Effects of Swimming on the Inflammatory and Redox Response in a Model of Allergic Asthma

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Key words
- swimming
- antioxidant system
- pulmonary inflammation

Abstract

In this study we hypothesized that swimming during sensitization phase could result in a preventive effect in mice with allergic asthma. Swiss mice were divided into 4 groups: Control and Swimming (non-sensitized), OVA and OVA + Swimming (sensitized). The allergic inflammation was induced by 2 intraperitoneal injections and 4 aerosol challenges using ovalbumin. Swimming sessions were performed at high intensity over 3 weeks. 48 h after the last challenge mice were euthanized. Swimming decreased OVA-increased total IgE, IL-1, IL-4, IL-5 and IL-6 levels, as well as the number of total cells, lymphocytes and eosinophils in bronchoalveolar lavage fluid, ($p < 0.05$). Simultaneously, swimming also increased IL-10 and glutathione levels in the Swimming and OVA + Swimming groups ($p < 0.05$). The levels of glutathione peroxidase and catalase were increased only in the Swimming group when compared to all groups ($p < 0.05$). 21 days of swimming resulted in an attenuation of pulmonary allergic inflammation followed by an increase of glutathione levels in the OVA group. Swimming only increased the levels of glutathione peroxidase and catalase in non-sensitized mice ($p < 0.05$). These data suggest that the pulmonary anti-inflammatory effects produced by 3 weeks of high-intensity swimming in this model of OVA-induced asthma may be, at least partly, modulated by reduced oxidative stress and increased IL-10 production.

Introduction

The beneficial effects of regular aerobic exercise at low and moderate intensity are extensively described in the literature [9]. These benefits include, for instance, reduction and maintance of blood pressure [9], reduction of total and LDL cholesterol and the associated reduced risk of developing atherosclerosis [20], reduced risk of developing diabetes and improved management of disease [15], reduced risk of obesity as well as improved body weight maintenance [15]. Benefits also include improved management of several pulmonary diseases, such as chronic obstructive pulmonary disease (COPD) [37] and asthma [26]. Furthermore, a growing number of experimental animal studies are showing that low- and moderate-intensity aerobic exercise result in reduced lung inflammation in models of asthma [42–11], COPD [39], acute respiratory distress syndrome [32], and even in lung injury induced by air pollution [41], pointing out the immunomodulatory and anti-inflammatory effects of low- and moderate-intensity aerobic exercise for respiratory diseases.

On the other hand, the literature is still conflicting concerning the effects of the high-intensity aerobic exercise. For example, Hafstad et al. (2013) showed that comparing moderate with high-intensity training in diet-induced obesity (DIO) mice, only the high-intensity training improved glucose tolerance, although both modes of exercise improved aerobic capacity and reduced obesity [17]. In the same way Hall et al. (2013) are showing that high-intensity aerobic exercise present the greatest reduction in insulin dosage compared to low-intensity treadmill training or moderate-intensity resistance training [16]. However, Camiletti-Moirón et al. (2013) have demonstrated deteriorated antioxidant response in the brains of rats trained at high-intensity [7]. Additionally, Balducci et al. (2010) shows that high-intensity aerobic exercise improves inflammatory status in diabetic patients [4].

According to Weisel et al. (2009), there are experimental and observational evidence from short-term studies that swimming is less asthmagenic

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than other types of vigorous exercise and had been recommended to asthmatic subjects. It may be because of the horizontal position of the body during swimming, which alters the breathing [45]. Nevertheless, there are few studies that show the negative role of physical exercise on allergic lung inflammation, as presented in a recent revision by Luks et al. (2013) [24].
Thus, these studies evaluating the effects of high-intensity aerobic exercise claim that high-intensity exercise may induce both anti-inflammatory and pro-inflammatory response [17,44].
Therefore, this study was designed to investigate the effects of prolonged practice of high-intensity swimming training during the sensitization phase in a model of ovalbumin-induced asthma.

Materials and Methods

This study was approved by the review board for animal studies of the Federal University of Santa Catarina. All animal care and experimental procedures followed the EU Directive 2010/63/EU for animal experiments [28]. This study also meets the ethical standards of the International Journal of Sports Medicine [18].

