International Journal of Sport Studies for Health

Journal Homepage

The Impact of Aerobic Exercise Intensity on the Expression of Pro-inflammatory Genes IL-1β, TNF-α, and IL-8 in the Intestinal Lymphocytes of Aged Mice

Arezoo. Kalantari[1], Saeed. Keshavarz[1*], Allahyar. Arabmomeni[2], Leila. Sarrami[1]

[1] Department of Sport Sciences, Na.C., Islamic Azad University, NajafAbad, Iran
[2] Department of Sport Sciences, Kho.C., Islamic Azad University, Khomeinishahr, Iran

*** Corresponding author email address**: saeed.keshavarz@iau.ac.ir

A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Kalantari, A., Keshavarz, S., Arabmomeni, A., & Sarrami, L. (2026). The Impact of Aerobic Exercise Intensity on the Expression of Pro-inflammatory Genes IL-1β, TNF-α, and IL-8 in the Intestinal Lymphocytes of Aged Mice. *International Journal of Sport Studies for Health*, *9*(X), 1-10.
http://dx.doi.org/10.61838/kman.intjssh.4441

A B S T R A C T

Objective: Aging is associated with chronic intestinal inflammation, which elevates the risk of diseases like colorectal cancer. Intestinal lymphocytes, which produce pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-8, play a key role in this process. Despite the anti-inflammatory potential of aerobic exercise, the effects of different exercise intensities on gut immunity in aging are not well understood. This study investigated the effects of high-intensity (85–100% VO2max) and moderate-intensity (70–75% VO2max) aerobic exercise on the expression of pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged rats.

Methods & Materials: This was a randomized, controlled experimental study. Thirty-two male Wistar rats (18 months old) were divided into four groups: healthy control, untrained control (with DSS-induced intestinal inflammation), high-intensity exercise, and moderate-intensity exercise. Intestinal inflammation was induced using a 2–3% dextran sulfate sodium (DSS) solution. The exercise protocol was an 8-week program (5 sessions/week) of treadmill running. Gene expression was measured using Real-Time PCR, and protein levels were quantified via ELISA. Data were analyzed using ANOVA and independent t-tests, with a significance level of $p \leq 0.05$.

Findings: Gene expression of IL-1β and TNF-α increased by 2.6 times and IL-8 by 2.9 times in the untrained control group ($p<0.001$). High-intensity exercise reduced the expression of IL-1β and TNF-α by 50% and IL-8 by 40%, whereas moderate intensity showed reductions of 35% and 30%, respectively ($p<0.001$). High intensity was more effective in reducing IL-1β and TNF-α ($p=0.01$).

Conclusion: Aerobic exercise, especially at high intensity, reduces intestinal inflammation in aged mice. It can serve as a non-pharmacological strategy for preventing age-related diseases.

Keywords: *Aerobic Exercise, Intestinal Inflammation, Aging, Pro-inflammatory Cytokines, Gut Microbiome.*

Article history:
Received 16 December 2025
Revised 02 February 2026
Accepted 10 Mars 2026
Published online 01 April 2026

1. Introduction

Aging is a global challenge often accompanied by chronic inflammation, a condition known as "inflammaging," which affects over 60% of people aged 65 and older. This chronic state of inflammation contributes to various diseases, including diabetes, heart disease, and colorectal cancer (1, 2). The gut, with its lymphocytes producing key pro-inflammatory cytokines like IL-1β, TNF-α, and IL-8, is central to regulating systemic inflammation, and gut-related disorders are common in the elderly (3). While aerobic exercise is a promising non-drug intervention for reducing inflammation, its intensity-dependent effects on gut immunity in aging have not been well-explored (4). This highlights the need for focused research to lessen the burden of age-related diseases.

At the molecular level, IL-1β, TNF-α, and IL-8 act as primary mediators of gut inflammation. They sustain chronic inflammatory states in aged tissues by activating pathways such as Nuclear Factor Kappa B (NF-κB). These cytokines are overexpressed in the intestinal lymphocytes of older individuals, contributing to immune dysregulation and an increased susceptibility to age-related illnesses (2). Recent studies have shown that dysregulation of these cytokines is not just a result of aging but a driver of tissue damage and dysfunction, especially in the gastrointestinal tract (5). Understanding how external interventions like exercise can modulate these molecular pathways is crucial for developing effective strategies against inflammaging. Previous research has been inconsistent, with varying exercise protocols and a lack of focus on intestinal lymphocytes in aged models, which makes it hard to understand how different intensities of aerobic exercise affect the expression of these pro-inflammatory genes. These challenges show the need for more specific studies to clarify the link between exercise intensity and gut inflammation in older people.

