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Resistance Training Improves the Immune Response, Mainly Associated with CD8$^+$ T Lymphocytes and B Lymphocytes, in Mice

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HIGHLIGHTS

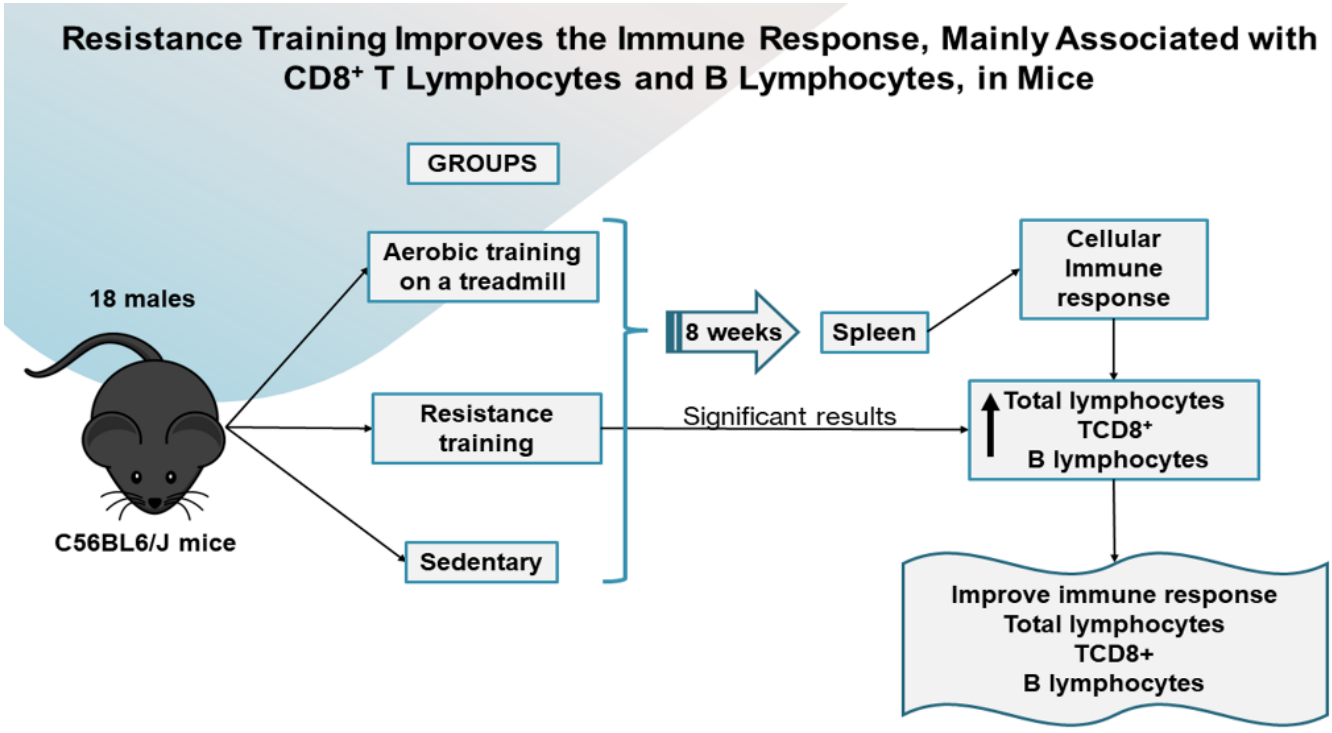
- Mice under aerobic training showed significantly higher workload after 4 and 8 weeks.
- Resistance exercises generate greater rates of total lymphocytes, CD8$^+$ and B lymphocytes.
- Maximum load of resistance training correlates with proliferation of total lymphocytes, CD8$^+$ and B cells.
- Resistance training promoted improvement to immune response associated with CD8$^+$ and B lymphocytes.

