Predictors of Airway Hyperresponsiveness in Elite Athletes

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ABSTRACT

TOENNESEN, L. L., C. PORSBJERG, L. PEDERSEN, and V. BACKER. Predictors of Airway Hyperresponsiveness in Elite Athletes. *Med. Sci. Sports Exerc.*, Vol. 47, No. 5, pp. 914–920, 2015. **Introduction**: Elite athletes frequently experience asthma and airway hyperresponsiveness (AHR). We aimed to investigate predictors of airway pathophysiology in a group of unselected elite summer-sport athletes, training for the summer 2008 Olympic Games, including markers of airway inflammation, systemic inflammation, and training intensity. **Methods**: Fifty-seven Danish elite summer-sport athletes with and without asthma symptoms all gave a blood sample for measurements of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α), completed a respiratory questionnaire, and underwent spirometry. Bronchial challenges with mannitol were performed in all 57 athletes, and 47 agreed to perform an additional methacholine provocation. **Results**: Based on a physician's diagnosis, 18 (32%) athletes were concluded to be asthmatic. Asthmatic subjects trained more hours per week than the 39 nonasthmatics (median (min–max): 25 h·wk^{-1} (14–30) versus 20 h·wk^{-1} (11–30), $P = 0.001$). AHR to both methacholine and mannitol (dose response slope) increased with the number of weekly training h ($r = 0.43$, $P = 0.003$, and $r = 0.28$, $P = 0.034$, respectively). Serum levels of IL-6, IL-8, TNF-α, and hs-CRP were similar between asthmatics and nonasthmatics. However, there was a positive association between the degree of AHR to methacholine and serum levels of TNF-α ($r = 0.36$, $P = 0.04$). Fifteen out of 18 *asthmatic* athletes were challenged with *both* agents. In these subjects, no association was found between the levels of AHR to mannitol and methacholine ($r = 0.032$, $P = 0.91$). **Conclusion**: AHR in elite athletes is related to the amount of weekly training and the level of serum TNF-α. No association was found between the level of AHR to mannitol and methacholine in the asthmatic athletes. **Key Words:** ELITE ATHLETES, AIRWAY HYPERRESPONSIVENESS, ASTHMA, SERUM CYTOKINES

Exercise-induced respiratory symptoms suggestive of asthma are common among elite athletes, and the prevalence of airway hyperresponsiveness (AHR) is higher than in sedentary individuals (22). However, the underlying pathogenetic mechanisms are unclear. Some elite athletes have classic eosinophilic asthma, but it seems that other inflammatory pathways are also involved. These may relate to airway epithelial damage due to the frequently repeated periods of increased ventilation during training and competition, and could involve innate immune pathways. Hence, elite athletes have been found to have a high prevalence of neutrophilic airway inflammation, particularly endurance athletes, although the existing evidence is sparse (8).

Neutrophilic airway inflammation has been found to be associated with systemic inflammation in sedentary asthmatics (34). The results concerning hs-CRP values found in asthma are however somewhat disperse, as some have shown elevated levels in steroid-naive subjects only (33), whereas other studies have shown increased levels of hs-CRP in steroid-treated asthmatic individuals (1). One large study have found a difference between allergic and nonallergic asthma, as only individuals with nonallergic asthma had increased levels of serum hs-CRP (27). This could suggest a relationship between increased hs-CRP levels and neutrophilic asthma, as the neutrophilic phenotype more frequently is found among nonallergic asthmatic subjects (12).

Moreover, other systemic biomarkers such as proinflammatory cytokines have been reported to be increased in individuals with asthma. Serum levels of tumor necrosis factor alpha (TNF-α) are found to be higher in subjects with severe asthma compared with healthy controls (18) and increased in

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subjects with uncontrolled asthma compared with periods with controlled asthma (21). Furthermore, sputum TNF-α has been shown to be associated with AHR in asthmatic subjects (26). Serum levels of interleukin-6 (IL-6) are elevated during asthma exacerbations (36), whereas circulating interleukin-8 (IL-8) is increased in individuals with severe asthma (31). This could suggest a relationship between neutrophilic asthma and increased inflammation markers, as neutrophilic airway inflammation is often seen during asthma exacerbations and in severe uncontrolled asthma (25).

