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Authors
Deborah Camargo Hizume-Kunzler[1], Flavia R. Greiffo[2], Bárbara Fortkamp[1], Gabriel Ribeiro Freitas[3], Juliana Keller Nascimento[3], Thayse Regina Bruggemann[4], Leonardo Melo Avila[1], Adenir Perini[5], Franciane Bobinski[6], Morgana Duarte Silva[6], Fernanda Rocha Lapa[7], Rodolfo Paula Vieira[8], Verônica Vargas Horewicz[9], Adair Roberto Soares dos Santos[10], Kelly Cattelan Bonorino[10]

Affiliations
1 State University of Santa Catarina, Physical Therapy, Florianopolis, Brazil
2 Nove de Julho University – UNINOVE, Research, São Paulo, Brazil
3 Universidade do Estado de Santa Catarina Centro de Ciencias da Departamento de Fisioterapia, Saude e do Esporte, Florianopolis, Brazil
4 School of Medicine of University of Sao Paulo, Internal Medicine, Sao Paulo, Brazil
5 Faculdade de Medicina da Universidade de São Paulo, Departamento de Clínica Médica, São Paulo, Brazil
6 Department of Biological Science, Federal University of Santa Catarina, Florianopolis, Brazil
7 Universidade do Contestado Campus Concordia, Ciências Biológicas, Concordia, Brazil
8 Nove de Julho University (UNINOVE), Laboratory of Pulmonary and Exercise Immunology (LABPEI) and Brazilian Institute of Teaching and Research in Pulmonary and Exercise Immunology (IBEPIPE), Sao Paulo, Brazil
9 Universidade Federal de Santa Catarina, Departamento de Farmacologia, Florianopolis, Brazil
10 Universidade Federal de Santa Catarina, Departamento de Ciências Biológicas, Florianópolis, Brazil

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Correspondence
Dr. Deborah Camargo Hizume-Kunzler, PhD
State University of Santa Catarina
Physical Therapy
Rua Pascoal Simone, 358
Florianopolis
Brazil
88080 350
Tel.: +51/483/3218 605, Fax: +51/483/321 8607
dehizume@gmail.com

ABSTRACT

Aerobic exercise (AE) reduces lung function decline and risk of exacerbations in asthmatic patients. However, the inflammatory lung response involved in exercise during the sensitization remains unclear. Therefore, we evaluated the effects of exercise for 2 weeks in an experimental model of sensitization and single ovalbumin-challenge. Mice were divided into 4 groups: mice non-sensitized and not submitted to exercise (Sedentary, n = 10); mice non-sensitized and submitted to exercise (Exercise, n = 10); mice sensitized and exposed to ovalbumin (OVA, n = 10); and mice sensitized, submitted to exercise and exposed to OVA (OVA + Exercise, n = 10). 24 h after the OVA/saline exposure, we counted inflammatory cells from bronchoalveolar fluid (BALF), lung levels of total IgE, IL-4, IL-5, IL-10 and IL-1ra, measurements of OVA-specific IgG1 and IgE, and VEGF and NOS-2 expression via western blotting. AE reduced cell counts from BALF in the OVA group ($p < 0.05$), total IgE, IL-4 and IL-5 lung levels and OVA-specific IgE and IgG1 titers ($p < 0.05$). There was an increase of NOS-2 expression, IL-10 and IL-1ra lung levels in the OVA groups ($p < 0.05$). Our results showed that AE attenuated the acute lung inflammation, suggesting immunomodulatory properties on the sensitization process in the early phases of antigen presentation in asthma.

Introduction

Asthma is defined as a chronic inflammatory airway disorder, and is associated with the activation of inflammatory cells such as eosinophils, mast cells, basophils, macrophages and lymphocytes, especially the cluster of differentiation 4 (CD4 +) subtype [6]. Recruitment and activation of these cell types in the airways of asthmatic patients depends on the expression and activation of several classes of proteins, such as cytokines, chemokines and adhesion molecules [2, 47].

During the early phase of an allergic asthma attack, an immediate reaction is triggered upon allergen contact, and involves the cross-linking of allergen-specific immunoglobulin (Ig) E to immunoglobulin E receptors (FcεRI) on submucosal pulmonary mast cells, resulting in the release of inflammatory mediators. The late phase is characterized by a sustained inflammatory response, and is triggered by cysteinyl leukotriene signaling, prostaglandins, and cytokines produced by CD4 + lymphocytes, interleukin (IL)-4, IL-5, IL-13 [20]. IL-4, for instance, can induce the recruitment and activation of eosino-

phils, stimulate mucus producing cells and perpetuate the release of histamine by mast cells. Additionally, IL-5 is highly specific for cytokine activation and eosinophil recruitment, and IL-13 stimulates airway hyperresponsiveness (AHR) and mucus overproduction [2, 20]. Chemokines initiate a complex signaling cascade that leads to the activation of adhesion molecules and their ligands on the cell surface of leukocytes, and are essential for the adhesion of immune cells to tissues involved in inflammatory processes [6, 20]. However, the initial step that triggers the release of inflammatory mediators in the airways is IgE dependent. IgE plays a central role in the pathogenesis of allergic asthma [6, 8, 20]. IgE mediates the immuno-regulatory effects of dendritic cells by driving T help cells (Th) 2 inflammatory responses. Furthermore, IgE is responsible for the clinical manifestations of asthma in both the early and late phases. In addition, elevated IgE levels are associated with persistent bronchial symptoms and increased AHR [6, 8, 20].

