# Aerobic Exercise Decreases Chronic Allergic Lung Inflammation and Airway Remodeling in Mice

**Rodolfo P. Vieira[1], Renata C. Claudino[2], Anna Cecília S. Duarte[3], Ângela B. G. Santos[1], Adenir Perini[3], Hugo C. C. Faria Neto[4], Thais Mauad[1], Mílton A. Martins[3], Marisa Dolhnikoff[1], and Celso R. F. Carvalho[2]**

[1]Department of Pathology, [2]Department of Physical Therapy, and [3]Department of Medicine, School of Medicine, University of São Paulo, São Paulo, Brazil; and [4]Departments of Physiology and Pharmacodynamics, Oswaldo Cruz Foundation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

***Rationale*: Aerobic conditioning improves exercise capacity and decreases symptoms in patients with asthma. However, its benefits in the context of allergic airway inflammation are poorly understood.**

***Objectives*: To evaluate the effects of two intensities of aerobic exercise on airway inflammation and remodeling in a model of chronic allergic lung inflammation.**

***Methods*: Mice were subjected to chronic ovalbumin (OVA) sensitization and to 4 weeks of low (OVA+Low) or moderate (OVA+Mod) exercise training in a treadmill. Airway inflammation and remodeling and expression of helper T-cell type 1 and 2 cytokines were evaluated.**

***Measurements and Main Results*: OVA-induced allergic airway inflammation and remodeling were characterized by an increase in collagen (288%), elastic fiber (56%), smooth muscle (380%), and epithelial (402%) contents ($P < 0.001$) when compared with the control group. OVA+Low and OVA+Mod groups presented a decrease in bronchoalveolar lavage fluid eosinophils (respectively, 84 and 75%; $P < 0.01$) and airway walls (respectively, 94 and 58%; $P < 0.001$) when compared with the OVA group. OVA+Low and OVA+Mod groups also presented a reduction in the number of peribronchial inflammatory cells expressing IL-4 (respectively, 85 and 75%; $P < 0.01$) and IL-5 (respectively, 88 and 89%; $P < 0.01$) when compared with the OVA group. Aerobic conditioning did not change the expression of either IFN-$\gamma$ or IL-2 by inflammatory cells or plasma levels of IgE or IgG1. OVA+Low and OVA+Mod groups presented an increase in the expression of IL-10 ($P < 0.001$). Low and moderate aerobic conditioning also reduced airway remodeling in OVA-sensitized mice when compared with the OVA group.**

***Conclusions*: We concluded that low and moderate aerobic exercise decreases airway inflammation and remodeling in a murine model of asthma.**

**Keywords:** asthma; aerobic exercise; lung inflammation; airway remodeling

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

Aerobic conditioning improves exercise capacity and decreases symptoms in patients with asthma. However, its benefits in the context of allergic airway inflammation are poorly understood.

### What This Study Adds to the Field

Low and moderate aerobic exercise intensities reduced allergic airway inflammation and remodeling and the expression of Th2 cytokines by lung inflammatory cells. These effects occurred independent of IgE/IgG1 production as well as Th1 cytokines.

The chronic airway inflammation present in asthma is a predominantly helper T-cell type 2 (Th2) response characterized by high levels of total and allergen-specific IgE, bronchial eosinophilia, $CD4^+$ T cell infiltrate in the airways, and Th2 cytokine production (e.g., IL-4, IL-5, and IL-13) (1–5). A persistent Th2 response leads to airway remodeling, characterized by collagen and elastic fiber deposition, smooth muscle hypertrophy and hyperplasia, hypertrophy of mucus-secreting glands, and increased vascularity (6, 7). Airway remodeling has been related to symptom severity and progression of asthma (6).

The role of aerobic exercise training in asthma pathophysiology and disease control has gained considerable attention. Patients with asthma have a unique response to physical activity. On the one hand, exercise can provoke an increase in airway resistance leading to exercise-induced asthma. On the other hand, regular physical activity and participation in sports are considered to be useful in asthma management (8). The benefits of physical conditioning in patients with asthma are related to the improvement of ventilatory capacity and lessening of asthma-related symptoms (9). Physical conditioning has been shown to improve physical fitness and work capacity and to decrease dyspnea, exercise-induced bronchospasm, peak expiratory flow variability, and daily use of inhaled steroids (9–15). However, the physiological effects of aerobic training in patients with asthma remain to be clearly delineated.

