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Airway inflammation in the elite athlete and type of sport

J Belda,¹ S Ricart,² P Casan,² J Giner,² J Bellido-Casado,² M Torrejon,² G Margarit,² F Drobnic³,⁴

ABSTRACT

Background: The prevalence of asthma and bronchial hyper-responsiveness is greater in elite athletes than in the general population, and its association with mild airway inflammation has recently been reported.

Objective: To study the relationship between the type of sport practised at the highest levels of competition (on land or in water) and sputum induction cell counts in a group of healthy people and people with asthma.

Material and methods: In total, 50 athletes were enrolled. Medical history, results of methacholine challenge tests and sputum induced by hypertonic saline were analysed.

Results: Full results were available for 43 athletes, who were classified by asthma diagnosis and type of sport (land or water sports). Nineteen were healthy (10 land and 9 water athletes) and 24 had asthma (13 land and 11 water athletes). Although the eosinophil counts of healthy people and people with asthma were significantly different (mean difference 3.1%, 95% CI 0.4 to 6.2, p = 0.008), analysis of variance showed no effect on eosinophil count for either diagnosis of asthma or type of sport. However, an effect was found for neutrophil counts (analysis of variance: F = 2.87, p = 0.04). There was also a significant correlation between neutrophil counts and both duration of training and bronchial hyper-responsiveness among athletes exposed to water (Spearman’s rank correlations, 0.36 and 0.47, p = 0.04 and 0.04, respectively).

Conclusions: Elite athletes who practice water sports have mild neutrophilic inflammation, whether or not asthma is present, related to the degree of bronchial hyper-reactivity and the duration of training in pool water.

The prevalence of asthma and bronchial hyper-responsiveness is greater among elite athletes than in the general population.¹ The prevalence is even higher among certain groups of athletes (long-distance runners, cyclists, skiers and swimmers) than among athletes in general.²

The underlying inflammation in such athletes is quite similar to the pattern seen in people with asthma. This eosinophilic inflammation in some athletes has been related to the presence of asthma, atopy, type of sport, and season and duration of training.³ The reason for inflammation is unknown, although a possible role for increased ventilation (of up to 20 l/min) has been suggested, as such an increase would extend the athlete’s exposure to cold air, airborne allergens, or environmental irritants such as chlorine derivatives at the surface of pool water in which swimmers train.³ Inflammation is reversible, at least partially, when the athlete’s period of active training comes to an end,⁴ suggesting that it is at least partly caused by participating in the sport.

Helenius et al⁵ found that increased ventilation in a group of cyclists was related to the appearance of bronchospasm, and we have shown that the prevalence of asthma and bronchial hyper-reactivity is higher among swimmers.⁶ However, the relationship between type of sport, amount of exposure to environmental allergens or irritants, and the type of bronchial inflammation has been insufficiently studied.

The aim of this study was to identify the effect of type of sport and duration of training in hours (as an indicator of level of exposure) on the features of airway inflammation in a group of healthy elite competition athletes and a similar group with asthma.

METHODS

Patients

In total, 50 elite athletes (23 healthy and 27 with asthma) were studied. The athletes with asthma were monitored by means of a specific questionnaire¹ and were stable during the study period. All were nonsmokers, were free of any other known diseases, and trained at the Centre d’Alt Rendiment (CAR; High Performance Center) in Sant Cugat, near Barcelona, Spain. The athletes agreed to enrol after a personal interview, at which their coaches were also present. Written informed consent was obtained from all the subjects, and the study was approved by the hospital ethics committee.

Methods

Part of the study was performed at the physiology laboratory of the CAR, where the subjects were recruited. There we obtained a medical and personal history (symptoms, history of asthma and allergies, treatment, sport practised and number of hours of training per week), and performed a physical examination and a methacholine bronchial challenge test. All participants also went to the laboratory at Hospital de la Santa Creu i de Sant Pau, where they were interviewed about control over their asthma,⁶ a skin-prick test was performed and sputum was induced using the procedure described below. The explorations were performed on two different days separated by a maximum of 2 weeks and ensuring clinical stability.

The patients were classified by diagnosis (healthy or having asthma), and by type of sport (exposed to water (22) or not exposed (land sports, 28)). A diagnosis of asthma was given if present or
past symptoms of asthma were reported and the forced expiratory volume in 1 second (FEV\textsubscript{1}) fell 20% from the baseline when the methacholine concentration was < 8 mg/mL.\textsuperscript{5} Land athletes were engaged in sports outside water (track and field, cycling, judo, Taekwondo, football), whereas water athletes practised sports that unfolded entirely or partially in pools (swimming, underwater hockey and water polo). All the sports were considered summer sports.

**Prick test**

A standard skin-prick test given to each athlete applied the respiratory allergens that are most common in Spain (Bial-Aristegui, Bilbao, Spain) and the number of aeroallergen-positive findings per subject was recorded. A test was considered positive when the weal was ≥3 mm in diameter in comparison with the negative control.

