The Role of Airway Inflammation and Bronchial Hyperresponsiveness in Athlete's Asthma

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[1]Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, NORWAY; [2]Institute of Clinical Medicine, University of Oslo, Oslo, NORWAY; [3]Department of Respiratory Medicine, Oslo University Hospital, Oslo, NORWAY; [4]Department of Clinical Sciences Lund, Respiratory Medicine and Allergology, Lund University, Lund, SWEDEN; and [5]Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, NORWAY

ABSTRACT

STANG, J., L. I. B. SIKKELAND, E. TUFVESSON, A. M. HOLM, T. STENSRUD, and K.-H. CARLSEN. The Role of Airway Inflammation and Bronchial Hyperresponsiveness in Athlete's Asthma. *Med. Sci. Sports Exerc.*, Vol. 50, No. 4, pp. 659–666, 2018. **Purpose**: Asthma is frequently reported in endurance athletes. The aim of the present study was to assess the long-term airway inflammatory response to endurance exercise in high-level athletes with and without asthma. **Methods**: In a cross-sectional design, 20 asthmatic athletes (10 swimmers and 10 cross-country skiers), 19 athletes without asthma (10 swimmers and 9 cross-country skiers), and 24 healthy nonathletes completed methacholine bronchial challenge, lung function tests, and sputum induction on two separate days. All athletes competed on a national or international level and exercised ≥10 $h \cdot wk^{-1}$. The nonathletes exercised ≤5 $h \cdot wk^{-1}$ and reported no previous lung disease. Bronchial hyperresponsiveness (BHR) was defined as a methacholine provocation dose causing 20% decrease in the forced expiratory volume in 1 s of ≤8 μmol. **Results**: BHR was present in 13 asthmatic athletes (62%), 11 healthy athletes (58%), and 8 healthy nonathletes (32%), and the prevalence differed among groups ($P = 0.005$). Sputum inflammatory and epithelial cell counts did not differ between groups and were within the normal range. Median (25th to 75th percentiles) sputum interleukin-8 was elevated in both asthmatic (378.4 [167.0–1123.4]) and healthy (340.2 [175.5–892.4]) athletes as compared with healthy nonathletes (216.6 [129.5–314.0], $P = 0.02$). No correlations were found between provocation dose causing 20% decrease and sputum cell counts. **Conclusion**: Independent of asthma diagnosis, a high occurrence of BHR and an increased sputum interleukin-8 were found in athletes as compared with nonathletes. Airway inflammation or epithelial damage was not related to BHR. **Key Words:** CROSS-COUNTRY SKIERS, EPITHELIAL DAMAGE, EXERCISE, INDUCED SPUTUM, METHACHOLINE, SWIMMERS

Asthma in athletes is frequently observed (1), and the clinical characteristics seem to differ from those observed among nonathlete asthmatics. For instance, exercise-induced respiratory symptoms are frequently reported among athletes, yet no associations to objective clinical findings are apparent (2,3). In fact, a distinct phenotype of "sport asthma" has recently been reported (4).

Bronchial hyperresponsiveness (BHR) is a well-known characteristic of asthma (5). Although swimmers and cold air endurance athletes do have increased BHR when compared with healthy nonathletes (6,7), there is no difference when comparing to asthmatic individuals (7). Interestingly, although swimmers have increased lung function compared with both nonathletes as well as athletes of other sports, they have also shown a large prevalence of BHR (3,6–8). The mechanisms of asthma in athletes are reportedly related to the accumulated strain from high ventilation rates upon the airways, in combination with unfavorable environmental exposures, such as inhalation of cold and dry air or chlorine-derivate of indoor swimming pools (9). In addition, increased parasympathetic activity due to systematic endurance exercise is suggested to influence bronchial tone and thus BHR in endurance-trained athletes (6). Bronchial epithelial damage is proposed to be an important feature of athlete's asthma (10), and increased sputum epithelial cells are shown in athletes as compared with both asthmatic and healthy nonathletes (3,7,11) as well as acutely postexercise (12).

The role of airway inflammation in athlete's asthma is not fully accounted for, and evidences of both acute and long-term inflammatory effects of exercise are conflicting. Some studies have shown an increased neutrophilic airway inflammation in athletes within different sport disciplines (3,11–15), whereas other studies show minimal or no airway inflammation (2,7,8,16). However, several studies report increased inflammatory mediators in plasma or sputum, such as club cell protein 16 (CC16) (3,17,18), interleukin (IL)-8 (19), IL-1β, and IL-6 (3,19). Notably, neither of these studies have stratified athletes

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by asthma diagnosis, and it is not clear if the airway inflammatory response to systematic endurance exercise is similar in athletes with and without asthma. Furthermore, there are gaps in the understanding of the long-term response to exercise regarding the role of airway inflammation and its relation to BHR. The aim of the present study was to assess the long-term effect of systematic endurance exercise upon airway inflammation and BHR in high-level asthmatic and nonasthmatic athletes within sports known to be of high-risk for asthma, namely, swimming and cross-country skiing (4,9). In addition, we wanted to examine the relationship between airway inflammation and BHR in these athletes.

