

## Airway responsiveness and inflammation in adolescent elite swimmers

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**Background:** Whereas increased airway hyperresponsiveness (AHR) and airway inflammation are well documented in adult elite athletes, it remains uncertain whether the same airway changes are present in adolescents involved in elite sport. **Objective:** To investigate airway responsiveness and airway inflammation in adolescent elite swimmers.

**Methods:** We performed a cross-sectional study on adolescent elite swimmers (n = 33) and 2 control groups: unselected adolescents (n = 35) and adolescents with asthma (n = 32). The following tests were performed: questionnaire, exhaled nitric oxide (FeNO), spirometry, induced sputum, methacholine challenge, eucapnic voluntary hyperpnea (EVH) test, and exhaled breath condensate pH.

**Results:** There were no differences in FeNO, exhaled breath condensate pH, cellular composition in sputum, or prevalence of AHR to either EVH or methacholine among the 3 groups. When looking at airway responsiveness as a continuous variable, the swimmers were more responsive to EVH than unselected subjects, but less responsive to methacholine compared with subjects with asthma. We found no differences in the prevalence of respiratory symptoms between the swimmers and the unselected adolescents. There was no difference in FeNO, cellular composition of sputum, airway reactivity, or prevalence

of having AHR to methacholine and/or EVH between swimmers with and without respiratory symptoms.

**Conclusion:** Adolescent elite swimmers do not have significant signs of airway damage after only a few years of intense training and competition. This leads us to believe that elite swimmers do not have particularly susceptible airways when they take up competitive swimming when young, but that they develop respiratory symptoms, airway inflammation, and AHR during their swimming careers. (*J Allergy Clin Immunol* 2008;122:322-7.)

**Key words:** Airway inflammation, airway hyperresponsiveness, airway responsiveness, asthma, elite swimmers, eucapnic voluntary hyperpnea, exercise-induced bronchoconstriction, methacholine, respiratory symptoms

Adult elite athletes have a high prevalence of respiratory symptoms and airway hyperresponsiveness (AHR),<sup>1-4</sup> which may result from excessive hyperpnea, as well as environmental factors related to elite sport.<sup>5-7</sup> In addition, many elite athletes have airway inflammation, although the type of inflammation seems to vary between different types of sport.<sup>8-11</sup> It is likely that an increase in neutrophils is a consequence of endurance training and that increased eosinophils and lymphocytes are a result of exposure to environmental factors related to the different types of sport.<sup>12</sup> Swimming is one sport that has attracted attention; many children with childhood asthma were recommended to undertake swimming as their daily exercise because swimming was found to be less likely to lead to exercise-induced asthma.<sup>13-15</sup> A prevalence of AHR in adult swimmers of up to 79% is much higher than in healthy controls.<sup>16-18</sup> Further, differences in the cellular characteristics of induced sputum have been documented, with swimmers having significantly higher differential cell counts of both neutrophils and eosinophils than healthy controls.<sup>16</sup>

Most studies on elite athletes have been performed in adults who have been involved in competitive sport over a long period. These individuals have therefore had years with hyperpnea in relation to their sport, as well as a prolonged exposure to environmental factors in their training environment. However, little is known about children and adolescents involved in elite sports. This group does not have the same long-term background of years of competition and strenuous exercise as adult elite athletes. It is unclear whether airway changes are already present before they start competing and training intensively or whether these develop during prolonged training. Thus, whereas increased AHR and airway inflammation seem to be well documented in adult elite athletes, it remains uncertain whether the same airway changes are present in children and adolescents involved in elite sport. The early presence of AHR is of importance because the

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#### Abbreviations used

AHR:	Airway hyperresponsiveness
EBC:	Exhaled breath condensate
EIB:	Exercise-induced bronchoconstriction
EVH:	Eucapnic voluntary hyperpnea
FeNO:	Fractional exhaled nitric oxide
FVC:	Forced vital capacity
ICS:	Inhaled corticosteroid
IOC-MC:	International Olympic Committee—Medical Commission
MVV:	Maximal voluntary ventilation

presence of asymptomatic AHR in childhood precedes development of asthma.<sup>19-21</sup>

We aimed to investigate AHR and airway inflammation in adolescent elite swimmers and compare the findings with those in adolescents with asthma and with those in unselected adolescents.

