Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

Allergy

ORIGINAL ARTICLE AIRWAY DISEASES

Damage-associated molecular pattern and innate cytokine release in the airways of competitive swimmers

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To cite this article: Seys SF, Hox V, Van Gerven L, Dilissen E, Marijsse G, Peeters E, Dekimpe E, Kasran A, Aertgeerts S, Troosters T, Vanbelle V, Peers K, Ceuppens JL, Hellings PW, Dupont LJ, Bullens DM. Damage-associated molecular pattern and innate cytokine release in the airways of competitive swimmers. *Allergy* 2015; **70**: 187–194.

Keywords
Clara cell protein-16; elite athlete; eucapnic voluntary hyperventilation; exercise-induced bronchoconstriction; uric acid.

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Accepted for publication 27 October 2014

DOI:10.1111/all.12540

Edited by: Marek Sanak

Abstract

Background: Daily intensive exercise by elite athletes can result in exercise-induced asthma especially in elite swimmers and this may be linked to epithelial damage.
Objective: To study airway epithelial damage and release of damage-associated molecular patterns (DAMPs) after intensive exercise in elite athletes and controls.
Methods: We recruited competitive swimmers (n = 26), competitive indoor athletes (n = 13) and controls (n = 15) without any history of asthma. Lung function was measured before, immediately after and 24 h after a 90-min intensive exercise protocol. Sputum induction was performed at baseline and 24 h after exercise. Exercise-induced bronchoconstriction (EIB) was assessed by the eucapnic voluntary hyperventilation test.
Results: Baseline sputum uric acid, high mobility group box-1, CXCL8 mRNA, sputum neutrophils and serum Clara cell protein-16 (CC-16) were significantly higher in competitive swimmers compared with controls. Intensive swimming for 90 min resulted in an increase of sputum IL-1β, IL-6 and TNF mRNA in competitive swimmers, and of sputum IL-6 mRNA and sputum neutrophils in controls. Although all participants were asymptomatic, seven competitive swimmers, one indoor athlete and one control met the criteria for EIB.
Conclusion: Our findings show that the intensive training combined with exposure to by-products of chlorination induces airway epithelial damage in competitive swimmers. This is associated with increased damage-associated molecular patterns, innate cytokine release and neutrophilic airway inflammation.

Elite athletes daily perform intensive physical exercise. A high prevalence of asthma and exercise-induced bronchoconstriction (EIB) has been reported in elite athletes (1,2). In particular, swimmers have a high risk of developing asthma (3). Competitive swimmers have the highest prevalence of airway hyper-reactivity (up to 76%) among elite athletes (4).

Mechanical stress of (sustained) extreme breathing during training in combination with environmental conditions in which training is performed may lead to airway epithelial cell damage and mediator secretion that could initiate or increase inflammatory processes in the airways, in the end leading to asthma (5,6). Bronchial epithelial cells have indeed been shown to be released in higher amounts into sputum (epithelial shedding) of cold air athletes (e.g. cross-country skiers) and swimmers (7). Serum Clara cell protein 16 has been used as a marker for disruption of the epithelial layer (8). Additionally, swimmers had higher sputum eosinophil percentages compared with controls (7), and those with airway hyper-reactivity showed increased sputum neutrophil percentages 24 h after a 1-h training session compared with baseline levels (9).

Unlike other elite athletes, swimmers are exposed to chlorine and chlorination by-products, such as trichloramine. Trichloramine in the air of indoor swimming pools may cause damage to the airways (10). In mice, it was shown that

the instillation of hypochlorite prior to ovalbumin induced airway hyper-reactivity as a result of neuroimmune interactions (11).

Damaged or dying cells release damage-associated molecular patterns (DAMPs) or danger signals: uric acid, high mobility group box-1 (HMGB-1), adenosine-5′-triphosphate (ATP) and others (12). Mice exposed to uric acid crystals develop airway inflammation that is dependent on IL-1/IL-1R signalling (13). In patients with asthma, uric acid is increased in broncho-alveolar lavage fluid after allergen challenge (14). We therefore hypothesize that intensive training induces DAMP secretion in the airways of elite athletes, which in turn induces airway inflammation.

In this study, we investigated sputum DAMP and cytokine expression in relation to markers of airway inflammation in competitive swimmers, competitive indoor athletes and controls before and after an intensive training session.

Methods

Subjects

Competitive swimmers and competitive indoor athletes (basketball or volleyball players) between 14 and 25 years of age were recruited among Belgian teams performing at national and international level (additional information: see Supporting Methods). Controls were required to be able to swim for 90 min and should not perform sports for more than 4 h a week (2 h sports at school and additional 2 h sports at recreational level were allowed).

