Aerobic exercise inhibits acute lung injury: from mouse to human evidence
Exercise reduced lung injury markers in mouse and in cells

Nicole Cristine Rigonato-Oliveira[1], BreAnne MacKenzie[2], Andre Luis Lacerda Bachi[2], Manoel Carneiro Oliveira-Junior[2], Alana Santos-Dias[2], Maysa Alves Rodrigues Brandao-Rangel[2,3], Humberto Delle[1], Tamara Costa-Guimaraes[2,4], Nilsa Regina Damaceno-Rodrigues[5], Larissa Hilário Dulley[2], Marcela Anhesini Benetti[2], Christiane Malfitano[6], Kátia de Angelis[7], Regiane Albertini[3], Ana Paula Ligeiro Oliveira[1], Asghar Abbasi[8], Hinnak Northoff[9*], Rodolfo Paula Vieira[2,3,4*]

[1] Nove de Julho University, São Paulo - SP, Brazil.
[2] Brazilian Institute of Teaching and Research in Pulmonary and Exercise Immunology (IBEPIPE), São José dos Campos - SP, Brazil.
[3] Federal University of São Paulo (UNIFESP), Post-graduation Program in Sciences of Human Movement and Rehabilitation, Santos - SP, Brazil.
[4] Universidade Brasil, São Paulo - SP, Brazil.
[5] University of Sao Paulo, School of Medicine, Department of Pathology (LIM 59), Sao Paulo - SP, Brazil.
[6] Science Department of Health, Universidade Federal de Lavras (UFLA), Lavras - MG, Brazil.
[7] Federal University of São Paulo (UNIFESP), Campus São Paulo, São Paulo - SP, Brazil.
[8] Division of Respiratory & Critical Care Physiology & Medicine, Los Angeles Biomedical Research Institute at Harbor - UCLA Medical Center, California - CA, USA.
[9] Institute of Clinical and Experimental Transfusion Medicine (IKET), University of Tübingen, Tübingen, Germany.

[*] HN and RPV have equally contributed to this work.

ABSTRACT

Acute respiratory distress syndrome (ARDS) is defined as hypoxemic respiratory failure with intense pulmonary inflammation, involving hyperactivation of endothelial cells and neutrophils. Given the anti-inflammatory effects of aerobic exercise (AE), this study investigated whether AE performed daily for 5 weeks would inhibit extra-pulmonary LPS-induced ARDS. C57Bl/6 mice were distributed into Control, Exercise, LPS and Exercise+LPS groups. AE was performed on a treadmill for 5x/week for four weeks before LPS administration. 24hours after the final AE physical test, animals received 100ug of LPS intra-peritoneally. In addition, whole blood cell culture, neutrophils and human endothelial cells were pre-incubated with IL-10, an anti-inflammatory cytokine induced by exercise. AE reduced total protein levels ($p<0.01$) and neutrophil accumulation in bronchoalveolar lavage (BAL) ($p<0.01$) and lung parenchyma ($p<0.01$). AE reduced BAL inflammatory cytokines IL-1β, IL-6 and GM-CSF ($p<0.001$), CXCL1/KC, IL-17, TNF-alpha and IGF-1 ($p<0.01$). Systemically, AE reduced IL-1β, IL-6 and IFN-gamma ($p<0.001$), CXCL1/KC ($p<0.01$) and TNF-alpha ($p<0.05$). AE increased IL-10 levels in serum ($p<0.001$) and BAL ($p<0.001$). Furthermore, AE increased superoxide dismutase SOD ($p<0.01$) and decreased superoxide anion accumulation in the lungs ($p<0.01$). Lastly, pre-incubation with IL-10 significantly reduced LPS-induced activation of whole blood cells, neutrophils and HUVECs, as observed by reduced production of IL-1β, IL-6, IL-8 and TNF-alpha. Our data suggest that AE inhibited LPS-induced lung inflammation by attenuating inflammatory cytokines and oxidative stress markers in mice and human cell culture via enhanced IL-10 production.

Key words: exercise immunology, lung inflammation, immune response, acute lung injury, cytokines.

INTRODUCTION

Acute and chronic exercise alters both the innate and adaptive immune response (38, 39). Anti-inflammatory effects are observed in individuals who perform low to moderate intensity exercise, as well as endurance exercise with marginal effects on innate immune cell numbers. In contrast, acute high intensity and/or long duration exercise induce profound increases in the number of neutrophils and lymphocytes (13, 38). The innate immune response is the first line of defense against infectious pathogens, while adaptive immune response corresponds to a pathogen-specific immune response (5).

In addition to exercise's ability to modulate the systemic immune response in healthy subjects (38, 39), a growing number of studies have shown that exercise also positively modu-

Corresponding author:
Prof. Dr. Rodolfo de Paula Vieira, rodrelena@yahoo.com.br
Universidade Brasil, Instituto de Pesquisa, Rua Carolina Fonseca 285, Itaquera, São Paulo - SP, Brazil, 08230-030

lates the innate and adaptive pulmonary immune response in the context of respiratory diseases, such as asthma (33-37), chronic obstructive pulmonary diseases (COPD) (37), lung fibrosis (26, 27), as well as acute respiratory distress syndrome (ARDS) (8, 28, 30). In general, these studies have shown that low and moderate intensity exercise when performed regularly, results in NF-kB deactivation, anti-inflammatory cytokine release, and reduced oxidative stress.

ARDS is characterized by hypoxemic respiratory failure, intense bilateral pulmonary inflammation, accumulation of proteinaceous exudate in the alveoli, and disruption of alveolar membrane barrier; processes which are mediated by pro-inflammatory cytokines (29). The mortality rate increases progressively with age, ranging from 24 percent among patients 15 to 19 years of age to 60 percent among patients 85 years of age older (overall 41%) (31). Survivors need special attention to non-pulmonary comorbidities, such as skeletal muscle wasting and weakness, in which physical and exercise therapy could present beneficial effects, as recently demonstrated in an experimental study (10). At the time of this submission no correlation studies have been performed linking physical fitness to ARDS outcome.

Given the ability of chronic, moderate AE to suppress systemic and pulmonary inflammation (13) as well as oxidative stress (7), the aim of the present study was to investigate whether chronic, moderate intensity aerobic exercise (AE) prior to injury attenuates extra-pulmonary LPS-induced ARDS.

METHODS

Animals and experimental design

This study was approved by the research ethics committee at the Nove de Julho University (UNINOVE), protocol: AN0035.2013.

