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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 (FiO_2 = 11%), which corresponds to pO_2 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 (O_2 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, O_2 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 gastro-intestinal symptoms, fatigue, dizziness and sleep disturbance on a 3-point scale. Peripheral 0_2 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 chemoluminiscence 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 & FEV_1 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 O_2 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.