Experimental groups

32 male Swiss mice (25–30 g) were divided into 4 groups: control (Control, n = 8); swimming (Sw, n = 8); OVA-sensitized (OVA, n = 8) and OVA-sensitized + swimming (OVA + Sw, n = 8).

Induction of chronic allergic inflammation

We used a modified OVA protocol from Arantes-Costa (2008) with 2 intraperitoneal injections (i.p) of ovalbumin (OVA) (10 µg per mouse) (SALTOS™ SP, Brazil) adsorbed with aluminum hydroxide for OVA-sensitized groups or with saline for control groups [2]. The OVA i.p. injections were performed on days 0 and 14, and the 4 aerosol challenges of OVA solution (1 %) or saline (for control groups) were performed over 30 min on days 23, 25, 27 and 29 (**Fig. 1**).

Exercise protocol

The swimming protocol was adapted from Kuphal, Fibuch and Taylor [8,21]. Swimming groups were adapted to the aquatic environment for 4 days, with gradually-increasing swimming periods being interspersed until the mice were able to swim for 30 min with no pause on fifth day. From the 5th to 21st day, animals from exercised groups were subjected to high-intensity swimming for 30 min with no pause. The Control and OVA groups did not perform the adaptation protocol.

Blood lactate measures

The intensity of the exercise was determined by an adapted protocol [13]. The blood lactate concentration in the Sw and OVA + Sw groups was measured every day over the 3-week exercise period specifically at the 10th and 30th minute of swimming. Control and OVA groups were subjected to exercise only once during the whole protocol period, and the measurement was taken at the 10th and 30th minute of the swimming session.

Anesthesia and euthanasia

48 h after the last allergen challenge, animals were anesthetized with ketamine (50 mg/Kg i.p) and xylazine (40 mg/Kg i.p), and a Tracheostomy was performed to collect the bronchoalveolar lavage fluid (BALF). Prior to BALF collection, mice were euthanized through exsanguination by sectioning the abdominal aorta.

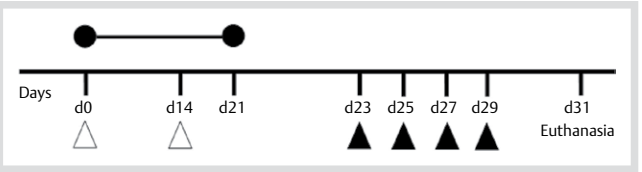

Fig. 1 Timeline of the experimental protocol. Mice received 2 intraperitoneal injections of either ovalbumin solution or vehicle on days 0 and 14 (open triangles). Aerosol challenges with either ovalbumin 1 % solution or vehicle were performed 4 times on days 23, 25, 27 and 29 (closed triangles). Swimming sessions were performed once a day beginning on day 0 until day 21 (closed circles). Animals were euthanized on day 31.

Table 1 Shows the values (mean ± SD) of blood lactate concentration, expressed in mmol/L. Control and OVA groups were subjected only once to exercise protocol in order to establish lactate blood basal control. * = significantly different from 10 min of respective group ($p < 0.05$).

Time	Control	Sw	OVA	OVA + Sw
10 min	3.53 ± 1.1	4.11 ± 0.74	4.4 ± 1.4	3.72 ± 1.6
30 min	5.62 ± 1.45 *	5.95 ± 0.35 *	6.24 ± 1.26 *	5.71 ± 0.82 *

Bronchoalveolar lavage fluid (BALF)

The lungs were gently washed with 3 instillations of 0.5 mL of phosphate-buffered saline (PBS, pH 7.2) via tracheal cannula. Total cells were counted in a Neubauer's hemocytometer chamber. Differential cell count of 300 cells/mouse was performed after Diff Quick staining of BALF prepared on slides. All measures were taken in a blinded fashion for specimen/group identification.

Enzyme-linked immunosorbent assay (ELISA)

Lung homogenate levels of IL-1, IL-4, IL-5, IL-6, and IL-10 and serum total levels of IgE were measured by ELISA according to the manufacturer's instructions (B&D, USA).