Aerobic physical training can modulate immune responses in healthy individuals: low and moderate intensities of aerobic training increase immune function and high-intensity aerobic training decreases it (16–18). Despite the known effects of physical exercise on healthy individuals, few studies have investigated the effect of physical training on allergic inflammatory responses (19, 20). Pastva and coworkers showed that aerobic exercise can decrease allergic lung inflammation in sensitized mice and suggested that this reduction may occur by inhibition of nuclear factor (NF)-κB activation; however, their model of airway allergic inflammation was predominantly neutrophilic (21). However, the effect of distinct exercise intensities on allergic airway response as well as the role of aerobic conditioning in airway remodeling remains unknown. In the present study we aimed at investigating the effects of two distinct intensities of aerobic exercise (low and moderate) on lung inflammation and

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Correspondence and requests for reprints should be addressed to Celso Ricardo Fernandes de Carvalho, Ph.D., School of Medicine, University of São Paulo, Av. Dr. Arnaldo, 455, 1° andar, sala 1216, São Paulo 01246-903, SP, Brazil. E-mail: cscarval@usp.br

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airway remodeling. For this purpose, total and differential cells in bronchoalveolar lavage fluid (BALF), serum levels of OVA-specific IgE and IgG1, airway structural changes, and inflammatory cells expressing Th2 cytokines (IL-4 and IL-5), Th1 cytokines (IL-2 and IFN-γ), and the immunoregulatory cytokine IL-10 were quantified in a model of chronic allergic lung inflammation in mice.

Some of the results of this study have been previously reported in the form of abstracts (22, 23).

## METHODS

This study was approved by the review board for human and animal studies of the School of Medicine of the University of São Paulo (São Paulo, Brazil). All animals in the study received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1985).

### Animals and Experimental Design

Forty-eight male BALB/c mice (20–25 g) were divided in six groups (n = 8 each): nonsensitized and nontrained animals (control group); nonsensitized and low-intensity aerobically trained animals (Low group); nonsensitized and moderate-intensity aerobically trained animals (Mod group); ovalbumin (OVA)-sensitized and nontrained animals (OVA group); OVA-sensitized and low-intensity aerobically trained animals (OVA+Low group); and OVA-sensitized and moderate-intensity aerobically trained animals (OVA+Mod group).

### Antigen Sensitization

BALB/c mice were sensitized by intraperitoneal injection of OVA (20 μg per mouse) adsorbed with aluminum hydroxide on Days 0, 14, 28, and 42 or with saline (0.9% NaCl), the diluent of OVA. Twenty-one days after the first intraperitoneal injection, the mice were challenged with aerosolized OVA (1%) or with saline, three times per week until Day 50. Challenging with aerosolized OVA (or saline) was performed in an acrylic box (30 × 15 × 20 cm) coupled to an ultrasonic nebulizer.

### Aerobic Exercise Treadmill Test and Exercise Conditioning

Animals were initially adapted to the treadmill for 3 days (15 min, 25% inclination, 0.2 km/h). After that, a maximal exercise capacity test was performed with a 5-minute warm-up (25% inclination, 0.2 km/h) followed by an increase in treadmill speed (0.1 km/h every 2.5 min) until animal exhaustion, that is, until they were not able to run even after 10 mechanical stimuli. The test was repeated after 30 days (before sacrificing). Maximal aerobic capacity (100%) was established as the speed reached by each animal. Mice were trained at low- or moderate-intensity exercise (respectively, 50 or 75% of maximal speed) for 60 minutes/day, 5 days/week. Aerobic conditioning began on Day 1 after OVA or saline inhalation and continued until Day 50. Figure 1 shows the time line of the experimental protocol.

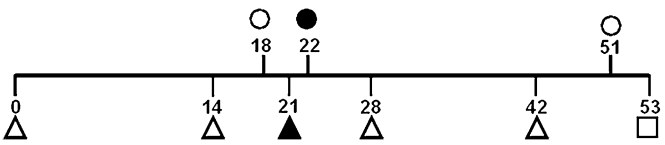


*Figure 1.* Time line of the experimental protocol. Mice received intraperitoneal injections of either ovalbumin solution (20 μg per animal) or vehicle on Days 0, 14, 28, and 42 (*open triangles*). Aerosol challenges with either ovalbumin (1% solution) or vehicle were performed three times per week starting on Day 21 (*solid triangle*) until Day 51. A maximal exercise capacity test was performed on Days 18 and 51 (*open circles*). Physical training (five times per week) was started on Day 22 (*solid circle*) and was performed until Day 50. Animals were killed and studied on Day 53 (*open square*).

