

# Airway remodeling and inflammation in competitive swimmers training in indoor chlorinated swimming pools

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**Background:** Airway disorders are common in regular chlorinated swimming pool attendees, particularly competitive athletes, but the impact of intense swimming training on airway function and structure remains unclear.

**Objective:** This study aimed to evaluate airway inflammation and remodeling in elite swimmers.

**Methods:** Twenty-three elite swimmers were tested during off-training season. All had exhaled nitric oxide measurement, methacholine test, eucapnic voluntary hyperpnea challenge, allergy skin prick tests, and bronchoscopy with bronchial biopsies. Clinical data and tissues from 10 age-matched mild-asthmatic and 10 healthy nonallergic subjects were used for comparison.

**Results:** Swimmers had increased airway mucosa eosinophil and mast cell counts than did controls ( $P < .05$ ). They had more goblet cell hyperplasia and higher mucin expression than did healthy or asthmatic subjects ( $P < .05$ ). A greater submucosal type I and III collagen expression and tenascin deposition was also observed in swimmers than in controls ( $P < .05$ ). Neither exhaled nitric oxide nor airway responsiveness to methacholine or eucapnic voluntary hyperpnea challenge correlated with these inflammatory and remodeling changes.

**Conclusion:** Intense, long-term swimming training in indoor chlorinated swimming pools is associated with airway changes similar to those seen in mild asthma, but with higher mucin

expression. These changes were independent from airway hyperresponsiveness. The long-term physiological and clinical consequences of these changes remain to be clarified. (*J Allergy Clin Immunol* 2011;■■■:■■■-■■■.)

**Key words:** Asthma, airway inflammation, airway remodeling, bronchial biopsies, swimmers

There is now indirect evidence showing a link between regular strenuous training and the development of airway disorders in endurance athletes.<sup>1</sup> The high level of ventilation sustained by athletes during repeated training sessions is believed to affect airway mucosa through dehydration and mechanical stress.<sup>2,3</sup> Exercise-induced increased ventilation also favors the penetration of pollutants, allergens, or irritants into the airways, and elite swimmers' airways seem to be particularly affected by the chlorine by-products used to disinfect swimming pools.<sup>4,5</sup> It is now recognized that airway hyperresponsiveness (AHR) to various bronchoprovocative challenges is highly prevalent in elite swimmers, with up to 76% being affected depending on the cutoff point chosen.<sup>6-8</sup> Recent observations suggest that many swimmers do not have or recognize asthma symptoms.<sup>6</sup> The significance of such AHR, often transient and variable in swimmers, is uncertain,<sup>9</sup> and the need to treat subjects who do not present symptoms may be questioned.

In asthma, the exact mechanisms underlying airway remodeling are still unclear, but chronic airway inflammation is considered a potential cause.<sup>10</sup> A previous study suggests the development of an airway inflammatory process in elite skiers that differs from that observed in classical mild asthma but that may lead to similar remodeling.<sup>11</sup> In swimmers, whether chronic airway inflammation persists in induced sputum after at least 12 hours without training is still debated,<sup>8,12</sup> and no data are currently available on airway remodeling. Whether or not elite swimmers develop a classical form of asthma and the type of changes occurring in the airways of these athletes remain to be documented. Thus, we aimed to characterize the morphologic changes in the bronchial mucosa of elite swimmers and to compare these changes with those in nonathletic mild asthmatic and healthy subjects.

## METHODS

### Subjects

Twenty-three competitive swimmers age 17 years and older were recruited. They were nonsmokers, nonobese, and free of any other disease. All gave written informed consent.

From <sup>a</sup>the Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec City, Québec, and <sup>b</sup>the Université Lille Nord de France, EA 4488, UDSL2, Lille.

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Disclosure of potential conflict of interest: L.-P. Boulet is on advisory boards for AstraZeneca, GlaxoSmithKline, MerckFrosst, and Novartis; receives lecture fees from 3M, AstraZeneca, GlaxoSmithKline, MerckFrosst, and Novartis; receives research support from AstraZeneca, GlaxoSmithKline, MerckFrosst, Schering, Altair, Asmacure, Boehringer-Ingelheim, Genentech, Pharmaxis, Wyeth; is an advisor for the Conseil du Médicament du Québec and AETMIS and a member of the Québec Workmen Compensation Board Respiratory Committee; is chair of the Canadian Thoracic Society Respiratory Guidelines Committee and chair of the GINA Guidelines Dissemination and Implementation Committee; is holder of the Laval University Chair on Knowledge Transfer, Prevention, and Education in Respiratory and Cardiovascular Health; and is a member of the Knowledge Translation (KT Canada) supported by the CIHR. The rest of the authors declare that they have no relevant conflicts of interest.

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**Abbreviations used**

AHR: Airway hyperresponsiveness

EVH: Eucapnic voluntary hyperpnea

PC<sub>20</sub> FEV<sub>1</sub>: Concentration of inhaled methacholine causing a 20% fall in FEV<sub>1</sub>

Bronchial biopsies and the associated clinical data from 10 age-matched mild-asthmatic and 10 healthy nonallergic subjects were obtained from the Québec Respiratory Health Network Tissue Bank at the Institut Universitaire de Cardiologie et de Pneumologie de Québec. These subjects had given their consent for clinical evaluation, bronchoscopy with bronchial biopsies, and keeping this information and their tissues at the tissue bank. Healthy subjects were free of respiratory disease and had a methacholine PC<sub>20</sub> FEV<sub>1</sub> (the concentration of inhaled methacholine causing a 20% fall in FEV<sub>1</sub>) of 16 mg/mL or more. Asthmatic subjects had well-documented mild asthma and were paired for atopic status with the swimmers. Control and asthmatic subjects did not train strenuously nor took part in sports competitions.

