Prolonged Endurance Challenge at Moderate Altitude*

Effect on Serum Eosinophil Cationic Protein, Eosinophil Dynamics, and Lung Function

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Background: Eosinophils contain granule proteins such as eosinophil cationic protein (ECP) that have proinflammatory effects on airways. ECP may be released on activation of eosinophils into the plasma and is widely used as a marker of bronchial hyperreactivity and allergic inflammation. Environmental factors as well as intense physical exertion may influence eosinophil-related bronchial hyperreactivity.

Study objectives: To investigate the effect of endurance exercise at moderate altitude on levels of circulating eosinophils, serum ECP, serum osmolality (sOS), and dynamic pulmonary function parameters in healthy mountaineers.

Setting: Alpine field study performed in the Alps of Upper Styria in Austria.

Type of exercise: Ascent of a mountain at maximal speed.

Participants: Thirty healthy male volunteers from a troop of military mountaineers.

Results: Mean ECP concentration increased by 66% at the summit checkpoint (H2) and remained at 63% above baseline (base checkpoint [H0]) after descent (H4), while the blood eosinophil count decreased concomitantly from 250/μL at H0 (preexercise) to 118/μL (53%) at H2 and to 22/μL (81%) at H4. The total serum ECP concentration adjusted to sOS correlated negatively with blood eosinophil count (r = −0.37; p < 0.0001) and PaO2 (r = −0.34; p < 0.001), but positively with the peak expiratory flow (PEF) [r = 0.45; p < 0.0001]. Although sOS correlated with serum ECP at H2 (r = 0.47; p = 0.02) and at 12 h after the start of the experiment (H12) [r = 0.57; p = 0.003], the relationship between total ECP and sOS (r = 0.19; p = 0.034) was less pronounced. FEV1 in percentage of FVC (%FEV1/FVC) [the Tiffenau test], forced expiratory flow rate at 25% of vital capacity, and PEF were significantly higher at H2 than at H0 and H4. %FEV1/FVC decreased to 85% (p < 0.01) and 83% (p < 0.001) predicted at H12 and 24 h after start of the experiment, respectively.

Conclusion: Results provide strong evidence for nonspecific activation of blood eosinophils during prolonged intense aerobic exercise at moderate altitude, modifying both eosinophil dynamics and regulation of ECP release in healthy subjects.

Key words: endurance exercise; eosinopenia; eosinophil cationic protein; eosinophil dynamics; lung function; moderate altitude; nonallergic eosinophil cationic protein release; serum osmolality

Abbreviations: ECP = eosinophil cationic protein; EIA = exercise-induced asthma; %FEV1/VC = forced expiratory flow rate at 25% of vital capacity; %FEV1/FVC = FEV1 in percentage of FVC; H0 = base checkpoint; H2 = summit checkpoint; H4 = after descent; H12 = 12 h after start of the experiment; H24 = 24 h after start of the experiment; PEF = peak expiratory flow; sOS = serum osmolality

Eosinophil granule-derived proteins such as eosinophil cationic protein (ECP) are considered important in the pathogenesis of allergic inflammation.1,2 When eosinophils degranulate in response to inhaled allergens, cytotoxic granule proteins are released. In asthma, ECP is released in response to allergen-IgE crosslinking in the bronchial wall.1 Se-

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Serum ECP is regarded as a highly sensitive indicator of eosinophil activation and, by extension, of the degree of bronchial hyperresponsiveness.\(^3,4\) The concentration of serum ECP usually correlates with activation of eosinophils in vivo. Eosinophils carrying cytotoxic peptides are known to mediate damage to the respiratory epithelium.\(^5\) Deposition of ECP and other granule proteins is also known to play an important role in the shedding of bronchial epithelium, which may lead to inflammatory asthmatic tissue damage.\(^5,6,7\) Correspondingly, studies using BAL revealed that BAL ECP correlates with the severity of lung damage and hypoxemia in patients with ARDS.\(^8\) However, it has been shown that blood eosinophils may also be activated by nonallergic mechanisms. Venge et al\(^9\) also observed an initial short-term rise in serum ECP in patients with exercise-induced asthma (EIA) after exercise challenge, but no change in serum ECP levels in asthmatics without EIA. Tsuda et al\(^10\) found that ECP levels in asthmatic children may rise during exercise and that this increase may even continue after exercise, but only in the EIA-positive group. Dufaux et al\(^11\) found an increase in ECP release after short, maximal bicycle exercise in healthy subjects, providing strong evidence that blood eosinophils may be activated after a short bout of maximal exercise. Varying release of eosinophil granule proteins has been previously reported in response to environmental factors.\(^12\) Altitudinal environmental factors such as tropospheric ozone may cause subclinical airway inflammation and temporally increase bronchial responsiveness, even in healthy adults.\(^13\) Moreover, ECP was found to be released on short maximal physical exertion, most likely due to a nonallergic mechanism of activation.\(^11\) In vivo data on nonimmunologic eosinophil activation are rare in the literature. There are only limited data on the mechanical properties, osmotic behavior, and stability of eosinophils,\(^4\) particularly in response to the physiologic challenge of altitude-related hypoxia and concomitant endurance exercise.