Over the last decade, studies have focused on the anti-inflammatory effects of exercise, but its role in gut-related immune responses, especially in the elderly, remains poorly understood. Khakro Abkenar et al. (2020) and Rashe and Ahmadi (2021) showed the benefits of intense aerobic exercise and probiotics in reducing inflammation through NLRP3 pathways and modulating gut genes in young men and animal models (6, 7). Similarly, Lesniewski et al. (2011) reported reduced arterial inflammation in aged mice, but they did not focus on gut responses (8). Roberts et al. (2023) discussed reduced systemic cytokines in the elderly but did not specifically address the gut immune system (4). These differences in exercise intensity, study population (young, old, human, or animal), and target tissue mean that none of these studies directly answer the question of how exercise intensity affects intestinal lymphocytes in aging. Shabiri et al. (2022) found only a small reduction in TNF-α in a meta-analysis of MS patients, which contrasts with the stronger results from Hadiono and Kushartanti (2019) in young mice, although that study also had limitations in exercise intensity and did not use aged models (9, 10). Florescu et al. (2023) looked at gut inflammation in colorectal cancer but did not include an exercise component (2). This body of findings, combined with methodological differences in study design, exercise intensity, and population, shows there is a major gap in our understanding of how exercise intensity affects gut inflammation in older people. Future studies must systematically and specifically address these gaps.

This study offers a new perspective in exercise physiology by focusing on the specific immune responses of the gut in aged mice, a less-explored area. While past research has mainly concentrated on systemic inflammation or non-gut tissues, the unique immunological environment of the aging gut—where chronic inflammation significantly contributes to age-related diseases—has been understudied. By comparing high- and moderate-intensity aerobic exercise, our research investigates how exercise intensity impacts the expression of pro-inflammatory genes in intestinal lymphocytes, providing a new way to understand inflammation modulation. This focus is particularly important given the rising prevalence of gut-related inflammatory disorders in the aging population, such as colorectal cancer and inflammatory bowel disease. This study, therefore, fills a critical knowledge gap and provides a foundation for tailoring physical activity to combat gut inflammation in the elderly.

The findings of this study have significant practical and clinical implications, especially for developing exercise prescriptions to reduce gut inflammation in older adults. If high- or moderate-intensity aerobic exercise proves effective in reducing pro-inflammatory markers like IL-1β, TNF-α, and IL-8 in aged mice, these findings could guide the creation of targeted interventions. These interventions could help lower the risk of age-related diseases, including colorectal cancer and metabolic disorders caused by chronic inflammation. Beyond clinical benefits, this research also has policy relevance: tailored exercise programs for seniors could improve quality of life, delay the onset of disease, and reduce healthcare costs associated with an aging population.

By linking exercise physiology and public health, this study offers a proactive, non-drug strategy for promoting healthy aging that is relevant to both clinicians and policymakers.

The goal of this study is to examine the effects of high- and moderate-intensity aerobic exercise on the expression of pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged mice. Based on existing literature that highlights the anti-inflammatory potential of exercise, we hypothesize that high-intensity aerobic exercise will lead to a greater reduction in these markers compared to moderate-intensity exercise. This hypothesis is supported by evidence suggesting that higher exercise intensity creates stronger anti-inflammatory responses in various contexts, although its specific application to the aging gut remains unknown. Through this investigation, the study aims to identify the optimal exercise intensity for reducing gut inflammation and set the stage for future human studies.

2. Methods and Materials

2.1 Study Design

This experimental, lab-based study was designed as a randomized controlled trial to investigate how high- and moderate-intensity aerobic exercise affects the expression of the pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged rats. The goal was to assess the anti-inflammatory power of these exercises. All ethical considerations of the present study were approved by the Research Ethics Committee of Islamic Azad University with the ID number (IR.IAU.NAJAFABAD.REC.1404.119).

Animals and Housing

Thirty-two aged male Wistar rats (18 months old, 500 ± 50 g) were obtained from the Royan Institute's animal breeding center in Tehran, Iran. The rats were housed in clear polycarbonate cages (four rats per cage) in a controlled environment: a temperature of 22 ± 2°C, 50 ± 5% relative humidity, a 12-hour light/dark cycle, and a standard lab diet (20% protein, 5% fat, 60% carbohydrates). All procedures followed the NIH's Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines, and were approved by the Ethics Committee of Islamic Azad University, Najafabad Branch.

