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Moderate-intensity Exercise Alleviates Rat's Systemic Inflammation Induced by Repeated Exposure to Lipopolysaccharide

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ABSTRACT

The protective impacts of physical activity against inflammatory and oxidative stress conditions have been demonstrated. In this study, the impacts of moderate-intensity exercise on oxidative stress-associated factors and proinflammatory cytokines levels as well as the count of white blood cells (WBC) were assessed in a lipopolysaccharide (LPS)-triggered model of inflammation.

Wistar rats were randomized into these groups (8 rats in each): (1) control; (2) LPS; (3) moderate exercise (EX); and (4) moderate exercise + LPS (EX+LPS). Exercise groups were trained for 8 weeks (30 min, 6 days/week) at 15 m/min speed. During the final week of the experiment, 1 mg/kg/day of intraperitoneal LPS was administered for 5 days. On day 56, from the rats' hearts, peripheral blood was taken for biochemical evaluation.

LPS enhanced serum levels of C-reactive protein (CRP), interleukin (IL)- 1β, tumor necrosis factor-α (TNF-α), metabolites of nitric oxide, and malondialdehyde (MDA), as well as the counts of total WBC, monocytes, neutrophils, and eosinophils, but decreased serum levels of thiol as well as superoxide dismutase (SOD) and catalase (CAT) activity versus the control rats. Moderate exercise reduced the levels of thiol, CAT, and SOD, but increased TNF-α level, and total WBC, neutrophils, eosinophils, and monocytes counts versus the control group. In the EX+LPS group, moderate exercise decreased cell counts and diminished MDA, TNF-α, IL-1β, and CRP levels, while increasing thiol level, CAT, and SOD versus the LPS group.

In our study, exercise preconditioning reduced inflammation induced by LPS by ameliorating inflammatory cytokine levels, WBC counts, and oxidative damage, while improving antioxidant defenses.

Keywords: Exercise; Inflammation; Lipopolysaccharide; Oxidative stress; White blood cells

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INTRODUCTION

Inflammation is an evolutionarily conserved process[1] that involves responses against harmful stimuli,

tissue damage, or infection, to protect the host.[2] However, severe and prolonged inflammation induces extreme tissue injury, leading to different disorders such as arthritis, cardiovascular disease, diabetes, chronic obstructive lung disease (COPD), and cancer.[3] Inflammation causes cytokines and chemokines secretion from immune cells, which induce recruitment of other immunomodulatory cells to the oxidative stress/infection site. On the other hand, the heightened production of reactive oxygen species (ROS) by the immune cells at the inflammation site worsens oxidative stress and tissue damage.[4]

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is known to cause excessive inflammatory responses and ROS production.[5] Repeated exposure to the circulatory levels of LPS has been shown as a contributing factor to chronic diseases such as type 2 diabetes, COPD, and gastrointestinal inflammation.[6] LPS can induce toll-like receptor 4 (TLR4) activation which in turn, induces the production of proinflammatory cytokines monocyte chemotactic protein (MCP)-1, interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α.[7] Secretion of these proinflammatory cytokines is related to the accumulation of neutrophils which exacerbate inflammation.[8] Hence, LPS is used to produce experimental models of inflammation.[9-11]

It is effectively established that exercise training enhances immunomodulatory cell responses by increasing pro- and anti-inflammatory mediators (IL-4, IL-5, and IL-1 receptor antagonist (IL-1ra)) secretion at the injury area.[12] The physical activity's anti-inflammatory effects might be mediated by suppression of cytokines and toll-like receptors expression.[13] Moreover, the exercise intensity and training duration have significant effects on immune system function.[12,14,15] Few studies have evaluated the impacts of different training protocols on inflammation following repeated exposure to LPS.[16-18] Therefore, the present study assessed the potential anti-inflammatory and anti-oxidative function of moderate exercise in LPS-challenged rats.

MATERIALS AND METHODS

Materials

Lipopolysaccharide (*Escherichia coli* O55:B5, LPS, Sigma-Aldrich Chemical Co., Germany), TNF-α enzyme-linked immunosorbent assay (ELISA) kit (Diaclone Co, France) and IL-1β and CRP ELISA kits (Zellbio Co, Germany) were purchased to conduct the present study.

Animals and Experimental Design

Thirty-two male Wistar rats (male, weight range 200–220 g, age 8 weeks old, from Mashhad University of Medical Sciences' animal house, Mashhad, Iran) were kept under controlled conditions (temperature 22 ± 2°C, 12 h/12 h light/dark cycles and free access to food and water). The NIH "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1996) was observed in this study and it was approved by Mashhad University of Medical Sciences' ethics committee (Ethics approval No. IR.MUMS.MEDICAL.REC.1400.069).