### Anesthesia and Killing of Animals

Seventy-two hours after the last inhalation day, animals were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (40 mg/kg), and tracheostomized to collect BALF. Animals were killed by exsanguination, and blood was collected through the abdominal vein for the quantification of OVA-specific immunoglobulins.

### Total and Differential Cell Counting in BALF

Lungs were gently lavaged with 1.5 ml of saline (administered as three 0.5-ml volumes) via the tracheal cannula. Total cell counts were performed automatically (automatic laser blood cell counter, model JXJ-402; Shanghai Odin Science and Technology, Shanghai, China). Differential cell counts were performed with May-Grünwald-Giemsa stain (300 cells per lamina) (24).

### OVA-specific Passive Cutaneous Anaphylaxis to IgE and IgG1

Blood serum was obtained and measurement of the titers of anaphylactic IgE and IgG1 OVA-specific antibody was performed by the passive cutaneous anaphylaxis (PCA) technique, as described by Ovary (25) and modified by Mota and Perini (26). The PCA titers were taken as being the highest dilution that presented a blue spot at least 10 mm in diameter.

### Lung Morphometry

Lungs were fixed in formalin and embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin and eosin for lung structure analysis, Weigert's resorcin–fuchsin with oxidation for elastic fibers, picrosirius for collagen fibers, and Luna staining for eosinophil detection (27, 28). Immunohistochemistry was performed with anti–IL-4, anti–IL-5, anti–IFN-γ, and anti–IL-2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), and with anti–IL-10 antibody (R&D Systems, Minneapolis, MN), by the biotin–streptavidin–peroxidase method. With a 50-line, 100-point grid connected to the ocular of the microscope, we assessed the peribronchial density of eosinophils, mononuclear cells, and cells positive for IL-4, IL-5, IFN-γ, and IL-2, using a point-counting technique (28, 29). Counting was performed in 25 fields of airway wall samples for each animal (5 airways per animal) at ×1,000 magnification. Results were expressed as cells per square millimeter (28, 29). The bronchoconstriction index was assessed as the numbers of intercepts between the lines of the grid and the basal membrane divided by the square root of the number of points hitting the airway lumen (30). Measurements were performed in five airways per animal. The airway smooth muscle area and epithelial thickness were assessed as the number of points hitting smooth muscle or epithelial cells, respectively, divided by the number of intercepts between the lines of the grid and the basal membrane. Measurements were performed in five airways from each animal at ×400 magnification.

### Statistical Analysis

Parametric and nonparametric data were expressed as means ± SD and as medians ± 95% confidence interval (95% CI), respectively. Comparisons among groups were performed by one-way analysis of variance followed by the Student-Newman-Keuls *post hoc* test (parametric data) or by one-way analysis of variance on ranks followed by Dunn's post-hoc test (nonparametric data); the significance level was adjusted to 5% ($P < 0.05$).

## RESULTS

### Cellular Changes in BALF after OVA Challenge and Exercise Training

Chronic OVA exposure increased by sixfold the total cell number in BALF compared with saline inhalation; aerobic exercise training did not decrease it (Figure 2A). The increase in total cell number was due mainly to eosinophils and mononuclear cells (Figures 2B and 2D). As compared with the OVA group, low and moderate exercise training decreased the