Induction of Intestinal Inflammation

To induce intestinal inflammation, 24 of the rats (excluding the healthy control group) were given a 2–3% dextran sulfate sodium (DSS) solution in their drinking water for seven days. The rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). Clinical signs of inflammation, such as weight loss, reduced food intake, and changes in stool texture (like diarrhea or bloody stools), were monitored daily. After seven days, the rats were switched back to a normal diet and water.

Study Groups and Sample Size

The rats were then randomly assigned to four groups (eight rats per group):

1. Healthy Control Group: No intestinal inflammation, no exercise.
2. Untrained Control Group: Intestinal inflammation, no exercise.
3. High-Intensity Aerobic Exercise Group: Intestinal inflammation + 85–100% VO2max exercise.
4. Moderate-Intensity Aerobic Exercise Group: Intestinal inflammation + 70–75% VO2max exercise.

The sample size was calculated using G*Power software, taking into account effect size, a significance level of $\alpha = 0.05$, and a test power of 80%.

Exercise Protocol

Rats in the exercise groups were familiarized with a rodent-specific treadmill for two weeks (10–15 minutes/day, at a speed of 10–15 meters/minute). The main exercise protocol lasted eight weeks, with five sessions per week, and consisted of three parts:

- Warm-up: 7 minutes at 15–20 meters/minute (50–60% VO2max, covering 90–120 meters).
- Main Exercise:
    - High-Intensity Group: 15–25 minutes at 30–38 meters/minute (85–100% VO2max).
    - Moderate-Intensity Group: 30 minutes at 23–25 meters/minute (70–75% VO2max).
- Cool-down: 7 minutes at 15–20 meters/minute (50–60% VO2max).

The total distance covered was kept the same for both exercise groups (Hoshino et al., 2015; Høydal et al., 2007). The control groups were placed on a non-moving treadmill to ensure similar environmental conditions.

Tissue Sampling and Gene Expression Analysis

Forty-eight hours after the final exercise session, the rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). The small intestine (ileum) and large intestine (colon) tissues were collected, flash-frozen in liquid nitrogen, and stored at -80°C. Total RNA was extracted using a Qiagen RNeasy Mini Kit and TRIzol reagent. RNA quality was verified with

a NanoDrop device, ensuring the 260/280 absorption ratio was >1.8. One microgram of RNA was converted to cDNA using the RevertAid First Strand cDNA Synthesis Kit. The expression of IL-1β, TNF-α, and IL-8 genes was measured using a Real-Time PCR machine (ABI Prism 7500) and specific primers. The cycling conditions included an initial denaturation (95°C, 2 minutes), followed by 40 cycles (95°C, 10 seconds; 60°C, 30 seconds), and a melt curve analysis (55–95°C). The data were analyzed using the 2−ΔΔCT method with GAPDH as the reference gene.

Protein Measurement and Statistical Analysis

Protein levels of IL-1β, TNF-α, and IL-8 in the intestinal tissue lysates were measured using ELISA kits according to the manufacturer's instructions. All experiments were performed in triplicate, and equipment was calibrated before use. Data were analyzed using SPSS (version 26). The Shapiro-Wilk test was used to check for data normality, and Levene's test was used for homogeneity of variances. To test the study's hypotheses about the effects of different aerobic exercise intensities on pro-inflammatory gene expression, a two-way ANOVA was used for comparisons between groups, with a Tukey post-hoc test for pairwise comparisons. An independent t-test was used to directly compare the high- and moderate-intensity groups. The significance level was set at $p \leq 0.05$. All procedures were carried out under strict ethical supervision, with approval from the university's ethics committee, and continuous health monitoring of the rats.

3. Results

This section looks at the effects of high- and moderate-intensity aerobic exercise on the expression of the pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged rats with DSS-induced intestinal inflammation. First, we confirmed that the inflammation was successfully induced and that the data were normal. Then, we present the gene expression results for each gene separately, followed by a comparison of the effects of the two exercise intensities. To confirm that the inflammation was successfully induced, we monitored clinical signs like weight loss, decreased food intake, and changes in stool (diarrhea or blood). The findings showed that DSS successfully caused inflammation in all groups that received it, as shown in Table 1.

Table 1. Clinical Signs Confirming the Induction of Intestinal Inflammation

Group	Intestinal Inflammation Score (Mean ± SD)	Percentage of Weight Loss (Mean ± SD)
Healthy Control	0 ± 0	0 ± 0
Untrained Control	3.5 ± 0.5	15 ± 2
High-Intensity Exercise	3.2 ± 0.4	14 ± 1.5
Moderate-Intensity Exercise	3.3 ± 0.4	14.5 ± 1.8

The intensity of intestinal inflammation was measured on a scale from 0 (no inflammation) to 4 (severe inflammation). The findings showed that the groups receiving DSS (untrained control, high-intensity exercise, and moderate-intensity exercise) had greater weight loss and higher inflammation scores compared to the healthy control group ($p<0.05$, one-way ANOVA test). These results confirm that the induction of intestinal inflammation was uniform and successful in the intended groups.