Exercise Protocol and Animal Groups

A motorized treadmill was employed for animal training. After a one-week familiarizing period, animals refusing to run voluntarily were excluded. Then, animals were distributed into 4 sets (each group had 8 rats) as follows: Control (sedentary and saline-injected), LPS (sedentary and LPS-injected), Exercise (EX; exercised but non-LPS-injected), and EX+LPS (exercised and LPS-injected). To acclimate to the device stressors, the LPS and control animals were placed on a silent treadmill for 8 weeks. The rats of exercise groups (i.e. EX and EX+LPS groups were primed with an increasing load of moderate training for two weeks and then, for six more weeks, animals were trained at 15 m/min speed (30 min/day, 6 days/week) (Figure 1).[19]

LPS Injection

During the final week of the study, for five consecutive days, the animals of the LPS and EX+LPS group were challenged with LPS (1 mg/kg, prepared in 0.2 mL saline) intraperitoneally (i.p.).[20] The control and exercise groups were received saline alone (0.2 mL, i.p.).

Blood Preparation, and Determination of Biochemical Parameters, and Total/differential White Blood Cells (WBC)

On the last day of the experiments, the animals were euthanized using deep anesthesia (xylazine (8 mg/kg) and ketamine (80 mg/kg), both administered i.p.) and blood (5 mL) was taken from the animals' hearts. Samples were subdivided and collected in two parts: one in tubes containing Ethylenediaminetetraacetic acid (EDTA) for

WBC counts, and the other in a clotting tube for serum isolation. Serums were isolated by 10 min centrifugation at 2000 rpm. Total and differential WBC counts were determined according to a previous study.[21] Serum was kept at -70°C for biochemical measurements. Oxidative stress indicator malondialdehyde (MDA) and antioxidant defense markers catalase (CAT), thiol, and superoxide dismutase (SOD) were assessed as previously reported.[22] The Griess reagent method was used for the determination of Nitric oxide (NO) metabolites (nitrite).[23] Using the above-noted ELISA kits, serum C-reactive protein (CRP), TNF-α, and IL-1β levels were measured.[24]

Statistical Analysis

Data have been shown as means ± SEM and SPSS version 22 software was used to analyze them. Statistical evaluation was done using one-way analysis of variance (ANOVA) and Tukey's post-test. Statistical significance was considered at $p<0.05$.

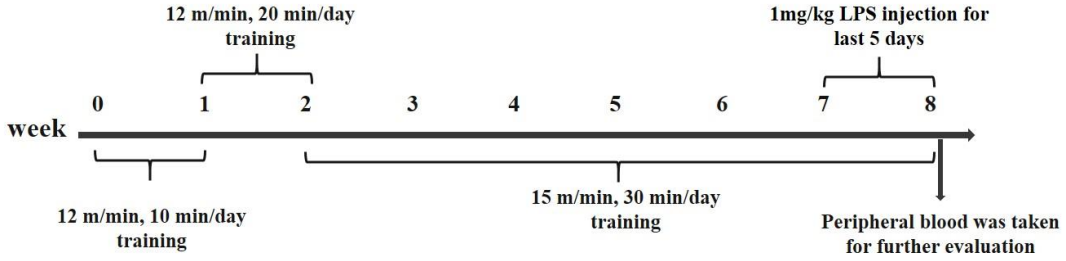

Figure 1. The study protocol. The animals of exercise preconditioning groups trained with an increasing load of moderate exercise 6 days per week for 8 weeks. During the final week, the animals of the LPS and EX+LPS group were challenged with LPS (1 mg/kg) intraperitoneally for 5 consecutive days.

RESULTS

Serum Inflammatory Markers

As indicated in Figure 2A-C, LPS significantly elevated levels of IL-1β, TNF-α, and CRP in serum ($p<0.05$, $p<0.001$, and $p<0.01$, respectively) versus the control animals. The serum level of TNF-α in the EX group was markedly higher than in the control rats ($p<0.05$). In the EX+LPS group, IL-1β, TNF-α, and CRP levels were markedly lower than in the LPS group ($p<0.01$ for all cases).

Serum Markers of Oxidative Stress

The levels of the oxidative indicator MDA, and total thiol content, CAT and SOD activity as markers of antioxidant defense were examined in all groups (Figure 3A-D). In response to LPS injection, thiol content, CAT and SOD activities were decreased, while the MDA level was elevated versus control animals ($p<0.001$ for all cases). Moderate-intensity exercise reduced total thiol content, SOD, and CAT activity in comparison with control rats ($p<0.001$, $p<0.01$, and $p<0.001$, respectively). In contrast, the EX+LPS group notably had increased levels of thiol, CAT and SOD activities but reduced MDA content versus the LPS group ($p<0.001$ for all, except SOD with $p<0.01$). The EX+LPS group had higher levels of MDA than the control and EX groups ($p<0.001$ for both cases), while CAT and SOD activity was remarkably lower in the EX+LPS group than the EX and control groups ($p<0.001$ for both cases).

Nitrite Concentration in Serum

Lower serum concentrations of NO metabolite (nitrite) in the control group were observed compared to the LPS and LPS+EX groups ($p<0.001$ and $p<0.01$, respectively). However, serum nitrite concentration was not statistically significantly different between the LPS and LPS+EX groups (Figure 4).