Forty male C57Bl/6 mice, 8 weeks of age,weighing between 20 and 25g were maintained in the animal center of the UNINOVE in standard conditions. Mice (n=10/group) were distributed into Control (Control), LPS (LPS), Exercise (Exe) and Exercise+LPS (Exe+LPS).

Treadmill aerobic exercise test and training

Exercise physical test and training was performed as previously described (34). Briefly, the animals from all groups were adapted to the treadmill training during three days (15 min, 25% grade, 0.3Km/h) before the first physical test. Then, maximum exercise capacity test was performed as follow; 5 minutes warm-up (25% grade, 0.3Km/h) followed by an increase in the treadmill speed (0.1Km/h each 2.5 min) until the animals' exhaustion, until they were not able to run even after 10 mechanical stimuli. Maximal exercise capacity (100%) was set as the maximum speed reached by each mouse. Mice were then trained using moderate intensity exercise (60% of average maximum speed) for 60 min/session, 5 days/week for 4 weeks. The maximum exercise capacity test was repeated for all animals after four weeks (34).

Extra-pulmonary model of LPS-induced ARDS

Twenty-four hours after the last maximum exercise capacity test, the animals were anesthetized (ketamine 100 mg/kg and xylazine 10 mg/kg) and received intra-peritoneal administration of 100 μg of Escherichia coli LPS (026:B6; L3755, Sigma Aldrich, St. Louis, MO, USA) suspended in phosphate buffered solution (PBS). Control and Exercise groups were submitted to intra-peritoneal injection of PBS. Treadmill training was performed for 4 weeks followed by the final physical test. Then, twenty-four hours after final physical test, animals received LPS administration, as stated above. Then, twenty-four hours later, animals were anesthetized and the following analysis were performed.

Blood collection and processing

One milliliter of blood was collected via cava vein and immediately centrifuged at 950g, 4ºC, during 7 min. The serum was collected and stored at -70ºC for cytokines measurement.

Bronchoalveolar lavage (BAL) fluid

To assess lung inflammation total and differential cells counts of BAL fluid was performed. BAL was obtained through a gentle washing of 3 x 0.5 ml PBS. The volume recovered was centrifuged at 900g, at 4ºC, for 7 min. The supernatant was stored at -70ºC for cytokines analysis and the cell pellet was resuspended in 1mL PBS. The total number of cells was counted using a hemocytometer and the differential cells count was performed through a cytospin preparation, stained with Diff Quick and 300 cells were counted according to the hematological criteria (28, 30).

Total proteins measurement in BAL

Total proteins in BAL fluid was quantified using the bicinchoninic acid (BCA) method to evaluate lung vascular permeability as previously described (30).

Cytokines measurements

The levels of IL-1β, IL-6, CXCL1/KC, IL-10, IL-17, TNF-α, GM-CSF, IFN-γ and IGF-1 were evaluated using ELISA kits from R&D Systems (Minneapolis, MN, USA) and BioLegend (San Diego, CA, USA) according to the manufacturer's instructions.

Histomorphometric study

To evaluate the effects of exercise on parenchymal inflammation, a hallmark of ARDS, lungs were collected, fixed in 10% formalin and submitted to histological routine. Briefly, 5μm thickness lung slices were stained with hematoxylin and eosin. 15 random fields of the lung parenchyma of each mouse were photographed. Image Pro Plus 4.0 software was used to measure the air and tissue area of all photomicrographs. Neutrophils were counted according to morphological criteria by an experienced researcher; groups were blinded. The number of neutrophils per square millimeter of lung tissue was presented (30, 35).

Oxidative stress and superoxide dismutase evaluation

The cranial lobe was washed with PBS and frozen at -70°C for analysis of oxidative stress. Approximately 50mg was used for the evaluation of superoxide dismutase (SOD) and QL (superoxide anion). After homogenization for 30 seconds in 1.15% phenyl methyl sulfonyl fluoride (PMSF) (100mmol/L) and in isopropanol 10uL/mL, the samples were centrifuged for 10min, 4ºC, at 950g and the supernatant was

frozen at -80°C. Protein content was measured by the Lowry's' method (21).

Chemiluminescence (QL): Lipid peroxidation was assessed by chemiluminescence. The chemiluminescence assay was performed with a liquid scintillation LKB Rack Beta 1215 spectrophotometer (LKB Producer AB, USA) in order to room temperature (25°C to 27°C) to out-of-coincidence. Supernatants were diluted in 140 mM KCl and sodium phosphate buffer 20 mM pH 7.4 and added to glass tubes, which were placed in scintillation vials; 3 mM tert-butyl hydroperoxide was added, and chemiluminescence was determined at the emission maximum (14, 17).

Superoxide Dismutase (SOD): SOD activity was measured by spectrophotometry in lung homogenates by inhibition of pyrogallol autoxidation rate at 420 nm. The enzymatic activity was reported as U/mg protein. The technique used was based on inhibition of superoxide radical reaction with piragalol. Since it is unable to determine the concentration of the enzyme or its activity in terms of substrate consumed per unit time, quantification was expressed in relative units. One unit of SOD is defined as the amount of enzyme that inhibits the reaction by 50% of the oxidation rate detector. The oxidation of pyrogallol leads to the formation of a colored product, which was detected at 420nm for 2min. The SOD activity was determined by measuring the rate of formation of the oxidized pyrogallol. In the reaction medium, 20uL of lung homogenate was used, 973uL Tris-Phosphate buffer 50 mmol/L (pH 8.2), 8uL pyrogallol (to 24 mmol/L) and 4uL of CAT (30umol/L). This curve obtained was used as a blank. A standard curve was also performed using three different concentrations of SOD (0,25U, and 0.5U 1U), and values were calculated based on the slope-intercept of the standard curve.