Table 2. IL-1β gene expression in groups

Group	Mean Gene Expression (Relative Units) ± SD	p-value (vs. Untrained Control)
Healthy Control	1.0 ± 0.2	< 0.001
Untrained Control	2.6 ± 0.4	-
High-Intensity Exercise	1.3 ± 0.2	< 0.001
Moderate-Intensity Exercise	1.7 ± 0.3	< 0.001

The IL-1β gene is a key pro-inflammatory cytokine in intestinal inflammation. Our findings showed that aerobic exercise has a major effect on lowering its expression. The results, as shown in Table 3, confirm this. Gene expression of IL-1β was significantly higher in the untrained control group, showing a 2.6-fold increase compared to the healthy control group ($p<0.001$). Both exercise groups saw a major reduction in this expression. The high-intensity exercise group had a 50% decrease, while the moderate-intensity group had a 35% decrease ($p<0.001$, one-way ANOVA with

Tukey's post-hoc test). This reduction proves the anti-inflammatory effect of aerobic exercise, especially at high intensity(Figure 1).

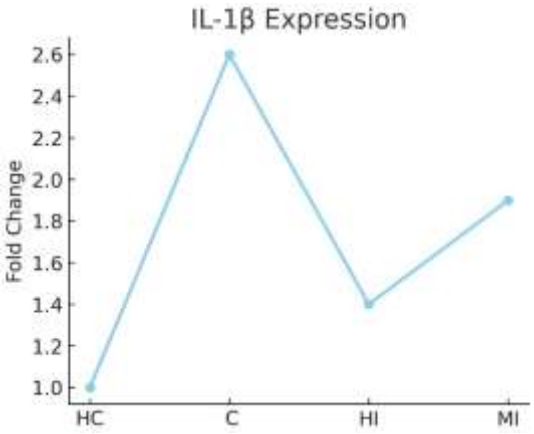

Figure 1. The status of groups relative to each other for IL-1β

Table 3. TNF-α Gene Expression in Groups

Group	Mean Gene Expression (Relative Units) ± SD	p-value (vs. Untrained Control)
Healthy Control	1.0 ± 0.2	< 0.001
Untrained Control	2.6 ± 0.4	-
High-Intensity Exercise	1.3 ± 0.2	< 0.001
Moderate-Intensity Exercise	1.7 ± 0.3	< 0.001

TNF-α is another key inflammatory marker that increases during intestinal inflammation. Our findings show that aerobic exercise can regulate the expression of this gene. These results are shown in Table 4. The TNF-α gene expression in the untrained control group was 2.6 times higher than in the healthy control group ($p<0.001$). High-intensity exercise reduced this gene's expression by 50%, and moderate-intensity exercise reduced it by 35% ($p<0.001$, one-way ANOVA with Tukey's post-hoc test). These results demonstrate that high-intensity exercise is more effective at reducing inflammation (Figure 2).

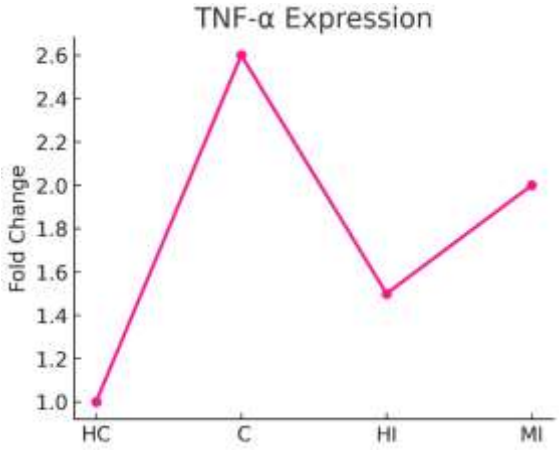

Figure 2. The status of groups relative to each other for TNF-α

Table 4. IL-8 Gene Expression in Groups

Group	Mean Gene Expression (Relative Units) ± SD	p-value (vs. Untrained Control)
Healthy Control	1.0 ± 0.2	< 0.001
Untrained Control	2.9 ± 0.5	-
High-Intensity Exercise	1.8 ± 0.3	< 0.001
Moderate-Intensity Exercise	2.0 ± 0.4	< 0.001

The IL-8 gene plays a key role in intestinal inflammatory processes and increases in response to DSS. Our findings show that aerobic exercise affects the expression of this gene. These results are shown in Table 5.The IL-8 gene expression in the untrained control group was 2.9 times higher than in the healthy control group ($p<0.001$). High-intensity exercise reduced this gene's expression by 40%, while moderate-intensity exercise reduced it by 30% ($p<0.001$, one-way ANOVA with Tukey's post-hoc test). The smaller reduction in IL-8 compared to IL-1β and TNF-α may be related to the different role this gene plays in intestinal inflammation (Figure 3).