While primary treatments for reducing chronic inflammation include pharmacotherapies such as anti-inflammatory agents, inhaled and oral steroids, and bronchodilators, there is no cure for the disease, leading to the necessity for complementary therapies [13].

Aerobic exercise (AE) training has also been shown to be beneficial in allergic asthma management. AE decreased dyspnea and exercise-induced bronchospasm, reduced lung function decline, decreased the risk of exacerbations, improved aerobic capacity, and improved the allergic asthma patient's quality of life [22, 23, 35].

In this way, the improvement in asthma control with supervised exercise highlights the importance of encouraging exercise in the population, for asthmatic children or adults. Exercise leads to improvements in medication use, and its regular practice can lead to a change in overall asthma control [10, 35].

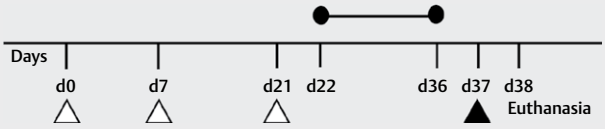
Mouse models of chronic allergic pulmonary inflammation suggest that AE decreases inflammation, lung remodeling, and improves respiratory mechanics by activating an immune-regulatory response in the airway epithelium [38, 42]. This leads to reduced AHR [38, 43], a decrease in the eosinophil count in bronchoalveolar lavage (BAL), a decrease in airway Th2 cytokines, and an increase in IL-10 production [21].

However, despite the increasing use of AE as an important tool in allergic asthma patient programs, the effects of AE during the sensitization process and after initial exposure to the allergen have been poorly explored in animal models.

Several experimental models in mice have shown that AE mitigated an OVA-induced lung inflammation and remodeling. However, in these studies, AE was not able to modify the levels of immunoglobulins, as shown by Pastva et al. (2004) and Silva et al. (2015) [31, 37]. Therefore, the aim of this study was to evaluate the effects of AE performed for 2 weeks following OVA sensitization and before a single allergen aerosol challenge.

2. Materials and Methods

This study was approved by the Review Board for Animal Studies of the Federal University of Santa Catarina under protocol number P00751. All animal care and experimental procedures followed the EU Directive 2010/63/EU for animal experiments [28] and the ethical standards of the International Journal of Sports Medicine [15].



▶ **Fig. 1** Timeline of experimental protocol. OVA and Saline intraperitoneal injections were performed on days 0, 7 and 21 (open triangles). Animals performed aerobic exercise on the treadmill from the 22nd to the 36th day of the experimental protocol. On day 37, we made a single aerosol challenge of OVA solution (1 %) or saline lasting 30 min (closed triangle), and on day 38 the mice were euthanized.

2.1 Animals and experimental groups

Male Swiss mice (30–40 g) from the Animal Facility of Santa Catarina Federal University were maintained in controlled conditions: temperature (22 ± 2 °C), humidity (70–75 %) and a 12 h dark/light cycle. Animals were distributed into 4 groups, as follows: Control (CON): sedentary, non-treated (n = 10); Aerobic Exercise group (AE): submitted to moderate treadmill exercise (n = 10) (see 2.3); OVA (OVA): animals sensitized with 10 µg OVA and exposed to aerosolized OVA 1 % once for 30 min (n = 10) (see 2.2); and finally OVA + Aerobic Exercise (OVA + AE): OVA sensitized, submitted to AE protocol (see 2.3) and exposed once for 30 min to aerosolized OVA (n = 10).

2.2 Induction of acute allergic lung inflammation

We used a modified OVA protocol from Arantes-Costa et al. (2008) with 3 intraperitoneal injections (i.p.) of OVA (10 µg per mouse) (SALTOS™ SP, Brazil) adsorbed with aluminum hydroxide [1]; CON and AE groups received saline injections [21]. OVA or Saline i.p. injections were performed on days 0, 7 and 21. One single aerosol challenge of OVA solution (1 %) or saline was performed for 30 min on day 37, following the exercise protocol (▶ **Fig. 1**).

2.3 Exercise protocol

Aerobic exercise was performed on a treadmill designed for human use (Athletic Advanced 2, Atleticway ®) and modified for mice. Mice ran from day (d) 22 to day 36 under the experimental protocol, for 30 min at a speed of 13.3 m/min (0.8 km/h) with no inclination, as described previously by Fló et al. (2006) [11]. Animals from AE and AE + OVA groups were subjected to 3 days of adaptation (d19–21), which consisted of 5 min of exercise at the same workout speed.

2.4 Anesthesia and euthanasia

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

2.5 Bronchoalveolar lavage fluid (BALF)

Lungs were gently rinsed with 3 instillations of 0.5 mL of phosphate buffered saline (PBS, pH 7.2) via tracheal cannula. Total cell number was counted in a Neubauer's hemocytometer chamber. Differential cell counts of 300 cells/mouse were obtained after Diff-Quik staining of BALF prepared on slides. All measurements were completed blindly [4, 29, 40, 42].