Abstract: Studies investigating the effects of different modalities of exercises on the immune system are scarce. Therefore, the aim of this study was to compare the effects of eight weeks of resistance and aerobic training on the proliferation of T and B lymphocytes from mice. Eighteen male C56BL6/J mice were divided into groups (n=6), sedentary, aerobic, and resistance training. After 8 weeks, animals were euthanized, and their splenocytes were labeled and cultured with and without stimulation. Lymphocyte proliferation (CD4$^+$, CD8$^+$ and CD21/CD35$^+$) was evaluated by flow cytometry. The mice subjected to resistance exercise exhibited greater proliferation for total, CD8$^+$ and B lymphocytes ($p<0.05$), but not CD4$^+$ cells ($p>0.05$),

compared with their sedentary counterparts. We found significant correlations between maximum load and total, $CD8^+$ and B lymphocytes proliferation rates ($p<0.05$). In conclusion, our results showed that resistance training promoted an improvement in the immune response associated with $CD8^+$ and B lymphocytes.

Keywords: mouse; immunology; lymphocyte proliferation; resistance training; aerobic exercises.

GRAPHICAL ABSTRACT



INTRODUCTION

The mammalian immune system is composed of several substances and cells that act in the defense of the organism, and which constitute the innate and adaptive phases of the immune response [1]. Physical exercise has been identified as a protective factor for health and well-being by increasing the immunological response [2], due to its anti-inflammatory activity [3,4]. Physical exercise of moderate intensity [5] induces a discharge of catecholamines (adrenaline and norepinephrine), leading to an increase in the concentration of T lymphocytes in the vascular compartment [6].

The classification of the level of effort (intensity) can be established using physiological and metabolic parameters [7]. In fact, regular physical activity improves resistance to infection and decrease in the risk of mortality in community-acquired infectious disease in the general population [8]. In addition, the skeletal muscles can be considered an endocrine organ as result of producing significant quantities of cytokines during exercise. These cytokines include IL-6 [9] and IL-7, which play important roles in signaling to naïve and memory $CD8^+$ lymphocytes, and IL-15, which is crucial to the proliferation of the same immunological cells [10]. Exercise of moderate intensity tends to polarize the immune response to the Th1 pattern [11], whereas exhaustive exercise may cause immunosuppression, thereby favoring infections or inflammation in athletes [12].

Although studies investigating the effects of different intensities of exercise on the immune system are extensive, comparisons of different modalities are scarce. Thus, this study aimed to evaluate the effects of different modalities of physical exercise (resistance and aerobic training) on the proliferation of lymphocyte subsets in C56BL6/J mice.

MATERIAL AND METHODS

The research project was approved by the Laboratory Animal Use Ethics Committee of the Federal University of Minas Gerais (UFMG) (registry number 292/18). Eighteen male C56BL6/J mice, aged 4 weeks and weighing approximately 15 g, were used. They were maintained at 22 ± 2o C, in 45% ± 15% humidity,

and 12 h light and 12 h dark cycle. The three groups were sedentary, aerobic training on a treadmill, and resistance training on a ladder (climbing with loads) [13]. Briefly, after five days of acclimation, animals were subjected to two types of fatigue tests: maximum incremental load/speed test and endurance test using 80% of maximum load/speed in order to evaluate each animal's performance. According to their initial performance, the mice were distributed into three groups composed of animals with similar physical capacity.

Animals were trained for 8 weeks, while sedentary animals were only exposed to the same laboratory conditions without any additional exercise. In the aerobic training, a motor-powered treadmill with electrical resistance at the end of the lane was used. For the resistance training, the animals were subjected to climbing sessions on a ladder with the following dimensions: 90 × 14 cm (grid between 1 cm steps), 80° angle, with a resting area on the upper part, adapted from previous research [14]. Fatigue tests were also performed by adapting the same models from the treadmill [13]. Both fatigue tests were conducted after four and eight weeks of training to indicate performance gains over time.