## METHODS

### Design

We performed a cross-sectional study on adolescents (age 12-16 years) involved in elite swimming (elite group). For comparison, we included 2 age-matched control groups: a group of unselected adolescents (unselected group) and a group of adolescents with a diagnosis of asthma (asthma group).

The study consisted of 2 visits (visits A and B) performed in random order on separate days. All participants had the following examinations performed: questionnaire, fractional exhaled nitric oxide (FeNO), lung function, and a skin prick test. At visit A, we performed a methacholine challenge followed by sputum induction,<sup>22</sup> and at visit B we collected exhaled breath condensate (EBC) followed by a eucapnic voluntary hyperpnea (EVH) test. The participants were asked not to take inhaled corticosteroids for the 4 weeks before the study, and they were asked to refrain from taking  $\beta_2$ -agonists 12 hours before testing and not to exercise 24 hours before the visits.

The study was approved by the local ethics committee (journal number KF 01 261528), and it was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov). All participants and their parents or guardians gave informed consent.

### Subjects

The study was composed of 100 adolescents in 3 groups: the elite group, the unselected group, and the asthma group. No subject had had an upper respiratory tract infection in the previous 4 weeks, none of the participants was a smoker, and none used any medication or dietary supplements other than those listed below (see "Elite group" and "Unselected group"). Except for those with asthma, none of the participants reported morbid conditions. None of the children was reimbursed in any way.

**Elite group.** We posted questionnaires to 55 swimmers who were all part of the National Swimming Association's talent program for young swimmers and living in the Greater Copenhagen area. The questionnaire was returned by 48 (87%), 1 of whom had discontinued swimming at competitive level; 6 (13%) had a previous diagnosis of asthma, of whom 2 did not use medication, 3 used only short-acting  $\beta_2$ -agonist when needed, and 1 used inhaled corticosteroids (ICSs) daily and was excluded from the study because of the undesirability of discontinuing ICSs for 4 weeks. A total of 33, including all 5 (15%) with asthma, volunteered to participate. The swimmers had been involved in competitive swimming for an average of 2.7 (SD, 1.3) years and trained 19.6 (5.4) hours per week; 28 swimmers completed both visits, 4 completed only visit A, and 1 completed only visit B. All swimmers not completing both visits gave lack of time as the reason.

**Unselected group.** This control group consisted of 2 unselected ninth grade school classes (average age, 14.4 years) with a total of 44 students, of whom 35 (19 girls) volunteered to participate. Three (9%) had doctor-diagnosed asthma, of whom 2 used only  $\beta_2$ -agonists as needed and 1 had had ICS prescribed but

was noncompliant at the time of the study. The unselected group was included to get an unselected sample from the general population. The participants were randomized either to visit A ( $n = 22$ ) or visit B ( $n = 13$ ) with an uneven distribution aiming at prioritizing sputum collection.

**Asthma group.** This control group consisted of 32 subjects with doctor-diagnosed asthma who were not involved in competitive sport. All were newly diagnosed or had had the diagnosis reconfirmed within the last year. The asthma group was included to have a group with expected airway hyperactivity and inflammatory changes. Fifteen participants were invited to and completed both visit A and visit B, whereas 17 were invited only to visit A (excluding sputum induction) because the visit was performed at a clinic without access to the EVH test and sputum collection; all 17 completed the visit. Thus, in total, 32 completed visit A (17 without having sputum induction done), and 15 completed visit B.

## Methods

**Questionnaire.** We used a modified version of a questionnaire normally used in elite athletes in our clinic, which is used in research.<sup>23</sup> The questionnaire focused on respiratory symptoms (wheezing, breathlessness, chest tightness, and cough) at rest and at exercise, use of asthma medication, doctor-diagnosed asthma, doctor-diagnosed hay fever, allergic symptoms, physical training, and childhood exposures. Subjects were classified as symptomatic if they had 1 or more respiratory symptoms at least once weekly within the last 4 weeks.