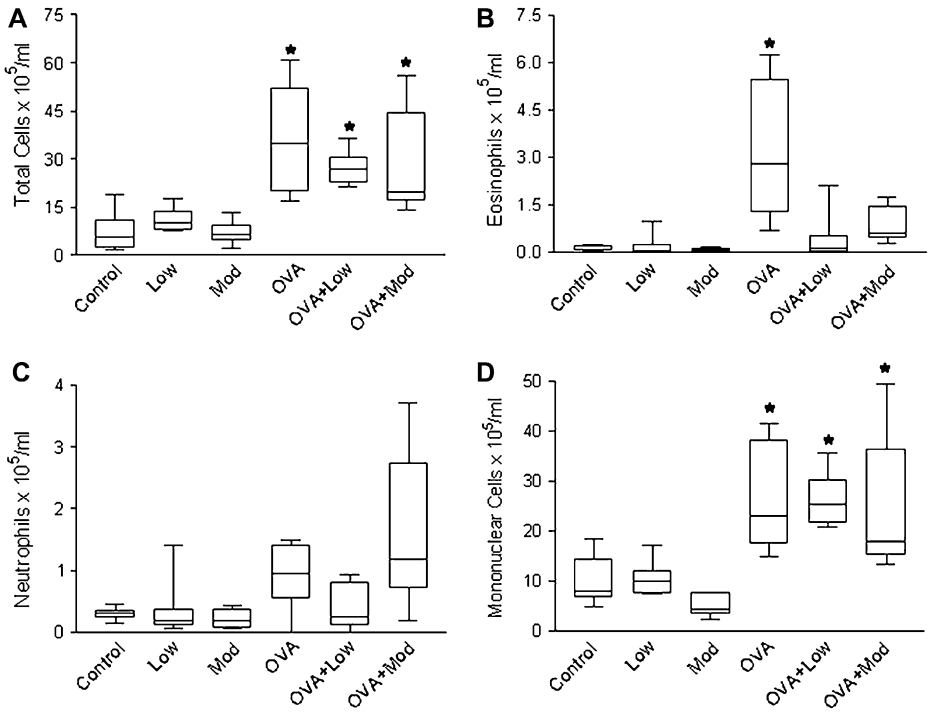


***Figure 2.*** *Box plots* of total cells (*A*), eosinophils (*B*), neutrophils (*C*), and mononuclear cells (*D*) in bronchoalveolar lavage fluid. *Boxes* show interquartile range, *whiskers* show range, and *horizontal lines* represent median values. In (*A*) and (*D*) an asterisk (*) indicates values significantly different from control, Low, and Mod groups. In (*B*) the asterisk indicates values significantly different from all groups ($P < 0.01$). Control = nonsensitized and nontrained animals; Low = nonsensitized and low-intensity aerobically trained animals; Mod = nonsensitized and moderate-intensity aerobically trained animals; OVA = OVA-sensitized and nontrained animals; OVA+Low = OVA-sensitized and low-intensity aerobically trained animals; OVA+Mod = OVA-sensitized and moderate-intensity aerobically trained animals.

eosinophil count in BALF but not the mononuclear cell count (Figures 2B and 2D, respectively) ($P < 0.01$). OVA sensitization and aerobic exercise training had no significant effect on the number of neutrophils in BALF (Figure 2C).

### Lung Tissue Inflammation

OVA sensitization increased peribronchial mononuclear cells and eosinophil density compared with saline groups. (Figures 3A and 3B). Both low and moderate aerobic exercise training substantially decreased eosinophil infiltration in airway walls, with no effects on mononuclear cells (Figures 3A and 3B).

### Effects of OVA Sensitization and Exercise Training on Cytokine Expression

The peribronchial density of cells positive for Th2 cytokines IL-4 and IL-5 was significantly increased in the OVA group compared with the saline groups (Figures 4A and 4B and Figures 5A and 5B). Both low and moderate aerobic exercise training resulted in a significant decrease in the density of cells positive for IL-4 and IL-5 (Figures 4C and 4D and Figures 5C and 5D). The density of positive cells staining for Th1 cytokines IFN-γ and IL-2 was similar in all studied groups (Figures 4C and 4D). The peribronchial density of cells positive for IL-10 was significantly increased in OVA+Low and OVA+Mod groups compared with all other groups (Figure 6).

### Effects of Chronic OVA Exposure and Exercise Training on Anaphylactic Antibodies IgE and IgG1

Chronic OVA exposure significantly increased OVA-specific IgE and IgG1 levels ($P < 0.001$); however, exercise training did not change OVA-induced immunoglobulin levels.

### Effects of Chronic OVA Exposure and Exercise Training on Volume Proportion of Collagen and Elastic Fibers, Airway Smooth Muscle Area, and Airway Epithelial Thickness

Chronic OVA exposure significantly increased the volume proportion of collagen and elastic fibers in airways. OVA+Low and OVA+Mod groups presented a significant reduction in the deposition of collagen and elastic fibers on airway walls (Figures 7A and 7B and Figures 8A–8D). The airway smooth muscle area increased 380% in the OVA group as compared with the control group. Both low and moderate aerobic exercise training led to a significant reduction in airway smooth muscle enlargement (Figure 7C). The epithelial thickness was increased 402% in the OVA group as compared with the control group. Both low and moderate aerobic exercise training led to a significant