### Materials and Methods

**Course of the Study**

Thirty healthy and physically fit male volunteers were recruited from a troop of military mountaineers; the demographics of these volunteers are given in Table 1. All subjects gave written informed consent and underwent a general health and physical fitness assessment 2 weeks before the study. Asthma or reactive airway diseases were ruled out at this time. Exclusion criteria also included ongoing medical treatment, acute or chronic disease, or physically limiting injuries. Subjects were asked to abstain from intense physical exercise 3 days before the start of the study. Food intake was standardized, and all participants consumed about 2,000 calories of food daily (75% carbohydrates, 15% fat, and 10% protein). Fluids were freely available immediately before but not during exercise. During ascent and descent, subjects carried a standard load of 20% of their individual body weight (12 to 19.5 kg). For blood sampling at the checkpoints, a venous cannula (TriCath; Codan; Esbjerg, Denmark) was inserted before the trial and filled with 1 mL of heparinized 0.9% sodium chloride. Starting from the base checkpoint (H0) at 632 m (Aigen, Styria, Austria), all participants climbed a mountain (Mount Grimming; Fig 1) to the summit checkpoint (H2) at 2,351 m above sea level. A schematic altitude profile is shown in Figure 2. Participants started their ascent at 10-min intervals. The first kilometer of the trail went up a gentle slope and provided a moderate warm-up, after which subjects increased their climbing pace to their maximal individual capacity and maintained that pace until they arrived at H2. After a 30-min rest period, they returned to H0 as quickly as possible.

**Clinical Variables and Dynamic Lung Function Parameters**

Laboratory data including serum ECP, serum osmolality (sOS), blood eosinophil count, and PaO\(_2\); lung function parameters were measured 30 min before exercise at H0, immediately after arrival at H2, after descent (H4), at 12 h after the start of the experiment (H12), and at 24 h after the start of the experiment (H24). Venous blood samples were drawn under anaerobic conditions using Vacutainer tubes (Plymouth, UK) at checkpoints along the ascent route. Samples were allowed to clot at room temperature for 60 min and were centrifuged at 2,000g for 10 min. Thereafter,

<table>
<thead>
<tr>
<th>Variables</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34 ± 8 (24–49)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 5 (170–186)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76 ± 7 (61–96)</td>
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<tr>
<td>Body mass index</td>
<td>24 ± 1 (19–25)</td>
</tr>
<tr>
<td>Additional weight, kg</td>
<td>15 ± 1.5 (12–19.5)</td>
</tr>
<tr>
<td>Time of ascent, min</td>
<td>170 ± 30 (130–230)</td>
</tr>
<tr>
<td>Time of descent, min</td>
<td>34 ± 8 (35–50)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD (range).*

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![Figure 1. Profile of the mountain.](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/ on 03/31/2017)
the sera were stored at 5°C in refrigerated chests before processing at the Karl-Franzens University School of Medicine in Graz. Serum ECP concentrations were determined by a radioimmunoassay technique (ECP-RIA; Pharmacia; Stockholm, Sweden) as described by Petersen et al.14 All concentrations were corrected for changes in plasma volume calculated from the sOs (normal values 250 to 300 mOsmol/kg), which was measured by osmometry (Fiske 100 Osmometer; Fiske Associates; Norwood, MA). Serum ECP was considered normal within the range of 2.3 to 16 µg/L. Blood eosinophil counts (normal range, 180 to 600/µL) were assessed by volume conductivity and scatter technology (STKS; Beckman Coulter; Miami, FL). Blood lactate concentration was measured from an earlobe blood sample (EBIO; AVL Micropuncture Set; AVL = List Medizintechnik; Graz, Austria) at each checkpoint of the trial according to a standardized method. Heparinized blood gas vials were refrigerated at 5°C and analyzed within 60 min after sampling in an automatic blood gas analyzer (AVL 995 Hb). Results were corrected for local atmospheric pressure and temperature. Pulmonary function was assessed using mobile battery-driven and flow-calibrated pneumotachographs at each of the checkpoints (Multipirio 100; Medical Graphics Corporation; St. Paul, MN); the devices were automatically calibrated for ambient temperature and atmospheric pressure. The best of three repeated flow-volume maneuvers was recorded.