**Methacholine challenge test**

Spirometry was performed with a Datospir 500 (Sibelmed SA, Barcelona, Spain) in accordance with the recommendations of the Spanish Society of Respiratory Diseases and Thoracic Surgery.\textsuperscript{6} The methacholine provocations were performed by adapting the continuous method described by the European Respiratory Society.\textsuperscript{7} A Hudson-type nebuliser was used to spray recently prepared solutions of methacholine in isotonic saline solution at concentrations ranging from 0.01 to 32 mg/mL. The mass median aerodynamic diameter of the nebuliser chamber was between 1.5 and 3 μm, and the airflow rate was 7 L/min. The chamber was filled with 2 mL of the appropriate solution with an oxygen flow of 7 L/min, and the device was connected to a unidirectional valve inserted into the mouth of the subject, who inhaled the circulating aerosol at a normal breathing rate for 2 minutes with nostrils occluded by a nose clip. Lung-function measurements were recorded at 30 seconds and were confirmed at 90 seconds after each inhalation. The process was repeated with increasing concentrations until we saw a fall in FEV\textsubscript{1} of 20% from the baseline value (PC\textsubscript{20}) or until the maximum concentration was reached. The PC\textsubscript{20} was computed from the methacholine dose–response curve in relation to the methacholine concentration by linear interpolation on a log scale. If a patient’s FEV\textsubscript{1} never fell 20% from baseline, the PC\textsubscript{20} that was assigned arbitrarily was one dose higher than the maximum methacholine concentration administered (52 mg/mL).

**Sputum sample induction and processing**

Sputum was induced by hypertonic saline solution to obtain cell counts according to the standard procedures of our laboratory.\textsuperscript{8} Briefly, we administered a short-acting inhaled β\textsubscript{2}-adrenergic agonist, and 10 minutes after bronchodilation the subjects inhaled hypertonic saline solutions at concentrations of 3% and 4% for 7 minutes each. The subject’s status was monitored spirometrically at the beginning and end of each inhalation period. Within 2 hours, mucus in the saliva was sampled and processed with dithiothreitol (Sputalysin, Calbiochem Corp., San Diego, CA, USA) and phosphate-buffered saline. The cell suspension was filtered and stained with trypan blue for cytometric evaluation of the number of cells per gram of sputum, the cell viability and the number of squamous cells contaminating from the upper airways. The suspension was spun in a centrifuge, and the sediment was used to obtain differential cell counts (macrophages, eosinophils, neutrophils, basophils, lymphocytes and bronchial epithelial cells) using a May–Grünwald–Giemsa stain. Cell count reference values were those previously described by Belda et al.\textsuperscript{9,10}

**Statistical analysis**

SPSS V.10.0 (SPSS Inc, Chicago, Illinois, USA), was used to analyse the data. Descriptive statistics were expressed as mean (SD) for each variable. An analysis of variance (ANOVA) was carried out to detect the effects of type of sport (land or water) and of diagnosis (healthy or having asthma). Spearman’s rank linear correlation coefficient (r) was used to determine relations between variables, and comparisons were made using the Mann–Whitney U test.

**RESULTS**

Induced sputum samples viable for processing were obtained from 43 of the initial 50 subjects. No significant differences were found between the studied subjects and the seven athletes (four healthy, three with asthma) who withdrew from the study. The induction procedure was well tolerated by all participants. The final population sample was composed of 19 healthy controls and 24 people with asthma. Of the healthy controls, 10 engaged in land sports and 9 in water sports. Of the people with asthma, 13 practised land sports and 11 participated in water sports. Atopy was present, with sensitivity to a mean of two agents, in 63% (27/43) of the study sample but only three healthy controls and five people with asthma had allergic rhinitis symptoms. All the people with asthma needed rescue doses of short-acting β\textsubscript{2}-

**Table 1** Anthropometric and clinical characteristics of both healthy subjects and subjects with asthma

<table>
<thead>
<tr>
<th></th>
<th>Healthy athletes</th>
<th>Athletes with asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Land</td>
<td>Water</td>
</tr>
<tr>
<td>Number of athletes (n = 43)</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/3</td>
<td>9/0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23 (5)</td>
<td>27 (10)</td>
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<tr>
<td>Methacholine (PC\textsubscript{20}, mg/mL)</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Training (hours/week)</td>
<td>21 (3)</td>
<td>10 (8)</td>
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<tr>
<td>FVC (%)</td>
<td>112 (20)</td>
<td>115 (2)</td>
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<tr>
<td>FEV\textsubscript{1} (%)</td>
<td>108 (19)</td>
<td>113 (11)</td>
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<tr>
<td>Prick +/−, n+</td>
<td>6/4, 2(2)</td>
<td>6/3, 2(2)</td>
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<tr>
<td>ACQ</td>
<td>0.4 (0.5)</td>
<td>0.3 (0.5)</td>
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<tr>
<td>Budesonide (μg/day)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Salmeterol (μg/day)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

ACQ, Asthma Control Questionnaire; FEV\textsubscript{1}, forced expiratory volume in 1 second; FVC, forced vital capacity; prick +/−, n+, number of subjects with a positive/negative prick test, and number of positive pricks per subject.