**Lung function measurements.** Spirometry was performed in accordance with American Thoracic Society/European Respiratory Society recommendations.<sup>24</sup> The FEV<sub>1</sub> and forced vital capacity (FVC) was measured using a 7-L dry wedge spirometer (Vitalograph, Buckingham, United Kingdom). Predicted values of FEV<sub>1</sub> and FVC were based on reference values according to Nysom et al.<sup>25</sup>

**EVH test.** The participants inhaled a dry gas containing 5% CO<sub>2</sub>, 20.93% O<sub>2</sub>, and balance N<sub>2</sub> at room temperature for 6 minutes with a target minute ventilation of  $30 \times \text{FEV}_{10}$ , equivalent to 85% of the maximal voluntary ventilation (MVV).<sup>26</sup> FEV<sub>1</sub> was measured before and 1, 3, 5, 7, 10, 15, and 20 minutes after hyperpnea. The lowest FEV<sub>1</sub> value after the test was used to determine the maximum decrease in FEV<sub>1</sub>. A positive EVH test was defined as a fall in FEV<sub>1</sub> of at least 10 percent of the prechallenge value. A cutoff point of 10% has been recommended as a proper criterion for judging a normal versus asthmatic response to the EVH challenge,<sup>27</sup> and in subjects without asthma, the average percentage drop in FEV<sub>1</sub> is less than 3% (mean  $\pm$  SE,  $2.4 \pm 0.6$ ).<sup>28</sup> In elite athletes, a similar cutoff point of 10% is recommended by the International Olympic Committee—Medical Commission (IOC-MC).<sup>29</sup>

**Methacholine challenge.** The methacholine challenge was performed with a Nebiheck nebulizer (#61 650; DeVilbiss, Gillingham, United Kingdom) according to the procedure described by Yan et al.<sup>30</sup> The participants were challenged first with saline (0.9%) and then increasing doses of methacholine. Measurements of FEV<sub>1</sub> were performed before the first inhalation and repeated after each inhalation. The challenge was terminated when a  $\geq 20\%$  decrease in FEV<sub>1</sub> from the postsaline value was measured or when a cumulative dose of methacholine (8  $\mu\text{mol}$ ) had been administered. AHR to methacholine was defined as a 20% fall in FEV<sub>1</sub> at a cumulative dose of methacholine of either  $\text{PD}_{20} \leq 2 \mu\text{mol}$  or  $\text{PD}_{20} \leq 8 \mu\text{mol}$  (equivalent to 4 mg/mL or 16 mg/mL or cumulative doses of 400 or 1600  $\mu\text{g}$ ).  $\text{PD}_{20} \leq 8 \mu\text{mol}$  is a cutoff point often used for defining a positive test result, and  $\text{PD}_{20} \leq 2 \mu\text{mol}$  ( $\sim 4 \text{ mg/mL}$ ) is the cutoff value used by IOC-MC at the Olympic Winter Games in 2006 in Turin and the value that will be used for Beijing 2008 for allowing use of  $\beta_2$ -agonists in subjects not taking ICSs.<sup>29</sup> The cumulative dose of methacholine required to provoke a 20% fall in FEV<sub>1</sub> was calculated by linear interpolation of the dose-response curve. The response dose ratio, an index of reactivity, was calculated as the percentage fall in FEV<sub>1</sub> after the last dose, divided by the cumulative dose of methacholine in micromoles.<sup>31</sup>

**Induced sputum.** The participants were pretreated with 0.2 mg salbutamol before they inhaled increasing doses of hypertonic saline (3%, 4%, and 5%) via an ultrasonic nebulizer (Easynb II, Flaemnuova, Italy) for 3 consecutive 7-minute periods. The samples were processed within 2 hours of collection. Sputum was processed according to the method described by Pavord et al.<sup>32</sup> Lung function was tested before and after every period of inhalation to detect