WBC Counts

Total and differential WBC counts in the blood samples are shown in Figure 5A-E. We found that LPS administration increased total WBC, lymphocytes, neutrophils, and monocytes counts compared to the control group ($p<0.001$ for all cases). Also, total WBC counts, and the number of neutrophils, monocytes, and eosinophils were significantly lower in the control group compared to the EX group ($p<0.05$ for eosinophils and $p<0.001$ for other cases). The EX+LPS group in

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comparison to the LPS group, had significantly lower total WBC, neutrophils, lymphocyte, and monocytes counts (p<0.001 for all cases). There was no statistical difference between the EX+LPS and LPS groups regarding the eosinophil count. However, the EX+LPS group compared to the EX group had significantly lower total WBC, monocytes, neutrophils (p<0.001 for all cases), and eosinophils (p<0.01) counts.

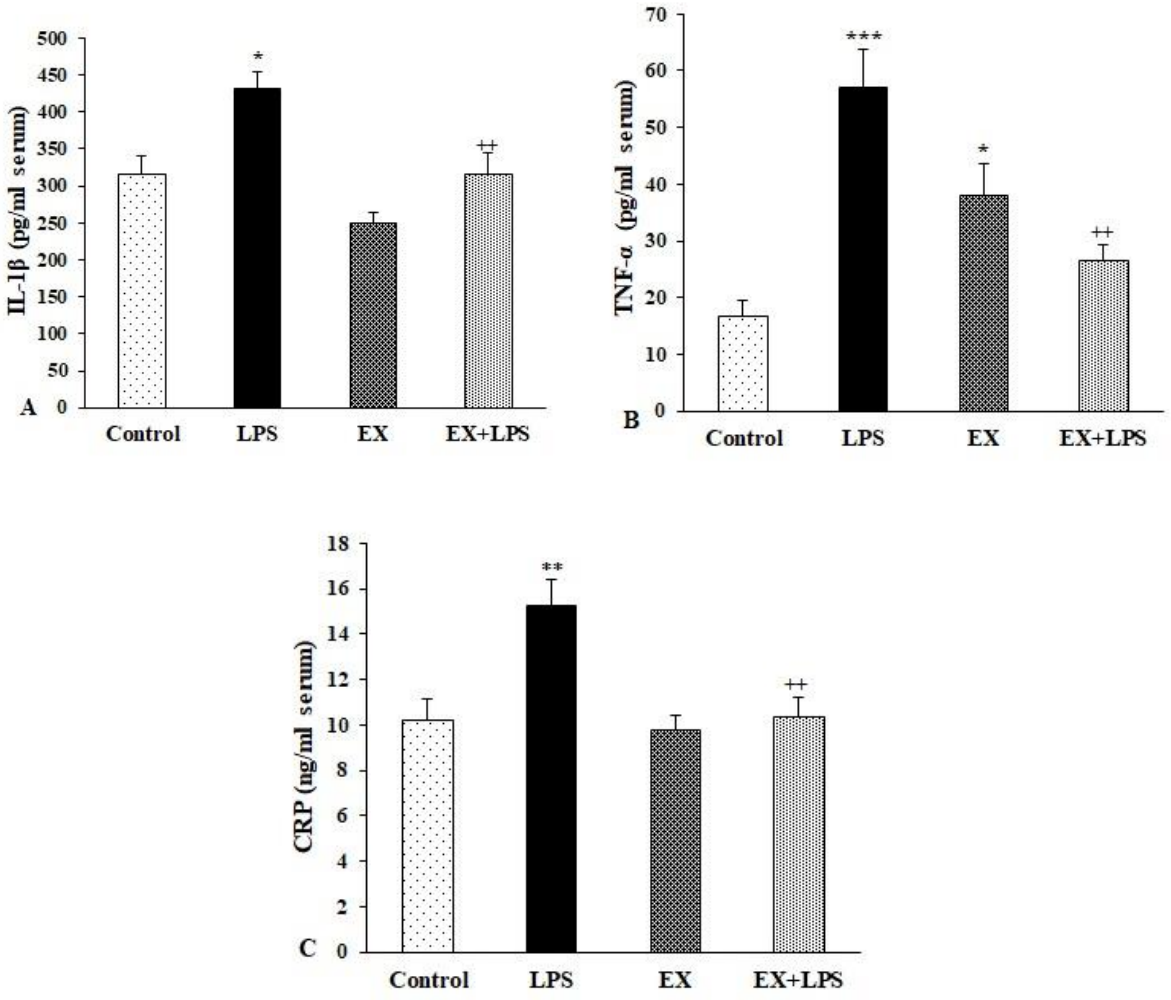

Figure 2. IL-1β (A), TNF-α (B), and CRP (C) levels in serum samples from the control, lipopolysaccharide (LPS), exercise (EX), and exercise+LPS (EX+LPS) groups. The results are expressed as mean±SEM (n=8 in each group). *p<0.05, **p<0.01, and ***p<0.001 show significant differences between the LPS and EX groups, and the control group. ^{++}p<0.01 shows a significant difference between the LPS+EX and the LPS group. IL-1β, interleukin (IL)- 1β; TNF-α, tumor necrosis factor-α; CRP, C-reactive protein.

