TITLE: Asthma, Airway Inflammation, and Epithelial Damage in Swimmers and Cold-air Athletes.

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Keywords: airway hyperresponsiveness; airway inflammation; asthma; athletes; epithelial damage;
ABSTRACT

Endurance athletes show an increased prevalence of airway hyperresponsiveness. The aim of this study was to evaluate the long-term effects of training on airway responsiveness, inflammation and epithelial damage in swimmers and cold-air athletes.

Sixty-four elite athletes (32 swimmers and 32 cold-air athletes), 32 mild asthmatic subjects and 32 healthy controls had allergy skin prick testing, methacholine challenge and induced sputum analysis.

Sixty-nine percent of swimmers and 28% of cold-air athletes had airway hyperresponsiveness. Sputum neutrophil count correlated with the number of training hours per week in both swimmers and cold-air athletes (r = 0.58 and 0.52 respectively). Eosinophil counts were higher in swimmers than in healthy subjects, although lower than in asthmatic subjects, and correlated with airway hyperresponsiveness in swimmers only (r = 0.64). Eosinophil count in cold-air athlete was similar to healthy subjects. Bronchial epithelial cell count was not correlated with airway hyperresponsiveness but was significantly increased in swimmers, compared with healthy and asthmatic controls.

In conclusion, we observed significant airway inflammation only in competitive athletes with airway hyperresponsiveness. The majority of elite athletes showed however evidences of bronchial epithelial damage that could possibly contribute to the development of airway hyperresponsiveness.

Word count: 193
INTRODUCTION

Exercise-induced asthma is frequently reported by endurance athletes [1]. During exercise, conditioning of large volumes of inspired air leads to drying and cooling of the conducting airways, the resulting airway fluid hyperosmolarity being considered the main mechanism involved in exercise-induced bronchoconstriction (EIB) [2]. EIB may also be enhanced by the constituents and characteristics of inhaled air [3]. Since swimmers and cold-air athletes spend many hours per week training in cold, dry air or a chlorinated environment, they are particularly affected by respiratory symptoms and AHR [3].

A mixed type of airway inflammation, mostly made of neutrophils and eosinophils, has been observed in swimmers and cross-country skiers [4-7]. However, whether such airway inflammation is associated or not with AHR in athletes has to be further documented [5-7]. Furthermore, airway damage, as evidenced by an increased bronchial epithelial shedding, may play a role in the development of AHR in asthma [8]. Such epithelial shedding may be involved in the development of asthma in athletes, and may largely result from specific environmental exposures. Epithelial damage may occur during intense exercise [9;10]. Epithelial damage has also been observed after long-term training in cold air and after as little as two hours of swimming activities in a chlorinated swimming pool [11]. It has been recently hypothesised that the inspired air conditioning process required during athletes’ training may render bronchial epithelial cells more susceptible to dehydration injuries following high levels of ventilation, particularly in cold air [2]. The epithelial cells could then release inflammatory mediators and be in part responsible for the airway neutrophilia observed in athletes [2]. The repair process of epithelial cells seems to involve plasma exudation, thus exposing airways to plasma-
derived products. When repeated many times per week and several times per day for a prolonged time-period, in the case of elite swimmers and cold-air athletes, the injury-repair process may affect the contractile properties of the airway smooth muscle and lead to AHR. Whether epithelial damage persists beyond training periods remains however to be documented, as a persistent altered or impaired repair process of the airway epithelium may possibly contribute to AHR.

We hypothesized that chlorine or cold air exposure could produce different persistent inflammatory and epithelial damage patterns in high-level athletes. In this regard, “swimmers’ asthma” and “skier’s asthma” may be different from the classical form of “asthma”, with respect to airway inflammatory features. We therefore aimed at comparing pulmonary function, airway responsiveness, and inflammation in swimmers and cold-air athletes.
METHODS

Subjects

One hundred high-level competitive athletes over 14 years of age were recruited. Nineteen cold-air athletes (37%) and 17 swimmers (35%) were excluded from the study due to their inability to produce sputum. We thus analyzed data from 64 competitive athletes from provincial to international level, able to produce sputum after induction, including 32 swimmers and 32 cold-air athletes (11 speed skaters training at an outdoor ice-rink, 16 cross-country skiers, and 5 biathletes). They were compared with 32 healthy non-athlete subjects without respiratory disease and with normal airway responsiveness, as shown by a PC$_{20}$ methacholine, the concentration of inhaled methacholine causing a 20% fall in forced expiratory flow in one second (FEV$_1$), equal or superior to 16 mg.ml$^{-1}$, in addition to 32 non-athlete subjects with symptomatic mild asthma and a PC$_{20}$ <16 mg.ml$^{-1}$. The control groups were matched for age, and atopy with the swimmers and cold-air athletes. All subjects were non-smokers, non-obese, and free of any disease which may have interfered with the study. All subjects gave their written informed consent and the study protocol was approved by our institutional ethics committee.

