Regular and moderate aerobic training before allergic asthma induction reduces lung inflammation and remodeling

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Experimental studies have reported that aerobic exercise after asthma induction reduces lung inflammation and remodeling. Nevertheless, no experimental study has analyzed whether regular/moderate aerobic training before the induction of allergic asthma may prevent these inflammatory and remodeling processes. For this purpose, BALB/c mice (n = 96) were assigned into non-trained and trained groups. Trained animals ran on a motorized treadmill at moderate intensity, 30 min/day, 3 times/week, for 8 weeks, and were further randomized into subgroups to undergo ovalbumin sensitization and challenge or receive saline using the same protocol. Aerobic training continued until the last challenge. Twenty-four hours after challenge, compared to non-trained animals, trained mice exhibited: (a) increased systolic output and left ventricular mass on echocardiography; (b) improved lung mechanics; (c) decreased smooth muscle actin expression and collagen fiber content in airways and lung parenchyma; (d) decreased transforming growth factor (TGF)-β levels in bronchoalveolar lavage fluid (BALF) and blood; (e) increased interferon (IFN)-γ in BALF and interleukin (IL)-10 in blood; and (f) decreased IL-4 and IL-13 in BALF. In conclusion, regular/moderate aerobic training prior to allergic asthma induction reduced inflammation and remodeling, perhaps through increased IL-10 and IFN-γ in tandem with decreased Th2 cytokines.

Asthma is characterized by airway obstruction, chronic inflammation, and remodeling (Global Initiative for Asthma (GINA), 2014) and is caused by unregulated production of cytokines secreted by allergen-specific type 2 T-helper (Th2) cells (Holloway et al., 2010). The incidence of asthma remains high, with an estimated 300 million people affected, resulting in high costs to society worldwide (Asher & Pearce, 2014). Different therapeutic strategies can mitigate inflammation and delay the development of airway histological and ultrastructural changes that characterize the remodeling process in asthma (Vanacker et al., 2001; Wang et al., 2011). However, to date, no therapy is effectively able to prevent the remodeling process.

Regular and moderate exercise exerts protective effects against many pathological conditions, including chronic lung disease, due to its anti-inflammatory effects (Petersen & Pedersen, 2005; Mussi et al., 2008; Loprinzi & Davis, 2015). Several experimental studies have reported that aerobic exercise after asthma induction reduces lung inflammation (Loverder et al., 2010; Silva et al., 2015a,b) and remodeling (Pastva et al., 2004; Vieira et al., 2007; Silva et al., 2010; Dugger et al., 2013), due to an increase in Th1 response and a subsequent suppression in Th2 cytokine levels (Lakier Smith, 2003).

Although the beneficial effects of aerobic exercise on asthma have been described, to date, no experimental study has analyzed whether regular and moderate aerobic training before the induction of allergic asthma may prevent and/or mitigate the inflammatory and remodeling processes. Therefore, in the present study, we tested the hypothesis that prior regular and moderate aerobic training might prevent airway inflammation and remodeling, thus...
improving lung mechanics, in a murine model of ovalbumin (OVA)-challenged allergic asthma.

Methods

This study was approved by the Health Sciences Centre Ethics Committee at the Federal University of Rio de Janeiro, Brazil (CEUA-CCS-019). All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the U.S. National Academy of Sciences.

Animal preparation and experimental protocol

Ninety-six male BALB/c mice (weighing 25–30 g) were kept under specific pathogen-free conditions in the animal care facility at the Laboratory of Pulmonary Investigation, Federal University of Rio de Janeiro. Of these, 48 (n = 12/group) were used to evaluate lung mechanics and histology, as well as echocardiographic parameters. Twenty animals (n = 5/group) were subjected to the same protocol described above to obtain aliquots of bronchoalveolar lavage fluid (BALF) and plasma for cytokine analysis. The remaining 28 animals (n = 7/group) were used to assess airway hyper-responsiveness.