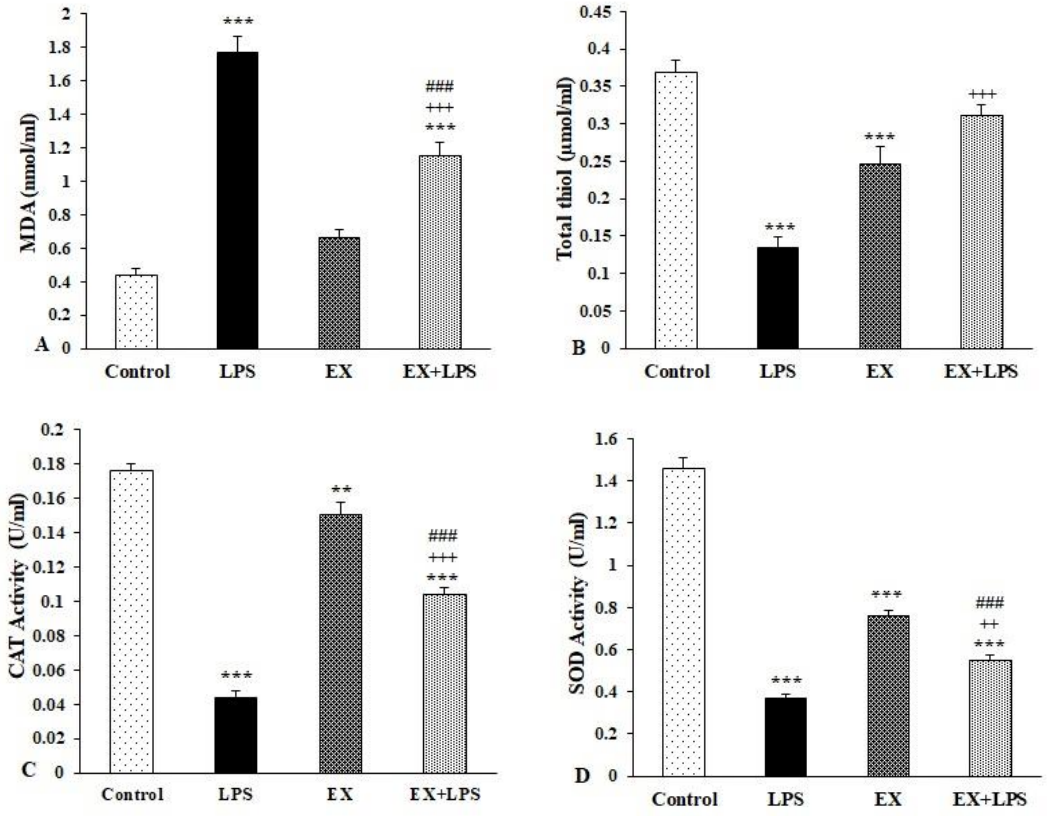

Figure 3. MDA (A), total thiol (B) and CAT (C), and SOD activity (D) in the control, lipopolysaccharide (LPS), exercise (EX), and exercise +LPS (EX+LPS) groups. The results are expressed as mean ± SEM (n=8 in each group). **p<0.01 and *** p<0.001 show marked differences between the LPS, EX and EX+LPS groups and the control group. $^{++}$ p<0.01 and $^{+++}$ p<0.001 show marked differences between the EX+LPS group and the LPS group. $^{###}$ p<0.001 shows a significant difference between the EX+LPS group and the EX group. MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase.

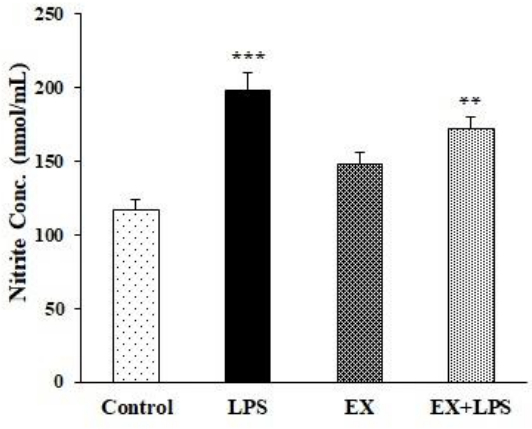

Figure 4. The Level of nitric oxide metabolite (nitrite) in the control, lipopolysaccharide (LPS), exercise (EX), and exercise +LPS (EX+LPS) groups. Data is expressed as mean ±SEM (n=8 in each group). ** p<0.01 and *** p<0.001 show marked differences between the LPS and EX+LPS groups and the control group.