Data are mean (SD), including log-transformed methacholine.
adrenergic agonists, 50% (12/24) of them were receiving regular doses of inhaled corticosteroids, and 37.5% (9/24) used long-lasting β2-adrenergic agonists. All subjects, including the people with asthma, had normal lung function, and 11 people with asthma were classified as having mild intermittent asthma, 6 as having mild persistent asthma and 7 as having moderate persistent asthma. Table 1 shows the characteristics of subjects in both groups.

The mean (SD) differential cell counts from sputum samples were 6.9 (8.8) million total cells/g for healthy subjects and 8.2 (8.1) million/g for people with asthma (p = 0.14). The percentage of eosinophils was 0.2 (0.3)% for healthy controls and 3.5 (7.8)% for people with asthma (Mann–Whitney U test, mean difference 3.1%; 95% CI 0.4 to 6.2, p = 0.008). The percentage of neutrophils was 40.3 (20.1)% in healthy controls and 51.1 (19.6)% for healthy athletes engaging in land sports (r = 0.35, p = 0.15) and 53.3 (21.9)% for athletes with asthma (r = 0.36, p = 0.04) but not in land athletes (r = 0.07, p = 0.30) (fig 2). The correlation between neutrophil counts and magnitude of bronchial hyper-responsiveness of water athletes as reflected by methacholine PC_{20} was r = 0.47 (p = 0.04) for water athletes and r = −0.35 (p = 0.15) in land athletes, but no correlation was found with eosinophil counts.

**DISCUSSION**

Our study found a neutrophilic inflammatory effect related to greater exposure to pool water measured by duration of training in hours, which was seen in elite athletes engaged in water sports. The observed inflammatory effect, which was probably caused by the pool environment, was greater than the theoretical intrinsic effect (eosinophilic or neutrophilic) produced by the asthma itself.

The inflammatory effect of environment was not reflected by eosinophil counts when adjusted for the fact that people with asthma had more eosinophils than controls. Two explanations may account for that observation, which was not corrected after adjusting by atopy or inhaled steroids use. One is that our sample size was insufficient to detect significant differences in eosinophil counts, a variable that varied widely among subjects with asthma. This could explain why Mann–Whitney analysis showed significant differences between diagnosis, but ANOVA, when considering the effect of environment, did not. A second explanation is that the asthma of all the patients under study was under control and 50% of them were taking regular inhaled corticosteroids, two factors that are associated with low eosinophilia.

Of great importance for verifying the effect of environment on neutrophilia was the significant relationship between degree of exposure to pool water measured in number of hours per week of training and neutrophil counts in sputum. This effect was present in spite of the relatively few hours of pool training undergone by healthy water sport athletes compared with water sport athletes with asthma.

The ultimate reason for neutrophil recruitment in the airways of subjects exposed long term to a water pool environment is unknown. It has been shown that chlorine derivatives are one of the main components of this environment when swimming in indoor water pools.15 14 In addition, we found that swimmers are exposed to huge amounts of chlorine derivatives during regular training, which is related to the degree of airway responsiveness.16 Unfortunately, we did not measure...
chlorine derivatives in this study, but we think that the cause of airway neutrophil recruitment associated with airway hyper-
responsiveness is chlorine derivatives. Supporting this speculation, it has been reported that the appearance of respiratory symptoms related to exposure to chlorine derivatives in the workplace or from pool water is a well-known phenomenon. Recent studies on the association between inflammation and lung injury in children after swimming pool attendance show a more energetic inflammation and are consistent with the hypothesis implicating pool chlorine in the rise of childhood asthma in industrialised countries.22–28 The effect is at its strongest among elite athletes, particularly during training in periods prior to competition, when they spend 8 hours/day in a microenvironment of chlorine gases equivalent to the maximum allowed threshold limited value.21 Similarly, the study by Helenius et al22 showed that the adjusted odds ratios for asthma and bronchial hyper-responsiveness were much higher among swimmers and long-distance runners than among athletes in sports demanding speed or power.

Increased counts of both eosinophils and neutrophils in the airways of elite athletes have been shown for various sports and situations: swimming,20 cross-country skiing22 and marathons.25 Our data are consistent with such findings and suggest a stronger role for the effect of pool water (exposure to chlorine) than for engaging in a sport of equal or greater intensity on land. The ANOVA of individual effects for each factor also shows that the direct irritative effect of chlorine from pools is greater than the effect of having asthma or not.