Analysis of oxidative stress

Samples of fresh lung tissue weighing approximately 40–50 mg were used for analysis of the levels of total glutathione (GSH) and non-protein thiols (NPSH). The actions of glutathione peroxidase (GPx) and catalase (CAT) were performed later via frozen lung tissue homogenate determined by the protocol used by Ellman (1959) [12]. The total glutathione was measured using the Tieze method modified by Akerboom and Sies (1981) [1].

Statistical analysis

Comparisons among groups were performed through a one-way analysis of variance (ANOVA) followed by Holm-Sidak test for multiple comparison. Significance levels were set at 5 % ($p < 0.05$). Values were expressed as mean ± SE.

Results

Swimming sessions increased blood lactate concentration

Swimming sessions resulted in an increase in blood lactate between 10° and 30° minute of exercise, measured weekly over the 21 days of the experimental protocol. These data set this model as high-intensity exercise, as shown in **Table 1**. All groups subjected to swimming sessions had an increase of more than 1 mmol/L of blood lactate concentration (**Table 1**).

Swimming sessions attenuate eosinophilia induced by OVA

OVA exposure resulted in a significant increase of total cells in BALF compared to all groups ($p < 0.01$) (**Fig. 2a**), while OVA + Sw group presented a reduced number of total cells compared with the OVA group ($p < 0.01$) (**Fig. 2a**). OVA exposure also increased the number of eosinophils ($p < 0.001$) and lymphocytes ($p < 0.01$) compared to all groups, while the OVA + Sw group presented a reduced number of eosinophils ($p < 0.001$) and lymphocytes ($p < 0.01$) compared to the OVA group (**Fig. 2b**).

Swimming sessions attenuated OVA-induced pro-inflammatory cytokines and IgE, while increasing anti-inflammatory cytokine IL-10 in lungs

Fig. 3a shows a significant increase of IgE levels in lung serum in the OVA group compared to all other groups ($p < 0.01$) followed by a significant decrease in IgE levels in the OVA + Sw group compared to the OVA group ($p < 0.01$). **Fig. 3b** through **Fig. 3e** show that OVA exposure resulted in a significant increase in the levels of IL-1, IL-4, IL-5 and IL-6, respectively, in lung homogenates compared to all other groups ($p < 0.05$), and also point out that the OVA + Sw group exhibited significantly reduced levels compared to the OVA group ($p < 0.01$). In addition, high-intensity swimming significantly increased IL-10 levels in lung homogenates in OVA-exposed animals compared to all others groups ($p < 0.013$) (**Fig. 3f**).

Swimming sessions increased the pulmonary levels of GSH, but had no effect on NPSH and antioxidants enzymes (GPx and CAT) in OVA-exposed mice

Fig. 4a shows the GSH levels in lung homogenates. We observed a significant increase in GSH levels in Sw and OVA + Sw groups compared to all other groups ($p < 0.01$). **Fig. 4b** shows the NPSH levels in lung homogenates. We observed no changes in NPSH levels compared to all groups ($p > 0.05$). **Fig. 4c, d** show that swimming training resulted in an increase of antioxidant enzymes (GPx and CAT) only in the non-sensitized swimming training group (Sw group) ($p < 0.01$). In addition, we also observed that the CAT levels were slightly but significantly lower in the OVA + Sw group compared to the OVA group ($p < 0.05$).