TABLE I. Subject characteristics

	Swimmers	Adolescents with asthma	Unselected adolescents
No. of subjects	33	32	35
Sex (female:male)	15:18	13:19	19:16
Age (y)	14.3 (1.2)	14.1 (1.2)	14.4 (0.5)
Height (cm)	172.1 (9.4)	166.9 (9.2)	169.7 (8.8)
Weight (kg)	63.6 (9.8)	59.9 (13.6)	57.5 (11.5)
Exercise, recreational (h/wk)	4.5 (3.4)	4.6 (2.3)	5.8 (3.2)
FEV <sub>1</sub> (L)	4.27 (0.82)*	3.27 (0.65)	3.41 (0.53)
FEV <sub>1</sub> , % predicted	119 (17)*	98 (12)	98 (11)
FVC (L)	5.03 (1.21)*	3.91 (0.83)	3.88 (0.76)
FVC, % predicted	121 (14)*	103 (12)	98 (11)
FEV <sub>1</sub> /FVC, % predicted	98 (10)	95 (8)	100 (6)
Respiratory symptoms, rest	5 (15.2%)*†	18 (60.0%)	9 (34.6%)
Respiratory symptoms, exercise	7 (21.9%)*‡	19 (63.3%)	3 (11.5%)*†
Allergic symptoms	7 (22.6%)*‡	10 (71.4%)	9 (33.3%)*§

Data are expressed as no. (%) or means (SDs) unless otherwise indicated.

\* $P < .001$  compared with adolescents with asthma and unselected adolescents.

† $P < .001$  compared with adolescents with asthma.

‡ $P < 0.01$  compared with adolescents with asthma.

§ $P < .05$  compared with adolescents with asthma.

any bronchoconstriction. A blind observer counted 400 nonsquamous cells. Differential cell counts are expressed as the percentage of nonsquamous cells.

**Exhaled nitric oxide.** Exhaled nitric oxide was measured according to American Thoracic Society/European Respiratory Society recommendations with an NO analyzer (NIOX MINO; Aerocrine, Stockholm, Sweden) at a flow of 50 mL/s.<sup>33</sup> FeNO measurements have been shown to be reproducible and free from diurnal and day-to-day variation.<sup>34</sup>

**EBC.** Exhaled breath condensate samples were collected by using RTubes (Respiratory Research, Inc, Charlottesville, Va). The collected samples were immediately stored at  $-80^{\circ}\text{C}$  for later measurement of pH. The pH was measured by using a pH meter (pH meter 350; Jenway, Dunmow, United Kingdom) before and after deaeration with argon for 10 minutes. Normative data from self-described healthy volunteers using the same collection device has shown a skewed distribution of pH with a median EBC pH of 8.0 and a minimum and maximum EBC pH of 4.5 and 8.4, respectively.<sup>35</sup>

**Blood samples.** We collected a blood sample from the median cubital vein for a blood cell count.

**Skin prick test.** We performed a skin prick test to 10 aeroallergens (birch, grass, mugwort, horse, dog, cat, house dust mites [*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*] and molds [*Alternaria iridis* and *Cladosporium herbarium*] with positive and negative references histamine 10 mg/mL in 50% glycerol and glycerol 50%, respectively [Soluprick SQ System; ALK-Abelló, Hørsholm, Denmark]).<sup>36</sup> Atopy was defined as a wheal of at least 3 mm in diameter to at least 1 of the allergens.

**Data analysis.** Normally distributed continuous data are presented as means (SDs) and analyzed by ANOVA, followed by  $t$  test. Skewed data are presented as medians (ranges). Differences in skewed data were assessed by the Kruskal-Wallis test followed by the Mann-Whitney test. Differences in categorical data were assessed by  $\chi^2$  tests and the Fisher exact test when appropriate. Current asthma among swimmers was defined as having respiratory symptoms and at least 1 positive challenge test result (EVH and/or methacholine with  $\text{PD}_{20} \leq 8 \mu\text{mol}$ ). Values of  $P < .05$  were considered statistically significant. Statistical analyses were performed by using the statistical software program SPSS 15.0 (SPSS Inc, Chicago, Ill).