All subjects were evaluated before, immediately after and 24 h after an intensive exercise session (swimming for swimmers and controls, an indoor training session for indoor athletes) of 90 min. The intensive exercise in all subjects consisted of a 60-min protocol at submaximal level (70–80% of maximal heart rate [HR_{max}]) followed by a 30-min protocol consisting of progressively increasing levels of exercise to maximal heart rate (>80% of HR_{max}). Individuals with a history of asthma and who are currently taking anti-asthmatic drugs (inhaled β_2-agonists and/or inhaled steroids) were excluded (Table 1). Baseline characteristics are presented in Table 2. The study was approved by the institutional review board and registered at clinicaltrials.gov (NCT01942096). Details on clinical evaluation and lung function evaluation can be found at the Supporting Methods S1.

Lung function evaluation

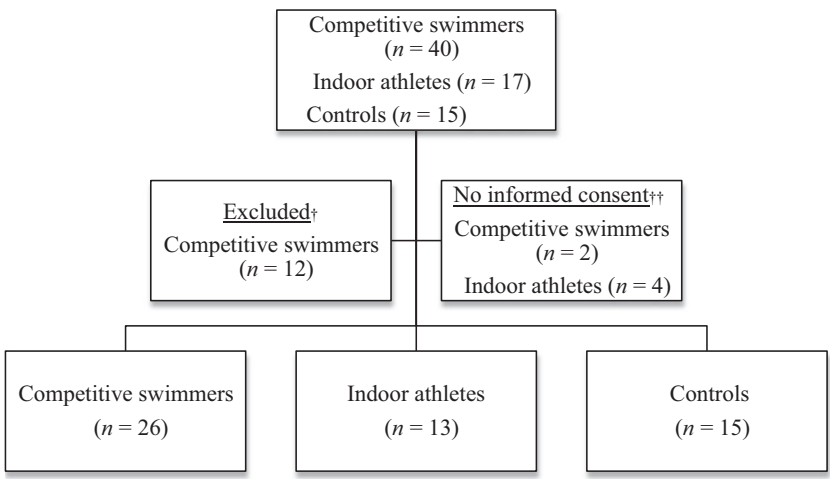
Eucapnic voluntary hyperventilation test (on a separate day) was used to assess exercise-induced bronchoconstriction (EIB) according to previous standards (15,16). The EVH test was considered positive if the fall in FEV_1 ≥10% at one of the time points after the test.

A fall in FEV_1 after the intensive training session compared with baseline was assessed immediately and 24 h later. Exercise-induced bronchoconstriction was suspected if there was a drop in FEV_1 ≥10% immediately after training. Reversibility of FEV_1 15 min after inhalation of 400 μg of salbutamol was assessed 24 h after exercise.

Sputum induction

Induced sputum was obtained by nebulizing a hypertonic salt solution (3%, 4% and 5% NaCl for 7 min) as previously described (17). Sputum samples were processed by selected plug method as previously described (see Supplementary Methods S1) (17,18).

Table 1 Enrollment to the study



†Twelve swimmers were excluded because of regular treatment with β_2-agonists (n = 4) and/or inhaled steroids (n = 8).
††Two swimmers and 4 indoor athletes denied informed consent.

Table 2 Subject characteristics

	Swimmers	Indoor athletes	Controls	*P* value
Number (*n*=)	26	13	15	
Age (years)	16.5 ± 2.2**	18.5 ± 1.8	19.2 ± 3.6	0.004
Gender (M/F)	16/10	8/5	7/8	0.61
Body mass index	21.0 ± 1.5	20.3 ± 1.8	22.1 ± 2.1	0.06
Atopy (*n*=)	5 (19%)	3 (23%)	4 (27%)	0.86
Training (years)	9.3 ± 2.0	9.5 ± 2.4	n.a.	0.40
Training/week (hours)	15.0 ± 3.7	14.8 ± 1.0	<4.0	0.24
FEV_1 (L)	4.9 ± 1.0**	4.5 ± 1.0	3.8 ± 1.1	0.01
$FEV_{1\%}$ predicted	108.2 ± 14.0	100.0 ± 20.0	98.8 ± 13.2	0.12
FVC (L)	6.3 ± 1.4***,†	4.6 ± 1.3	4.6 ± 1.3	0.0002
FVC % predicted	119.8 ± 18.6**,‡	99.7 ± 18.0	99.6 ± 13.5	0.0004

Patient is defined as atopic if skin prick tests were positive for at least one allergen. **, $P < 0.001$ compared to controls; ***, $P < 0.0001$ compared to controls; †, $P < 0.01$ compared to indoor athletes; and ‡, $P < 0.001$ compared to indoor athletes, n.a.: not applicable.

Analysis of induced sputum and blood analysis

A selected panel of cytokines known to be involved in asthma was analysed (17,19–21). This includes the following: Th1 (IFN-γ), Th2 (IL-4, IL-5), Th22 (IL-22), epithelial cell-derived (IL-25, IL-33, TSLP) and innate (IL-1β, IL-6, CXCL8, TNF) cytokines. Sputum cytokine mRNA levels were measured using RT-PCR (17,19,20). Primer and probe sequences were listed in the supporting information. Sputum uric acid levels were quantified by Amplex Red Uric Acid/Uricase Assay kit from Life Technologies (Carlsbad, USA) according to the manufacturers' protocol. Samples were diluted 1/5 to reduce interference of DTT in the sputum.