In elite athletes, the phenotype of asthma is frequently noneosinophilic (9), with a variable response to inhaled steroids (32), a feature which is typical for neutrophilic asthma (16). Therefore, it has been suggested that asthma in elite athletes most often resembles the neutrophilic phenotype rather than the classic eosinophilic asthma (3). However, systemic inflammation among elite athletes with asthma symptoms has not yet been reported.

Because systemic low-grade inflammation measured with hs-CRP, IL-6, IL-8, and TNF-α seems to be characteristic for the neutrophilic phenotype, it could be hypothesized that these markers would be elevated in elite athletes with asthma. However, it has also been demonstrated that intense physical exercise reduces resting serum levels of inflammatory markers such as hs-CRP (24). If asthma in elite athletes is caused by epithelial damage due to hyperventilation, a relationship between the number of weekly training hours and the presence of asthma and AHR as well as the decreased serum levels of systemic inflammatory markers could be expected. Because both theories could be argued, we sought to investigate the serum levels of markers of systemic inflammation in elite athletes with and without asthma and their relationship with measurements of airway pathophysiology, fractional exhaled nitric oxide (FeNO) as a marker of eosinophilic airway inflammation, and training volume.

METHODS

Design. A cross-sectional design was chosen to study Danish elite athletes less than 6 months before participating in the 2008 Olympic Games in Beijing, China. All athletes who qualified for the Olympics were invited to take part in a research study concerning asthma and elite sport.

Materials. Inclusion criteria were participation at the summer Olympic Games in August 2008 in Beijing, China, representing Denmark, and accepting to undergo spirometry and having a venipuncture done. Exclusion criteria were any chronically conditions besides asthma, atopy, or rhinitis or the use of any other medicine than antiasthma medicine. Of the 84 who qualified, 57 met the inclusion and exclusion criteria. All athletes gave their written informed consent and the study was approved by the Regional Ethics Committee of Copenhagen and Frederiksberg County (Committee D) (J nr H-D-2008-021).

All participants were seen and interviewed by a senior pulmonary physician who made a diagnosis of asthma, based on symptoms, lung function, and hyperresponsiveness. Asthma-like symptoms were defined as wheeze, abnormal breathlessness, cough, or chest tightness either on exertion or at rest. The diagnosis of asthma was based on asthma-like symptoms combined with at least one positive bronchial provocation test with methacholine and/or mannitol.

In case of athletes using antiasthma medicine, they were asked not to use short-acting beta-2 agonists less than 6 h before all tests and long-acting beta-2 agonists (LABA) and/or a combination of LABA and inhaled corticosteroids (ICS) less than 12 h before pulmonary function tests and bronchial provocation tests.

Questionnaires. An Allergy Questionnaire for Athletes (AQUA) questionnaire (7), which is a validated questionnaire developed to asses allergic symptoms in athletes, was completed in a modified version by all athletes. It included questions on asthma-like symptoms, symptoms from nose or eyes, doctor-diagnosed asthma, atopy or rhinitis, competitive sport, training hours, and smoking habits. Sports were classified as endurance/nonendurance in accordance with the energy delivery systems for sports, as done by Lund et al. (23)

Blood analysis. Blood were drawn from a cubital vein and serum was kept at -80°C until analyzed. Serum levels of IL-6, IL-8, and TNF-α were measured by enzyme-linked immunosorbent assays according to the manufacturer's instructions (R&D systems). All analyses were done using high-sensitivity kits. The lower detection limits were 1 $pg \cdot mL^{-1}$ (IL-8), 0.156 $pg \cdot mL^{-1}$ (IL-6) and 0.11 $pg \cdot mL^{-1}$ (TNF-a). Hs-CRP concentrations were measured by high-sensitivity particle-enhanced turbidimetric immunoassays provided by Roche (Roche Diagnostics GmbH; Mannheim, Germany). The lower detection limit of the assay was 0.1 $mg \cdot L^{-1}$.