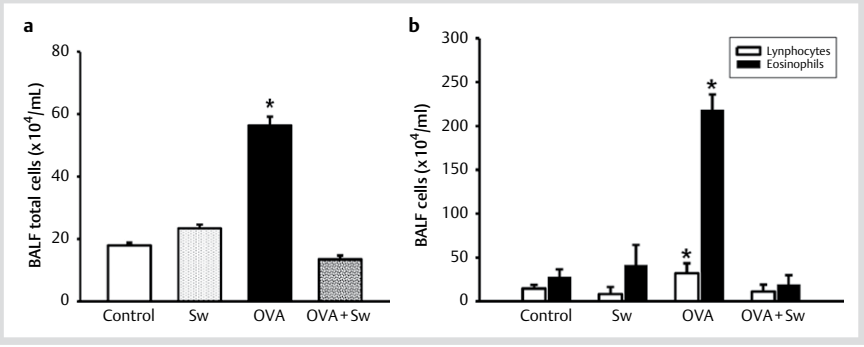

Fig. 2 Shows the total number of cells **a**, eosinophils and lymphocytes **b** from BALF. Values are expressed as mean ± SE. For **a** * = significantly different from all groups ($p < 0.009$). For **b** * = significantly different from all groups for lymphocytes and eosinophils ($p < 0.01$ and $p < 0.009$), respectively.

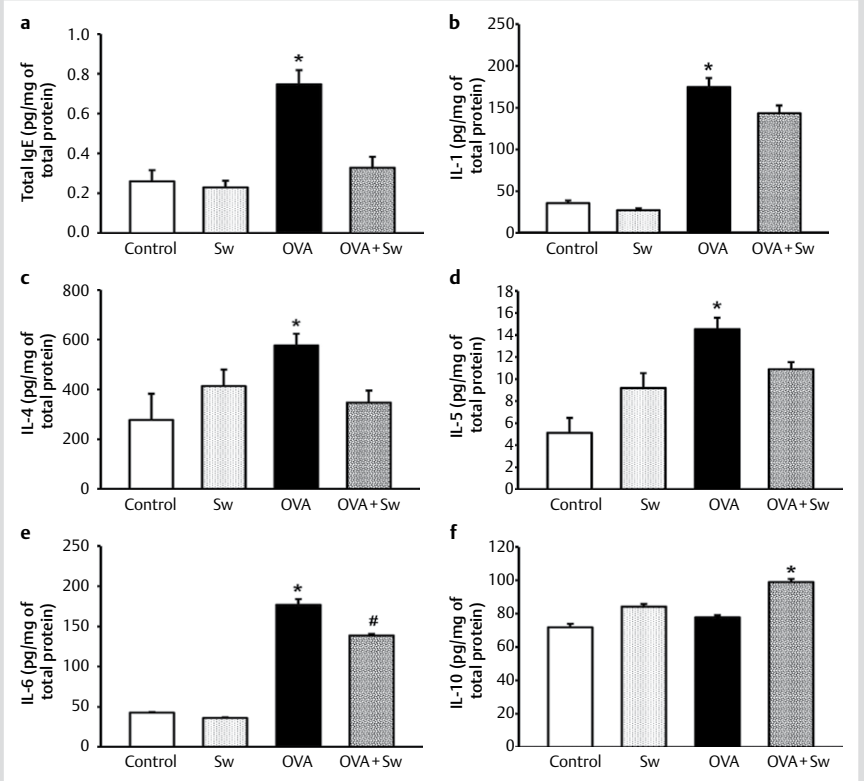

Fig. 3 Shows the total IgE serum levels **a**, and IL-1 **b**, IL-4 **c**, IL-5 **d**, IL-6 **e** and IL-10 **f** in the lung proteins. Results are expressed as pg/mg of total protein and expressed as mean ± SE. For **a–f** * = $p < 0.01$ when compared with all groups. For **e** # = $p < 0.01$ when compared with Control and Swimming groups.

