

Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers

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To investigate respiratory symptoms, increased bronchial responsiveness, and signs of airway inflammation in elite swimmers, we examined 29 swimmers from the Finnish national team and 19 healthy control subjects (nonasthmatic, symptom-free). They answered a questionnaire and were interviewed for respiratory symptoms. Lung volumes were measured and bronchial responsiveness assessed by a histamine challenge test. Induced sputum samples were also collected. Fourteen (48%) of the swimmers and three (16%) of the control subjects showed increased bronchial responsiveness ($P < 0.05$). The sputum cell differential counts of eosinophils (mean 2.7% vs 0.2%) and neutrophils (54.7% vs 29.9%) from swimmers were significantly higher than those from controls ($P < 0.01$). Eosinophilia (sputum differential eosinophil count of $> 4\%$) was observed in six (21%) of the swimmers and in none of the controls ($P < 0.05$). Symptomatic swimmers had significantly more sputum eosinophils than did the symptom-free. The concentrations of sputum eosinophil peroxidase (EPO) and human neutrophil lipocalin (HNL) were significantly higher in swimmers than control subjects ($P < 0.001$ and $P = 0.05$). We conclude that elite swimmers had significantly more often increased bronchial responsiveness than control subjects. Sputum from swimmers contained a higher percentage of eosinophils and neutrophils, and higher concentrations of EPO and HNL than sputum from controls. Long-term and repeated exposure to chlorine compounds in swimming pools during training and competition may contribute to the increased occurrence of bronchial hyperresponsiveness and airway inflammation in swimmers.

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High prevalence rates for asthma and symptoms compatible with asthma have been recently found in elite athletes (1–10). Various mechanisms may explain these findings. In elite swimmers, exposure to chlorine compounds from swimming-pool disinfectants may provoke asthmatic symptoms (1–3). In cross-country skiers, self-reported asthma (4, 5) and increased bronchial responsiveness (6, 7) are common. The postulated mechanism of increased bronchial responsiveness and common appearance of symptoms compatible with asthma in skiers is increased and prolonged breathing of cold, dry air (6). Of Finnish long-distance runners, 17%, and of speed and power track-and-field athletes, 8% had physician-diagnosed asthma (8). In track-and-field athletes, not

only cold air but also extensive exposure to airborne allergens in the pollen season may increase the risk of asthma (8–10). Despite the strong functional evidence of asthma in athletes, pulmonary pathophysiology has not yet been studied in any athletic group.

Asthma – even in patients with newly diagnosed disease – is characterized by airway inflammation. Increased numbers of eosinophils, mast cells, and lymphocytes can be observed in the bronchial mucosal biopsies obtained by bronchoscope (11). Recently, the method of induced sputum has been introduced as a noninvasive and safe way to investigate airway inflammation (12–14). We applied this method to study airway inflammation in elite swimmers and healthy controls.

Material and methods

Subjects

Twenty-nine elite swimmers (23 belonging to the Finnish national teams) and 19 control subjects (medical students, laboratory staff) volunteered to participate in the study. The swimmers had competed for a mean of 9.1 years (range 3–17 years) and had swum a mean distance in training of 1670 km (range 800–3380 km) in the previous year. Six (21%) of the swimmers had a history of asthma as diagnosed by a physician, and five of them had been diagnosed during their competitive swimming career. Four (14%) of the swimmers used currently antiasthmatic medication, and three had been treated previously, but had stopped the medication for more than 3 months before this study. The control subjects were healthy and nonasthmatic, and did not report bronchial symptoms or use of antiasthmatic medication. None of the subjects (swimmers or controls) were smokers, and none had a history of cardiopulmonary disease other than asthma. The clinical characteristics of the study subjects are shown in Table 1. All subjects gave their written informed consent, and the protocol was approved by the local ethics committee.

Protocol

The study was carried out from February to May 1996. Swimmers were recruited from the Finnish national team and from one swimming club in the city of Helsinki during the World Cup Race, held in Espoo, Finland, on 27–28 January 1996. The swimmers were briefed on the purpose of the study. Forty-two swimmers of the 53 (79%) who attended the information session participated in the respiratory function testing and skin prick tests. They represented 49% of the swimmers in the national team or in the one swimming club. Eleven (21%) of those 53 eligible swimmers did not have the opportunity to participate in further studies. Three of them reported having asthma, which they considered to be well taken care of. Twenty-nine (69%) of the 42 swimmers participated in the present study, and gave induced sputum and blood samples.