The study protocol was approved by our institutional Ethics Committee and was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT00686452).

**Study design**

Swimmers were evaluated during a period of relative rest with no to low-intensity exercise, at least 3 days after the last competition or strenuous training, either at the end of summer 2008, mostly in September, or in March–April 2009. For subjects with seasonal allergies, the tests were done outside the symptomatic period. They underwent a physical examination, an exhaled nitric oxide measurement (fraction of exhaled nitric oxide), allergy skin prick tests, methacholine and eucapnic voluntary hyperpnea (EVH) challenges, and a bronchoscopy with bronchial biopsies.

**Subjects' evaluation tests**

Spirometry was carried out according to the American Thoracic Society criteria.<sup>13</sup> Predicted spirometry values were defined according to Knudson et al.<sup>14</sup>

Skin prick tests were performed with extracts of 26 common aeroallergens.<sup>4</sup>

Exhaled nitric oxide was measured by using a NOA 280 analyzer (Siever Instruments, Boulder, Colo), with a single-breath online technique that used a target exhaled pressure of 20 cm H<sub>2</sub>O.<sup>15</sup>

Airway responsiveness to methacholine was measured by using the tidal-breathing method according to the standards recommended by the American Thoracic Society.<sup>6,13</sup> The PC<sub>20</sub> FEV<sub>1</sub> was obtained by linear interpolation on the log concentration response curve. AHR was defined as a PC<sub>20</sub> FEV<sub>1</sub> of less than 16 mg/mL, and we also included a subanalysis with the cutoff of 4 mg/mL as suggested by sports authorities.<sup>16</sup>

AHR to EVH was measured in swimmers, according to the method described by Anderson and Brannan.<sup>17</sup> A swimmer was considered as having AHR to EVH if he or she had a fall of more than 10% in FEV<sub>1</sub> from baseline that was sustained for more than 5 minutes after EVH.

Bronchoscopy was performed by M.L., and 6 to 10 biopsies were taken from subsegmental, segmental, or lobar divisions by using a previously validated technique.<sup>18</sup> Our institution's tissue bank processed the biopsies by using the same standardized methodology: tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5  $\mu$ m) were cut by using a microtome (Surgipath Medical Industries, Richmond, Ill).

The slides were examined by experienced blinded evaluators (P.J. and C.C.). Qualitative evaluation (score) of bronchial tissues was done for the following criteria: general morphology, presence of smooth muscle bundles, inflammation, cartilage, mucus glands, basement membrane, and epithelial integrity.<sup>18</sup> Biopsies of poor quality were not kept. For morphological evaluation, sections were cut and stained with hematoxylin and eosin. Goblet cell hyperplasia, basement membrane denudation, and presence of smooth muscle bundles were semiquantified by using a 0 to 3 score.

Inflammatory cells (neutrophils, T cells, eosinophils, and mast cells) were identified by using the following mAbs: antihuman neutrophil elastase, antihuman CD3, antihuman major basic protein (Bioscience, Cincinnati, Ohio), and antihuman tryptase (Dako, Mississauga, Ontario, Canada). Mucin 5A was stained by using mouse antihuman mucin 5A (Bioscience).<sup>18–20</sup> Sections were also stained with mouse antihuman type-I collagen, mouse antihuman type-III collagen (Calbiochem, Gibbstown, NJ), and mouse antihuman tenascin-c (Leica Microsystems, Bannockburn, Ill). Positive cells were counted in a blinded fashion at  $\times 400$  magnification. For mucin 5A and extracellular matrix proteins, submucosal areas were measured with a calibrated image analysis system (Image Pro-Plus software, Media Cybernetics, Bethesda, Md) as previously reported.<sup>18</sup> The mean intraobserver variability (3 repeated cell counts) was between 4% and 8% for all studied antibodies. Mucin 5A was quantified by using Image Pro-plus software. Types I and III collagen and tenascin layer thickness below the basement membrane were measured by using the Image Pro-Plus software.<sup>19</sup> These data were expressed as the mean of 3 measurements.

**TABLE I.** Subjects' characteristics

|                                                                 | Controls<br>(n = 10) | Swimmers<br>(n = 23)   | Asthmatic<br>subjects<br>(n = 10) | Swimmers with<br>AHR to methacholine<br>(n = 12) | Swimmers without<br>AHR to methacholine<br>(n = 11) |
|-----------------------------------------------------------------|----------------------|------------------------|-----------------------------------|--------------------------------------------------|-----------------------------------------------------|
| Sex, male:female                                                | 2:8                  | 12:11                  | 2:8                               | 5:7                                              | 7:4                                                 |
| Age (y)                                                         | 21 $\pm$ 2           | 21 $\pm$ 2             | 23 $\pm$ 6                        | 21 $\pm$ 2                                       | 20 $\pm$ 2                                          |
| Atopy (%)                                                       | 0 (0)                | 18 (82)                | 8 (80)                            | 11 (92)                                          | 7 (64)                                              |
| Training (y)                                                    | –                    | 11 $\pm$ 3             | –                                 | 11 $\pm$ 4                                       | 11 $\pm$ 3                                          |
| FEV <sub>1</sub> (% predicted)                                  | 99 $\pm$ 9           | 115 $\pm$ 12*,†        | 93 $\pm$ 11                       | 111 $\pm$ 14                                     | 118 $\pm$ 9                                         |
| FVC (% predicted)                                               | 106 $\pm$ 11         | 126 $\pm$ 12*,†        | 100 $\pm$ 8                       | 128 $\pm$ 15                                     | 126 $\pm$ 7                                         |
| Methacholine PC <sub>20</sub> FEV <sub>1</sub> (mg/mL), (range) | 93.7<br>(33.4–128.0) | 12.5*,†<br>(1.0–128.0) | 1.3‡<br>(0.3–19.7)                | 4.7<br>(0.97–11.7)                               | 43.0<br>(17.3–128.0)                                |
| FENO (ppb)                                                      | –                    | 18.8 $\pm$ 11.3        | –                                 | 23.4 $\pm$ 13.2                                  | 13.1 $\pm$ 4.6                                      |
| Post-EVH fall in FEV <sub>1</sub>                               | –                    | 11 $\pm$ 6             | –                                 | 14 $\pm$ 6                                       | 7 $\pm$ 3                                           |
| Previous physician-diagnosed asthma (%)                         | 0 (0)                | 3 (14)*                | 10 (100)‡                         | 3 (25)                                           | 0 (0)                                               |
| Current use of inhaled $\beta_2$ -agonists when needed (%)      | 0 (0)                | 0 (0)*                 | 9 (90)‡                           | 0 (0)                                            | 0 (0)                                               |
| Current use of inhaled corticosteroids (%)                      | 0 (0)                | 0 (0)                  | 0 (0)                             | 0 (0)                                            | 0 (0)                                               |