## RESULTS

The baseline characteristics of the 3 groups are shown in Table I. The swimmers had significantly higher lung function values

TABLE II. AHR and atopy

	Swimmers	Adolescents with asthma	Unselected adolescents
Atopy	7 (24.1)*	22 (71.0)	13 (37.1)*
EVH	8 (27.6)	6 (40.0)	1 (7.7)
Methacholine ( $\text{PD}_{20} \leq 8 \mu\text{mol}$ )	8 (25.0)	16 (50.0)	6 (27.3)
Methacholine ( $\text{PD}_{20} \leq 2 \mu\text{mol}$ )	2 (6.3)**	10 (31.3)	0 (0)*

Data are expressed as no. (%).

\* $P < .01$  compared with adolescents with asthma.

\*\* $P < .05$  compared with adolescents with asthma.

TABLE III. Airway responsiveness, FeNO, and EBC pH

	Swimmers	Adolescents with asthma	Unselected adolescents
EVH	8.1 (1.1-48.2)*	8.8 (0-43.8)*	3.6 (-2.0-13.8)
Methacholine, response dose ratio	0.9 (-0.1-28.2)**	2.2 (-0.3-190.1)	1.5 (-0.1-6.6)
FeNO, parts per billion	15 (4-35)	18.5 (4-127)	15.5 (9-69)
pH, deaerated	8.13 (5.03-8.36)	8.21 (7.99-8.49)	8.14 (4.55-8.37)
pH, not deaerated	6.17 (4.92-7.00)	6.28 (5.71-8.73)	6.50 (4.55-7.07)

Results are expressed as medians (ranges).

\* $P < .01$  compared with unselected adolescents.

\*\* $P < .05$  compared with adolescents with asthma.

than the subjects in both the asthma group and the unselected group, with the swimmers' FEV<sub>1</sub> and FVC values 21% and 19% higher than expected.

We found no differences in the prevalence of respiratory or allergic symptoms between the swimmers and the unselected adolescents. The swimmers had significantly fewer respiratory symptoms (both related to and not related to exercise) and allergic symptoms than the subjects in the asthma group.

There was no difference in the prevalence of AHR to either EVH or methacholine ( $\text{PD}_{20} \leq 8 \mu\text{mol}$ ) among the 3 groups, but both swimmers and unselected adolescents had a lower prevalence of AHR to methacholine than the subjects with asthma using  $\text{PD}_{20} \leq 2 \mu\text{mol}$  as cutoff (Table II). When looking at airway responsiveness as a continuous variable, there was a difference with both swimmers and subjects with asthma more responsive to EVH than the unselected subjects, and the subjects in the asthma group having greater airway responsiveness to methacholine than the swimmers (Table III). There were no differences in FeNO or EBC pH among the 3 groups. We found no difference in atopy between the swimmers and the unselected adolescents, but significantly more subjects with asthma than swimmers had atopy (Table II).

There were no differences in the cellular composition of the sputum between the groups (Table IV). Neither were there differences in the cellular composition when comparing subjects with or without a positive EVH test or with or without a positive methacholine challenge within the groups (data not shown). No adverse events were registered during sputum induction.

The swimmers had significantly fewer eosinophils in the blood than the subjects with asthma and significantly more lymphocytes and monocytes than unselected subjects.

TABLE IV. Induced sputum

	Swimmers	Adolescents with asthma	Unselected adolescents	P value (between groups)
Usable samples	24 (75)	12 (80)	14 (64)	NS
Squamous cells	20.5 (0-79)	9.1 (1-64)	4.3 (1-67)	NS
Eosinophils	0.4 (0-3.0)	1.3 (0-93.8)	0.3 (0-5.8)	NS
Lymphocytes	2.6 (0-8.3)	1.6 (0-5.3)	1.8 (0-5.3)	NS
Macrophages	58.9 (10.6-96.8)	76.1 (6.3-98.5)	73.4 (1-96.3)	NS
Neutrophils	29.1 (0-81.7)	10.0 (0-57.0)	18.8 (1-98.5)	NS
Columnar epithelial cells	3.3 (0-13.3)	0.8 (0-12.8)	1.0 (0-14.8)	NS

NS, Not significant.