MATERIALS AND METHODS

Subjects and Design

In the present cross-sectional study, one group of athletes with a previous asthma diagnosis (n = 27, 13 swimmers and 14 cross-country skiers), one group of athletes without doctor diagnosed asthma (n = 26, 13 swimmers and 13 cross-country skiers), and one group of healthy nonathletes (n = 27) completed methacholine bronchial challenge, lung function tests, and sputum induction. Athletes were grouped on whether they had current asthma or not. Current asthma was defined as a doctor's diagnosis of asthma, combined with the presence of either current BHR to methacholine (provocation dose causing 20% decrease [PD_{20met}] ≤ 8 μmol) or the current use of asthma medication. Only the subjects with eligible sputum samples (as described in induced sputum section) were included in the present study. The final study population consisted of 20 athletes with asthma (10 swimmers), 19 healthy athletes (10 swimmers), and 24 healthy nonathletes. All subjects were nonsmokers, 16–35 yr of age, and both men and women were included.

Athletes were recruited from regional sport clubs, as well as through the National Olympic Center in Oslo, Norway. Control subjects were recruited from the Norwegian School of Sport Sciences, University of Oslo, and from local high schools through online advertisements on social media channels. Inclusion criteria for athletes were competition at high national or international levels and more than 10 h (h) of exercise per week. Control subjects were not to take part in competitive sports and not to exercise more than 5 $h \cdot wk^{-1}$.

Data collection was conducted from September 2013 to September 2014. The subjects with known or suspected allergies were not tested during the pollen season. Inhaled short-acting β_2-agonists were withheld for 8 h before testing; inhaled long-acting β_2-agonists, oral theophylline, and leukotriene antagonists were withheld for the last 72 h; antihistamines were withheld for the last 7 d; and orally administered glucocorticosteroids were withheld for the last month. Inhaled corticosteroids were not to be used on the day of testing (20). The subjects had to be free from any acute respiratory illness for the last 3 wk and refrain from exercise on the day of testing (>12 h). All subjects attended the laboratory at Norwegian School of Sport Sciences on two different visits, separated by < 3 wk and >24 h. At the first visit, measurements of fractional exhaled nitric oxide (FE_{NO}), spirometry, and skin prick test (SPT) followed by a methacholine bronchial challenge was performed. On the second visit, blood sample was collected, and induced sputum induction was conducted. A questionnaire was administered to document the subjects' past or present history of asthma and allergy (21). All subjects gave their written informed consent for participation, and an additional signed consent was acquired by parent or guardian for subjects who were younger than 18 yr. The present study was approved by the Regional Committee for Medical and Health Research Ethics (2013/167).

Test Protocols

FE_{NO}. FE_{NO} was measured with a single-breath online technique at a constant expiratory flow rate of 50 $mL \cdot s^{-1}$ in accordance to the manufactures instructions (EcoMedics AG, Duerten, Switzerland) (22). Mean values of three measurements with a <10% difference were used in the analysis.

Lung function. Lung function was measured by maximal expiratory flow volume curves (MasterScreen Pneumo Jäger ®, Würzburg, Germany) according to current guidelines (23) and recorded as forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and forced expiratory flow at 25%–75% of FVC. Predicted spirometry values were defined according to Quanjer et al. (24).

Allergy skin prick test. Test was conducted with extracts of 10 common allergens (ALK-Abelló as, Hørsholm, Denmark): dog, cat, horse dander, birch, timothy, mugwort pollens, mold (*Cladosporium herbarum*), house dust mite (*Dermatophagoides pteronyssinus*), cow's milk, and hen's egg white. A subject was classified as atopic if at least one allergen caused a weal of ≥3 mm in diameter greater than the negative control, in the presence of a negative saline control and a positive histamine (25).

Methacholine provocation challenge. Challenge was performed, using an inspiration-triggered Aerosol Provocation System Jäger nebulizer (Würzburg, Germany), according to guidelines of the American Thoracic Society (26). After baseline measurement of lung function, subjects inhaled doubling doses of methacholine chloride (32 $mg \cdot mL^{-1}$) from a starting dose of 0.25 μmol and until a fall in FEV_1 of ≥20% (PD_{20met}) or if the maximal dose of methacholine (24.48 μmol or 4.8 mg) was reached. A subject was considered to have clinical BHR if their methacholine PD_{20} was < 8 μmol (1.6 mg).