All animals were randomly assigned to one of two groups: non-trained (NT) or trained (T). In the T group, aerobic training was performed on a motorized treadmill (Insight, Brazil) with lanes for the mice to run in individualized compartments. Mice were trained 30 min per day, three times a week, at a speed of 8–12 m/min, and a 5% grade (de Araújo et al., 2012) which corresponded to approximately 65–70% of their maximal oxygen uptake (VO2max) (Lu et al., 1999; Lowder et al., 2005). Mice ran without electric shock or prodding. Non-trained animals were treated identically to the T group, except for treadmill running, in order to eliminate the confounding effects of handling stress on lung and systemic data. Upon completion of the 8 weeks of exercise training or non-training, mice from each group were divided into two groups.

In the OVA group, mice were sensitized using an adjuvant-free protocol by intraperitoneal injection of sterile ovalbumin (10-µg OVA in 100-µL vehicle) on seven alternate days. Forty days after the start of sensitization, 20-µg OVA in 20-µL saline was instilled intratracheally. This procedure was performed three times with 3-day intervals between applications (Xisto et al., 2005). The control group (SAL) received saline instead of OVA during both sensitization and challenge (Fig. 1). Aerobic training continued until the last challenge. All efficacy data were collected and analyzed 24 h after the last challenge, as described in the following sections.

Echocardiography

The beneficial effects of regular and moderate aerobic training on the cardiovascular system are well documented (Arbab-Zadeh et al., 2014; Fernandes et al., 2015); however, whether these cardiac benefits remain after asthma induction requires investigation. Therefore, to evaluate cardiac adaptation to aerobic training before and after asthma induction, echocardiography (using a 30 MHz mechanical transducer, Visual Sonics, Toronto, Ontario, Canada) was performed before the training protocol, at 4 and 8 weeks, and at 1 and 47 days of the asthma protocol. After induction of anesthesia with 1.5–2.0% isoflurane by mask, the chest was shaved and the animal placed in the supine position. Images were obtained from parasternal views. Systolic volume and ejection fraction were calculated from long-axis B-mode tracings according to Simpson’s method. The measurements were obtained according to the American Society of Echocardiography Guidelines (Chetlin et al., 2003).

Airway responsiveness

Twenty-four hours after the last challenge, airway responsiveness was measured. One dose of saline (baseline) was administered, followed by increasing doses of methacholine (Sigma Chemical Co., Saint Louis, Missouri, USA) (0, 3, 10, 30, 100, 300, and 1000 µg/kg) via a Silastic cannula placed into the jugular vein. Data were recorded 30 s, 1 min, 3 min, and 5 min after agonist injection. Shortly after each i.v. infusion of methacholine, the maximal increase in P0.1 was reached, and the respective airflow was measured at this moment (Antunes et al., 2009). Respiratory system resistance (R) was obtained using the equation of motion of the respiratory system: P0.1(t) = E · V(t) + R · V′(t), where (t) is time.

Mechanical parameters

Twenty-four hours after the last intratracheal challenge with saline or OVA, animals were sedated (diazepam 1 mg i.p.), anesthetized (thiopental sodium 20 mg/kg i.p.), tracheotomized, paralyzed (vecuronium bromide, 0.005 mg/kg i.v.), and ventilated mechanically with a constant flow ventilator (Samay VR15; Universidad de la Republica, Montevideo, Uruguay) set to the following parameters: respiratory rate 100 bpm, tidal volume (VT) 0.2 mL, and fraction of inspired oxygen (FIO2) 0.2 L. The anterior chest wall was surgically removed and a positive end-expiratory pressure of 2 cm H2O applied. Airflow and tracheal pressure (Ptr) were measured (Burburan et al., 2007). Lung mechanics were analyzed by the end-inflation occlusion method (Bates et al., 1988). In an open chest preparation, Ptr reflects transpulmonary pressure (PL). Briefly, after end-inspiratory occlusion, there is an initial rapid decline in PL (ΔP1) from the pre-occlusion value down to an
inflection point (Pi), followed by a slow pressure decay (AP2), until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the lung (Pel). AP1 selectively reflects the pressure used to overcome airway resistance. AP2 reproduces the pressure spent by stress relaxation, or the viscoelastic properties of the lung, together with a small contribution of pendelluft. Static lung elastance (Est, L) was determined by dividing Pel by V T. Lung mechanics measurements were performed 10 times in each animal. All data were analyzed using ANADAT data analysis software (RHT-InfoData, Inc., Montreal, Quebec, Canada). Laparotomy was performed immediately after determination of lung mechanics and heparin (1000 IU) was injected into the vena cava. The trachea was clamped at end-expiration and the abdominal aorta and vena cava were sectioned, producing massive hemorrhage and rapid terminal bleeding.