Data are expressed as medians (ranges) except "Usable samples," which is expressed as no. (%). Percentage squamous cells are expressed as a percentage of the total cell count. Differential counts for other cells are expressed as a percentage of lower airway cells, excluding squamous cells.

Four (80%) of the 5 swimmers with a history of asthma had respiratory symptoms, but only 2 (40%) of the 5 had a positive challenge. Of 13 swimmers with AHR to EVH and/or methacholine ( $PD_{20} \leq 8 \mu\text{mol}$ ), only 4 had symptoms (current asthma), whereas 5 with symptoms did not have AHR to either EVH or methacholine (see this article's Table E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). Of the 5 swimmers with a history of asthma before the study, only 2 had current asthma. There was no difference in FeNO, cellular composition of sputum, airway reactivity, or prevalence of having AHR to methacholine and/or EVH between swimmers with and without respiratory symptoms (data not shown). Neither were there differences in FeNO or cellular sputum composition between swimmers with and without a positive challenge test result (data not shown).

Excluding swimmers and unselected subjects with an asthma diagnosis before the study did not change the results. Neither did the results change when excluding the 5 swimmers who did not complete both visits.

## DISCUSSION

This research attempted to assess respiratory symptoms, airway responsiveness, and airway inflammation in adolescents involved in competitive swimming and to compare the findings with those found in unselected adolescents not involved in competitive sport and adolescents with asthma. We found that adolescents who had been involved in competitive swimming for about 2 years and trained approximately 20 hours per week did not show major differences from adolescents not involved in competitive swimming in terms of prevalence of respiratory symptoms, airway responsiveness, or airway inflammation. Only when looking at indirect airway responsiveness to EVH did we find a significant difference, with the swimmers more responsive to EVH than the unselected adolescents.

Our findings in adolescents involved in endurance sport are not in line with the findings in adult elite athletes. Adult elite swimmers, as well as other endurance athletes, such as cross-country skiers and long-distance runners, have an increased prevalence of respiratory symptoms compared with subjects not involved in elite sport.<sup>4,6,23</sup> In our study of adolescent elite swimmers, we found no difference in the prevalence of respiratory symptoms or allergic symptoms between the swimmers and the group of unselected children. Further, the prevalence of AHR and the degree of airway responsiveness have been reported to be increased in adult elite athletes, including elite swimmers, compared with healthy control subjects. A Finnish study on 23 adult elite swimmers showed that 48% of the swimmers had

increased airway responsiveness to a histamine challenge test compared with 16% of the healthy control subjects without asthma.<sup>16</sup> In our study, we found no difference between the swimmers and the unselected adolescents when looking at the response to the airway challenge tests as being either positive or negative. However, there was a difference in airway responsiveness between elite swimmers and the unselected group, with the swimmers more responsive to EVH than the unselected adolescents. We found that adolescents with asthma had greater airway responsiveness to methacholine than the elite swimmers. Many of the swimmers with AHR did not have respiratory symptoms, whereas others with respiratory symptoms did not have AHR.

There were no differences in the cellular composition of sputum between the swimmers and the unselected school children. This result is also in disagreement with results in adult elite athletes. Bronchial biopsies from elite cross-country skiers have shown increased T-lymphocyte, macrophage, and eosinophil counts compared with findings from healthy nonathletic controls, as well as higher neutrophil counts compared with those of nonathletic subjects with asthma.<sup>8</sup> In both ice hockey players and adult elite swimmers, sputum cell counts of neutrophils and eosinophils have been shown to be higher than in the control groups of randomly selected university students and laboratory staff.<sup>9,16</sup> Whereas adult elite athletes thus seem to have a special type of airway inflammation compared with healthy control subjects, this seems not to be the case for adolescent elite swimmers after 2 years of elite swimming.