Induced sputum. Induced sputum was collected and processed as described by Alexis et al. (27). All subjects were pretreated with inhaled salbutamol (0.1 $mg \cdot mL^{-1}$ per 10 kg body mass) mixed in 1 mL isotonic NaCl before the sputum induction. Subjects inhaled 3% (w/V), 4%, and 5% hypertonic saline for 7 min via an ultrasonic nebulizer (DeVilbiss Healthcare Ltd., West Midlands, UK), respectively. After each inhalation, the subjects were asked to blow their nose, rinse their mouth, and perform a chesty-type cough. Expectorate was collected into a sterile container, and lung function tests were

TABLE 1. Characteristics of athletes with asthma, healthy athletes, and healthy nonathletes.

	Asthmatic Athletes (n = 20)	Healthy Athletes (n = 19)	Healthy Nonathletes (n = 24)
Sex (male:female)	13:7	14:5	11:13
Sport type (s:XC)	10:10	10:9	NA
Age, yr	20.3 (18.3, 22.3)*	18.6 (17.6, 19.6)*	27.3 (24.9, 29.7)
FEV_1 (% of predicted)	108.2 (103.3, 113.1)*	106.4 (101.5, 111.4)*	97.6 (93.6, 101.6)
FVC (% of predicted)	115.0 (109.9, 120.1)*	110.2 (103.8, 116.6)*	102.0 (97.6, 106.3)
Training hours per week	18.2 (16.0, 20.3)	18.5 (15.7, 21.5)	<5
FE_{NO}	21.3 (15.3, 27.4)*	15.5 (12.7, 18.3)	13.6 (11.0, 16.2)
Allergy (%)	9 (45%)	6 (32%)	11 (46%)

Data are presented as mean (95% confidence interval) unless otherwise stated.
*Significantly different from nonathletes ($P < 0.05$).
NA, not analyzed; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; FE_{NO}, fractional exhaled nitric oxide; s, swimming; XC, cross-country skiing.

repeated. Sputum was processed within 2 h after induction. Mucus plugs were selected from saliva and weighed and dissolved in phosphate buffered saline (PBS, Dulbecco's PBS Invitrogen, Burlington, ON, Canada) containing 0.1% (w/V) dithiothreitol (Sigma, St. Louis, MO). The sample was mixed for 15 min, washed with PBS, filtered through a 48-μm pore mesh filter (Sintab, Oxie, Sweden), and centrifuged. Supernatants were frozen at −80°C. Total cell count and cell viability was determined with a Bürker chamber using the trypan blue (0.4%) (Sigma) exclusion method. Calculation of cell differentiation was conducted on blinded cytocentrifuged preparations stained with Diff-Quik (Merz-Dade, Dudingen, Switzerland) expressed as percentage of total. At least 400 cells per slide were counted by two investigators. The sputum sample was considered adequate if it was contaminated by <50% squamous epithelial cells and/or >50% viability.

Protein analysis in blood plasma and induced sputum supernatant. IL-1β and IL-8 were measured with a DuoSet ELISA kit obtained from R&D (Minneapolis, USA). The analyses were performed according to instructions from the manufacturers. The kits used in the analysis were tested for dithiothreitol. CC16 was measured using the Human Club Cell Protein ELISA kit (detection limit 46 $pg \cdot mL^{-1}$) from BioVendor (Modrice, Czech Republic) according to the manufacturers protocol.

Statistical Analysis

Continuous data are presented as means with 95% confidence intervals after tests for normality, unless otherwise stated. Categorical variables are presented as counts (*N*) with percentages. Subjects with a PD_{20met} of >24.48 μmol were assigned a PD_{20met} of 25 μmol, and subjects with PD_{20met} of <0.1 μmol were assigned a PD_{20met} of 0.1 μmol. One-way ANOVA or Kruskal–Wallis tests were used to compare the three groups after tests for normality on continuous data. *Post hoc* tests (Tukey's multiple comparisons technique) were applied to determine within-group differences on normally distributed data. Independent-sample Mann–Whitney *U* test was used to compare two groups of nonnormally distributed data. Chi-square tests were used to assess group differences of categorical variables. Correlations were calculated by Spearman's rank order correlation (ρ). *P* values below 0.05 were considered significant. Statistical analyses were performed using IBM SPSS Statistics version 21.0 (SPSS Inc., Chicago, IL).