Lung histology

The right lung was removed, fixed in 4% buffered formalin, paraffin-embedded, and sliced into 4-µm-thick sections, which were stained with hematoxylin and eosin (Vetec Quimica Fina, Rio de Janeiro, Brazil). Fraction areas of collapsed and normal lung were determined by the point-counting technique at a magnification of 200× across 10 random, non-coincident microscopic fields (Hsia et al., 2010). Points falling on collapsed or normal pulmonary areas were counted and divided by the total number of points in each microscopic field. Poly-morphonuclear (PMN) and mononuclear (MN) cells and lung tissue were evaluated at 1000× magnification. Points falling on PMN and MN cells were counted and divided by the total number of points falling on lung tissue in each microscopic field (Xisto et al., 2005; Burburan et al., 2007). Histologic sections were stained for eosinophils and neutrophils with Llewellyn’s Sirius Red (Direct Red 80, CI 35780; Aldrich, Milwaukee, Wisconsin, USA) (Serra et al., 2012). Eosinophil and neutrophil infiltrates were evaluated around the airway as well as between the bronchial epithelium and adventitia through an integrating eyepiece (10⁴ per µm² total area). Determinations were made in four randomly selected fields at a magnification of ×1000 and expressed as eosinophils and neutrophils/unit area (µm²).

The airway contraction index was determined by counting the points falling on the airway lumen and those falling on airway smooth muscle and on the epithelium, at a magnification of 400×. The number of intercepts (NI) of the lines with the epithelial basal membrane is proportional to the airway perimeter, and the number of points (NP) falling on the airway lumen is proportional to airway area; thus, the magnitude of bronchoconstriction was computed as CI = NI/NP⁴. Measurements were performed in five airways from each animal at 400× magnification.

Collagen fibers (stained by the Picrosirius-polarization method) (Montes, 1996) were quantified in alveolar septa and airways with the aid of a digital analysis system and specific software (Image-Pro® Plus 5.1 for Windows® Media Cybernetics – Silver Spring, Maryland, USA) under 200× magnification. The area occupied by fibers was determined by digital densitometric recognition. To avoid any bias due to alveolar collapse, the areas occupied by collagen fibers in each alveolar septum were divided by the total area. The results were expressed as the percentage of collagen fiber content per tissue area (%). Collagen fiber content was quantified in the whole circumference of the two largest, transversally cut airways present in the sections. Results were expressed as the area of collagen fibers divided by the perimeter of the basement membrane (µm²/µm).