The pathogenic mechanism by which neutrophils from blood are attracted to the airways is not well understood. Kivity et al20 showed a clear relationship between the concentration of cysteinyl leukotrienes (C4, D4 and E4) in supernatants from sputum preparations and the eosinophil and neutrophil counts in induced sputum from people with asthma with exercise-induced bronchospasm, whereas such a relationship was not seen for other types of asthma, suggesting that cell attraction is attributable to these mediators. However, Bonsignore et al23–27 and Kanazawa et al28–30 suggested that nitric oxide should be considered responsible for this type of inflammation, whereas other authors have shown that the magnitude of exercise-induced bronchoconstriction in a group of people with asthma was not related to either intensity of inflammation or bronchial hyper-responsiveness.29 The explanation for such discrepancies may lie in differences in the subjects studied, the different sports involved, the environments in which they take place, or the clinical and medical situation of the subjects.31

In this study, we saw more marked eosinophilia and neutrophilia in athletes with asthma, but statistical analysis suggested a greater role for neutrophilia for the training environment than for the underlying disease. We conclude that ongoing, steady exposure to a water pool environment (probably due to chlorine gases), reflected in our study by hours of training, may be the source of predominantly neutrophilic inflammation. Further studies designed to measure concentrations of environmental chlorine and of mediators that are theoretically implicated in this type of inflammation are needed to elucidate the pathogenic processes. In addition, these theoretical mechanisms suggest that exploring treatment approaches involving antileukotrienes would be justified.

Acknowledgements: This investigation was supported in part by the Consejo Superior de Deportes (01/EP10/00 of 25 July 2000), and the “Red Respira” group (Redes Temáticas de Investigación Cooperativa del Instituto de Salud Carlos III) and the Spanish Society of Respiratory Diseases and Thoracic Surgery (SEPAR).

Figure 2 Relationship between the number of hours of training and neutrophil concentrations (%) in sputum from athletes engaged in land sports (white symbols) or water sports (black symbols). The lines show the estimated linear regressions.

Figure 3 Relationship between the methacholine bronchial challenge test findings (PC_{20}) for athletes participating in land sports (white symbols) or water sports (black symbols). The lines show the estimated linear regressions.

What is already known on this topic

- The prevalence of bronchial hyper-responsiveness is greater among elite athletes than in the general population.
- The underlying inflammation in such athletes is quite similar to the pattern seen in people with asthma, involving a predominance of eosinophils, and has been related to the presence of atopy, type of sport, and season and duration of training.

What this study adds

This study draws attention to the presence of inflammation mediated by neutrophils, not eosinophils, and its relationship to the degree of bronchial hyper-reactivity and the duration of training in the swimming pool in the athlete who practices aquatic sports whether or not asthma is present, perhaps due to exposure to chlorine derivatives.
was awarded the National Award for Sports Medicine 2005 granted by the University of Oviedo. We thank M E Kenans for her translation of the manuscript into English.

Competing interests: None.

REFERENCES

Commentary 1
The prevalence of exercise-induced asthma and bronchial hyperreactivity is very high in elite athletes, especially in those who train in a microenvironment of chlorine gases (indoor swimming pools). This paper explores the role of neutrophilia in these conditions, comparing healthy athletes and athletes with asthma, for both land and water sports. The authors highlight that this neutrophilic inflammatory effect is related to the hours of water pool training in elite water-sports athletes. It is greater than that expected for asthma alone, so that the neutrophilic inflammation exists in these athletes whether or not asthma is present.

The data in this paper suggest that the role of exposure to chlorine is greater than that of performing land or water sports, and even greater that the presence or not of asthma.

This paper opens new approaches for the study of bronchial hyper-reactivity, particularly in those sports where athletes are exposed to chlorine gases.

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Commentary 2
The paper of Belda and co-workers describes the interesting finding that swimmers have an increased number of neutrophils in induced sputum. A mixed type of neutrophilic–eosinophilic inflammation has been already reported in asthmatic swimmers by Hellenius et al.1

Interestingly, this inflammation persists whether athletes continue practising sports, whereas it attenuates after retirement from competition and training. In addition, our group recently described in swimmers a similar form of non-allergic form of rhinitis with an intense neutrophilia seen in nasal scrapings.2

Chlorine exposure has been often invoked to explain airways inflammation in swimmers. Certainly, the high prevalence of asthma in swimmers should stimulate further research on environmental factors causing inflammation and airways symptoms in swimmers (and preventive measures). In addition, the results might also raise concern about the widespread tendency to recommend swimming as the most appropriate sport for people with asthma.

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REFERENCES

Commentary 3
The available evidence suggests an increased risk of asthma and bronchial hyperreactivity in elite athletes. Plausible biological