RESULTS

Characteristics of the subjects are presented in Table 1. The nonathlete group was older than both athlete groups ($P < 0.001$). The asthmatic athletes and healthy athletes exercised the same amount of hours per week. However, swimmers (22.2 h [20.8, 23.6], mean [95% confidence interval]) exercised more than cross-country skiers did (14.3 h [12.8, 15.8]) ($P < 0.001$). No differences were observed in prevalence of atopy between the groups.

BHR. Clinical BHR ($PD_{20met} \leq 8$ μmol) was found in 67% of the asthmatic athletes, 58% of the healthy athletes, and 33% of the nonathletes (Fig. 1) ($P = 0.005$). *Post hoc* analyses revealed no difference in BHR prevalence between asthmatic and healthy athletes ($P = 0.07$). However, severe BHR ($PD_{20met} \leq 2$ μmol) was more frequent in swimmers ($n = 8$, of which 7 had asthma) compared with cross-country skiers ($n = 1$, $P = 0.05$) (Fig. 2).

Sputum inflammatory and epithelial cell counts. Total sputum cell counts were similar among the three groups, and no significant differences were observed when analyzing the different types of leukocytes by number or percentage (Table 2). All subjects had eosinophils ≤2% of total cell counts of bronchial epithelial cells and leukocytes. Bronchial epithelial cells in induced sputum varied from 1.2% to 2.0% of total cells, with no significant differences between groups. No differences were observed when comparing the percentage of the different leukocytes in sputum between subjects with or without BHR

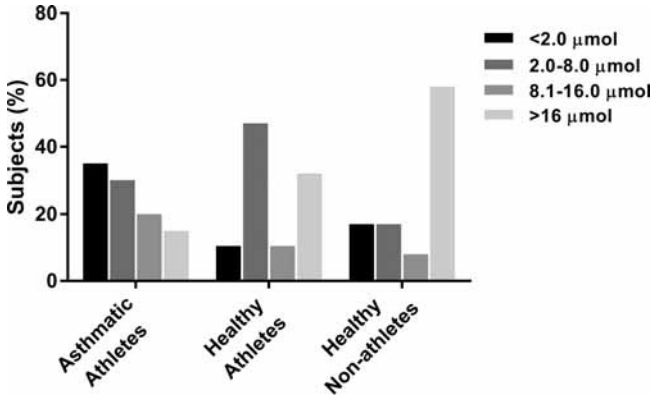

FIGURE 1—Severity of BHR defined as the methacholine dose (μmol) causing ≥20% decrease in FEV_1 (PD_{20met}) in 20 athletes with asthma, 19 healthy athletes, and 24 healthy nonathletes. The distribution in PD_{20met} differed among groups ($P = 0.005$).

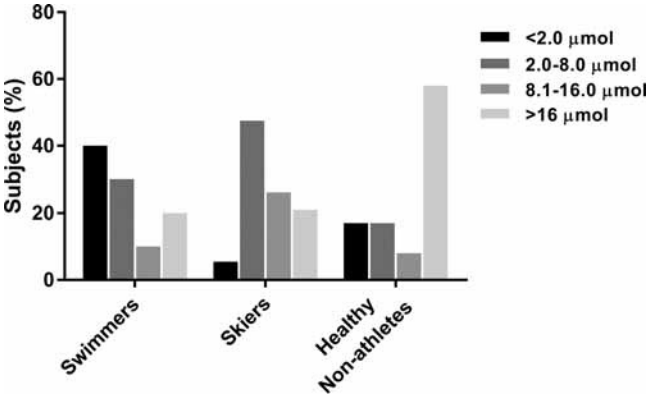

FIGURE 2—Severity of BHR defined as the methacholine dose (μmol) causing ≥20% decrease in FEV_1 (PD_{20met}) in 20 swimmers, 19 cross-country skiers, and 24 healthy nonathletes. The distribution in PD_{20met} differed among groups ($P = 0.007$).

(defined as PD_{20met} <8 μmol, <4 μmol, or <2 μmol) (Table 3). Sputum inflammatory or epithelial cell counts did not correlate to PD_{20met} (data not presented). No significant correlations were observed between weekly hours of exercise or years of sport participation and sputum neutrophils or epithelial cells among the athletes. Nonatopic subjects ($n = 39$) showed similar sputum cell counts as the 26 atopic subjects (9 asthmatic athletes, 6 healthy athletes, and 11 nonathletes) (data not presented).