Immunohistochemistry

Strips of right lung tissue (2 × 2 × 10 mm) were fixed in 4% paraformaldehyde and embedded in paraffin for immunohistochemistry using a monoclonal antibody against α-smooth muscle actin (Dako, Carpinteria, California, USA) at a 1:500 dilution. Sections were then rinsed with Tris-buffered saline and sequentially incubated with biotinylated rabbit anti-mouse IgG (Dako, Cambridge, UK) at a dilution of 1:400, followed by streptavidin combined in vitro with biotinylated horseradish peroxidase at a dilution of 1:1000 (Dako). The reaction product was developed with 3,3′-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin for 1 min, dehydrated through a graded alcohol series, and mounted in resinous medium. Known positive controls were included with each run, and negative controls had the primary antibody omitted (Dolnikoff et al., 1998). Analysis was performed by means of the point-counting technique (Hsia et al., 2010) on slides stained for α-smooth muscle actin. Using a 121-point grid, we calculated the volume proportion of smooth muscle-specific actin in terminal bronchioles and alveolar ducts as the ratio between the number of points falling on actin-stained and non-stained tissue. Measurements were obtained at 400× magnification on each slide.

Transmission electron microscopy

Slices (2 × 2×2 mm) were obtained from three different segments of the left lung and fixed in 2.5% glutaraldehyde and phosphate buffer 0.1 M (pH 7.4) for electron microscopy analysis (JEOL 1010 Transmission Electron Microscope, Tokyo, Japan). In each micrograph (n = 12/animal), the following structural changes were analyzed: (a) basement membrane; (b) collagen deposition; (c) smooth muscle cells; and (d) organellar changes (Antunes et al., 2010). The pathologic findings were graded on a five-point, semi-quantitative, severity-based scoring system as follows: 0 = normal lung parenchyma, 1 = changes in 1–25% of examined tissue, 2 = changes in 25–50% of examined tissue, 3 = changes in 51–75% of examined tissue, and 4 = changes in 76–100% of examined tissue.

Bronchoalveolar lavage fluids and blood

Interleukin (IL)-4, IL-10, IL-13, interferon (IFN)-γ, and transforming growth factor (TGF)-β were quantitated in BALF and blood by ELISA, according to the manufacturer’s instructions (Duo Set, R&D Systems, Minneapolis, Minnesota, USA).

Statistical analysis

The normality of data (Kolmogorov–Smirnov test with Lilliefors’ correction) and the homogeneity of variances (Levene’s median test) were tested. If both conditions were satisfied, two-way ANOVA followed by Tukey’s post-hoc test was used to compare differences among the groups. Non-parametric data were analyzed using two-way ANOVA on ranks followed by Tukey’s test, and two-way ANOVA for repeated measures followed by Tukey’s test was used to compare echocardiographic findings. Parametric data were expressed as mean ± standard deviation, while non-parametric data were expressed as median (interquartile range). All tests were performed in PASW Statistics for Windows® version 18.0 (SPSS Inc., Chicago, Illinois, USA). Statistical significance was established as P < 0.05.
Results

Regular and moderate aerobic training induced cardiac adaptations, as demonstrated by significant increases in ejection fraction and systolic volume over time (Fig. 2a, b Weeks 4 and 8). The allergic response was unable to modify the ejection fraction in non-trained animals; however, there was a significant reduction of ejection fraction in OVA-challenged trained animals (Fig. 2a). Systolic volumes increased during the course of the experiment, but were not modified by OVA challenge (Fig. 2b).

When challenged with OVA, non-trained animals exhibited a marked increase in all lung mechanics parameters analyzed (Est,L, ΔP1, ΔP2). Meanwhile, exposure to the aerobic training regimen inhibited antigen-induced modification in Est,L (~70%), ΔP1 (~60%), and ΔP2 (~70%) when compared to non-trained animals (Fig. 3a and b). Furthermore, non-trained, sensitized mice, when challenged with OVA (NT-OVA), were hyper-responsive to subsequent methacholine injection compared to NT-SAL animals. Aerobic training completely abrogated this hyper-responsiveness, inasmuch as the T-OVA group was not statistically different from SAL-challenged groups (Fig. 4).

