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Aerobic exercise inhibits obesity-induced respiratory phenotype

Jefferson Comin Jonco Aquino-Junior[a,1], BreAnne MacKenzie[b,1], Ana Roberta Almeida-Oliveira[a,1], Ana Carolina Martins[g], Manoel Carneiro Oliveira-Junior[a,b], Auriléa Aparecida Britto[a], Fernanda Magalhaes Arantes-Costa[c], Nilsa Regina Damaceno-Rodrigues[d], Elia Garcia Caldini[d], Ana Paula Ligeiro de Oliveira[a,b], Dioze Guadagnini[e], Luiz Osorio Leiria[e], Djalma Rabelo Ricardo[f], Mario Jose Abdalla Saad[e], Rodolfo Paula Vieira[b,f,g,*]

[a] Nove de Julho University, Rua Vergueiro 235/249, São Paulo, SP 01504-001, Brazil
[b] Brazilian Institute of Teaching and Research in Pulmonary and Exercise Immunology (IBEPIPE), Rua Pedro Ernesto 240, Sao José dos Campos, SP 12245-520, Brazil
[c] Laboratory of Experimental Therapeutics (LIM 20), School of Medicine, University of Sao Paulo (USP), Avenida Doutor Arnaldo 455, Cerqueira Cesar, Sao Paulo, SP 01246-903, Brazil
[d] Laboratory of Cellular Biology (LIM 59), School of Medicine, University of Sao Paulo (USP), Avenida Doutor Arnaldo 455, Cerqueira Cesar, Sao Paulo, SP 01246-903, Brazil
[e] Department of Internal Medicine, State University of Campinas (UNICAMP), Rua Tessália Vieira de Camargo 126, Cidade Universitária, Campinas, SP 13083-887, Brazil
[f] School of Medical Sciences of Sao Jose dos Campos Humanitas, Avenida Brigadeiro Faria Lima 811, Sao José dos Campos, SP 12227-000, Brazil
[g] Universidade Brasil, Rua Carolina Fonseca, 584 - Itaquera, São Paulo, SP 08230-030, Brazil

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ABSTRACT

Purpose: Obesity results in decreased lung function and increased inflammation. Moderate aerobic exercise (AE) reduced lung inflammation and remodeling in a variety of respiratory disease models. Therefore, this study investigated whether AE can attenuate a diet-induced obesity respiratory phenotype; including airway hyperresponsiveness (AHR), remodeling and inflammation.

Methods: Sixty C57Bl/6 male mice were distributed into four groups: control lean (CL), exercise lean (EL), obese (O) and obese exercise (OE) groups (2 sets of 7 and 8 mice per group; n = 15). A classical model of diet-induced obesity (DIO) over 12 weeks was used. AE was performed 60 min/day, 5 days/week for 5 weeks. Airway hyperresponsiveness (AHR), lung inflammation and remodeling, adipokines and cytokines in bronchoalveolar lavage (BAL) was determined.

Results: A high fat diet over 18 weeks significantly increased body weight ($p < .0001$). Five weeks of AE significantly reduced both AHR and pulmonary inflammation. AHR in obese mice that exercised was reduced at the basal level ($p < .05$), vehicle (PBS) ($p < .05$), 6.25 MCh mg/mL ($p < .05$), 12.5 MCh mg/mL ($p < .01$), 25 MCh mg/mL ($p < .01$) and 50 MCh mg/mL ($p < .05$). Collagen ($p < .001$) and elastic ($p < .001$) fiber deposition in airway wall and also smooth muscle thickness ($p < .001$) were reduced. The number of neutrophils ($p < .001$), macrophages ($p < .001$) and lymphocytes ($p < .01$) were reduced in the peribronchial space as well as in the BAL: lymphocytes ($p < .01$), macrophages ($p < .01$), neutrophils ($p < .001$). AE reduced obesity markers leptin ($p < .001$), IGF-1 ($p < .01$) and VEGF ($p < .001$), while increased adiponectin ($p < .01$) in BAL. AE also reduced pro-inflammatory cytokines in the BAL: IL-1β ($p < .001$), IL-12p40 ($p < .001$), IL-13 ($p < .01$), IL-17 ($p < .001$, IL-23 ($p < .05$) and TNF-alpha ($p < .05$), and increased anti-inflammatory cytokine IL-10 ($p < .05$).

Conclusions: Aerobic exercise reduces high fat diet-induced obese lung phenotype (AHR, pulmonary remodeling and inflammation), involving anti-inflammatory cytokine IL-10 and adiponectin.

* Corresponding author at: Brazilian Institute of Teaching and Research in Pulmonary and Exercise Immunology (IBEPIPE), School of Medical Sciences of São José dos Campos Humanitas and Universidade Brasil, Rua Pedro Ernesto 240, São José dos Campos, SP 12245-520, Brazil.
E-mail address: rodrelena@yahoo.com.br (R.P. Vieira).
[1] JCJAJ, BM and ARAO have equally contributed to this study.

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1. Introduction

According to the World Health Organization increased intake of energy-dense foods high in fat in combination with significantly decreased physical activity has resulted in a doubling of the incidence of overweight and obesity since 1980. Currently, 1.9 billion people or 13% of the world is overweight [25]. Overweight and obesity diagnoses indicate abnormal or excessive fat accumulation that may result in impaired overall health. Body mass index (BMI) is a commonly used index to classify adults as overweight or obese. Weight in kilograms is divided by height squared in meters (kg/m^2); a BMI greater or equal to 25 is considered overweight while BMI greater to or equal to 30 is considered obese. Though the mechanisms remain unclear, health care professionals have reached the consensus that heightened BMI, especially when the excess weight is concentrated in the thorax rather than peripheral regions of the body, has a negative impact on respiratory health [1,12].