Airway inflammatory markers. Both athlete groups had increased sputum IL-8 as compared with nonathletes ($P = 0.02$) (Fig. 3). However, no significant differences were observed in sputum IL-1β between asthmatic athletes, healthy athletes, or nonathletes (Table 2). Neither IL-1β nor IL-8 correlated with PD_{20met}. However, sputum neutrophils (%) correlated with both IL-1β ($\rho = 0.389$, $P = 0.002$) and IL-8 ($\rho = 0.481$, $P < 0.001$). No group differences in either sputum or plasma CC16 were observed (Table 2). Neither sputum nor plasma CC16 correlated with PD_{20met} or sputum inflammatory or epithelial cell counts or differed between subjects with different PD_{20met} (Table 3). However, sputum CC16 correlated inversely to years of sport participation ($\rho = -0.367$, $P = 0.039$) in the athletes. A weak correlation between sputum and plasma CC16 was observed ($\rho = 0.281$, $P = 0.024$).

FE_{NO} was significantly increased in athletes with asthma as compared with nonathletes ($P = 0.018$), but not healthy athletes (Table 1). Furthermore, cross-country skiers had increased FE_{NO} (21.7 [15.9, 27.5]) as compared with swimmers (15.1 [12.0, 18.2]). No differences were observed between atopic (19.5 [15.6, 23.5]) and nonatopic subjects (15.0 [12.1, 17.9]). FE_{NO} correlated with sputum eosinophils ($\rho = 0.509$ [$P = 0.026$]).

Lung function. Athletes, both asthmatic and healthy, showed increased FVC (% pred. $P = 0.009$) and FEV_1 (% pred. $P < 0.001$) as compared with healthy nonathletes (Table 1). Furthermore, swimmers had increased FVC (124.0 % of predicted [117.3, 130.7]) as compared with cross-country skiers (115.3 % of predicted [109.7, 121.9]), $P = 0.02$). No lung function variables correlated with weekly hours of exercise, years of sport participation, sputum inflammatory or epithelial cells, or PD_{20met}.

TABLE 2. Differential cell counts in induced sputum, presented as proportion (%) and absolute numbers, and protein markers from athletes with and without asthma and healthy nonathletes given in medians (25th to 75th percentiles) unless otherwise stated.

	Asthmatic Athlete			Healthy Athlete			
	All (n = 20)	Swimmers (n = 10)	Cross-Country Skiers (n = 10)	All (n = 19)	Swimmers (n = 10)	Cross-Country Skiers (n = 9)	Nonathlete (n = 24)
Total cells[a] per milligram of sputum	2217 (1036–5141)	4478 (1647–8863)	1733 (2028–2943)	2066 (981–2949)	2792 (1912–3345)	1241 (651–2258)	1790 (1454–2610)
Neutrophil granulocytes							
Pct.[b]	38 (27–50)	34 (18–50)	42 (23–60)	36 (27–44)	38 (29–48)	31 (14–48)	31 (22–40)
Cells per milligram of sputum	970 (244–1825)	1568 (317–2757)	735 (234–1297)	913 (240–1377)	1105 (719–1466)	360 (92–1194)	502 (302–772)
Airway macrophages							
Pct.[b]	60 (50–72)	65 (49–81)	57 (38–75)	63 (55–72)	61 (51–70)	68 (51–85)	68 (58–77)
Cells per milligram of sputum	1065 (668–3098)	2068 (1049–5693)	693 (648–1334)	1155 (670–1854)	168 (1056–2070)	699 (482–1786)	1333 (766–2028)
Lymphocytes							
Pct.[b]	1.2 (0.6–1.8)	1.0 (0.4–1.6)	1.4 (0.4–2.5)	0.9 (0.6–1.2)	0.9 (0.5–1.4)	0.8 (0.4–1.2)	1.0 (0.7–1.3)
Cells per milligram of sputum	22 (9–39)	31 (15–40)	15 (4–42)	13 (5–33)	19 (6–61)	6 (3–22)	12 (1–24)
Eosinophils							
Pct.[b]	0.2 (0.0–0.4)	0.1 (0.0–0.2)	0.3 (0.0–0.7)	0.1 (0.0–0.2)	0.1 (0.0–0.3)	0.2 (0.0–0.3)	0.1 (0.0–0.3)
Cells per milligram of sputum	0.0 (0.0–2.0)	0.0 (0.0–2.8)	0.0 (0.0–2.6)	0.0 (0.0–2.1)	0.0 (0.0–5.5)	0.0 (0.0–6.2)	0.0 (0.0–0.0)
Protein markers							
Sputum IL-8 ($pg \cdot mL^{-1}$)	378 (167–1123)	462 (169–1737)	356 (161–787)	340 (176–892)	863 (195–1127)	194 (168–446)	217 (130–314)
Sputum IL-1β ($pg \cdot mL^{-1}$)	9.6 (6.1–30.8)	10.2 (5.7–41.3)	8.9 (6.5–15.7)	12.6 (9.7–20.0)	13.1 (11.2–21.6)	11.6 (7.0–20.4)	9.0 (5.7–18.2)
Sputum CC16 ($ng \cdot mL^{-1}$)	2208 (642–4907)	2701 (635–6588)	2208 (959–3856)	2775 (871–3813)	3292 (1505–3974)	1837 (767–2847)	1332 (489–4043)
Plasma CC16 ($ng \cdot mL^{-1}$)	8.1 (6.3–9.6)	6.5 (3.3–8.1)	8.8 (7.8–10.4)	6.2 (5.3–8.3)	5.7 (4.3–9.4)	6.2 (5.4–7.7)	7.5 (6.5–8.8)

