

RETRACTION

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Model animals

Review article

Effects of physical exercise on lung inflammation in animal models of chronic allergic lung inflammation: a systematic review

Exercise and animal models of asthma

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ABSTRACT

We performed a systematic review to identify and summarize the effects of distinctive aerobic exercise (AE) protocols on chronic allergic lung inflammation reported in asthma animal models. We identified 255 preclinical studies of asthma and AE protocols by comprehensive searches in PubMed, ScienceDirect and LILACS for peer-reviewed journals, using the search components “exercise,” “lung inflammation,” and “asthma,” without restrictions on publication date. Twenty-two studies were selected that met all eligibility criteria. Most studies used an ovalbumin experimental model and performed experiments with BALB/c mice. Most studies performed treadmill AE, using protocols of 30 to 60 minutes, 3 to 5 times/week, for 4 to 8 weeks. The results showed that the effects of AE on lungs were protocol dependent, but generally, there was a decrease in inflammatory cell influx and Th2 cytokines, as well as an increase in anti-inflammatory defenses. However, the immunoregulation of globulins and nitric oxide levels in asthma seemed to be associated with the onset of AE. The results suggest that AE plays an important immunoregulatory role in animal models of asthma, with effects mainly mediated by increased anti-inflammatory defenses and reduced Th2 response. In parallel, prophylactic and therapeutic AE also played important roles in decreasing bronchial responsiveness and attenuating lung remodeling in animal models of asthma, secondary outcomes in this systematic review. The review protocol was published for free access in October 2017 on the Systematic Review Facility (SyRF) platform (<http://syrf.org.uk/protocols/>).

Key words: animal model, asthma, lung inflammation, physical exercise.

INTRODUCTION

Asthma is a heterogeneous disease of the airways that affects approximately 300 million people worldwide and is characterized by chronic inflammation, hyperresponsiveness, and airway remodeling [5, 10, 36]. Aerobic exercise (AE) has been pointed out as an important tool for treatment and management of asthmatic patients, since regular aerobic exercise is closely related to improvement of ventilatory capacity improvement, decrease of symptoms, and reduction of inhaled medications [4, 7, 8, 33, 42, 43].

In this context, Mendes et al., 2011, demonstrated that patients with moderate and severe asthma who underwent an AE program showed decreased eosinophil influx in the lungs and a significant decrease in exhaled nitric oxide, important markers of the asthma inflammatory process [26]. Additionally, these same patients presented a significant increase in the number of days without asthmatic symptoms.

In parallel, AE has been shown in animal models of asthma to result in striking decreases in inflammatory cell influx and pro-inflammatory cytokines, mitigating lung remodeling. Additionally, AE also increased levels of anti-inflammatory cytokines and antioxidant defenses [7, 8, 17, 24, 40, 45, 47].

Therefore, these results suggest that AE plays an influential role as an adjuvant therapeutic strategy in the treatment of asthma [45]. However, the results concerning the benefits of aerobic training in chronic allergic lung inflammation may vary according to the experimental model applied, as well as to the protocol and exercise intensity proposed.

An analysis of previous AE protocols used in experimental models of asthma may help us to understand the mechanisms involved in pulmonary

immunoregulation, as well as the process of translating these results into clinical practice.

Thus, the objective of this systematic review (SR) was to investigate the effects of AE on major components of pulmonary inflammation in animal models of asthma. In addition, we also aimed to synthesize distinctive protocols of asthma induction and aerobic training, and to analyze the bias risk and the quality of manuscript reports.

MATERIALS AND METHODS

Protocol and registration

This systematic review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. The review protocol was developed using the guidelines of the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE), published for free access in October 2017 on the Systematic Reviews Facility (SyRF) platform (<http://syrf.org.uk/protocols/>) [21, 48].

Eligibility criteria, information sources and search

Preclinical studies were identified by comprehensive searches in three online databases (PubMed, ScienceDirect and LILACS) using the search components “exercise,” “lung inflammation,” and “asthma” (Supplementary Appendix 1) as eligibility criteria. We performed the electronic search from October 2017 until August

2019, without language or publication date restrictions. Reference lists from previous reviews were searched manually to identify additional relevant studies.

Study selection

The screening phase was based on title and abstract, and both were selected and exported to Excel, in order to remove duplicates and perform an examination for triage relevance related to the predefined eligibility criteria. At this stage, no restrictions were applied to language or publication date. The text of the articles that meet inclusion criteria were obtained and assessed for eligibility.

We included studies that evaluated the effects of AE on any variable related to pulmonary inflammation in animal models of chronic allergic lung inflammation. In the eligibility phase, studies that did not meet the inclusion criteria were described in an appropriate manner in a table. In both phases of selection, references were evaluated by two independent reviewers. Disagreements were resolved by a third party.

Extraction of study characteristics and outcome data

Data were extracted from each study based on their identity (first author and year of publication), characteristics of study design (animal strain, sex and age, groups, number of animals/groups and presence of control group), animal model protocol (type, initial period, session, duration, frequency, and intensity,) and evaluations of pulmonary inflammation (main outcome), bronchial

hyperresponsiveness, lung remodeling, and oxidative stress in the lungs (secondary outcomes).

During data extraction, we identified individual comparisons between exercised and sedentary animals with chronic allergic lung inflammation in terms of mean, standard deviation (SD), interquartile range, median or confidence interval, and number of animals per group (n) from tables, graphs, and text. All results were presented as absolute and/or relative frequencies in relation to the total number of articles included in the systematic review after evaluation of eligibility criteria.

Bias risk assessment

The SYRCLE risk of bias tool for animal studies contains 10 domains related to selection bias, performance, detection, attrition, reporting, and other biases. The objective of the adoption and use of this tool is to improve the critical evaluation of evidences found in animal studies, with more consistency and less discrepancies [18].

Two independent reviewers evaluated the bias risk in each study, which was flagged with a plus sign (+) for low risk and a minus sign (-) for moderate or high risk. A question mark symbol (?) was used to indicate lack of clarity for properly assessment. In this review, studies with low bias risk in at least half of the classification criteria were considered to have the best quality.

Reporting quality

The data reporting quality was evaluated according to the ARRIVE (Animals in Research: Reporting of In Vivo Experiments) guidelines, published in the journal PLOS Biology in June 2010, from the initiative of the National Center for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) [20].

In this systematic review, for evaluation of the quality reports, articles were divided according to the year of publication of the ARRIVE guidelines, that is, according to whether they were published before or after 2010. Each ARRIVE item was classified into three descriptive levels: complete, meaning that all recommendations were met for the evaluated item described; partial, meaning that half or more of the recommendations were met for the evaluated item; and incomplete, meaning that less than half or none of the recommendations were met for the evaluated item.