2.7 Analysis of lung total ige and cytokines levels

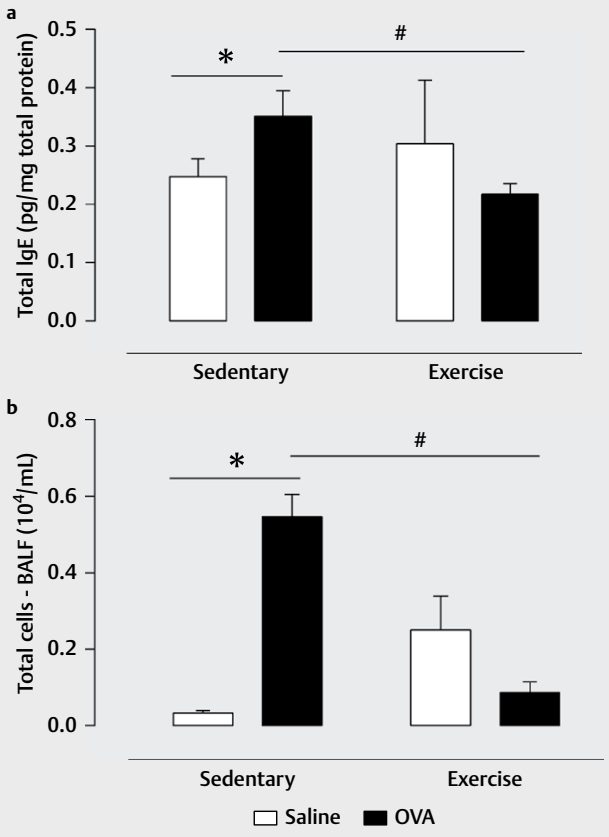
After BALF collection, the chest was opened and the heart-lung block was removed. The left lung was homogenized in a tissue processor (Ultra-Turrax IKA T18 basic, IKA®, Germany) with a PBS solution containing: Tween-20 (0.05 %), phenylmethylsulfonyl fluoride (PMSF) 0.1 mM, ethylenediaminetetraacetic acid (EDTA) 10 mM, Aprotinin 2 ng/ml and benzethonium chloride 0.1 mM. The homogenates were transferred to 1.5 mL Eppendorf tubes, centrifuged at 3 000 × g for 10 min at 4 °C, and the supernatant obtained was stored at − 80 °C until cytokines analysis. Total protein content was measured in the supernatant using the Bradford method. Lung tissue levels of total IgE, IL-4, IL-5, IL10 and IL 1-ra were measured by the ELISA technique, according to manufacturer's instructions (DuoSet ELISA R&D Systems, Minneapolis, MN, USA). The levels of cytokines were estimated by interpolation from a standard curve by colorimetric measurements at 450 nm (correction wavelength 540 nm) in an ELISA plate reader (Berthold Technologies – Apollo 8 – LB 912, KG, Germany). All results were expressed as pg/mg of protein.

2.8 IgG1 and IgE anti OVA antibodies titration

Passive cutaneous anaphylaxis (PCA) was performed as described by Ovary (1964) and modified by Mota and Perini (1970) in Wistar furth rats and BALB/c mice, for anti-OVA IgE and IgG1 measurements, respectively [26, 30]. The backs of the animals were shaved and injected intradermally (i.d.) with different plasma dilutions obtained from mice submitted to the different protocols of immunization. The animals were challenged intravenously with 1 mg of OVA in 0.5 % Evans Blue solution, after a sensitization of 18–24 h in rats, for IgE titration; and 2 h in mice, for IgG1 titration. PCA titer was expressed as the reciprocal of the highest dilution that gave a blue spot greater than 10 mm in diameter in triplicate of test. The detection threshold of the technique was established at 1:5 dilution.

2.9 Western blotting of VEGF (vascular endothelial growth factor) and nitric oxide synthase 2 (NOS-2)

Lung tissues were quickly frozen in liquid nitrogen, homogenized in ice-cold lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM EDTA, 50 mM NaF, 5 % glycerol, 1 % Triton X-100, 0.2 mM Na_3VO_4, 40 mM beta-glycerophosphate containing 10 µg/ml each of aprotinin, leupeptin, soy bean trypsin inhibitor, and 1 mM PMSF), centrifuged at 10 000 × g and the supernatant collected. The bicinchoninic acid assay (BCA) protein kit was used to determine protein concentrations. Protein samples (50 µg/well) were denatured and gel electrophoresis (SDS/PAGE, 7 % gel) was performed in the Mini-PROTEAN® Tetra cell apparatus connected to a PowerPac® HC power supply (both from Bio-Rad, CA, USA). After electrophoresis, proteins were electro-transferred to nitrocellulose membranes (Hybond; Amersham Biosciences, NJ). Membranes were blocked for 2 h at room temperature with 5 % non-fat dry milk (prepared in TBS-T buffer, pH 7.4; concentration in mmol/L: 20 Tris-HCl, 137 NaCl; and 0.1 % Tween 20), and subjected to incubation (overnight, at 4 °C) with primary antibodies against NOS-2 (1:1000, Sigma-Aldrich, St. Louis, MO, USA), anti-VEGF (1:500, Abcam Inc, Cambridge, Massachusetts, USA) or actin (1:35,000, Sigma-Aldrich). Following washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000, Amersham Biosciences, Piscataway, NJ, USA) for 1 h at room temperature. The membranes were exposed to HRP substrate (Thermo Fisher Scientific Inc., Rockford, IL, USA) and immune complexes were visualized by chemiluminescence using Chemidoc MP System (Bio-Rad Laboratories). Bands were quantified by densitometry using the software from the manufacturer.