Study design

Each examination was performed when the athletes had not trained for at least 12 hours. Most tests were conducted in the fall. Short-acting inhaled β2-agonists, leukotriene receptor antagonists, and inhaled corticosteroids were stopped at least 12, 24, and 48 hours, respectively, before the visits. A physical examination and allergy skin prick tests were performed. A locally developed standardized questionnaire was administered to the
athletes; it documented their past or present history of asthma and exercise-induced asthma-like symptoms (EIS). A methacholine challenge and sputum induction were also carried out. Asthma was defined as the presence of asthma symptoms with proven airway obstruction [12].

We previously described the methodology used for allergy skin prick tests, baseline expiratory flows and methacholine challenge [13]. A subject was classified as atopic if at least one allergen caused a wheal of at least 3mm in diameter, in the presence of a negative saline control and positive histamine. A subject was considered to have AHR if his/her methacholine PC20 was less than 16 mg.ml⁻¹.

Sputum was induced and processed as described by Pizzichini et al. [14]. Briefly, concentrations of 3%, 4%, and 5% hypertonic saline were inhaled for 7 minutes each with a Medix electronic nebuliser (Medix, Catthorp, England). After each inhalation, subjects were asked to blow their nose and rinse their mouth with water to minimize postnasal drip and squamous epithelial cell contamination, respectively, and to expectorate into a sterile container. Sputum was processed within two hours after induction. Mucus was selected from saliva, weighted and four equal volumes of 0.1% dithiothreitol (DTT) (Sputolysin; Calbiochem-Novabiomech, San Diego, CA, USA) were added. Samples were rocked for 15 minutes. The reaction was stopped by adding four equal volumes of Dulbecco’s phosphate-buffered saline 1 ×, pH 7.1 (D-PBS) (Invitrogen, Burlington, ON, Canada). Following filtration, total cell count and viability were determined using the trypan blue exclusion method. Cell suspension was centrifuged at 800 ×g for 4 min. Cells were resuspended to a final concentration of 1 × 10⁶ cells.ml⁻¹. Cells were well mixed before being transferred into cytofunnels for cytospin preparation.
Slides were prepared with approximately \(0.07 \times 10^6\) cells for differential count. The cell count was made by two investigators blinded to the clinical characteristics of the subjects. The sputum sample was considered adequate if it was contaminated by less than 20% squamous epithelial cells from saliva. Reproducibility of the technique was previously described [15].

**Analysis**

Data were expressed as mean ± SD except for airway inflammatory cells, expressed as median and range. Analysis of variance (ANOVA) was used to compare the 4 groups. Subjects with a \(PC_{20} > 128\) mg.ml\(^{-1}\) were assigned a \(PC_{20}\) of 128 mg.ml\(^{-1}\) for the statistical analyses. Statistical results from these parameters were expressed with the log-transformed values. The Tukey’s multiple comparisons technique was applied *post-hoc* to the ANOVA. The univariate normality assumptions were verified with the Shapiro-Wilk test, and the Brown-Forsythe’s modification of the Levene’s test was used to verify the homogeneity of variances. Stepwise regression analyses were performed to determine the relationship between inflammatory cells and the variables (sex, allergies, physician-diagnosed asthma, AHR, training level, hours and years, swimmers or cold-air athletes, and EIS). The results were considered significant at a \(p\) value of \(\leq 0.05\). The data were analyzed using the statistical package program SAS, version 9.1.3 (SAS Institute Inc., Cary, NC).
RESULTS

Characteristics of the subjects

Subjects’ characteristics are presented in Table 1. Athletes were mostly national and international levels Canadian athletes (20 swimmers and 27 cold-air athletes) while some were at provincial level (12 swimmers and 5 cold-air athletes). Three swimmers (9%) and 2 cold-air athletes (6%) had a diagnosis of asthma during childhood, all before the age of 12 and before initiating their respective sports’ training program.