RESULTS

Selection of studies

A flow diagram of the search procedure is shown in Figure 1. A total of 255 articles were identified. After the screening and abstracts, 24 studies were evaluated for eligibility, and due to failure to meet the eligibility criteria, 2 of them were excluded, resulting in the inclusion of 22 full-text articles (Supplementary Table 1).

Studies features

Mice and groups: Among the 22 articles included in this review, mice (90.91%) were the most commonly used animals (BALB/c (68.18%), male mice (68.18%), adults mice (50%)) and were divided into four main groups, control, trained, ovalbumin non-trained and ovalbumin trained, with 8 animals per group in 45.45% of the studies.

Experimental protocol of chronic allergic lung inflammation: The induction of lung inflammation by ovalbumin (OVA) intraperitoneal injections was described in 90.91% of studies, with 2 (45.45%) or 4 (36.36%) doses of 20 μ g (36.36%) or 10 μ g per animal (22.73%), in the majority of cases, and this is typically followed by aerosol challenges (90.91%) with 1% OVA (50%), most frequently for 4 weeks (31.82%). Sixteen studies (72.73%) reported the use of aluminum hydroxide as an adjuvant, and OVA purity was reported in only 5 articles (22.73%), as greater than or equal to grade 5. Only one article induced the lung inflammation orotracheally with house dust mite (HDM).

AE protocols: Treadmill exercise was the most used protocol intervention (95.45%), with its onset after a sensitization period in 90.91% of articles. Sixty-minute sessions were described in 59.10% of studies, followed by 45- and 30-minute sessions (18.20% each), with a frequency of 5 times/week (50%) or 3 times/week (27.30%). The overall duration was 4 (54.54%) or 8 weeks (13.64%), at a low or moderate exercise intensity (54.54% and 50%, respectively). These characteristics are shown in Table 1.

Maximal physical capacity test (MPCT): Twelve articles (54.54%) evaluated MPEC with the aim of determining the training velocity, and only 5 of them (22.73%)

performed initial and final tests in order to compare groups before and after the AE protocol.

Lung damage: All studies evaluated some component of lung inflammation, such as the inflammatory cell count in bronchoalveolar lavage fluid (BALF; 68.20%) and inflammatory cytokine levels in BALF or lung tissue (90.91%). Among the selected studies, 8 (36.36%) evaluated some feature of hyperresponsiveness, 18 (81.82%) evaluated markers of pulmonary tissue remodeling, and 9 articles (40.91%) evaluated oxidative stress in lungs.

Bias risk unclear

From the 22 articles included in this review, 19 (86.36%) presented insufficient information to judge the domains in 50% or more of the classification criteria, resulting in the risk of bias being unclear. Only 2 (10.53%) articles presented a risk of moderate or high bias in 60% of the classification criteria, because they did not report the criteria in the article or did not classify it during the study.

Among the domains evaluated, baseline characteristics, concealment of allocation, and incomplete results were topics classified with moderate or high risk of bias in more than 50% in studies. More than 80% of the articles classified the risks of selectivity of important results and other forms of bias as low. The individual scores of all the studies are shown in Figure 2.

Reporting Quality

In this systematic review, 4 studies (21.05%) were published before 2010 and classified as completed in terms of the introduction, objectives, ethical statement, methodology of experimental results, and generalization or translation. These studies were also complete in terms of reporting how experimental procedures were performed, the order used to treat or observe each animal, the units used in analyses, methods used to assess whether the results met statistical requirements, and data interpretation.

In contrast, studies published since 2010 were only completed in terms of the report introduction, experimental results, the units used in each analysis, and methods used to assess statistical requirements, as well as data interpretation and generalization. Detailed results of the evaluations of study quality are presented in Tables 2 and 3.

Effects of AE on chronic allergic lung inflammation

Among the studies that evaluated the total number of inflammatory cells, eleven studies (50%) showed that high-, moderate-, or low-intensity AE decreased cell influx into lungs in OVA experimental models. The eosinophil lung infiltration in sensitized and trained animals was reduced in 17 (77.27%) of the 22 evaluated articles, while the increased numbers of neutrophils, lymphocytes, and macrophages in OVA groups were reversed by AE in 8 (36.36%), 10 (45.45%), and 5 (26.31%) studies, respectively. Only 3 papers (13.64%) did not evaluate lung cell migration.

Aerobic exercise also decreased levels of pro-inflammatory cytokines in the lungs, such as interleukin (IL)-4, IL-5, IL-6, and IL-13, in the OVA groups in all studies. Twelve articles (54.55%) showed a concomitant increase of anti-inflammatory cytokines, such as IL-10 and IL-1ra, induced by AE in OVA animals. Interestingly, Olivo et al., 2012, observed that the expression of regulatory cytokines remained unchanged by either OVA exposure or by low exercise training, while Lowder et al., 2010, reported that moderate exercise decreased IL-10 production in OVA-sensitized mice.

The total immunoglobulin (Ig) E levels were evaluated only in 3 studies (15.79%); 2 articles (66.67%) showed that both moderate- and high-intensity exercise decreased the IgE levels in sensitized animals, while 1 article (33.33%) showed that moderate training did not change OVA-induced immunoglobulin antibody levels in serum. Among the studies, 6 (31.58%) evaluated OVA-specific IgE and IgG1 titers; in 3 of them (50%), AE at low and moderate intensity decreased the Ig-specific antibody titers in OVA groups, and 3 (50%) articles observed that the increased OVA-induced antibody titers unchanged by exercise.

Finally, 4 articles (21.05%) evaluated the effects of exercise on nitric oxide (NO) isoform. Hizume-Kunzler et al., 2017, and Olivo et al., 2012, reported that moderate and low aerobic exercise did not change lung levels of NOS-2 and ENO, respectively, but that both were increased after an allergen challenge. In contrast, Vieira et al., 2011 and 2014, showed that low-intensity AE significantly reduced iNOS expression in OVA groups. The detailed results, with significance level values, are presented in Table 4.

DISCUSSION

This systematic review aimed to summarize the main findings regarding the inflammatory mechanisms involved in experimental animal models of chronic allergic inflammation of the lungs, relating them to a powerful tool in the treatment and management of asthma, AE. Thus, the synthesis of the experimental protocols for asthma, as well as the aerobic training employed, may contribute to an all-encompassing view of the disease in future experiments.