After 8 weeks of training, the mice were euthanized, and their spleens were collected to evaluate the cellular immune response. Splenocytes were obtained as previously described [12]. The tubes containing the splenocytes were centrifuged at 200 × g for 10 min at 4° C and the pellets (containing the cells) were resuspended in RPMI 1640 (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum (Sigma Aldrich, USA). Then, the splenocytes were labeled with CFSE [5(6)-carboxyfluorescein diacetate N-succinimidyl ester] (Life Technologies, USA), according to the manufacturer's instructions. An aliquot of each cell suspension was diluted in trypan blue to assess cell viability using light microscopy. The cells were cultured in 48-well culture plates for 3 days (72 h) (1 × 106 cells/well) at 37 °C and 5% CO2. Cultures were stimulated with phytohemagglutinin-L (PHA-L) (Medicago, Sweden) (5 µg/mL) (positive control) or RPMI 1640 (Sigma Aldrich, USA) only (negative control). After incubation, cells were labeled with previously standardized amounts of monoclonal antibodies (mAbs), including anti-mouse CD4 (clone RM4-5), anti-mouse CD8 (clone 53-6.7), and anti-mouse CD21 / CD35 (clone 7G6), conjugated with peridinin chlorophyll protein complex (PerCP), phycoerythrin (PE), and allophycocyanin (APC), respectively (all purchased from Becton Dickinson, USA). A minimum of 30,000 cells per sample were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, USA). FlowJo 7.6.1 software (Tree Star, USA) was used for all analyses of flow cytometry data. Lymphocytes were identified based on their size and granularity. Specific lymphocyte proliferation was determined by considering the percentage of lymphocytes that express CD4, CD8, or CD21/CD35 that proliferated divided by the total lymphocytes of that subpopulation.

The data were first tested for normality and variance of the data sets using the Shapiro-Wilk test. The Kruskal-Wallis test followed by Dunn´s test was used to analyze all flow cytometry data, considering its non-parametric nature. The two-way ANOVA followed by the Bonferroni test was used to compare the exercise modalities for the analysis of Work (J) performed in the tests with constant load, and one-way ANOVA followed by the Tukey test to analyze the maximum load and speed among the groups. Pearson correlation coefficients were calculated for the maximum speed/load and cell proliferation rates. All analyses were performed using GraphPad Prism 8.0.1 software (GraphPad Software, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

After eight weeks of training, animals in both the aerobic and resistance trained groups showed significant improvement in physical performance (Figure 1). Aerobic trained animals showed significantly superior performance in the incremental test of maximum speed after four and eight weeks of training in relation to the initial test (Figure 1 A). Similarly, the animals in the group subjected to resistance training also improved their performance, supporting a greater maximum load after four and eight weeks of training, with a significant difference between the evaluation points (4 and 8 weeks) and their initial condition (Figure 1 B). In the analysis of the workload (J) performed, when comparing the three groups we observed that the group subjected to aerobic training performed at a significantly higher workload (J) than the other groups (resistance training and control groups) after 4 and 8 weeks of training (Figure 1 C).