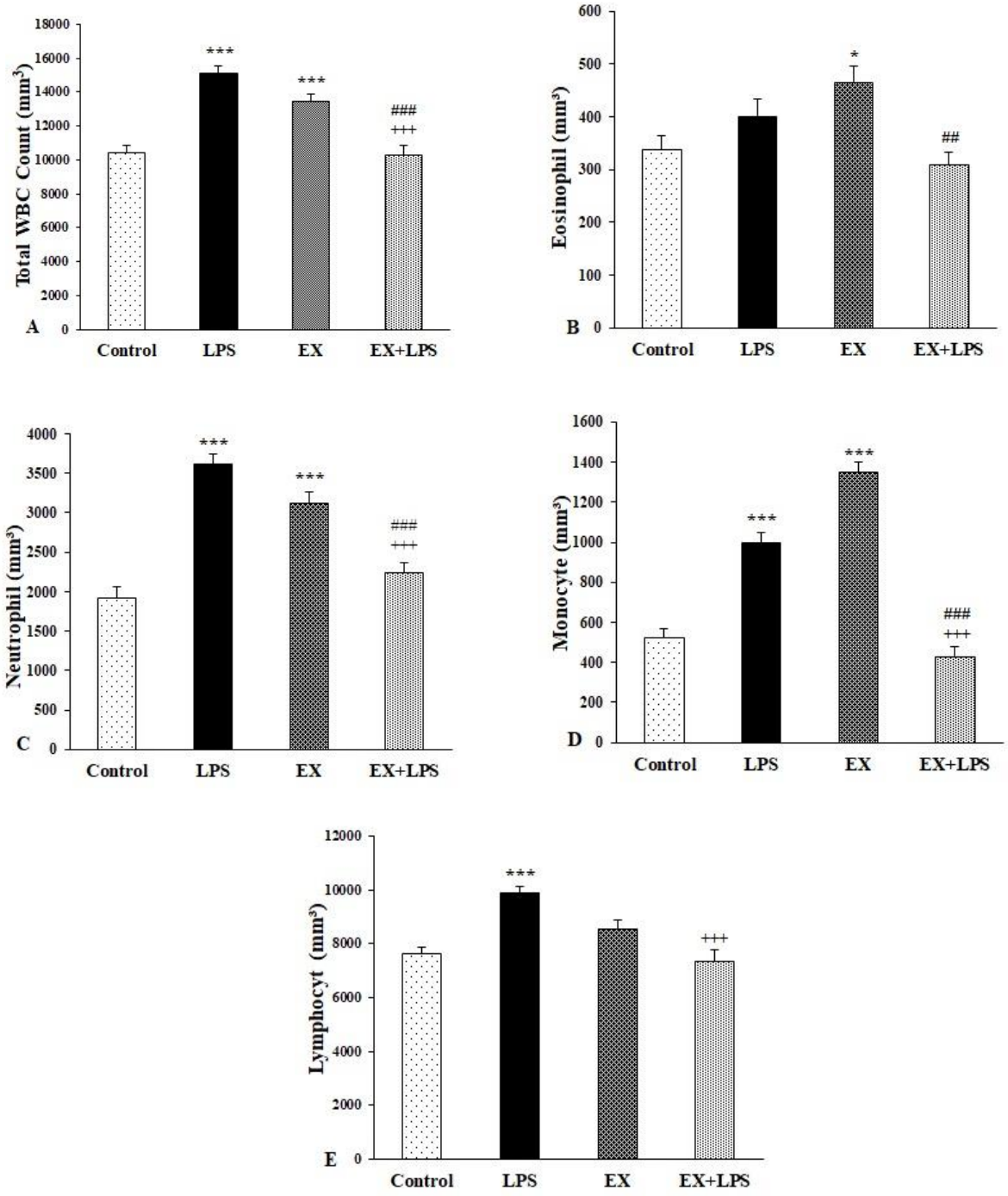

Figure 5. The number of total WBC (A), eosinophil (B), neutrophil (C), monocyte (D), and lymphocyte (E) in serum samples from the control, lipopolysaccharide (LPS), exercise (EX), and exercise +LPS (EX+LPS) groups. The data has been expressed as mean ± SEM (n=8 in each group). * p<0.05 and *** p<0.001 indicate marked differences between the LPS and EX groups and the control group. $^{+++}$ p<0.001 indicates a marked difference between the EX+LPS group and the LPS group, $^{\#\#}$ p<0.01 and $^{\#\#\#}$ p<0.001 indicate marked differences between the EX+LPS group and the EX group. WBC, white blood cells.

DISCUSSION

This study investigated the impact of moderate-intensity exercise preconditioning in rats treated with LPS as an experimental model of inflammation. LPS administration caused an increment in serum levels of TNF-α, IL-1β, and CRP which were ameliorated by exercise preconditioning. In agreement with the present findings, it has been established that three weeks of moderate exercise causes a reduction in levels of IL-6, TNF-α, IL-1β, and CRP in diabetic rats with pro-inflammatory status.[25] Similarly, a recent review showed that generally, exercise decreases TNF-α and CRP levels.[26] This finding might be due to the effect of exercise on reducing toll-like 4 receptor (TLR) expression in monocytes and macrophages which are involved in the induction of inflammatory cytokines release.[27] Another possible explanation is that training decreases the pro-inflammatory monocytes numbers in the blood circulation.[28]

In this study, exercise training increased serum TNF-α levels but did not markedly alter the CRP and IL-1β levels in comparison to the control rats. Some studies showed no changes in serum levels of IL-1β and TNF-α after moderate exercise, however, after prolonged or high-intensity training, elevation of these cytokines was indicated.[29] In a study, a two-fold increase in IL-1β and TNF-α and a 50-fold increment in serum level of IL-6 were reported in athletes after marathon races.[30] Therefore, the exercise duration (prolonged or acute) and intensity and time lag between training and cytokine analysis have been shown to affect the serum levels of pro- and anti-inflammatory cytokines.[15,31] In the present study, although there was an elevation in serum levels of TNF-α in the exercise group, exercise preconditioning prevented LPS-induced TNF-α production. Good physiological adaptation to exercise might cause anti-endotoxin capability of the immune system by enhancing leucocyte binding to endotoxin (kupffer cell phagocytosis) and anti-inflammatory cytokines production. It has been shown that during exercise, a marked increase in systemic IL-6 and catecholamine concentrations might increase IL-10 and inhibit LPS-induced TNF-α release.[32]