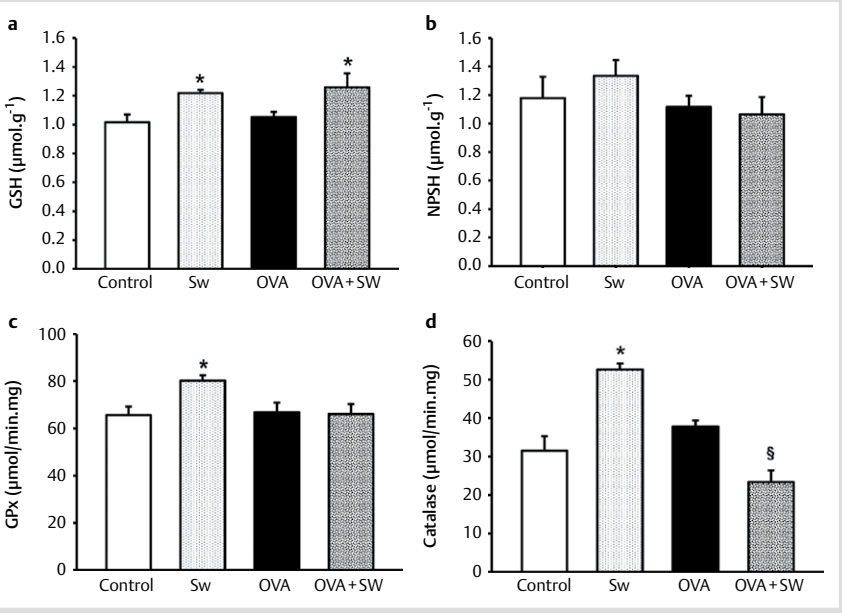

Fig. 4 Shows the pulmonary levels of GSH **a**, NPSH **b**, GPx **c**, CAT **d**. For **a, b**, the results are expressed as µmol.g^{-1}, while for **c, d**, the results are expressed as µmol/min.mg and also expressed as mean ± SE. For **a** * = p < 0.009 when compared with Control group. For **c** * = p < 0.013 when compared with all groups. For **d** * = p < 0.013 when compared with all groups and § p < 0.017 when compared with OVA group.

Discussion

Our study showed for the first time that high-intensity swimming training was able to reduce OVA-induced increases in total IgE, IL-1, IL-4, IL-5 and IL-6 in lung homogenate, as well as the number of total cells, lymphocytes and eosinophils in bronchoalveolar lavage fluid. The study also demonstrated that these anti-allergenic and anti-inflammatory effects were mediated or influenced by IL-10 and glutathione levels in the lungs increased through high-intensity swimming.

Swimmers are more likely to have asthma than any other group of athletes [19] because they aspirate water droplets and chemicals when breathing the air floating just above the water surface. Pools are disinfected with chlorine, the derivatives of which can induce asthma symptoms [30]. The water used in this study was tap water, and thus has a different composition than that of pool water, which differentiates the experimental model used from the results of clinical studies involving swimmers. Furthermore, the high-intensity swimming in this experimental model may not be exactly the same that swimmers use to train. The OVA model is a well-established model of asthma, which reproduces many phenotypes of the disease in mice, despite showing few differences in immunologic responses for example.

In experimental research, several studies have shown a significant reduction in total number of eosinophils and lymphocytes from BALF in exercised OVA-sensitized mice [22, 42]. These studies have suggested that the beneficial effects of physical exercise in asthma phenotype can be directly related to its anti-inflammatory effects [36]. To date, it is unclear whether the benefits of exercise seen in asthma come predominantly from a direct impact on lung airway inflammation, or if they stem from improved cardiac and peripheral muscle conditioning, or both [24].

In this study, we observed that mice with allergic pulmonary inflammation induced by ovalbumin and subjected to high-intensity swimming presented an attenuation of pulmonary inflammation, demonstrated by a decrease in cells in BALF. This finding points out that aerobic exercise presents anti-inflammatory effects even when performed at high intensity. Specifically regarding the effects of aerobic exercise in allergic inflammation, our study has furthermore shown that high-intensity aerobic exercise performed during allergic sensitization process retains its anti-inflammatory effects.

An increasing proportion of athletes are atopic, i.e. they show signs of IgE-mediated allergy, which is a major risk factor for asthma and respiratory symptoms in athletes [19]. Our results corroborate those of Patsva et al. (2004) that showed decreased airway inflammatory responses as well as IgE levels in OVA-sensitized animals submitted to exercise [29]. On the other hand, however, the present study shows for the first time anti-inflammatory effects resulting from high-intensity swimming training. In experimental asthma models and in clinical studies with asthmatic populations, the increase of pro-inflammatory cytokines such as IL-4 and IL-5 is directly related to promoting differentiation, proliferation, increased recruitment and survival of inflammatory cells in allergic inflammation [18, 34].

