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Early-onset airway damage in early-career elite athletes: a risk factor for exercise-induced bronchoconstriction

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Title: Early-onset airway damage in early-career elite athletes: a risk factor for exercise-induced bronchoconstriction

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Conflict of interest

The authors have no conflicts of interest to disclosure.

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41 **Keywords:** exercise-induced bronchoconstriction, elite athletes, epithelial damage, eucapnic
42 voluntary hyperventilation test

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44 **Capsule Summary**

45 Exercise-induced bronchoconstriction is present in 24.5% of 12-13 years old early-career elite
46 athletes. Epithelial damage might be an early phase hallmark in the development of EIB, in the
47 absence of overt cellular inflammation.

48 **Word count:** 1206

49 To the editor,

50 Recent data indicate that elite athletes are at risk of developing exercise-induced

51 bronchoconstriction (EIB)⁽¹⁻³⁾. EIB is defined as the acute narrowing of the airways during or

52 immediately after exercise⁽²⁾. The prevalence of EIB in athletes ranges between 3.7% and 54.8%

53 depending on the sport discipline and the environment in which the athletes exercise⁽¹⁾. The

54 pathophysiological mechanisms driving EIB are not fully understood. One of the hypotheses states

55 that high ventilatory rates in combination with environmental factors such as cold dry air, air

56 pollution and/or chlorine by-products, can lead to an increased mechanical load to the airways

57 resulting in epithelial shedding and damage⁽⁴⁾. In line with this hypothesis, we showed that in

58 adolescent and young adult elite swimmers epithelial damage with increased sputum uric acid and

59 serum CC16 levels is present⁽⁵⁾. As damage-associated molecular patterns (DAMPs) result from

60 epithelial damage, airway innate immune cells, such as macrophages, can get activated, resulting in

61 release of pro-inflammatory cytokine such as TNF- α , IL-6, IL-8, IL-1 β and IL-1 α ⁽⁵⁾. Only few

62 studies have investigated the prevalence of EIB in early-career elite athletes. Pedersen *et al.* did not

63 find increased EIB or signs of airway inflammation in 13-16 years old elite swimmers⁽⁶⁾. This led to

64 the conclusion that elite swimmers develop EIB throughout their career. This urges for screening

65 tools to detect EIB in young elite athletes early in and during their sports career. We hypothesize

66 that markers of epithelial damage, which might be the first pathological event upon airway

67 triggering, could be suitable biomarkers for detection of EIB. Therefore, we tested 117 12-13 years

68 old early-career elite athletes performing more than 12 hours of sports per week from different

69 Flemish prevalent sport disciplines (swimming, basketball, football) and compared them to control

70 subjects (see article's online repository). We investigated the prevalence of EIB with the eucapnic

71 voluntary hyperventilation (EVH) test adapted for young elite athletes⁽⁷⁾ as well as different markers

72 of airway inflammation and epithelial damage (see article's online repository). Subject

73 characteristics can be found in Table 1. Interestingly, swimmers showed significantly higher FEV₁

74 and FVC compared to control subjects (Table 1). Symptoms during exercise were investigated in
75 the sport disciplines (Fig E1). In this study, we found that 24.5% of the tested early-career elite
76 athletes (12-13 years old) suffer from EIB. This is slightly higher than the mean prevalence (15%)
77 of EIB in young elite athletes (5-18 year old) as reported in a meta-analysis by de Aguiar *et al.*⁽⁸⁾.
78 Besides the differences in age group, this higher prevalence can be due to the use of the EVH test
79 instead of an exercise challenge test, such as a 6-minute running test at 85%-90% of the maximal
80 heart rate, which is used in most of the previous studies in athletes between 12 and 18 years old. We
81 opted to use the EVH test because it is performed in a standardized setting similar for all sport
82 disciplines and it is accepted by the International Olympic Committee to diagnose EIB. In our
83 study, early-career elite swimmers (33.3%) have the highest prevalence of EIB followed by
84 basketball (27.3%) and football players (12.8%) (Fig 1). Several studies in adult elite athletes have
85 already shown that EIB is more common in swimmers as well as in athletes performing sports in a
86 cold environment⁽³⁾. We now show that already at the start of their sports career, swimmers have a
87 higher EIB prevalence compared to others, probably because of the long-term exposure to chlorine
88 by-products during intensive exercise. Additional to the presence of EIB in early-career elite
89 athletes, signs of epithelial damage were also detected in 12-13 years old athletes. Indeed, in all
90 athletes, both in swimmers as well as basketball and football players, baseline serum CC16 was
91 increased compared to age-matched controls (Fig 1). No significant differences were found in the
92 percentages of sputum neutrophils and eosinophils between athletes and control subjects (Fig E3)
93 leading to believe that at this young age epithelial damage is already present, but cellular
94 inflammation is still missing in almost all the athletes (Table E3). Interestingly, higher serum CC16
95 levels correlated significantly with higher maximal fall amplitude in FEV₁ after the EVH test ($r = -$
96 0.1937 ; $P = 0.0262$; Fig 1). Sputum uric acid was increased in all athletes compared to the age-
97 matched controls, but most striking in basketball players (Fig 1). In contrast, no significant
98 differences in serum uric acid levels were found amongst the different athletes and control subjects

(data not shown). However, EIB+ athletes have higher serum uric acid, but not CC16 levels compared to EIB- athletes ($P = 0.044$, Fig 1). No significant differences were observed in sputum pro-inflammatory cytokine mRNA levels (IL-1 α , IL-1 β , IL-6, IL-8, IL-17A and TNF- α) between the four groups (Fig E2). However, sputum IL-8 mRNA levels (but none of the other pro-inflammatory cytokine levels) were significantly increased in athletes with EIB compared to those without EIB ($P = 0.009$, Fig 1).

