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SHORT COMMUNICATION

Airway vascular damage in elite swimmers

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Summary
We postulated that high level swimming can promote airway inflammation and thus asthma by enhancing local vascular permeability. We aimed to test this hypothesis by a cross-sectional study comparing *swimmers* ($n = 13$, 17 ± 3 years, competing 7 ± 4 years, training 18 ± 3 h per week), *asthmatic-swimmers* ($n = 6$, 17 ± 2 years, competing 8 ± 3 years, training 16 ± 4 h per week), and *asthmatics* ($n = 19$, 14 ± 3 years).

Subjects performed induced sputum and had exhaled nitric oxide, lung volumes, and airway responsiveness determined. Airway vascular permeability index was defined as the ratio of albumin in sputum and serum.

Results from the multiple linear regression showed each unit change in airway vascular permeability index was associated with an increase of 0.97% (95%CI: 0.02 to 1.92; $p = 0.047$) in sputum eosinophilis, and of 2.64% (95%CI:0.96 to 4.31; $p = 0.006$) in sputum neutrophils after adjustment for confounders. In a general linear model no significant differences between airway vascular permeability between index study groups existed, after controlling for sputum eosinophilis and neutrophils.

In conclusion, competitive swimmers training in chlorine-rich pools have similar levels of airway vascular permeability than asthmatics. Although competitive swimming has been associated with asthma, airway inflammation and airway hyperesponsiveness do not seem to be dependent on increased airway vascular permeability.

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Introduction

Elite swimmers have been shown to put themselves at increased risk of asthma.[1] This has been attributed to airway inflammation and increased airway responsiveness induced by high-intensity long-term exercise and repeated exposure to chlorine-rich atmosphere in swimming pools.[2] In animal models, repeated exercise-induced hyperpnea causes airway inflammation, obstruction and bronchiolar epithelium damage.[3] These changes may origin not only airway smooth-muscle contraction but also airway edema due to bronchial microvascular phenomena such as vascular engorgement and plasma leakage.[4]

Higher levels of airway vascular permeability have been shown to be a good predictor of the severity of exercise-induced bronchoconstriction in asthmatics.[5] This has lead to the microvascular theory of exercise-induced bronchoconstriction based on functional abnormalities of endothelial cells in newly generated microvessels in asthmatic airways.[6] However, effects of elite swimming on the airway microvascular leakage are unknown.

We postulated that elite swimming can promote airway inflammation and thus asthma by enhancing local vascular permeability. We aimed to test this hypothesis by a cross-sectional study comparing healthy swimmers with subjects with asthma.

Methods

Subjects

Athletes from the main swimming team of FC Porto, training in a chlorinated pool and consecutive non swimming asthmatic teenagers attending an outpatient clinic of a University Hospital were invited to participate. The study was explained to parents and patients with informed consent obtained from those willing to participate. The study was approved by the hospital ethical committee. All swimmers and 19 non-athlete asthmatics were recruited and gave their informed consent to participate. Subjects were classified by their asthma and training status as *swimmers* ($n = 13$), *asthmatic-swimmers* ($n = 6$), and *asthmatics* ($n = 19$). Table 1 shows the descriptive characteristics of the entire sample.

Study design and procedures

During two visits to the clinic, maximum one week apart, subjects performed induced sputum and had exhaled nitric oxide, lung volumes, and airway responsiveness to methacholine ($PD_{20}M$) determined. Sputum was examined as described previously.[7] Briefly, after induction using an inhalation of hypertonic saline, sputum was selected and treated with dithiothreitol (Sputolysin®; Calbiochem Corporation, San Diego, USA). The suspension was centrifuged and the cell pellet was resuspended. Cytospins were prepared and stained using May-Grünwald Giemsa. Differential cell counts were made by counting a minimum of 500 nonsquamous cells. Sputum supernatant was stored at −70 °C for subsequent laser nephelometry assay for albumin. We then calculated the airway vascular permeability index defined as the ratio of albumin concentrations in induced sputum and serum.[8]

Exhaled nitric oxide (NO) was determined by chemiluminescence (NIOX; Aerocrine; Stockholm, Sweden), spirometry and airway responsiveness to metacholine ($PD_{20}M$) were determined using a computerized pneumotachograph spirometer (SensorMedics Vmax 22, SensorMedics, Yorba Linda, USA) and standard procedures.