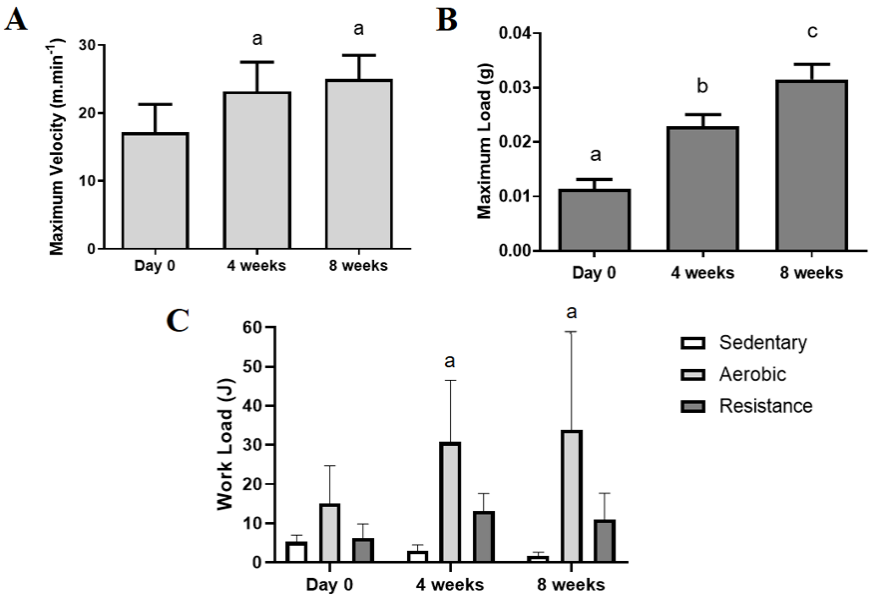


Figure 1. Bar graphs demonstrating the fatigue tests (A and B) and the workload (C) of C56BL6/J mice divided in three experimental groups, sedentary, aerobic trained and resistance trained, at day 0, and after 4 and 8 weeks of training. Error bars represent the standard error of the mean. Same letter indicates no statistical differences ($P < 0.05$).

After PHA-L in vitro stimulation, splenocytes recovered from mice subjected to resistance exercise exhibited greater proliferation rates for total lymphocytes and $CD8^+$ and B lymphocytes ($p<0.05$), but not $CD4^+$ cells ($p>0.05$), compared to splenocytes from their sedentary counterparts. Proliferation of splenocytes from animals subjected to aerobic training did not differ significantly from those of either sedentary or resistance-trained animals (Figure 2).

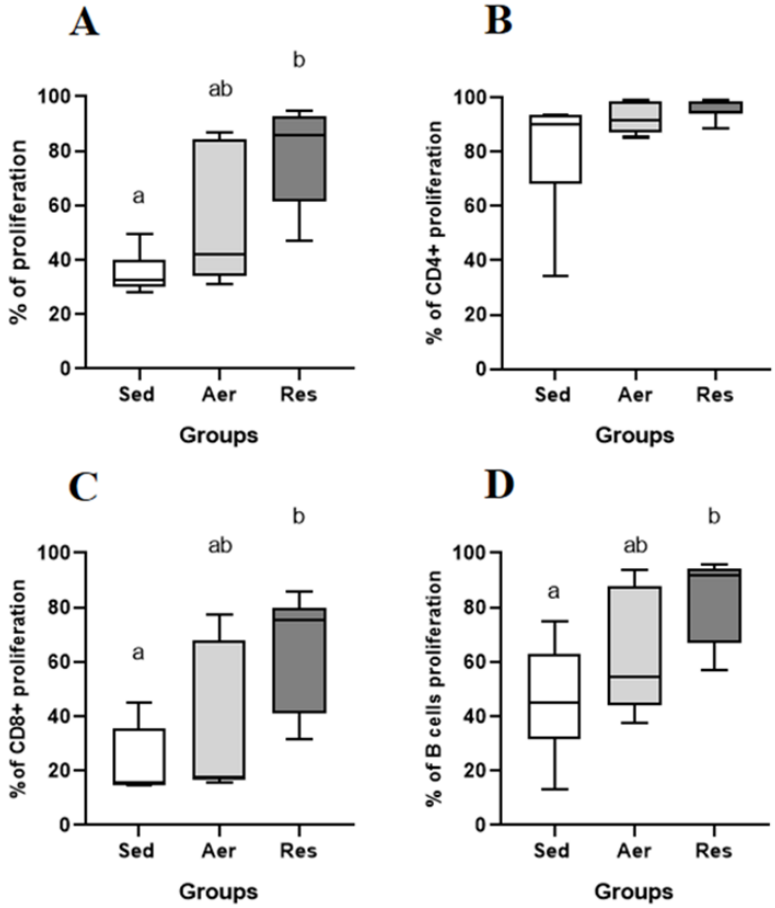


Figure 2. Bar graphs comparing the proliferation of total immune cells (A), $CD4^+$ T cells (B), $CD8^+$ T cells (C) and B cells (D) of C56BL6/J mice from the sedentary, aerobic and resistance groups. Sed: sedentary group; Aer: aerobic trained group; Res: resistance trained group. Error bars represent the standard error of the mean. Same letter indicates no statistical differences ($P < 0.05$).