Also, it has been documented that regular exercise can modulate the balance of oxidants to antioxidants by enhancing the antioxidant system's radical scavenging activity.[23,33] Based on the present findings, moderate exercise led to increased concentrations of thiol, and activity of CAT and SOD in the EX and EX+LPS groups, but decreased levels of MDA only in the LPS-treated rats. Similarly, another study showed that moderate exercise diminished ROS release by neutrophils and macrophages via attenuation of leucocytes responsiveness.[25] It was reported that voluntary wheel running by diabetic rats improved SOD, CAT, and glutathione peroxidase (GPX) activities, but reduced levels of MDA in blood.[34] Additionally, aerobic exercise for 6 weeks (treadmill running for 30 min/day at 18 m/min speed) promoted CAT and GPX activities and enhanced MDA scavenging in Wistar rats with liver oxidative stress.[35] Moreover, aerobic exercise might enhance cell oxidative adaptation and capacity by elevation the levels of mitochondrial oxidative enzymes and improving cellular insulin sensitivity .[36] Of note, intense exercise in 18 soccer players diminished serum CAT activity, while increasing MDA serum concentration, probably via exhausting the antioxidant defense system.[37] Bloomer et al. found that oxidative stress parameters in serum increased to the highest level after 120 minutes of exercise.[38] In brief, these results appear to oppose the present study's findings, and exercise triggers oxidative stress only when the intensity goes beyond a certain level. However, the pathophysiological mechanism by which enhanced intensity or a long duration of exercise surges oxidative stress, remains unidentified. Altogether, these results accentuate the importance of unravelling optimal exercise intensity to benefit from exercise under inflammatory/oxidative conditions.

The present study showed that repeated exposure to LPS increases the total and differential WBC count. It was indicated that two weeks of LPS injection at a dose of 1 mg/kg caused an increase in the total WBC and percentage of eosinophils, neutrophils and monocytes in the blood of rats, reflecting the induction of systemic inflammation.[39] In another study, a single injection of LPS (3, 5 and 10 mg/kg) resulted in the reduction of blood WBC at high doses and an elevation of WBC count at low doses in comparison to control rats.[40] The difference in the dose and frequency of LPS administration may explain discrepancies in the WBC count. In the present study, in the exercise group, the total WBC, neutrophils and monocytes counts increased. There are different reports about changes in the number of WBC after sports training of different intensities. In a

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previous study, the total and differential WBC count did not change significantly immediately and 24 hours after moderate-intensity exercise.[41] However, in human studies, an increase in the leukocytes following acute exercise or after competition in professional athletes, and an increase in neutrophils and a decrease in lymphocytes in marathon runners are indicated.[42] Also, it was revealed that high-intensity exercise results in considerable leukocytosis, despite the inter individual variability, whereas low–intensity exercise could not modulate the immune system.[43] Our results showed a decrease in total WBC, neutrophils, eosinophils, and monocytes counts following moderate exercise in LPS-treated rats. The results of a randomized clinical trial on 390 obese and sedentary postmenopausal women showed a decrease in blood total WBC and neutrophils counts after four, eight, and twelve kcal/kg/week of aerobic exercise training recommended for 6 months; however, no changes were found in monocyte, lymphocyte, basophil or eosinophil counts among the different exercise levels.[44] Another study identified that acute high-intensity exercise might increase the levels of epinephrine and norepinephrine which subsequently resulted in mobilization of white immune cells.[43] Our findings showed that in the EX+LPS group, the total WBC and eosinophil, neutrophil, and monocyte counts were lesser than in the exercise group. There are several possible explanations for the lower total WBC counts observed in the present work and some reports following exercise training.[44,45] Exercise may affect bone marrow hematopoiesis and thereby, influences WBC count.[46] Moreover, it has been suggested that exercise may alter the leukocyte subsets trafficking between blood and secondary lymphoid organs or inflamed tissues.[44]According to the literature, it seems that the employed exercise protocol in terms of type, timing of implementation of aerobic exercise, and frequency of LPS exposure can produce diverse effects on different WBC counts in human and animal models.[40-42]

As a limitation, we did not analyze lymphoid organs, or serum levels of pro-inflammatory IL-6 and anti-inflammatory IL-10, to achieve a better judgment on how exercise preconditioning affects the immune system in LPS-treated rats.

Taken together, our findings show that moderate exercise could alleviate inflammatory status. The exercise preconditioning modulatory effects might be mediated by decreased levels of inflammatory cytokines, an enriched arsenal of anti-oxidative defenses, and decreased leukocytes counts, suggesting that moderate exercise can modulate inflammatory/oxidative conditions.