Each subject completed a 2-day protocol. During the first visit, they filled in a respiratory-symptom questionnaire, were interviewed, and underwent a resting spirometry, a histamine challenge test, and skin prick tests. On the second visit (1–2 weeks after the first visit), induced sputum and blood samples were obtained.

Questionnaire, spirometry, histamine challenge, and skin prick test

The questionnaire covered occurrence of asthma, asthma medication, family history of asthma, and exercise-induced bronchial symptoms (8). The last were considered to occur if symptoms of cough, wheeze, or breathlessness had been observed repeatedly during the previous year. Answers were confirmed in personal interviews by the author (I. H.).

Resting spirometry was carried out according to the American Thoracic Society (15) with a flow-volume spirometer (Medikro, Medikro Oy, Kuopio, Finland). Values are expressed as percentages of reference (predicted) values from adult Finns (16).

Histamine challenges were performed by the dosimetric method with controlled tidal breathing (17). Noncumulative histamine doses of 0.025, 0.1, 0.4, and 1.6 mg were inhaled, and FEV₁ values were measured 90 s after each inhalation. The dose of histamine that caused a 15% fall in FEV₁ (PD₁₅FEV₁) was determined by logistic interpolation.

Skin prick tests were done with 10 allergen extracts (Soluprick SQ, 10 HEP, ALK, Denmark) and positive (histamine dihydrochloride, 10 mg/ml) and negative (solvent) control solutions. The allergens used were birch, timothy, meadow fescue, and mugwort pollen; horse, cat, dog, and cow dander; the mite *Dermatophagoides pteronyssinus*; and spores of the mold *Cladosporium herbarum*. A subject was classified as atopic if any allergen caused a wheal of 3 mm or more in diameter while control solutions gave expected results.

Sputum induction

Sputum was induced without pretreatment by inhalation of hypertonic saline generated by an ultrasound nebulizer (Spira Ultra, Hengityshoitokeskus, Hämeenlinna, Finland) until sputum was obtained or 15 min had passed (14). Subjects were asked to rinse their mouths and blow their noses before coughing. The nebulizer was on maximum settings and was charged with 4 ml of 5% NaCl. According to the manufacturer, most particles generated have a diameter of 1.1–2.2 µm.

Sputum examination

Sputum was examined as described by Pizzichini et al. (18). Briefly, sputum was collected into a sterile plastic container and transferred into a Petri dish. All portions that macroscopically appeared free of salivary contamination were selected and placed in an Eppendorf tube, and the weight was recorded.

This selected proportion was treated with four times the volume of dithiotreitol (DTT) (Sputolysin 10%, CalbioChem Corp., San Diego, CA, USA), freshly diluted to 0.1%. The tube was then placed in a bench rocker and rocked for 15 min. A further four volumes of PBS (phosphate-buffered saline, pH 7.4) was added and rocked for 5 min more. The suspension was filtered through 50- μ m nylon gauze, and was thereafter centrifuged at 500 *g* for 10 min. The supernatant was stored in Eppendorf tubes at -20°C for later assays.

The pellet was resuspended in a volume of 1000 μ l PBS, and a total cell count of leukocytes was calculated in a Bürker chamber. The total and absolute number of cells per milligram of processed sputum was calculated. The cell viability was determined by the trypan blue exclusion method and was always over 80%. The cytopspins were made by a Shandon III cytocentrifuge (Shandon Southern Instruments, Sewickley, PA, USA), and were air-dried and stained with May-Grünwald-Giemsa. Differential cell counts of intact bronchial epithelial cells and leukocytes involved counting at least 400 nonsquamous cells by two investigators (I. H. and P. R.) blinded to the clinical characteristics of the study subjects. The cell counts of the two investigators were averaged to yield the final percentages reported here. Sputum eosinophilia was defined as a sputum differential eosinophil count of $>4\%$. This selection was based on the upper limit of the 95% confidence interval (3.2%) in the sputum of 14 normal subjects studied by Pin et al. (13).