Up till now, an exercise or EVH test to diagnose EIB is not included in the standard medical screening of early-career elite athletes, although EIB might seriously affect their future career. It remains very difficult to screen all early-career elite athletes yearly for EIB. Therefore, one of the goals of this research was to define risk factors and/or biomarkers that might predict EIB with high sensitivity and to screen early-career athletes from different sport disciplines who might be more susceptible to have EIB. Therefore, we looked at the atopic state (determined via skin prick test) and the AQUA[®]-score⁽⁹⁾ of our athletes and correlated this with maximal fall in FEV₁ and EIB status. In our cohort of early-career elite athletes, being atopic or having an AQUA-score ≥ 6 on its own does not indicate the presence of EIB. Secondly, sputum cytokine mRNA levels and serum DAMPs were investigated to predict EIB. Using ROC analysis, cut-off values were determined which were able, with a high sensitivity, to predict the presence of EIB in early-career elite athletes (Fig E4). Athletes with sputum IL-8 mRNA levels lower than 190 or serum uric acid levels below 92 μ M have a very low risk to develop EIB ($P=0.003$ and $P= 0.044$ respectively, Table E1). On the other hand, athletes with sputum IL-1 β mRNA levels higher than 300 are more prone to have or develop EIB ($P = 0.0331$, PPV = 0.85, Table E1).

To conclude, EIB and markers of potential epithelial damage are already present in early-career elite athletes of 12-13 years old, in absence of overt cellular inflammation. We suggest that in early-career elite athletes, EIB can be screened by searching for “wheezing during exercise” and using the AQUA[®] questionnaire on a first level to look for athletes who are at risk for EIB development.

However, this would lead to a substantial number of non-atopic yet EIB+ athletes that would be missed in screening. On a second level, potential biomarkers such as serum CC16, serum uric acid, sputum IL-8 and IL-1 β mRNA levels might be of use to identify athletes that are more prone to develop EIB, independent of their atopy status. This however needs to be validated in a larger cohort. This selected group of athletes should then be referred to specialized centers to undergo an exercise challenge test or an EVH test to confirm the presence of EIB.

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LEGEND TO FIGURE

Figure 1: A) Maximal fall in FEV₁ after EVH test. EVH test was performed in competitive swimmers (n=45), basketball players (n=22), football players (n=39) and 17 age-matched control subjects. Open dots represent athletes with a physician-based asthma diagnosis. **B)** Serum CC16 levels in basketball players (n=23), football players (n=33), swimmers (n=44) and control subjects (n=9). **C)** A spearman correlation was used to correlate serum CC16 levels with the maximal fall in FEV₁ (%) after the EVH test. **D)** Sputum uric acid levels were measured in sputum supernatans after the EVH test in all athletes and control subjects. **E, F)** Sputum IL-8 (F) mRNA levels and serum uric acid levels (E) in EIB+ and EIB- athletes. Red dots represent EIB+ athletes. Data are represented as median with interquartile range. Mann-Whitney test or Kruskal-Wallis test was used to compare the different group.

193 **Table 1:** Subject characteristics

	Basketball players	Football players	Swimmers	Control subjects	P-value
Number (n=)	26	44	47	17	
Age (years)	12.2 ± 0.6	13.1 ± 0.9	12.1 ± 0.8	12.9 ± 1.8	< 0.0001
Gender (M/F)	14/12	37/7	36/11	5/12	0.0001
BMI	18.4 ± 1.7	19.0 ± 2.9	18.0 ± 1.7	17.7 ± 2.2	0.2194
Atopy (n=)	6 (23.1%)	14 (31.8%)	19 (40.4%)	4 (23.5%)	0.3056
FEV1 (L)	3.2 ± 0.5	3.3 ± 0.6	3.5 ± 0.6**	2.9 ± 0.9	0.0101
FEV1% predicted	101.9 ± 10.6	110.9 ± 17.1	114.1 ± 15.7*	97.5 ± 18.7	0.0008
FVC (L)	3.7 ± 0.7	3.8 ± 0.8	4.3 ± 0.8**	3.5 ± 1.0	0.0007
FVC% predicted	100.7 ± 14.9	108.5 ± 18.8	117.9 ± 13.6***	97.5 ± 20.9	< 0.0001
Hours of sports a week (median)	12***	13***	12***	3.5	< 0.0001
Sputum total cell count (x 10 ⁶)	0.3 – 0.6 – 1.6	0.2 – 0.5 – 1.1	0.3 – 0.4 – 0.9	0.6 – 0.96 – 1.9	0.0764
Sputum macrophages (%)	69.5 – 93.1 – 96	42 – 81.5 – 99.1	58.4 – 94.4 – 99.7	40 – 80 – 93	0.1518
Sputum neutrophils (%)	1.8 – 4 – 14.7	0.9 – 21.5 – 57.5	0 – 4 – 37.4	7 – 20 – 60	0.1346
Sputum eosinophils (%)	0 – 0 – 0	0 – 0 – 0.07	0 – 0 – 0	0 – 0 – 0	0.1601
Sputum lymphocytes (%)	0 – 0.4 – 1.2	0 – 0 – 0	0 – 0 – 1.1	0 – 0.8 – 1.8	0.1836

194 Normally distributed data are represented as mean ± SD and analysed via one-way ANOVA. Non-

195 parametric data are represented as median with interquartile range and analysed with Kruskal-

196 Wallis test. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001 compared to control subjects