STATEMENT OF ETHICS

The ethics committee of Mashhad University of Medical Sciences approved the present work (Approval No. IR.MUMS.MEDICAL.REC.1400.069).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25(12):1822-32.
2. Li W, Wu X, Yu J, Ma C, Zhuang P, Zeng J, et al. Magnesium sulfate attenuates lipopolysaccharides-induced acute lung injury in mice. Chin J Physiol. 2019;62(5):203-9.
3. Roth GA, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159):1736-88.
4. Chatterjee S. Chapter Two - Oxidative Stress, Inflammation, and Disease. In: Dziubla T, Butterfield DA, editors. Oxidative Stress and Biomaterials: Academic Press; 2016. p. 35-58.5.
5. Zhang Y, Fang F, Tang J, Jia L, Feng Y, Xu P, et al. Association between vitamin D supplementation and mortality: systematic review and meta-analysis. Bmj. 2019;366.

6. Stewart I, Schluter PJ, Shaw GR. Cyanobacterial lipopolysaccharides and human health - a review. Environ Health. 2006;5:7.
7. Cardoso GH, Petry DM, Probst JJ, de Souza LF, Ganguilhet G, Bobinski F, et al. High-intensity exercise prevents disturbances in lung inflammatory cytokines and antioxidant defenses induced by lipopolysaccharide. Inflammation. 2018;41(6):2060-7.
8. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. J. pathol., Clin. res. 2004;202(2):145-56.
9. Wu XX, Huang XL, Chen RR, Li T, Ye HJ, Xie W, et al. Paeoniflorin Prevents Intestinal Barrier Disruption and Inhibits Lipopolysaccharide (LPS)-Induced Inflammation in Caco-2 Cell Monolayers. Inflammation. 2019;42(6):2215-25.
10. Mendez ME, Sebastian A, Murugesh DK, Hum NR, McCool JL, Hsia AW, et al. LPS-Induced Inflammation Prior to Injury Exacerbates the Development of Post-Traumatic Osteoarthritis in Mice. J Bone Miner Res. 2020;35(11):2229-41.
11. Yu R, Li Q, Feng Z, Cai L, Xu Q. m6A reader YTHDF2 regulates LPS-induced inflammatory response. Int J Mol Sci. 2019;20(6):1323.
12. Suzuki K, Hayashida H. Effect of exercise intensity on cell-mediated immunity. Sports. 2021;9(1):8.
13. Collao N, Rada I, Francaux M, Deldicque L, Zbinden-Foncea H. Anti-Inflammatory Effect of Exercise Mediated by Toll-Like Receptor Regulation in Innate Immune Cells - A Review. Int Rev Immunol. 2020;39(2):39-52.
14. Nobari H, Cholewa JM, Pérez-Gómez J, Castillo-Rodríguez A. Effects of 14-weeks betaine supplementation on pro-inflammatory cytokines and hematology status in professional youth soccer players during a competition season: a double blind, randomized, placebo-controlled trial. J Int Soc Sports Nutr. 2021;18(1):42.
15. Gholamnezhad Z, Boskabady MH, Hosseini M, Sankian M, Khajavi Rad A. Evaluation of immune response after moderate and overtraining exercise in wistar rat. Iran J Basic Med Sci. 2014;17(1):1-8.
16. Nunes PRP, Martins FM, Souza AP, Carneiro MAS, Orsatti CL, Michelin MA, et al. Effect of high-intensity interval training on body composition and inflammatory markers in obese postmenopausal women: a randomized controlled trial. Menopause. 2019;26(3):256-64.
17. Baygutalp F, Buzdağlı Y, Ozan M, Koz M, Kılıç Baygutalp N, Atasever G. Impacts of different intensities of exercise on inflammation and hypoxia markers in low altitude. BMC Sports Sci Med Rehabil. 2021;13(1):145.
18. Giraldo E, Garcia JJ, Hinchado MD, Ortega E. Exercise intensity-dependent changes in the inflammatory response in sedentary women: role of neuroendocrine parameters in the neutrophil phagocytic process and the pro-/anti-inflammatory cytokine balance. Neuroimmunomodulation. 2009;16(4):237-44.
19. Khoshkhouy F, Farshbaf A, Mahmoudabady M, Gholamnezhad Z. Effects of moderate exercise on lipopolysaccharide-induced inflammatory responses in rat's cardiac tissue. Cytokine. 2021;138:155409.
20. Hou S, Ding H, Lv Q, Yin X, Song J, Landén NX, et al. Therapeutic effect of intravenous infusion of perfluorocarbon emulsion on LPS-induced acute lung injury in rats. PloS one. 2014;9(1):e87826.
21. Saadat S, Beheshti F, Askari VR, Hosseini M, Roshan NM, Boskabady MH. Aminoguanidine affects systemic and lung inflammation induced by lipopolysaccharide in rats. Respir. Res. 2019;20(1):1-13.
22. Amin F, Roohbakhsh A, Memarzia A, Kazerani HR, Boskabady MH. Immediate and late systemic and lung effects of inhaled paraquat in rats. J. Hazard. Mater. 2021;415:125633.
23. Jahangiri Z, Gholamnezhad Z, Hosseini M, Beheshti F, Kasraie N. The effects of moderate exercise and overtraining on learning and memory, hippocampal inflammatory cytokine levels, and brain oxidative stress markers in rats. J Physiol Sci. 2019;69(6):993-1004.
24. Joshi L, Ponnana M, Sivangala R, Chelluri LK, Nallari P, Penmetsa S, et al. Evaluation of TNF-α, IL-10 and IL-6 cytokine production and their correlation with genotype variants amongst tuberculosis patients and their household contacts. PloS one. 2015;10(9):e0137727.
25. Belotto MF, Magdalon J, Rodrigues HG, Vinolo MA, Curi R, Pithon-Curi TC, et al. Moderate exercise improves leucocyte function and decreases inflammation in diabetes. Clin. Exp. Immunol. 2010;162(2):237-43.
26. Alizaei Yousefabadi H, Niyazi A, Alaee S, Fathi M, Mohammad Rahimi GR. Anti-inflammatory effects of exercise on metabolic syndrome patients: a systematic review and meta-analysis. Biol Res Nurs. 2021;23(2):280-92.
27. Gleeson M, McFarlin B, Flynn M. Exercise and Toll-like receptors. Exerc Immunol Rev. 2006;12(1):34-53.
28. Timmerman KL, Flynn MG, Coen PM, Markofski MM, Pence BD. Exercise training-induced lowering of inflammatory (CD14+ CD16+) monocytes: a role in the