Prevalence is expressed as number of subjects (% of subjects), and other data are expressed as mean  $\pm$  SD. Methacholine PC<sub>20</sub> FEV<sub>1</sub> is expressed as geometrical mean (range).

\* $P < .005$  swimmers versus asthmatic subjects.

† $P < .05$  swimmers versus controls.

‡ $P < .005$  asthmatic subjects versus controls.

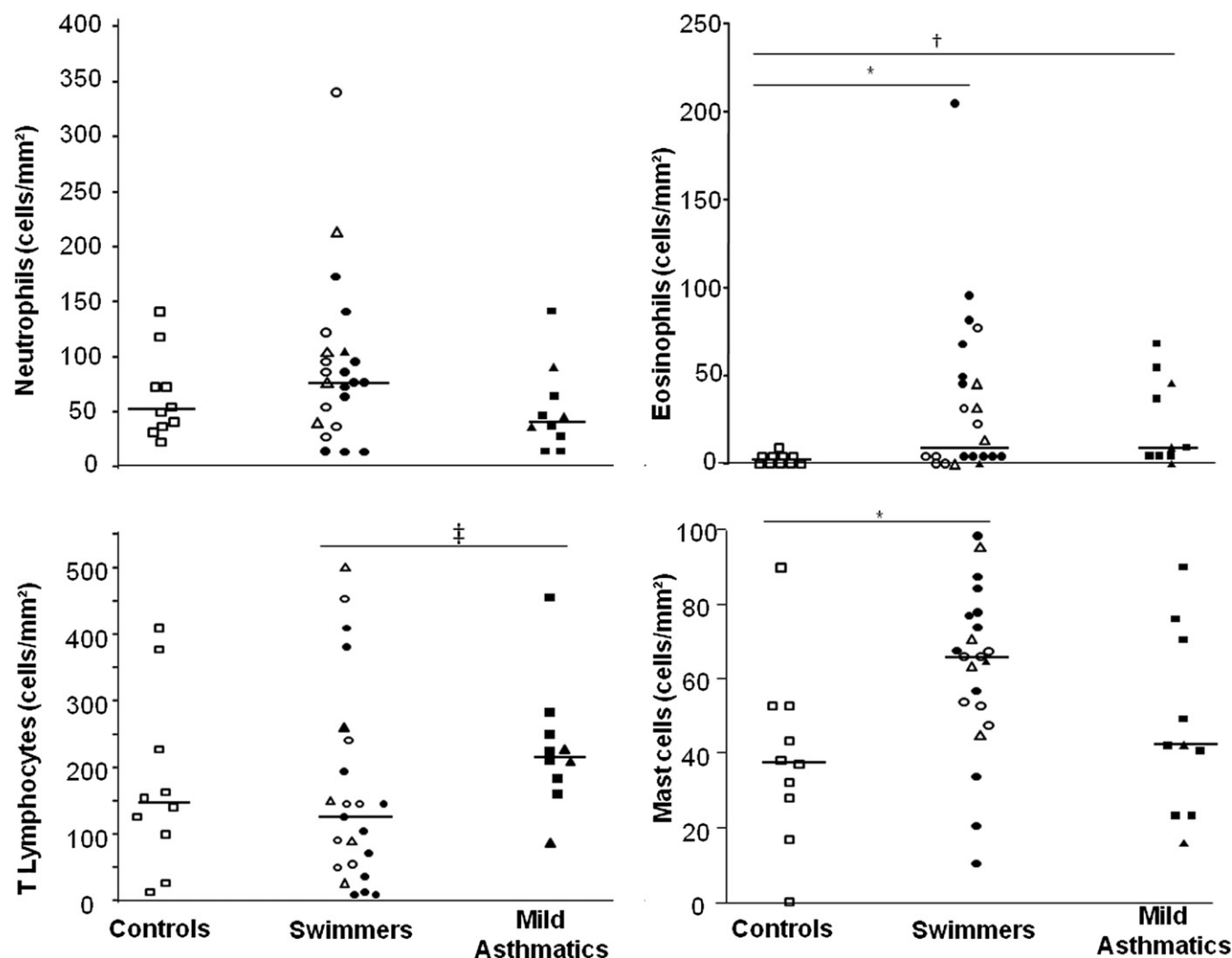


**FIG 1.** Quantification of inflammatory cells (number of cells per square millimeter of connective tissue) in bronchial biopsy specimens from controls, swimmers, and asthmatic subjects. Horizontal bar represents median value. Subjects with methacholine AHR are represented with a full symbol, and those without AHR are represented with an empty symbol. Subjects without allergies, either swimmers or asthmatic subjects, are represented with a triangle in their respective group. \* $P < .05$  entire swimmer group versus controls. † $P < .05$  asthmatic subjects versus controls. ‡ $P < .05$  entire swimmer group versus asthmatic subjects.