Sputum myeloperoxidase (MPO) levels (Hycult Biotech, Uden, the Netherlands), sputum HMGB-1 levels (Mybiosource, San Diego, CA, USA) and serum Clara cell protein-16 (CC-16) (Biovendor, Modrice, Czech Republic) were quantified by ELISA according to the manufacturers' protocol.

Analysis of swimming pool water and air

Details can be found at the Supporting Methods S1 and Table S1.

Statistical analysis

Details on statistical analyses can be found at the Supporting Methods S1.

Results

Exercise-induced bronchoconstriction

Twenty-three percentage of swimmers (6/26; $\chi^2 = 1.2$, $P = 0.14$) had a positive eucapnic hyperventilation (EVH) test (≥10% fall in FEV_1) compared with none of the indoor athletes (0/13) and one of the controls (Fig. 1A). Two swimmers had ≥10% fall in FEV_1 immediately after intensive exercise, of whom one also had a positive EVH test (Fig. 1B). A FEV_1 increase ≥12%, 15 min after inhalation of 400 μg salbutamol, was found in 7 swimmers at 24 h after exercise, but in none of the indoor athletes or controls (data not shown). However, these swimmers had decreased FEV_1 levels 24 h after exercise compared with baseline.

Baseline markers of airway inflammation and DAMPs

None of the controls had sputum eosinophil counts ≥3% or neutrophil counts >40%. Baseline sputum eosinophil ($P = 0.02$; Fig. 2A) and neutrophil ($P = 0.008$; Fig. 2B) counts were significantly higher in swimmers compared with controls. Baseline sputum neutrophils ($P = 0.0005$; Fig. 2B) were also significantly higher in indoor athletes compared with controls. Five (19%) swimmers and three (23%) indoor athletes had sputum eosinophils ≥3%. Nine (35%) swimmers and seven (54%) indoor athletes had sputum neutrophils >40% (Fig. 2B). No significant differences in baseline sputum MPO levels, representing activation of neutrophils, were observed between swimmers, indoor athletes or controls (data not shown). Swimmers had significantly higher baseline sputum CXCL8 mRNA levels compared with controls ($P = 0.006$; Fig. 2C).

Baseline sputum IFN-γ mRNA levels (Th1 cytokine) were significantly higher in swimmers ($P = 0.031$; Fig. S1 in the supporting information) and indoor athletes ($P = 0.039$; Fig. S1) compared with controls. Baseline sputum IL-1β, IL-4, IL-5, IL-6, IL-25 and TSLP mRNA levels were not significantly different between swimmers, indoor athletes and controls (data not shown). Baseline sputum uric acid and HMGB-1 levels were higher in swimmers ($P = 0.009$ and $P = 0.0003$) but not in indoor athletes ($P = 0.24$ and $P = 0.22$) compared with controls (Fig. 2D, E).

Effect of intensive exercise on airway inflammation and cytokine release

Sputum eosinophil and neutrophil counts were not altered by intensive exercise in swimmers and indoor athletes (data not shown). In contrast, sputum neutrophils were higher

