



Systemic and airway oxidative stress in competitive swimmers

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ABSTRACT

Background: The environment in swimming pools, which contain chlorine, might interact with the airway epithelium, resulting in oxidative stress and/or inflammation during high intensity training periods.

Methods: We evaluated pulmonary functional (metacholine challenge test, FEV1 and VC), cellular (eosinophils and neutrophils), inflammatory (FeNo, IL-5, IL-6, IL-8 and TNF- α), oxidative (8-isoprostanes) and angiogenesis factors (VEGF) in induced sputum and peripheral blood of 41 healthy non-asthmatic elite swimmers (median 16 years) during the period of high intensity training before a national championship. The second paired sampling was performed seven months later after training had been stopped for one month.

Results: There was a ten-fold increase (median 82–924 pg/ml; $P < 0.001$) in 8-isoprostanes in induced sputum and five-fold increase (median 82–924 pg/ml; $P < 0.001$) in sera during training in comparison to the period of rest. However, there was no difference in FEV1 (113 vs 116%), VC (119 vs 118%), FeNo (median 34 vs 38 ppb), eosinophils (2.7 vs 2.9% in sputum; 180 vs 165 cells/ μ l in blood), neutrophils, different cytokines or VEGF in induced sputum or sera. The only exception was TNF- α , which was moderately increased in sera (median 23 vs 40 pg/ml; $P = 0.02$) during the peak training period. Almost half (18 of 41) of swimmers showed bronchial hyperresponsiveness during the peak training period (PC20 cutoff was 4 mg/ml). There was no correlation between hyperresponsiveness and the markers of oxidative stress or inflammation.

Conclusions: High intensity training in healthy, non-asthmatic competitive swimmers results in marked oxidative stress at the airway and systemic levels, but does not lead to airway inflammation. However, we could not confirm that oxidative stress is associated with bronchial hyperresponsiveness (AHR), which is often observed during the peak exercise training period.

1. Introduction

The mechanisms of airway disorders associated with competitive swimming are not fully understood. The high level of ventilation during exercise is believed to affect the airway mucosa through two mechanisms: dehydration and mechanical stress [1,2]. Chemical treatment with chlorine is used to disinfect water in indoor swimming pools, and the gaseous chlorine by-products above the water level, such as tri-chloramines or trihalometans, may be especially important. However, there are conflicting data concerning the impact of the chlorinated pool environment on the airways of competitive swimmers [3–5]. The chlorine hypothesis suggests that the interaction between chlorine and the airway epithelium results in oxidative stress and airway inflammation. When inhalation of chlorine by-products repeatedly occurs, such as in elite swimmers, it may result in an impairment of antioxidant activity and/or the pro-inflammatory response, contributing to increased airway responsiveness or asthma [6,7].

In previous studies of induced sputum in swimmers, there were no

significant differences in neutrophil counts between swimmers and mild asthmatic subjects and controls. Eosinophils were increased in both swimmers and asthmatic subjects compared with controls [4]. Furthermore, no significant differences in inflammatory cell counts were observed between swimmers with a PC20 FEV1 of less than 4 mg/mL and those with a PC20 FEV1 of more than 4 mg/mL [5]. In addition, adolescent swimmers had increased IL-6 and TNF- α levels after exercise [8]. Regarding the cytokine response to exercise, swimmers, but not the controls, showed a decrease in the amount of some cytokines, including IL-6 and TNF- α [9].

Oxidative stress is defined as the presence of active oxygen species in excess of the available antioxidant buffering capacity. Major reactive oxygen species belong to free radicals, and reactive oxygen species may damage proteins, lipids, DNA and carbohydrates by changing their structure and function [10,11]. They are involved in non-enzymatic processes that involve the peroxidation of membrane phospholipids, resulting in the generation of isoprostanes. Isoprostanes are thus major oxidative stress markers that are generated from polyunsaturated fatty

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acids, mainly from arachidonic acid. Isoprostanes are stable compounds that are present in urine, plasma, cerebrovascular fluid and exhaled breath condensate. They are unaffected by the lipid content and diet [12]. Oxidative stress is known to be associated with many acute and chronic diseases, such as cancer and cardiovascular, neurodegenerative and lung diseases. There are several reports highlighting oxidative stress as a part of the pathogenesis of asthma [13–15]. Balanza and colleagues found elevated concentrations of isoprostane levels in exhaled breath condensate in children with moderate persistent asthma compared to healthy controls [13].

The aim of our study was to monitor pulmonary function, airway hyperresponsiveness (AHR), FeNO and respiratory symptoms in healthy non-asthmatic elite swimmers during the period of high intensity training before a national championship and then during the period of rest after training had stopped for one month.

We therefore aimed to characterise systemic and airway (induced sputum) oxidative stress and inflammation in competitive swimmers during two different swimming periods: the period of high intensity training just before a national championship and after training had stopped for one month. At both time points, we evaluated systemic and airway (induced sputum) inflammation (eosinophils, neutrophils, IL-5, IL-6, IL-8 and TNF cytokines), oxidative stress (8-isoprostane), chemokines (MCP1) and angiogenesis factors (VEGF).