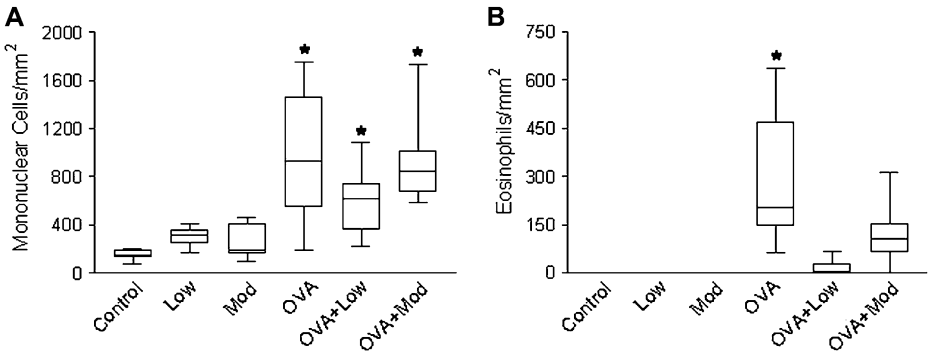


***Figure 3.*** *Box plots* of mononuclear cell (*A*) and eosinophil (*B*) density in airway wall. In (*A*) an asterisk (*) indicates values significantly different from control, Low, and Mod groups ($P < 0.05$). In (*B*) the asterisk indicates values significantly different from all other groups ($P < 0.001$).

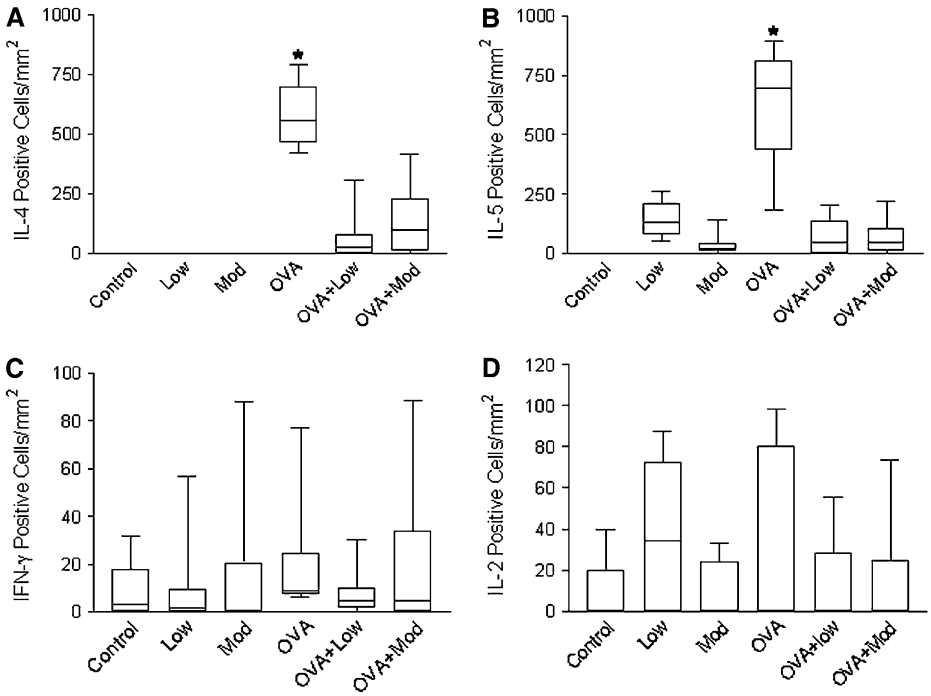


***Figure 4.*** *Box plots* of density of inflammatory cells expressing IL-4 (*A*), IL-5 (*B*), IFN-γ (*C*), and IL-2 (*D*) in airway walls. In (*A*) and (*B*) an asterisk (*) indicates values significantly different from all groups ($P < 0.01$). In (*C*) and (*D*) no statistically significant differences were found between the groups.

reduction in airway epithelial thickness to control levels (Figure 7D). The bronchoconstriction index was significantly increased in the OVA group as compared with the saline groups. Both low and moderate aerobic exercise training were associated with a reduction in the bronchoconstriction index to control levels (Figure 7E).

## DISCUSSION

In the present study, we showed that both low and moderate exercise training inhibit OVA-induced eosinophil infiltration in airway walls, as well as the expression of Th2 cytokines IL-4 and IL-5. We also showed that both exercise intensities prevent structural airway alterations induced by allergic inflammation. These results suggest that exercise training programs might play an important role as an adjunct therapeutic strategy in the treatment of lung diseases with chronic allergic airway inflammation such as asthma.