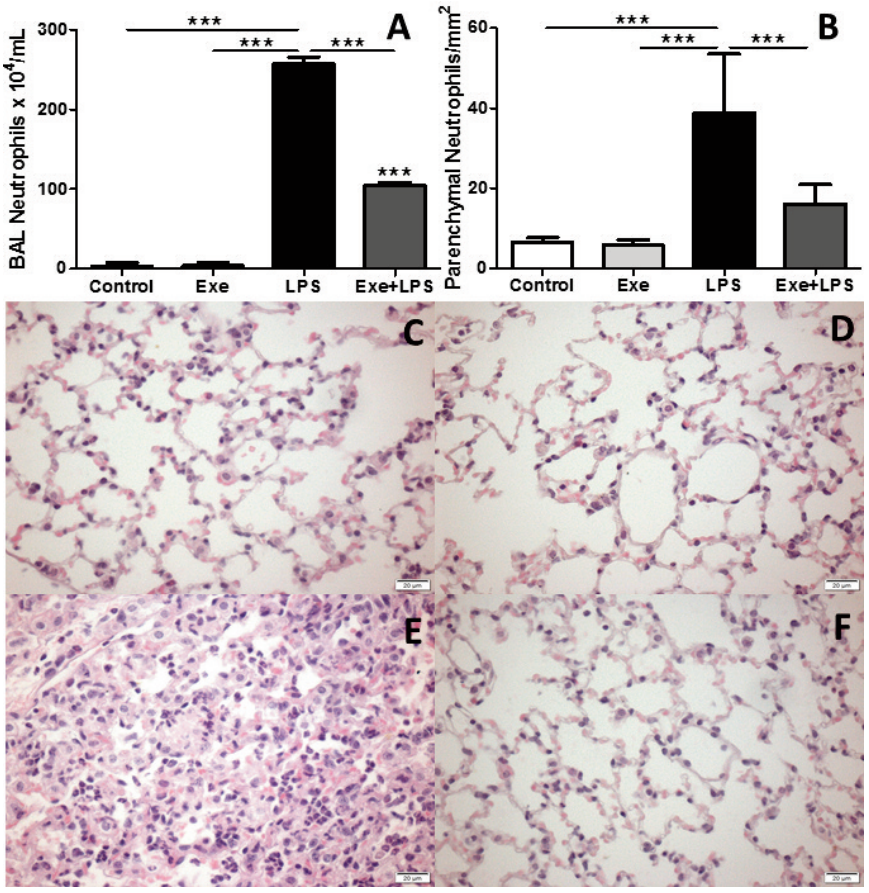

Figure 1. Neutrophils count in BAL and parenchyma. Results expressed as the number of neutrophils x 10^5 in BAL per mL (Figure 1A) and as number of neutrophils per mm2 of lung parenchyma (Figure 1B). *** p<0.001. Figures 1C-F are representative photomicrographs of lung parenchyma stained with hematoxylin and eosin, respectively from Control (Figure 1C), Exercise (Figure 1D), LPS (Figure 1E) and Exercise+LPS (Figure 1F) groups. *** p<0.001.

Human Cell Cultures Studies

For all *in vitro* studies, IL-10 was used in order to mimic the effects observed in the *in vivo* exercise protocol, which was performed prior to LPS inoculation and resulted in a strong release of anti-inflammatory cytokine IL-10. In summary, we cell cultures (whole blood cells, neutrophils and endothelial cells) were pre-incubated with human recombinant IL-10 (10 ng/mL) for one hour and followed by stimulation with lipopolysaccharide (LPS) (Escherichia coli 026:B6; L3755, Sigma Aldrich, St. Louis, MO, USA; final concentration 10 ng/mL) for 4 hours, at 37ºC, 5% CO2 at humid atmosphere. Therefore, we had four final conditions in our cell culture performed in triplicate: Control (medium stimulated only), IL-10 (stimulated with IL-10), LPS (only LPS-stimulated) and IL-10+LPS (incubated with IL-10 for 1 hour + LPS stimulation and recovery after 4 hours). After 4 hours of LPS stimulation, the cells were centrifuged for 7 minutes, at 4ºC. IL-1β, IL-6, IL-8 and TNF-alpha; indicators of cellular activation were measured in both the serum and supernatant.

Whole blood cell culture

Blood samples were drawn from each mouse and were cultured using whole blood culture system as described previously (1). Briefly, venous blood samples were drawn from the antecubital vein in a sitting position and collected into endotoxin-free K3-EDTA tubes (Vacuette, Greiner bio-one-Frickenhausen, Germany). 3 × 4-ml whole blood were stimulated with or without IL-10 and lipopolysaccharide (LPS) in 6 well plate in humid atmosphere as described above.

Human umbilical vein endothelial cells (HUVEC) culture

HUVEC (3 x 10^4 cells/300µL medium) were cultivated in vascular cell basal media (ATCC PCS100030) supplemented with endothelial cell growth kit (ATCC PCS100040) components. The stimulation protocol was applied as described above.

Human neutrophils

Five milliliter of venous blood were collected and the neutrophils were isolated by using the EasySep™ Human Neutrophil Enrichment Kit (Stemcell, Cod 19257, Cambridge, MA, USA), according to the manufacturer's instructions. 5 x 10^4/mL neutrophils per well per mL were seeded in 24 well plate and stimulated as described above.

Statistical analysis

Graph pad prism 5.0 software was used to perform two-way ANOVA, followed by Student–Newman–Keuls post hoc test. Differences were considered significant for $p< 0.05$.

RESULTS

Aerobic exercise inhibits pulmonary inflammation

During lung injury, cytokines hone neutrophils to the lung to clear and kill invading pathogens. Neutrophil influx was measured in both the bronchial alveolar lavage fluid (BAL) and lung parenchyma. AE reduced neutrophil influx in the BAL of Exercise+LPS mice (Figure 1A). Likewise, the number of neutrophils was reduced in the lung parenchyma (Figure 1B). Representative photomicrographs of lung parenchyma stained with hematoxylin and eosin (H+E) reveal reduced neutrophil number in the alveolar spaces and in the parenchyma of Exercise+LPS animals (Figure 1F) compared to LPS alone (Figure 1E). Neutrophil numbers were low in sedentary (Figure 1C) and exercised animals (Figure 1D).

Aerobic exercise modulates pulmonary cytokine and growth factor release

A pro-inflammatory cytokine "storm" is implicated in death due to ARDS. Therefore, pro and anti-inflammatory cytokine levels were assessed in the BALF using ELISA technique. AE reduced BAL levels of inflammatory cytokines including interleukin (IL) IL-1β (Figure 2A), IL-6 (Figure 2B), chemokine (C-X-C motif) ligand 1 (CXCL1)/KC (Figure 2C), IL-17 (Figure 2E), tumor necrosis factor (TNF)-alpha (Figure 2F), Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Figure 2G), and IGF-1 ($p<0.01$) (Figure 2I). In contrast, anti-inflammatory cytokine IL-10 was increased in both exercise only and Exe+LPS mice (Figure 2D). Interferon-gamma was not significantly regulated in this model (Figure 2H). The results are expressed as picograms of cytokines per mL of BAL. *** $p<0.001$; ** $p<0.01$; * $p<0.05$. In summary, pro-inflammatory cytokines were reduced in exercised mice and anti-inflammatory cytokine IL-10 was increased.