► **Fig. 2** **a** shows the levels of total IgE. Values are expressed as mean ± SE. * p = 0.026 when Control and OVA groups were compared. #p = 0.019 when OVA and OVA + AE were compared. **b** shows the total number of cells from BALF. Values are expressed as mean ± SE. * p = 0.0001 when Control and OVA groups were compared. # p = 0.002 when OVA and OVA + AE were compared.

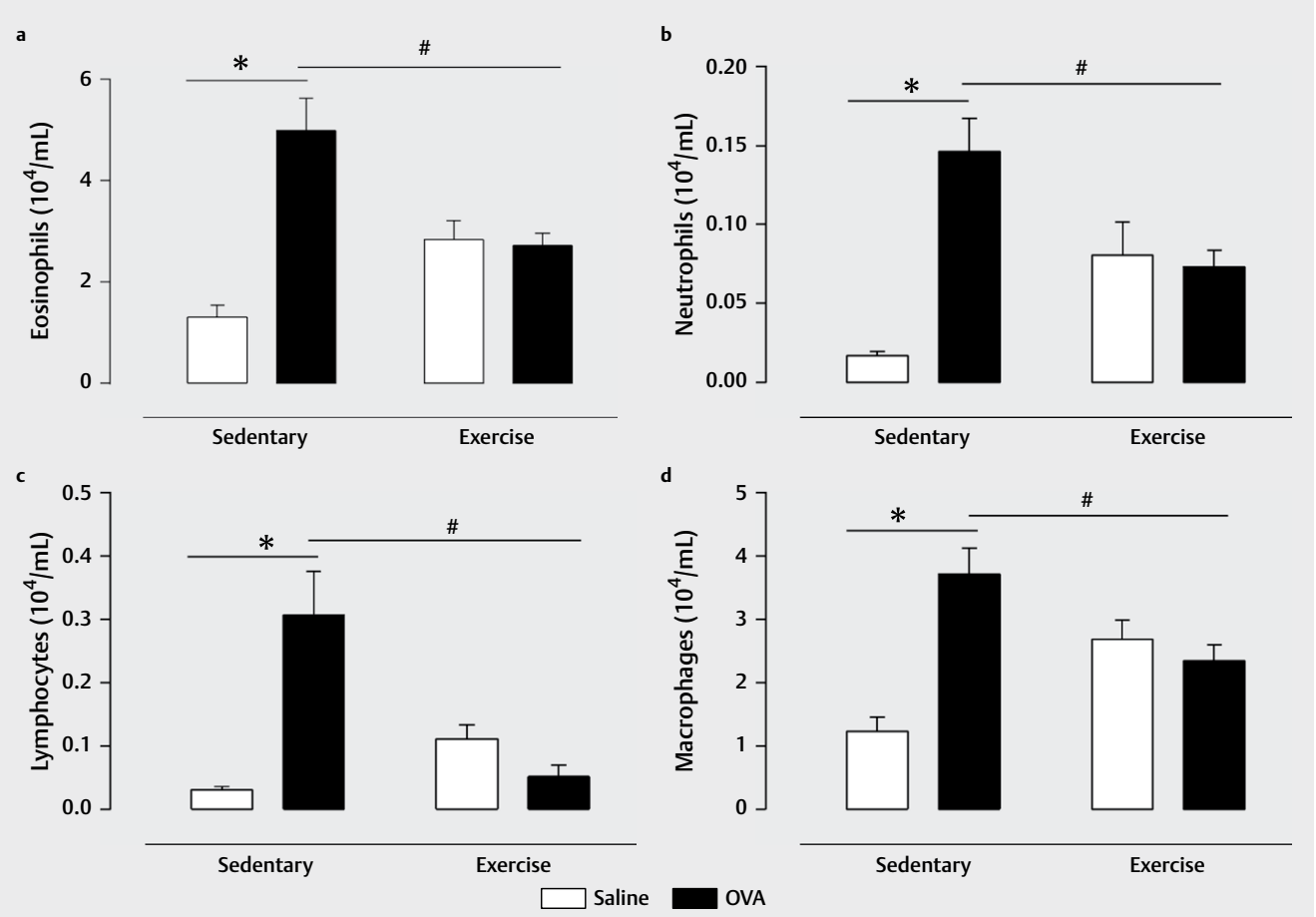
2.10 Statistical analysis

Comparisons among groups were performed using Sigma Stat 3.5 software (California, EUA, 2005) by a 2-way analysis of variance (ANOVA), followed by the Holm-Sidak test for multiple comparisons. Data showed a normal distribution, as analyzed by the Kolmogorov-Smirnov test. Significance levels were set at 5 % ($p < 0.05$). Values were expressed as the mean ± standard error (SE).