Our model of chronic allergic lung inflammation was characterized by a persistent cellular infiltrate of eosinophils and mononuclear cells in BALF and the peribronchial compartment. A large number of previous studies showed that these inflammatory cells play a pivotal role in asthmatic airway inflammation, contributing to airway remodeling and the development of airway obstruction (1–7). The increased expression of Th2 cytokines IL-4 and IL-5 by airway inflammatory cells observed in our animal model is in agreement with previous experimental and clinical studies, which showed that these cytokines contribute either directly or indirectly to promoting the differentiation, survival, and function of key allergic effector cells (4, 31, 32). Interestingly, our OVA-sensitized mice also presented features of airway remodeling, such as collagen

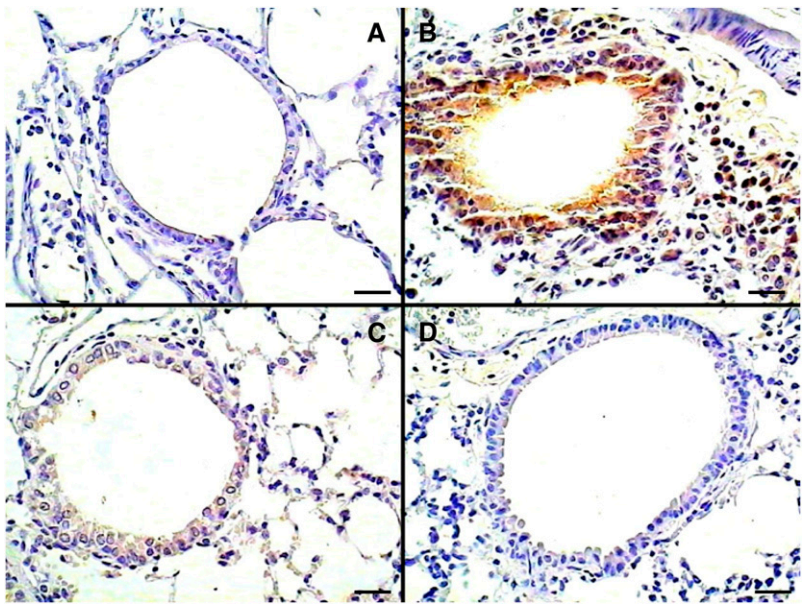


***Figure 5.*** (*A–D*) Representative photomicrographs of airways stained with anti–IL-4 in control, ovalbumin (OVA), OVA+Low, and OVA+Mod groups, respectively. Note positive inflammatory cells in airway wall stained for IL-4. *Scale bars*: 25 μm.

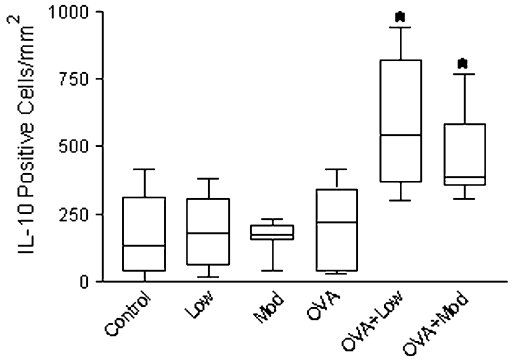


***Figure 6.*** *Box plots* of density of inflammatory cells expressing IL-10. *Values significantly different from all other groups ($P < 0.001$).

and elastic fiber deposition and enlargement of the smooth muscle layer and epithelial thickness, also documented in some experimental models of chronic allergic lung inflammation. These airway structural alterations are thought to be secondary to chronic inflammation and are likely to explain the functional abnormalities in experimental models of chronic allergic lung inflammation (33–35). We are aware that animals do not develop asthma, and understand the intrinsic limitations of animal studies. However, in rodent models of allergic sensitization, several studies have shown the development of structural airway alterations after 2, 4, or more weeks after the onset of OVA sensitization (28, 30, 33–35). These alterations partially mimic the remodeled airways in asthma, and therefore have been extensively used, as in the present study, to better understand disease pathogenesis and to assess therapeutic interventions in a broad sense.

We observed that low and moderate exercise training specifically inhibited eosinophil migration to the airways. Pastva and coworkers showed that exercise reduced airway allergic inflammation in a mouse model. Although these authors reported that exercise reduced both lymphomononuclear and polymorphonuclear cells in BALF, including eosinophils, their model of allergic inflammation was predominantly neutrophilic. Concerning the levels of IL-4 and IL-5 in BALF, Pastva and coworkers observed that exercise determined a reduction only in IL-4 levels and not in IL-5, suggesting a decreased Th2 response by exercise (21). Our model of allergic lung inflammation showed increased expression of IL-4 and IL-5 by inflammatory cells and also that low and moderate aerobic exercise decreased this expression. Because IL-4 and IL-5 have been shown to mediate IgE production and airway eosinophilia (35), we also evaluated the levels of OVA-specific IgE and IgG1, which were not modified by aerobic exercise training. This finding suggests that the effect of exercise on airway allergic inflammation was not mediated by changes in the levels of OVA-specific immunoglobulins.