[a]Leukocytes.
[b]Data are presented as mean (95% confidence interval).
CC16, club cell protein 16.

TABLE 3. Differential cell counts in induced sputum (presented as proportion) and protein markers from asthmatic and nonasthmatic swimmers (n = 20) and cross-country skiers (n = 19).

	PD_{20met} <2 μmol (n = 9)	PD_{20met} 2–4 μmol (n = 5)	PD_{20met} >4–8 μmol (n = 10)	PD_{20met} <8 μmol (n = 15)
Neutrophil granulocytes (%)	34 (20–48)	25 (10–41)	49 (33–64)*	33 (21–45)
Airway macrophages (%)	65 (50–79)	72 (58–89)	51 (35–66)*	65 (53–77)
Lymphocytes (%)	1.2 (0.5–1.8)	1.2 (0.3–2.0)	0.7 (0.0–1.4)	1.1 (0.5–1.8)
Eosinophils (%)	0.2 (0.0–0.4)	0.1 (0.0–0.3)	0.0 (0.0–0.1)	0.2 (0.0–0.5)
Protein markers				
Sputum IL-8 ($pg \cdot mL^{-1}$)**	354 (166–1090)	437 (190–580)	547 (187–1227)	320 (170–982)
Sputum IL-1β ($pg \cdot mL^{-1}$)**	9.0 (5.6–27.7)	11.5 (5.6–15.2)	11.7 (5.8–29.3)	11.6 (7.6–26.9)
Sputum CC16 ($pg \cdot mL^{-1}$)**	811 (559–6710)	1996 (1851–2825)	2847 (1611–4472)	2738 (809–3473)
Plasma CC16 ($pg \cdot mL^{-1}$)**	5.8 (3.6–7.7)	8.8 (6.8–9.4)	7.0 (6.2–9.2)	8.2 (5.6–9.2)

Data are presented as mean (95% confidence interval) unless otherwise stated.
*Significantly different from PD_{20met} 2–4 μmol ($P < 0.05$).
**Data are presented as median (25th to 75th percentiles).
PD_{20met}, inhaled dose of methacholine causing a 20% decrease in the FEV_1; CC16, club cell protein 16; IL, interleukin.

Drug use. Eight of the 20 athletes with asthma reported regular use of inhaled bronchodilators (β_2-agonist or ipratropium bromide). Use of inhaled corticosteroids was reported in seven asthmatic athletes of which four had BHR (PD_{20met} <8 μmol). No differences were observed between athletes reporting use of inhaled corticosteroids compared with athletes who did not used inhaled corticosteroids regarding lung function (FEV_1 and FVC), BHR (PD_{20met}), leukocytes, epithelial cell counts, or inflammatory markers in sputum or plasma (IL-1β, IL-8, or CC16). The use of antihistamines was reported in eight athletes with asthma, three healthy athletes, and six healthy nonathletes. One healthy athlete and two healthy nonathletes with a history of allergy/rhinitis reported use of bronchodilators, but not during testing.

DISCUSSION

The main findings of the present study were the high occurrence of BHR to methacholine in both asthmatic and nonasthmatic swimmers and cross-country skiers as compared with healthy nonathletes. Yet, increased airway inflammatory cells were not observed in either group. However, we found an increased level of sputum IL-8 among the athletes, independently of asthma diagnosis, as compared with healthy nonathletes. IL-8 correlated with neutrophils in induced sputum.