3. Results

3.1 Aerobic exercise decreased IgE and IgG1 OVA-specific titers

30 min of aerobic exercise (AE) per day was performed for a period of 14 days, before a single OVA challenge in sensitized mice, which resulted in a decrease in the average titers of OVA-specific IgE (90–30) and IgG1 (340–10) in the OVA + AE groups ($p < 0.05$).



▶ **Fig. 3** Shows the differential count of BALF cells: eosinophils **a**, neutrophils **b**, lymphocytes **c** and macrophages **d**, respectively. Values are expressed as mean ± SE. **a** shows * $p < 0.001$, and # $p < 0.001$; Fig. 3 **b** shows * $p < 0.001$, and # $p = 0.006$; **c** shows * $p < 0.001$, and # $p < 0.001$; and **a** shows * $p < 0.001$, and # $p = 0.01$.

3.2 Aerobic exercise decreased total IgE lung levels in lung homogenate and total cells from BALF

AE decreased total lung homogenate levels of IgE (▶ **Fig. 2a**). A 2-way ANOVA for the total IgE lung levels showed a statistically significant effect for OVA ($F(3,36) = 2.84$; $p = 0.026$).The OVA + AE interaction was also statistically significant ($F(3,36) = 7.47$; $p = 0.019$) (▶ **Fig. 2a**). Additionally, AE also attenuated the OVA-induced increase in the number of total cells from BALF. A 2-way ANOVA for the total cells showed a significant effect for OVA ($F(3,36) = 17.548$; $p = 0.0001$). The OVA x AE interaction ($F(3,36) = 20.088$; $p = 0.002$) was also significant (▶ **Fig. 2b**).

3.3 Aerobic exercise decreased differential cells from BALF

AE attenuated the OVA-induced increase of eosinophils, neutrophils, lymphocytes and macrophages in the OVA group (▶ **Fig. 3a–d**).

A 2-way ANOVA for the eosinophils (▶ **Fig. 3a**) showed a significant effect for OVA ($F(3,36) = 42.148$; $p < 0.001$), and for OVA × AE ($F(3,36) = 69.679$; $p < 0.001$). A 2-way ANOVA for the neutrophils (▶ **Fig. 3b**) showed a significant effect for OVA ($F(3,36) = 16.362$; $p < 0.001$) and for the OVA x AE interaction ($F(3,36) = 20.460$; $p = 0.006$).

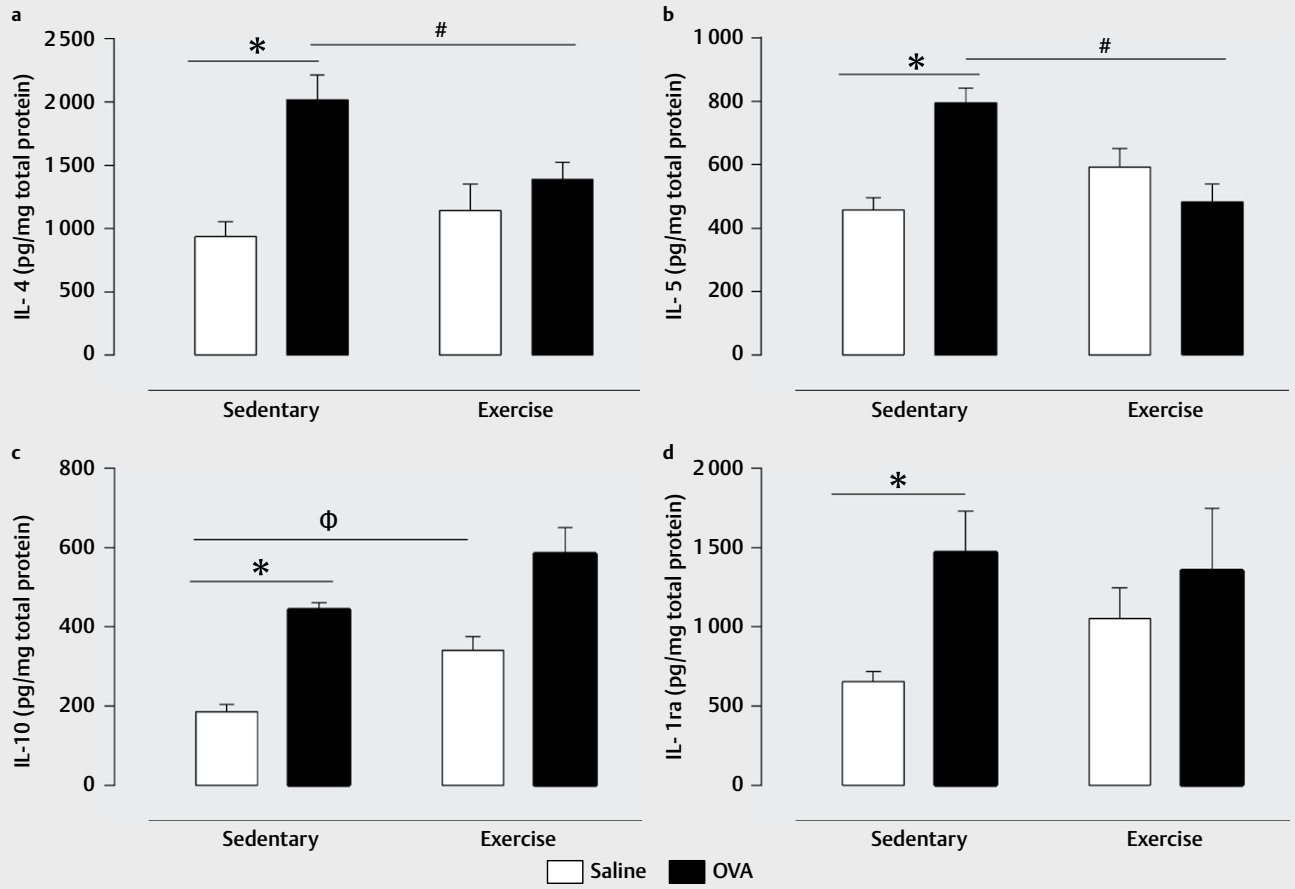
The 2-way ANOVA for the lymphocytes showed a significant effect for OVA ($F(3,36) = 13.064$; $p < 0.001$) and for OVA × AE ($F(3,36) = 20.944$; $p < 0.001$) (▶ **Fig. 3c**). Finally, a 2-way ANOVA for the macrophages also showed a significant effect for OVA ($F(3,36) = 12.162$; $p < 0.001$) and for the OVA x AE interaction ($F(3,36) = 20.901$; $p < 0.01$) (▶ **Fig. 3d**).