It has been suggested that moderate exercise gears the immune system toward a more Th1-type cytokine response, whereas strenuous exercise induces an increase in Th2-type cytokines (36, 37); however, exercise-induced changes in the Th1–Th2 balance was not previously investigated in allergic inflammation. We hypothesized that exercise-induced reduction in Th2 cytokines in our experimental model could represent a Th1–Th2 imbalance, with an increase in the expression of Th1 cytokines. On the basis of that, we also investigated the effect of exercise on the expression of Th1 cytokines IFN-γ and IL-2 by inflammatory cells (38, 39). We did not observe any changes in Th1 cytokine expression, either by OVA sensitization or after exercise. In addition, we also investigated the expression of the antiinflammatory cytokine IL-10. It has been suggested that the immunoregulatory effects of aerobic exercise are mediated by increased release of IL-10 (40, 41). Activated skeletal muscle releases increased amounts of IL-10 during aerobic training sessions, underlying part of the antiinflammatory effects of aerobic exercise in cardiovascular disease and type 2 diabetes (42). IL-10 also presents antiinflammatory effects in experimental models of chronic allergic lung inflammation (43, 44). Our results demonstrated that in sensitized animals, aerobic exercise training increased the expression of IL-10 by inflammatory cells, representing a possible mechanism of exercise-induced decrease in allergic inflammation.

Airway remodeling is a term applied to describe the dynamic process that leads to airway structural changes in asthma. These structural changes are thought to be secondary to chronic inflammation and to result in an irreversible component of the

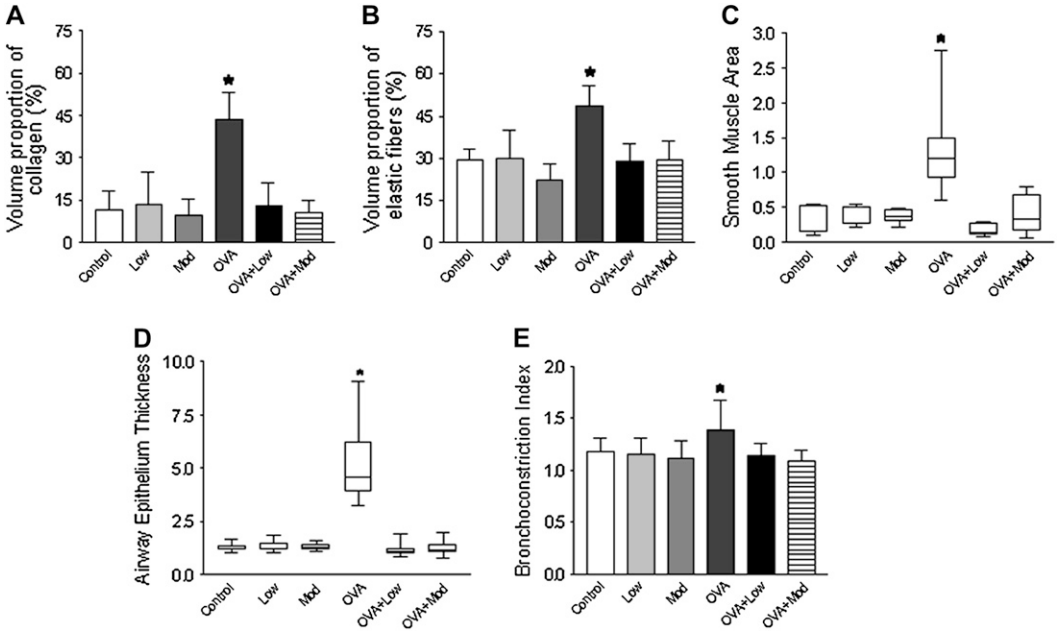


***Figure 7.*** (*A* and *B*) Collagen and elastic fiber content in airway walls, respectively. Results are expressed as volume proportion (%) and as means ± SD. *Values significantly different from all groups ($P < 0.001$). (*C* and *D*) *Box plots* of airway smooth muscle area and airway epithelial thickness, respectively. *Values significantly different from all groups ($P < 0.001$). (*E*) Airway constriction index. *Significantly different from control group ($P < 0.05$).