Lung morphometry analysis confirmed previous results from our group (Antunes et al., 2009, 2010;
suggesting that this asthma model elicits a marked increase in the fraction area of alveolar collapse in antigen-challenged animals (Fig. 5). Aerobic training reduced alveolar collapse by approximately 80% in T-OVA compared to NT-OVA animals (Fig. 5). Additionally, NT-OVA animals manifested a larger contraction index (smaller mean alveolar and central airway diameters) when compared with T-OVA (Fig. 6a and b). NT-OVA mice also exhibited greater collagen fiber content in the airways and alveolar septa (Fig. 7a and b) and a greater volume proportion of smooth muscle-specific actin in terminal bronchioles and alveolar ducts (Fig. 8a and b) compared with T-OVA.

In the NT-OVA group, electron microscopy revealed several ultrastructural changes in terminal bronchioles that are characteristic of asthma: smooth muscle hypertrophy, eosinophil infiltration, collagen deposition, basement membrane thickening, mucous cell hyperplasia, and organelle abnormalities. These ultrastructural changes were minimized by pre-treatment with regular and moderate aerobic training (Table 1 and Fig. 9).

In addition, antigen-induced accumulation of PMN cells was completely abrogated in T-OVA group when compared to NT-OVA (Fig. 10a). Peribronchial eosinophil and neutrophil infiltration was higher in NT-OVA compared to NT-SAL animals (Fig. 10b). Regular and moderate aerobic training markedly reduced eosinophil infiltration, with no significant reduction in the number of neutrophils (Fig. 10b).

Production of cytokines involved in modulation of the inflammatory process was analyzed in BALF and plasma (Figs 11 and 12). There was a marked reduction in IL-4 and IL-13 levels in T-OVA when compared to NT-OVA animals. IFN-γ levels increased in the BALF of trained animals after OVA challenge (T-OVA), while the reduction in IL-10 levels observed after OVA challenge in non-trained animals was markedly inhibited in trained animals, indicating a modification in the allergic immune response profile. OVA challenge also induced an increase in TGF-β levels that was markedly inhibited in T-OVA, reinforcing the potential role of aerobic

Prior exercise reduces lung inflammation

Fig. 4. Airway hyper-responsiveness induced by increasing methacholine doses (0, 3, 10, 30, 100, 300, 1000 mg/mL). NT, non-trained; T, trained; OVA, mice sensitized and challenged with ovalbumin; SAL, mice given saline in a protocol identical to OVA sensitization and challenge. Values are mean (±SD) of 7 animals in each group (P < 0.05). *Significantly different from NT-SAL (P < 0.05). **Significantly different from NT-OVA (P < 0.05).

Fig. 5. Lung morphometry. (a) Fraction area of normal and collapsed alveoli. All values were computed in 19 random, non-coincident microscopic fields per animal. Values are mean (±SD) of 12 animals in each group. NT, non-trained; T, trained; OVA, mice sensitized and challenged with ovalbumin; SAL, mice given saline in a protocol identical to OVA sensitization and challenge. *Significantly different from NT-SAL (P < 0.05). **Significantly different from NT-OVA (P < 0.05). #Significantly different from T-SAL (P < 0.05). (b) Representative photomicrographs of lung parenchyma stained with hematoxylin and eosin. Original magnification: 200×.
training in the remodeling process. The same overall profile of modification of the allergic immune response by aerobic training was observed in the analysis of circulating cytokine levels. There were two notable exceptions: no significant IFN-γ production could be detected after OVA challenge in either group; and aerobic training had no impact on the allergen-induced increase in plasma levels of IL-13.

**Discussion**

The present study demonstrated that regular, moderate aerobic training prior to the induction of experimental allergic asthma prevented airway and lung parenchyma remodeling, as demonstrated by reductions both in collagen fiber content and in percentage of smooth muscle-specific actin in terminal bronchi-oles and alveolar ducts, as well as reductions in smooth cell hypertrophy and mechanical stress on organelles. Prevention of ultrastructural changes by prior aerobic training resulted in improved pulmonary function when compared to no training, as assessed by lung mechanics and airway hyperresponsiveness. Furthermore, aerobic training acted on the balance between prototypical Th1 and Th2 cytokines, increasing IFN-γ and decreasing IL-4 and IL-13 levels in BALF.