3.4 Aerobic exercise decreased the induction of lung pro-inflammatory cytokines after allergen challenge

AE attenuated the OVA-induced increase in lung homogenate levels of IL-4 (▶ **Fig. 4a**) and IL-5 (▶ **Fig. 4b**). A 2-way ANOVA showed a significant effect for OVA for IL-4 ($F(3,36) = 16.319$; $p < 0.001$) and IL-5 ($F(3,36) = 5.117$; $p < 0.001$), as well as for the OVA x AE interaction, ($F(3,36) = 6.52$; $p = 0.0013$) and ($F(3,36) = 19.752$; $p < 0.001$), for IL-4 and IL-5, respectively.

3.5 Effects of aerobic exercise on the lung anti-inflammatory cytokines after allergen challenge

Aerobic exercise resulted in an increase of lung levels of IL-10 in the AE group when compared to sedentary controls ($p = 0.0013$). An ANOVA for IL-10 (▶ **Fig. 4c**) showed a significant effect for AE



▶ **Fig. 4** Shows the total levels of IL-4 **a**, IL-5 **b**, IL-10 **c** and IL-1ra **d**, respectively, from homogenate lung tissue. Values are expressed as mean ± SE. shows * $p < 0.001$, and # $p = 0.013$; shows * $p < 0.001$, and # $p < 0.001$; shows * $p < 0.001$, and $^{\Phi} p = 0.0013$; and Fig. 4d shows * $p = 0.03$.

($F(3,36) = 15.94$; $p = 0.0013$) and OVA ($F(3,36) = 45.64$; $p < 0.001$), but did not show any significant effect for the OVA × AE interaction ($F(3,36) = 0.03$; $p = 0.85$). Additionally, a 2-way ANOVA also showed that there was a significant effect only for OVA ($F(3,36) = 5.41$; $p = 0.03$) in IL-1ra lung levels (▶ **Fig. 4d**), but not for AE ($F(3,36) = 0.35$; $p = 0.56$) or the OVA × AE interaction ($F(3,36) = 1.14$; $p = 0.3$).