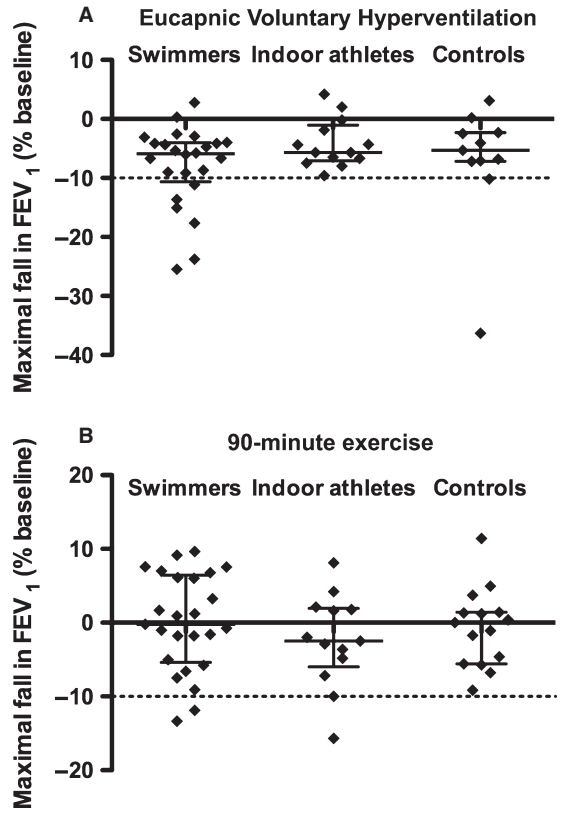


Figure 1 Exercise-induced bronchoconstriction tests at baseline. (A) Competitive swimmers ($n = 24$), competitive indoor athletes ($n = 13$) and controls ($n = 11$) performed eucapnic voluntary hyperventilation test for 6 min at a target rate of 85% of maximal voluntary ventilation (MVV). Spirometry was performed at baseline and 1′, 5′, 10′ and 15′ after MVV. The test was considered positive if there was a fall in FEV_1 ≥10% on at least one time point. (B) Competitive swimmers ($n = 26$), competitive indoor athletes ($n = 13$) and controls ($n = 15$) performed an intensive training session for 90 min. During the last 30 min, intensity was progressively increased to reach a maximum at the end of the test. Spirometry was performed at baseline and 1′ after, and the test was considered positive if there was a fall in FEV_1 ≥10%. Chi-square test was used to compare proportions of patients with positive EVH test or intensive exercise protocol test.

after intensive exercise in controls ($P = 0.02$; data not shown). Intensive exercise did not induce a significant increase of sputum uric acid (UA) or HMGB-1 in any of the groups (data not shown). However, swimmers with a positive EVH test showed a significant increase in sputum uric acid levels 24 h after swimming compared with baseline (Fig. S2a). Sputum IL-1β mRNA levels were significantly increased in swimmers 24 h after swimming ($P = 0.01$; Fig. 3A) but not in indoor athletes (Fig. S3a) or controls (Fig. S3b). Sputum IL-6 ($P = 0.01$; Fig. 3B) and TNF ($P = 0.04$; Fig. 3C) mRNA levels also significantly increased after intensive exercise in swimmers, and sputum IL-6 mRNA levels ($P = 0.01$; Fig. S4b) significantly increased in controls. Sputum TNF mRNA levels did not change after intensive exercise in indoor athletes and controls (Fig. S5a, b). No significant induction of epithelial cell-derived cytokines (IL-25, IL-33 and TSLP) was observed in sputum 24 h after sport-specific training in any of the three groups (data not shown).

Serum biomarker for epithelial damage

Baseline serum Clara cell protein-16 was significantly higher in swimmers ($P = 0.001$; Fig. 4) and indoor athletes ($P = 0.02$; Fig. 4) compared with controls. No significant induction of serum CC-16 after intensive exercise was seen in swimmers or controls (data not shown). Surprisingly, serum CC-16 levels decreased after intensive exercise in indoor athletes ($P = 0.005$; data not shown).

Discussion

In the present study, we found that 27% of competitive swimmers, without history of asthma and who were not on regular treatment with anti-asthmatic medication, had either a positive eucapnic voluntary hyperventilation (EVH) or a positive field exercise test. Twelve competitive swimmers were *a priori* excluded from the study because of history of asthma and regular treatment with anti-asthmatic medication. Most previous studies assessed airway hyper-reactivity in elite athletes by histamine or methacholine challenge. Elite swimmers were shown to have the highest prevalence of airway hyper-reactivity (up to 76%) among elite athletes (4). Histamine or methacholine challenge tests, however, although being helpful for the diagnosis of asthma, are not specific to the diagnosis of EIB. Recently, an official ATS (American Thoracic Society) clinical practice guideline for exercise-induced bronchoconstriction was published (15), recommending serial lung function measurements after exercise or hyperpnea challenge (EVH). Both tests have advantages and disadvantages. The field exercise test can be performed in the environment in which exercise typically occurs but is less sensitive compared with the EVH test to detect EIB in athletes (22). On the other hand, the EVH test can be performed in a standardized manner but is laboratory dependent and can only be performed in specialized centres (23). A study by Martin and colleagues showed that 72% of pool-based athletes with symptoms of exercise-induced asthma (not taking inhaled corticosteroids) had a positive EVH test compared with 39% of nonpool-based athletes (24). The lower prevalence of EIB in our study might be due to the *a priori* selection of nonasthmatic swimmers. In a study of adolescent (12–16 years of age) elite swimmers who stopped inhaled steroids 4 weeks prior to the study, 27.6% of swimmers had a positive EVH test (25).

Besides these signs of airway hyper-reactivity and EIB, we found neutrophilic airway inflammation (>40% sputum neutrophils) in both swimmers ($n = 9$) and indoor athletes ($n = 7$) at baseline. Thus, sustained intensive training, irrespective of exposure to by-products of chlorination, induces