Statistical analysis

Measurements not normally distributed were log transformed prior to analysis. Linear regression was initially fitted to analyze the associations between airway vascular permeability (independent variable) and the asthma outcomes (dependent variables). Multiple regression models were performed separately for FEV_1, $PD_{20}M$, sputum eosinophils and neutrophils, exhaled NO and ECP to analyze the effect of airway vascular permeability after adjusting for confounders. Gender, age, current use of inhaled corticosteroid were all analyzed as potential confounders of asthma outcomes. Only the variables significant associated with each of the asthma outcome were considered confounders in the respective final models. Differences in airway vascular permeability levels between groups were tested with analysis of covariance after adjustment for sputum eosinophils and neutrophils. The relative proportion of subjects with higher than the median airway vascular permeability index was compared between groups using chi-square test. A 0.05 level of significance (p-value) and 95% confidence intervals (95%CI) were considered. The data analysis was performed using the statistical package PASW Statistics ®, 18.0 version (SPSS Inc; Chicago, IL).

Results

Results from the linear and multiple linear regressions showed that airway vascular permeability was related to sputum eosinophils and neutrophils even after adjustment for confounders (Table 2). Each unit change in airway vascular permeability index was associated with an average increase of 0.97% (95%CI: 0.02 to 1.92; $p = 0.047$) in sputum eosinophilis after adjusting for lung function, airway responsiveness and serum levels of ECP, and of 2.64% (95%CI: 0.96 to 4.31; $p = 0.006$) in sputum neutrophilis after adjustment for inhaled steroids use, atopy, airway hyperesponsiveness, and sputum epithelial cells counts. No other significant associations between airway vascular permeability and asthma outcomes were observed.

The proportion of subjects with higher than the median levels of airway vascular permeability index was higher in asthmatic subjects (68% vs. 32%) than in swimmers with (33% vs. 67%) or without asthma (31% vs. 69%) although non-significantly ($p = 0.075$) (Fig. 1). In the general linear model no significant differences between the airway vascular permeability index in swimmers, with or without asthma, and asthmatics existed after controlling for sputum eosinophilia and neutrophilia (respectively mean, 0.07 [95%

Table 1 Characteristics of sputum cell counts, exhaled nitric oxide, airway responsiveness and lung function in swimmers, asthmatic-swimmers and asthmatics.

	Swimmers, $n = 13$	Asthmatics, Swimmers, $n = 6$, $n = 13$	Asthmatic, $n = 19$
Age (years)	$17 \pm 2.7^{£}$	$16 \pm 1.3^{¥}$	$14 \pm 3.7^{£\ ¥}$
Gender (female/male)	4/9	2/4	7/19
Swimming hours/week	15 (8.0)	12 (14.8)	NA
Years of competition	6 (5.5)	6 (4.8)	NA
Atopic, n (%)	6 (46)$^{\alpha}$	6 (100)	19 (100)$^{\alpha}$
ECP, mcg/L	15 (18)$^{\%}$	33 (35)	32.6(23.9) $^{\%}$
Total IgE, kUA/L	41 (103)*$^{\alpha}$	341 (4944)*	402(629) $^{\alpha}$
hsCRP, mg/dL	0.03 (0.04)	0.04 (0.06)	0.07 (0.13)
$PD_{20}M$, mcmol	8 (4.60)$^{\beta\ \gamma}$	0.20 (2.29)$^{\beta}$	0.60(7.77) $^{\gamma}$
FEV_1, % pred	$110 \pm 16.3^{\&}$	99 ± 27.0	$85 \pm 10.2^{\&}$
Sputum			
Eosinophils, %	0.6 (1.2)$^{\$}$	0.4 (7.5)	2.6 (10.0)$^{\$}$
Neutrophils, %	41.0 (31.3)$^{\alpha}$	48.0 (61.9)	15.5 (25.6)$^{\alpha}$
Lymphocytes, %	2.4 (3.2)	2.4 (3.1)	4.0 (4.3)
Macrophages, %	30.0 (29.3)	20.0 (31.4)	51.4 (30.8)
Epitelial cells, %	11.4 (16.7)	17.8 (38.1)	17.1 (10.8)
Exhaled NO, ppb	18 (24.5)$^{\#}$	37 (50.5)	42 (38.2)$^{\#}$

