometry were performed and venous blood samples were obtained before and after exposure to hypoxia.

24-hour exposure to cold air
Patients stayed for 24 h in an indoor ski area (≤−5°C). FeNO, spirometry and induced sputum were analysed before and immediately after the time spent in the indoor ski area and spirometry was also performed after 12 h in the indoor ski area.

Expedition
The altitude profile is shown in figure 1. Baseline measurements were carried out 5 days before departure and at the start of the expedition (spirometry, heart rate, blood pressure, oxygen saturation). All measurements were repeated 72 h after the ascent. During the expedition at the different altitudes, patients reported symptoms of AMS by means of the Lake Louise self-report questionnaire. Peripheral oxygen saturation and heart rate were measured as well as daily asthma symptoms and use of asthma rescue medication. FeNO and spirometry were measured at different altitudes and compared with baseline levels. Partial pressure of nitric oxide in exhaled gas (PeNO) values were calculated as described previously.

Statistical analyses
Statistical analyses were performed with Graphpad Prism V for Macintosh. Normality was analysed by the Kolmogorov–Smirnoff test and data were analysed by the paired Student t test, repeated measures ANOVA or the Friedman test where appropriate (Bonferroni’s or Dunn’s multiple comparison tests were used as a post test). Correlation was studied by the Spearman or Pearson test as appropriate.

RESULTS
Baseline characteristics of patients
The baseline characteristics of the patients are shown in table 1. At screening, five patients had a prebronchodilator (pre-BD) FEV₁ of <80% predicted (table 1). Seventeen patients had well-controlled asthma (Asthma Control Test (ACT) ≥ 20/25). Ten patients had a FeNO value of >25 ppb, indicating that these patients possibly had more airway inflammation. In these patients the inhaled steroid dose was increased after the first visit. Subsequent treatment was kept unchanged in all subjects during the preparation. Mean pre-BD FEV₁% predicted (January: 91.8%±21.5%, December: 90.2%±12.1%) and ACT scores (January: 22.1±1.4, December: 22.0±1.9) remained unchanged when evaluated at the end of the preparation period prior to the expedition. Median FeNO levels decreased during the year (January: 27.8 ppb (IQR 17.2–35.3), June: 19.0 ppb (IQR 12.3–27.6) and December: 18.1 ppb (IQR 14.4–24.3), p=0.009) and only four patients had FeNO values >25 ppb at the end of the year of preparation compared with 10 patients at screening.

Hypoxic exercise test
Oxygen saturation decreased (mean oxygen saturation at 30 min 79.3%±8.7%; p<0.0001) and heart rate increased (mean heart rate at 30 min 80.4±15.1 bpm; p=0.004) significantly after 30 min at rest in the hypoxic chamber (figure 2A,B). A further decrease in oxygen saturation and increase in heart rate was seen during exercise (figure 2A,B). FEV₁% predicted did not
change (p=0.48) after 30 min at rest in hypoxia (FiO2 11%) and decreased after maximal exercise in hypoxia (mean change 2% (95% CI −0.3% to 4.3%), p=0.04; table 2). Median FeNO values were slightly but significantly lower after maximal exercise in hypoxia compared with baseline (17.5 ppb vs 19.0 ppb, p=0.03; table 2). None of the patients reported an increase in asthma symptoms during subsequent days at the same altitude, suggestive of an acclimatisation effect (figure 3A). FeNO values did not significantly change (figure 3B). Total cell count in sputum significantly increased (mean change 737×10^3 cells (95% CI 1198×10^3 to 277×10^3), p=0.002; data not shown). Median sputum neutrophil percentages were also significantly higher after 24 h of cold air exposure than at baseline (55% vs 9.5%, p=0.01; figure 3C). Sputum interleukin (IL)-17A (p=0.001) and IL-5 (p=0.002) mRNA levels increased significantly and sputum IL-4 (p=0.001) mRNA levels decreased significantly after cold exposure (figure 3D). Eight patients reported an increase in asthma symptoms during cold exposure which necessitated additional bronchodilator treatment with salbutamol in five cases.