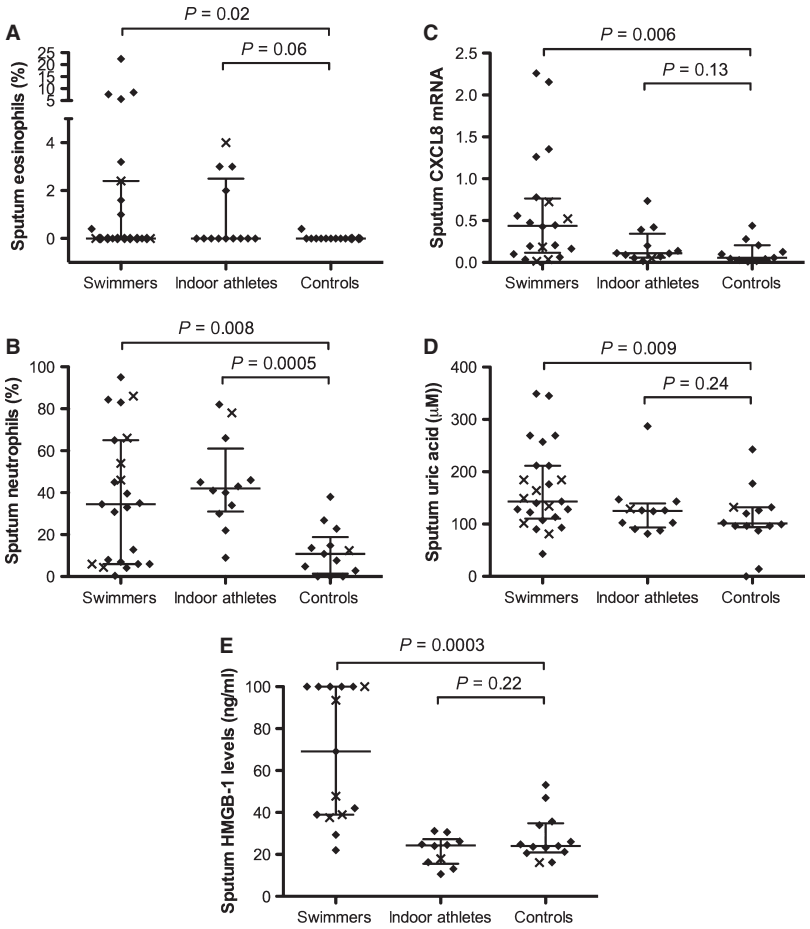


Figure 2 Airway inflammation at baseline. Sputum differential cell counts (eosinophils (A) and neutrophils (B)) were counted on cytospins. Sputum CXCL8 mRNA was measured on lysed sputum cells and normalized to β-actin (C). Sputum uric acid (D) and sputum HMGB-1 (E) were measured on sputum supernatant. Individuals with a positive eucapnic voluntary hyperventilation test were marked (cross). Mann–Whitney test was used to compare swimmers and indoor athletes with controls.

neutrophilic airway inflammation. In line with these results, we previously reported increased sputum neutrophils, IL-17A and MPO levels after exposure to intensive exercise, cold air and high altitude (expedition to the Aconcagua mountain) in patients with asthma (26). In our present study, there was no further increase in sputum neutrophilia 24 h after intensive exercise in swimmers or indoor athletes, while in controls, we did notice a significant increase in sputum neutrophil counts 24 h after swimming. These data suggest that an acute exercise protocol at a high level induces neutrophilic airway inflammation. We hypothesize that no further increase of sputum neutrophils in swimmers and indoor athletes is seen because of already high baseline levels.

Baseline sputum CXCL8 levels were significantly higher in swimmers compared with controls. This was not observed in indoor athletes. Local CXCL8 can be released in the airways by bronchial epithelial cells, fibroblasts and airway smooth muscle cells. We recently reported a highly significant positive correlation between sputum CXCL8 mRNA levels and sputum neutrophil counts in allergic and nonallergic patients (19). Although this correlation did not prove that neutrophils are attracted to the airways by local CXCL8, a cause–effect relationship seems plausible.

We were also able to demonstrate increased presence of damage-associated molecular patterns in the sputum of the athletes, which may feature as early inducers of the pro-inflammatory cytokines. Bronchial epithelial cells as well as alveolar macrophages release DAMPs, which was shown in the airways of COPD subjects (27). Bougault and colleagues showed that bronchial epithelial cells in sputum are significantly higher in elite swimmers compared with controls at baseline suggesting epithelial shedding in elite swimmers (28). A damaged airway epithelium could then result in release of DAMPs in the airways of athletes, which was confirmed by our data. Sputum uric acid and High Mobility Group Box (HMGB)-1 were higher at baseline in swimmers compared with controls. Sputum HMGB-1 has been proposed as a marker for neutrophilic airway inflammation (29), which may help to explain the higher sputum neutrophil count in swimmers compared with controls. HMGB-1 may induce the transition of epithelial cells to mesenchymal structures (30). In line with this, increased airway remodelling has