Atopy was defined by a positive skin prick test (wheal $\geq$3 mm when the control solutions gave expected results) to at least one aeroallergen (house dust mites, pollens, animal dander, moulds).Values are shown as media (IQR) or mean $\pm$ sd were appropriate; ECP: eosinophil cationic protein; FEV1: forced expiratory volume in the first second; hsCRP: high sensitivity C reactive protein; NO: nitric oxide; $PD_{20}M$: methacholine causing a 20% fall from the post-saline value; significant differences between groups using Mann–Whitney or *T*-test were appropriate flagged by same symbols as follows: *:$p = 0.021$; $^{\alpha}$:$p < 0.001$; $^{\beta}$:$p = 0.004$; $^{\gamma}$:$p = 0.009$; $^{\&}$:$p < 0.001$; $^{\$}$:$p = 0.041$; $^{\#}$:$p = 0.005$; $^{\%}$:$p = 0.008$; $^{£}$:$p = 0.045$; $^{¥}$:$p = 0.03$.

Table 2 Associations between airway vascular permeability and allergic inflammation, lung function, airway inflammation and airway hyperesponsiveness.

	Airway vascular permeability index	
	Unadjusted Model[a]	Confounders-adjusted Model
	β (95%CI)	β (95%CI)
FEV_1, % pred	−6.781 (−17.448to3.886)	5.057 (−3.310to13.423) [b]
$PD_{20}M$, mcmol	−0.540 (−1.729to0.648)	1.434 (−0.541 to 3.408) [c]
Eosinophils, %	1.061 (0.252−1.870); $p = 0.012$	0.966 (0.016−1.916); $p = 0.047$ [d]
Neutrophils, %	−0.859 (−1.359to−0.359); $p = 0.001$	2.636 (0.962−4.310); $p = 0.006$ [e]
Exhaled NO, ppb	7.885 (−5.943 to 21.713)	−7.272 (−25.911 to 11.366) [f]
ECP	0.389 (0.003−9.776); $p = 0.024$	8.751 (−1.506 to 19.008) [g]

[a] Linear regression between airway vascular permeability index (independent variable) and each one of the asthma outcomes (dependent variable).

[b] Multiple linear regression between airway vascular permeability index and FEV_1, adjusted for confounders (age, inhaled steroids use, $PD_{20}M$, hsCRP, IgE, sputum eosinophils, ECP).

[c] Multiple linear regression between airway vascular permeability index and $PD_{20}M$, adjusted for confounders (inhaled steroids use, FEV_1, IgE, sputum eosinophils and neutrophils, ECP).

[d] Multiple linear regression between airway vascular permeability index and sputum eosinophils, adjusted for confounders (FEV_1, $PD_{20}M$, ECP).

[e] Multiple linear regression between airway vascular permeability index and sputum neutrophils, adjusted for confounders (inhaled steroids use, atopy, PD_{20}Msputum epithelial cells).

[f] Multiple linear regression between airway vascular permeability index and exhaled NO, adjusted for confounders (inhaled steroids use, atopy, sputum eosinophils and neutrophils).