The benefits of aerobic training in asthma inflammation and remodeling have been studied (Pastva et al., 2004; Vieira et al., 2007, 2014; Lowder et al., 2010; Olivo et al., 2012); however, in most studies, the training protocol was initiated simultaneously with sensitization (Pastva et al., 2004; Vieira et al., 2007, 2014), which does not mimic what occurs in...
asthmatic patients (Wenzel & Holgate, 2006). To the best of our knowledge, this is the first study to report the efficacy of prior regular and moderate aerobic training in attenuating the inflammatory and remodeling processes in experimental allergic asthma.

BALB/c mice were chosen because of their greater ability to develop airway hyper-responsiveness after chronic sensitization and challenge with ovalbumin (Xisto et al., 2005; Antunes et al., 2009). The model of chronic allergic inflammation used in the present study was previously described and demonstrated to reproduce some aspects of human chronic asthma, such as airway hyper-responsiveness, eosinophilia, increased basement membrane thickness, mucous cell hyperplasia, and smooth muscle hypertrophy (Xisto et al., 2005; Burburan et al., 2007; Antunes et al., 2009). This model was chosen because of its greater ability to develop airway hyper-responsiveness after chronic sensitization and challenge with ovalbumin (Antunes et al., 2009). The model of chronic allergic inflammation used in the present study was previously described and demonstrated to reproduce some aspects of human chronic asthma, such as airway hyper-responsiveness, eosinophilia, increased basement membrane thickness, mucous cell hyperplasia, and smooth muscle hypertrophy (Xisto et al., 2005; Burburan et al., 2007; Antunes et al., 2009). This model was chosen because of its greater ability to develop airway hyper-responsiveness after chronic sensitization and challenge with ovalbumin (Antunes et al., 2009).

**Table 1. Semi-quantitative analysis of electron microscopy**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basement membrane thickening</th>
<th>Collagen deposition</th>
<th>Eosinophil infiltration</th>
<th>Smooth muscle hypertrophy</th>
<th>Abnormal mitochondria</th>
<th>Enlarged endoplasmic reticulum</th>
<th>Mucous cell hyperplasia</th>
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<tr>
<td>T</td>
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<td>4 (2.75–4)*</td>
<td>3 (2.75–3.25)*</td>
<td>4 (3–4)*</td>
<td>3 (3–4)*</td>
<td>4 (3–4)*</td>
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<tr>
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<td>2 (1.75–2.25)***,#</td>
<td>2 (1.75–2.25)***,#</td>
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Values are median (interquartile range) of five animals per group. Pathologic findings were graded on a five-point, semi-quantitative, severity-based scoring system: 0 = normal lung parenchyma, 1 = changes in 1–25% of examined tissue, 2 = 26–50% of examined tissue, 3 = 51–75% of examined tissue, and 4 = 76 to 100% of the tissue.

*Significantly different from NT-SAL (P < 0.05).
**Significantly different from NT-OVA (P < 0.05).
#Significantly different from T-SAL (P < 0.05).