**Asthma and the environment**

### Table 1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Before expedition</th>
<th>After expedition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (M/F)</td>
<td>13/5</td>
<td>16 (89%)</td>
<td>83.6±10.3†</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.6±11.3</td>
<td>3 (17%)</td>
<td>5 (28%)</td>
</tr>
<tr>
<td>Inhaled steroids, n (%)</td>
<td>13 (72%)</td>
<td>15 (83%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>High dose</td>
<td>2 (11%)</td>
<td>2 (11%)</td>
<td>5 (28%)</td>
</tr>
<tr>
<td>Medium dose</td>
<td>3 (17%)</td>
<td>5 (28%)</td>
<td>5 (28%)</td>
</tr>
<tr>
<td>Low dose</td>
<td>8 (44%)</td>
<td>12 (67%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>LABA, n (%)</td>
<td>10 (59%)</td>
<td>10 (59%)</td>
<td>10 (59%)</td>
</tr>
<tr>
<td>SABA alone, n (%)</td>
<td>3 (8%)</td>
<td>5 (28%)</td>
<td>5 (28%)</td>
</tr>
<tr>
<td>LTRA, n (%)</td>
<td>7 (41%)</td>
<td>7 (41%)</td>
<td>7 (41%)</td>
</tr>
<tr>
<td>Pre-BD FEV1, (% pred)</td>
<td>92.0±21.5</td>
<td>90.2±12.0</td>
<td>83.6±10.3†</td>
</tr>
<tr>
<td>Pre-BD FVC (% pred)</td>
<td>116.0±14.4</td>
<td>116.8±14.4</td>
<td>102.7±15.9††</td>
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<tr>
<td>FEV1/FVC (%)</td>
<td>66.0±14.5</td>
<td>66.0±12.0</td>
<td>69.5±9.5</td>
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<tr>
<td>FeNO (ppb)</td>
<td>17.2–27.8</td>
<td>14.4–18.1–24.3t</td>
<td>8.0–13.5–23.8</td>
</tr>
<tr>
<td>ACT</td>
<td>22.0±1.4</td>
<td>22.0–1.9</td>
<td>18.2±4.3</td>
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<tr>
<td>History of allergy</td>
<td>18 (100%)</td>
<td>18 (100%)</td>
<td>18 (100%)</td>
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<tr>
<td>Total IgE (kU/L)</td>
<td>45.2–93.1</td>
<td>45.2–93.1</td>
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</table>

### Table 2 Sputum analysis

<table>
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<th></th>
<th>Baseline</th>
<th>Before expedition</th>
<th>After expedition</th>
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</thead>
<tbody>
<tr>
<td>Total cell count ×10^6/mL</td>
<td>0.4–0.8–1.4</td>
<td>0.6–0.9–1.9</td>
<td>1.0–1.7–1.8</td>
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<tr>
<td>Eosinophils (%)</td>
<td>0.0–2.0–6.3</td>
<td>0.0–0.0–0.05†</td>
<td>0.0–0.0–0.0*</td>
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<tr>
<td>Neutrophils (%)</td>
<td>2.3–9.5–20.8</td>
<td>16.8–30.5–51.8</td>
<td>40.0–51.0–74.0**</td>
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<td>Macrophages (%)</td>
<td>77.0–87.0–95.8</td>
<td>44.0–64.0–85.0</td>
<td>26.0–45.0–54.0**</td>
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<tr>
<td>Lymphocytes (%)</td>
<td>0.0–0.0–1.0</td>
<td>0.0–1.0–8.0</td>
<td>2.0–5.0–7.0**</td>
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### Table 2 Hypoxic exercise test

<table>
<thead>
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<th>Baseline</th>
<th>After 30 min</th>
<th>After exercise</th>
<th>p Value</th>
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<tbody>
<tr>
<td>FEV1, (% pred)</td>
<td>100.3±15.3</td>
<td>104.2±15.3t</td>
<td>99.7±15.0t††</td>
<td>0.05</td>
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<tr>
<td>FeNO (ppb)</td>
<td>13.0–19.0–27.3</td>
<td>11.1–17.5–22.0</td>
<td>0.03</td>
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<tr>
<td>α1-AT</td>
<td>1.2–1.3–1.5</td>
<td>1.3–1.4–1.6</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>C3</td>
<td>0.9–1.0–1.2</td>
<td>1.0–1.0–1.3</td>
<td>&lt;0.0001</td>
<td></td>
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<tr>
<td>hs-CRP</td>
<td>0.4–0.5–1.4</td>
<td>0.5–0.7–1.4</td>
<td>0.047</td>
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**Cold exposure test**