Sputum fluid-phase measurements were performed from 27 of the 29 samples from swimmers, and from 14 of the 19 samples from control subjects because of the limited amount of sample. The concentrations of eosinophil peroxidase (EPO, $\mu\text{g/l}$) and human neutrophil lipocalin (HNL, $\mu\text{g/l}$) in the thawed supernatant were determined (19). A prototype CAP System FEIA for EPO and HNL (Pharmacia Upjohn, Diagnostics, Uppsala, Sweden) was used, according to the manufacturer's instructions. The principle of the assay for EPO (HNL) is as follows. Anti-EPO (HNL) coupled to ImmunoCAP reacts with EPO (HNL) in the sample. After washing, β -galactosidase-anti-EPO (HNL) reacts with bound EPO (HNL). After another wash, bound enzyme-anti-EPO (HNL) reacts with 4-methylumbelliferyl- β -D-galactosidase, forming a fluorescent product. EPO used in the standard was purified according to Carlsson et al. (20), and HNL was purified according to Xu et al. (21) but with some modifications. The EPO were highly purified according to the 415/280 nm ratio of 1.14. Monoclonal antibodies were produced by the hybridoma technique. One of them was

Table 1. Clinical characteristics of study subjects*

Characteristic	Swimmers	Control subjects
Subjects (<i>n</i>)	29	19
Age (years)	19.0 (15–25)	24.4 (21–28)
Sex (M/F)	12/17	14/5
History of physician-diagnosed asthma (<i>n</i>)	6	—
Atopic (<i>n</i> †)	15	7
FEV ₁ (% predicted‡)	102.1 (81–130)	95.8 (61–121)
FEV ₁ /FVC (% predicted‡)	93.5 (77–113)	94.7 (74–103)
PEF (% predicted‡)	103.1 (72–137)	100.6 (79–135)
FVC (% predicted‡)	110.1 (72–133)	101.1 (64–128)
Increased bronchial responsiveness, (<i>n</i> §)	14	3
Current inhaled steroid (<i>n</i>)	3	—
Family history of asthma (<i>n</i>)	4	2

* Data are expressed as mean and range. † At least one positive (≥ 3 mm) skin prick test reaction. ‡ Reference values for adult Finns (16). § Slightly or moderately increased responsiveness ($\text{PD}_{15}\text{FEV}_1 \leq 1.6$ mg) (17).

selected and covalently coupled to the ImmunoCAP, and the other was enzyme labeled. The lowest nonzero values for EPO and HNL were 4.5 $\mu\text{g/l}$ and 180 $\mu\text{g/l}$, respectively. Intra- and interassay variations were, for EPO, less than 9%, and for HNL less than 8%.

Blood sampling

Blood was collected by venipuncture. The erythrocyte sedimentation rate (ESR) and blood eosinophils were determined by methods previously described (14).

Statistical analysis

Data are presented as mean and range. The comparisons between groups were performed by the Mann–Whitney *U*-test or the chi-square test (22). For correlations, the Spearman rank-order test was used. A two-tailed *P* value of <0.05 was considered statistically significant.

Results

Clinical and pulmonary function results

Eight (28%) of the 29 swimmers, including four of the six with asthma previously diagnosed by a physician, had a history of exercise-induced bronchial symptoms. The resting spirometric values were within normal limits for all the swimmers (Table 1). Fourteen (48%) of the swimmers, and three (16%) of the control subjects showed increased bronchial responsiveness in the histamine challenge test ($\text{PD}_{15}\text{FEV}_1 \leq 1.6$ mg) ($P < 0.05$). The $\text{PD}_{15}\text{FEV}_1$ values were significantly lower in

Table 2. Comparison of sputum differential cell counts and fluid-phase measurements between swimmers and control subjects*

	Swimmers	Control subjects	P value
Total cell count ($10^3/\text{mg}$)	6.6 (0.1–47.5)	4.6 (0.1–14.6)	0.87
Eosinophils (%)	2.7 (0–25.4)	0.2 (0–1.3)	0.0044
Neutrophils (%)	54.7 (1.7–92.5)	29.9 (0.5–86.2)	0.0056
Lymphocytes (%)	0.7 (0–3.5)	0.6 (0–1.5)	0.78
Macrophages (%)	38.4 (1.7–93.0)	66.2 (13.2–99.5)	0.0049
Bronchial epithelial cells (%)	0.3 (0–2.7)	0.2 (0–2.6)	0.62
EPO ($\mu\text{g/l}$)	28.1 (4.5–437.6)	4.5 (4.5–4.9)	0.0007
HNL ($\mu\text{g/l}$)	12443 (1845–48846)	7110 (1271–21499)	0.05

* Data are expressed as mean and range.

the swimmers than in the controls ($P=0.01$). Fifteen (52%) of the swimmers and seven (39%) of the control subjects were atopic according to skin prick tests. Mean blood eosinophils were $0.14 \times 10^9/\text{l}$ (range $0.04\text{--}0.51 \times 10^9/\text{l}$) for swimmers, and $0.12 \times 10^9/\text{l}$ (range $0.02\text{--}0.39 \times 10^9/\text{l}$) for controls.