[g] Multiple linear regression between airway vascular permeability index and ECP, adjusted for confounders (inhaled steroids use, FEV_1, hsCRP, IgE); ECP: eosinophil cationic protein; FEV_1: forced expiratory volume in the first second; hsCRP: high sensitivity C reactive protein; NO: nitric oxide.

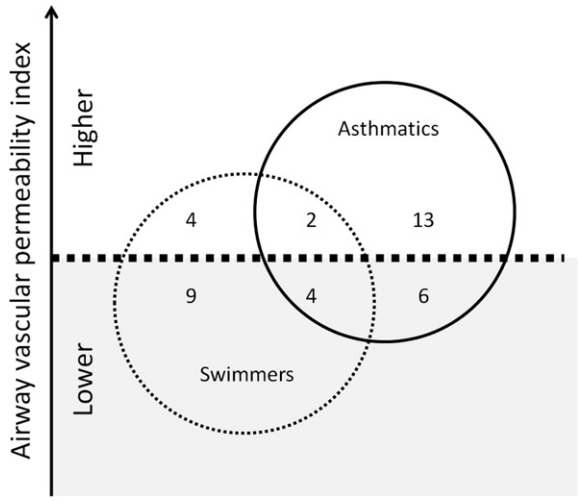

Figure 1 Distribution of subjects according with the median level of airway vascular permeability index and study groups. Horizontal bar represents median level of airway vascular permeability index.

CI, 0.04 to 0.10] and mean, 0.05 [95%CI, 0.04 to 0.06] vs. mean, 0.11 [95%CI, 0.08 to 0.14]).

Discussion

In the present study, competitive swimmers, independent of their asthmatic status, had similar levels of airway microvascular permeability compared to asthmatics. Furthermore, our results suggest the increased levels of inflammatory cells observed in the airways of elite athletes are associated with the airway vascular leakage which, however, does not correlate with lung function or airway hyperesponsiveness. This provides further support to the hypothesis of a "frustrated' inflammatory process in the airways of elite swimmers,[9] suggesting that increased inflammatory cells airway cells in these athletes may represent a training adaptation, not necessarily implying detrimental effects on respiratory health.

Our study has some limitations. First, the direct interpretation of the degree of airway vascular permeability. Airway edema and inflammation are recognized as cardinal features of asthma, resulting from increase microvascular permeability of the bronchial circulation with the exudation of plasma and inflammatory cells into the airway lumen. However, the ratio of albumin concentrations in induced sputum and serum is specific to the bronchial vasculature rather than airway inflammation and is not a marker of the epithelial barrier function. Second, the cross-sectional nature of our study does not allow us to establish causal relationships. Although swimmers were already involved in active competition for an average of 8 years, a longer period of training and competition could eventually be associated with further changes in the airway vascular permeability. Third, control subjects were asthmatics on regular treatment. Inhaled corticosteroids are able to down regulate several airway inflammatory cytokines, to reduce airway vascularity and to inhibit vascular permeability. This effect may have shortened the difference between levels of airway vascular permeability between groups. Finally it can be argued we failed to include a control group with healthy subjects. However, it has been consistently shown that angiogenesis and microvascular remodelling are known features of chronic inflammatory diseases such as asthma and chronic bronchitis, so our control group were subjects with known disease with or without the risk factor exposure (asthmatics swimmers and non-swimmers).

We have previously shown that induced sputum samples of asthmatic-swimmers have increased numbers of eosinophils and neutrophils compared both with healthy subjects and asthmatic patients, respectively.[10] Moreover, asthmatic-swimmers had similar magnitude of exhaled nitric oxide but significantly more pronounced airway responsiveness than asthmatics.[10] We suggested sputum neutrophils may result from the daily exposure of the pool training environment while increased bronchial responsiveness could be the result of both allergic inflammation and training, as it occurred similarly in swimmers and asthmatics. The present study shows these changes were related with the degree of the airway inflammation assessed by numbers of neutrophils and eosinophils in sputum. Moreover, they did not correlate with lung function nor airway responsiveness, sustaining our hypothesis that elite swimming can promote airway inflammation by enhancing local vascular permeability.