FEV1% predicted decreased significantly after 12 and 24 h of cold air exposure compared with baseline measurements (mean change in FEV1 after 12 h 11.9% (95% CI 6.8% to 17.1%), p<0.0001; mean change in FEV1 after 24 h 5.5% (95% CI 0.2% to 10.9%), p≤0.02; figure 3B). FeNO values did not change significantly (figure 3B). Total cell count in sputum significantly increased (mean change 737×10^3 cells (95% CI 1198×10^3 to 277×10^3), p=0.002; data not shown). Median sputum neutrophil percentages were also significantly higher after 24 h of cold air exposure than at baseline (55% vs 9.5%, p=0.01; figure 3C). Sputum interleukin (IL)-17A (p=0.001) and IL-5 (p=0.002) mRNA levels increased significantly and sputum IL-4 (p=0.001) mRNA levels decreased significantly after cold exposure (figure 3D). Eight patients reported an increase in asthma symptoms during cold exposure which necessitated additional bronchodilator treatment with salbutamol in five cases.

**Expedition**

A decline in oxygen saturation and an increase in heart rate were observed after each increase of altitude followed by recovery during subsequent days at the same altitude, suggestive of an acclimatisation effect (figure 4A,B). A significant decline in sputum neutrophil percentages was observed during the expedition; the maximal decrease in FEV1 and FVC of −12.3% and −18.9%, respectively, occurred on December 30, 2013. doi:10.1136/thoraxjnl-2013-203280
respectively, occurred at 4300 m and did not appear to decrease further at higher altitude (FEV₁ and FVC 98.2% and 94.1% of baseline, respectively at 5600 m; figure 4A,C,D).

The pre-BD FEV₁% predicted was significantly lower when evaluated 72 h after the expedition compared with the measurement before the expedition (mean change 6.6% (95% CI 2.0% to 11.2%), p=0.004; table 1). A decrease of more than 10% was observed in four of the 18 patients with asthma. During the expedition there was a small but significant increase in the daily asthma symptom score (from 0.2 at 750 m to 0.8 at 6000 m; p=0.0124). One patient who experienced a severe exacerbation at 4300 m was treated with oral steroids and did not climb to higher altitude. There was an increase in the use of asthma rescue medication at 5600 m and 6000 m. The mean ACT score was significantly lower when assessed after the expedition (ACT=18/25; p=0.002).

PeNO values did not change significantly during the expedition except for a small drop in the PeNO value at 5600 m (figure 4E). Sputum neutrophil percentages (mean change 15.2% (95% CI −3.4% to 33.8%), p=0.049; figure 5A) and median myeloperoxidase levels (23.1 vs 102.0 ng/ml, p=0.002; data not shown) increased significantly after the expedition accompanied by an increase in sputum IL-17A mRNA (p=0.007; figure 5B). Sputum IL-5 mRNA levels also increased.

Figure 3 Cold exposure test. (A) Forced expiratory volume in 1 s (FEV₁) was measured by spirometry (n=16) at baseline, after 12 h (at the indoor ski run) and after 24 h. (B) Fractional expired nitric oxide (FeNO) levels and induced sputum (percentage neutrophils (C), interleukin (IL)-17A mRNA (D)) were obtained at baseline and after 24 h. Repeated measures ANOVA (with Bonferroni’s multiple comparison test as a post test) (A), paired t test (B, C) or Wilcoxon signed rank test (D) were used to analyse the results.
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after the expedition but no difference was found for IL-4 and interferon γ mRNA levels (p=0.52 and p=0.086, respectively; figure 5C) and sputum eosinophil count (p=0.50; data not shown). We could not detect higher levels of serum Clara Cell 16 (CC16) protein, a marker for epithelial damage,12 after the expedition compared with baseline measurements (p=0.36; data not shown), but there was a positive correlation between sputum IL-17A mRNA and serum CC16 protein (Spearman r=0.57, p=0.008; data not shown). Both serum and sputum vascular endothelial growth factor A (VEGF-A) levels were significantly increased after the expedition compared with baseline measurements (serum protein levels: 57.3 pg/mL (95% CI 14.2 to 100.3), p=0.02; figure 5D; mean change in sputum mRNA levels: 2.5 copies (95% CI 0.2 to 4.7), p=0.018; figure 5E).

There was an increase in the Lake Louise score (LLS) with increasing altitude (figure 4F). Symptoms increased on the first day at 3400 m and 4300 m and tended to decrease on the following days at the same altitude. Three patients experienced severe symptoms of AMS (LLS>5) which prevented them from climbing to higher altitude. These three patients all had an oxygen saturation of ≤60% during the hypoxic exercise test, while only 2 of the 15 patients with asthma who did not develop severe AMS had an oxygen saturation of ≤60% during the hypoxic exercise test (Fisher exact test, p=0.01). The minimal oxygen saturation during the hypoxic exercise test in the preparation period correlated positively with the maximal LLS score during the expedition (Pearson r=−0.68, p=0.0009; figure 6).