## Data analysis

Nominal variables were analyzed by using the chi-square test. Results of representative measures from continuous variables were expressed by using mean  $\pm$  SEM or median (interquartile range) according to the data distribution. One-way ANOVA was used to analyze data. *Posteriori* comparisons among groups were performed by using the Tukey's technique. For CD3 and neutrophil elastase markers, the logarithm was the estimate form of the required transformation associated with these variables. Some values were expressed with transformed values, and statistical results arise from these transformations. The normality assumption was verified with the Shapiro-Wilk test, and the Brown and Forsythe's variation of Levene's test statistic was used to verify the homogeneity of variances. When needed, some variables were log transformed. All assumptions were fulfilled except for major basic protein. For this variable, 1-way ANOVA was performed on its rank. Relationships between parameters were expressed with the Pearson's correlation coefficients. The results were considered significant with  $P$  values of .05 or less. The data were analyzed by our local statistician

by using the statistical package program SAS, version 9.2 (SAS Institute, Inc, Cary, NC).

## RESULTS

### Subjects

Subjects' characteristics are presented in Table I. Swimmers competed at the provincial ( $n = 11$ ) or national level ( $n = 12$ ). The mean rest period since the last strenuous training or competition was  $45 \pm 55$  days.

Twelve swimmers (52%) had a PC<sub>20</sub> FEV<sub>1</sub> of less than 16 mg/mL, and 5 of them (22%) had a PC<sub>20</sub> FEV<sub>1</sub> of less than 4 mg/mL. The FEV<sub>1</sub> and forced vital capacity were higher in swimmers than in asthmatic and control groups ( $P < .05$ ). Three swimmers had a previous physician-diagnosed asthma but used no current medication. Two swimmers did not undergo the EVH test, because of the

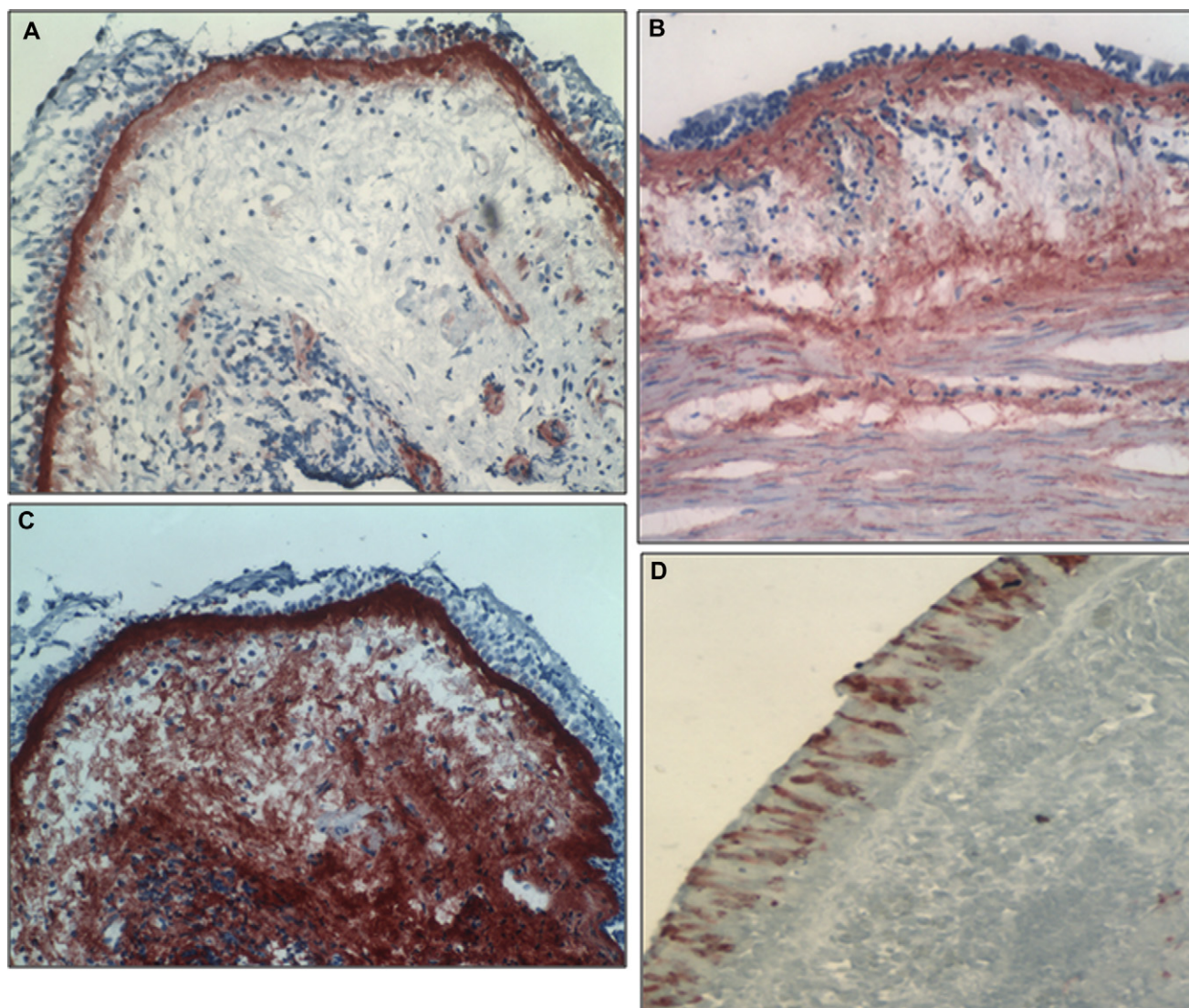


**FIG 2.** Representative bronchial tissue from a nonatopic swimmer without methacholine AHR. **A**, Tenascin deposition under the basement membrane. **B**, Collagen III deposition. **C**, Collagen I deposition. **D**, Mucin 5 immunostaining.