The results described in the studies, however, may be directly associated with the asthma induction protocols, and their heterogeneity may compromise the comparisons among the studies [24]. Additionally, the variety, duration, and intensity of AE also seemed to result in distinctive lung inflammation outcomes. Therefore, we described each one of these variables, correlating them to the pulmonary inflammation status.

Animal strains and asthma induction protocols

In this review, we observed that male BALB/c mice were the most common animals adopted in the experimental models. In fact, the response involved in asthma is antigen specific and is mediated by T helper 2 (Th2) cells [3] and BALB/c mice, aside from being isogenic, are animals with the best response to this kind of experimental protocol [11]. In addition to these factors, the choice of males takes into consideration the hormonal modulation of asthma, since the estrous and diestrus cycles in females could interfere directly on inflammatory response [15].

Ovalbumin was the most common means of experimental asthma induction and perpetuation observed in this review. The use of OVA results in an allergic inflammatory response in the airways with an immunoglobulin profile that is quite consistent with that observed in humans [17, 22, 24]. However, the degree of purity of the allergen used was only described in a few studies, which limits the ability to develop a standardized animal model for allergic diseases.

The number of injections, interval between them, weekly frequency of inhalational challenges, and the doses used differed greatly among articles. Nevertheless, in all the included studies, regardless of the protocol adopted, the parameters evaluated for airway inflammation, hyperresponsiveness and remodeling were sufficient to characterize the model as a typical allergic response.

AE on a treadmill as a therapeutic intervention

AE has been applied as a therapeutic tool in many kinds of lung diseases, like asthma [9, 26]. The first studies regarding exercise practice as a therapeutic strategy emerged in 1968 in the United States with the proposal of making regular swimming training part of complementary therapy for asthmatic children. Among the results, it was found that the physical fitness of asthmatic children increased significantly and that medication prescription decreased, as did the severity of disease [13].

Regarding preclinical trials, most of the studies gathered in this SR performed AE on a treadmill and used protocols of 30-60 minutes, 3 to 5 times/week, for 4 to 8 weeks. Among the included studies, 21 of the studies used a low to moderate training intensity, and just one study used high-intensity swimming, with the intensity

confirmed by lactate measurements [7]. These findings are in line with the AE protocols adopted in pulmonary rehabilitation programs.

The recommendation of the American College of Sports Medicine (ACSM) for individuals with chronic respiratory disease is the execution of AE on a treadmill or bicycle 3 to 5 times a week, 20 to 60 minutes per session, with a minimum duration of 6 weeks. Nevertheless, exercise intensity is another important parameter to be defined, and according to ACSM the ideal intensity is the highest possible that allows continuous exercise without any harm to the individual [41].

Interestingly, the outcomes of the distinctive protocols used in the studies included in this review showed improvements related to lung inflammation status regardless of type or intensity of the protocols applied, and this might suggest that the timing with respect to the challenges, the AE, and sacrifice of the animals might have had significant effects on the results.

Effects of AE on lung inflammation, bronchial responsiveness, and remodeling

After antigen inhalation, bronchial epithelial cells initiate the production of cytokines, chemokines, and adhesion molecules, activating receptors in leukocytes and initiating a complex cascade of signaling, resulting in an intense cell influx in the lungs, which is a precise marker of the inflammatory response to allergen induction, as observed in the studies included in of this SR [16, 34].

The included studies focused on evaluation of the effects of AE on cell influx into lungs, especially eosinophil and lymphocyte numbers, as well as Th2 profile

cytokines like IL-4, IL-5, and IL-13, in addition to regulatory cytokine levels, such as those of IL-10 and IL-1ra.

Some of the studies evaluated the expression of chemokines and adhesion molecules, as well as total and specific immunoglobulin profiles [16, 38, 46]. In contrast to human studies, evaluations were performed preferentially on bronchoalveolar lavage fluid or directly in lung tissue.

According to the articles, AE was capable of mitigating lung inflammation and decreasing cell influx. These data reinforce findings in the literature, such as the reduced sputum cell counts in trained asthmatic patients [26].

The studies also showed an increase in pulmonary pro-inflammatory cytokines, and the findings unanimously verified that cytokine levels or expression levels in the lungs were attenuated by AE, especially those of IL-4, IL-5, IL-6, and IL-13. In general, IL-4 and IL-5 are responsible for eosinophil recruitment to the inflammation site. IL-4 also stimulates mucus production, activation of mast cells, and production of immunoglobulin by B lymphocytes, while IL-13 acts directly on the bronchial epithelium and is responsible for fibroblast proliferation and collagen production [3, 35].

The production of IL-10 by regulatory T cells counteracts allergic inflammation in the lungs and is increased under the influence of AE [14], as described in 12 articles in this review. Interestingly, increased levels or expression of IL-10 in the lungs were shown in animal protocols from both prophylactic and therapeutic perspectives. [2, 7, 38, 39].

Immunoglobulin levels were evaluated in 7 studies and were also increased by OVA, as expected. However, AE showed a heterogeneous effect on this outcome, regardless of the intensity or protocol applied. In 2 articles, AE decreased total IgE

levels in lung homogenate, and in 3 studies, the AE decreased the Ig-specific titers in the OVA groups. On the other hand, 2 studies suggested that the anti-inflammatory effects of exercise could be more related to other factors not involving the production of immunoglobulins [29, 45].

Mediators of the inflammatory cascade, such as NO and its isoforms (NO_x, iNOS, and NOS-2), may increase vascular capillarity, accentuating cell influx and inflammatory markers and contributing to tissue damage in the lungs [8]. Among NO isoforms, iNOS-2, which is expressed by macrophages, neutrophils, and the airway epithelium, is considered the most important source of NO [8, 19].

In 2011 and 2014, Vieira et al. demonstrated that AE reduced significantly the iNOS expression in lung tissue of asthmatic animals [46, 47], and in parallel, Mendes et al. reported in 2011 that there was a striking decrease of exhaled nitric oxide fractions (FeNO) in patients with moderate or severe persistent asthma after 3 months of aerobic training [26].

In contrast, Hizume-Kunzler et al., 2017, and Olivo et al., 2012, described that AE did not alter the increased expression of NOS-2 and eNO, respectively, in the lungs induced by OVA [8, 30]. These results corroborate the findings of Moreira et al., 2008, who showed that AE performed by asthmatic children did not alter FeNO levels [28]. Bonsignore et al., 2008, also observed that in a clinical trial with 50 children with mild persistent asthma, FeNO levels did not change after AE training [6]. According to some authors, these distinctive outcomes may be due to the severity of asthma inflammation, since the regulation of these markers by AE was mainly associated with patients with well-established disease [8, 23].