Prevalence of AHR for each group is reported on Figure 1. Twenty-two swimmers (69%) and 9 cold-air athletes (28%) were hyperresponsive to methacholine if we consider the criteria of PC$_{20}$ < 16mg.ml$^{-1}$. No significant correlation was observed between age, years of training, and AHR in athletes.

Exercise-induced respiratory symptoms were reported by 15 swimmers (47%), 14 with AHR and one without AHR, and 20 cold-air athletes (63%), 5 with AHR and 15 without AHR (Figure 2). Cold-air athletes reported more cough than swimmers (p<0.05).

In swimmers, exercise-induced symptoms were negatively correlated with the PC$_{20}$ (r=-0.48, p<0.05). This was not the case however in cold-air athletes (r=-0.04, p>0.05). Sputum production was a respiratory symptom rarely reported by the athletes after training.

Inflammatory cell counts
Total cell counts and viability were similar in the 4 groups (Table 2). No significant differences were noted for the total number of each type of inflammatory cell per gram of sputum, except for macrophages, more numerous in the cold-air group than in swimmers or in controls with asthma \( (p<0.05) \). Compared with the 49 atopic athletes, the 15 without atopy showed no significant difference in inflammatory cell counts.

Asthmatic controls had a higher percentage of eosinophils than healthy controls \( (p<0.001) \), swimmers \( (p<0.005) \), or cold-air athletes \( (p<0.001) \). Swimmers had a higher percentage of eosinophils than healthy controls \( (p<0.01) \), while cold-air athletes had a similar eosinophil count compared to healthy controls. The whole group of athletes had lower percentages of eosinophils than did controls with asthma \( (p<0.005) \). The sputum eosinophil count was correlated with AHR in swimmers only \( (r=0.64, p<0.0001) \).

Neutrophil count was similar in athletes with or without AHR (Table 3). However, both swimmers and cold-air athletes with AHR had a higher percentage of sputum eosinophils than athletes without AHR \( (p<0.05) \). Athletes with or without AHR had similar neutrophil counts compared to healthy controls (Table 2 and 3).

Cold air athletes without EIS had a higher percentage of neutrophils than cold-air athletes with EIS \( (p<0.01) \) and swimmers without EIS \( (p<0.01) \) (Table 4). Swimmers with EIS had a higher percentage of eosinophils than swimmers without EIS and cold-air athletes with EIS \( (p<0.001) \). Furthermore, percentages of sputum neutrophils were correlated with the number of training hours per week in the two groups of athletes \( (p<0.005) \) (Figure 3).

**Epithelial cells**
The percentage of bronchial epithelial cells was increased in swimmers compared to healthy controls and controls with asthma (p<0.05) (Table 2 and Figure 4). In regard to cold air athletes, there is a slight increase in epithelial cells in sputum compared with healthy controls, but it did not reach statistical significance (p=0.13). The percentage of bronchial epithelial cells was high in swimmers with or without AHR or exercise-induced symptoms compared with controls (p<0.05). We observed no correlation between epithelial cell counts and eosinophil or neutrophil cell counts. Epithelial cell counts were not correlated with AHR.
DISCUSSION

We found minimal or no airway inflammation in high-level swimmers and cold-air athletes who had not exercised in the previous 12 hours, except for those with AHR, who had a slight but significant increase in airway eosinophil count, particularly in the swimmers group. A key original finding of this study was however the high degree of persistent epithelial cell shedding in induced sputum observed in athletes, particularly marked in swimmers. Such increase in sputum bronchial epithelial cell count was not associated with airway inflammation but suggests significant underlying airway damage.

In the present study, neutrophil count in the athletes was in the range observed in healthy subjects. This had also been reported by Belda et al. [16], showing that neutrophils may come back within the normal range in the 12 hours following the last training session, eosinophils remaining slightly elevated in athletes with AHR. We can not also exclude that airway remodelling in athletes following a repeated intense exercise could be protective and constitute a mechanism limiting the inflammatory process [17]. Belda et al. attributed the neutrophilic inflammation seen in swimmers to the effects of chlorinated pool exposure rather than to the number of training hours [5]. We observed however that airway neutrophil count was strongly correlated with the number of training hours, whatever the specific environment. Our observations are in keeping with the report of Bonsignore et al. [18], showing a correlation between the presence of neutrophilic inflammation and the number of training hours, and between eosinophilic or lymphocytic inflammation and environmental exposure during training.