3.6 Aerobic exercise did not change lung levels of VEGF and blunted NOS-2 increase after allergen challenge

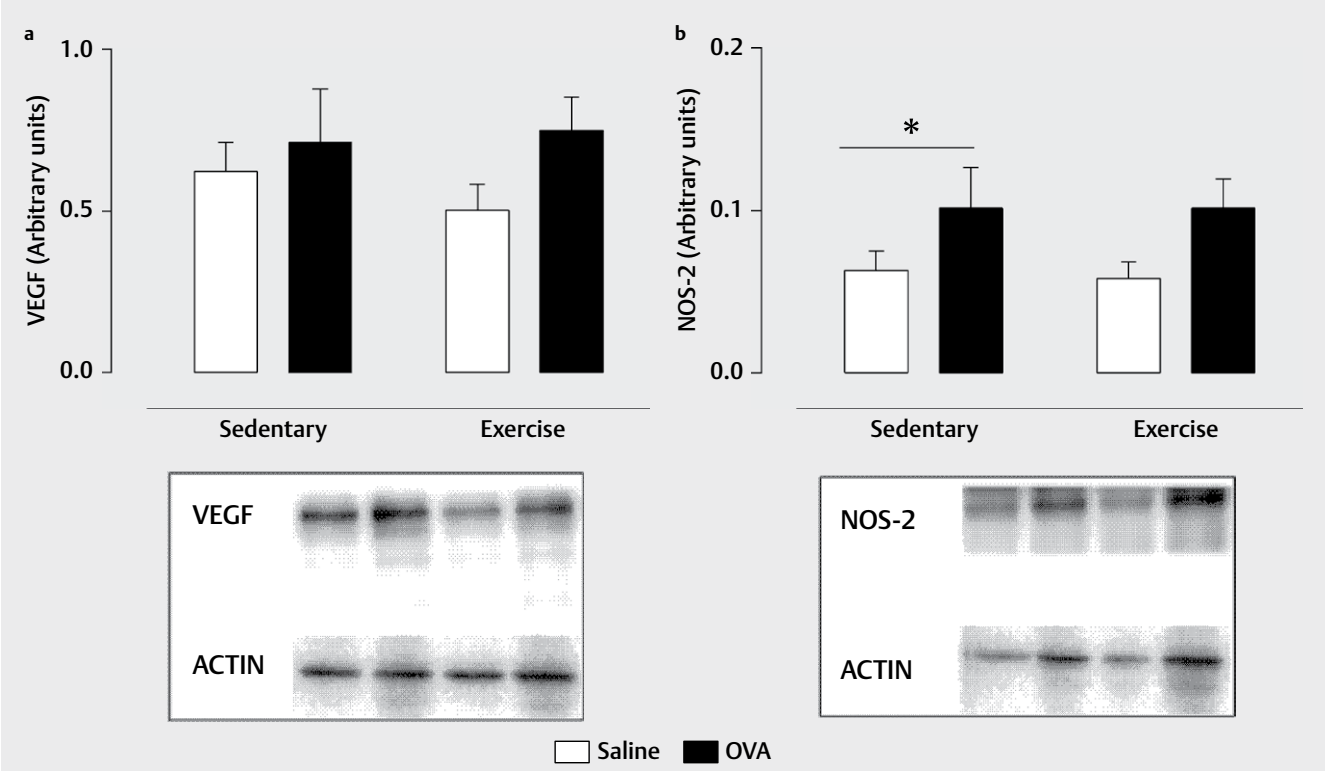
In this experimental model, AE did not result in significant changes in lung levels of VEGF (▶ **Fig. 5a**) ($p > 0.05$). A 2-way ANOVA showed that there was not any significant effect for OVA ($F(3,36) = 2.182$; $p = 0.155$), AE ($F(3,36) = 0.134$; $p = 0.718$) or OVA × AE interaction for VEGF ($F(3,36) = 0.472$; $p = 0.50$).

However, OVA-sensitized and single challenged mice showed an increase in iNOS-2 expression in lung tissue ($p = 0.02$). A 2-way ANOVA for iNOS-2 expression (▶ **Fig. 5b**) showed a significant effect for OVA ($F(3,36) = 5.655$; $p = 0.02$), but not for the OVA + AE effect ($F(3,36) = 0.02$; $p = 0.88$).

4. Discussion

Using an OVA model of low to moderate acute inflammatory asthma, this study demonstrated that aerobic exercise (AE), performed for 2 weeks at moderate intensity following OVA sensitization and before aerosol antigen challenge, reduced the number of total cells, lymphocytes, macrophages, eosinophils and neutrophils in the BALF of OVA + AE animals compared to OVA (sedentary) animals. Total lung levels of IgE as well as OVA-specific IgE and IgG1 serum titers were also reduced. Furthermore, significant decreases in IL-4 and IL-5 cytokines were observed. Like the AE-only group, OVA groups also showed an increase in IL-10 and IL-1ra lung levels, though this effect was cumulative for IL-10 production in the AE + OVA group. There was also a significant increase of NOS-2 post challenge in sedentary mice, contrasting with a non-significant change in the AE group. In summary, these results suggested that AE attenuated the primary phases of acute allergic lung inflammation by modulating both lymphocyte and humoral immune responses before being subjected to a single allergic challenge.

The inflammatory process characteristic of allergic asthma consists of an increase in vascular permeability and exudate, as well as an influx of mast cells, macrophages, neutrophils, lymphocytes (es-



▶ **Fig. 5** Shows VEGF and NOS-2 expression in lung tissues. Panel **a** VEGF densitometry and representative immunoelectrophoresis. Panel **b** NOS-2 densitometry and representative immunoelectrophoresis. Bars represent the mean ± SE. shows $p > 0.05$ and shows * $p = 0.02$.

pecially CD4 + subtype) and eosinophils into extravascular compartments, a mucus content increase, and bronchial epithelium surface desquamation [2, 6, 8]. Though mice do not develop asthma as humans do, and experimental models have intrinsic limitations, animal models of allergic lung inflammation constitute acceptable alternatives for investigating mechanisms and disease progression, in order to better understand disease pathogenesis and to evaluate therapeutic interventions [9, 19, 31, 38, 42, 45].