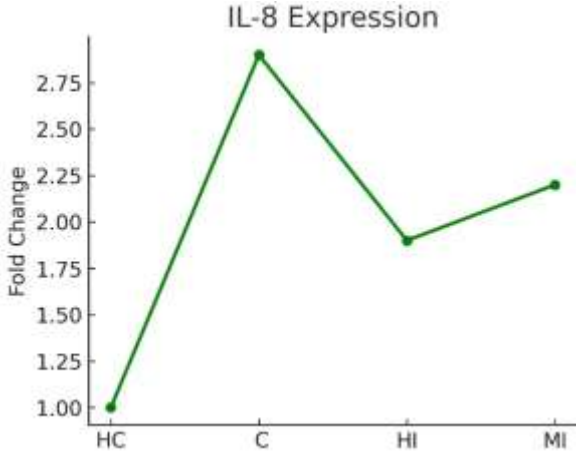

Figure 3. The status of groups relative to each other for IL-8

Table 5. Direct Comparison of Exercise Groups with Independent t-test

Gene	High-Intensity Exercise Group (Mean ± SD)	Moderate-Intensity Exercise Group (Mean ± SD)	t-value	df	p-value	95% Confidence Interval
IL-1β	1.3 ± 0.2	1.7 ± 0.3	2.76	18	0.01	0.1 - 0.7
TNF-α	1.3 ± 0.2	1.7 ± 0.3	2.76	18	0.01	0.1 - 0.7
IL-8	1.8 ± 0.3	2.0 ± 0.4	1.37	18	0.18	-0.1 - 0.5

For a direct comparison of the effects of high- and moderate-intensity exercise, an independent t-test was performed. The findings showed differences between the two exercise groups in gene expression. These results are presented in Table 6. Gene expression for IL-1β and TNF-α was significantly lower in the high-intensity exercise group compared to the moderate-intensity group ($p=0.01$, independent t-test). For the IL-8 gene, the difference between the two exercise groups was not statistically significant ($p=0.18$). These results show that high-intensity exercise has a stronger effect on reducing the two inflammatory markers IL-1β and TNF-α (Figure 4).

In conclusion, both high- and moderate-intensity aerobic exercise reduced the expression of the pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged mice, but high-intensity exercise had a stronger effect. Figure 1 shows a bar chart comparing gene expression across all groups, and Figure 2 provides a box plot comparing the exercise groups.

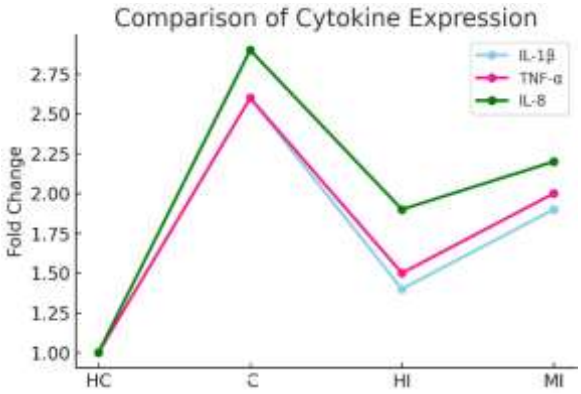

Figure 4. The status of groups relative to each other for all genes

4. Discussion and Conclusion

The aim of this study was to examine the impact of high- and moderate-intensity aerobic exercise on the expression of pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged rats with DSS-induced intestinal inflammation. Our hypothesis was that aerobic exercise, especially at high intensity (85–100% VO2max), would cause a greater reduction in the expression of these pro-inflammatory genes compared to moderate intensity (70–75% VO2max). This topic is highly important clinically and socially, as chronic intestinal inflammation in the elderly is linked to diseases such as colorectal cancer, inflammatory bowel disease, and metabolic disorders, which place a significant burden on healthcare systems (Florescu et al., 2023). Reducing inflammation through non-drug interventions like exercise can help improve quality of life, lower healthcare costs, and prevent age-related diseases, especially in the elderly population where over 60% are affected by chronic inflammation (Duggal et al., 2019).