Spirometry. Using a 7-L dry wedge spirometer (Vitalograph, Buckingham, UK), which was calibrated weekly, lung function measurements were performed according to the standards specified by the European Respiratory Society and the American Thoracic Society (4).

AHR. Methacholine and mannitol bronchial challenges were performed.

Methacholine provocation tests were performed in accordance with the Yan method (35) by tidal inhalation of doubling doses of methacholine using nebulizer Spira® (Spira Respiratory Care Centre Ltd., Hämeenlinna, Finland) until forced expiratory volume in 1 s (FEV_1) had decreased with a 20% fall from baseline (after inhaled isotonic saline) or when a cumulative dose of methacholine (>16 μmol) had been administrated. A positive response indicating AHR was defined as a 20% fall or more in FEV_1 at a cumulative dose of methacholine (PD_{20}) of less than 8 μmol.

The dose response slope (DRS) values were calculated as the percentage decrease in FEV_1 after the last dose divided by the cumulative dose of methacholine in micromoles. To meet normal distribution, log-transformed values of DRS were used. To eliminate negative values, an additional of 3% was added to the percentage decrease in FEV_1 in the calculation of DRS values.

TABLE 1. Basic variables.

	Asthma[a]	Nonasthma[a]	Total	*P* Value (Asthma/Nonasthma)
Subjects, no. (%)	18 (32%)	39 (68%)	57 (100%)	
Age, yr (mean (SD))	26.3 (4.6)	28.0 (5.4)	27.5 (5.2)	0.26
Sex, no. (%)				0.04
Male	10 (56%)	32 (82%)	42 (73.7%)	
Female	8 (44%)	7 (18%)	15 (26.3%)	
BMI, $kg \cdot m^{-2}$ (mean (SD))	21.8 (2.0)	23.4 (3.2)	22.9 (3.0)	0.05
ICS use, no. (%)				<0.001
Yes	8 (44%)	1 (3%)	9 (16%)	
No	10 (56%)	38 (97%)	48 (84%)	
Rhinitis, no. (%)				0.71
Yes	5 (17%)	9 (23%)	14 (24.6%)	
No	13 (83%)	30 (77%)	43 (75.4%)	
Atopy, no. (%)				0.91
Yes	3 (17%)	7 (18%)	10 (17.5%)	
No	15 (83%)	32 (82%)	47 (82.5%)	
Training hours per week (mean (SD))	24.2 (4.2)	20.0 (4.4)	21.3 (4.4)	0.01
FEV_1 %pred. (mean (SD))	117.0% (14.96)	117.3% (11.8)	117.2% (12.7)	0.95
FVC %pred.	127.5% (16.3)	119.5% (12.0)	122.0% (13.9)	0.04
FEV_1/FVC (SD)	0.77 (0.08)	0.82 (0.06)	0.81% (0.07)	0.02
Metabolism, no. (%)				0.002
Endurance	16 (89%)	18 (46%)	34 (59.6%)	
Nonendurance	2 (11%)	21 (54%)	23 (40.4%)	
Positive mannitol test,[b] no. (%)	9 (50%)	3 (7.7%)	12 (21%)	<0.001
Positive methacholine test,[c] no. (%)	15 (94%)	1 (3%)	16 (34%)	<0.001
High FeNO,[d] no. (%)	2 (13.5%)	7 (21.2%)	9 (18.8%)	0.70

[a]Based on a physician's diagnosis.
[b]PD_{15} <635 mg.
[c]PD_{20} <8 μmol.
[d]>25 ppb.
FEV1 %pred, forced expiratory volume in one second in percent of predicted; FVC, forced vital capacity.

Mannitol provocation tests were done using the method described by Anderson et al. (2): The participants inhaled an empty capsule followed by increasing doses of mannitol (5, 10, 20, 40, 80, 80, 160, 160, and 160 mg) until reaching an accumulated dose of 635 mg or achieving a 15% reduction in FEV_1. A positive response indicating AHR was defined as a 15% fall or more in FEV_1 at a cumulative dose of mannitol (PD_{15}) of less than 635 mg.

The DRS values were calculated as the percentage decrease in FEV_1 after the last dose divided by the cumulative dose of mannitol in micromoles. To meet normal distribution, log-transformed values of DRS were used, and to eliminate negative values, an additional of 1% was added to the percentage decrease in FEV_1 in the calculation of DRS values.