Our results showed decreased IL-4 and IL-5 levels in lung tissue in the OVA + Sw group. These data corroborate those described by Vieira et al., (2007), showing that in an experimental model of chronic allergic pulmonary inflammation, mice undergoing moderate aerobic treadmill training present decreased eosinophilic infiltration and a reduced count of positive cells for IL-4 and IL-5 in the airway wall [42]. We also showed that despite levels of blood lactate pointing to no training evidence, OVA mice subjected to high-intensity exercise showed a significant decrease in IL-1 and IL-6 levels from lung tissue, reinforcing the anti-inflammatory effects of exercise performed prior to OVA challenges. In the airways of asthmatic individuals, IL-1 cell expression is increased and activates many inflammatory genes expressed in asthma. This pro-inflammatory cytokine amplifies pulmonary inflammation by activating several chemokines that also attract circulating cells into the lungs [5].

IL-6 is a cytokine produced by inflammatory cells and by primary lung epithelial cells in response to a variety of different stimuli including allergens, respiratory viruses and exercise [6, 24]. The pleotropic nature of this immunoregulatory cytokine suggests that IL-6 could be a potential wide-ranging contributor to asthma as well as other pulmonary diseases involving damage

to the pulmonary epithelium [25,33]. In the present study, we observed that high-intensity swimming reduced the pulmonary levels of IL-6, pointing out the anti-inflammatory effect of aerobic exercise in this OVA model of asthma.

It has been suggested that the immunoregulatory effects of aerobic exercise are mediated by increased release of IL-10 [27,38]. Our results demonstrated that during sensitization, high-intensity swimming increased IL-10 lung levels, suggesting a possible mechanism of exercise-induced decrease in pulmonary allergic inflammation and corroborating the data presented by Vieira and Silva [36,40].

In a situation where there is an increased oxygen consumption, such as exercise, there is also an associated acute state of oxidative stress. Therefore, repeated exposure to increases in reactive oxygen species (ROS) caused by exercise results in an increase of antioxidant defense system either [14]. In addition, there is an associated change in redox balance, which elicits a reducing environment, resulting in an increase in antioxidant defenses. This mechanism results in an adaptive protection of ROS during subsequent sessions of exercise [14], such as an increase of catalase and glutathione peroxidase. However, in an experimental rat model, Prada, et al., (2004), found no increasing levels of CAT and glutathione reductase, even after 4 weeks of swimming and an improvement in aerobic fitness of the animals [31]. Our results corroborate those findings, given that in this experimental model no significant changes were found in CAT and GPx levels in the OVA + Sw group. However, swimming by itself was able to induce an increase in anti-oxidant enzymes levels in non-sensitized animals.

These results may be explained, in part, by the fragile balance existing between the anti-oxidant and oxidant systems in response to exercise in the context of asthma. According to Wang (2003), the nature of radical species in inflammation can usually amplify the primary lesion, which hampers an establishment of a reliable index of substances able to eliminate the free radicals [43]. In this way, a possible explanation for our findings showing that swimming did not increase CAT levels in the lungs of OVA exposed mice could be partly explained by the high levels of nitric oxide normally found in asthmatic lungs, which may impair enzymes activity [10].

On the other hand, in this experimental model, swimming performed before OVA challenges increased the levels of GSH. Glutathione is a biomolecule widely used in several vital functions, including electrophilic detoxification, maintenance of thiol balance, free radical scavenging and modulation of immune function [23]. GSH is the reduced form of glutathione and acts directly or indirectly in many important biological processes, including protein synthesis and cell metabolism during exercise [35]. Because this GSH response results from an increase in H_2O_2 levels, GSH is also required to maintain the balance between oxidant production and the antioxidant system, which in our study may suggest an additional anti-inflammatory role on asthma [3]. Therefore, the present study showed for the first time that high-intensity swimming training performed during the sensitization phase present anti-inflammatory effects in this experimental model of allergic asthma, which may be attributed to a reduction in the release of pro-inflammatory cytokines (IL-1, IL-4, IL-5, IL-6), increases in the release of anti-inflammatory cytokine (IL-10) and increases in the anti-oxidant (GSH) levels.

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Conflict of interest: The authors have no conflict of interest to declare.

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