Sputum results

One swimmer and one control subject failed to give an adequate sputum sample (Table 2, Figs. 1 and 2). None of the subjects experienced any adverse effects during induction. None of them had a history of upper or lower respiratory tract infection

4 weeks before sputum induction, and the ESR was below 10 in men and below 20 in women.

No significant differences in total nonsquamous sputum cell counts (TCC) existed between swimmers and controls (Table 2). The differential cell counts of lymphocytes and bronchial epithelial cells were also similar in both groups. Sputum from swimmers had significantly higher differential cell counts of eosinophils and neutrophils than sputum from control subjects ($P<0.005$) (Table 2, Figs. 1 and 2). Eosinophilia (sputum differential eosinophil count of $>4\%$) was observed in six (21%) of the swimmers and in none of the controls ($P<0.05$).

Sputum from swimmers contained a higher concentration of EPO and HNL than sputum from controls ($P<0.001$, $P=0.05$, respectively) (Table 2, Figs. 1 and 2). The concentration of sputum EPO correlated significantly with the sputum eosinophil cell count ($r_s=0.47$, $P<0.005$), as did the sputum HNL with the sputum neutrophil cell count ($r_s=0.58$, $P<0.0001$).

Comparisons between clinical and sputum results

The eight swimmers with a history of exercise-induced bronchial symptoms (mean 7.6%, SD 8.8%) had significantly higher sputum eosinophil differential cell counts than symptom-free swimmers (mean 0.7%, SD 0.1%) ($P<0.01$). No correlations appeared between sputum eosinophil

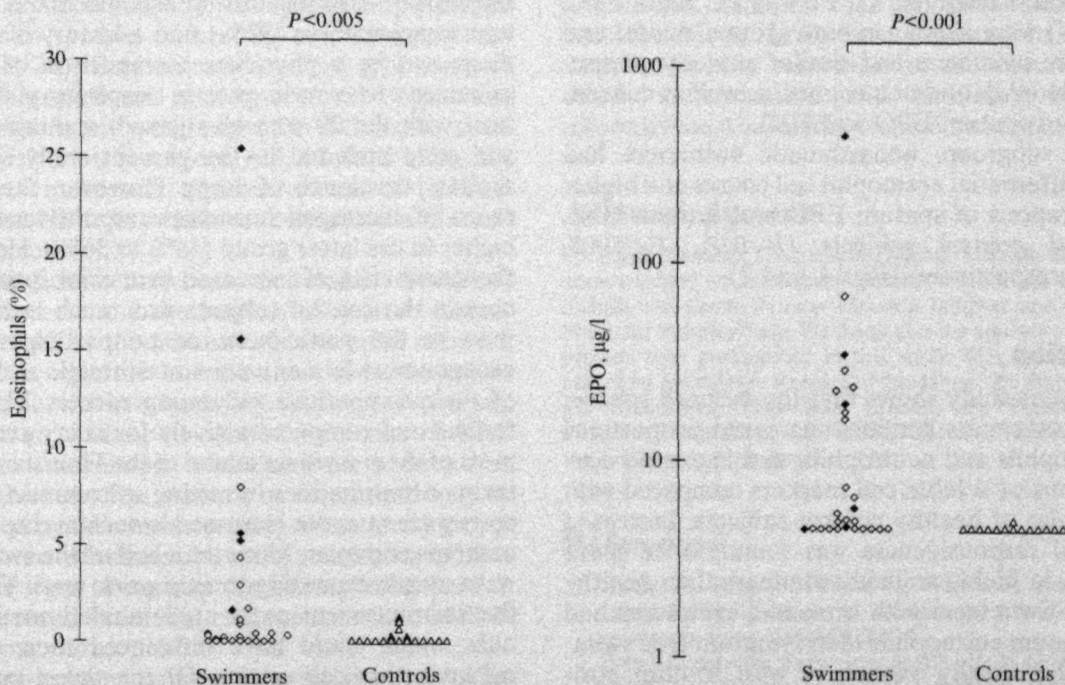


Fig. 1. Differential sputum cell counts of eosinophils and concentrations of sputum eosinophil peroxidase (EPO) in swimmers and healthy control subjects. ◆: swimmers with physician-diagnosed asthma; ◇: nonasthmatic swimmers; △: control subjects.