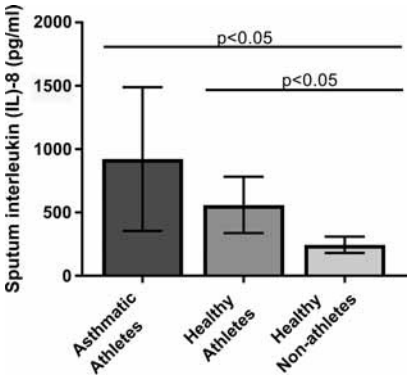

FIGURE 3—Sputum IL-8 in three groups; athletes with asthma (*n* = 20), healthy athletes (*n* = 19), and healthy nonathletes (*n* = 24) presented as median with interquartile range. Error bars represent maximal and minimal values. *P* values show difference between healthy nonathletes and the other groups.

The proportion (differential) and the absolute number of sputum inflammatory cells counts did not differ between asthmatic athletes, nonasthmatic athletes, and nonathletes. Our results are in agreement with similar studies showing no to minimal airway inflammation present in swimmers and cold weather athletes (7,16) and suggest that that the potential acute inflammatory response to exercise is reversible or that the long-term effect of endurance exercise does not involve airway inflammation. However, in the present study, we found increased levels of IL-8 among the athletes compared with healthy nonathletes, suggesting that systematic endurance exercise may induce an inflammatory response in the airways, independently of asthma diagnosis. In the present study, the proportion of sputum neutrophils correlated significantly to both supernatant IL-8 and IL-1β, yet the correlations were moderate. It is conceivable that the stress of intensive exercise or cold air exercise may cause unspecific damage of bronchial epithelium that is associated with increased secretion of IL-8 and influx of neutrophils (12,27). Similarly, Belda et al. (13) found a mild neutrophilic inflammation in the airways of both asthmatic and nonasthmatic athletes practicing water sports. IL-8 is a chemoattractant, and we could have expected an increase in the neutrophil level in sputum in the athletes of the present study that reflected the IL-8 level. However, no such differences were found. We found a correlation between the concentrations of IL-8 and the proportion of neutrophils cells. In sputum, the proportion of newly and old recruited neutrophils differs (28). It is therefore possible that IL-8 is a more sensitive marker than proportion of neutrophils when studying the activity level of the inflammation process in the lung. Increased plasma IL-8 was found in swimmers with BHR after a swim ergometer sprint, but not in swimmers without BHR (19), which may suggest a relationship between IL-8 and BHR. Yet, despite a large prevalence of BHR in the current sample, we found no association to sputum concentrations of IL-8, and conversely increased IL-8 was found in athletes both with and without BHR. The role of IL-8 in athletes with asthma and BHR thus needs further studies.

Increased sputum bronchial epithelial cells are found after a half-marathon run in nonasthmatic subjects (12), as well as >12 h after exercise in swimmers (but not cold air athletes) (7), and it is suggested to reflect epithelial damage with subsequent shedding of epithelial cells into the airway lumen (7).

Serum CC16 has been used as a marker for epithelial damage in relation to chlorine exposure (29), and urinary CC16 is shown to increase after a swimming exercise (17) and after an eucapnic voluntary hyperventilation (EVH) challenge in both athletes and nonathletes with and without BHR (18). In the present study, we found no increase in sputum epithelial cells or CC16 in plasma or sputum in athletes as compared with nonathletes. Furthermore, no difference between asthmatic and nonasthmatic athletes was found. Possibly, our results may be related to the fact that the athletes in our study had not performed any exercise on the day of the sputum sampling. However, there are reports showing increased levels of serum CC16 in swimmers as compared with controls before exercise (3).

The presence of BHR with no increase in airway inflammatory cells is frequently found in endurance athletes (2,7,8). Although BHR is a feature of asthma and a majority of asthmatics have BHR, this state is not exclusive for asthma and may be present in healthy subjects as well (5). However, the large number of nonasthmatic athletes with BHR and increased plasma IL-8 in the present study may suggest undiagnosed asthma. At the same time, evidence of increased inflammatory mediators in sputum of nonasthmatic athletes with exercise-induced bronchoconstriction (EIB) is previously reported (30). In the present study, we set the methacholine cutoff for BHR at 8 μmol (1.6 mg), a higher cutoff than commonly used as recommendation for medical treatment of asthma in athletes (31). However, this is a cutoff commonly used as cutoff for BHR in asthmatics (5). We also analyzed our data using stricter cutoffs of 4 or 2 μmol, which did not change our results (Table 3). In the present study, no correlations were found between PD_{20met} and sputum inflammatory cells, questioning the link between airway inflammation and BHR in athletes. Instead, it is conceivable that the BHR observed may be caused by delayed repair of airway epithelial damage (10), epithelial dysfunction (32), or increased parasympathetic tone (6). However, allergy, as measured by an SPT, was frequently observed among the asthmatic athletes, which suggest that mechanisms involving atopy could be involved in asthma pathogenesis in the athletes.