DISCUSSION

In this study we evaluated a small group of patients with asthma during an expedition to extreme altitude as well as during the preparation phase. The main findings of our study were: (1) patients with asthma with adequate asthma control are able to climb to high altitude (>4000 m); (2) exposure to environmental conditions at high altitude (hypoxia, exercise, cold) was associated with a moderate loss of asthma control and neutrophilic airway inflammation; (3) the cold temperature probably features as the most important contributing factor, as 24-hour exposure to cold by itself induced increased airway obstruction and increased airway neutrophilia; (4) climbing to altitudes >4000 m was associated with a small restrictive impairment of dynamic lung volumes and 17% of the patients with asthma experienced severe AMS; (5) asthma patients with the lowest oxygen saturation at the end of a maximal exercise test during hypoxia were more prone to develop AMS during the expedition.

In our study we found a loss of asthma control after the expedition at high altitude that was associated with increased asthma symptoms and rescue medication use as well as a reduction in pre-BD FEV1. Nevertheless, most of the patients with asthma were able to climb to high altitude (>5000 m) and only one patient experienced a severe asthma exacerbation. As a result, we feel that climbing to high altitude should not be considered as a contraindication for patients with asthma who are well-controlled and who take appropriate preparatory measures. This agrees with previous data in the literature, although limited information was available about the exposure of subjects with asthma to high altitude and the suitability of mountaineering as an appropriate form of sport for them. In a study by Golan et al.,13 147 patients with asthma were identified who had engaged in high altitude trekking. Two independent risk factors for attacks during travel were identified: frequent use (>3 times weekly) of inhaled bronchodilators before travel and participation in intensive physical exertion during treks. Cogo et al14 studied the effect of high altitude on bronchial hyperresponsiveness both at Capanna Regina Margherita (4559 m) and Pyramid Laboratory in the Himalayas (5050 m) in a group of 11 patients with mild asthma in a stable condition and with normal respiratory function at sea level. None of the patients participating in these studies experienced a severe asthma exacerbation. A significant reduction of bronchial responsiveness to both challenges

Figure 4 Clinical parameters during expedition. (A) Oxygen saturation, (B) heart rate, (C) forced expiratory volume in 1 s (FEV1)% at baseline, (D) forced vital capacity (FVC)% at baseline and (E) partial pressure of nitric oxide in exhaled gas (PeNO) were measured regularly at different altitudes during the expedition. Data are represented as mean values with SD (A–E). (F) Individual and mean values of Lake Louise score are represented by dots and as a horizontal bar, respectively. Data were analysed by Dunn’s multiple comparison test: *p<0.01; **p<0.001; ***p<0.0001.
was demonstrated at the highest altitude.\textsuperscript{14, 15} Huismans \textit{et al} evaluated 24 patients with asthma during trekking at high altitude in the Tibetan Everest region.\textsuperscript{5} Asthma symptoms did not significantly increase during the expedition. Similar to our data, two of the 24 subjects in this study experienced a severe asthma exacerbation and 40% had increased medication use. There may be a number of reasons for the relatively low incidence of acute asthma exacerbations in our study and previous studies. The patients with asthma who participated were selected on the basis of adequate asthma control and asthma medication was optimised several months prior to the expedition. Allergen avoidance at higher altitude with subsequent improvement of bronchial responsiveness and airway inflammation may also result in a favourable effect on asthma symptoms.\textsuperscript{6, 16, 17} A few studies documented higher levels of catecholamines and corticosteroids during the first 2 weeks of altitude exposure.\textsuperscript{18, 19} Increased plasma epinephrine and steroid concentrations, in addition to the anti-inflammatory treatment, can also reduce hyperresponsiveness and subsequent symptoms in subjects with asthma.\textsuperscript{20–22}

Despite the fairly moderate increase in asthma symptoms and medication use as well as a limited reduction in pre-BD FEV\textsubscript{1}, we have shown that the expedition resulted in increased airway inflammation before and after the expedition. Induced sputum cells (% neutrophils, A) and peripheral blood were obtained before and after return from the expedition. Sputum interleukin (IL)-17A (B), IL-4, IL-5, interferon (IFN)-γ (C) and vascular endothelial growth factor A (VEGF-A) (E) mRNA was quantified by real-time PCR and normalised to β-actin. Serum VEGF-A (D) protein levels were measured by ELISA. Data were analysed by paired t test (A, D–E) or Wilcoxon signed rank test (B–C) where appropriate.