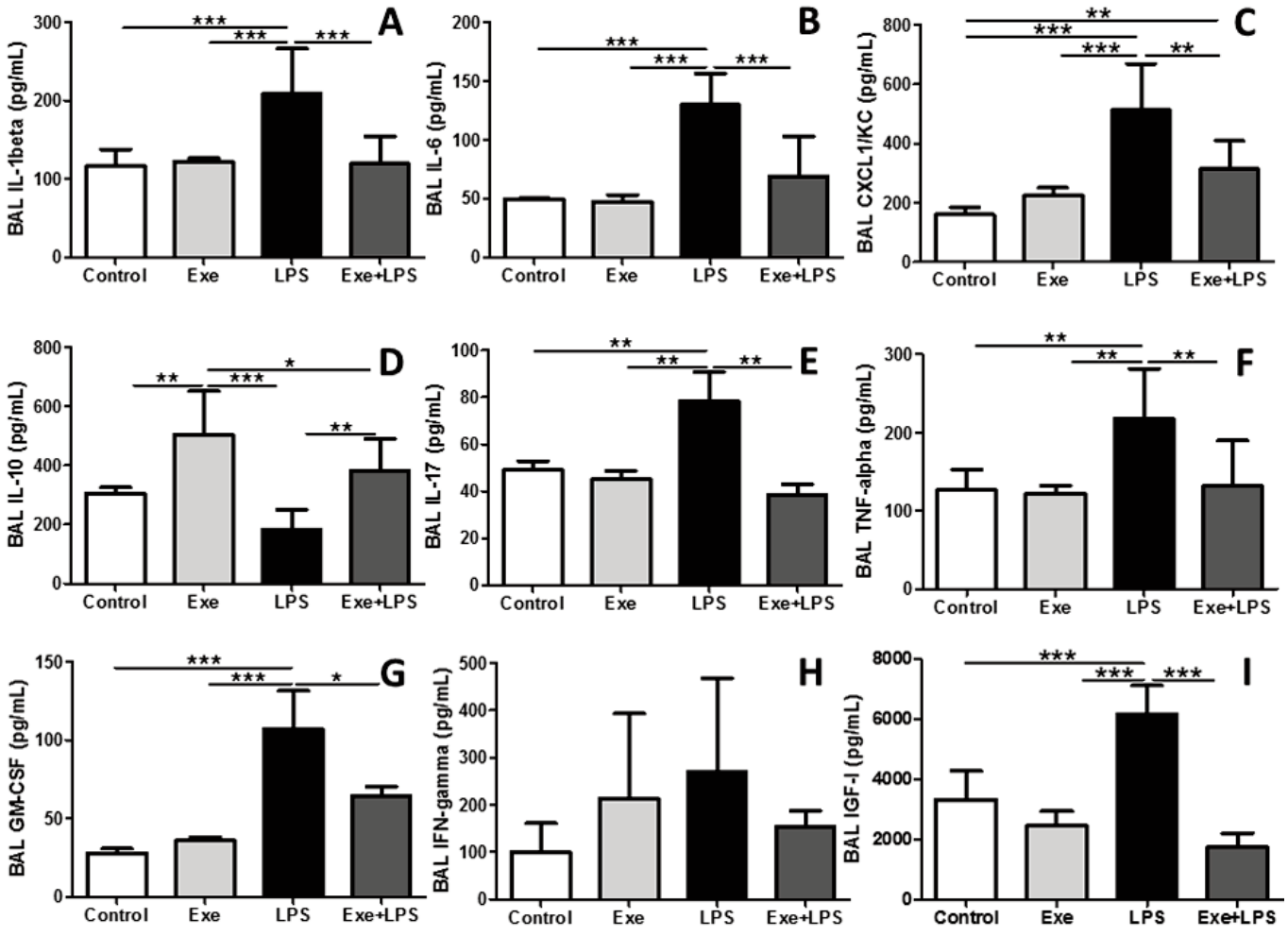

Figure 2. Pro and anti-inflammatory cytokines in BAL. The results are expressed as picograms of cytokines per mL of BAL. *** $p<0.001$; ** $p<0.01$; * $p<0.05$.

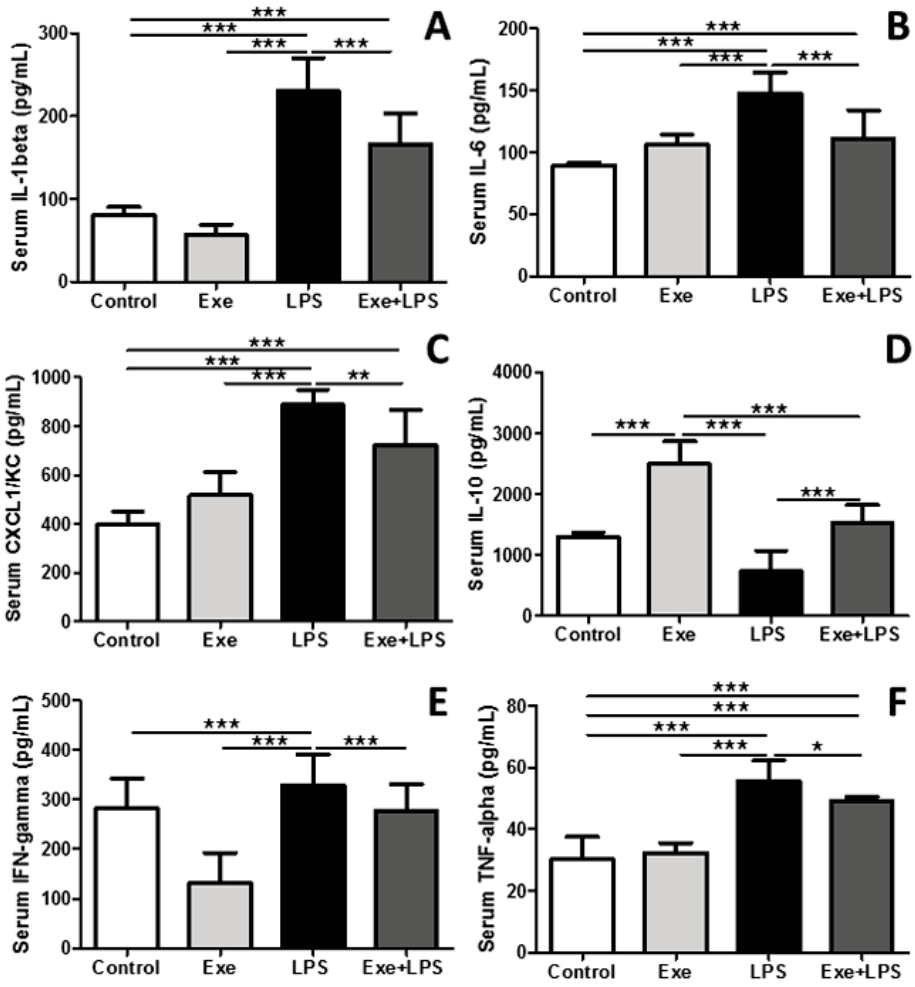

Figure 3. Pro and anti-inflammatory cytokines in serum. The results are expressed as picograms of cytokines per mL or serum. *** p<0.001; ** p<0.01; * p<0.05.