Loss of epithelial integrity can be observed in asthmatic airways [19]. However, we found no increase in bronchial epithelial cell count in induced sputum in the controls.
with asthma, probably due to their mild disease, while we found significant evidences of epithelial damage in athletes. Such damage, more marked in swimmers, may be due to mechanical stress of breathing at large volume during training and/or repeated intense hyperventilation and resultant dehydration of the mucosa and shear stress on the airway wall [9;10;20]. The release of fibrogenic factors has been observed when epithelial cells are submitted to repeated external pressure [20]. The epithelial shedding may also result of specific environmental exposures, particularly in chlorinated swimming pools. Also, cold, dry air may be an important stimulus for epithelial cell shedding in the human nose at rest [21] and in horses airways during exercise [22]. Repeated cold air inhalation may also be responsible for AHR in cold-air athletes, through a repeated plasma-derived products exposure, resulting from the repair process induced by airway dehydration [2].

We believe that exposure to chlorine derivatives can enhance exercise-induced bronchial epithelial cell shedding and AHR, explaining the difference in epithelial cell counts observed between cold-air athletes and swimmers. Recent studies have shown that occasional exposure to low level of chlorine in recreational swimmers or children attending swimming lessons causes transient lung epithelial damage [23]. Moreover, during acute accidental chlorine exposure, severe epithelium destruction and desquamation usually occurs [24].

Epithelial shedding may reflect alterations in the airway epithelium and this epithelial damage may possibly play a role in the development of AHR. It has been previously shown that bronchial epithelium modulates the responsiveness of airway smooth muscle and that the dysfunction or absence of the epithelium could contribute to abnormal responses of the bronchial tone [25]. Airway epithelium is the first line of
defence protecting sensory nerves and smooth muscle from stimulation by inhaled irritants. When the epithelial layer is damaged, the sensory nerves are more directly exposed, releasing neuropeptides that may induce a bronchoconstriction [26]. A disruption of the epithelial layer may be responsible for an increased permeability of the diffusion barrier, which may facilitate the penetration of inhaled irritants and allergens into the underlying smooth muscle or blood [27]. Bernard et al. reported that cumulative school pool attendance was positively correlated with the serum concentration of blood pneumoproteins, indicating increased alveolo-capillar permeability and thus epithelial damage [11;23]. Epithelial damage may also contribute to AHR through the loss of epithelium-derived relaxing factor synthesis (EpDIF), such as prostaglandin E2 and nitrous oxide (NO), which normally prevent the airways to constrict [25]. Another mechanism possibly contributing as much or even more than underlying inflammation to AHR is the remodelling process. Airway remodelling is characterized by changes in the quantity, composition, and organization of the cellular and molecular components of the airway wall, alterations considered secondary to chronic injury and repair of the airway epithelial – mesenchymal trophic unit [28]. Disruption of this trophic state, for example following airway injury, may lead to airway remodelling [20;28]. Epithelial cell count in induced sputum, as measured in our study, reflect epithelial shedding and thus an injury to bronchial epithelium, which may be responsible for the activation of a process leading to airway remodelling. In support of this, significant airway remodelling has been documented in cross-country skiers, even without asthma, and with variable degrees of airway inflammation [6;7]. It is therefore possible that swimmers and cold-air athletes
develop AHR and asthma through a mechanism somewhat different from the classical forms of asthma.

Our observations may explain the reduced effectiveness of asthma drugs observed in athletes [29;30]. Exercise-induced cough, the predominant symptom in cold-air athletes, is probably not due primarily to asthma or to airway inflammation, but rather to a neurogenic reflex-mediated, lower-airway response to cooling of the skin or upper airways, or to the dryness of cold air [31]. The mechanisms of skiers’ cough also remain to be investigated as do the effects of exercise-induced symptoms and AHR on athletic performance and quality of life.