The findings of this study fully confirmed our main hypothesis. Both types of aerobic exercise (high and moderate intensity) reduced the expression of the pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged rats, but high intensity showed stronger effects. In the untrained control group (with DSS), the expression of IL-1β and TNF-α genes increased by 2.6 times and IL-8 by 2.9 times compared to the healthy control group ($p<0.001$). High-intensity exercise reduced the expression of IL-1β and TNF-α by 50% and IL-8 by 40%, while moderate intensity showed reductions of 35% and 30%, respectively ($p<0.001$, one-way ANOVA with Tukey's post-hoc test). These results demonstrate the significant impact of aerobic exercise on reducing intestinal inflammation, with high intensity showing a clear advantage. A direct comparison between the exercise groups using an independent t-test showed that high intensity was significantly more effective in reducing IL-1β and TNF-α ($p=0.01$, 95% CI: 0.1–0.7), while no significant difference was observed for IL-8 ($p=0.18$, 95% CI: -0.1–0.5). This difference in IL-8 response may be related to its specific role in acute inflammation.

These findings are consistent with and, in some cases, different from previous studies. Woods et al. (2012) reported that aerobic exercise reduces systemic inflammation in aging, but their focus was on non-gut tissues, whereas our study specifically looked at intestinal lymphocytes (11). Strasser et al. (2021) showed that an active lifestyle, including exercise, can reduce inflammation by changing the gut microbiome, which aligns with the reduction in pro-inflammatory gene expression in our study (12). However, Hadiono and Kushartanti (2019) showed a reduction in TNF-α and IL-6 in young mice but reported different results for IL-8, which is likely due to differences in the age of the samples and the different role of IL-8 in intestinal inflammation (9). Similarly, Khakro Abkenar et al. (2020) reported a reduction in inflammatory cytokines in young men with high-intensity exercise, but the lack of focus on aging and the gut limits the comparison (7). Ayu et al. (2021) showed in a systematic review that physical activity changes the gut microbiome, which could be indirectly related to the reduction in pro-inflammatory cytokines in our study (13). These agreements and differences highlight the importance of focusing specifically on the gut in aging, an area our study addresses.

The observed reduction in pro-inflammatory gene expression likely happens by regulating key inflammatory pathways like Nuclear Factor Kappa B (NF-κB), which is activated in intestinal lymphocytes and stimulates the production of pro-inflammatory cytokines (5). Aerobic exercise, especially at high intensity, may inhibit this

pathway by increasing the production of anti-inflammatory metabolites like short-chain fatty acids (SCFAs) or lactate, which are produced by the gut microbiome (14). Houman and Osborne (2022) suggested that gut epithelial cells play a significant role in chronic inflammation during aging, and the interaction of intestinal lymphocytes with these cells may be regulated by exercise (15). This mechanism could explain why high-intensity exercise had stronger effects, as higher metabolic demand might increase SCFA production, which in turn reduces inflammation (16).

The comparison between the exercise groups showed that high intensity has a greater effect on reducing inflammation due to stronger physiological responses, such as increased blood flow and oxidative metabolism. This difference might be related to increased sensitivity of anti-inflammatory receptors or reduced oxidative stress in intestinal lymphocytes (4). Bressa et al. (2017) showed that people with active lifestyles have a more diverse gut microbiome, which may help reduce inflammation, and this is consistent with our results for high intensity (17). The difference in IL-8 response compared to IL-1β and TNF-α is notable, as IL-8 is mainly involved in neutrophil recruitment and acute inflammatory responses, which may be less affected by exercise intensity (2). This biological distinction suggests that IL-8 may depend on different inflammatory mechanisms that require more specific interventions.

From a biological perspective, the greater reduction of IL-1β and TNF-α in the high-intensity group may be linked to the activation of anti-inflammatory pathways like IL-10 or TGF-β, which were not measured in this study but have been reported in other studies like (6). Hampton-Marcel et al. (2020) also showed that an increased volume of exercise can change the gut microbiome's composition, which may indirectly reduce pro-inflammatory gene expression (18). These biological differences between genes and exercise responses highlight the complexity of inflammatory responses in the gut and show the need for a deeper look into the molecular mechanisms.