Aerobic exercise modulates systemic cytokine release

Serum cytokine levels can also indicate extent of severity of ARDS. Therefore, serum was collected and cytokines were measured by ELISA (Figure 3). IL-β (Figure 3A), IL-6 (Figure 3B), CXCL1 (Figure 3C), and TNF-alpha (Figure 3F) were reduced in Exercise+LPS animals. Anti-inflammatory cytokine IL-10 was increased in serum of exercised animals (Figure 3D). IFN-gamma was slightly reduced in Exe+LPS mice versus LPS mice (Figure 3E). In summary, exercising before injury resulted in reduced levels of pro-inflammatory cytokines in the serum.

Aerobic exercise inhibits oxidative stress and increases antioxidant enzyme (SOD)

Increased protein levels in BAL fluid indicates alveolar damage and leakage. Animals that performed AE before LPS injury, had less total protein in BAL fluid (Figure 4A). The reactive oxygen species, superoxide anion, expressed as anion units per mg of lung protein was also reduced in BAL fluid of Exe+LPS mice (Figure 4B). Exercise increased the antioxidant superoxide dismutase (SOD) (Figure 4C). In summary, AE decreased oxidative stress and alveolar damage.

IL-10 pre-incubation inhibits whole blood cells, endothelial cells and neutrophils activation

To mimic the observed exercised-induced production of IL-10 post-exercise in vivo, human whole blood cells, endothelial cells and neutrophils were incubated with IL-10 prior to LPS administration. Pre-incubation with IL-10 resulted in reduced production of IL-1β ($p<0.001$), IL-6 ($p<0.001$), IL-8 ($p<0.001$) and TNF-α ($p<0.001$) by human whole blood cell culture (Figure 5A to D, respectively). Similarly, pre-incubation with IL-10 also reduced the production of IL-1β ($p<0.001$), IL-6 ($p<0.01$), IL-8 ($p<0.001$) and TNF-α ($p<0.001$) by human endothelial cells (Figure 5 E through H, respectively). In addition, pre-incubation with IL-10 also inhibited the production of IL-1β ($p<0.001$), IL-6 ($p<0.01$), IL-8 ($p<0.001$) and TNF-α ($p<0.01$) by human neutrophils (Figure 5 I through M, respectively). Taken together, increased IL-10 significantly inhibited the activation of human structural and inflammatory cells that play a pivotal role in the pathophysiology of ARDS.

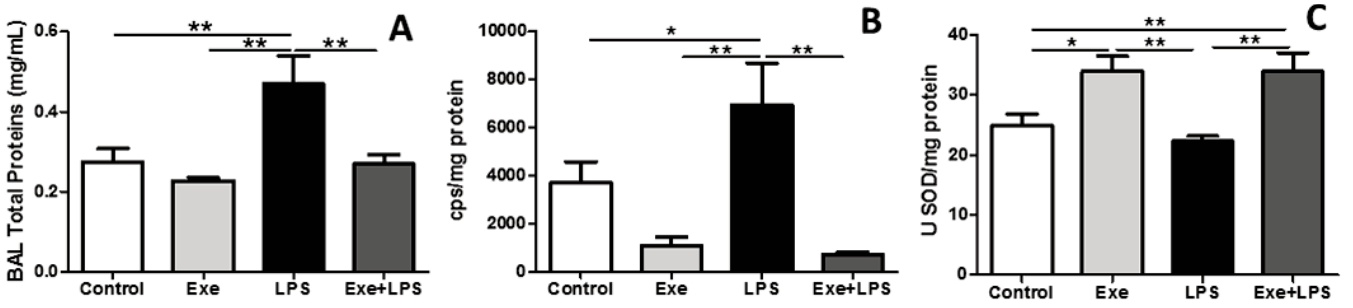

Figure 4. Total proteins and reactive oxygen specie and antioxidant enzyme in the lungs. Total protein (Figure 4A). Reactive oxygen species (ROS), mainly superoxide anion, expressed as anion units per mg of lung protein (Figure 4B). Total superoxide dismutase (SOD) per mg of lung protein (Figure 4C). ** p<0.01 and * p<0.05.