Hyperresponsiveness and lung remodeling were also widely evaluated through respiratory mechanics and lung tissue analysis, respectively. These data

were related to prostaglandin levels, collagen content and increased tissue growth factors in the lung [16, 17, 22, 46]. In this context, prophylactic and therapeutic AE are also able to decrease bronchial responsiveness and attenuate lung remodeling [2, 17, 39].

In contrast, oxidative stress pathways were poorly explored in asthma models in this SR, despite the fact that they play fundamental roles in lung inflammation and expose or point out possible biochemical mechanisms related to asthma and AE. Nevertheless, Vieira et al., 2014, showed that AE reduced reactive oxygen and nitrogen species (GP91phox and 3-nitrotyrosine) and in 2015, Brüggemann et al. observed that high-intensity exercise did not alter levels of CAT and GPx in an OVA group and that this was partially explained by high levels of NO, which may impair the activity of antioxidant enzymes. On the other hand, in this experimental model, swimming before OVA challenges increased GSH levels, suggesting an additional anti-inflammatory role in asthma [7, 46].

Evaluation of physical fitness and functional tests

In most articles, the MPCT was performed only to determine the training speed, even though it is widely used in clinical research and pulmonary rehabilitation programs as a pivotal outcome of physical fitness [1, 30, 40, 44, 47].

Unclear risks of bias and incomplete investigation reports

In this review, 19 articles presented insufficient information to judge 50% or more of the domains, and 2 studies were classified as having a risk of bias in 60% of

competencies. According to the ARRIVE evaluation guidelines, all studies had incomplete reporting of in more than 50% of items.

These data reinforce the need to improve the quality of the methodological description of studies to allow proper translation of experimental data to clinical practice, as well as to ensure the replicability of experimental asthma protocols [20].

Limitations and Conclusions

Mean and standard deviation values were not presented for components of lung inflammation that were discussed in these studies, and even after contacting the corresponding authors, it was not possible to collect these data. Thus, it was not feasible to perform a meta-analysis in this SR.

Based on the results described, AE was able to attenuate lung inflammation, as well as tissue hyperresponsiveness and remodeling, and this was partially modulated by an increase in anti-inflammatory defenses, a decrease in Th2 response, and an influx of inflammatory cells. These results reinforce the role of AE as an adjuvant treatment for asthmatic patients, despite some apparent contradictions.

Conflict of interest statement

The authors have no conflicts of interest to declare, and each contributed equally to this study.

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Retracted

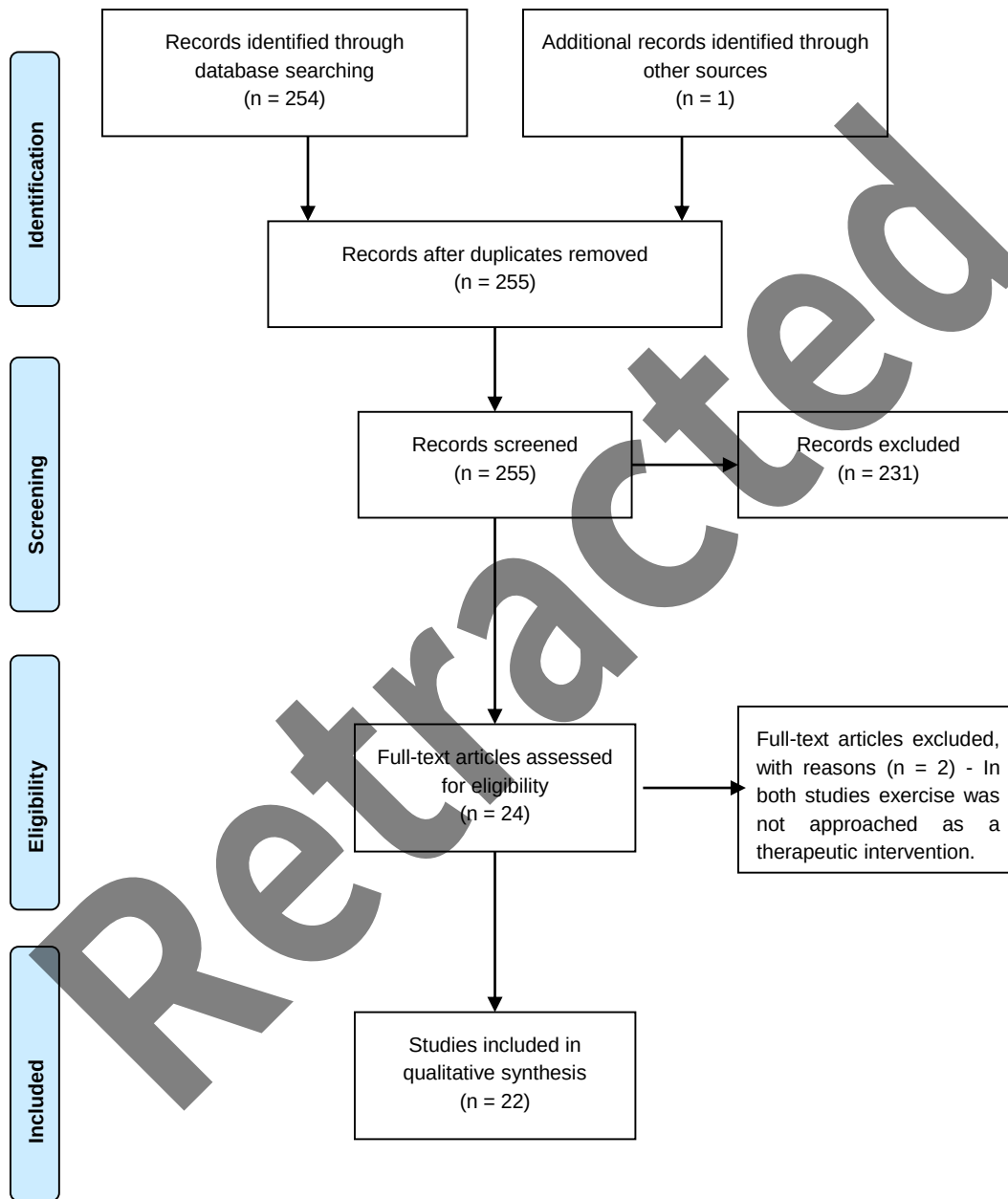
FIGURE LEGENDS

Figure 1. Flow diagram of search procedure

Source: PRISMA Flow Diagram, 2018.