In Belgian schoolchildren, cumulated pool attendance has been related with asthma prevalence, exercise-induced bronchoconstriction and, in a dose dependent manner, with markers of lung epithelial damage.[11] Studies in athletes are in line with this finding. We observed a high number of epithelial cells even in sputum of healthy swimmers. Recently Bougault et al. showed a high degree of persistent epithelial cell shedding especially in swimmers. Such increase was not associated with airway inflammation but suggested significant underlying airway damage in a wear-and-tear effect caused by increased ventilation during endurance training.[12]

In conclusion, our results showed airway vascular permeability was similar in swimmers, with or without asthma, and asthmatics; was positively associated with sputum eosinophilis or neutrophilis; did not correlate with lung function, airway responsiveness or exhaled nitric oxide. While we wait for prospective larger studies aiming to assess the impact of recreational swimming on respiratory health, our results suggest we should not refrain subjects to engage on competitive swimming, irrespective of their asthma status.

Conflict of interest statement

None.

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References

1. Helenius I, Haahtela T. Allergy and asthma in elite summer sport athletes. *J Allergy Clin Immunol* 2000 Sep;**106**(3): 444—52.
2. Haahtela T, Malmberg P, Moreira A. Mechanisms of asthma in olympic athletes—practical implications. *Allergy* 2008 Jun; **63**(6):685—94.
3. Chimenti L, Morici G, Paterno A, Bonanno A, Siena L, Licciardi A, et al. Endurance training damages small airway epithelium in mice. *Am J Respir Crit Care Med* 2007 Mar 1; **175**(5):442—9.
4. Kanazawa H, Hirata K, Yoshikawa J. Involvement of vascular endothelial growth factor in exercise induced bronchoconstriction in asthmatic patients. *Thorax* 2002 Oct;**57**(10):885—8.
5. Kanazawa H, Asai K, Hirata K, Yoshikawa J. Vascular involvement in exercise-induced airway narrowing in patients with bronchial asthma. *Chest* 2002 Jul;**122**(1):166—70.
6. Kanazawa H. VEGF, angiopoietin-1 and -2 in bronchial asthma: new molecular targets in airway angiogenesis and microvascular remodeling. *Recent Pat Inflamm Allergy Drug Discov* 2007;**1**(1):1—8.
7. Luís Araújo AM, Carmo P, Marília Beltrão, João Fonseca, Luís Delgado. Induced sputum in children: success determinants, safety and cellular patterns. *J Investig Allergol Clin Immunol* 2011;**21**(3):216—21.
8. Kanazawa H, Nomura S, Yoshikawa J. Role of microvascular permeability on physiologic differences in asthma and eosinophilic bronchitis. *Am J Respir Crit Care Med* 2004 May 15; **169**(10):1125—30.
9. Bonsignore MR, Morici G, Riccobono L, Profita M, Bonanno A, Paterno A, et al. Airway cells after swimming outdoors or in the sea in nonasthmatic athletes. *Med Sci Sports Exerc* 2003 Jul; **35**(7):1146—52.
10. Moreira A, Delgado L, Palmares C, Lopes C, Jacinto T, Rytila P, et al. Competitive swimmers with allergic asthma show a mixed type of airway inflammation. *Eur Respir J* 2008 May; **31**(5):1139—41.
11. 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 Jun;**119**(6):1095—103.
12. Bougault V, Turmel J, St-Laurent J, Bertrand M, Boulet LP. Asthma, airway inflammation and epithelial damage in swimmers and cold-air athletes. *Eur Respir J* 2009 Apr;**33**(4):740—6.