To understand the environmental impact on these processes, we compared swimmers in two different swimming pools with different exposures to chlorine and its by-products.

2. Methods

2.1. Study subjects

We included 41 healthy competitive swimmers. The exclusion criteria included a previous diagnosis of asthma or any other chronic disease. All subjects were prospectively followed for 8 months: first during high intensity training before the beginning of the national championship in February 2015 and then after training had stopped for one month in September 2015. At the first time point in February, we performed pulmonary function tests, metacholin testing, induced sputum and peripheral blood sampling. At the second time point in September, we repeated all of the testing and sampling, except metacholin testing. Swimmers were trained in two different pools with different levels of ventilation: 23 swimmers were trained in pool 1 and 18 swimmers were trained in pool 2.

2.2. Spirometry, metacholin and FeNO

Spirometry was carried out according to American Thoracic Society Criteria [16] on a spirometer (Vyntus CPX, CareFusion Germany 234 GmbH). Airway responsiveness to metacholine was measured during the high intensity training period by using the tidal breathing method according to the standards recommended by the American Thoracic Society [16]. The PD20 FEV1 (provocation dose of inhaled metacholine causing a 20% fall in FEV1) was obtained by linear interpolation on the log concentration response curve. A cutoff of a maximum of 2 mg of metacholine was used to determine the PD20.

FeNO was measured with the online chemiluminescence FeNO analyser CLD 88 Series (ECOMEDICS, Duernten, Switzerland) according to published guidelines from the European Respiratory Society (ERS) and American Thoracic Society (ATS) [17].

2.3. Induced sputum

Sputum was induced as previously described in detail [18]. Briefly, subjects inhaled 4.5% hypertonic saline, nebulized via an ultrasonic nebulizer (PARI MASTER Type 84.0100, PARI GmbH, Starnberg, Germany), during three 5-min periods, and at least 2 ml of sputum was

collected into a sterile container. The sputum was immediately processed and homogenized with 0.1% dithiothreitol (Sputolysin, Calbiochem, San Diego, CA, USA), and cell-free supernatants were frozen at -80°C until subsequent analysis. The total number of nonsquamous cells (TNNC) per ml of sputum sample was assessed using a hemocytometer. Cytospins were stained according to the May-Grünwald-Giemsa and Papanicolaou methods. Differential cell counts were performed by one observer counting 200 nonepithelial cells. The quality of the induced sputum was assessed according to the recommendations of Pizzichini E et al. [19], and only samples with a score of 7 or more were used for further analysis.

2.4. Cytokines, MCP1 and VEGF

The detection of pro-inflammatory cytokines and VEGF was performed as reported previously [18]. Briefly, IL-5, IL-6, IL-8, TNF, MCP1 and VEGF (subtypes 165 and 121), were measured with cytometric bead arrays (BD Biosciences, San Diego, CA, USA) containing micro-particles dyed to different fluorescence intensities. The captured beads were incubated with standards (purified from human plasma) or test samples (serum or induced sputum supernatant), followed by a wash and incubation with phycoerythrin-conjugated detection antibodies to form sandwich complexes. Flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD Biosciences). Data were acquired and analysed using cytometric bead array software.

2.5. 8-Isoprostanes

8-isoprostane in serum or induced sputum supernatant was measured with a competitive enzyme immunoassay for the quantification of 8-isoprostane (8-isoprostane EIA KIT, Cayman Chemical Company, MI, USA) according to the manufacturer's instructions. Immediately after the collection of the sample, we added 0.005% butylated hydroxytoluene stabilizer. The detection limit of this immunoassay is in the pg/ml range.

2.6. Chlorine and trihalometans

We determined the free chlorine and total chlorine in swimming pools by using a colorimetric method with *N,N*-diethyl-1,4-phenylenediamine (according EN ISO 7393:2000 standard). Trihalometans were determined with Electron Capture Detectors and HP-5 columns according to SIST EN ISO 10301:1998 standard (Agilent, Santa Clara, CA, USA) in the air 5 cm above the water level.

3. Results

3.1. Study subjects

There were 23 (56.1%) females and 18 (43.9%) males. The median age was 16 years (IQR 4 years). All subjects were healthy, without any previous or current diagnosis of asthma, and they were not taking any inhaled or systemic therapies. Twenty-seven of the 41 swimmers had a history of mild and occasional respiratory symptoms during the high training period.

3.2. Pulmonary function

There was no difference in FEV1 (median 116% in high training vs. 113% in rest; $P = 0.248$) or VC (median 118% in high training vs. 119% in rest) (Table 1). However, almost half (18 of 41) of the swimmers showed bronchial hyperresponsiveness during the peak training period (PD20 cutoff was 4 mg/mL, median 0.59 mg/mL). There was no difference in FeNO measurements (median 39 ppb in high training vs 34 ppb in rest).

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