unavailability of the apparatus at the time of the visit. Among the remaining 21 swimmers, 9 (43%) had AHR to EVH. Individual data are shown in the Online Repository (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

The PC<sub>20</sub> FEV<sub>1</sub> was lower in asthmatic subjects than in controls or swimmers ( $P < .0001$ ) and in swimmers than in controls ( $P < .0001$ ) (Table I). Swimmers with AHR to methacholine had a higher fall in FEV<sub>1</sub> after EVH compared with those without AHR to methacholine ( $P = .006$ ).

### Assessment of airway inflammation

Inflammatory cell counts on bronchial biopsies are represented in Fig 1. Swimmers with and without AHR to methacholine had similar inflammatory cell counts. There were no significant differences in neutrophil counts between swimmers and mild asthmatic subjects and controls. Eosinophils were increased in both swimmers and asthmatic subjects compared with controls, 13.6

(4.5-47.8), 9.1 (4.5-43.2), and 2.3 (0-4.5) cells/mm<sup>2</sup>, respectively ( $P \leq .04$ ); no significant difference was observed between asthmatic subjects and swimmers. T lymphocytes were increased in asthmatic subjects compared with swimmers, 215.9 (188.6-244.3) and 115.9 (51.1-184.1) cells/mm<sup>2</sup>, respectively ( $P = .04$ ), and these groups were not different from controls: 147.7 (106.8-211.4) cells/mm<sup>2</sup> ( $P = .04$ ). Mast cell counts were higher in swimmers than in controls, 65.8 (52.8-75.9) and 37.4 (29.1-50.2) cells/mm<sup>2</sup>, respectively ( $P = .02$ ). No difference in mast cell counts was observed between the 2 groups compared with asthmatic subjects: 42.0 (27.5-64.8) cells/mm<sup>2</sup>.

No significant differences in inflammatory cell counts were observed between swimmers with a PC<sub>20</sub> FEV<sub>1</sub> of less than 4 mg/mL and those with a PC<sub>20</sub> FEV<sub>1</sub> of more than 4 mg/mL, nor between those with a FEV<sub>1</sub> fall post-EVH of more than 10% and those with a fall of less than 10% (data not shown).

Atopic and nonatopic swimmers, represented, respectively, by circles and triangles, had similar inflammatory features.

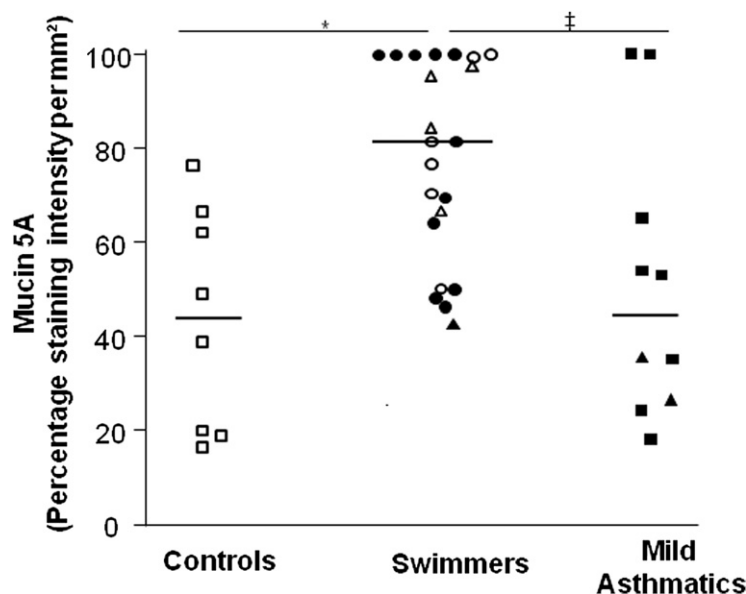


**FIG 3.** Mucin 5A (percentage staining intensity per square millimeter of connective tissue) staining in controls, swimmers, and asthmatic subjects. Horizontal bar represents median value. Subjects with methacholine AHR are represented with a full symbol, and those without AHR are represented with an empty symbol. Subjects without allergies, either swimmers or asthmatic subjects, are represented with a triangle in their respective group. \* $P < .01$  entire swimmer group versus controls. ‡ $P < .05$  entire swimmer group versus asthmatic subjects.

### Assessment of airway remodeling

Fig 2 shows distinctive subepithelial fibrosis and mucin secretion observed in swimmers. Semiquantitative analysis showed no significant difference in the presence of smooth muscle and in basement membrane denudation between the 3 groups (data not shown). However, compared with the other groups, swimmers had significant goblet cell hyperplasia, 0 (0-0) and 0 (0-1.8), respectively ( $P < .005$ ).

Swimmers with or without AHR had similar mucin 5A staining. The swimmers' mucin 5A expression was increased by 1.8-fold compared with both controls ( $P = .01$ ) and mild asthmatic subjects ( $P = .04$ ), respectively (Fig 3). Fig 4 shows collagen I and III deposition and tenascin thickness underneath the basement membrane; there were no significant difference between swimmers and patients with mild asthma or between swimmers with AHR to methacholine or EVH and swimmers without AHR to methacholine or EVH. However, collagen I and III were increased in swimmers compared with in controls,  $9.3 \pm 0.7 \mu$  and  $6.7 \pm 0.4 \mu$  for collagen I ( $P = .001$ ) and  $14.5 \pm 1.3 \mu$  and  $5.7 \pm 0.6 \mu$  for collagen III ( $P = .02$ ), respectively. Tenascin deposition was also increased in swimmers ( $6.9 \pm 0.2 \mu$ ) compared with controls ( $6.2 \pm 0.3 \mu$ ) ( $P = .02$ ).