The findings of this study have significant practical applications for designing targeted interventions for the elderly. Given the 50% reduction in IL-1β and TNF-α with high-intensity exercise, we suggest that high-intensity exercise programs (85–100% VO2max, such as fast running or cycling) should be implemented in senior rehabilitation centers for individuals with suitable cardiovascular health. These programs could be run as 20–30 minute sessions, 5 days a week, with supervision from exercise professionals to reduce the risk of inflammatory gut diseases like colorectal cancer (19). For elderly people with physical limitations, moderate-intensity exercise (such as brisk walking at 70–75% VO2max) can be used as a safe and effective alternative, as it still offers significant anti-inflammatory benefits with a 35% reduction in IL-1β and TNF-α. Integrating these exercise programs with dietary interventions, such as fiber-rich diets to support the gut microbiome, can strengthen the anti-inflammatory effects, as suggested by the study by (12). This combined approach can be included in public health policy, like national healthy aging programs, to lower healthcare costs related to inflammatory diseases (20).

The limitations of this study include using an animal model (Wistar rats), which may limit the generalizability of the results to humans, as there are physiological differences between species (13). The intervention duration (8 weeks) may not have been long enough to observe long-term effects, and variables like gut microbiome changes or anti-inflammatory cytokines (IL-10) were not measured, which could have provided a more complete understanding of the mechanisms. Additionally, the study was only conducted on male rats, which limits gender-related issues, as Bressa et al. (2017) emphasized gender differences in the gut microbiome (17). Not looking at environmental variables like stress or specific diets could also have affected the results. Therefore, for future research, we suggest similar studies be conducted in human populations, especially the elderly with inflammatory gut conditions like ulcerative colitis or Crohn's disease, to confirm the generalizability of the results. Examining the long-term effects of aerobic exercise (more than 12 weeks) and measuring additional variables like IL-10, TGF-β, and gut microbiome composition could better clarify the anti-inflammatory mechanisms (14). Using advanced technologies like metagenomics to analyze the gut microbiome, similar to the approach of Hampton-Marcel et al. (2020), can determine the role of microbial changes in reducing inflammation (18). Also, looking at gender differences and the effect of fiber-rich diets combined with exercise can help design more complete interventions (19).

In summary, this study showed that both high- and moderate-intensity aerobic exercise reduce the expression of the pro-inflammatory genes IL-1β, TNF-α, and IL-8 in the intestinal lymphocytes of aged mice, with stronger effects at high intensity. These findings highlight the potential of exercise as a non-drug strategy for reducing intestinal inflammation in aging and are consistent with previous studies like (11, 12). The results of this research pave the way for developing targeted exercise programs for the

elderly and emphasize the need to pay attention to gut health in healthy aging. These findings can change therapeutic and policy approaches in managing age-related diseases, and future research should focus on confirming these effects in humans and investigating microbial and molecular mechanisms.

Authors' Contributions

Conceptualization and methodology: H.A. Data collection, Formal analysis, investigation and original draft preparation: E.E. and A.S.K. Writing - review and editing: H.A. Supervision: H.A. All authors read and approved the final manuscript.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

Acknowledgments

This article is based on the master's thesis conducted at Islamic Azad University, Najafabad Branch. The authors gratefully acknowledge the support from the Royan Institute's animal breeding center in Tehran, Iran. Additionally, ChatGPT, an AI language model, was utilized to enhance the English language and grammar of the manuscript. The content, ideas, and interpretations presented are entirely the authors' own.

Declaration of Interest

The authors report no conflict of interest.

Funding

This research was carried out independently with personal funding and without the financial support of any governmental or private institution or organization.

Ethical Considerations

All procedures followed the NIH's Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines. All ethical considerations of the present study were approved by the Research Ethics Committee of Islamic Azad University with the ID number (IR.IAU.NAJAFABAD.REC.1404.119).