Figure 2. Risk of bias summary

Legend: The bias risk in each study was flagged with a pluss sign (+) for low risk or a minus sign (-) for moderate or high risk. A question mark (?) was used to indicate lack of clarity for proper assessment. Review of the judgments of authors regarding each risk of bias item for each included study, in alphabetic order. The graph was prepared in the Review Manager 5.3 software.



	Random sequence generation (selection bias)	Baseline characteristics (selection bias)	Allocation concealment (selection bias)	Random housing (performance bias)	Blinding of participants and personnel (performance bias)	Random outcome assessment	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Almeida-Oliveira et al., 2019	?	?	?	?	-	-	?	?	+	+
Araújo et al., 2016	?	-	-	?	-	?	-	?	+	+
Brüggemann et al., 2015	?	-	-	?	-	?	?	?	+	+
Dugger et. al., 2018	?	+	?	?	-	-	?	?	+	+
Dugger et al., 2013	?	-	-	?	?	?	?	-	+	+
Hewitt et al., 2009	?	-	-	?	?	?	?	-	+	+
Hewitt et al., 2010	?	-	-	?	?	?	?	?	+	?
Hizume-Kunzler et al., 2017	?	?	-	-	-	?	?	?	+	+
Lowder et al., 2010	?	-	-	+	?	?	?	?	+	+
Mackenzie et al., 2016	?	-	-	?	?	?	?	?	+	+
Mokhtari-Zaer et al., 2018	?	?	?	?	-	-	?	?	+	+
Olivo et al., 2012	?	-	-	?	?	?	?	?	+	+
Pastva et al., 2014	?	-	?	?	?	?	?	-	+	?
Qin et al., 2014	?	-	-	?	?	?	?	-	+	+
Silva et al., 2010	?	+	-	?	?	?	?	-	+	+
Silva et al., 2015	?	-	-	?	-	-	-	-	+	+
Silva et al., 2016	?	-	-	?	?	?	-	?	+	+
Silva et al. 2015	?	-	-	?	?	?	?	-	+	+
Vieira et al., 2007	?	-	-	?	-	-	-	-	+	+
Vieira et al., 2008	?	?	-	?	?	?	?	-	+	+
Vieira et al., 2011	?	-	-	?	?	?	?	-	+	?
Vieira et al., 2014	?	-	-	?	?	?	?	-	+	?

Table 1. Summary finding characteristics

Reference	Animals	Asthma model	OVA Experimental groups	Exercise intervention
Almeida-Oliveira et al., 2019 [1]	C57BL/6 mice, male, adult	HDM, orotracheally administered	HDM non-trained, n=30 HDM trained, n=30	Type: treadmill Start: after HDM sensitization Duration: 60 min/session, 5 times/week, 4 weeks Intensity: moderate intensity
Dugger et al., 2018 [12]	BALB/c mice, female, adult	2 i.p. injections of OVA (200 µL/mouse); aerosol challenge of OVA solution (5 mg/mL), 4 weeks	OVA non-trained, n=ND OVA trained, n=ND	Type: treadmill Start: after OVA sensitization Duration: 45 min/session, 3 times/week, 3 weeks Intensity: moderate intensity
Mokhtari-Zaer et al., 2018 [27]	Wistar rats male, juvenile	2 i.p. injections of OVA (10 µg/mouse); aerosol challenge of OVA solution (1%), 4 weeks	OVA non-trained, n=7 OVA trained, n=7	Type: treadmill Start: after OVA sensitization Duration: 30 min/session, 5 times/week, 4 weeks Intensity: low intensity
Camargo Hizume-Kunzler et al., 2017 [8]	Swiss mice, male, age ND	3 i.p. injections of OVA (10 µg/mouse) and single aerosol challenge of OVA solution (1%)	OVA non-trained, n=10 OVA trained, n=10	Type: treadmill Start: after OVA sensitization Duration: 30 min/session, 15 consecutive days Intensity: moderate intensity
Araújo et al., 2016 [2]	BALB/c mice, male, adult	7 i.p. injections of OVA (10 µg per/mouse); instilled intratracheally of OVA (20 µg/mouse), 3 days	OVA non-trained, n=24 OVA trained, n=24	Type: treadmill Start: before OVA sensitization Duration: 30 min/session, 3 times/week, 8 weeks Intensity: moderate intensity
Mackenzie et al., 2016 [25]	C57BL/6 mice, male, adult	4 i.p. injections of OVA (20 µg/mouse); aerosol challenge of OVA solution (1%), 5 weeks	OVA non-trained, n=20 OVA trained, n=20	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, 4 weeks Intensity: low intensity
Brüggemann et al., 2015 [7]	Swiss mice, male, age ND	2 i.p. injections of OVA (10 µg/mouse); aerosol challenges of OVA solution (1%), 4 days	OVA non-trained, n=8 OVA trained, n=8	Type: swimming Start: after OVA sensitization Duration: 30 min/session, 21 consecutive days Intensity: high intensity
Silva et al., 2016 [38]	BALB/c mice, male, age ND	3 i.p. injections of OVA (20 µg/mouse); aerosol challenge of OVA solution, 2 weeks	OVA non-trained 1 st day, n=8 OVA trained 1 st day, n=8 OVA non-trained 3 rd day, n=8 OVA trained 3 rd day, n=8 OVA non-trained 7 th day, n=8 OVA trained 7 th day, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 7 consecutive days Intensity: low intensity
Qin et al., 2014 [32]	C57BL/6 mice, male, adult	2 i.p. injections of OVA (100 mg/mouse); aerosol challenge of OVA solution, 6 weeks	SRA, n=8 SRA trained, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, 8 weeks Intensity: low intensity
Silva et al., 2015 [37]	BALB/c mice, gender and age ND	4 i.p. injections of OVA (20 µg/mouse); aerosol challenge of OVA solution (1-3%), 5 weeks	OVA non-trained 7 th day, n=8 OVA trained 7 th day, n=8 OVA non-trained 15 th day, n=8 OVA trained 15 th day, n=8 OVA non-trained 30 th day, n=8 OVA trained 30 th day, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, one week (/ days around 3 weeks (15 days around) and 6 weeks (30 days around) Intensity: low intensity
Silva et al., 2015 [39]	BALB/c mice, male, adult	ACBS group: 2 i.p. injections of OVA (20 µg/mouse); aerosol challenge of OVA solution ACAS group: 4 i.p. injections of OVA (10 µg /mouse); aerosol challenge of OVA solution	OVA non-trained ACBS, n=8 OVA trained ACBS, n=8 OVA non-trained ACAS, n=8 OVA trained ACAS, n=8	Type: treadmill Start: after OVA sensitization in ACBS group and after OVA Duration: 60 min/session, 5 times/week, 8 weeks Intensity: low intensity
Vieira et al., 2014 [46]	BALB/c mice, male, age ND	4 i.p. injections of OVA (20 µg/mouse); aerosol challenge of OVA solution (1%), 4 weeks	OVA non-trained, n=8 OVA trained, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, 4 weeks Intensity: low intensity
Dugger et al., 2013 [11]	BALB/c mice, gender and age ND	2 i.p. injections of OVA (50 µg/mouse); aerosol challenge of OVA solution (5 mg/mL), 5 consecutive days; and	OVA non-trained, n=7 OVA trained, n=7	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 3 times/week, 4 weeks Intensity: moderate intensity
Olivo et al., 2012 [30]	Hartley guinea pigs, male, age ND	Aerosol challenge of OVA solution (1-20 mg/mL/mouse), 8 weeks	OVA non-trained, n=8 OVA trained, n=8	Type: treadmill Start: after OVA sensitization Duration: 6 weeks, session and frequency ND Intensity: low intensity
Vieira et al., 2011 [47]	BALB/c mice, male, age ND	4 i.p. injection of OVA (20 µg/mouse); and aerosol challenge of OVA solution (1%), 4 weeks	OVA non-trained, n=8 OVA trained, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, 4 weeks Intensity: low intensity
Hewitt et al., 2010 [17]	BALB/cJ mice, female, adult	2 i.p. injection; aerosol challenge of OVA solution, 3 days	OVA non-trained, n=ND OVA trained, n=ND	Type: treadmill Start: after OVA sensitization Duration: 45 min/session, 3 times/week, 4 weeks Intensity: moderate intensity
Lowder et al., 2010 [22]	BALB/cJ mice, female, adult	2 i.p. injections of OVA (200 µL/mouse); aerosol challenge of OVA solution (5 mg/mL), 5 consecutive days; and	OVA non-trained, n=ND OVA trained, n=ND	Type: treadmill Start: after OVA sensitization Duration: 45 min/session, 3 times/week, 4 weeks Intensity: moderate intensity
Silva et al., 2010 [40]	BALB/c mice, male, adult	4 i.p. injection of OVA (10 µg/mouse); and aerosol challenge of OVA solution (1%), 4 weeks	OVA non-trained, n=7 OVA trained, n=7	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times /week, 4 weeks Intensity: low intensity
Hewitt et al., 2009 [16]	BALB/cJ mice, female, adult	2 i.p. injection of OVA; aerosol challenge of OVA solution, 3 days	OVA non-trained, n=ND OVA trained, n=ND	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, single bout Intensity: moderate intensity
Vieira et al., 2008 [44]	BALB/c mice, male, age ND	4 i.p. injection of OVA (20 µg/mouse); aerosol challenge of OVA solution (1%), 4 weeks	OVA non-trained, n=8 OVA trained low intensity, n=8 OVA trained moderate intensity, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, 4 weeks Intensity: low and moderate intensity
Vieira et al., 2007 [45]	BALB/c mice, male, age ND	4 i.p. injection of OVA (20 µg/mouse); aerosol challenge of OVA solution (1%), 4 weeks	OVA non-trained, n=8 OVA trained low intensity, n=8 OVA trained moderate intensity, n=8	Type: treadmill Start: after OVA sensitization Duration: 60 min/session, 5 times/week, 4 weeks Intensity: low and moderate intensity
Pastva et al., 2004 [31]	BALB/cJ mice, female, adult	2 i.p. injections of OVA (50 µg/mouse); aerosol challenge of OVA solution (5 mg/mL), 5 consecutive days; and	OVA non-trained, n=15 OVA trained, n=15	Type: treadmill Start: after OVA sensitization Duration: 45 min/session, 3 times/week, 4 weeks Intensity: moderate intensity