![Fig. 8. (a) Smooth muscle-specific actin in the terminal bronchioles and alveolar ducts. Values are mean (±SD) of 12 animals in each group. NT, non-trained; T, trained; OVA, mice sensitized and challenged with ovalbumin; SAL, mice given saline in a protocol identical to OVA sensitization and challenge. *Significantly different from NT-SAL (P < 0.05). **Significantly different from NT-OVA (P < 0.05). Original magnification: 400×. Bars = 100 μm.](image)
**Fig. 9.** Electron micrographs of ultrastructural changes in the airway. NT, non-trained; T, trained; OVA, mice sensitized and challenged with ovalbumin; SAL, mice given saline in a protocol identical to OVA sensitization and challenge. (a, b) hypertrophy of smooth muscle cells (SM) is visible in continuity with the basement membrane (BM) in bronchioles from NT-SAL animals. (c, d) Small mitochondria (MIT) and endoplasmic reticulum (ER) cisternae (arrows). (e) Bronchiole from a T-SAL animal, lined by mucous (MUC) and ciliated (CIL) cells. (f) Resting over slightly irregular BM in continuity with SM. (g, h) At high magnification, SM cells exhibit small MIT with discrete cristae concentrated near the nucleus. (i, j) Besides BM changes and collagen (COL) deposition, a bronchiole from an NT-OVA animal displays prominent SM hypertrophy, reflecting the effect of induced mechanical stress on organelles. (k, l) Note the prominence of MIT, irregular mitochondrial cristae, and dilated ER cisternae. (m, n) ER cisternae are regular and ribosomes evident (arrows). Bronchiole from a T-OVA animal still displays prominent BM merged with COL deposition. (o, p) asterisks: MIT are concentrated at the cytoplasm periphery and exhibit irregular cristae. (o, p) arrows: Note regular and narrow ER cisternae and poorly loaded ribosomes. Arrows indicate ER cisternae. Asterisks indicate MIT cristae. Photomicrographs are representative of data obtained from lung sections derived from 12 animals.
et al., 2010). Additionally, the model used also promoted a stereotypical Th2 cytokine profile, with increases in cytokines related to airway and lung parenchyma inflammation and remodeling, such as IL-4 and IL-13 and the growth factor TGF-β (Xisto et al., 2005; Hamid & Tulic, 2009; Antunes et al., 2010).

In this study, animals in the trained group were subjected to 30-min sessions of motorized treadmill running, at a speed of 8–12 m/min and 5% grade, for 8 weeks (de Araújo et al., 2012) before induction of allergic asthma. Aerobic training continued until the last challenge. This intensity corresponds approximately to 65–70% of maximal oxygen uptake (VO_2_{max}), as determined in a previous experimental study (Lowder et al., 2005). This mode of training was chosen because intensity and duration could be manipulated experimentally, unlike in swimming or voluntary running (Pastva et al., 2004). Additionally, some benefits of treadmill use include the repeatability of training volume and the consistency of many physiological adaptations, such as changes in skeletal and cardiac muscle, which are observed after 8 weeks of training (Moraska et al., 2000). As expected, the trained group exhibited increases in ejection fraction and systolic volume over time when compared to non-trained animals, suggesting cardiac adaptation to aerobic training (de Araújo et al., 2012). However, this beneficial effect did not persist in the OVA groups, which may be attributed to inflammation and remodeling in the airway and lung parenchyma preventing cardiac adaptation and thus keeping systolic volume and ejection fraction unchanged (Alioglu et al., 2007).

Regarding functional parameters, the increases in lung static elastance and viscoelastic/inhomogeneous pressure observed in the non-trained group may be associated with increased alveolar collapse and inflammatory cell infiltration. Additionally, the increase in resistive pressure could be explained by a reduction in bronchial diameter caused by airway inflammation, eosinophil infiltration, increased collagen deposition, α-smooth muscle actin content, and ultrastructural airway damage. Lung static elastance, resistive and viscoelastic pressures, and ultrastructural airway changes were attenuated in trained compared to non-trained OVA animals.

Moreover, prior aerobic training significantly inhibited the methacholine-induced airway hyperresponsiveness that is a hallmark of asthma (Boyce & Austen, 2005). Improvement of airway function was paralleled by inhibition of airway remodeling. The percentage of α-smooth muscle-specific actin in terminal bronchioles and alveolar ducts was lower in T-OVA than in NT-OVA animals, which may be associated with the observed reduction in collagen deposition (Lowder et al., 2010; Silva et al., 2015a, b). A previous study demonstrated that, in OVA-treated mice, repeated bouts of aerobic training at a
moderate intensity decreased total lung resistance and airway smooth muscle thickness (Hewitt et al., 2010).