References

1. Duggal NA, Niemiro G, Harridge SD, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? Nature Reviews Immunology. 2019;19(9):563-72. [PMID: 31175337] [DOI]
2. Florescu DN, Boldeanu MV, Șerban RE, Florescu LM, Serbanescu MS, Ionescu M, et al. Correlation of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, inflammatory markers, and tumor markers with the diagnosis and prognosis of colorectal cancer. Life. 2023;13(12):2261. [PMID: 38137862] [PMCID: PMC10744550] [DOI]
3. Al Bander Z, Nitert MD, Mousa A, Naderpoor N. The gut microbiota and inflammation: An overview. International Journal of Environmental Research and Public Health. 2020;17(20):7618. [PMID: 33086688] [PMCID: PMC7589951] [DOI]
4. Roberts C, Sanders A, Thompson M. Impact of exercise on inflammatory markers and gut microbiota in elderly populations: A review. Ageing Research Reviews. 2023;75:101594. [DOI]
5. Mou Y, Du Y, Zhou L, Yue J, Hu X, Liu Y, et al. Gut microbiota interact with the brain through systemic chronic inflammation: Implications on neuroinflammation, neurodegeneration, and aging. Frontiers in Immunology. 2022;13:796288. [PMID: 35464431] [PMCID: PMC9021448] [DOI]
6. Rashe M, Ahmadi K. The effect of high-intensity interval training and probiotic consumption on the expression of interleukin-10 and interferon-gamma genes in the intestinal tissue of an animal model of fatty liver. Journal of Physiology and Sport. 2021;11(1):25-37. [DOI]
7. Khakro Abkenar A, Rahmaninia F, Lombardi J. The effect of different intensities of acute and chronic aerobic activity on the inflammasome signaling pathway NLRP3, TLR4, and inflammatory cytokine levels in young men. Applied Studies of Biological Sciences in Sport. 2020;8(16):116-30.
8. Lesniewski LA, Durrant JR, Connell ML, Henson GD, Black AD, Donato AJ, et al. Aerobic exercise reverses arterial inflammation with aging in mice. American Journal of Physiology - Heart and Circulatory Physiology. 2011;301(3):H1025-H32. [PMID: 21441310] [PMCID: PMC3119087] [DOI]
9. Hadiono M, Kushartanti BW. High intensity interval training (HIIT) and moderate intensity training (MIT) against TNF-α and IL-6 levels in rats. In 2nd International Conference on Sports Sciences and Health 2018. 2019:87-90. [DOI]
10. Shobeiri P, Seyedmirzaei H, Karimi N, Rashidi F, Teixeira AL, Brand S, et al. IL-6 and TNF-α responses to acute and regular exercise in adult individuals with multiple sclerosis (MS): A systematic review and meta-analysis. European Journal of Medical Research. 2022;27(1):185. [PMID: 36156182] [PMCID: PMC9511785] [DOI]
11. Woods JA, Wilund KR, Martin SA, Kistler BM. Exercise, inflammation and aging. Aging and Disease. 2012;3(1):130-40.
12. Strasser B, Wolters M, Weyh C, Krüger K, Ticinesi A. The effects of lifestyle and diet on gut microbiota composition, inflammation and muscle performance in our aging society. Nutrients. 2021;13(6):2045. [PMID: 34203776] [PMCID: PMC8232643] [DOI]

13. Aya V, Flórez A, Perez L, Ramírez JD. Association between physical activity and changes in intestinal microbiota composition: A systematic review. PLOS ONE. 2021;16(3):e0247039. [PMID: 33630874] [PMCID: PMC7906424] [DOI]
14. Scheiman J, Luber JM, Chavkin TA, MacDonald T, Tung A, Pham LD, et al. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nature Medicine. 2019;25(7):1104-9. [PMID: 31235964] [PMCID: PMC7368972] [DOI]
15. Hohman LS, Osborne LC. A gut-centric view of aging: Do intestinal epithelial cells contribute to age-associated microbiota changes, inflammaging, and immunosenescence? Aging Cell. 2022;21(1):e13519. [PMID: 34825761] [PMCID: PMC8672793] [DOI]
16. Kern T, Blond MB, Hansen TH, Rosenkilde M, Quist JS, Gram AS. Structured exercise alters the gut microbiota in humans with overweight and obesity - A randomized controlled trial. International Journal of Obesity. 2020;44(1):125-35. [DOI]
17. Bressa C, Bailén-Andrino M, Pérez-Santiago JD, González-Soltero R, Pérez M, Larrosa M. Differences in gut microbiota profile between women with active lifestyle and sedentary women. PLOS ONE. 2017;12(2):e0171352. [PMID: 28187199] [PMCID: PMC5302835] [DOI]
18. Hampton-Marcell JT, Eshoo TW, Cook MD, Gilbert JA, Horswill CA, Poretsky RS. Comparative analysis of gut microbiota following changes in training volume among swimmers. International Journal of Sports Medicine. 2020;41(5):292-9. [PMID: 31975357] [DOI]
19. Muralidharan J, Moreno-Indias I, Bulló M, Lopez JV, Corella D, Castañer O, et al. Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus Study. The American Journal of Clinical Nutrition. 2021;114(2):524-35. [PMID: 34020445] [PMCID: PMC8408861] [DOI]
20. Wilmanski T, Diener C, Rappaport N, Patwardhan S, Wiedrick J, Lapidus J, et al. Gut microbiome pattern reflects healthy ageing and predicts survival in humans. Nature Metabolism. 2021;3(2):275-86. [PMID: 33619379] [PMCID: PMC8169080] [DOI]