The order in which the bronchial provocation tests were performed was the same as described and validated by Gade et al. (15). The athletes performed the mannitol provocation test first, and after FEV_1 had returned to baseline, the athletes were challenged with methacholine.

Atopy. Skin prick tests were performed according to European standards with the GA2LEN Pan-European panel of allergen extracts (13). A positive response, indicating atopy, was calculated with a cut-off value of 5 mm.

FeNO. Using the nitric oxide analyzer (NIOX; Aerocrine AB, Solna, Sweden), measurements of FeNO were performed following the recommendations of the European Respiratory Society and the American Thoracic Society (6). A high FeNO level, indicating airway inflammation, was calculated with a cut-off value of 25 parts per billion (ppb).

Statistical analyses. Data were stored and analyzed using the statistical software program SPSS 20.0 (SPSS Inc., Chicago, IL). To compare two independent groups, two-sample *t*-tests were used for data that were normally distributed, and the Mann–Whitney test was used for data that were not normally distributed. Log-transformed values of serum levels of hs-CRP, IL-6, IL-8, and TNF-a were used to meet normal distribution. Univariate correlations between parameters that were not normally distributed were assessed using Spearman's rank method. To assess predictors of AHR, multiple regression analyses were performed, including parameters that were significantly associated with levels of AHR to mannitol and methacholine (DRS) in the univariate analyses. *P* values <0.05 were considered statistically significant.

RESULTS

The general characteristics of the asthmatic ($n = 18$) and the nonasthmatic athletes ($n = 39$) are summarized in Table 1. The athletes in the asthma group had a higher number of weekly training hours ($P = 0.001$), and a larger part of asthmatic subjects were females ($P = 0.035$). Moreover, significantly more endurance athletes were found in the asthma group compared with those in the nonasthma group ($P = 0.002$).

A combination of ICS and LABA was used by 16% of the athletes who additionally used short-acting beta-2 agonists

TABLE 2. Sports classifications of athletes.

Sport Classification	Sport (*n*)
Endurance (*n*) (asthmatic/nonasthmatic)	Rowing (4/11), swimming (8/2), cycling (4/1), canoeing (0/2), triathlon (0/2)
Nonendurance (*n*) (asthmatic/nonasthmatic)	Handball (1/7), badminton (0/6), sail sport (0/4), fencing (1/0), table tennis (0/1), shot put (0/1), wrestling (0/1), javelin throw (0/1)

TABLE 3. Univariate correlations between AHR and serum inflammatory markers, FEV_1 %pred.

	DRS-Methacholine *(No. of Subjects)*	DRS-Mannitol *(No. of Subjects)*
hs-CRP ($mg \cdot L^{-1}$)	0.08 ($P = 0.63$) *(45)*	0.17 ($P = 0.28$) *(55)*
IL-6 ($pg \cdot mL^{-1}$)	−0.05 ($P = 0.78$) *(41)*	0.01 ($P = 0.93$) *(48)*
IL-8 ($pg \cdot mL^{-1}$)	−0.06 ($P = 0.67$) *(46)*	−0.21 ($P = 0.13$) *(56)*
TNF-α ($pg \cdot mL^{-1}$)	0.36 ($P = 0.04$)* *(46)*	0.009 ($P = 0.95$) *(56)*
BMI ($kg \cdot m^{-2}$)	−0.30 ($P = 0.04$)* *(47)*	−0.28 ($P = 0.04$)* *(57)*
FEV_1 %pred. (%)	−0.16 ($P = 0.28$) *(47)*	−0.076 ($P = 0.58$) *(57)*
FeNO (ppb)	−0.02 ($P = 0.91$) *(38)*	0.028 ($P = 0.85$) *(48)*
Weekly training (h)	0.43 ($P = 0.003$)* *(47)*	0.28 ($P = 0.034$)* *(57)*
DRS-mannitol	0.55 ($P = 0.000$)* *(47)*	—

FeNO and the number of weekly training hours in the overall group of athletes.
*Significant at a 5% level.

as rescue medication. No athletes used any other kinds of antiasthma medicine.