Statistics

All statistical data given in Tables 1 to 4 were calculated on a personal computer and analyzed using software (StatView 4.5; Abacus Concepts; Berkeley, CA). Data were expressed as mean ± SD. Differences between the checkpoints were tested for significance using Wilcoxon’s signed rank test. Relationships between laboratory and lung function parameters were assessed by Pearson product moment correlation coefficients. Values of \( p < 0.05 \) were considered significant.

RESULTS

The ascent to H2 took 130 to 230 min (mean ± SD, 170 ± 30 min), while the descent to H0 took 35 to 50 min (34 ± 8 min; Table 1). The mean lactate concentration measured at H2 was far below the inflection point of 4 mmol/L, indicating aerobic endurance challenge during ascent. However, the lactate concentration rose slightly from 1.1 mmol/L prechallenge (at H0) to 2.7 mmol/L after arrival at H2.

![Figure 2. Schematic profile of the trail, time intervals, and checkpoints.](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/)
Clinical Laboratory Parameters

Clinical laboratory results and lung function data at all checkpoints are presented in Table 2. sOS values and concentrations of serum ECP remained within normal limits at all checkpoints. Blood eosinophils decreased by 53% from checkpoints H0 to H2 (p < 0.0004), by 81% from H2 to H4 (p < 0.0001), and returned to normal at H12 (Table 2). In contrast, serum ECP levels rose significantly (66%) from H0 to H2 (p < 0.0001) and remained elevated at H4 by 63% (p < 0.0001). During recovery and rehydration (H12 to H24), serum ECP returned to near-baseline levels. sOS rose by 3% from H0 to H2 (p < 0.0001) and by 5% at H4 (p < 0.0001). At H12, sOS was still significantly higher than H0 (p < 0.05), but returned to normal after sufficient rehydration at H24 (280 ± 9 mOsmol/kg). sOS was significantly correlated to ECP at all checkpoints (H2, H4, H12, and H24). There was also a weak but significant correlation between cumulative serum ECP and sOS (r = 0.19, p < 0.04; Table 3). A strong correlation among these variables was found only for cumulative serum ECP and eosinophils (r = -0.37, p < 0.0001). Furthermore, serum ECP was strongly correlated to the peak expiratory flow (PEF) at H0, H2, and H4 (Table 4).

Ventilatory Response and Ventilatory Parameters

The cumulative FEV₁ in percentage of FVC (%FEV₁/FVC) [the Tiffenau test] correlated weakly with sOS (p < 0.02) but not with serum ECP (r = 0.07, p < 0.5; Table 3). %FEV₁/FVC rose from 92 ± 10% predicted at H0 to 101 ± 15% at H2 (p < 0.0001) and returned to near-baseline levels at H4 (93 ± 13%, p < 0.32). The postexercise %FEV₁/FVC decreased to 88% (p < 0.01) and 83% (p < 0.0001) of the predicted values at H12 and H24, respectively. The PEF rate increased significantly by 23% from H0 to H2 (p < 0.0001). There was a 130% increase in respiration rate from H0

### Table 3—Correlations Between the Cumulative Laboratory Values, Pulmonary Function Parameters (Σ H0–H24) and Serum ECP

<table>
<thead>
<tr>
<th>Y-Axis</th>
<th>X-Axis, Serum-ECP (H0 to H24)</th>
<th>r</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sOS</td>
<td>r, H0</td>
<td>0.19</td>
<td>&lt; 0.04</td>
</tr>
<tr>
<td>%FEV₁/FVC</td>
<td>r, H2</td>
<td>0.07</td>
<td>&lt; 0.48</td>
</tr>
<tr>
<td>PaO₂</td>
<td>r, H4</td>
<td>-0.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>%FEF₂/FVC</td>
<td>r, H12</td>
<td>0.31</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Blood eosinophil count</td>
<td>r, H24</td>
<td>-0.37</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PEF</td>
<td>r, H0</td>
<td>0.45</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>r, H2</td>
<td></td>
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<tr>
<td></td>
<td>r, H4</td>
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<tr>
<td></td>
<td>r, H12</td>
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<td></td>
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<tr>
<td></td>
<td>r, H24</td>
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</table>

*EO = eosinophil count.
†Arterial blood gas examination refused by most of the subjects at H24.