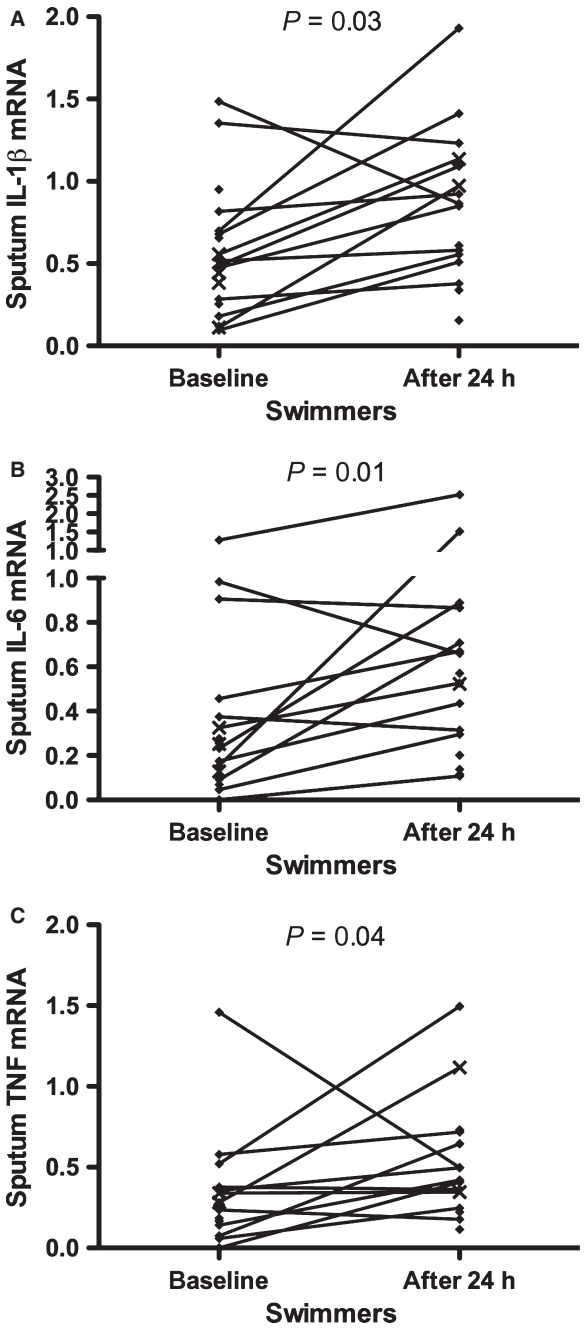


Figure 3 Sputum innate cytokines at baseline and 24 h after intensive training (swimming: swimmers and controls, basketball or volleyball: indoor athletes). Sputum IL-1β, IL-6 and TNF mRNA was measured on lysed sputum cells and normalized to β-actin. Individuals with exercise-induced bronchoconstriction were marked (cross). Wilcoxon's signed rank test was used to compare results at two time points.

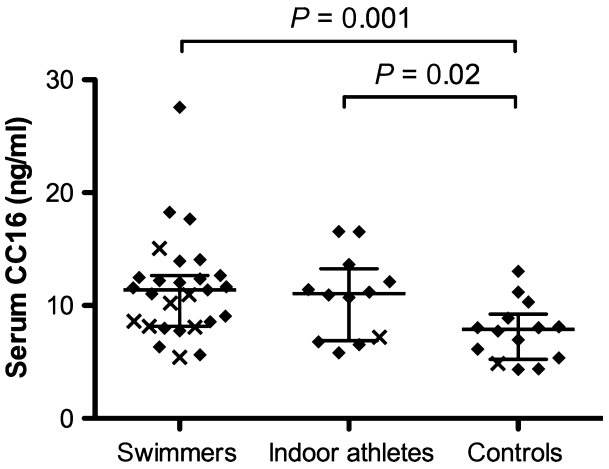


Figure 4 Serum Clara cell protein-16 was measured at baseline in swimmers, indoor athletes and controls. Individuals with exercise-induced bronchoconstriction were marked (cross). Mann–Whitney test was used to compare swimmers and indoor athletes with controls.

been shown in elite swimmers compared with controls (31). Remarkably, uric acid was induced after intensive swimming only in swimmers with a positive EVH test. No further increase in sputum uric acid or HMGB1 levels could be observed in any of the total groups after exercise, but an increase in IL-1β mRNA levels (downstream to uric acid) was found in swimmers 24 h after the training session. It has previously been shown that uric acid is released upon allergen challenge in allergic asthmatics and contributes to the mounting of Th2 immunity (14). Release of uric acid causes activation of NALP3 inflammasome and cleavage of pro-IL-1β to IL-1β via caspase 1 (32). IL-1β then creates a pro-inflammatory environment with release of IL-6 and chemokines (like CXCL8), facilitating recruitment of neutrophils and a Th17 response (32). We also found higher baseline serum CC-16 levels in swimmers and indoor athletes compared with controls. Unexpectedly, serum CC-16 levels decreased in indoor athletes 24 h after intensive exercise. We, however, cannot explain this finding. Serum Clara cell protein-16 has been proposed as a biomarker for airway epithelial damage (8). Thus, epithelial damage is induced by sustained intensive exercise, irrespective of exposure to by-products of chlorination. This is consistent with results of Chimenti and colleagues who showed increased serum CC-16 levels and bronchial epithelial cells after a half-marathon in nonasthmatic amateur runners (33). However, only few bronchial epithelial cells were detected in sputum samples of our study. This might be explained by the time point of sampling that was chosen for our study and which was not optimal to detect epithelial damage at the cellular level.