Atopic and nonatopic swimmers, represented, respectively, by circles and triangles, had similar remodeling features.

### Correlation analyses

We found no significant correlation between years of training, time between the last swimming training and the first evaluation visit, degree of airway obstruction, fraction of exhaled nitric oxide level, EVH and methacholine AHR, and inflammatory or remodeling features.

The results remained unchanged when the 3 athletes with a previous diagnosis of asthma were excluded.

### DISCUSSION

We observed inflammatory and remodeling changes in bronchial biopsies of competing swimmers, with or without AHR, similar to nonathletes with mild asthma. Interestingly, swimmers showed greater goblet cell hyperplasia and increased mucin expression than did asthmatic subjects. Therefore, our results suggest that swimming training in chlorinated pools affects the structure of the airway in elite swimmers.

In our study, a majority of swimmers had atopy (18 of 23), which may have contributed to the changes in airway inflammation and remodeling, as previously reported in atopic asthmatic subjects and controls.<sup>21-23</sup> In atopic controls, Djukanovic et al showed that these changes are similar in nature but less marked compared with those in atopic asthmatic subjects.<sup>23</sup> In elite winter athletes, increases in airways' tenascin thickness or inflammatory cell counts were observed, independently of atopy, having significant airway remodeling and inflammation compared with controls.<sup>11</sup> We and others previously underlined the high prevalence of atopy among elite swimmers when tested with 20 to 26 common aeroallergens.<sup>6,24</sup> This is an important point, as a recent hypothesis stated that atopy may develop in swimmers because of an increasing exposure to chlorination products.<sup>25,26</sup> Exposure to chlorine compounds in indoor swimming pools has been shown to cause epithelial hyperpermeability and damage,<sup>5</sup> which, in turn, may facilitate mucosal penetration by allergens and induce allergic sensitization.<sup>25</sup> In our study, the high prevalence of atopy in swimmers may be a consequence of repeated training in indoor chlorinated swimming pools. However, when comparing nonatopic and atopic swimmers (Figs 1-4), the presence of atopy did not seem to be associated with more marked effects on airway inflammation or remodeling in our swimmers.

High-level sport training may also induce airway changes through the high ventilatory demand sustained during repeated