Studies have shown that subjects with asthma have acidic EBC pH levels compared with healthy controls, and EBC pH has been suggested as a noninvasive marker of airway inflammation.<sup>37-40</sup> We found no differences in EBC pH between the swimmers and the unselected adolescents, which also indicates that the swimmers do not have significant airway inflammation. Surprisingly, there was no difference between the EBC pH of subjects with asthma and that found in swimmers and unselected adolescents. This could be because the subjects with asthma predominantly had mild asthma, in which EBC pH values do not differ from those observed in healthy controls.<sup>37</sup> Another possible explanation is that EBC pH is low only in a subpopulation of subjects with asthma.

The lack of differences in FeNO between swimmers and unselected adolescents in our study is consistent with findings in adult elite athletes, although the findings in adult athletes vary somewhat. Sue-Chu et al<sup>41</sup> showed that FeNO is not elevated in cross-country skiers compared with healthy controls, and neither do adult elite swimmers have increased FeNO compared with controls.<sup>42</sup> In general, it seems that measuring FeNO does not add



much in the evaluation of either adolescent or adult elite athletes. Elevated FeNO primarily reflects an eosinophilic airway inflammation.<sup>43</sup> This type of inflammation is characteristic of asthma but might not be present in elite athletes with respiratory symptoms and AHR. We expected subjects with asthma to have higher FeNO values than the other 2 groups, but this was not the case. One reason could be that most of the subjects in the asthma group had very mild asthma because of the inclusion criteria used.

We believe that one of the strengths of our study is that we used both a direct (methacholine challenge) and an indirect challenge (EVH test). The EVH test is currently the test recommended by the IOC-MC when diagnosing asthma and exercise-induced bronchoconstriction (EIB).<sup>44,45</sup> It is also the most sensitive diagnostic test for identifying EIB in elite athletes.<sup>3,46</sup> We believe it is important to include an indirect test when evaluating EIB in elite athletes, and currently the EVH test must be the first choice, but other indirect tests can be used. The sensitivity of a direct test such as the methacholine challenge in identifying EIB and AHR in elite athletes is questionable and seems to depend on the cutoff value used.<sup>3</sup> In this study, we used 2 cutoff values:  $PD_{20} \leq 2 \mu\text{mol}$  and  $PD_{20} \leq 8 \mu\text{mol}$ . The first is the cutoff used by the IOC-MC for allowing  $\beta_2$ -agonists. We chose to include the higher cutoff value of  $PD_{20} \leq 8 \mu\text{mol}$  because we were interested in identifying even mild AHR, which has been shown to increase the risk of developing asthma as an adult.<sup>47</sup> Another important reason we used the methacholine challenge was that the test is one of most if not the most widely used test for AHR in respiratory departments and within respiratory research.

Our study has some potential limitations. Firstly, we included only subjects who had not used corticosteroids 4 weeks before the study. This inclusion criterion might have led primarily to subjects with intermittent or mild persistent asthma being enrolled, and when classifying asthma severity in accordance with the Global Initiative for Asthma,<sup>48</sup> 16 (50%) had intermittent asthma, 8 (25%) had mild persistent asthma, 6 (18.8%) had moderate persistent asthma, and 2 (6.3%) had severe persistent asthma. However, when looking only at lung function, 31 (97%) of the subjects with asthma had a  $FEV_1 \geq 80\%$  predicted, and just 1 had a  $FEV_1 < 80\%$  predicted. This could at least partly explain why the subjects with asthma did not have elevated FeNO, elevated eosinophil count in sputum, lower EBC pH, or a higher prevalence of AHR to methacholine and EVH compared with the swimmers and the unselected adolescents. Factors such as amount of training, competitive level, and training environment are elements of insecurity when performing studies on elite athletes. It is difficult to adjust for differences in these parameters. In our study, the swimmers trained approximately 20 hours per week, and they were all involved in a well organized elite program for young swimmers run by the National Swimming Association, which leads us to believe that the group of swimmers is representative of adolescent elite swimmers. It has been suggested that regularly attending an indoor swimming pool, especially before 6 to 7 years of age, increases the risk of developing asthma, and in this study infant swimming was not taken into account.<sup>49</sup> Neither were differences in the swimming pools and the pool area—for example, height of swimming pool edges, ventilation, pH in the pool, and concentrations of chlorine. Finally, the ventilation achieved in all 3 groups (elite group, 63%; unselected group, 57%; and asthma group, 58% of the MVV) was below 65% MVV, which has been proposed as the minimum threshold for an adequate challenge. This may

have resulted in false-negative responses to the EVH test in all 3 groups.