\textbf{Figure 5} Airway inflammation before and after the expedition. Induced sputum cells (% neutrophils, A) and peripheral blood were obtained before and after return from the expedition. Sputum interleukin (IL)-17A (B), IL-4, IL-5, interferon (IFN)-γ (C) and vascular endothelial growth factor A (VEGF-A) (E) mRNA was quantified by real-time PCR and normalised to β-actin. Serum VEGF-A (D) protein levels were measured by ELISA. Data were analysed by paired t test (A, D–E) or Wilcoxon signed rank test (B–C) where appropriate.

\textbf{Figure 6} Inverse relation between lowest oxygen saturation after hypoxic exercise test and maximal Lake Louise score during the expedition. Oxygen saturation was continuously measured during 30 min at rest followed by a maximal incremental exercise protocol under hypoxic conditions (fractional inspired oxygen 11%). The Lake Louise score was measured daily during the expedition to assess acute mountain sickness. The Pearson test was used to evaluate the association between the two parameters.
inflammation with a predominant neutrophilic infiltration into the airways. FeNO values were measured during the expedition and tended to decrease slightly. Similarly, sputum eosinophils evaluated after the expedition did not increase significantly. However, the absolute and relative number of neutrophils in sputum significantly increased, which is an important observation in our study. There are no previous studies evaluating airway inflammation in subjects with asthma or normal subjects when exposed to high altitude. We hypothesise that the combination of cold air (lowest temperature measured inside tent during the expedition was −16°C) and high ventilation rate may feature as the most important contributing factor in provoking airway obstruction and inflammation, as we showed in these patients with asthma that short-term exposure to cold air (24 h at ≤−5°C in normoxia) prior to the expedition by itself also induced airway obstruction with a significant increase in sputum neutrophils without affecting FeNO levels. These findings are in agreement with studies evaluating airway inflammation in athletes with asthma performing sports that require high ventilation.28–29 A high ventilation rate or long-term exposure to cold air leads to hypoxic exercise test and, together with an altered permeability of the blood—air barrier, predisposes to cerebral oedema. Performing a hypoxic exercise test may be helpful in trying to identify those individuals with asthma who are most likely to develop AMS.

Conclusions
Patients with asthma can travel to high altitude when their asthma is well-controlled. Close follow-up is advised because loss of asthma control may occur, the exposure to cold temperatures could promote a neutrophilic airway inflammation and some patients may experience an acute exacerbation. A hypoxic exercise test may identify those patients with asthma who are likely to suffer from AMS when climbing to high altitude.

Acknowledgements
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Funding
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Contributors
SFS, ED, RVT, MD and LJD performed the experiments. SFS, PH, DMAB and LJD were responsible for the study design, analysed the data and wrote the paper.

Competing interests
None.

Ethics approval
Ethics approval was obtained from the Institutional Review Board, University Hospital Leuven, Belgium.

Provenance and peer review
Not commissioned; externally peer reviewed.

REFERENCES

Asthma and the environment

Online depository

Subjects

Potential candidates were non-smokers who were referred by their treating physicians on the basis of a previous diagnosis of asthma (of at least 1 year) requiring treatment, adequate compliance with the treatment (as assessed by physician), stable asthma control and willingness to participate to this expedition. Final selection was done by 2 pulmonologists (MD & LD) after review of clinical records, asthma control scores and test results. Approval was obtained from the local ethical committee of the UZ Leuven and patients gave informed consent.

Hypoxic exercise test

A hypoxic exercise test was performed in a hypoxic facility at KU Leuven (SportingEdge UK, Sherfield on Loddon, UK). Participants were exposed to normobaric (760 mm Hg) hypoxia (FiO₂ = 11%), which corresponds to pO₂ existing at ~5200 m altitude. They entered the room and remained seated on a chair for 30 min. After 30 min of rest, patients performed an incremental maximal exercise test on a treadmill in the altitude chamber. They started at a speed of 6 km/hour for 10 min followed by a 1 km/hour increase every 3 min until exhaustion. Patients were continuously monitored at rest for 30 minutes and during exercise (O₂ saturation, heart rate, ECG). FeNO and spirometry were measured and venous blood samples were obtained.