Abbreviation: HDM, house dust mite; ND, not described; OVA, ovalbumin; ACBS, aerobic conditioning before sensitization; ACAS, aerobic conditioning after sensitization; SRA, steroid-resistant asthma non-trained; MPCT, maximal physical capacity test.

Table 2. Proportion of complete (%) ARRIVE items per year of publication

	Item	References published before 2010	References published since 2010
Introduction	1 Title	75	38.9
	2 Abstract	0	0
	3a Background	100	100
	3b Background	100	100
	4 Objectives	100	77.8
Methods	5 Ethical statement	100	66.7
	6a Study design	75	94.4
	6b Study design	0	0
	6c Study design	50	13.3
	7a Experimental procedures	100	77.8
	7b Experimental procedures	25	6.7
	7c Experimental procedures	25	6.7
	7d Experimental procedures	0	13.3
	8a Animals	0	22.2
	8b Animals	50	27.8
	9a Housing and husbandry	0	0
	9b Housing and husbandry	0	13.3
	9c Housing and husbandry	0	0
	10a Sample size	75	77.8
	10b Sample size	0	0
Results	10c Sample size	0	0
	11a Allocation	0	0
	11b Allocation	100	77.8
	12 Experimental results	100	100
	13a Statical methods	0	27.8
Discussion	13b Statical methods	100	100
	13c Statical methods	100	100
	14 Baseline data	0	0
	15a Numbers analyzed	50	33.3
	15b Numbers analyzed	0	0
	16 Results and estimates	0	0
	17a Adverse events	0	0
	17b Adverse events	0	0
	18a Interpretation	100	100
	18b Interpretation	0	20
	18c Interpretation	0	0
	19 Generalizability/ translation	100	100
	20 Funding	50	72.2