The distribution of specific types of sports is summarized in Table 2, which shows that the majority of athletes performed endurance sports.

All 57 athletes had a mannitol provocation test performed, but because of refusal or technical reasons in this real-life study, 10 athletes did not undergo the following methacholine provocation, and measurements of FeNO failed in 9 subjects. There were no significant differences in the prevalence of asthma, levels of AHR to mannitol, body mass index (BMI), FEV_1 in percent of predicted (FEV_1 %pred.), serum levels of inflammatory markers, the number of weekly training hours, age, or the use of ICS in the athletes who had a methacholine provocation test performed when compared with those who did not (data not shown).

Of the 18 athletes categorized as being asthmatic, 9 had a positive mannitol test and 16 had an additional methacholine provocation performed, and of these, 15 subjects had AHR to methacholine. In eight athletes, both tests were positive.

Considering the 47 athletes who had a methacholine challenge performed, 16 (34%) athletes had a positive test. Of those, 8 (50%) had a positive mannitol test and 50% had a negative test. Of the 12 athletes with a positive mannitol test, 8 had a positive methacholine tests.

In the overall group of athletes (asthmatic and nonasthmatic), the univariate correlations between levels of AHR to mannitol and methacholine (DRS), serum inflammatory markers, FeNO, FEV_1 %pred., and the number of weekly training hours are summarized in Table 3.

The number of weekly training hours correlated positive with levels of AHR to methacholine (Fig. 1) as well as to mannitol (Fig. 2) ($r = 0.43$, $P = 0.003$, and $r = 0.28$, $P = 0.034$, respectively).

Fifteen out of 18 asthmatic athletes successfully performed provocation tests with both mannitol and methacholine. In these subjects, no association was found between levels of AHR to mannitol and methacholine ($r = 0.032$, $P = 0.91$). In 15 out of 18 asthmatic subjects who underwent FeNO measurement as well as mannitol provocation test, a significant correlation was found between FeNO and the level of AHR to mannitol ($r = 0.54$, $P = 0.04$).

To assess predictors of AHR to methacholine and mannitol in the overall group of athletes, multiple regression analyses were performed including the variables that were significantly associated with AHR to methacholine and mannitol, respectively, in the univariate analyses.

In the model build to asses predictors of AHR to methacholine, the variables included were the number of weekly training hours, BMI, and TNF-α. Eleven subjects were excluded from the analysis because of missing data. The number of weekly training hours but not BMI and TNF-α significantly predicted levels of AHR to methacholine ($\beta = 0.37$, $t = 2.30$, $P = 0.03$; $\beta = -0.16$, $t = -0.97$, $P = 0.34$; $\beta = 0.17$, $t = 1.23$, $P = 0.27$), respectively, $R^2 = 0.24$.

Likewise, a multiple regression model including BMI and the amount of weekly training hours as independent variables,

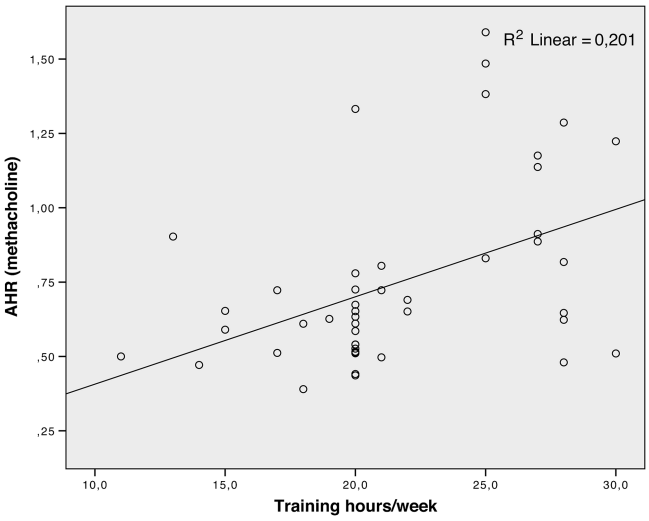

FIGURE 1—The univariate correlation between the number of weekly training hours and AHR to methacholine in the 47 athletes (asthmatic and nonasthmatic) who had a methacholine provocation test performed. Spearman's rho = 0.43 ($P = 0.003$). *Log DRS (methacholine).