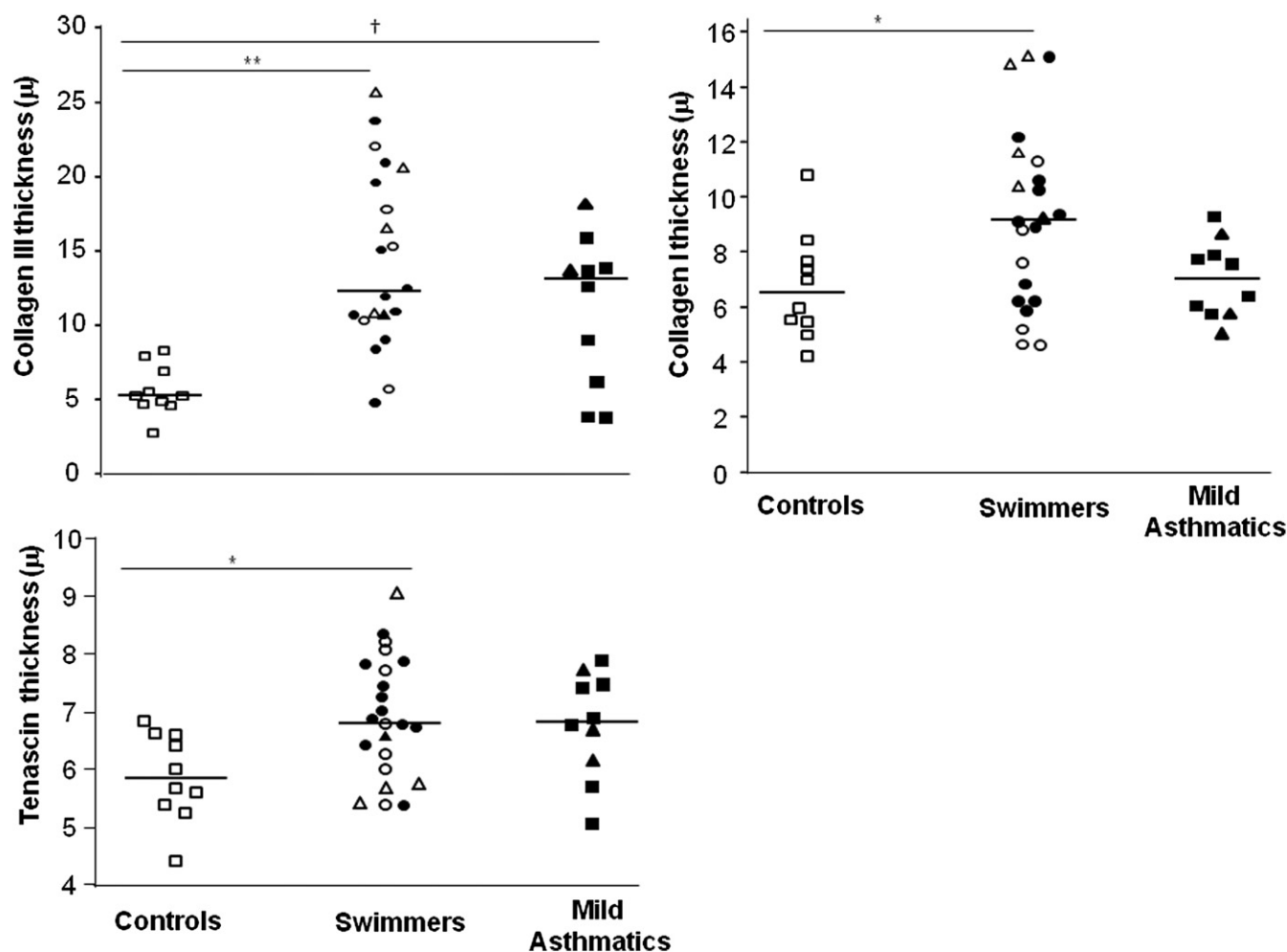


**FIG 4.** Thickness of tenascin immunoreactive band and collagen I and collagen III deposition in subepithelial basement membrane zone in controls, swimmers, and asthmatic subjects. Horizontal bar represents median value. Subjects with allergies, either swimmers or asthmatic subjects, are represented with a triangle in their respective group. Subjects with methacholine AHR are represented with a full symbol, and those without AHR are represented with an empty symbol. Subjects without allergies, either swimmers or asthmatic subjects, are represented with a triangle in their respective group. \* $P < .05$  entire swimmer group versus controls. \*\* $P < .005$  entire swimmer group versus controls. † $P < .05$  asthmatic subjects versus controls.

strenuous period. This is in agreement with a previous study that reported increased bronchial inflammation and tenascin expression in elite cross-country skiers.<sup>11</sup> Exercise-induced hyperpnea may directly stimulate mucin secretion to counteract the drying of the protective lining of the airways and possibly increase the provision of water.<sup>27</sup> Moreover, we cannot exclude the role of mechanical stress induced by the repeated high ventilatory demand on the bronchial epithelium.<sup>28</sup> Increased airway movements and increased airway pressure changes during intense breathing could stimulate epithelial cells to release fibrogenic cytokines, as shown in *in vitro* studies by Tschumperlin and Drazen.<sup>28</sup> In this regard, an increase in induced sputum mucin 5A and mast cells has been shown following exercise in asthmatic subjects who developed exercise-induced bronchoconstriction.<sup>29</sup> Remodeling of the airway epithelium to a secretory phenotype, which would be a response to injury, has been previously hypothesized to be implicated in the physiopathology of asthma<sup>30</sup> and the development of exercise-induced bronchoconstriction.<sup>29</sup> Chimenti et al<sup>31</sup>

showed evidence that goblet cells were not affected by endurance training at low to moderate intensity in mice without AHR. The characteristics of inhaled air as well as the presence of asthma diagnosis probably explain the discrepancies in mucin-secreting cell changes observed between these studies.

Repeated exposure to chlorine by-products may also have played a role in airway changes observed in elite swimmers. As previously mentioned, chlorine by-products' exposure may predispose swimmers to develop epithelial damage and atopy.<sup>5,25,26</sup> While eosinophil infiltration was previously reported in athletes, neutrophils and T cells seem more abundant in winter-sports athletes' airways and mast cells seem more abundant in swimmers.<sup>11</sup> These differences are likely because winter-sport athletes breathe high volumes of dry and cold air, whereas swimmers repeatedly inhale hot and humid air. In addition, swimmers are exposed to chlorine by-products that are significant airway irritants,<sup>32,33</sup> which may induce airway inflammation, as well as epithelial damage and changes in airway responsiveness.<sup>5,34,35</sup> As a

consequence of repeated exposures, swimmers may develop airway mast cell and eosinophil infiltration, as previously observed after repeated exposure to occupational agents.<sup>36,37</sup> We cannot exclude that mucin secretion may also be a defense mechanism against repeated inhaled chlorine by-products. Consistent with previous studies on athletes,<sup>8,11,12</sup> we found no correlation between the severity of AHR and inflammatory cell counts in the airways.

Because of a lack of report of respiratory symptoms in many swimmers with AHR,<sup>6,38</sup> the clinical significance of our results remains uncertain, and consequently it is unclear whether this condition should be treated. It seems, however, reasonable to suggest that preventive measures should be taken to reduce the impact of the environment on athletes' airways.<sup>39</sup>

In conclusion, we have demonstrated inflammatory and remodeling changes in airways of elite swimmers, which were not associated with AHR. Atopy, strenuous training, and chlorine by-product inhalation may have contributed to these features. These changes could constitute a normal adaptation process to intense training; furthermore, as demonstrated for AHR, these are possibly reversible, at least in part, after the end of the swimming career, although this remains to be demonstrated. Consequently, the physiological and clinical consequences of these changes remain indefinite and whether recreational swimmers and children attending regularly indoor chlorinated swimming pools have a higher risk to develop similar airway changes remains to be determined. Although preventive measures such as reduction of chloramines exposure in pool environments should be considered, it is still uncertain whether we should offer preventative treatments to swimmers, in order to try to reduce airways inflammation and structural changes.

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### Key message

- Regular intense swimming in a chlorinated environment seems to induce airway inflammatory and remodeling changes that are not always associated with AHR.
- A particular attention should be paid to the respiratory health of competitive swimmers and further studies should be conducted to dissociate the role of chlorine by-products and intense training on the airways.

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## RESULTS

Individual data from the swimmers are presented in Table E1.