Table 3. Proportions of reported ARRIVE items

		% ARRIVE		
		Complete	Partially	Incomplete
References published before 2010	Hewitt et al., 2009 [16]	39.47	36.84	23.68
	Vieira et al., 2008 [44]	42.11	18.42	39.47
	Vieira et al., 2007 [45]	39.47	21.05	39.47
	Pastva et al., 2004 [31]	44.74	26.32	28.95
	Almeida-Oliveira et al., 2019 [1]	39.47	23.68	36.84
	Dugger et al., 2018 [12]	31.58	31.58	36.84
	Mokhtari-Zaer et al., 2018 [27]	36.84	31.58	31.58
	Hizume-Kunzler et al., 2017 [8]	36.84	23.68	39.47
	Araújo et al., 2016 [2]	42.11	23.68	34.21
	Mackenzie et al., 2016 [25]	36.84	23.68	39.47
References published since 2010	Brüggemann et al., 2015 [7]	31.58	23.68	44.74
	Silva et al., 2016 [38]	34.21	23.68	42.11
	Qin et al., 2014 [32]	39.47	26.32	34.21
	Silva et al., 2015 [37]	39.47	7.89	52.63
	Silva et al., 2015 [39]	42.11	15.79	42.11
	Vieira et al., 2014 [46]	36.84	18.42	44.74
	Dugger et al., 2013 [11]	34.21	31.58	34.21
	Olivo et al., 2012 [30]	36.84	23.68	39.47
	Vieira et al., 2011 [47]	34.21	21.05	44.74
	Hewitt et al., 2010 [17]	44.74	28.95	26.32
	Lowder et al., 2010 [22]	42.11	28.95	28.92
	Silva et al., 2010 [40]	44.74	18.42	36.84

Table 4. Effects of exercise on lung inflammation

Reference	Exercise intensity	Cells	Th2 proinflammatory cytokines	Anti-inflammatory cytokines	Total IgE	Anti-OVA IgG1 and IgE	NO/isoforms
Almeida-Oliveira et al., 2019 [1]	Moderate	Reduced total and differential cells in BALF	Reduced IL-4, IL-5, IL-13, IL-17, IL-23, and IL-33 in BALF	Increased IL-10 in BALF	Not rated	Not rated	Not rated
Dugger et al., 2018 [12]	Moderate	Not rated	Reduced IL-4, IL-5, and IL-13 in BALF	Not rated	Not rated	Not rated	Not rated
Mokhtari-Zaer et al., 2018 [27]	Low	Reduced total and differential cells in BALF	Not rated	Not rated	Not rated	Not rated	Not rated
Hizume-Kunzler et al., 2017 [8]	Moderate	Reduced total and differential cells in BALF	Reduced IL-4 and IL-5 in lung homogenate	Increased IL-10 of lung levels	Reduced total IgE in lung homogenate	Reduced OVA-specific IgE and IgG1 titers	No change in lung levels of blunted NOS-2
Araújo et al., 2016 [2]	Moderate	Reduced peribronchial eosinophil infiltration	Reduced IL-4 and IL-13 in BALF and plasma	Increased IL-10 in BALF and plasma	Not rated	Not rated	Not rated
Mackenzie et al., 2016 [25]	Low	Reduced total and differential cells in BALF	Reduced IL-4, IL-5, IL-6, IL-13, and TNF- α in BALF	Increased IL-10 in BALF	Not rated	Not rated	Not rated
Brüggemann et al., 2015 [7]	High	Reduced total and differential cells in BALF	Reduced IL-4, IL-5, and IL-6 in lung homogenate	Increased IL-10 of lung levels	Reduced total IgE in lung serum	Not rated	Not rated
Silva et al., 2016 [38]	Low	Reduced eosinophil migration in BALF	Reduced IL-4 and IL-5 in lung homogenate	Increased IL-10 and IL-1ra of lung levels	Not rated	Not rated	Not rated
Qin et al., 2014 [32]	Low	Reduced total cells and neutrophils in BALF	Reduced IL-17 in BALF	Not rated	Not rated	Not rated	Not rated
Silva et al., 2015 [37]	Low	Reduced total and differential cells in BALF	Reduced IL-5 in lung homogenate	Not rated	Not rated	Not rated	Not rated
Silva et al., 2015 [39]	Low	Reduced peribronchial eosinophil infiltration	Reduced IL-4, IL-5, and IL-13 in lung homogenate	Increased IL-10 in lung tissue	Not rated		Not rated
Vieira et al., 2014 [46]	Low	Not rated	Reduced IL-4, IL-5, and IL-13 in BALF and serum	Increased IL-10 in BALF	Not rated	Not rated	Reduced iNOS expression
Dugger et al., 2013 [11]	Moderate	Reduced the number of lymphocytes in lung tissue	Reduced IL-4 and increased IL-13 in BALF	Not rated	Not rated	Not rated	Not rated
Olivo et al., 2012 [30]	Low	Reduced density of peribronchial eosinophils and lymphocytes	Reduced IL-4 and IL-13 in lung tissue	Did not change	Not rated	No change	No change the ENO levels
Vieira et al., 2011 [47]	Low	Reduced total cells and eosinophils in BALF	Reduced epithelial expression of IL-13, IL-4, and IL-5	Increased epithelial expression of IL-10	Not rated	Not rated	Reduced expression of iNOS
Hewitt et al., 2010 [17]	Moderate	Not rated	Not rated	Not rated	Not rated	Not rated	Not rated
Lowder et al., 2010 [22]	Moderate	Reduced total and differential cells in BALF	Reduced IL-4, IL5, and IL-17 in CD4+CD25+Foxp3+ Treg cell suppression cultures	Reduced IL-10	Not rated	Not rated	Not rated
Silva et al., 2010 [40]	Low	Reduced peribronchial eosinophils and CD3+ and CD4+ lymphocytes	Reduced IL-4, IL-5, and IL-13 peribronchial expression	Increased IL-10 and IL-1ra in lung tissue	Not rated	No change	Not rated
Hewitt et al., 2009 [16]	Moderate	Reduced total and differential cells	Reduced IL-5 and IL-13 in BALF	Not rated	Not rated	Not rated	Not rated
Vieira et al., 2008 [44]	Low and moderate	Reduced density of eosinophils in perivascular tissue and in lung parenchyma	Reduced IL-4 and IL-5 perivascular and parenchymal density	Increased IL-10 perivascular and parenchymal density	Not rated	Not rated	Not rated
Vieira et al., 2007 [45]	Low and moderate	Reduced eosinophil count in BALF and infiltration in airway walls	Reduced IL-4 and IL-5 density of cells positive	Increased IL-10 peribronchial density	Not rated	No change	Not rated
Pastva et al., 2004 [31]	Moderate	Reduced total cells and differential cells in BALF	Reduced IL-4 in BALF	Not rated	No change	Reduced OVA-specific IgE titers	Not rated

Abbreviations: IgE, Immunoglobulin E; IgG1, Immunoglobulin G subclasse; NO, nitric oxide BALF, bronchoalveolar lavage fluid; OVA, Ovalbumin; AE, aerobic exercise; IL, interleukin; NOS-2, nitric oxide synthase 2; TNF- α , tumor necrosis factor alpha; Th, T helper cells; ENO, exhaled nitric oxide; iNOS, induced nitric oxide synthase; CD, cluster of differentiation; Foxp3, forkhead box P3.