In contrast to resistance training, aerobic training did not improve the lymphocyte proliferation rate compared to that of cells from sedentary mice (P = 0.07) (Fig. 2 B). We found significant correlations between maximum load (in the resistance training group) and total lymphocyte, $CD8^+$ T lymphocyte, and B lymphocyte proliferation rates (Fig.3). Correlations regarding workload (both training modalities) and maximum speed (aerobic group) were not significant.

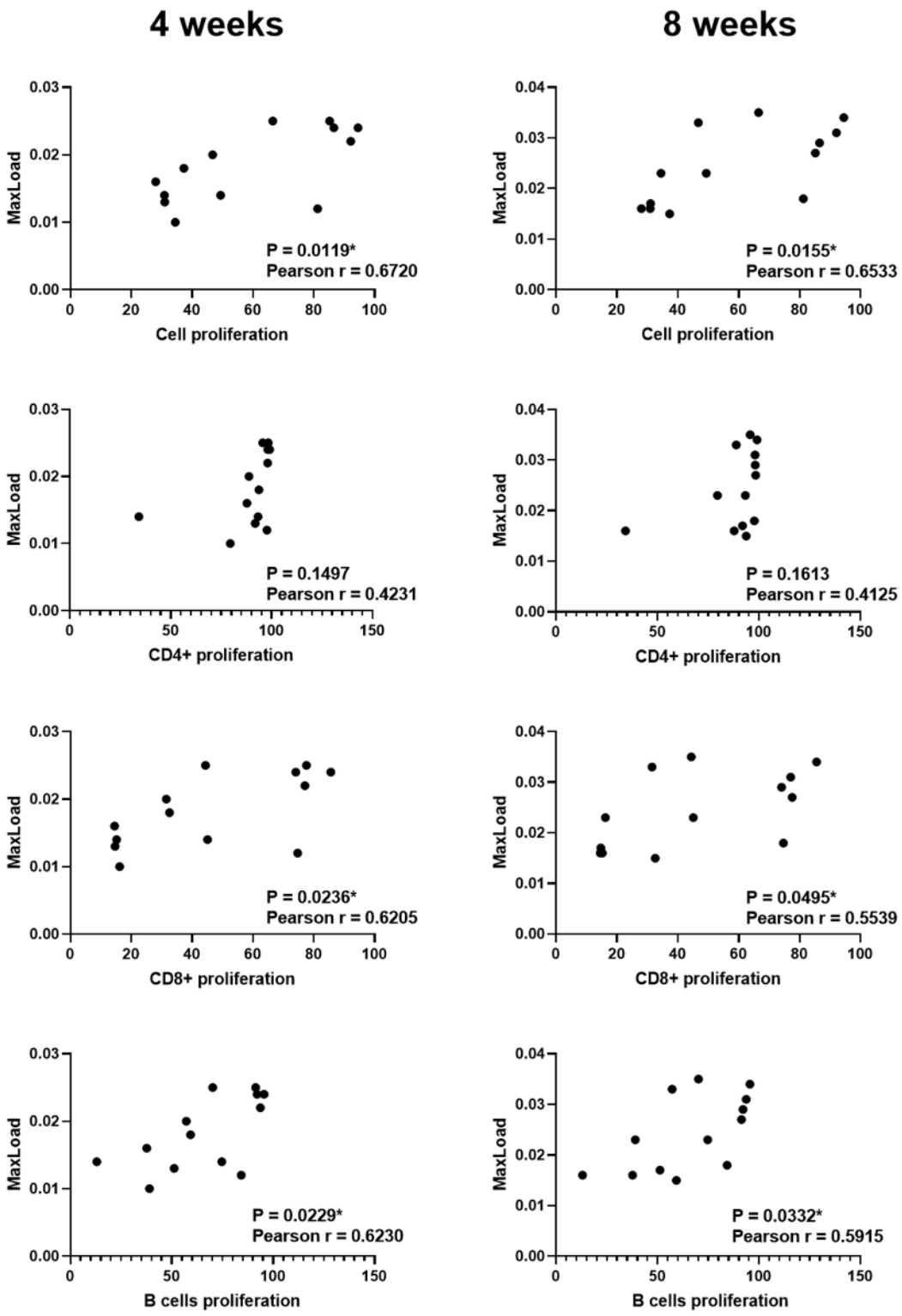


Figure 3. Correlation analysis of maximum load on resistance training with the proliferation rates for total lymphocytes, B lymphocytes, $CD8^+$ and $CD4^+$ cells of C56BL6/J mice.

DISCUSSION

Studies comparing resistance to aerobic training in immune responses are increasing over the years. A previous study in humans demonstrated that a 3-month aerobic exercise program (60% of heart rate reserve) did not significantly increase natural killer cell cytotoxicity, T lymphocyte mitogenesis, natural killer cells, or T cell subsets in previously sedentary women [15]. This lack of an effect was attributed to the short period of evaluation and corroborates our findings for aerobic training. In contrast, more recent study shows that aerobic training improves endothelial activation immunological markers and inflammatory cytokines in elderly people [16].

In contrast, the animals in the resistance training group showed enhanced performance at the three assessment points, exhibiting a progressive improvement and a better physical performance compared with that of the aerobic group. Indeed, as shown in Fig.3, we found significant correlations between maximum load and total lymphocyte, $CD8^+$ T lymphocyte, and B lymphocyte proliferation rates ($P<0.05$). This improvement was coincident with an increase in the blastogenic capacity of the total, $CD8^+$ T-, and B lymphocytes, which are important cells in the defense of the organism against pathogens and in the damage repair response [16]. In this context, it is tempting to speculate that physical activity practitioners who undertake resistance training would be more likely to respond to infections or environmental challenges than those who perform aerobic sports. Indeed, the