A limitation of the current study is the low number of individuals analysed in the study. A strong selection was, however, made to include athletes in the study. We therefore cannot be certain whether the lack of significance is due to small sample sizes or represents a real lack of difference between the groups.

In conclusion, a quarter of elite swimmers without history of asthma were found to have exercise-induced bronchoconstriction. Elite swimmers had higher levels of sputum DAMP

molecules (uric acid and HMGB-1), innate cytokines (IL-1β, IL-6 and TNF) and neutrophils in the airways. Also, the biomarker Clara cell protein-16 was found to be higher in serum of swimmers and indoor athletes compared with controls. We thus suggest that intensive exercise, especially in combination with exposure to by-products of chlorination, induces damage of the airway epithelium, thereby releasing DAMP molecules and activating a cascade of inflammation resulting in a recruitment of predominantly neutrophils in the airways.

Author contributions

SS, VH, LVG, GM, ED, AK, SA and VV contributed to recruiting of the patients and performing of the experiments. SS, VH, LVG, GM, KP, JC, PH, LD and DB contributed to analysis and interpretation. SS, VH, LVG, GM, KP, JC, PH, LD and DB contributed to drafting the manuscript for important intellectual content.

Acknowledgments

We are very grateful to Lode Grossen and Kim Van Malderen of the elite sport high school for their cooperation to the project.

Funding

The study was funded by the Climbing for life research fund, Belgium, the Interuniversity Attraction Poles Program, Belgian State, Belgian Science Policy, project P7/30 and the Study Centre for Allergy Projects (SCAP), Haarlem, the Netherlands. Lieven Dupont and Dominique Bullens are recipients of a senior researcher fellowship from the Fund for Scientific Research Flanders (FWO).

Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Analysis of swimming pool water and air.

Methods S1. Supporting methods.

Figure S1. Baseline sputum IFN-γ mRNA levels in swimmers, indoor athletes and controls.

Figure S2. Sputum uric acid levels at baseline and 24 h after intensive exercise.

Figure S3. Sputum IL-1β mRNA levels at baseline and 24 h after intensive exercise in indoor athletes (A) and controls (B).

Figure S4. Sputum IL-6 mRNA levels at baseline and 24 h after intensive exercise in indoor athletes (A) and controls (B).

Figure S5. Sputum TNF mRNA levels at baseline and 24 h after intensive exercise in indoor athletes (A) and controls (B).

References

1. Carlsen KH, Anderson SD, Bjermer L, Bonini S, Brusasco V, Canonica W et al. Exercise-induced asthma, respiratory and allergic disorders in elite athletes: epidemiology, mechanisms and diagnosis: part I of the report from the Joint Task Force of the European Respiratory Society (ERS) and the European Academy of Allergy and Clinica. *Allergy* 2008;**63**:387–403.
2. Fitch KD. An overview of asthma and airway hyper-responsiveness in Olympic athletes. *Br J Sports Med* 2012;**46**:413–416.
3. Bernard A, Nickmilder M, Voisin C, Sardella A. Impact of chlorinated swimming pool attendance on the respiratory health of adolescents. *Pediatrics* 2009;**124**:1110–1118.
4. Bougault V, Boulet L-P. Airway dysfunction in swimmers. *Br J Sports Med* 2012;**46**:402–406.
5. Anderson S. The mechanism of exercise-induced asthma is …. *J Allergy Clin Immunol* 2000;**106**:453–459.
6. Hallstrand TS. Inflammatory basis of exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2005;**172**:679–686.
7. Bougault V, Turmel J, St-Laurent J, Bertrand M, Boulet L-P. Asthma, airway inflammation and epithelial damage in swimmers and cold-air athletes. *Eur Respir J* 2009;**33**:740–746.
8. Bernard A, Carbonnelle S, Dumont X, Nickmilder M. Infant swimming practice, pulmonary epithelium integrity, and the risk of allergic and respiratory diseases later in childhood. *Pediatrics* 2007;**119**:1095–1103.
9. Boulet L-P, Turcotte H, Langdeau J-B, Bernier M-C. Lower airway inflammatory responses to high-intensity training in athletes. *Clin Invest Med* 2005;**28**:15–22.
10. Catto C, Sabrina S, Ginette C-T, Manuel R, Robert T. Occurrence and spatial and temporal variations of disinfection by-products in the water and air of two indoor swimming pools. *Int J Environ Res Public Health* 2012;**9**:2562–2586.
11. Hox V, Vanoirbeek JA, Alpizar YA, Voedisch S, Callebaut I, Bobic S et al. Crucial role of transient receptor potential ankyrin 1 and mast cells in induction of nonallergic airway hyperreactivity in mice. *Am J Respir Crit Care Med* 2013;**187**:486–493.
12. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 2003;**425**:516.
13. Chen C-J. MyD88-dependent IL-1 receptor signaling is essential for gouty inflammation stimulated by monosodium urate crystals. *J Clin Invest* 2006;**116**:2262–2271.
14. Kool M, Willart MAM, van Nimwegen M, Bergen I, Pouliot P, Virchow JC et al. An unexpected role for uric acid as an inducer of T helper 2 cell immunity to inhaled antigens and inflammatory mediator of allergic asthma. *Immunity* 2011;**34**:527–540.
15. Parsons JP, Hallstrand TS, Mastronarde JG, Kaminsky DA, Rundell KW, Hull JH et al. An official American Thoracic Society clinical practice guideline: exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2013;**187**:1016–1027.
16. Anderson SD. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med* 2001;**35**:344–347.
17. Truyen E, Coteur L, Dilissen E, Overbergh L, Dupont LJ, Ceuppens JL et al. Evaluation of airway inflammation by quantitative