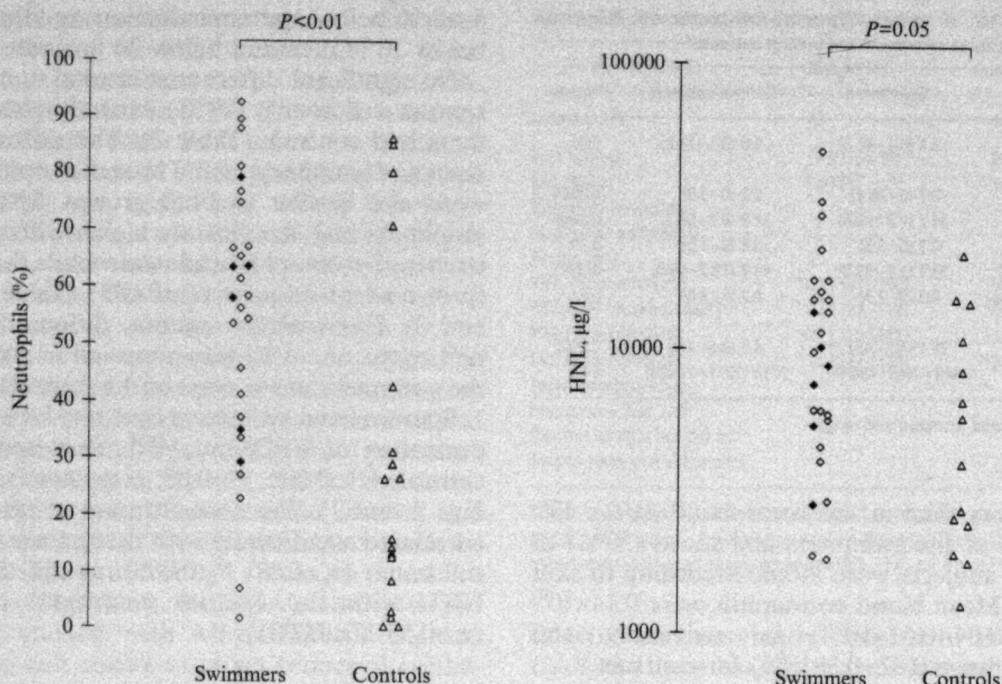


Fig. 2. Differential sputum cell counts of neutrophils and concentrations of sputum neutrophil lipocalin (HNL) in swimmers and healthy control subjects. Symbols as in Fig. 1.

differential cell counts or sputum EPO and $PD_{15}FEV_1$ values (in the 14 swimmers with increased bronchial responsiveness). However, the six swimmers with sputum eosinophilia had significantly ($P < 0.05$) lower $PD_{15}FEV_1$ values than the 22 swimmers without eosinophilia, but resting spirometric values (FEV_1 , FEV_1/FVC ratio, FVC, and PEF) were similar in both groups. Atopic and nonatopic swimmers had similar amounts of sputum eosinophils and neutrophils, as well as concentrations of sputum EPO and HNL.

As a subgroup, nonasthmatic swimmers had higher differential eosinophil cell counts and higher concentrations of sputum EPO and sputum HNL than did control subjects ($P < 0.01$, $P < 0.005$, $P < 0.05$, respectively) (Figs. 1 and 2).

Discussion

The present study shows that the induced sputum of elite swimmers contains increased proportions of eosinophils and neutrophils, and increased concentrations of soluble cell markers compared with the sputum of healthy control subjects. Increased bronchial responsiveness was found to be more common in highly trained swimmers than healthy controls. Swimmers with bronchial symptoms had more sputum eosinophils than symptom-free swimmers. Additionally, swimmers with sputum eosinophilia had more responsive airways than those without eosinophilia. However, correlations were

not found between increased bronchial responsiveness and the percentage of sputum eosinophils or the amount of EPO.