Supplementary data

TITLE: Effects of physical exercise on lung inflammation in animal models of chronic allergic lung inflammation: a systematic review

Supplementary Appendix 1 - Search Strategy

SEARCH COMPONENT 1 (SC1) Physical exercise

1. Physical exercise
2. Aerobic exercise
3. Exercise
4. Training
5. Physical training
6. Swimming
7. Treadmill
8. Treadmill training
9. Running

SEARCH COMPONENT 2 (SC2) Lung inflammation

10. Lung inflammation
11. Lung injury
12. Inflammation
13. Immunomodulation
14. Airway inflammation
15. Pulmonary inflammation
16. Pulmonary disease
17. Immunology
18. Chronic airway inflammation
19. Airway remodeling

SEARCH COMPONENT 3 (SC3) Asthma

20. Asthma
21. House dust mite induce allergic asthma
22. Allergic asthma
23. Ovalbumin induced allergic asthma
24. Chronic allergic lung

MEDLINE PUBMED

((("Aerobic exercise"[All Fields] OR "Exercise"[All Fields] OR "Physical exercise"[All Fields] OR "Training"[All Fields] OR "Physical training"[All Fields] OR "Swimming"[All Fields] OR "Treadmill"[All Fields] OR "Treadmill training"[All Fields] OR "Running"[All Fields]) AND ("Lung inflammation"[All Fields] OR "Lung injury"[All Fields] OR "Inflammation"[All Fields] OR "Immunomodulation"[All Fields] OR "Airway inflammation"[All Fields] OR "Pulmonary inflammation"[All Fields] OR "Pulmonary disease"[All Fields] OR "Immunology"[All Fields] OR "Chronic airway inflammation"[All Fields] OR "Airway remodeling"[All Fields]) AND ("Asthma"[All Fields] OR (("pyroglyphidae"[MeSH Terms] OR "pyroglyphidae"[All Fields] OR ("house"[All Fields] AND "dust"[All Fields] AND "mite"[All Fields]) OR "house dust mite"[All Fields]) AND induce[All Fields] AND allergic[All Fields] AND ("asthma"[MeSH Terms] OR "asthma"[All Fields])) OR "Allergic asthma"[All Fields] OR "Ovalbumin induced allergic asthma"[All Fields] OR "Chronic allergic lung"[All Fields])) AND "animals"[MeSH Terms:noexp]

Filters: Other animals

SCIENCE DIRECT

((("Aerobic exercise" OR "Exercise" OR "Physical exercise" OR "Training" OR "Physical training" OR "Swimming" OR "Treadmill" OR "Treadmill training" OR "Running") AND ("Lung inflammation" OR "Lung injury" OR "Inflammation" OR "Immunomodulation" OR "Airway inflammation" OR "Pulmonary inflammation" OR "Pulmonary disease" OR "Immunology" OR "Chronic airway inflammation" OR "Airway remodeling") AND ("Asthma" OR "House dust mite induce allergic asthma" OR "Allergic asthma" OR "Ovalbumin induced allergic asthma" OR "Chronic allergic lung"))

Filters: Journals and all years.

Supplementary Table 1 - Eligibility table

Identification	Eligibility criteria				Inclusion for analysis	
	Asthma animal model	Physical exercise intervention	Lung inflammation assessment		YES	NO, reason
Effects of Aerobic Exercise on Molecular Aspects of Asthma: Involvement of SOCS-JAK-STAT Beta-2 adrenergic receptors increase TREG cell suppression in an OVA-induced allergic asthma mouse model when mice are moderate aerobically exercised	Yes	Yes	Yes		YES	
The effects of exercise on depressive- and anxiety-like behaviors as well as lung and hippocampus oxidative stress in ovalbumin-sensitized juvenile rats	Yes	Yes	Yes		YES	
Aerobic Exercise Decreases Lung Inflammation by IgE Decrement in an OVA Mice Model	Yes	Yes	Yes		YES	

Lack of desensitization of the cough reflex in ovalbumin-sensitized rabbits during exercise	Yes	Not	Yes	NO, did not use physical exercise as therapeutic intervention
Regular and moderate aerobic training before allergic asthma induction reduces lung inflammation and remodeling.	Yes	Yes	Yes	YES
Dendritic Cells Are Involved in the Effects of Exercise in a Model of Asthma	Yes	Yes	Yes	YES
Effects of Swimming on the Inflammatory and Redox Response in a Model of Allergic Asthma	Yes	Yes	Yes	YES
Exercise reverses OVA-induced inhibition of glucocorticoid receptor and increases anti-inflammatory cytokines in asthma	Yes	Yes	Yes	YES
Low-intensity aerobic exercise training attenuates airway inflammation and remodeling in a rat model of steroid-	Yes	Yes	Yes	YES

resistant asthma				
Airway remodeling is reversed by aerobic training in a murine model of chronic asthma	Yes	Yes	Yes	YES
Comparison of the effects of aerobic conditioning before and after pulmonary allergic inflammation	Yes	Yes	Yes	YES
Exercise deactivates leukocytes in asthma	Yes	Yes	Yes	YES
Moderate aerobic exercise alters migration patterns of antigen specific T helper cells within an asthmatic lung	Yes	Yes	Yes	YES
High-intensity training induces EIB in rats through neuron transdifferentiation of adrenal medulla chromaffin cells	Yes	Not	Yes	NO, did not use physical exercise as therapeutic intervention
Effects of aerobic exercise on chronic allergic airway inflammation and remodeling in guinea pigs	Yes	Yes	Yes	YES
Airway epithelium mediates the anti-	Yes	Yes	Yes	YES

inflammatory effects of exercise on asthma

Repeated bouts of moderate-intensity aerobic exercise reduce airway reactivity in a murine asthma model

Yes

Yes

Yes

YES

Repeated bouts of aerobic exercise enhance regulatory T cell responses in a murine asthma model

Yes

Yes

Yes

YES

Aerobic training reverses airway inflammation and remodelling in an asthma murine model

Yes

Yes

Yes

YES

Acute exercise decreases airway inflammation, but not responsiveness, in an allergic asthma model

Yes

Yes

Yes

YES

Aerobic conditioning and allergic pulmonary inflammation in mice. II. Effects on lung vascular and parenchymal inflammation and

Yes

Yes

Yes

YES

remodeling				
Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice	Yes	Yes	Yes	YES
Aerobic exercise attenuates airway inflammatory responses in a mouse model of atopic asthma	Yes	Yes	Yes	YES