Finally, the subjects excluded from the analysis because of their inability to produce sputum were not different from the athletes included in our study in regard to airway responsiveness. A majority of our subjects were atopic, and this could have influenced baseline airway inflammation. However, in our study, control subjects were matched for atopy with athletes and the study was mostly performed in the fall, when the pollen season was over. The lack of correlation between exercise-induced respiratory symptoms and inflammatory cell counts of induced sputum in athletes could be explained by the fact that measurements were obtained at rest and not immediately after exercise. However, the increased bronchial epithelial cell counts at rest may indicate a persistent epithelial shedding after exercise that may have been responsible for exercise-induced respiratory symptoms or may be involved in airway remodelling. Because our first aim was to observe the persistence of inflammation and epithelial shedding in induced sputum of athletes, and to compare this last to the classical form of asthma, we did not perform an analysis of biochemical markers in sputum.
Although this has to be studied, Helenius et al. have shown a reversibility of airway responsiveness in swimmers who stopped their sport career, and that may be associated to a restore process of the epithelium after repeated injuries [4]. Our study suggests an epithelial shedding at least 12h after the last training session. The time-course of changes in epithelial damage, airway cell composition, and AHR after a rest period remains thus to be documented in athletes to better understand the mechanisms of AHR.

In conclusion, analysis of induced sputum in elite athletes during a period without recent training uncovered slight persistent airway inflammation as indicated by an increase in eosinophil counts but only in athletes with AHR. Airway neutrophil counts were correlated to the current weekly duration of training. Exercise-induced symptoms, especially cough, were not associated with either AHR or airway inflammation in cold-air athletes. Epithelial desquamation was documented in athletes, particularly in swimmers, while it was minimal or absent in healthy subjects or controls with asthma. These results suggest that bronchial epithelial damage, following repeated high-ventilation episodes in cold air or in chlorinated pools, may contribute to airway remodelling that, in turn, may cause AHR in susceptible athletes.
REFERENCES


Table 1: Subjects’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=32)</th>
<th>Controls with asthma (n=32)</th>
<th>Swimmers (n=32)</th>
<th>Cold-air athletes (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 2</td>
<td>21 ± 3</td>
<td>20 ± 2</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>24 (75)</td>
<td>27 (84)</td>
<td>25 (78)</td>
<td>24 (75)</td>
</tr>
<tr>
<td>Years of training</td>
<td>—</td>
<td>—</td>
<td>10 ± 3</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Hours of training per week</td>
<td>—</td>
<td>—</td>
<td>21 ± 6</td>
<td>16 ± 7</td>
</tr>
<tr>
<td>Previous physician-diagnosed asthma (%)</td>
<td>—</td>
<td>—</td>
<td>7 (22)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Using inhaled β2-agonists (%)</td>
<td>0</td>
<td>20 (63)</td>
<td>5 (16)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>Using inhaled corticosteroids (%)</td>
<td>0</td>
<td>3 (9)</td>
<td>0 (0)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>4.1 ± 0.8</td>
<td>3.9 ± 0.8</td>
<td>4.7 ± 0.8**††</td>
<td>4.7 ± 0.7**††</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>101 ± 14</td>
<td>99 ± 15</td>
<td>115 ± 13**††</td>
<td>112 ± 13*†</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>5.0 ± 1.1</td>
<td>4.9 ± 1.1</td>
<td>5.9 ± 1.2**††</td>
<td>5.7 ± 0.9**††</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>106 ± 14</td>
<td>106 ± 15</td>
<td>127 ± 14**††</td>
<td>119 ± 13**††$</td>
</tr>
</tbody>
</table>

Age and spirometric values are expressed as mean ± SD and atopic status, training parameters, and drug utilization are expressed as number of subjects (percentage).

* p<0.05, ** p<0.01, between athletes (cold-air or swimmers) and healthy controls
† p<0.05, †† p<0.001 between athletes (cold-air or swimmers) and controls with asthma
$ p<0.05 between swimmers and cold air athletes.
**Table 2:** Sputum cell counts in controls, asthmatics, swimmers and cold-air athletes.