The differential sputum cell counts did not differ between the asthmatic and nonasthmatic swimmers and cross-country skiers (Table 2). However, the low number of subjects in each group limits the present study's power to disguise possible differences between types of sport. In line with previous studies (7,8,16), the swimmers of the present study had increased lung function compared with nonathletes and more severe BHR (<2 μmol) compared with cross-country skiers. FE_{NO} was increased in cross-country skiers as compared with swimmers. There were no differences in the occurrence of atopy between sport types. Six (32%) of 19 cross-country skiers and 9 (45%) of 20 swimmers had a positive SPT. However, two cross-country skiers had an FE_{NO} >50 ppb, one of whom was allergic, which influence the mean in this group. In contrast to our results, Bougault et al. (7) found a mild eosinophilic inflammation in swimmers, but not in cold-air athletes (including cross-country skiers), as compared with healthy control subjects. However, similar to our study, Martin et al. (8) found no difference in sputum eosinophils between swimming pool–based athletes and non-pool-based athletes, despite a markedly higher incidence of BHR in the pool-based athletes. Notably, the swimmers in the present study exercised more weekly hours than the cross-country skiers did, yet they were younger than the cross-country skiers and thus had accumulated fewer years with systematic exercise as active athletes. It has previously been found that both sputum eosinophils and neutrophils correlate to the amount of weekly exercise performed in swimmers and cold weather athletes, although the degree of sputum inflammatory cells is not increased (7,13). Such associations were not found in the present study. Inhalation of chlorine derivate from indoor swimming pools may affect the airway epithelial layer that may make them more prone to methacholine or other substances that influence the smooth muscles surrounding the bronchi (10).

A strength of the present study was that we studied airway inflammation using induced sputum cells provided directly from the lower airways (33). We found increased levels of IL-8 among the athletes but did not find any differences in sputum cells between the groups. However, the present study was not originally powered to detect differences in sputum inflammatory cells (6). In addition, our measurements were made >12 h postexercise, which may explain the lack of inflammatory cells found in sputum. This is a limitation of the current study, as both pre- and postexercise samples would have allowed for a more complete assessment of the inflammatory response to exercise in athletes. Data collection was conducted throughout a year, including the competitive seasons for cross-country skiers (November–March) as well as for swimmers who compete throughout the year. Thus, recent competitions and high-intensity training activity, as well as seasonal variations, may influence BHR and airway inflammation (34). The results of the present study will not reflect postexercise conditions but the general state of the airways in competitive swimmers and cross-country skiers who exercise >10 $h \cdot wk^{-1}$. However, our results may be affected by the use of inhaled corticosteroids in 7 of the 20 asthmatic athletes, which may influence both inflammatory cell distribution and BHR (35). The nonathletes in the present study had sputum neutrophil and eosinophil levels comparable with low exposed or nonexposed healthy nonathletes in previous studies (7,14).

Sputum is mainly collected from the central airways (28,33), whereas EIB is known as a phenomenon that occurs in the peripheral airways (36). This may explain the lack of association between the sputum result and the PD_{20met}. The use of impulse oscillometry might have provided interesting insight into the bronchial response to methacholine as impulse oscillometry is shown to be more sensitive than spirometry in detecting EIB in athletes after indirect provocations challenges. Thus, it might even detect additional cases of airway dysfunction in athletes (37,38). A high proportion of healthy swimmers are shown to be positive to mannitol (39), suggesting that a mannitol test (or another indirect provocation challenge) performed in our individuals could have provided another access to the inflammation, although indirect tests as Mannitol bronchial

provocation are usually less sensitive than direct tests such as methacholine bronchial challenge (5). The airway response to indirect as compared with direct bronchial provocation challenges may vary between subjects. This lack of agreement may reflect the different underlying mechanisms of BHR in the airways. As we did not include an indirect test in the present study, our results are limited to those athletes with a positive response to a methacholine bronchial challenge. Furthermore, it has been stated that methacholine bronchial provocation is more related to airway remodeling, being a direct challenge test for BHR as opposed to indirect tests, such as exercise tests or the mannitol or EVH test, which have been regarded as more related to airway inflammation (40).

CONCLUSION

The results from the present study show that the long-term response to systematic endurance exercise (as measured >12 h postexercise) in competitive swimmers and cross-country skiers is characterized by BHR and increased IL-8, but not increased airway inflammatory cells. BHR is frequent in both asthmatic and nonasthmatic athletes as compared with healthy nonathletes and is not related to airway inflammation or sputum epithelial cells. Sputum IL-8 may be a marker of the long-term airway inflammatory response of systematic exercise among high-level swimmers and cross-country skiers.

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