TABLE E1. Swimmers' individual data

| Swimmer | Years of training | Atopies        |                   |            | Rhinitis/nasal symptoms during the year |          |      | ENO (ppb) | Baseline spirometry (%) |     |                      | EVH (% fall in FEV <sub>1</sub> ) | MCh PC <sub>20</sub> FEV <sub>1</sub> (mg/mL) | Blood IgE (UI/mg) | Childhood asthma |
|---------|-------------------|----------------|-------------------|------------|-----------------------------------------|----------|------|-----------|-------------------------|-----|----------------------|-----------------------------------|-----------------------------------------------|-------------------|------------------|
|         |                   | Animal danders | Mixed trees/grass | House dust | During effort                           | Allergic | nPIF |           | FEV <sub>1</sub>        | FVC | FEF <sub>25-75</sub> |                                   |                                               |                   |                  |
| A       | 14                | No             | Yes               | Yes        | No                                      | Yes      | 280  | 26.5      | 101                     | 120 | 105                  | 16.0                              | 3.2                                           | 1000              | No               |
| B       | 12                | Yes            | No                | Yes        | No                                      | No       | 180  | 15.4      | 116                     | 120 | 111                  | 8.4                               | 5.98                                          | 64                | No               |
| C       | 14                | Yes            | Yes               | Yes        | No                                      | Yes      | 130  | 17.1      | 110                     | 119 | 88                   | 11.2                              | 17.3                                          | 139               | No               |
| D       | 10                | No             | No                | No         | Yes                                     | No       | 170  | 8.2       | 106                     | 130 | 87                   | 7.1                               | 18.4                                          | 28                | No               |
| E       | 13                | No             | Yes               | Yes        | Yes                                     | No       | 120  | 19.9      | 109                     | 130 | 88                   | 7.8                               | 3.3                                           | NA                | No               |
| F       | 14                | Yes            | Yes               | Yes        | No                                      | Yes      | 150  | 26.0      | 132                     | 155 | 108                  | 29.3                              | 0.97                                          | 590               | No               |
| G       | 11                | Yes            | No                | Yes        | No                                      | Yes      | 90   | 53.6      | 129                     | 135 | 121                  | 8.8                               | 9.7                                           | 59                | No               |
| H       | 11                | No             | No                | Yes        | Yes                                     | Yes      | 140  | 11.2      | 118                     | 119 | 127                  | 2.4                               | 48.6                                          | 78                | No               |
| I       | 8                 | Yes            | Yes               | Yes        | Yes                                     | Yes      | 150  | 13.0      | 100                     | 107 | 104                  | 11.7                              | 5.4                                           | 463               | Yes              |
| J       | 13                | No             | No                | No         | No                                      | No       | 190  | 16.2      | 135                     | 138 | 133                  | 6.6                               | 92.7                                          | 33                | No               |
| K       | 8                 | Yes            | Yes               | Yes        | No                                      | Yes      | 140  | 14.4      | 106                     | 126 | 89                   | 17.0                              | 3.4                                           | 276               | Yes              |
| L       | 14                | No             | No                | No         | NA                                      | NA       | NA   | NA        | 110                     | 117 | NA                   | NA                                | 3.5                                           | NA                | No               |
| M       | 12                | Yes            | Yes               | Yes        | NA                                      | NA       | NA   | NA        | 116                     | 117 | NA                   | NA                                | 33.0                                          | 611               | No               |
| N       | 8                 | No             | No                | No         | No                                      | No       | NA   | 7.6       | 112                     | 123 | 85                   | 7.4                               | 72.3                                          | 189               | No               |
| O       | 1                 | Yes            | Yes               | Yes        | No                                      | No       | NA   | 26.5      | 97                      | 124 | 66                   | 17.7                              | 8.9                                           | 611               | Yes              |
| P       | 6                 | Yes            | Yes               | Yes        | No                                      | Yes      | NA   | 10.4      | 132                     | 131 | 154                  | 3.3                               | 79.9                                          | 21                | No               |
| Q       | 13                | Yes            | No                | Yes        | No                                      | No       | 235  | 21.3      | 117                     | 122 | 113                  | 4.8                               | 53.2                                          | 65                | No               |
| R       | 10                | No             | Yes               | Yes        | No                                      | No       | NA   | 9.7       | 98                      | 112 | 93                   | 11.9                              | 5.2                                           | 31                | No               |
| S       | 14                | Yes            | Yes               | Yes        | No                                      | No       | NA   | 13.5      | 99                      | 130 | 82                   | 13.9                              | 11.7                                          | 32                | No               |
| T       | 8                 | No             | No                | No         | No                                      | No       | NA   | NA        | 122                     | 125 | 112                  | 8.7                               | 28.0                                          | 16                | No               |
| U       | 10                | Yes            | Yes               | Yes        | No                                      | No       | NA   | 39.0      | 133                     | 156 | 117                  | 9.7                               | 5.1                                           | 244               | No               |
| V       | 13                | No             | No                | No         | No                                      | No       | NA   | 15.3      | 117                     | 124 | 91                   | 11.9                              | 128                                           | 16                | No               |
| W       | 14                | Yes            | Yes               | Yes        | Yes                                     | No       | 190  | 10.3      | 116                     | 137 | 116                  | 7.5                               | 17.8                                          | 244               | No               |

Rhinitis was reported at the evaluation by the presence of nasal symptoms during the previous year. The biopsy and respiratory challenges were performed outside the upper airways' symptomatic period. The 3 swimmers with a diagnosis of asthma made during childhood by a clinician did not take inhaled corticosteroids or  $\beta_2$  agonists.

eNO, Exhaled nitric oxide; EVH % fall in FEV<sub>1</sub>, maximum percentage fall in FEV<sub>1</sub> after the eucapnic voluntary hyperpnea test; FVC, forced vital capacity; FEF<sub>25-75</sub>, forced expiratory flow from 25% to 75% of FVC; NA, not available; nPIF, nasal peak inspiratory flow.