Th1/Th2 cytokine mRNA measurement in sputum of asthma patients. *Thorax* 2006;**61**:202–208.
18. Pizzichini E, Pizzichini MM, Efthimiadis A, Hargreave FE, Dolovich J. Measurement of inflammatory indices in induced sputum: effects of selection of sputum to minimize salivary contamination. *Eur Respir J* 1996;**9**:1174–1180.
19. Bullens D, Truyen E, Coteur L, Dilissen E, Hellings P, Dupont L et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res* 2006;**7**:135.
20. Seys SF, Grabowski M, Adriaensen W, Decraene A, Dilissen E, Vanoirbeek JA et al. Sputum cytokine mapping reveals an "IL-5, IL-17A, IL-25-high" pattern associated with poorly controlled asthma. *Clin Exp Allergy* 2013;**43**:1009–1017.
21. Finkelman FD, Hogan SP, Hershey GKK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. *J Immunol* 2010;**184**:1663–1674.
22. Pedersen L, Winther S, Backer V, Anderson SD, Larsen KR. Airway responses to eucapnic hyperpnea, exercise, and methacholine in elite swimmers. *Med Sci Sports Exerc* 2008;**40**:1567–1572.
23. Weiler JM, Hallstrand TS, Parsons JP, Randolph C, Silvers WS, Storms WW et al. Improving screening and diagnosis of exercise-induced bronchoconstriction: a call to action. *J Allergy Clin Immunol Pract* 2014;**2**:275–280.
24. Martin N, Lindley MR, Hargadon B, Monteiro WR, Pavord ID. Airway dysfunction and inflammation in pool- and non-pool-based elite athletes. *Med Sci Sports Exerc* 2012;**44**:1433–1439.
25. Pedersen L, Lund T, Barnes P, Kharitonov S, Backer V. Airway responsiveness and inflammation in adolescent elite swimmers. *J Allergy Clin Immunol* 2008;**122**:322–327.
26. Seys SF, Daenen M, Dilissen E, Van Thienen R, Bullens DMA, Hespel P et al. Effects of high altitude and cold air exposure on airway inflammation in patients with asthma. *Thorax* 2013;**68**:906–913.
27. Ferhani N, Letuve S, Kozhich A, Thibaudeau O, Grandsaigne M, Maret M et al. Expression of high-mobility group box 1 and of receptor for advanced glycation end products in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;**181**:917–927.
28. Bougault V, Turmel J, St-Laurent J, Bertrand M, Boulet L-P. Asthma, airway inflammation and epithelial damage in swimmers and cold-air athletes. *Eur Respir J* 2009;**33**:740–746.
29. Watanabe T, Asai K, Fujimoto H, Tanaka H, Kanazawa H, Hirata K. Increased levels of HMGB-1 and endogenous secretory RAGE in induced sputum from asthmatic patients. *Respir Med* 2011;**105**:519–525.
30. Lynch J, Nolan S, Slattery C, Feighery R, Ryan MP, McMorrow T. High-mobility group box protein 1: a novel mediator of inflammatory-induced renal epithelial-mesenchymal transition. *Am J Nephrol* 2010;**32**:590–602.
31. Bougault V, Loubaki L, Joubert P, Turmel J, Couture C, Laviolette M et al. Airway remodeling and inflammation in competitive swimmers training in indoor chlorinated swimming pools. *J Allergy Clin Immunol* 2012;**129**:351–358.
32. Besnard A-G, Togbe D, Couillin I, Tan Z, Zheng SG, Erard F et al. Inflammasome-IL-1-Th17 response in allergic lung inflammation. *J Mol Cell Biol* 2012;**4**:3–10.
33. Chimenti L, Morici G, Paterno A, Santagata R, Bonanno A, Profita M et al. Bronchial epithelial damage after a half-marathon in nonasthmatic amateur runners. *AJP Lung Cell Mol Physiol*2010;**298**:L857–L862.