(17 ± 3 breaths/min) to H2 (39 ± 7 breaths/min), which remained elevated at H4 (30 breaths/min, +76%), and H12 (24 breaths/min, +41%). The cumulative serum ECP concentration, the sum of serum ECP concentrations of all subjects at checkpoints H0 to H24, correlated significantly with PaO₂ (r = 0.33, p < 0.001; Fig 3) and with PEF (r = 0.45, p < 0.0001; Table 3, Fig 4). Figure 5 is a graphical summary of the main results correlating blood eosinophil count, ECP in serum, and %FEV₁/FVC at all checkpoints (H0 to H24).

### Discussion

Mountaineering is a popular recreational activity, and remote sites at moderate and high altitudes are
becoming increasingly accessible to the public. Unlike athletes who undergo altitude-training regimens, recreational mountaineers are often not acclimatized. Our study specifically investigated effects of mountaineering (a prolonged endurance challenge) in nonacclimatized subjects.

We observed a severe drop in the blood eosinophil count accompanied by a drastic increase in serum ECP following endurance exercise at moderate altitude (Fig 5). Our data also provide evidence of moderate generation of bronchial hyperreactivity reflected as a transient ECP increase during exercise, and a delayed, significant decrease in %FEV<sub>1</sub>/FVC during the recovery period (Table 2). Our endurance exercise study involved intense, prolonged mouth breathing of cold, dry air. Under these circumstances, the inhaled air cannot be conditioned by the upper airways and this task is shifted to the peripheral airways. We propose that increased respiratory water loss dehydrates the periciliary fluid; the increased osmolality then indirectly triggers the release of ECP from eosinophils via an osmotic challenge to the bronchial epithelium.

Our data suggest a subclinical, nonallergic inflammation within the airways leading to an increase in bronchial reactivity. Lung function measurements during the study showed that the %FEV<sub>1</sub>/FVC was generally unaffected during exercise, but decreased significantly during the recovery period. We suggest that airway resistance increased as a delayed response to eosinophil degranulation products and that the deterioration of the dynamic lung function parameters, %FEV<sub>1</sub>/FVC, PEF, and forced expiratory flow at 25% of vital capacity (%FEF<sub>25</sub>/VC) during the recovery period (H12 to H24), is due to increased airway resistance rather than respiratory muscle fatigue. It is possible that during exercise, the airflow was improved due to the lower air density at altitude and that this effect compensated for or exceeded any increase in airway resistance during the exercise period, so that the %FEV<sub>1</sub>/FVC remained substantially unaffected until the recovery period.

It has been shown that circulating eosinophils are attracted to the airway mucosa by enhanced cytokine and mediator release from activated bronchial epithelial cells, principally by interleukin-5<sup>16</sup> and tumor necrosis factor-α. An enhanced migration of blood eosinophils to airway tissues can explain the marked eosinopenia during and immediately after the endurance challenge. Similar profound drops in the blood eosinophil count down to complete disappearance of eosinophils from the circulation have been described in acute inflammatory conditions,<sup>15</sup> acute physiologic stress,<sup>4</sup> and physical exercise.<sup>18,19</sup> In contrast to these findings, Gabriel et al<sup>20</sup> reported increased circulating eosinophils after short supramaximal exercise on a bicycle ergometer; this may be explained by mobilization of eosinophils due to the short duration of exercise.
the physical effort. Investigations by Venge et al\textsuperscript{3} clearly point out a positive relationship between serum ECP concentrations and cellular damage to airway tissue in patients with severe asthma. While the effect of relapsing episodes of nonallergic ECP rise on the expression of bronchial hyperreactivity in healthy subjects has not been established, it is conceivable that frequent episodes of ECP peaks create a higher risk for developing exercise-induced and allergic asthma. Recurrent enhancements of eosinophil adhesion, migration, and release may even cause high-altitude cough or high-altitude pulmonary edema. An increase in serum ECP may also be involved in the rise of vascular permeability at altitude due to liberation of histamine from mast cells and eosinophils,\textsuperscript{21} causing acute altitude-related disorders. Although a potential link between tissue eosinophils and plasma exudation mechanisms \textit{in vivo} has been observed,\textsuperscript{22} such a relationship remains speculative.

In summary, our results may indicate a general relationship between strenuous endurance exercise and ECP release from eosinophils. We acknowledge that in addition to the mechanism we propose, environmental factors could have had additive systemic effects on ECP release and may have enhanced the propensity of eosinophils to degranulate in our healthy subjects. Whether prolonged exercise-induced hyperventilation in a dry, hypoxic, and cold environment can lead to a certain type of airway pathology remains unclear.

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