24-hour exposure to cold air

Patients stayed for 24 hours in an indoor ski area (SnowWorld, Landgraaf, the Netherlands). Walking exercises and equipment training were performed in preparation to the high altitude expedition, and patients also spent the night in the
indoor ski area. During this 24-hour period, daytime temperature was kept at -5°C and night time temperature at -8°C. In order to evaluate the effects of cold exposure FeNO, spirometry and induced sputum were analyzed before and immediately after the time spent in the indoor ski area. In addition, spirometry was also performed after 12 hours inside the indoor ski area.

**Expedition**

During the expedition the patients were exposed to a progressively increasing altitude: 750 m at the start of the expedition (Mendoza, Argentina), 2600 m (Los Penitentes), 3400 m (Camp Confluenzia), 4300 (Basecamp Plaza de Mulas), 5000m (Camp Canada), 5600m (Camp Nido de Condores) and 5963 m (Camp Colera, final high altitude camp before the top of the Aconcagua mountain at 6959 m). In addition, during the 3 weeks of the expedition, the group made several high altitude trekkings. The altitude profile is shown in Figure 1. Baseline measurements were carried out 5 days before departure in Leuven, Belgium (sputum induction, venous blood sample) and in Mendoza at the start of the expedition (FeNO, spirometry, heart rate, blood pressure, O₂ saturation, clinical examination). All measurements were repeated 72 hours after the ascent in Brussels, Belgium. During the expedition at the different altitude levels, patients reported symptoms of acute mountain sickness by means of the Lake Louise self-report questionnaire, scoring symptoms of headache gastrointestinal symptoms, fatigue, dizziness and sleep disturbance on a 3-point scale. Peripheral O₂ saturation and heart rate were measured as well as daily asthma symptoms (on a 5-point scale in which a score of 0 represented no asthma-related symptoms and a score of 4 represented the highest discomfort) and use of asthma rescue medication. FeNO and spirometry were measured at the different altitude levels and compared to baseline values at 750 m. PeNO (partial pressure of NO in
exhaled gas) values were calculated as described previously by multiplying FeNO values by ambient pressure minus water vapor pressure at 37°C.

None of the patients were treated with acetazolamide or any medication to prevent symptoms of high altitude sickness. Asthma treatment remained unchanged during at least 3 months before departure as well as during the expedition. Salbutamol DPI was provided as rescue treatment, instead of salbutamol MDI at altitudes >3400m.

**Measurements**

**Lung function**

Spirometry and bronchial challenge test was performed according to ATS/ERS guidelines in the pulmonary function lab of the university hospital Gasthuisberg. Measurement of the fraction of exhaled nitric oxide (FeNO) was performed at a flow rate of 50 ml/s with a chemoluminescence analyser (NIOX Flex, Aerocrine, Sweden).

**Sputum induction, processing and analysis**

Sputum induction and processing was performed as described previously. Sputum total and differential cell counts were obtained by cytospin (Shandon cytocentrifuge, Block Scientific). Cytokine mRNA levels (IL-4, IL-5, IL-17A and IFN-γ) were measured by real-time (RT)-PCR. Sputum MPO and VEGF-A levels were measured by Enzyme Linked Immunosorbent Assay (ELISA) according to the manufacturers’ protocol (Hycult biotech and R&D Systems).

**Serum analysis**

Serum samples were analyzed for high-sensitive CRP, α1-antitrypsin, complement factor 3 and total IgE (ImmunoCAP, Phadia) as detailed in Figure 1. Serum Clara cell
protein 16 was analyzed by ELISA according to the manufacturers’ protocol (Biovendor).

Spirometry, FeNO, Oxygen saturation and heart rate during the expedition

During the expedition, spirometry was performed using a Microloop turbine spirometer (Micro Medical, UK). This spirometer has been shown not to be affected by altitude, temperature changes and humidity (Pedersen, Miller, Sigsgaard et al. ERJ 1994 and Pollard, Mason, Barry et al. Thorax 1996) and has been used in a previous high altitude study (Huismans, Douma, Kerstjens et al. J of Asthma 2010). Absolute measurements of FVC & FEV₁ were recorded from 2 to 3 maximal expiratory flow volume curves, the best values were used for analysis. FeNO was measured using the NObreath analyser (Bedfont Scientific Ltd), before the spirometry. Peripheral O₂ saturation and heart rate were recorded with a Nonin Onyx 9500 fingertip pulse oximeter at the warm index of the middle finger.

All measurements during the expedition were performed by the same 2 pulmonologists (MD & LD) between 3 and 7 pm after at least 10 min of rest while the subject was seated in a tent.