As described in Lucks et al. (2013), the differences in the ability of AE to attenuate IgE production in experimental models of allergic asthma, are likely attributable to the differences in the timing of the implementation of the AE protocol and the level of inflammation induced by the model [21]. Vieira et al. (2011) submitted mice to AE during the establishment of allergic lung inflammation while the studies of Silva et al. (2010) and Pastva et al. (2004) submitted mice to AE protocols following the completion of an allergic asthma protocol [31, 38, 45]. Interestingly, only Pastva et al. (2004) showed a decrease in OVA-induced IgE levels in the lung, and no perceptible effect was observed on IgE titers [31].

In contrast to previous studies, the aim of the present study was to investigate whether implementing AE before the first antigen lung challenge would attenuate inflammation, particularly IgE production. The results from this study still corroborate, in part, those shown by Vieira et al. (2007), Silva et al. (2010) and Pastva et al. (2004), since AE resulted in a significant decrease of eosinophils, neutrophils, lymphocytes and macrophages from BALF, as well as decreases in IL-4 and IL-5 levels, and increases in anti-inflammatory IL-10 [31, 38, 43]. However, unlike the previous studies, IgE and IgG_1 titers decreased in OVA + AE group as well as total lung IgE. These results may be explained by the ability of AE to attenuate macrophage activation and antigen presentation to B lymphocytes [37]. Besides antigen presentation modulation, the improvements promoted by AE in asthma are attributed to the increase in anti-inflammatory cytokines. In fact, in our experimental OVA-model, the AE blunted the induction of lung-pro-inflammatory cytokines by the allergen challenge, but we did not observe for the anti-inflammatory cytokines, since they already had a higher basal level of these in the lung.

The role of nitric oxide (NO) in clinical studies of asthma as well as in animal models of allergic asthma, are well described [31–34, 44]. Asthmatic patients show increased levels of NO in exhaled air and increased expression of iNOS2 in the bronchial epithelium [14, 17]. iNOS2 is considered the most important source of NO as it is capable of producing NO in a very efficient way compared to other NOS isoforms. iNOS is expressed by macrophages, neutrophils, and airway epithelium [16]. Significant amounts of NO secreted by activated macrophages and neutrophils can lead to surrounding tissue damage and to increasing inflammatory cell influx into airspaces [12, 16]. Mendes et al. (2011), evaluated the effects of an AE program on eosinophilic inflammation and NO released in the breath of patients with moderate or severe persistent asthma [22]. Following 3 months of training, eosinophil counts in sputum were reduced, a decrease in the fraction of exhaled nitric oxide (FeNO) was observed, and the number of asthma symptom-free days increased. Though an increase in iNOS2 expression in OVA-challenged mice was observed, AE did not decrease iNOS2 ex-

pression after OVA challenged. In contrast to the study by Mendes et al. (2011), the results in this study corroborate results shown by Moreira et al. (2008) [22, 24]. While AE performed by asthmatic children did not change the FeNO concentration in exhaled breath, a reduction in total IgE levels and house dust mite (HDM)-specific IgE levels was observed. In addition, Bonsignore et al. (2008), also evaluated the levels of FeNO in 50 children with mild persistent asthma and did not observe a reduction in FeNO after a protocol of AE [7]. The differences in these studies were attributed to the differences in the severity of asthma, as improvements due to AE were more observed to a greater extent in patients with chronic and well-established inflammation [20].

Another inflammatory inducer in asthma pathogenesis is VEGF, a growth factor involved in lung remodeling, epithelial cell survival, and the regulation of vascular permeability [39]. VEGF is thought to be responsible for increasing vascular permeability to an even greater extent than histamine, by inducing fenestration of endothelial cells, in vivo and in vitro [3, 48]. VEGF also enhances Th2-mediated sensitization and inflammation [18]. Bronchial biopsies from patients with severe and moderate asthma also showed an increase in the number of blood vessels expressing the intercellular adhesion molecule (ICAM)-1, which induces the migration of inflammatory cells to tissue, along with VEGF activity [5, 46]. In contrast to this study, animal models of repeated allergen exposure to OVA or house dust mite (HDM), showed higher VEGF expression in lung tissue, which was associated with increased vascular remodeling and inflammatory cell influx [36, 41]. Given the relatively low level of inflammation induced by the OVA model used in this study compared to other models, VEGF expression was only slightly increased [27].

While managing the inflammatory components of allergic asthma is essentially pharmacologic, AE may serve as a complementary therapy, as results demonstrate increases in ventilation and respiratory capacities, an improvement in the patient's quality of life and decreased asthma symptoms such as dyspnea [22, 23, 25, 31].

Our results suggest that AE performed during the sensitization process can attenuate the early phases of acute allergic lung inflammation by modulating the Th2 response, decreasing specific IgE and IgG1 levels, reducing inflammatory cell influx into lungs, and attenuating the release of pro-inflammatory cytokines. Although we observed a decrease in levels of IgE and IgG1 24 h after a single allergen challenge, we assume the timeframe as a limitation of our study. There is a need to study the IgE and IgG1 levels for longer periods after the last antigen challenge, in order to investigate the duration of the inhibitory effects of aerobic exercise observed in our study.

These findings may indicate that aerobic exercise might be especially advantageous in allergic patients and that, once performed during allergen sensitization, it has a preventive role in the progress of allergic inflammation.

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Conflict of interest

The authors have no conflict of interest to declare.

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