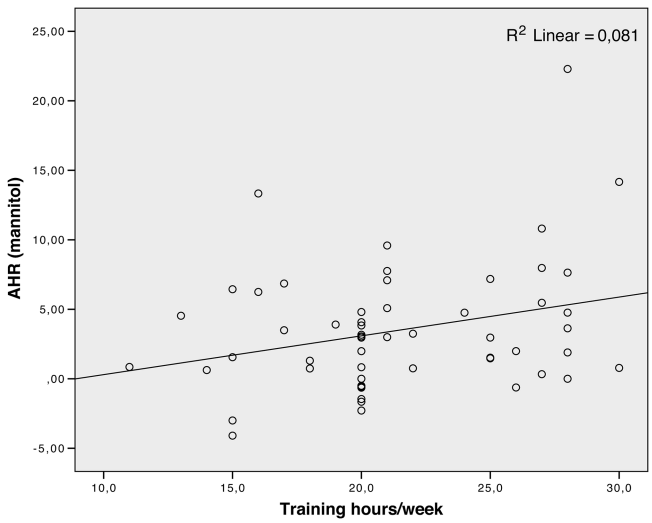

FIGURE 2—The univariate correlation between the number of weekly training hours and AHR to mannitol in the 57 participating athletes. Spearman's rho = 0.28, $P = 0.034$. *Log DRS (mannitol).

and AHR to mannitol as the dependent variable, showed that the number of weekly training hours, but not BMI, significantly predicted AHR to mannitol ($\beta = 0.31$, $t = 2.10$, $P = 0.041$, and $\beta = 0.06$, $t = 0.38$, $P = 0.70$), respectively, $R^2 = 0.08$. All 57 athletes were included in this model.

Because of technical reasons, measurements of serum hs-CRP failed in two of the participants, measurements of IL-6 failed in nine participants, IL-8 and TNF-α measurements failed in one participant.

Serum levels of hs-CRP did not differ between the athletes with and without asthma (median (interquartile range)/(number of subjects): 0.35 $mg \cdot L^{-1}$ (0.37)/(17) versus 0.20 $mg \cdot L^{-1}$ (0.40)/(38), $P = 0.30$). Likewise, serum levels of IL-6 and IL-8 were similar to the asthma/nonasthma group (median (interquartile range)/(number of subjects): 0.49 $pg \cdot mL^{-1}$ (0.34)/(17) versus 0.52 $pg \cdot mL^{-1}$ (1.19)/(31), $P = 0.91$, and 3.15 $pg \cdot mL^{-1}$ (1.19)/(18) versus 3.82 $pg \cdot mL^{-1}$ (3.10)/(38), $P = 0.12$, respectively). Lastly, serum levels of TNF-α did not differ significantly between athletes with asthma and those without (median (interquartile range)/(number of subjects): 1.00 $pg \cdot mL^{-1}$ (0.69)/(18) versus 0.70 $pg \cdot mL^{-1}$ (0.48)/(38), $P = 0.24$).

When considering only the endurance athletes, still no significant differences in serum levels of hs-CRP, IL-6, IL-8, or TNF-α between the asthma/nonasthma group were found (data not shown).

DISCUSSION

In the present study, we found that levels of AHR to both methacholine and mannitol in elite athletes were associated to the number of weekly training hours in the overall group of athletes.

Additionally, we found that levels of AHR to methacholine increased with serum levels of TNF-α. Therefore, TNF-α may play a role in the inflammatory pathways leading to AHR in elite athletes. The expression of this proinflammatory cytokine has been reported to be unaffected by steroid treatment (19), and our results could thereby reflect a steroid-resistant pathway involved in asthma in elite athletes. Our results support what have been described by others, that TNF-α directly alters the contractility of airway smooth muscle cells probably through alterations in calcium flux and bronchoconstrictor sensitivity, thereby possibly inducing AHR (5).