Self-selection may have operated if healthier or symptom-free subjects were less likely to take part. Of the 11 swimmers who did not participate in respiratory function testing and did not give sputum samples, three (27%) had a history of asthma diagnosed by a physician. Comparison of the 42 swimmers who took part in respiratory function tests with the 29 who also gave a sputum sample and were included in the present study shows a similar prevalence of atopy. However, the occurrence of increased bronchial responsiveness was higher in the latter group (48% vs 36%). However, the occurrence of increased bronchial responsiveness in the control subjects was much less (16%) than in the participants or nonparticipants. All except one swimmer were nonasthmatic at the start of their competitive swimming careers. They had trained and competed actively for many years, and most of them were members of the Finnish national team. Abnormalities in resting spirometric parameters were rare, but increased bronchial responsiveness was common. More than half of the swimmers were atopic according to skin prick tests. Three of the swimmers currently used inhaled corticosteroids, which could have influenced their sputum inflammatory cell counts. Of the three, only one showed eosinophilia in the sputum sample. Control subjects were healthy nonsmokers free of bronchial

symptoms, and had not used antiasthmatic medication. Induced sputum samples were obtained and processed by well-documented methods (14, 18, 23).

Comparison with earlier data

Our results confirm earlier findings on the high prevalence of increased bronchial responsiveness and atopic sensitization in elite swimmers (1–3). Zwick et al. (1) found that 11 out of 14 swimmers had increased bronchial responsiveness, and nine reacted to airborne allergens in skin tests. Potts (2) found increased bronchial responsiveness in one-third of the swimmers studied. Among track-and-field athletes, atopic allergy seems to be the most important risk factor for asthma (8–10). In the present study, respiratory symptoms, increased bronchial responsiveness, sputum eosinophil and neutrophil counts, and concentrations of soluble inflammatory cell markers did not differ between atopic and nonatopic swimmers. It may be that in swimmers exposure to airborne allergens is not the reason for increased bronchial responsiveness and signs of airway inflammation.

An increased share of eosinophils and neutrophils was found in induced sputum from competitive swimmers. Previous studies on the cellular characteristics of induced sputum from asthmatics have observed higher differential cell counts of eosinophils and neutrophils than in healthy controls (13, 18, 23, 24). Eosinophils were very few in our control subjects, in agreement with the findings in sputum of healthy, nonasthmatic subjects (18, 24). Concentrations of soluble markers from eosinophils and neutrophils have been found to be significantly increased in asthmatics compared with control subjects (12, 14, 18, 24, 25). We observed increased concentrations of sputum EPO and HNL in swimmers compared with control subjects.

Correlations between increased bronchial responsiveness and the number of sputum eosinophils, as well as the amount of eosinophil markers, have been reported in asthmatics in some studies (13, 18), but not in others (26). In the present study, the sputum eosinophil count or amount of EPO was not correlated with increased bronchial responsiveness. The absence of correlation may be explained by the characteristics of our study group: not chronic asthmatics, but elite swimmers, in most of whom signs of eosinophilic airway inflammation and increased bronchial responsiveness, as well as symptoms, were mild.

Irritant-induced asthma is a variant of occupational asthma occurring in subjects who are acutely or repeatedly exposed to high concentrations of an

irritant product, commonly a gas. The patients develop respiratory symptoms without a latency period (27). Asthma symptoms, airflow obstruction, and increased bronchial responsiveness may arise as a result of repeated exposure to chlorine gas (28, 29). Histologic analysis of the bronchial mucosa has shown a thickened basement membrane, eosinophilic inflammation, and a relative lack of T cells (28). The much increased ventilation during training and competition in elite swimmers results in long-term and repeated exposure to chlorine derivatives from swimming-pool disinfectants. Alveolar chloroform in exhaled air, which is a marker of chlorine gas and organic substances in swimming pools, has been found to be significantly higher in competitive swimmers than ordinary visitors to swimming pools (30). Drobnik et al. (31) measured chlorine gas at breathing level from four different swimming pools, and observed a mean concentration of 0.42 g/m³. They calculated that during a 2-h training period, the swimmer may be exposed to chlorine to an extent (4–6 g) exceeding the US recommendation for a worker with 8-h exposure. The eosinophilic and neutrophilic inflammation observed in sputum from the elite swimmers may represent a form of irritant-induced asthma.

In conclusion, increased bronchial responsiveness was more common in swimmers than in control subjects. Sputum from elite swimmers contained a higher proportion of eosinophils and neutrophils, as well as a higher concentration of soluble cell markers, than sputum from healthy control subjects. Long-term exposure to chlorine compounds may contribute to the occurrence of airway inflammation and increased bronchial responsiveness in highly trained swimmers.

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