Obesity is linked to a wide range of respiratory conditions including aspiration pneumonia, asthma, chronic obstructive pulmonary disease, obstructive sleep apnea and pulmonary embolic disease [12]. An inverse relationship between BMI and forced expiratory volume in 1 s (FEV1) has been observed in obese people [1]. Poor lung function is also associated with a low-grade inflammatory state including elevated levels of interleukins (ILs) 6 and 8, tumor necrosis factor α (TNF-α), C reactive protein (CRP), leptin, and depressed levels of adiponectin which plays a role in regulating insulin sensitivity [26].

In a variety of models of respiratory diseases that also present as obesity comorbidities including allergic asthma [8,18,21] and COPD [19], mice that performed moderate intensity exercise five days a week for 5 weeks showed significantly reduced pulmonary inflammation and lung remodeling compared to sedentary controls. Taken together, this study investigated whether moderate aerobic exercise can attenuate respiratory remodeling and inflammation induced via a high fat diet that led to obesity in mice.

2. Methods

2.1. Ethics

This study was approved by the animal ethics committee of the Nove de Julho University (AN0034.2014). Experiments were carried out in accordance to the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH publication No. 85-23, revised 1996). Animals did not present any alteration in health status, which was monitored one week before and during physical training sessions. No mice died due to training or injury.

2.2. Animals

As the estrus cycle of female mice can significantly influence inflammation, male mice were used for all experiments. Mice were housed under specific pathogen-free conditions on a 12-h light and dark cycle with free access to food and water. The animals were housed three per cage and fed either a standard formula: (10 kcal% fat) or a high-fat diet that induces obesity (60 kcal% fat) gently given by the laboratory of Prof. Dr. Mario José Abdalla Saad, from University of State of Campinas.

2.3. Exercise protocol

For a complete schematic illustrating the exercise and diet-induced obesity protocol, see Fig. 1. Briefly, male mice were fed either high-fat chow from 7 weeks of age or normal chow for the duration of the experiment. The exercise protocol began when mice reached 20 weeks of age. Following 3 days of adaptation (15 min/day, 25° incline, 0.2 km/h), animals were submitted to a physical test (beginning at 0.2 km/h, increasing 0.1 km/h every 2.5 min) until exhausted. Exhaustion was defined as failure to run following 10 gentle mechanical stimuli [21,22]. Moderate intensity aerobic exercise (AE), defined as 60% maximal speed attained during the pre-protocol physical test. Treadmill training (light run) was performed by mice in the lean exercise (EL) and obese exercise (OE) groups for 60 min/day, 5 days a week for 5 weeks. Twenty-four hours before euthanasia (ketamine 200 mg/kg and xylazine 20 mg/kg i.p.), the final physical test was performed [21,22].

2.4. Measurement of airway hyperresponsiveness (AHR)

AHR to methacholine (MCh) was assessed in conscious mice using a whole body plethysmograph (Buxco Europe, Winchester, UK), as previously described performed [9]. Briefly, spontaneously breathing mice were placed into a plethysmography chamber and the box pressure was recorded. Expiratory (TE) and relaxation (TR), peak inspiratory and expiratory pressures were recorded. Peak expiratory (PEF) and inspiratory (PIF) flows were also calculated, as well as the Penh, which has a theoretical relationship with airway obstruction, serving as a direct index of AHR to growing doses of nebulized methacholine (6.25, 12.5, 25, 50 mg/ml) [9].

2.5. Histology

The left lung of 15 mice/group were fixed in 4% PFA, embedded in paraffin and sliced into 5 μm sections. Sections were stained with hematoxylin and eosin to quantify immune cell influx into the airways wall. Five airways of each animal per group were imaged at 400× magnification using an Olympus BX40 microscope, and Image Pro-Plus 4.0 software. The area between the airway basal membrane and the adventitia was quantified using the Image Pro-Plus software and the number of lymphocytes, macrophages and neutrophils was quantified in this area according to morphological criteria. Results were expressed as the number of cells per square millimeter.

2.6. Preparation of cytospin and ELISA

2.6.1. Cytospin

Lungs were flushed with phosphate-buffered saline (PBS) and the recovered fluid was centrifuged (500G for 10 min at 4 °C). BAL supernatant was stored at −80 °C for ELISA experiments. The cell pellet was resuspended in 200 μL of PBS, differential (Diff-Quick stain) cell counts were performed for lymphocytes macrophages and neutrophils.

2.6.2. ELISA

ELISAs were performed according to manufacturer's instructions: Adiponectin, DY1119 (R&D Systems), Leptin, DY498 (R&D Systems) IGF-1, DY791 (R&D Systems), VEGF, DY493 (R&D Systems), IL-1β 432601 (Biolegend), IL-12p40 431601 (Biolegend), IL-13 DY413 (R&D Systems), IL-17 DY421 (R&D Systems), IL-23 DY1887 (R&D Systems), TNF-α 430901 (Biolegend) and IL-10 431411 (Biolegend).

2.6.3. Statistical analysis

Data are presented as the means ± SEM of n experiments. The GraphPad Prism software 5.0 was used for statistical analysis. One-way anova followed by Newman-Keuls post hoc test was used A value of $p < .05$ was accepted as significant.

3. Results

In total, sixty C57Bl/6 male mice were distributed equally into four experimental groups that controlled for diet and exercise. The first set of experiment used 8 mice per group and the second set of experiment used 7 mice per group. Lean mice received normal chow (10% fat) throughout the experiment while obese mice were fed high fat chow