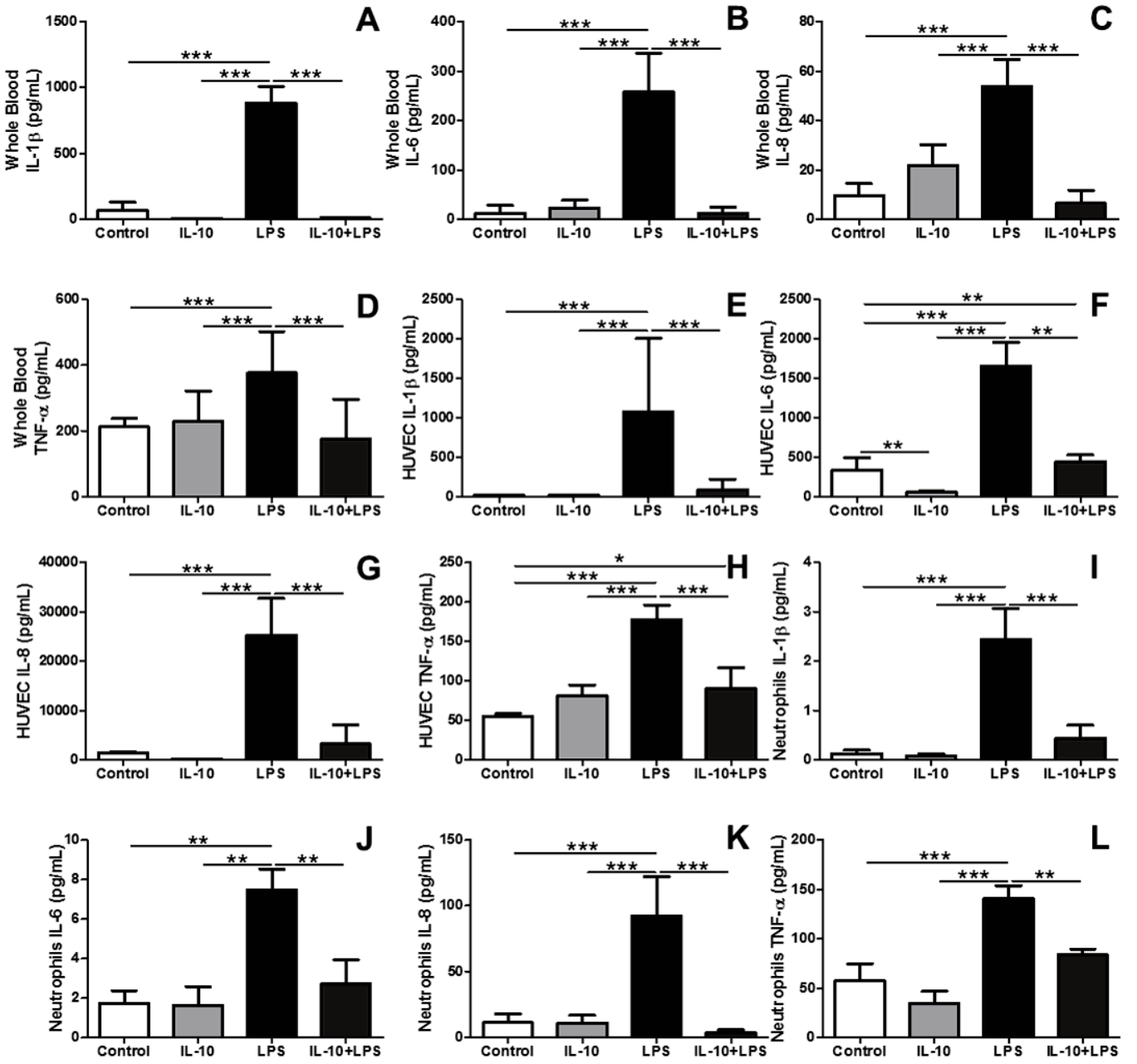

Figure 5. Pro-inflammatory cytokines produced by human whole blood cells (Figure 5 A, B, C and D), HUVEC cells (Figure 5 E, F, G and H) and neutrophils (Figure 5 I, J, K and L). The results are expressed as picograms of cytokines per mL of BAL. *** p<0.001; ** p<0.01; * p<0.05.

DISCUSSION

This study demonstrated for the first time that chronic, moderate intensity aerobic exercise (AE) may have prophylactic effects against ARDS from extra pulmonary origin, a disease that often occurs unexpectedly and for which there is no cure. While pro-inflammatory cytokines were reduced, as well as oxidative stress levels, anti-inflammatory cytokine IL-10, was increased in LPS+Exe mice compared to LPS (sedentary) animals providing additional evidence that chronic, moderate, AE is an effective, cost-efficient, preventative, medicine against pulmonary disease.

In contrast to sedentary mice receiving LPS, Exe+LPS mice showed decreased neutrophil accumulation both in BAL fluid and in the parenchyma as well as less damage to the alveolar epithelium as measured by protein content in the BAL. Reduced injury in Exe+LPS mice was likely due to the reduction of neutrophils and neutrophil chemoattractants including reduced: IL-1β, IL-6, (CXCL1)/KC, IL-17, TNF-α, GM-CSF, and IGF-1.

While exercise alone does not regulate CXCL1/KC, increased CXCL1/KC in BAL is related to endothelial damage and contributes to leukocyte recruitment (22). Thus decreased CXCL1/KC in Exe+LPS animals compared to LPS animals was likely due to decreased neutrophil damage in the endothelial compartment. Likewise, increased IGF-1 is associated with ARDS and has been demonstrated to activate fibroblasts (repair) and permit epithelial cell death (32). Like

CXCL1/KC, IGF-1 was not regulated by exercise alone, however, levels were reduced in Exe+LPS animals indicating decreased injury compared to LPS animals.

Recent studies have implicated that IL-17 may play an important role in ARDS. In addition to forming a positive feedback loop involving IL-1β and IL-6 (24), IL-17 promotes IL-6, IL-8, TNF-α, IL-1β, G-CSF, and monocyte chemo-attractant protein (MCP)-1 (11, 23). IL-17 is increased in ARDS patient BAL fluid and has been shown to increase permeability across alveolar epithelial monolayers (19). Furthermore, IL-17 knockout mice exposed to acute lung injury showed reduced inflammation (20). While exercise alone did not regulate IL-17, IL-1β, or IL-6, these cytokines were reduced in Exe+LPS groups compared to LPS alone, confirming the old notion that exercise can suppress endotoxin-stimulated inflammatory cytokines in blood culture (40). However, exercise alone increased IL-10 which has been demonstrated to repress the release of cytokines by macrophages and lymphocytes, including IL-17 (15). In addition, although it was not evaluated in the present study, exercise-induced IL-10 release have been attributed to increased mobilization of T-regulatory cells (21) and also by IL-10 derived from bronchial epithelial cells (38). Furthermore, low concentrations of IL-10 are associated with poor prognosis for ARDS. (3, 9).

GM-CSF has been shown to prime the neutrophilic response to LPS by up-regulating toll-like receptors (TLR2 and TLR4), the main receptors for LPS. In addition, the over-production of GM-CSF in ARDS contributes to pulmonary destruction by neutrophils, as GM-CSF is also a neutrophil chemo-attractant. In horses that underwent moderate, chronic AE, TLR expression was reduced in pulmonary alveolar macrophages compared to unconditioned animals (10). In this model, moderate AE blunted GM-CSF production, which likely repressed LPS-induced TLR expression and contributed to reduced inflammation in the Exe+LPS group. While GM-CSF was not significantly regulated by exercise alone, increases in the anti-inflammatory cytokine IL-10 (observed in both the Exe and Exe+LPS groups) may have had a repressive effect on GM-CSF and other cytokine production.