Our study adds an important component to our knowledge about the development of airway inflammation, airway responsiveness, and respiratory symptoms in elite swimmers. Whereas adult elite swimmers have more AHR and respiratory symptoms and show signs of airway inflammation compared with healthy volunteers, adolescent elite swimmers do not have significant signs of airway damage after approximately 2 years of intense training and competition. This leads us to believe that elite swimmers do not have particularly susceptible airways when they take up competitive swimming when young, but that they develop respiratory symptoms, airway inflammation, and AHR during their swimming careers. Our findings forward the theory that respiratory symptoms and asthma in swimmers result from years of intense swimming activity.<sup>50</sup>

One of the next steps should be to perform a follow-up on our study groups. This will help us understand what happens to the airways during years of intense swimming with strenuous physical exercise and long-term exposure to chloride-containing gases in swimming pool arenas. More data on the acute effect of swimming on the airways are also needed, as are studies on treatment of elite swimmers, and elite athletes in general, with asthma and AHR.

In conclusion, we found only minor differences between adolescent elite swimmers and age-matched unselected adolescents, and the swimmers had no signs of airway inflammation. It seems that adolescent swimmers, who have been involved in competitive swimming for only a few years, have not developed respiratory symptoms, signs of airway inflammation, or AHR except for a slight increase in airway responsiveness to EVH.

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#### Key messages

- Whereas adult elite swimmers have a high prevalence of AHR and respiratory symptoms and show signs of airway inflammation, adolescent elite swimmers do not have significant signs of airway damage after a few years of intense training and competition.
- These findings suggest that elite swimmers do not have particularly susceptible airways when they take up competitive swimming when young, but that they develop respiratory symptoms, airway inflammation, and AHR during their swimming careers.

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**TABLE E1.** Case summaries (swimmers)

Subject no.	Respiratory symptoms (rest)	Respiratory symptoms (exercise)	Atopy	FeNO (parts per billion)	PD <sub>20</sub> methacholine (μmol)	Decrease in FEV <sub>1</sub> after EVH (%)	Percent of MVV (EVH)
1	No	No	Yes	30	>8	6.0	53
2	No	No	No	15	>8	8.1	80
3	No	No	No	14	6.6	8.0	65
4	No	No	No	14	>8	6.7	37
5	Yes	Yes	No	14	>8	4.4	—
6	No	No	No	14	5.7	4.6	—
7	No	No	No	15	>8	6.6	77
8	Yes	No	No	8	5.6	6.5	59
9	No	No	No	15	>8	15.1	60
10	No	No	No	22	>8	2.9	62
11	No	No	Yes	28	>8	9.8	51
12	No	No	—	—	1.1	—	—
13	Yes	Yes	No	21	>8	8.2	51
14	No	No	No	20	>8	7.7	45
15	No	No	No	8	>8	8.3	63
16	No	—	No	25	>8	8.5	54
17	No	No	No	16	0.7	21.8	86
18	Yes	No	Yes	20	>8	10.9	72
19	No	No	No	14	>8	48.2	61
20	No	No	Yes	40	>8	16.2	84
21	No	Yes	No	14	>8	1.5	58
22	No	No	No	16	—	18.1	64
23	No	No	Yes	17	7.2	14.2	68
24	No	No	—	—	>8	—	—
25	No	No	—	—	>8	—	—
26	No	No	—	—	>8	—	—
27	No	Yes	Yes	17	>8	8.3	51
28	No	No	No	22	>8	4.4	53
29	No	Yes	No	12	>8	4.7	77
30	No	Yes	No	12	5.6	17.8	75
31	No	No	No	35	>8	1.2	57
32	No	No	No	17	>8	8.8	74
33	Yes	Yes	Yes	4	4.8	3.1	52