We found that the overall prevalence of asthma in our group of athletes was 32%. This is higher than what has earlier been described in Olympic summer-sport athletes in general (14). However, what is most likely to explain our findings is the fact that a large proportion of the athletes performed endurance sport, especially swimming. Endurance athletes in general and swimmers in particular have been found to have the highest prevalence of asthma when compared to elite athletes who do not perform endurance sports (14,23). In active elite swimmers, the prevalence of asthma/AHR has been reported to be up to 50% (17). Swimmers are among the athletes with the longest training hours, and this supports the theory that asthma/AHR in elite athletes, especially endurance athletes, at least in part, is driven by epithelial damage mediated by repeated heavy breathing leading to cooling and drying of the airways (20). In addition, environmental factors are likely to contribute to the development of AHR and asthma in athletes who perform certain types of sports such as swimming. Swimmers, who in addition to long training hours, are exposed to by-products of chlorine-disinfected swimming pools such as chloramines, which they probably inhale in high concentrations from the air just above the water (10). Lastly, the mean age of the participating athletes was relatively high, reflecting that they had been performing their sports at a high level for a substantial number of years. This could, at least in part, contribute to the high prevalence of AHR, which was found in our group of athletes. It has been shown that in adolescent elite swimmers, the levels of airway inflammation and AHR are not increased when compared to sedentary adolescence. This suggests that the level of AHR in elite athletes increases over time doing high-level sport (28).

What also distinguishes asthma and AHR in elite athletes is that it seems to improve or even disappear when high-intensity training is terminated (17). This is probably a result of the reduction of the most important stimulus for the development of asthma in elite athletes, the heavy physical training.

We found that AHR to mannitol were associated with levels of AHR to methacholine in the overall group of athletes, but no correlation was found when considering only the asthmatic athletes. The last mentioned finding is in contrast to what is described in the general population, where asthmatics often have AHR to multiple stimuli (30).

We did not find any differences in serum levels of hs-CRP, IL-6, IL-8, or TNF-α between elite athletes with and without asthma. As asthma in elite athletes has been suggested to resemble that of the neutrophilic phenotype (3), one could expect serum markers of inflammation to be increased in the asthmatic athletes, as this has previously been found in general asthma patients with neutrophilic asthma (34). However, our results suggest that asthma in elite athletes does not share the same systemic inflammatory characteristics as the neutrophilic phenotype.

The lack of increased systemic inflammation markers in the asthmatic athletes could also be due to the effect of supraintense training on immune function. It is well documented that endurance exercise affects metabolism as well as markers of systemic inflammation and that regular physical activity reduces resting low-grade inflammation (29). Another explanation could be that the inflammation caused by training in athletes is predominantly local.

We found that one athlete had asymptomatic AHR to methacholine and three athletes had asymptomatic AHR to mannitol. These subjects were categorized as nonasthmatic in the present paper because they did not report any respiratory symptoms. In a previous study of elite swimmers and winter sport athletes, 7%–24% of athletes had asymptomatic AHR, with the highest prevalence found among the

swimmers (11). Asymptomatic AHR in these athletes could be mediated by a mild degree of epithelial damage; however, it is not enough to cause respiratory symptoms.

One limitation of our study is that we did not obtain information on the intensity of the training the athletes undertook and that we did not challenge the athletes with a eucapnic voluntary hyperpnea test. We found that one athlete, who in our study was concluded to be nonasthmatic because this athlete had no AHR to either methacholine or mannitol, used anti-inflammatory medicine when entering the study. In addition, this subject had a PD_{20} >16 μmol so the methacholine provocation test would still be interpreted as negative even if the PD_{20} cut-off value was increased because of the use of ICS. However, it is likely that this subject would have a positive test result if challenged with a eucapnic voluntary hyperpnea test, which would perhaps explain why this athlete was prescribed antiasthma medicine.

In conclusion, we found that AHR assessed by methacholine and mannitol challenge in elite athletes with and without asthma was associated to the volume of weekly training and the level of AHR to methacholine increased with the serum level of TNF-α. No differences were found in serum levels of hs-CRP, IL-6, IL-8, or TNF-α between elite athletes with and without asthma.

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The authors declare no conflicts of interest.

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