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=32)</th>
<th>Controls with asthma (n=32)</th>
<th>Swimmers (n=32)</th>
<th>Cold-air athletes (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count (cells/g)</td>
<td>4.9 ± 5.0</td>
<td>3.5 ± 5.1</td>
<td>4.2 ± 3.3</td>
<td>5.4 ± 4.6</td>
</tr>
<tr>
<td>Cell viability (%)</td>
<td>67 ± 14</td>
<td>71 ± 17</td>
<td>68 ± 19</td>
<td>69 ± 27</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>24.4 (2.0-95.3)</td>
<td>21.6 (1.0-76.3)</td>
<td>34.8 (7.0-68.8)</td>
<td>21.6 (4.3-80.5)†</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.1 (0-3.5)</td>
<td>2.63 (0-36.1)‡‡</td>
<td>0.8 (0-6.8)††</td>
<td>0.1 (0-7.8)††</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>71.6 (2.0-89.5)</td>
<td>68.3 (5.5-88.0)</td>
<td>52.4 (20.0-92.0)</td>
<td>59.0 (7.3-91.5)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>1.5 (0-4.8)</td>
<td>1.4 (0-8.8)</td>
<td>1.3 (0-6.8)</td>
<td>1.0 (0-3.3)</td>
</tr>
<tr>
<td>Bronchial epithelial cells (%)</td>
<td>0.8 (0-11.8)</td>
<td>1.1 (0-11.9)</td>
<td>2.9 (0.5-20.4)††</td>
<td>2.5 (0.5-11.8)</td>
</tr>
</tbody>
</table>

Data are expressed as percentages except for total cell count, and as median (range) except for cell viability expressed as mean ± SD.

* p<0.05, ** p<0.01 between athletes (cold-air or swimmers) and healthy controls
† p<0.05, †† p<0.001 between controls with asthma and athletes (cold-air or swimmers)
‡‡ p<0.001 between healthy controls and controls with asthma
Table 3: Sputum neutrophil and eosinophil percentages in swimmers and cold-air athletes, according to airway responsiveness.

<table>
<thead>
<tr>
<th></th>
<th>Swimmers</th>
<th>Cold-air athletes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With AHR (n=22)</td>
<td>Without AHR (n=10)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>37.5 (34.9-40.1)</td>
<td>36.5 (31.2-41.7)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.8 (1.5-2.1)*</td>
<td>0.3 (0.2-0.3)</td>
</tr>
</tbody>
</table>

Inflammatory cell data are expressed as mean percentages (95% confidence interval range).

*p<0.05 between athletes (cold-air or swimmers) with AHR and those without AHR
Table 4: Sputum neutrophil and eosinophil cell percentages in swimmers and cold-air athletes, according to the exercise-induced respiratory symptoms

<table>
<thead>
<tr>
<th>Sputum indices</th>
<th>Swimmers With EIS (n=17)</th>
<th>Without EIS (n=15)</th>
<th>Cold-air athletes With EIS (n=20)</th>
<th>Without EIS (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils (%)</td>
<td>35.3 (32.0-38.7)$</td>
<td>39.2 (35.8-42.7)$</td>
<td>27.1 (25.1-29.2)$*</td>
<td>56.7 (52.9-60.5)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.6 (0.4-0.8)</td>
<td>2.1 (1.7-2.5)$§§</td>
<td>0.3 (0.3-0.4)</td>
<td>1.0 (0.5-1.4)</td>
</tr>
</tbody>
</table>

Data are expressed as mean percentages (95% confidence interval range). Exercise-induced respiratory symptoms include wheezing, breathlessness, chest tightness, and/or cough.

$p<0.05$, $**p<0.001$ between athletes (cold-air or swimmers) with and without exercise-induced respiratory symptoms

$§p<0.05$, $§§p<0.001$ between swimmers and cold-air athletes
LEGENDS OF THE FIGURES:

**Figure 1:** Airway responsiveness of the 4 groups studied. Geometrical means (range) are represented through a horizontal line for each group.

Methacholine PC\textsubscript{20}: concentration of inhaled methacholine causing a 20% fall in forced expiratory flow in one second (FEV\textsubscript{1}).

![Graph showing airway responsiveness of 4 groups](image)

**Figure 2:** Exercise-induced asthma-like symptoms reported by swimmers and cold air athletes, with or without airway hyperresponsiveness, defined as PC\textsubscript{20} <16mg.ml\textsuperscript{-1}.

AHR: airway hyperresponsiveness.

* p<0.05 between cold air athletes and swimmers.
**Figure 3**: Relationship between the number of hours of training per week and percentage of neutrophils in the airways of swimmers and cold-air athletes; ■: Cold-air athletes; □: Swimmers.
**Figure 4:** Bronchial epithelial cells in induced sputum for the 4 groups. Median is represented through a horizontal line for each group and is (median (range)): 0.8 (0 to 11.8%) in healthy controls, 1.1 (0 to 11.9%) in controls with asthma, 2.9 (0.5 to 20.4%) in swimmers and 2.5 (0.5 to 11.8%) in cold-air athletes.

* p<0.05 compared with healthy or asthmatic controls.