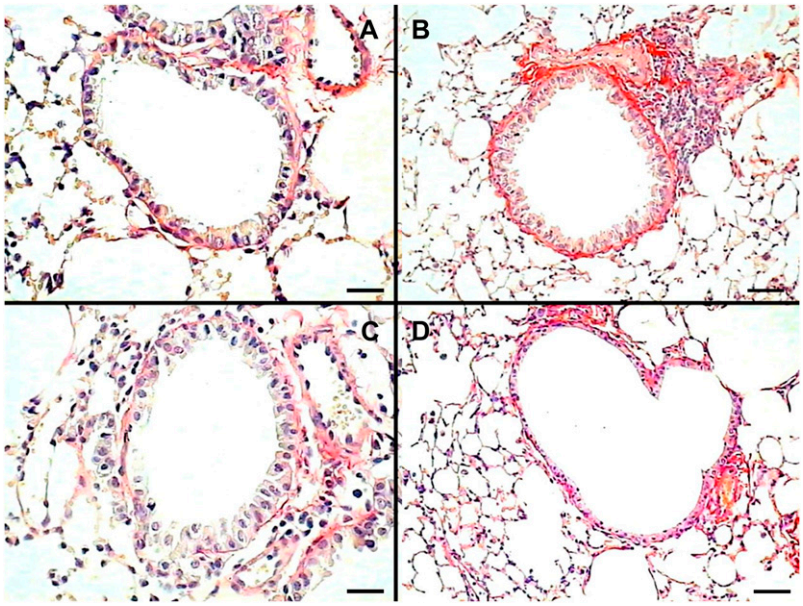


***Figure 8.*** (*A* to *D*) Representative photomicrographs of collagen content in airways stained with Sirius red in control, ovalbumin (OVA), OVA-Low, and OVA-Mod groups, respectively. Note collagen fibers in airway wall (*red*). *Scale bars*: 25 μm.

airway obstruction seen in patients with asthma, especially in severe asthma (35, 45). We observed in the OVA group an increase in airway collagen and elastic fiber content, as well as an enlargement of the airway smooth muscle layer and an augmentation in epithelium thickness. There is evidence suggesting that IL-4 increases airway eosinophilia and the development of subepithelial fibrosis and goblet cell hyperplasia in mice (35). The same study also suggested that IL-5 is implicated in eosinophilic inflammation, but it is not critical for the development of airway dysfunction or some aspects of airway remodeling. We do recognize that an analysis of the cytokine network that regulates allergen-induced airway remodeling is complex and that our study cannot explain mechanisms involved in airway remodeling. However, we can speculate that the exercise-induced reduction in IL-5 expression might decrease the ongoing sustained eosinophil inflammation, and that the decrease in IL-4 expression might be related to the reduction of some airway-remodeling features.

Previous clinical studies have evaluated the impact of exercise in patients with asthma and a systematic review has shown that physical training resulted in a significant increase in cardiorespiratory fitness, work capacity, and dyspnea improvement (46). These effects of aerobic physical conditioning on patients with asthma have been related to the reduction in ventilatory threshold. The effect of physical training intensity on patients with asthma has never been evaluated, but there is evidence suggesting that it may result in different responses in exercise-induced bronchoconstriction. In fact, a decrease in exercise-induced bronchoconstriction after training was more likely to be found in studies with patients who were trained at moderate intensity (15, 47–49), whereas patients who underwent low-intensity exercises did not present changes in exercise-induced bronchoconstriction after training (13, 50). The effect of exercise training on the pathophysiology of asthma remains to be elucidated. On the basis of our findings, it is appealing to postulate that patients with asthma, who are physically conditioned at both low and moderate exercise intensity, would experience a beneficial effect, with a reduction in airway inflammation and remodeling. However, the extent to which the results obtained in this murine model of allergic airway inflammation can be transposed to patients with asthma is unclear. Interestingly, decreased physical activity seems to contribute to persistence of asthma because lower levels of physical activity in asthmatic children are correlated with a history of wheezing, diagnosis of asthma, and presentation to the emergency room (51).

In conclusion, our results have suggested that exercise aerobic training at low and moderate intensities presents similar results and could provide a protective effect against allergic lung inflammation and airway remodeling in a murine model of asthma.

***Conflict of Interest Statement***: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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