Prior regular and moderate aerobic training prevented asthma-associated alterations through modification of the adaptive immune response, which led to reduced levels of the Th2 cytokines IL-4 and IL-13 in BALF, but not in blood, suggesting an important effect of this type of training on pro-inflammatory cytokines involved in airway inflammation and remodeling (Silva et al., 2010; Silva et al., 2015a; Olivo et al., 2012). Indeed, an exacerbation of the Th2 response leads to changes in airway responsiveness and structure, as well as functional impairment, in asthma (Hamid & Tulic, 2009). Interestingly, levels of the pro-fibrogenic mediator TGF-β (Halwani et al., 2011) were reduced both in BALF and in blood.

The finding that regular and moderate aerobic training reduced Th2 pro-inflammatory mediators in BALF, but not in blood, suggests that such training may act on local inflammatory response and remodeling but have no significant effect on OVA sensitization. Lowder et al. (2010) demonstrated that exercise may increase Tregs in lungs without modifying their numbers in the spleen; thus, exercise may have an important immunomodulatory and anti-inflammatory effect that is restricted to the lungs. Airflow-induced shear stress has been demonstrated to increase epithelial barrier function (Sidhaye et al., 2008). Thus, the beneficial effects of exercise reported herein may represent a combination between modifications of the inflammatory milieu of the lung and some form of airway pre-conditioning. Likewise, moderate exercise improves the immune response by elevating levels of Th1 cytokines (Pedersen & Toft, 2000) or IL-10 (Petersen & Pedersen, 2005; Silva et al., 2010), an anti-inflammatory cytokine whose expression is inhibited in asthmatic patients and seems to potentiate the chronic allergic response (Silva et al., 2015a,b). Additionally, our results have shown that prior aerobic training modifies expression both of the Th1 cytokine IFN-γ in BALF and

![Fig. 11. Interleukin (IL)-5, IL-10, IL-13, interferon (IFN)-γ, and transforming growth factor (TGF)-β levels in bronchoalveolar lavage fluid. Values are mean (±SD) of 5 animals in each group. NT, non-trained; T, trained; OVA, mice sensitized and challenged with ovalbumin; SAL, mice given saline in a protocol identical to OVA sensitization and challenge. *Significantly different from NT-SAL (P < 0.05). **Significantly different from NT-OVA (P < 0.05).](image)
of IL-10 in BALF and plasma. IFN-γ is considered the main antagonist of IL-4 in the regulation of Th2 cytokines (Hamelmann et al., 1999), while the immune regulatory effects of aerobic exercise are mediated by increased levels of IL-10 (Vieira et al., 2007). These data further support previous reports which suggested an anti-inflammatory effect of aerobic training at reducing the Th2 immune response, by decreasing Th2 cytokine levels, in a murine model of allergic asthma (Pastva et al., 2004; Lowder et al., 2010; Dugger et al., 2013).

In conclusion, prior regular and moderate aerobic training attenuated lung histological changes, airway and lung parenchyma chronic inflammation, and remodeling, resulting in improvement of pulmonary mechanics, in the murine model of allergic asthma used herein. These beneficial effects may be related to an increase in the production of IL-10 and IFN-γ in tandem with a decrease in Th2 cytokines (IL-4 and IL-13) and TGF-β. These findings suggest that regular and moderate aerobic training induces favorable changes in the immune system and may protect against development of asthma in clinical settings.

**Perspectives**

This experimental study suggests that prior regular and moderate aerobic training attenuated lung histological changes, airway and lung parenchyma inflammation, and remodeling, resulting in improvement of lung function in allergic asthma. Therefore, we hypothesize that regular and moderate aerobic exercise might play a protective role in human subjects presenting a genetic tendency toward development of asthma. Further clinical studies are required to analyze the impact of aerobic exercise on the natural history of asthma in populations predisposed to this condition.

**Key words:** airway hyper-responsiveness, cytokines, collagen, electron microscopy, aerobic training.

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