Recently, SNPs in genes involved in oxidative stress have been identified in ARDS patients and may contribute to patient prognosis (2). Levels of oxidative stress and anti-oxidant activity were maintained at "reduced" exercise-only levels (compared to uninjured mice) in Exe+LPS mice. Importantly, these results indicated that at least for rodents, chronic, moderate AE significantly represses oxidative stress levels and enhances anti-oxidant activity after 24 hours of basal, sedentary activity. Further studies exploring the length of "sedentary" time for which AE modulates both oxidative stress and cytokine activity would help to further define a general, prophylactic exercise program against a variety of pulmonary diseases.

While chronic, moderate AE in rodents has been repeatedly demonstrated to improve outcomes in a variety of lung disease models, in order to assess the extent by which physical fitness at the time of injury effects ARDS prognosis, longitudinal studies correlating with previous physical fitness, not just body mass index, should be performed.

REFERENCES

1. Abbasi A, Hauth M, Walter M, Hudemann J, Wank V, Niess AM, Northoff H. Exhaustive exercise modifies different gene expression profiles and pathways in LPS-stimulated and un-stimulated whole blood cultures. Brain Behav Immun: 39:130–41, 2014.
2. Acosta-Herrera M, Pino-Yanes M, Blanco J, Ballesteros JC, Ambrós A, Corrales A, Gandía F, Subirá C, Domínguez D, Baluja A, Añón JM, Adalia R, Pérez-Méndez L, Flores C, Villar J Common variants of NFE2L2 gene predisposes to acute respiratory distress syndrome in patients with severe sepsis. Crit Care: 19:256, 2015.
3. Armstrong L, Millar AB. Relative production of tumour necrosis factor alpha and interleukin 10 in adult respiratory distress syndrome. Thorax 1997; 52:442–6, 1997.
4. Brüggemann TR, Ávila LC, Fortkamp B, Greiffo FR, Bobinski F, Mazzardo-Martins L, Martins DF, Duarte MM, Dafre A, Santos AR, Silva MD, Souza LF, Vieira RP, Hizume-Kunzler DC. Effects of Swimming on the Inflammatory and Redox Response in a Model of Allergic Asthma. Int J Sports Med, 2015.
5. Cayrol C, Girard J-P. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr Opin Immunol: 31:31–7, 2014.
6. Dal-Pizzol F, Ritter C. Functional disability 5 years after ARDS. N Engl J Med: 365:274-5-6, 2011.
7. Debevec T, Pialoux V, Mekjavic IB, Eikein O, Mury P, Millet GP. Moderate exercise blunts oxidative stress induced by normobaric hypoxic confinement. Med Sci Sports Exerc:46:33–41, 2014.
8. de Araújo CC, Silva JD, Samary CS, Guimarães IH, Marques PS, Oliveira GP, do Carmo LG, Goldenberg RC, Bakker-Abreu I, Diaz BL, Rocha NN, Capelozzi VL, Pelosi P, Rocco PR. Regular and moderate exercise before experimental sepsis reduces the risk of lung and distal organ injury. J Appl Physiol: 112:1206–14, 2012.
9. Donnelly SC, Strieter RM, Reid PT, Kunkel SL, Burdick MD, Armstrong I, Mackenzie A, Haslett C. The association between mortality rates and decreased concentrations of interleukin-10 and interleukin-1 receptor antagonist in the lung fluids of patients with the adult respiratory distress syndrome. Ann Intern Med: 125:191–6, 1996.
10. Files DC, Liu C, Pereyra A, Wang ZM, Aggarwal NR, D'Alessio FR, Garibaldi BT, Mock JR, Singer BD, Feng X, Yammani RR, Zhang T, Lee AL, Philpott S, Lussier S, Purcell L, Chou J, Seeds M, King LS, Morris PE, Delbono O. Therapeutic exercise attenuates neutrophilic lung injury and skeletal muscle wasting. Sci Transl Med. 2015 Mar 11;7(278):278ra32.
11. Frellstedt L, Waldschmidt I, Gosset P, Desmet C, Pirottin D, Bureau F, Farnir F, Franck T, Dupuis-Tricaud MC, Lekeux P, Art T. Training modifies innate immune responses in blood monocytes and in pulmonary alveolar macrophages. Am J Respir Cell Mol Biol: 51:135–42, 2014.
12. Gaffen SL. Recent advances in the IL-17 cytokine family. Curr Opin Immunol: 23:613–9, 2011.
13. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol:11:607–15, 2011.