lymphocyte subsets ($CD8^+$ and B) that showed significant proliferation against an unspecific stimulus (PHA-L) are critically involved in the cellular and humoral immune responses that are triggered by pathogens and damage [18]. Furthermore, it is important to highlight that our results showed a different response capacity (blastogenic) of the immunological cells of the animals (B and T $CD8^+$ lymphocytes) depending on the type of physical exercise practiced, which is at variance with the transient changes observed in the number of peripheral lymphocytes which occur as result of exercise-induced cell redistribution observed elsewhere [19].

The differences in lymphocyte proliferation according to exercise modality (resistance vs. aerobic) may be associated with changes in circulating levels of stress hormones, since it was previously demonstrated that acute/intense muscular exercise increases the plasma concentrations of cortisol [20]. Cortisol produced during acute/intense exercise contributes to apoptosis, lymphocytopenia, and reduced immunity post-exercise [21, 22]. Hypothetically, considering that the total work performed by the aerobic group was significantly greater than that performed by the animals in the resistance training, it would be reasonable to state that these animals were subjected to a more intense and stressful training protocol. This hypothesis explains the inferior immunological performance of splenocytes from the aerobic group in the proliferation assay. Overall, our findings point to different responses of the immune system as consequence of practicing different exercise' modalities, showing an adjuvant role of resistance exercise on the immune response in mice.

CONCLUSION

In conclusion, our results showed that resistance exercise promoted, a significant improvement in the immune system response in C57BL6/J mice, which was predominantly associated with $CD8^+$ T lymphocytes and B lymphocytes. However, the group of animals undergoing 8-weeks of aerobic training failed to show clear improvements.

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Conflicts of Interest: The authors declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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