anti-inflammatory influence of exercise? J Leukoc Biol. 2008;84(5):1271-8.
29. Starkie RL, Rolland J, Angus DJ, Anderson MJ, Febbraio MA. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. Am J Physiol Cell Physiol. 2001;280(4):C769-74.
30. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol. 1999;515 (Pt 1)(Pt 1):287-91.
31. Docherty S, Harley R, McAuley JJ, Crowe LAN, Pedret C, Kirwan PD, et al. The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. BMC Sports Sci Med Rehabil. 2022;14(1):5.
32. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. Faseb j. 2003;17(8):884-6.
33. Moghaddasi M, Javanmard SH, Reisi P, Tajadini M, Taati M. The effect of regular exercise on antioxidant enzyme activities and lipid peroxidation levels in both hippocampi after occluding one carotid in rat. J Physiol Sci. 2014;64(5):325-32.
34. Naderi R, Mohaddes G, Mohammadi M, Ghaznavi R, Ghyasi R, Vatankhah AM. Voluntary exercise protects heart from oxidative stress in diabetic rats. Adv Pharm Bull. 2015;5(2):231.
35. Li K, Zhu X, Wang Y, Zheng S, Dong G. Effect of aerobic exercise intervention on DDT degradation and oxidative stress in rats. Saudi J Biol Sci. 2017;24(3):664-71.
36. Gholamnezhad Z, Mégarbane B, Rezaee R. Molecular Mechanisms Mediating Adaptation to Exercise. Adv Clin Exp Med. 2020;1228:45-61.
37. Kiyici F, Kishali NF. Acute effect of intense exercises on serum superoxide dismutase, catalase and malondialdehyde levels in soccer players. J Sports Med Phys Fitness. 2012;52(1):107-11.
38. Bloomer R, Davis PG, Consitt L, Wideman L. Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. Int J Sports Med. 2007;28(01):21-5.
39. Boskabady M, Khazdair MR, Bargi R, Saadat S, Memarzia A, Mohammadian Roshan N, et al. Thymoquinone Ameliorates Lung Inflammation and Pathological Changes Observed in Lipopolysaccharide-Induced Lung Injury. Evid Based Complement Alternat Med. 2021;2021:6681729.
40. Fodor RS, Georgescu AM, Cioc AD, Grigorescu BL, Cotoi OS, Fodor P, et al. Time- and dose-dependent severity of lung injury in a rat model of sepsis. Rom J Morphol Embryol. 2015;56(4):1329-37.
41. Gholamnezhad Z, Boskabady MH, Hosseini M, Aghaei A, editors. Effect of different loads of exercise and Nigella sativa L. seed extract on serologic and hematologic parameters in rat. Indian J Exp Biol. 2019; 57:21-29.
42. Gleeson M. Immune function in sport and exercise. J Appl Physiol. 2007;103(2):693-9.
43. Neves PRDS, Tenório TRDS, Lins TA, Muniz MTC, Pithon-Curi TC, Botero JP, et al. Acute effects of high- and low-intensity exercise bouts on leukocyte counts. J Exerc Sci Fit 2015;13(1):24-8.
44. Johannsen NM, Swift DL, Johnson WD, Dixit VD, Earnest CP, Blair SN, et al. Effect of different doses of aerobic exercise on total white blood cell (WBC) and WBC subfraction number in postmenopausal women: results from DREW. PLoS One. 2012;7(2):e31319.
45. Michishita R, Shono N, Inoue T, Tsuruta T, Node K. Effect of exercise therapy on monocyte and neutrophil counts in overweight women. Am J Med Sci. 2010;339(2):152-6.
46. Chen J, Zhou R, Feng Y, Cheng L. Molecular mechanisms of exercise contributing to tissue regeneration. Signal Transduct. Target. 2022;7(1):1-24.