14. Gleeson M, Walsh NP. The BASES expert statement on exercise, immunity, and infection. J Sports Sci: 30:321–4, 2012.
15. Gonzalez-Rothi RJ, Straub L, Cacace JL, Schreier H. Liposomes and pulmonary alveolar macrophages: functional and morphologic interactions. Exp Lung Res: 17:687–705, 1991.
16. Gu Y, Yang J, Ouyang X, Liu W, Li H, Yang J, Bromberg J, Chen SH, Mayer L, Unkeless JC, Xiong H. Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. Eur J Immunol: 38:1807–13, 2008.
17. Herridge MS, Tansey CM, Matté A, Tomlinson G, Diaz-Granados N, Cooper A, Guest CB, Mazer CD, Mehta S, Stewart TE, Kudlow P, Cook D, Slutsky AS, Cheung AM; Canadian Critical Care Trials Group. Functional disability 5 years after acute respiratory distress syndrome. N Engl J Med: 364:1293–304, 2011.
18. Irigoyen M-C, Paulini J, Flores LJF, Flues K, Bertagnolli M, Moreira ED, Consolim-Colombo F, Belló-Klein A, De Angelis K. Exercise training improves baroreflex sensitivity associated with oxidative stress reduction in ovariectomized rats. Hypertension: 46:998–1003, 2005.
19. Li JT, Melton AC, Su G, Hamm DE, LaFemina M, Howard J, Fang X, Bhat S, Huynh KM, O'Kane CM, Ingram RJ, Muir RR, McAuley DF, Matthay MA, Sheppard D. Unexpected Role for Adaptive αβTh17 Cells in Acute Respiratory Distress Syndrome. J Immunol: 195:87–95, 2015.
20. Li Q, Gu Y, Tu Q, Wang K, Gu X, Ren T. Blockade of Interlukin-17 restrains the development of acute lung injury. Scand J Immunol 2015.
21. Lowder T, Dugger K, Deshane J, Estell K, Schwiebert LM. Repeated bouts of aerobic exercise enhance regulatory T cell responses in a murine asthma model. Brain Behav Immun. 2010 Jan;24(1):153-9.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem: 193:265–75, 1951.
23. Maniatis NA, Orfanos SE. The endothelium in acute lung injury/acute respiratory distress syndrome. Curr Opin Crit Care: 14:22–30, 2008.
24. Memtsoudis SG, Bombardieri AM, Ma Y, Walz JM, Chiu YL, Mazumdar M. Mortality of patients with respiratory insufficiency and adult respiratory distress syndrome after surgery: the obesity paradox. J Intensive Care Med: 27:306–11, 2012.
25. Moon H-G, Kang CS, Choi J-P, Choi DS, Choi HI, Choi YW, Jeon SG, Yoo JY, Jang MH, Gho YS, Kim YK. Acetyl salicylic acid inhibits Th17 airway inflammation via blockade of IL-6 and IL-17 positive feedback. Exp Mol Med: 45:e6, 2013.
26. Orme J, Romney JS, Hopkins RO, Pope D, Chan KJ, Thomsen G, Crapo RO, Weaver LK. Pulmonary function and health-related quality of life in survivors of acute respiratory distress syndrome. Am J Respir Crit Care Med: 167:690–4, 2003.
27. Pereira PR, Oliveira-Junior MC, MacKenzie B, Chiovatto JE, Matos Y, Greiffo FR, Rigonato-Oliveira NC, Brugemman TR, Delle H, Idzko M, Albertini R, Ligeiro Oliveira AP, Damaceno-Rodrigues NR, Caldini EG, Fernandez IE, Castro-Faria-Neto HC, Dolhnikoff M, Eickelberg O, Vieira RP. Exercise Reduces Lung Fibrosis Involving Serotonin/Akt Signaling. Med Sci Sports Exerc 2016.
28. Prata LO, Oliveira FMS, Ribeiro TMS, Almeida PW, Cardoso JA, Rodrigues-Machado Mda G, Caliari MV. Exercise attenuates pulmonary injury in mice with bleomycin-induced pulmonary fibrosis. Exp Biol Med (Maywood): 237:873–83, 2012.
29. Ramos DS, Olivo CR, Quirino Santos Lopes FDT, Toledo AC, Martins MA, Lazo Osório RA, Dolhnikoff M, Ribeiro W, Vieira RP. Low-intensity swimming training partially inhibits lipopolysaccharide-induced acute lung injury. Med Sci Sports Exerc:42:113–9, 2010.
30. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS Acute respiratory distress syndrome: the Berlin Definition. JAMA: 307:2526–33, 2012.
31. Reis Gonçalves CT, Reis Gonçalves CG, de Almeida FM, Lopes FD, dos Santos Durão AC, dos Santos FA, da Silva LF, Marcourakis T, Castro-Faria-Neto HC, Vieira RP, Dolhnikoff M. Protective effects of aerobic exercise on acute lung injury induced by LPS in mice. Crit Care: 16:R199, 2012.
32. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD Incidence and outcomes of acute lung injury. N Engl J Med: 353:1685–93, 2005.
33. Schnapp LM, Donohoe S, Chen J, Sunde DA, Kelly PM, Ruzinski J, Martin T, Goodlett DR. Mining the acute respiratory distress syndrome proteome: identification of the insulin-like growth factor (IGF)/IGF-binding protein-3 pathway in acute lung injury. Am J Pathol: 169:86–95, 2006.
34. Silva RA, Vieira RP, Duarte ACS, Lopes FD, Perini A, Mauad T, Martins MA, Carvalho CR. Aerobic training reverses airway inflammation and remodelling in an asthma murine model. Eur Respir J : 35:994–1002, 2010.
35. Vieira RP, Claudino RC, Duarte ACS, Santos AB, Perini A, Faria Neto HC, Mauad T, Martins MA, Dolhnikoff M, Carvalho CR. Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice. Am J Respir Crit Care Med: 176:871–7, 2007.
36. Vieira RP, de Andrade VF, Duarte ACS, Dos Santos AB, Mauad T, Martins MA, Dolhnikoff M, Carvalho CR. Aerobic conditioning and allergic pulmonary inflammation in mice. II. Effects on lung vascular and parenchymal inflammation and remodeling. Am J Physiol Lung Cell Mol Physiol: 295:L670-9, 2008.
37. Vieira RP, Duarte AC, Santos ABG, Medeiros MC, Mauad T, Martins MA, Carvalho CR, Dolhnikoff M. Exercise reduces effects of creatine on lung. Int J Sports Med: 30:684–90, 2009.
38. Vieira RP, Toledo AC, Ferreira SC, Santos AB, Medeiros MC, Hage M, Mauad T, Martins Mde A, Dolhnikoff M, Carvalho CR. Airway epithelium mediates the anti-inflammatory effects of exercise on asthma. Respir Physiol Neurobiol. 2011 Mar 15;175(3):383-9.
39. Vieira R de P, Toledo AC, Silva LB, Almeida FM, Damaceno-Rodrigues NR, Caldini EG, Santos AB, Rivero DH, Hizume DC, Lopes FD, Olivo CR, Castro-Faria-Neto HC, Martins MA, Saldiva PH, Dolhnikoff M. Anti-inflammatory effects of aerobic exercise in mice exposed to air pollution. Med Sci Sports Exerc: 44:1227–34, 2012.

40. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, Simon P Position statement. Part one: Immune function and exercise. Exerc Immunol Rev: 17:6–63, 2011.
41. Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, Oliver SJ, Bermon S, Kajeniene A. Position statement. Part two: Maintaining immune health. Exerc Immunol Rev: 17:64–103, 2011.
42. Weinstock C, König D, Harnischmacher R, Keul J, Berg A, Northoff H. Effect of exhaustive exercise stress on the cytokine response. Med Sci Sports Exerc: 29:345–54, 1997.

Acknowledgements: Sao Paulo Research Foundation (FAPESP) grant 2012/15165-2. Conselho Nacional de Pesquisa e Desenvolvimento (CNPq) grant 311335-2015-2. Comissão de Aperfeiçoamento de Pessoal de Nivel Superior (CAPES) grants 12804/13-4 and 1303/13-9. NCRO holds a MSc fellowship from FAPESP 2013/24076-6. BM holds a postdoctoral fellowship from FAPESP 2014/23196-0. MCOJ holds a PhD fellowship from FAPESP 2012/14604-8. ASAS holds a MSc fellowship from CAPES. LHD holds a scientific initiation fellowship from FAPESP 2012/25435-7. MAB holds a scientific initiation fellowship from FAPESP 2012/24880-7.